Vol. 10 No. 2 **July-December 2016**

Tungs' Medical Journal

CONTENTS IN BRIEF

EDITORIAL

1 **EPA-based Assessment in Residency Training Program**
Ching-Shiang Chi

REVIEW ARTICLE

5 **Anti-Programmed Death-1 Antibody: Review of a Novel Promising Treatment for Advanced Non-Small Cell Lung Cancer**
Sheng-Han Cheng, Kee-Ching Jeng, Kuo-Shu Chen

ORIGINAL ARTICLE

12 **Apple Polyphenols Inhibit Lipopolysaccharide-induced Inflammation via Regulation of COX, iNOS, and MAPK Signaling Pathways**
Yi-Chen Lee, Huei-Jane Lee

21 **Percutaneous Endoscopic Foraminoplasty for Lumbar Spinal Lateral Stenosis**
Shao-Keh Hsu, Chia-Hsiao Keo, Sheng-Chi Chen

CASE REPORT

29 **Infantile Hypertrophic Pyloric Stenosis: A Case Report and Literature review**
Ssu-Wen Chen, Pin-Ho Pan, Peng-Nien Tuan, Chien-Hung Kuo1 Min-Yung Kuo, Chiao-Yon Huang

34 **Two viral- and Pneumocystis Jiroveci-Related Pneumonia Infections in a Patient with Dermatomyositis: A Case Report**
Kuo-Hsi Lin, Hau-Hsin Wu, Chih-Ming Chen, Bei-Rong Su, Mao-Wang Ho

39 **General Anesthesia in a Pediatric Patient with Leigh Syndrome: A Case Report and Literature Review**
Ching-Hsin Chang, Chun-Lai Ma, Kuo-Sheng Huang, Liang-Chieh Wang, Yiu-Sion Chen, Kee-Ming Man, Yi-Chun Hung

43 **Anaphylactic Shock During Central Venous Catheter Insertion: A Case Report**
Liang-Chieh Wang, Chun-Lai Ma, Yiu-Sion Chen, Kee-Ming Man, Kam-Sun Cheung, Kuo-Sheng Huang

PATHOLOGY PAGE

48 **Malignant Peritoneal Mesothelioma**
Guo-Dong Chen, Tzu-Ming Chang, Tang-Yi Tsao, Jong-Shiaw Jin

52 **Intraductal Papillary Mucinous Neoplasm of the Pancreas**
Ji-Kuen Yu, Lien-Fu Lin, Jong-Shiaw Jin

IMAGE

56 **Perforated Appendicitis: KUB Findings in a Patient with Atypical Presentation**
Leo Sun, Xi-Shuo Qiu, Meng-Yu Shi

Editorial

EPA-based Assessment in Residency Training Program

Ching-Shiang Chi

Tungs' Taichung Metroharbor Hospital Taichung, Taiwan

Received: Nov. 25, 2016; Accepted: Nov. 28, 2016

Abstract

Entrustable Professional Activities (EPAs) are specific tasks selected by members of professional fields to assess the competency of knowledge and skills of their program trainees. Those activities should be observable, measurable and based on daily clinical works . Entrustable Professional Activities can be used to decide if a trainee can be trusted to complete a selected task or still need a certain level of supervision. Usually each EPA contains several different aspects of competencies; and five developmental milestones are used to evaluate a trainee's competency of the EPA selected. When a trainee reaches at milestone level 4 or above, he or she will gain an entrustment of the EPA.

Key words: EPA (Entrustable Professional Activity), Milestone, Entrustment

Patient safety is essential in health care. To enhance patient safety and quality of residency training program, an ongoing assessment aligned to suitable levels of supervision is needed. In 2005, ten Cate first developed the concept of Entrustable Professional Activities (EPAs). He mentioned that entrustable professional activities are "tasks or responsibilities to be entrusted to the unsupervised execution by a learner once he or she has attained sufficient specific competence"[1]. In EPAs process, clinical supervisors observe and evaluate trainees' performance and provide feedbacks. If the trainee obtains the entrustment of competencies, he/she will be able to perform the professional activity independently. On the contrary, if the trainee has not reached entrustable level, they will develop their professional skills with the supervisors' support. As such, patients are still provided high quality health care with the appropriate level of supervision. Entrustable professional activities (EPAs) are important to the medical practice, which requires competency in specific domains.

Nevertheless, using EPAs in a medical residency training program has both benefits and challenges. Implementation might be a challenge in current clinical environment. It is very difficult to measure clinical performance from a written assessment. Therefore, there is great demand for developing work-based evaluation instruments for medical education. Based on Miller's framework for clinical assessment, the development of progression for a trainee follows the sequence as such: knows, knows how, shows how, and does [2,3]. Assessment in the first two stages, knows and knows how, can be easily assessed by using a reliable written test. However, the next two stages, shows how and does, are related to performance and action; the evaluation should be administered as a workplace-based assessment. Competency might be shown on a written assessment, but the trainee may lack competency in clinical performance. Therefore, EPAs have been built in medical education training programs internationally. Medical educators believe that EPAs can target certain competency domains of practice that are not easily assessed in a traditional written assessment format. As the result, supervisors using EPA-based assessment to evaluate competences of what trainees do on day-to-day based work should be more reliable. Nowadays, many medical associations, schools, and hospitals have developed EPAs for their training programs. For example, the American board of Pediatrics has also listed 17 EPAs for their members [4].

EPAs do not cover every professional activity

*Correspondence to: Dr. Ching-Shiang Chi, Tungs' Taichung Metroharbor Hospital, No. 699, Sec. 1, Chungchi Road, Wuchi, Taichung, 435, Taiwan, (R.O.C.)

that a resident engages in. They are activities related to daily professional tasks that are observable, measurable, and can be executed independently[1]. Usually, EPAs are selected because they are crucial in the practice of a medical field; they require important knowledge and skills for daily work; or they are involved in a set of performance across major roles that a trainee plays [5]. An EPA can be implemented for assessing trainees to perform the task in different training stages but different milestones are required for entrustments. Ten Cate suggested that EPAs in most specialties can be grouped into no more than 20-30 EPAs [3,6]. Generally, an EPA includes a group of competency domains and five levels of milestone (Fig. 1)[1,4]. Each level of competency milestone is linked to a tailored level of supervision. At level 1 "Not ready for entrustment", trainees cannot execute a particular EPA because of insufficient skills or knowledge. They need to observe the performance modelled by supervisors or other trainees. At level 2 and 3, trainees perform their daily work under different levels of supervision. At level 4, trainees are permitted to practice independently without supervision. If a trainee has reached at level 5, he/she may act as a supervisor and instructor to help colleagues in workplace [5]. The assessor of an EPA usually is the supervisor of trainees and must be recognized as appropriately skilled and experienced in the area of EPA. An EPA is achieved when a trainee demonstrates proficiency of the knowledge, skills and attitude required by the task[1]. Once a trainee attains an EPA, it will be recorded on the trainee's Confirmation of Entrustment (COE) form.

The challenges of implementing EPA used as an evaluation tool has been raised in many hospital-based training programs. To effectively use EPA as a clinical assessment, it relies on a flexible learning environment and a positive culture of assessment. In the current configuration of hospital-based residency program, the workload of each faculty is pretty heavy. However, the observation and data collection of an EPA-based assessment is very time consuming. Therefore, how to distribute time between clinical and teaching duties is a critical factor for executing EPA successfully. In addition, a positive culture of EPA assessment requires a trusting relationship and common vision of the assessment between both supervisors and trainees. We need to have a very clear picture and comprehensible description of each level of milestone in each EPA. Supervisors must be capable to administer an assessment without bias and avoid possible grading mistakes. Insufficient training and experience of EPA-based assessment is another significant obstacle for implementation. As a result,

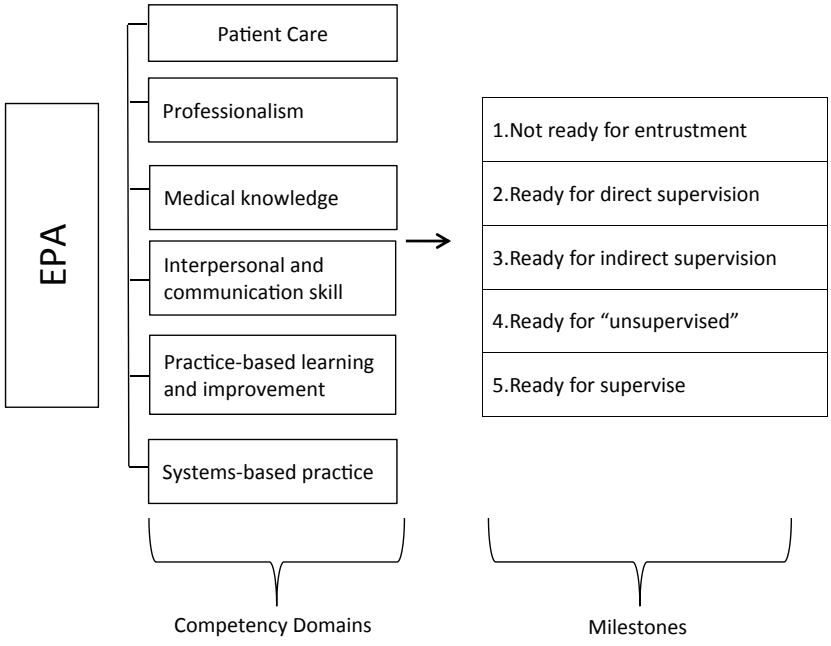

Fig. 1 Sample structure of EPA(Entrustable Professional Activity)

providing appropriate and adequate training for assessors is necessary. In order to inspire both supervisors and trainees buy-in, they must be involved in entire development process. Bi-directional feedbacks must be mutually linked to make every faculty accountable.

The acceptance of EPA-based assessment has been increasing in medical education. It shifts the assessment focus to trainees' performance in their daily work. The initial goal under the concept of EPAs was to increase patient safety. Situate competencies and milestones designed in clinical context for daily practice are the important features of EPA to connect medical education and clinical practice. The major challenge of EPA-based assessment is implementation. Providing proper faculty development, developing positive assessment culture, and creating flexible learning environment are key components for executing EPA assessment successfully.

References

1. Ten Cate O. Entrustability of professional activities and competency-based training. Med Educ 2005;39: 1176-77.
2. Miller GE. The assessment of clinical skills/competence/performance. Acad Med 1990; 65: 63-7.
3. El-Haddad C, Damondaran A, McNeil HP, Hu W. The ABCs of entustable professional activities: An overview of "entrustable professional activities" in medical education. 2016; Royal Australasian College of Physicians, 1006-10.
4. Carraccio C, Englander R, Gilhooly J. EPAs for Pediatrics. 2014, November, 13. Retrieved from https://www.abp.org/entrustable-professional-activities-epas
5. Caccia N, NaKajima A, Scheele F, and Kent N. Competency-based medical education: Developing a framework for obstetrics and gynaecology. J Obstet Gynaecol Can 2015; 37: 1104-12.
6. Ten Cate O. Nuts and bolts of entrustable professional activities. J Grad Med Educ 2013; 5: 157-58.

可信任專業活動（EPAs）測驗在住院醫師訓練的運用

遲景上

童綜合醫院

受文日期：民國 105 年 11 月 25 日；接受刊載：民國 105 年 11 月 28 日

摘要

可信任專業活動（Entrustable Professional Activities, EPAs）是由專業訓練課程的成員由日常工作項目中制定出的專業活動，這些活動是可明確的由觀察中評估學員的知識及技能。從學員在一可信任專業活動（EPA）的表現，課程督導可以依照評估標準決定學員是否能有不須督導的獨立作業能力，或者還需要各種不同程度的督導或支援。一般而言，一項可信任專業活動中會包括數項不同層面的能力（competency），而每一種能力皆以五階段的里程碑（Milestone）來標示，學員必須達到至少第四階里程碑才能得到該專業活動獨立作業的信任度（Entrustment）。

關鍵詞：可信任專業活動、里程碑、信任度

* 通訊作者：遲景上醫師　童綜合醫療社團法人童綜合醫院　43503 臺中市梧棲區臺灣大道八段 699 號

Review Article

Anti-Programmed Death-1 Antibody: Review of a Novel Promising Treatment for Advanced Non-Small Cell Lung Cancer

Sheng-Han Cheng[1], Kee-Ching Jeng[2], Kuo-Shu Chen[1,*]

[1]Division of Allergy, Immunology and Rheumatology and [2]Department of Medical Research, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan

Received: Sep. 08, 2015; Accepted: Jan. 27, 2015

Abstract

Programmed death 1 (PD-1) protein is a negative coreceptor expressed on antigen-stimulated T cells and B cells that serves as an immune checkpoint. The novel therapy of anti-PD-1 antibody has many advantages over the current treatments: applicable to almost all types of cancer at any stages, long duration, and weak side-effects. Nivolumab, a fully human IgG4 PD-1 immune-checkpoint inhibitor antibody has been approved by FDA for several advanced cancers including lung cancer. The majority (85%) of lung cancer is non-small cell lung cancer (NSCLC) which consists of squamous (30%) and non-squamous (50%) NSCLC, both are difficult to treat. In the phase III CheckMate-017 trial with nivolumab, the one-year overall survival (OS) for squamous NSCLC is 41% vs. 24% of docetaxel (18% better). Similarly in the phase III CheckMate-057 trial, the one-year OS for nivolumab vs. docetaxel is 51% vs. 39%, respectively and the 18 month OS, 39% vs. 23%, respectively (16% better) in patients with non-squamous NSCLC. Both studies enrolled patients who had progressed following platinum-based doublet chemotherapy. The results show that therapy with nivolumab has a better result of OS and safety than docetaxel. Therefore we review this novel cancer treatment and discuss the future perspective of this application.

Key words: anti-PD-1 antibody, squamous and non-squamous non-small cell lung cancer, survival

Programmed death 1 (PD-1) function

Programmed death 1 (PD-1) protein, a negative coreceptor expressed on antigen-stimulated T cells and B cells, seems to serve as a 'rheostat' of the immune response[1]. The function of PD-1 is immune regulation which is antigen specific and cell intrinsic, as shown in the mild, chronic and strain-specific autoimmune phenotypes of PD-1-deficient mice[2]. PD-1 (*Pdcd1*) belonging to the CD28/CTLA-4 family. It regulates antigen receptor signaling negatively by recruiting protein tyrosine phosphatase, SHP-2 upon interacting with either of two ligands, PD-L1 or PD-L2. Because of the wide range of ligand distribution in the body, the biological significance of PD-1 pervades almost every aspect of immune responses including autoimmunity, tumor immunity, infectious immunity, transplantation immunity, allergy and immunological privilege[1]. Immune-checkpoint-inhibitor antibody that disrupts the PD-1 signaling may restore anti-tumor immunity. Such unique properties make PD-1 a powerful target for immunological therapy, with highly effective clinical applications for cancer treatments (Fig 1).

*Correspondence to: Dr. Kuo-Shu Chen, Division of Allergy, Immunology and Rheumatology, Department of Internal Medicine Tungs' Taichung MetroHarbor Hospital, No. 699, Sec. 8, Taiwan Blvd., Wuqi Dist., Taichung 435, Taiwan (R.O.C.)

Advantages of anti-PD-1 cancer therapy

Immune checkpoint blockade with anti-PD-1 has revolutionized the cancer therapy with many advantages over the current treatments. It is applicable to

almost all types of cancer at any stages, and it has a long duration and weak side-effects. Therefore, anti-PD-1 is predicted as the first choice of cancer treatment in the future[3]. The striking effects of anti-PD-1 depend on three basic principles: (a) the immune system can recognize mutated cancer antigens, (b) the diversity of the immune repertoire is much larger than variations generated by mutations in tumor cells, and (c) the immune system is tolerated in tumor patients by excessive negative regulations of the immune system[3].

Current treatments for advanced NSCLC

Lung cancer is the most common cause of cancer mortality globally, representing 13% of all cancer diagnoses each year and nearly 1 in 5 cancer-related deaths. The majority of lung cancer patients are diagnosed with advanced disease (stage IIIB/IV)[4]. Current treatment including surgery, chemotherapy, and radiotherapy are unlikely to result in cure, although they may significantly improve survival and provide symptom relief[5-7]. Patients with specific genetic mutations may benefit from targeted therapies such as the EGF receptor blocker but unfortunately, the tumor develops resistance eventually[8-10]. Recent studies are focusing on combining an epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) with chemotherapy agent for advanced NSCLC. A randomized phase II study has been designed to select a combination regimen of TKI and platinum-based doublet chemotherapy for phase III evaluation on patients with EGFR-mutant NSCLC[11]. On the other hand, immunotherapies currently in development may offer significant benefit to lung cancer patients, including those whom conventional treatments are ineffective. New treatments that harness the immune system enable patients to fight lung cancer. Monoclonal antibodies (mAbs) are currently used in cancer treatment, and some appear to generate an immune response. For instance, bevacizumab (Avastin), which targets vascular endothelial growth factor (VEGF), is FDA-approved for the treatment of non-squamous NSCLC. Other gene mutations have been identified in NSCLC are also served as targets for therapy. In adenocarcinoma, the mutations are: K-RAS (25-35%), EGFR (15-20%), Anaplastic Lymphoma Kinase (ALK, 7%), Mesenchymal-Epithelial Ttransition (MET, 4%), HER2/MEK (2%), Rearranged during Transfection (RET, 1.7%), and ROS1 (1.5%)

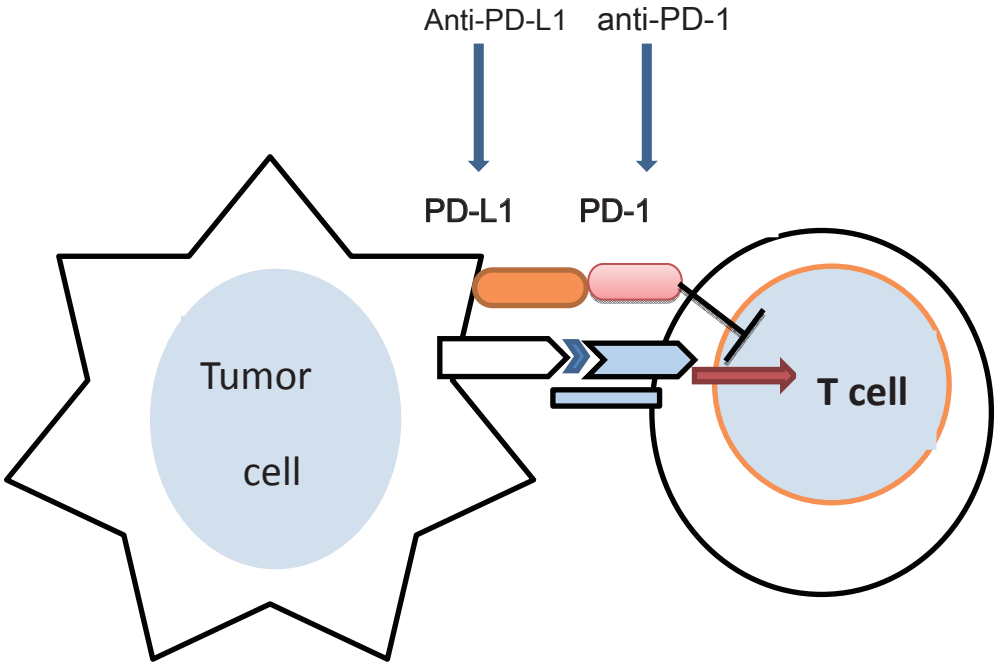

Fig 1. Programmed death 1 (PD-1) protein, a negative coreceptor expressed on antigen-stimulated T cells and B cells, is served as a checkpoint of the immune response. When PD-1 encounters with its ligand PD-L1 from dendritic cells (APC) or tumor cells, T cell response is suppressed. Anti-PD-1 or anti-PD-L1 antibody treatment can restore the function of T effector by disrupting the PD-1/PD-L1 suppression.

and in squamous cell cancer: PIK3CA (33%), FGFR1 (22%), MET (6%), Discoidin Domain Receptors (DDR, 4%), and BRAF (2%)[12]. However, the efficacies of these target therapy trials are not met our expectations. For instance the progression-free survival (PFS) of advanced NSCLC are ranged from 2.7 months to 13.1 months[12]. Efforts to use antibody to block the immune-checkpoint-inhibition between the PD-1 receptor have also ushered in a new era of immunotherapy (Figure 1)[1].

Efficacy

Nivolumab, a fully human IgG4 PD-1 inhibitor antibody has been approved for squamous and non-squamous NSCLC, which affects a third of all lung cancer patients[13-16]. Patients were selected from those who had progressed after treatment with platinum-based doublet chemotherapy in the CheckMate-017 and CheckMate-057 studies[14, 15]. They were randomly assigned to subsequent treatment with nivolumab (3 mg/kg every 2 weeks; 135 and 292 patients, respectively for 017 and 057 trials) or docetaxel (75 mg/m^2 every 3 weeks; 137 and 290 patients, respectively); both drugs were continued until progression or discontinuation due to toxicity[14,15].

The CheckMate-017 and CheckMate-057 clinical trials mark a milestone in the development of new treatment options for lung cancer. Nivolumab (Opdivo) treatment showed a significant improvement in overall survival (OS) in both subtypes of NSCLC Phase III trials compared with the current standard of care, docetaxel[14, 15]. In the CheckMate-017 trial[14], the patients with nivolumab treatment have a median OS of 9.2 months (95% confidence interval [CI], 7.3 to 13.3) vs. 6.0 months (95% CI, 5.1 to 7.3) of the docetaxel treatment. The risk of death in these patients is 41% lower with nivolumab than docetaxel treatment (hazard ratio [HR], 0.59; 95% CI, 0.44 to 0.79; p <0.001). The patients' OS rate with nivolumab treatment at 1 year is 42% (95% CI, 34 to 50) vs. 24% (95% CI, 17 to 31) of docetaxel treatment (Table 1). The response rate with nivolumab treatment is 20% vs. 9% of docetaxel (p = 0.008). The median progression-free survival with nivolumab treatment is 3.5 months vs. 2.8 months of docetaxel (HR for death or disease progression, 0.62; 95% CI, 0.47 to 0.81; p <0.001).

In the CheckMate-057 trial[15], the median OS for the patients with nivolumab treatment is significantly higher than that of the docetaxel treatment: 12.2 vs. 9.4 months (HR 0.73, 95% CI 0.59, 0.89; p = 0.0015). Patients with nivolumab treatment reduce 27% of the risk of death and their 1-year OS is 51% as compared with 39% of docetaxel (Table 1). Survival benefits are seen for all subgroups of patients, except those whose tumors had *EGFR* mutations (CM057). Objective response rate (ORR) of the nivolumab treatment is also significantly higher than that of the docetaxel: 19% vs. 12%, p = 0.0246). There is a significant difference in the progression-free survival (PFS): 19% vs. 8%, for nivolumab and docetaxel, respectively at 1 year treatment (Table 1).

Table 1. Efficacy of nivolumab versus docetaxel in patients with advanced non-small cell lung cancer (NSCLC)

Trial[a]	Overall survival (at 12 month)	Response	Progression-free survival (at 12 month)
CheckMate 017 for squamous NSCLC			
Nivolumab	42% (95% CI 34-50)	63% (17/27 responder)	21% (95% CI 14-28)
Docetaxel	24% (95% CI 17-31)	33% (4/12 responder)	6% (95% CI 3-12)
CheckMate 057 for nonsquamous NSCLC			
Nivolumab	51% (95% CI 45-56)	52% (29/56 responder)	19% (95% CI 14-23)
Docetaxel	39% (95% CI 33-45)	14% (5/36 responder)	8% (95% CI 5-12)

[a]CheckMate 017 clinical trial for squamous NSCLC[14] and CheckMate 057 trial for non-squamous NSCLC[15]. Patients with stage IIIB or IV NSCLC after one prior platinum-based doublet chemotherapy regimen were randomly assigned to receive nivolumab (3 mg/Kg) every 2 weeks or docetaxel (75 mg/cm^2 body-surface area) every 3 weeks. The primary end point is overall survival (at one year). The secondary efficacy end points included the rate of investigator-assessed confirmed objective response, progress-free survival, efficacy according to tumor PD-L1 expression level, and patient-reported outcomes. The 18 month OS for nivolumab is 39% vs. 23% of docetaxel in patients with non-squamous NSCLC.

Correlation with tumor PD-L1 expression

Patients with PD-L1–positive tumors have a great of benefit by nivolumab treatment, and PD-L1 expression is a predictor of response[13]. Patients with non-squamous NSCLC having tumors staining for PD-L1 that are 1% or higher, 5% or higher, and 10% or higher, correlate with 17.2, 18.2, and 19.4 months of the median OS from the nivolumab treatment, respectively as compared with 9, 8.1, and 8.0 months of docetaxel treatment[15]. However, patients with tumors having less than 1%, less than 5%, and less than 10% of cells staining for PD-L1, the median OS is similar between the nivolumab and docetaxel treatments (range 9.7 to 10.4 months vs.10.1 to 10.3 months, respectively)[15]. Early-stage trials have suggested that PD-L1 expression on tumor cells or tumor-infiltrating lymphocytes (or both) may increase the likelihood of response to PD-1–directed or PD-L1–directed therapies[13, 17-20]. The response rates can be as high as 83% depending on the chosen assay and PD-L1 expression levels[20]. However, responses are consistently seen in patients with tumors or tumor-infiltrating lymphocytes that are not positive for PD-L1.

However, in patients with squamous-cell NSCLC, the expression of the PD-1 ligand (PD-L1) is neither prognostic nor predictive of benefit for the efficacy of nivolumab[14]. Because the PD-L1 expression was assessed in archival tumor tissue, which may not have reflected tumor PD-L1 status at the time of treatment, and that only 83% of the patients had quantifiable PD-L1 expression. The lack of an association between PD-L1 expression and efficacy may be explained by the complex interactions between tumors and the immune system[14].The CheckMate-017 study indicates that PD-L1 testing is not required in order to inform treatment decisions for use of nivolumab in second-line therapy of squamous-cell NSCLC and suggests that patients may have a survival benefit that is independent of PD-L1 expression level.

Safety

Treatment-related adverse events of grade 3 or 4 were reported in 7% of the patients with squamous NSCLC in the nivolumab group as compared with 55% of those in the docetaxel group (Table 2). Adverse events of any grade occurred in 58% of patients receiving the nivolumab treatment and in 86% of patients receiving docetaxe[14]. Similarly, adverse events of grade 3-5 were reported in 10.5% of the patients with non-squamous NSCLC in the nivolumab group as compared with 53.7% of the docetaxel group (Table 2). Adverse events of any grade occurred in 69% of patients receiving nivolumab and 88% of

Table 2. Safety of nivolumab or docetaxel therapy in patients with advanced non-small cell lung cancer

Adverse events (Grade 3 or 4)	CheckMate 017[a] Nivolumab (N = 131)	Docetaxel (N = 129)	CheckMate 057 Nivolumab (N = 287)	Docetaxel (N = 268)
Any event	9 (7%)	71 (55%)	30 (10%)	144 (54%)
Fatigue	1 (1%)	10 (8%)	3 (1%)	13 (5%)
Nausea	0	2 (2%)	2 (1%)	2 (1%)
Decreased appetite	1 (1%)	1 (1%)	0	3 (1%)
Asthenia	0	5 (4%)	1 (<1%)	6 (2%)
Diarrhea	0	3 (2%)	2 (1%)	3 (1%)
Anemia	0	4 (3%)	1 (<1%)	7 (3%)
Leukopenia	1 (1%)	3 (2%)	0	22 (8%)
Neutropenia	0	38 (30%)	0	73 (27%)
Febrile neutropenia	0	13 (10%)	0	26 (10%)
Myalgia	0	0	1 (<1%)	0
Alopecia	0	1 (1%)	0	0

[a]Safety analysis included all the patients at least one dose of study drug. In CheckMate 017 trial: three treatment-related deaths (grade 5 events) were reported and in CheckMate 057 trial: one treatment-related death was reported in the Docetaxel group (recorded as grade 4 febrile neutropenia before the database lock)[14,15].

patients, docetaxel[15].

The safety profile of nivolumab is more favorable than that of docetaxel. Nivolumab has less frequencies of both hematologic and non-hematologic adverse events, including severe toxic events than docetaxel and less adverse events leading to discontinuation. It has no new safety concerns and no deaths are reported in these two trials. However, attention should be given to rapid evaluation and initiation of treatment because immune-mediated adverse events with immunotherapies such as nivolumab is totally differ from traditional cytotoxic therapies. These adverse events, including pneumonitis, are infrequent and of low severity in these studies and can be managed with the use of established guidelines.

Further improvement for immune-checkpoint-inhibitors

Although nivolumab has a better survival rate and safety than docetaxel, the response rate is about 20% (95% CI, 14-28) vs. 9% (95% CI, 5-15) for squamous NSCLC[14] and 19% (95% CI, 15-24) vs. 12% (95% CI, 9-17) for non-squamous NSCLC[15]. This indicates 80% of NSCLC patients are not responded to the treatment. Another multicenter phase 1 trial with antibody-mediated blockage of PD-L1 is also promising in patients with advanced cancer[17]. Anti-PD-L1 induced durable tumor regression (response rate 6-17%) and prolonged stabilization of disease (12 to 41% at 23 weeks) in advanced cancer including NSCLC, melanoma, and renal-cell cancer[18-20]. Current thinking of combined therapy for different targets may improve the efficacy of these checkpoint-inhibitors[21]. For instance, a phase I trial of nivolumab is studied with or without ipilimumab (CTLA-4 antibody) for patients with advanced or metastatic solid tumors, including SCLC (NCT01928394) (Table 3) or a phase I trial of MPDL3280A with bevacizumab (Avastin), or with bevacizumab and chemotherapy, for patients with advanced NSCLC (NCT01633970). Recently, a randomized phase II study (NEJ005/TCOG0902) is reported to compare the concurrent versus sequential alternating gefitinib and chemotherapy in untreated NSCLC patients with sensitive EGFR mutations [22]. There are 41 patients in the concurrent group and 39 in sequential alternating regimen group. The result shows that median PFS is 18.3 months for the concurrent regimen and 15.3 months for the sequential alternating regimen (HR: 0.71 (0.42-1.20), P = 0.20). The median survival times are 41.9 and 30.7 months, respectively [HR 0.51 (0.26-0.99); P = 0.042]. Response rates are similar in both groups (87.8% and 84.6%) and adverse events are common and reversible. Both regimens has promising efficacy with predictable toxicities, although concurrent regimens might provide better OS[22].

Table 3. List of immune-checkpoint-inhibitors for advanced cancer[13-23]

Antibody	Therapeutic evaluation
CTLA-4 antibodies	
Ipilimumab (Yervoy)	Extend survival in patients with metastatic melanoma. Phase III trials for NSCLC (NCT01285609) and for SCLC (NCT01450761).
Tremelimumab	Phase II clinical trial for patients with mesothelioma (NCT01655888).
PD-1 antibodies	
Nivolumab (Opdivo)	US FDA approved in October 2015 for the treatment of advance (metastatic) squamous and nonsquamous NSCLC that failed chemotherapy.
Pembrolizumab (Keytruda)	US FDA approved in September 2014 for the treatment of advanced melanoma and was granted a US FDA approved in October 2014 for advance (metastatic) NSCLC.
PD-L1 antibodies	
BMS-936559	Phase I trial (NCT00729664) in patients with advanced NSCLC, melanoma, renal cell cancer (RCC), ovarian, gastric, pancreatic or breast cancers.
MPDL3280A	Phase II trial (NCT01846416) in patients with PD-L1- positive locally advanced or metastatic NSCLC. Melanoma and RCC.
MEDI4736	Phase I trial (NCT01693562) in patients with solid tumors and phase III for advanced NSCLC.
NSB0010718C(Avelumab)	Phase I trial (NCT01772004) for advance solid tumors; Phase II Merkel cell carcinoma

Conclusion

Nivolumab is a PD-1 checkpoint inhibitor that has a clinically meaningful survival benefit and a safety profile, over the current therapy for patients with advanced, previously treated squamous and non-squamous NSCLC. The benefit is particularly related with PD-L1 expression level in the non-squamous NSCLC. Further studies are needed to identify relevant biomarkers to predict which patients are most likely to benefit for the immune-checkpoint inhibition and which combined therapy may offer a better OS.

References

1. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. Int Immunol 2007; 19: 813-24.
2. Nishimura H, Minato N, Nakano T, HonjoT. Immunological studies on PD-1 deficient mice: implication of PD-1 as a negative regulator for B cell responses. Int Immunol 1998; 10: 1563-72.
3. Honjo T. Cancer immunotherapy by PD-1 blockade. 2015 SCBA Proceedings, Sinica Academia, Taipei, Taiwan: Abstract 1.
4. Travis WD. Pathology of lung cancer. Clin Chest Med 2011; 32: 669-92.
5. Fossella F, Pereira JR, von Pawel J, et al. Randomized, multinational, phase III study of docetaxel plus platinum combinations versus vinorelbine plus cisplatin for advanced non-small-cell lung cancer: the TAX 326 study group. J Clin Oncol 2003; 21: 3016-24.
6. Shepherd FA, Dancey J, Ramlau R, et al. Prospective randomized trial of docetaxel versus best supportive care in patients with non-small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol 2000; 18: 2095-103.
7. Taxotere (docetaxel) U.S. prescribing information. Bridgewater, NJ: Sanofi-Aventis, May 2010. (http://www.accessdata.fda.gov/ drugsatfda_docs/label/ 2010/020449s059lbl.pdf).
8. National Comprehensive Cancer Network (NCCN). Non-small cell lung cancer guidelines, v4. 2015 (http://www.nccn.org/professionals /physician_gls/ f_ guidelines.asp#nscl).
9. Maemondo M, Inoue A, Kobayashi K, et al. Gefitinib or Chemotherapy for Non–Small-Cell Lung Cancer with Mutated EGFR. N Engl J Med 2010; 362: 2380-8.
10. Mok TS, Wu YL, Thongprasert S, et al. Gefitinib or Carboplatin–Paclitaxel in pulmonary adenocarcinoma. N Engl J Med 2009; 361: 947-57.
11. Soria JC, Wu YL, Nakagawa K, et al. Gefitinib plus chemotherapy versus placebo plus chemotherapy in EGFR-mutation-positive non-small-cell lung cancer after progression on first-line gefitinib (IMPRESS): a phase 3 randomised trial. Lancet Oncol 2015; 16: 990-8.
12. Boolell V, Alamgeer M, Watkins DN, Ganju V. The evolution of therapies in non-small cell lung cancer. Cancers 2015; 7: 1815-46.
13. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 2012; 366: 2443-54.
14. Brahmer JR, Reckamp KL, Baas P, et al. Nivolumab versus Docetaxel in advanced squamous-cell non-small-cell lung cancer. N Engl J Med 2015; 373: 123-35.
15. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J 2015; 373: 1627-39.
16. Paz-Ares L, Horn L, Borghaei H, et al. Phase III, randomized trial (CheckMate 057) of nivolumab versus docetaxel in advanced non-squamous cell non-small cell lung cancer. J Clin Oncol 2015; 33(Suppl): 109.
17. Brahmer JR, Tykodi SS, Chow LQM, et al. Safety, activity, and immune correlates of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012; 366: 2455-65.
18. Garon EB, Gandhi L, Rizvi N, et al. Antitumor activity of pembrolizumab (Pembro; MK-3475) and correlation with programmed death ligand 1 (PD-L1) expression in a pooled analysis of patients (pts) with advanced non-small cell lung carcinoma (NSCLC). Ann Oncol 2014; 25(Suppl 5): 1-41.
19. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014; 515: 563-7.
20. Soria JC, Cruz C, Bahleda R, et al. Clinical activity, safety and biomarkers of PD-L1 blockade in non-small cell lung cancer (NSCLC): additional analyses from a clinical study of the engineered antibody MPDL3280A (anti-PDL1). Eur J Cancer 2013; 49(Suppl 2): abstract 3408
21. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology: mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015; 348: 124-8.
22. Sugawara S, Oizumi S, Minato K, et al. Randomized phase II study of concurrent versus sequential alternating gefitinib and chemotherapy in previously untreated non-small cell lung cancer with sensitive EGFR mutations: NEJ005/TCOG0902. Ann Oncol 2015; 26: 888-94.
23. Philips GK, Atkins M. Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies. Int Immunol 2015; 27: 39-46.

抗 PD-1 抗體：對晚期非小細胞肺癌的一種新治療的回顧

鄭聖翰[1] 鄭啟清[2] 陳國書[1,*]

童綜合醫院 [1]免疫風濕科 [2]醫學研究部

受文日期：民國 104 年 09 月 08 日；接受刊載：民國 105 年 01 月 27 日

摘要

計畫性死亡蛋白質 -1（PD-1）是一抑制性複合受體，表現在抗原刺激的 T 及 B 細胞上，作用為調節免疫反應。以 PD-1 抗體來阻斷腫瘤抑制免疫作用的治療相對於其他治療方法很具革命性，它適於各種細胞型態各期的腫瘤、長效及較少的副作用。Nivolumab 是一種全部人類 IgG4 抗 PD-1 的抗體能阻斷腫瘤的免疫抑制作用，已被美國食品藥物署批准使用於多種晚期腫瘤，包括了肺癌。而肺癌中非小細胞肺癌所占的比率最高達 85%，其中有鱗狀（30%）及非鱗狀（50%）的非小細胞肺癌，皆屬難治之癌。Nivolumab 第三期的臨床試驗（Checkmate-017）的結果，其一年總存活率對鱗狀非小細胞肺癌的病人比歐洲紫杉醇更佳，為 42% 比 24%（多 18%）。同樣療法在 Checkmate-057 試驗的結果，對非鱗狀（上皮）非小細胞肺癌的病人，Nivolumab 一年總存活率為 51% 比紫杉醇的 39% 好，而一年半總存活率則是 39% 比 23%（多了 16%）。兩項研究召募對象，皆為曾以鉑類為主雙藥化療無效之病患，結果都顯示 Nivolumab 優於紫杉醇的總存活率，且副作用較小，故回顧此創新治療及前景，供臨床參考以期造福病患。

關鍵詞：計畫性死亡蛋白質 -1 抗體、非鱗狀及鱗狀上皮非小細胞肺癌、存活率

* 通訊作者：陳國書 童綜合醫療社團法人童綜合醫院 免疫風濕科 43503 臺中市梧棲區臺灣大道八段 699 號

Tungs' Medical Journal 10 (2016) 12-20

Original Article

Apple Polyphenols Inhibit Lipopolysaccharide-induced Inflammation via Regulation of COX, iNOS, and MAPK Signaling Pathways

Yi-Chen Lee[1], Huei-Jane Lee[2,*]

[1]*Department of Nutrition Therapy, E-DA Hospital, Kaohsiung City, Taiwan*
[2]*Department of Biochemistry, School of Medicine, Medical College, Chung Shan Medical University, Taichung City, Taiwan*

Received: Oct. 28, 2015; Accepted: Mar. 12, 2016

Abstract

Background and purpose: We previously reported that polyphenols extracted from unripe apples exert antioxidant and anti-inflammatory effects on lipopolysaccharide (LPS)-treated animals. In this study, we further examined the anti-inflammatory mechanism of apple polyphenols (AP) using in vitro and in vivo models.
Methods: The effect of AP on cyclooxygenase-2 (COX-2), inducible NO synthase (iNOS) expression, and their products was tested in LPS-induced macrophages. Sprague–Dawley rats were treated with AP for five days; they were then injected with 5 mg/kg LPS. The protein expression in LPS-induced signaling pathways was examined in liver specimens.
Results: AP suppressed the expression of both COX-2 and iNOS and their products in LPS-induced macrophages. Furthermore, LPS induction of COX-2, JNK, and p38 expression in rat liver tissue was also inhibited by AP treatment.
Conclusion: The anti-inflammatory effect of apple polyphenol involves the regulation of COX, iNOS, JNK, and P38 MAPK signaling pathways.

Key words: Lipopolysaccharide; Inflammation; Apple polyphenol; Signaling pathways

Introduction

Inflammation protects an individual from injury or infection and is characterized by redness, heat, pain, and swelling, with the occasional impairment or loss of normal function. Recent studies have shown that chronic inflammation can mediate numerous conditions, including cardiovascular diseases, cancer, diabetes, arthritis, Alzheimer's disease, pulmonary diseases, and autoimmune diseases [1]. It is also involved in tumorigenesis at different steps including cellular transformation and promotion. The cytokines produced from inflammation regulate cancer cell survival, proliferation, invasion, angiogenesis, and metastasis [2].

*Correspondence to: Huei-Jane Lee, Department of Biochemistry, School of Medicine, Medical College, Chung Shan Medical University, No. 110, Section 1, Jianguo N. Road, Taichung city 402, Taiwan (R.O.C.)

Cyclooxygenase-2 (COX-2) is induced in immune cells, such as macrophages, in response to infection, injury, or other stresses. COX-2 is induced by certain growth factors and pro-inflammatory stimulators such as lipopolysaccharide (LPS). The mechanisms of COX-2 action are similar to those underlying stimulation by several activated oncogenes [3]. COX-2 activity is responsible for the synthesis of prostaglandin, a compound that causes the symptoms of inflammation. Because COX-2 is usually specific to inflamed tissue, COX-2 inhibitors can be used to block the synthesis of prostaglandin E_2 (PGE_2), thereby inhibiting inflammation and providing analgesia [4]. However, the selectivity of COX-2 inhibitors does not seem to outweigh the side-effects of NSAIDs, most notably an increased risk of renal failure. COX-2 inhibitors also increase the risk of heart attack, thrombosis, and stroke by increasing thromboxane unbalanced by prostacyclin. One study showed that a

homozygous deletion in the COX-2 gene relieves the hepatocellular toxicity caused by LPS administration [5]. In 2015, the extract of grape seed was shown to inhibit oxidation and nitrite production in LPS-stimulated Raw 264.7 cells [6]. Nair et al. also found that phenolic compounds extracted from the Butterfly Pea have activity against LPS-induced inflammation in macrophages [7]. These reports suggest that COX-2 protein expression can be used as an index of the anti-inflammatory effects of synthetic drugs or natural products.

Nitric oxide (NO), produced by inducible nitric oxide synthase (iNOS), is an important messenger and effector molecule involved in inflammation and sepsis in a variety of tissues [8,9]. Although the half-life of NO is very short, it mediates several intracellular signaling molecules, including JNK and MAPK. Therefore, NO production may reflect the degree of inflammation and provide a measure for assessing the effectiveness of drugs on the inflammatory process. iNOS is often induced after exposure to endogenous and exogenous stimulators in cells such as macrophages, smooth muscle cells, and hepatocytes to trigger disadvantageous cellular responses leading to inflammation, sepsis, and stroke [10]. In the liver, LPS activates iNOS activity in Kupffer cells, endothelial cells, and hepatocytes, increasing NO production [11,12]. Several natural antioxidants, including curcumin [13], resveratrol [14], and plant polyphenols inhibit LPS-induced iNOS activity and prevent hepatic damage in vivo [15].

A large number of phenolic compounds present in edible and medicinal plants are reported to have anticarcinogenic and antimutagenic activities. The majority of naturally-occurring phenolics possess antioxidative and anti-inflammatory properties, which appears to contribute to their chemopreventive or chemoprotective activity [16]. Many types of polyphenols are present in apples, with oligomeric procyanidins being the most abundant. Applephenon is an apple polyphenol extract produced commercially from unripe Fuji apples. This product has been used as a food additive to prevent the oxidation of food components. It contains abundant phenolic compounds, including procyanidin, epicatechin, catechin, p-coumaroyl quinic acid, chlorogenic acid, rutin, and phloridzin [17, 18]. Epicatechin and catechin are among the most abundant apple polyphenols (AP) [19]. These phenolic substances are reported to display anti-allergic effects [20], anti-hyperlipidemic properties [21], anti-oxidant effects [22], and anti-tumor effects [23]. We previously observed that AP reduces LPS-induced inflammation in the liver via regulation of the oxidative status. This study aims to elucidate the mechanism whereby AP exerts anti-oxidant and antiinflammatory effects on LPS-treated macrophages and animals. The expression of proteins in the COX2, iNOS, and MAPK signaling pathways was examined.

Materials and Methods

Chemicals

AP (purity, 98.7%) was purchased from Asahi Co. (Japan). LPS (endotoxin from *Escherichia coli*, serotype 0127:B8), Prostaglandin E_2 immunoassay kit (R&D Systems, USA), anti-iNOS, COX-2, phospho-JNK, phosphor-P38, anti-α-tubulin antibody (Transduction Laboratories, Lexington, KY), and a protein assay kit (Bio-Rad Lab. Ltd., Watford, Herts, UK) were purchased from the indicated manufacturers.

Activity of xanthine oxidase assay

Because the bacterial nitrate/nitrite reductases are structurally similar to xanthine oxidase, they can reduce nitrite to nitric oxide. To examine the anti-oxidant activity of AP, xanthine oxidase activity was evaluated spectrophotometrically by assaying the formation of uric acid from xanthine [24]. Xanthine (0.2 mM, 0.88 mL) in phosphate buffer (0.1 M, pH 7.8) with xanthine oxidase (0.12 μM/mL) was incubated for 15 min at room temperature and read at 295 nm against a blank sample without the enzyme. AP (0.01–0.5 mg/mL, 0.1 mL) was added to the sample before enzyme addition, and the effect on xanthine oxidase was compared according to the absorbance data. The percent inhibition of xanthine oxidase activity was calculated.

Cell Culture

Rat macrophages (RAW 264.7 cell) were cultured in a humidified atmosphere of 95% air-5% carbon dioxide at 37°C in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum, 1% glutamine, and 1% penicillin- streptomycin.

Cell Viability Assay

RAW 264.7 cells were plated in 6-well plates at

2×10^5 cells per well, allowed to adhere to the plate overnight, and the medium refreshed. Cells were treated with AP (0–0.5 mg/mL) for 24 hours, followed by replacement of the medium with fresh medium containing 5 mg/mL MTT (3-[4,5-dimethylthiazol-2-yl]-2,5- diphenyltetrazolium bromide) and incubation for 4 hours. The medium was then removed, and 1 mL of isopropanol was added to the wells to solubilize crystals. The optical density of each sample was read at 563 nm against a blank prepared from cell-free wells.

Nitrite Measurement

Total nitrites in the culture medium were measured using the Griess reagent as described [25]. After incubation of RAW 264.7 cells with or without LPS and/or AP (0–0.5 mg/mL) at 37°C for 12 hour, 100 μL of each culture medium was centrifuged and then mixed with an equal volume of Griess reagent (1g/L sulfanilamide and 0.1g/L N-1-naphthyl-ethylenediamine in 2.5% phosphoric acid solution) and incubated at room temperature for 10 min. Absorbance at 540 nm was then read and compared with known standard solutions of $NaNO_2$.

Production of PGE_2

RAW 264.7 cells were plated in 24-well plates at 1×10^6 cells per well. After cells adhered to the plates, fresh medium without phenol red was added. After incubation with or without LPS and/or AP (0–0.5 mg/mL) at 37°C for 12 hours, the amount of PGE_2 in the culture medium was determined using a commercial competitive PGE_2 ELISA kit.

Animal Treatment

The male Sprague–Dawley (SD) rats (260 ± 10 g) used in this study were purchased from BioLASCO Taiwan Co Ltd. (Yilian County, Taiwan). All animals were housed in laboratory conditions (18–23 °C, humidity 55–60%, 12 h light/dark cycle) for at least one week before each study. The studies were conducted under the guidelines for the care and use of laboratory animals approved by Chung Shan Medical University's Institute of Animal Care and Use Committee (IACUC). The rats were provided with food and water *ad libitum* and divided into five groups (five rats per group). Apple polyphenol (50, 100, or 150 mg/kg) was given to the animals daily for five consecutive days via gavage. On day 5, one hour after the apple polyphenol treatment, LPS (5 mg/kg) or distilled water as a solvent control was injected (i.p.) into each animal. The rats were decapitated six hours later, and blood samples were collected for assay.

Immunoblots analysis

Liver tissue was extracted at 4°C by homogenization in buffer containing 20 mM HEPES, 1 mM dithiothreitol, 50 μM antipain, 50 μM leupeptin, 50 μM chymostatin, and 50 μM pepstatin (PH7.4). The homogenates were then centrifuged at 25 000 × g for 30 min at 4°C. The protein content of the supernatant was determined using the Bio-Rad protein assay reagent with bovine serum albumin as a standard. To perform western blotting, 50 μg of protein was resolved on SDS-PAGE gels along with pre-stained protein molecular weight standards (Bio-Rad). Proteins were then blotted onto an NC membrane (Sartorius) and reacted with primary antibodies (anti-COX2, -iNOS, -phospho-JNK, -phospho-P38 and -α-tubulin, and -actin as an internal control). The secondary antibody was a peroxidase-conjugated goat anti-mouse antibody. The reacted bands were revealed by enhanced chemiluminescence using an ECL commercial kit.

To investigate the effect of AP on iNOS and COX-2 mediated by LPS, RAW 264.7 cells (5×10^6) were plated onto 10-cm plates, allowed to adhere overnight, and the medium refreshed. The cells were then treated with 100 ng/mL of LPS and various concentrations of AP (0.01–1.0 mg/mL) for 18 hr. Total proteins (50 μg/lane) were prepared and subjected to western blot analysis as described.

Statistical Analysis

The data are reported as the mean ± standard deviation of 3 repeated determinations and evaluated by one-way ANOVA and Student's *t*-test. Differences with $P < 0.05$ were considered statistically significant.

Results

Effect of AP on xanthine oxidase activity in vitro

The antioxidant activity of AP was determined using the *in vitro* xanthine oxidase activity test. As shown in Fig. 1A, AP displayed a strong inhibitory effect on xanthine oxidase activity (EC_{50}, 0.589 mg/mL).

Effect of AP on LPS-induced Nitrite and PGE_2 Production in Macrophages

The viability of AP-treated RAW 264.7 cells was examined before testing the effects of AP on LPS-induced nitrite and PGE_2 production. The results presented in Fig 2A demonstrate that the concentration of 0.5 mg/mL did not affect with the survival of macrophages ($P > 0.05$). This non-toxic concentration was used in the following experiments. First, the effect of AP on LPS-induced nitrite production in RAW 264.7 macrophages was investigated. Nitrite accumulation in the culture medium was estimated using the Griess reaction as an index for NO release from the cells. After treating with LPS (100 ng/mL) for 12 hours, the nitrite concentration in the medium increased remarkably. When RAW264.7 macrophages were treated with different concentration of AP with LPS, nitrite production was significantly inhibited (Fig 2B). Increased PGE_2 production was demonstrated in LPS-treated cells. Therefore, we investigated the effects of AP on LPS-induced PGE_2 production in macrophages. After treatment with LPS (100 ng/mL) for 12 hours, the amount of PGE_2 in the medium clearly increased. Co-treatment of cells with LPS and different concentrations of AP significantly suppressed the production of LPS-induced PGE_2 (Fig 2C).

Effect of AP on LPS-induced iNOS and COX-2 Protein Expression in Macrophages

Because LPS activates iNOS, promoting NO production, AP pretreatment likely regulates LPS-induced iNOS protein expression in macrophages. Immunoblotting of iNOS protein shows that LPS treatment (100 ng/mL) induced the expression of this enzyme (Fig 3A) as compared to the control; iNOS expression was decreased by AP treatment. LPS treatment resulted in the activation of COX-2 gene expression. Therefore, we also investigated the effects of AP on LPS-induced

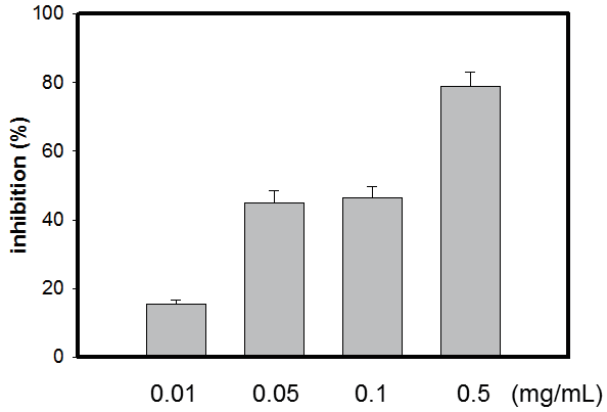

Fig. 1 AP inhibits xanthine oxidase activity in vitro. Concentrations of AP (0.01-0.5 mg/ml) were added to determine xanthine oxidase activity. The results were analyzed by measuring the absorbance at 295 nm. Data are analyzed and represented as mean±SD, n=6.

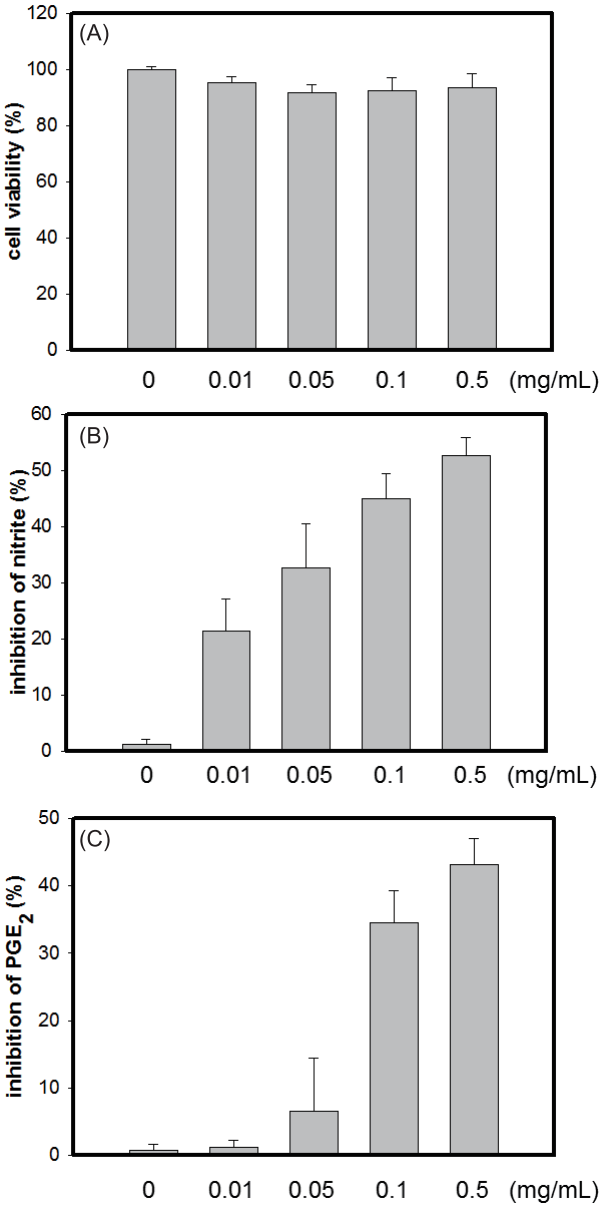

Fig. 2 AP reduces the level of nitrite and PGE2 in LPS-treated Raw264.7 cell. (A) the cell viability was detected by MTT assay as describe in Method. Various concentrations of AP were added in LPS-treated Raw264.7 cells, then (B) nitrite level was detected by using Griess reagent and (C) the results were analyzed for triple repeats by PGE2 ELISA kit. The data are shown as mean±SD, n=6.

COX-2 protein expression. In response to treatment with LPS (100 ng/mL), COX-2 protein expression increased; AP treatment inhibited this LPS-induced COX-2 protein expression (Fig 3A). Because COX-2 plays a crucial role in LPS-induced inflammation in the rat, we assessed the effect of AP on COX-2 protein expression. COX-2 protein expression increased upon stimulation with LPS and decreased in a dose-dependent manner after treatment with AP (Fig 3B). To further investigate whether MAPK proteins (JNK and p38), which are upstream of COX-2, are involved in the mechanism of AP attenuation of inflammation, rat liver protein was analyzed by immunoblotting. Pretreatment with AP for 5 days decreased LPS-induced p-JNK and p-P38 protein expression (Fig. 4A and B).

Discussion

We observed that the treatment of macrophages with AP suppressed the LPS-induced expression of COX-2, iNOS, and their products. The LPS-induced expression of COX-2, JNK, and p38 in rat liver tissue was also inhibited by AP treatment. These results indicate that the anti-inflammatory effect of AP involves the regulation of COX, iNOS, JNK, and P38 MAPK signaling pathways.

Polyphenol extracts from various plants have been used as supplements and food ingredients. These compounds are reported to have diverse physiological functions, including the regulation of lipid metabolism and inflammation. In our previous study, we showed that polyphenols extracted from Hibiscus sabdariffa L. possess anti-oxidant activity in vitro and suppress LPS-induced NO and PGE2 production and COX-2/iNOS protein expression in macrophages [15]. In the present study, AP was found to reduce LPS-induced liver dysfunction and to improve the oxidation status of cells. Other studies have also shown that AP has anti-inflammatory effects in various systems. In 2015, Xu et al. fed AP (obtained from JF-Natural, purity >75%) to ApoE-/- and found that AP improves atherosclerosis and hepatic steatosis via suppression of the ROS/MAPK/NF-κB signaling pathway [32]. In 2014, Sekhon-Loodu reported that flavonols from apple combined with fish oil can lower inflammation in hypercholesterolemia and acute inflammation [33]. In addition, apple (Ralls) polyphenol extract was shown to prevent aluminum-induced liver oxidative stress in rats [34]. AP was proved to possess

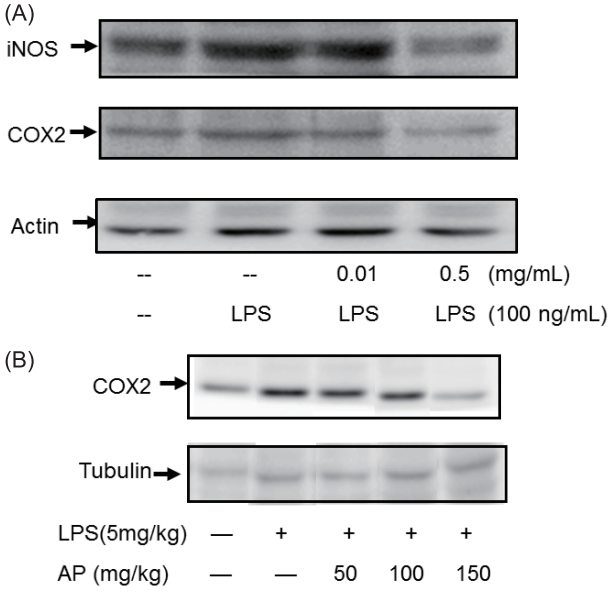

Fig. 3 AP inhibits the protein expression of iNOS and COX-2. (A) macrophages were stimulated with LPS accompanied with AP for 18 h. total cellular protein was analyzed as described in "Materials and Methods". (B) rats were fed with AP for 5 days, after the last AP given for 1 h, 5 mg/kg of LPS treated for 6 hrs. After sacrificing the animals, liver specimen was obtained to analyze the proteins. Expression of COX-2 was examined as described in the text. The data are done for three independent examinations.

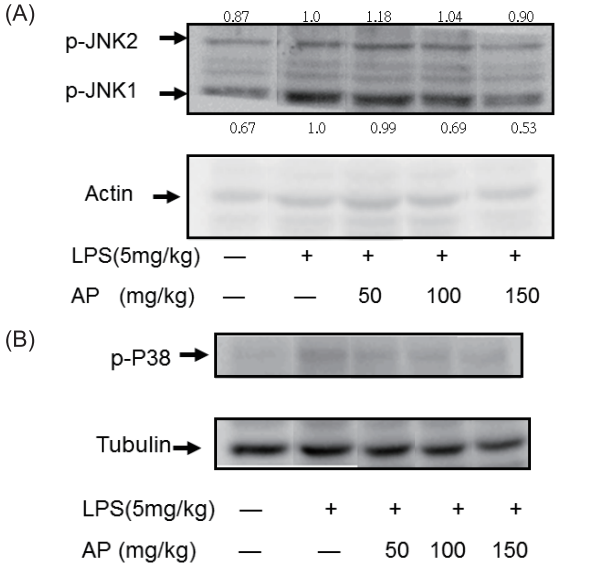

Fig. 4 AP inhibits JNK and p38 expression in liver specimen of LPS-induced rats.
Rats were fed with AP for 5 days, after the last AP given for 1 h, 5 mg/kg of LPS treated for 6 hrs. After sacrificing the animals, liver specimen was obtained to analyze the proteins. Expression of JNK and p38 was examined as described in the text. The data are done for three independent examinations.

anti-inflammatory activity in an *in vitro* system and to regulate the inflammatory response via MAPK signaling. JNK and p38 MAPK are known to play a role in the LPS-induced signal transduction pathway involving NFκB activation [35]. Under LPS-induction, this transcription factor is necessary to regulate the promoter of iNOS and COX-2 [36]. The relationship between AP and NFkB regulation in the LPS induction model is still unknown and should be clarified.

The feasible medicinal use of AP requires that effective concentrations are not toxic. The catechins of green tea and proanthocyanidins of grape seeds extracts have shown no toxicity in mutagenicity tests. Even though these extracts were applied daily, safety still remained a concern. The toxicity of Applephenon has been evaluated in previous studies [17]. Shoji et al. reported that AP did not cause chromosomal aberration in CHL/IU cells and represent significant mutagenicity. In rats, a study of oral acute toxicity showed that the lethal dose of AP is > 2000 mg/kg. Fujiwara et al. also reported that rats given diets containing AP at 0%, 1.25%, 2.5%, or 5.0% for 90 days did not appear to have toxicological responses. However, it is important to note the occurrence of some physiological adaptive responses to oral stimuli caused by the lower pH of AP-containing diets [26].

LPS induces iNOS and COX-2 gene expression in rat liver. The COX enzyme possesses both cyclooxygenase and peroxidase functions. The prostaglandins produced via COX activity impair immune surveillance and modulate the proliferation of various types of cells [27,28]. The peroxidase function contributes to the activation of procarcinogens [29]. During infection and inflammation, elevated NO production causes DNA damage as well as mutations *in vivo* [28]. Overexpression of either COX-2 or iNOS may be intimately involved in the pathogenesis of cancer [30], multiple sclerosis, neurodegenerative diseases, and heart infarction [31].

In addition to regulating signaling molecules, LPS in the liver binds to LPS-binding-proteins facilitating its transfer to CD14 receptors on the surface of Kupffer cells, the resident macrophages of liver. Macrophage activation by bacterial LPS promotes the secretion of proinflammatory cytokines such as tumor necrosis factor- α (TNF-α) and interleukin-1β (IL-1β) and of secondary mediators such as leukotrienes and prostaglandins (PGs). These substances are important regulators of innate and adaptive immunity to cause acute or chronic inflammatory syndromes. Until now, there has been little evidence to prove the relationship between AP and immune regulation under the model of LPS induction.

Phenolic compounds are secondary plant metabolites that play important roles in fruit flavor and color characteristics [37, 38]. Unripe fruits have an abundance of these compounds, which rapidly decrease during maturation. After harvesting, the total phenol concentration remains essentially constant or decreases slightly; however, individual phenolic compounds are reported to vary in their browning rates. Although the efficiency of different extraction methods varies, AP from unripe apples contains a high fraction of catechin (a phenolic compound) that differs from other sources of apple polyphenols [39, 40]. Catechins from green tea are reported to reduce neurodegeneration, cardiovascular disease, and gastric ulcer due to their anti-inflammatory effects. However, similar profiles for AP have not yet been elucidated. Given the abundance of polyphenols in unripe apples, exploitation of this potential natural source could improve the economic value of apples and also help in protecting the environment. Further study of the polyphenols in unripe apples is therefore required [41].

Taken together, these results suggest that AP has anti-inflammatory capability. In addition to its anti-oxidative profile, AP inhibits COX-2 induction by down-regulating JNK and p38. Therefore, we recommend further studies that pursue the development and application of AP as an anti-inflammatory agent.

Acknowledgments

This work was supported by an E-DA Hospital Grant (EDAHT103009).

References

1. Tabas I, Glass CK.Anti-Inflammatory Therapy in Chronic Disease: Challenges and Opportunities. Science 2013; 339: 166-72.
2. Nowarski R, Gagliani N. Huber S, Flavell RA. Innate Immune Cells in Inflammation and Cancer. Cancer Immunol Res 2013; 1: 77-84.
3. Barrios-Rodiles M, Tiraloche G, Chadee K. Lipopolysaccharide modulates cyclooxygenase-2 transcriptionally and posttranscriptionally in human macrophages independently from endogenous IL-1b and TNF-a. J Immunol 1999; 163: 963-9.

4. Meade EA, Smith WL, DeWitt DL. Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J Biol Chem 1993; 268: 6610-4.
5. Dinchuk JE. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. Nature 1995; 378: 406-9.
6. Pérez C, del Castillo MLR, Gil C, Blanch GP, Flores G. Supercritical fluid extraction of grape seeds: extract chemical composition, antioxidant activity and inhibition of nitrite production in LPS-stimulated Raw 264.7 cells. Food Funct 2015; 6: 2607-13.
7. Nair V, Bang WY, Schreckinger E, Andarwulan N, Cisneros-Zevallos L.Protective Role of Ternatin Anthocyanins and Quercetin Glycosides from Butterfly Pea (Clitoria ternatea Leguminosae) Blue Flower Petals against Lipopolysaccharide (LPS)-Induced Inflammation in Macrophage Cells. J Agric Food Chem 2015; 63: 6355-65.
8. Wheeler AP, Bernard GR.Treating patients with severe sepsis. New Eng J Med 1999; 340: 207-14.
9. Lowenstein CJ, Hill SL, Lafond-Walker A, Wu J, Allen G, Landavere M, et al. Nitric oxide inhibits viral replication in murine myocarditis. J Clin Invest 1996; 97: 1837-43.
10. Duval DL, Miller DR, Collier J, Billings RE. Characterization of hepatic nitric oxide synthase: identification as the cytokine-inducible form primarily regulated by oxidants. Mol Pharmacol 1996; 50: 277-84.
11. Rockey DC, Chung JJ. Regulation of inducible nitric oxide synthase in hepatic sinusoidal endothelial cell. Am J Physiol 1996; 271: G260-7.
12. Zhang C, Walker LM, Hinson JA, Mayeux PR. Oxidant stress in rat liver after lipopolysaccharide administration: effect of inducible nitric oxide synthase inhibition. J Pharmacol Exp Ther 2000; 293: 968-72.
13. Brouet I, Ohshima H: Curcumin, an anti-tumor promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. Biochem Biophys Res Commun 1995; 206: 535-40.
14. Tsai SH, Lin-Shiau SY, Lin JK.Supression of nitric oxide synthase and the down-regulation of the activation of NF-kB in macrophages by resveratrol. Br J Pharmacol 1999; 126: 673-80.
15. Kao ES, Hsu JD, Wang CJ, Yang SH, Cheng SY, Lee HJ: Polyphenols extracted from Hibiscus sabdariffa L. inhibited lipopolysaccharide-induced inflammation by improving antioxidative conditions and regulating cyclooxygenase-2 expression. Biosci Biotechnol Biochem 2009; 73: 385-90.
16. Banerjee S, Rajamani P: Cellular, molecular, and biological perspective of polyphenols in chemoprevention and therapeutic adjunct in cancer. Nature Prod 2013; 2175-54.
17. Shoji T, Akazome Y, Kanda T, Ikeda M: The toxicology and safety of apple polyphenol extract. Food Chem Toxicol 2004; 959-67.
18. Akazome, Y: Characteristics and physiological functions of polyphenols from apples. Biofactors 2004; 22: 311-4.
19. Sugiyama H, Akazome Y, Shoji T, Yamaguchi A, Yasue M, Ohtake Y.Oligomeric Procyanidins in Apple Polyphenol Are Main Active Components for Inhibition of Pancreatic Lipase and Triglyceride Absorption. J Agric Food Chem 2007; 55: 4604-9.
20. Kojima T, Akiyama H, Sasai M, Taniuchi S, Goda Y, Toyoda M, et al. Anti-allergic effect of apple polyphenol on patients with atopic dermatitis: Aa ppilot study. Allergol Internat 2000; 49: 69-73.
21. Hu SH, Liang ZC, Chia YC, Lien JL, Chen KS, Lee MY, et al: Antihyperlipidemic and antioxidant effects of extracts from Pleurotus citrinopileatus. J Agric Food Chem 2006; 5: 2103-10.
22. Sluis AA, Dekker M, Jager A, WMF Jongen.Activity and concentration of polyphenolic antioxidants in apple: effect of cultivar, harvest year, and storage conditions. J Agric Food Chem 2001; 49: 3606-13.
23. Yang KC, Tsai CY, Wang YJ, Wei PL, Lee CH, Chen IH, et al. Apple polyphenol phloretin potentiates the anticancer action of paclitaxel through induction of apoptosis in human hep G2 cells. Mol Carcinogen 2009; 48: 420-31.
24. Masuokaa N, Niheib K, Maetaa A, Yamagiwac Y, Kubo I: Inhibitory effects of cardols and related compounds on superoxide anion generation by xanthine oxidase. Food Chem 2015; 166: 270-274.
25. Shafiei MS, Lui S, Rockey DC. Integrin-linked kinase regulates endothelial cell nitric oxide synthase expression in hepatic sinusoidal endothelial cells. Liver Int 2015; 35: 1213-21.
26. Fujiwara K, Nakashima S, Sami M, Kanda T: Ninety-day dietary toxicity study of apple polyphenol extracts in Crl: CD (SD) rats. Food Chem Toxicol 2013; 56: 412-22.
27. James PE, Madhani M, Roebuck W, Jackson SK, Swartz HM. Endotoxin-induced liver hypoxia: defective oxygen delivery versus oxygen consumption. Nitric Oxide 2002; 6: 18-28.
28. Marnett LJ: Aspirin and the potential role of prostaglandins in colon cancer. Cancer Res 1992; 52: 5575-89.
29. Eling TW, Thompson DC, Foureman GL, Curtis JF, Hughes MF. Prostaglandin H synthase and xenobiotic oxidation. Annu Rev Pharmacol Toxicol 1990; 30: 1-45.
30. Nguyen T, Brunson D, Crespi CL, Penman BW, Wishnok JS, Tannenbaum SR. DNA damage and mutation in human cells exposed to nitric oxide in vivo. Proc Natl Acad Sci USA 1992; 89: 3030-4.
31. Good PF, Werner P, Hsu A, Olanow CW, Perl DP. Evidence for neuronal oxidative damage in Alzheimer's disease. Am J Pathol 1996; 149: 21-8.
32. Xu ZR, Li JY, Dong XW, Tan ZJ, Wu WZ, Xie QM, Yang YM. Apple Polyphenols Decrease Atherosclerosis and Hepatic Steatosis in ApoE−/− Mice through the ROS/MAPK/NF-κB Pathway. Nutrients 2015; 7: 7085-105.
33. Sekhon-Loodu S, Catalli A, Kulka M, Wang YW, Shahidi F, Rupasinghe HPV. Apple flavonols and n-3 polyunsaturated fatty acid-rich fish oil lowers blood C-reactive protein in rats with hypercholesterolemia and acute inflammation. Nutri Res 2014; 34: 535-43.
34. Cheng D, Zhu C, Wang C, Xu H, Cao J, Jiang W. Hepatoprotective effects of apple polyphenol extract on aluminum-induced liver oxidative stress in the rat. Canad J Physiol Pharmacol 2014; 92: 109-16.
35. Xie QW, Kashiwabara Y, Nathan C. Role of transcription factor NF-kappa B/Rel in induction of nitric oxide synthase. J Biol Chem 1994; 269: 4705-8.
36. Surh YJ, Chuna KS, Chaa HH, Hana SS, Keuma YS, Park KK, et al. Molecular mechanisms underlying chemopreventive activities of anti-inflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-kB activation. Muta Res 2001; 480-481: 243-68.
37. Zocca A, Ryugo K. Changes in polyphenoloxidase activity and substrate levels in maturing "Golden Delicious" apple

and other cultivars. HortScience 1975; 10: 586-7.
38. Coseteng MY, Lee CY. Changes in apple polyphenoloxidase and polyphenol concentrations in relation to degree of browning. J Food Sci 1987; 52: 985-9.
39. Murata M, Tsurutani M, Tomita M, Honmma S, Kaneko K. Relationship between apple ripening and browning: changes in polyphenol content and polyphenol oxidase. J Agric Food Chem 1995; 43: 1115-21.
40. Yue T, Shao D, Yuan Y, Wang Z, Qiang C. Ultrasound-assisted extraction, HPLC analysis, and antioxidant activity of polyphenols from unripe apple. J Sep Sci 2012; 35: 2138-45.
41. Rocha AM, Morais AM. Polyphenoloxidase activity and total phenolic content as related to browning of minimally processed 'Jonagored' apple. J Sci Food Agric 2001; 82: 120-6.

蘋果多酚對脂多醣誘導之發炎作用與調節環氧化酶、一氧化氮合成酶及微管活化磷酸化酶訊息路徑有關

李宜臻[1] 李彗禎[2,*]

[1]義大醫院 營養治療科
[2]中山醫學大學醫學系 生化科

受文日期：民國 104 年 10 月 28 日；接受刊載：民國 105 年 03 月 12 日

摘要

背景與目的：蘋果多酚在先前文獻中被證實在體內試驗具有抗氧化及抗發炎作用，為了進一步探究其抗發炎機轉，以脂多醣誘導體內及體外發炎作用，並檢測其相關蛋白以釐清機轉。
方法：蘋果多酚處理脂多醣誘導的巨噬細胞，並檢測細胞中 COX-2 及 iNOS。動物實驗則是預先給予動物 5 天的蘋果多酚，之後腹腔注射 5 mg/kg 脂多醣，6 小時後犧牲動物，取肝臟組織測定 COX-2、JNK 及 p38 的變化。
結果：本結果顯示在體外發炎模式中，蘋果多酚可降低巨噬細胞中 COX-2 及 iNOS 的表現；並可降低 LPS 誘導發炎肝組織中的 COX-2、JNK 及 p38 表現量。
討論：結果指出蘋果多酚在體內及體外系統中皆具有抗發炎作用，且其機轉可能是經由調節 COX 、iNOS 及 MAPK 訊息傳遞路徑所致。

關鍵詞：蘋果多酚、脂多醣、抗發炎、訊息路徑

* 通訊作者：李彗禎 中山醫學大學醫學系 生化科 40201 台中市建國北路一段 110 號

Original Article

Percutaneous Endoscopic Foraminoplasty for Lumbar Spinal Lateral Stenosis

Shao-Keh Hsu*, Chia-Hsiao Keo, Sheng-Chi Chen

Department of Orthopedic, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan

Received: Jun. 26, 2014; Accepted: Mar. 11, 2015

Abstract

Background : This article reports the preliminary results from a study on the efficacy and feasibility of percutaneous endoscopic foraminoplasty (PEF) in the treatment of lumbar spinal lateral stenosis.
Methods : This prospective study included 38 patients (mean age, 73 years; range, 62–88 years) fulfilling the following inclusion criteria: (1) degenerative lumbar foraminal stenosis with or without lateral recess stenosis; (2) neurological claudication with sciatica; (3) MR/CT confirmation of the pathology; or (4) failed conservative management of at least 3 months. These patients underwent PEF under local, aware-state anesthesia.
Results : On evaluation with the MacNab scale, the results for patients in the foraminal and lateral recess stenosis sub-group were less satisfactory than those for patients in the foraminal stenosis sub-group (65% vs. 83%). The overall success rate was 76%. The VAS pain scale score showed an average improvement of 4.5 points. The Oswestry Disability Index score showed a >20% improvement in 30 patients (30/38: 79%) and a <20% improvement in eight patients (8/38: 21%) at the 2-year follow-up. No neurovascular injuries were reported. Two patients had transient postoperative dysesthesia.
Conclusions : PEF is a safe and effective method for the treatment of lumbar spinal foraminal stenosis, particularly in elderly and medically-compromised patients in whom general anesthesia is not recommended. Careful preoperative evaluation and appropriate patient selection is crucial to obtaining satisfactory outcomes, following arthroscopic foraminal decompression. However, patients with severe degenerative spondylosis associated with advanced bony lateral recess stenosis would not benefit from this operative procedure.

Key words: Percutaneous, endoscope, foraminoplasty, transforaminal, lateral recess stenosis, foraminal stenosis

Introduction

In the present decade, percutaneous endoscopic transforaminal approach has found application as a surgical technique in spinal surgery. The percutaneous transforaminal route facilitates the correction of problems in the foraminal and extraforaminal zones and the epidural space, which are difficult to approach by conventional operative techniques. Two fundamental differences make this minimally invasive technique unique. First, the surgery can be done in the state of consciousness, allowing communication with the patient during surgery, thereby helping with more accurate identification of the source of pain. Second, using the posterolateral approach, the pathology around the intervertebral foramen, foraminal, and extraforaminal areas can be easily accessed. Endoscopic aware-state studies and anatomical studies of foramina have indicated various factors that cause the compressive entrapment or irritation of the nerve root[1-3] and the presence of other painful foraminal tissues in a degenerative spine. This article reports the results from an early prospective study of percutaneous endoscopic foraminoplasty (PEF) in patients with lumbar spinal lateral stenosis.

*Correspondence to: Shao-Keh Hsu, Department of Orthopedic, Tungs' Taichung MetroHarbor Hospital, No.699, Sec. 8, Taiwan Blvd., Wuqi Dist., Taichung City 43503, Taiwan (R.O.C.)

Materials and Methods

From February 2005 to February 2007, 38 patients (17 men and 21 women; average age, 73 years; range, 62–88 years) were included in this prospective study. Twenty-six patients were medically compromised (five patients with angina, 12 patients with DM, seven patients with COPD, and two patients with colon cancer) The following were the inclusion criteria: (1) degenerative lumbar foraminal stenosis with or without lateral recess stenosis; (2) neurological claudication with sciatica; (3) magnetic resonance (MR)/computed tomography (CT) confirmation of the pathology; and (4) failed conservative management of at least 3 months. The following were the exclusion criteria: (1) previous spinal surgery; (2) vascular claudication; (3) grade II degenerative spondylolisthesis; (4) unfit for local anesthesia; and (5) cauda equina syndrome. Thirty cases were at the L45 level and eight at the L5S1 level. Percutaneous endoscopic foraminoplasty was applied in these cases under local anesthesia. The clinical outcomes were measured using the MacNab criteria, VAS pain scale, and Oswestry Disability Index score.

Operative Technique

Patients were placed in the prone position with the lumbar spine in mild flexion on a radiolucent table. Aware-state analgesia, with a focus on patient feedback, was initiated with 1–2 ampoules of 2–5 μg/kg fentanyl and ketamine administered intravenously. A bolus of 1 gm cefazolin was administered 30 minutes before the surgery. The skin and subcutaneous tissues were infiltrated with a local anesthetic (1% lidocaine) solution. The needle entry was selected at a point 10–14 cm from the midline, depending on the patient's size, and was situated just above the facet joint on lateral view and at an angle of 10–25° from the horizontal plane. A needle guide wire was placed on the facet joint at the junctional point of the upper endplate of the adjacent vertebral body, and a facet block was carried out through the superolateral synovial recess using 1% lidocaine (Fig 1). Under biplanar X-ray guidance, the dilator tube was railroaded to the facet joint and a beveled working sleeve was applied. Thereafter, an 8.0 mm Richard Wolf endoscope with an eccentrically-placed 4.1-mm working channel and two irrigation channels was inserted. A 2000 mL gravity-aided normal saline irrigation system was suspended 200 cm above the patient and connected to the scope irrigation channel. A 2.1-mm diameter side-firing Holmium-YAG laser probe with an internal irrigation system was inserted through the endoscope to facilitate tissue and bone cutting. An Ellman bipolar was used as the coagulator. Throughout the procedure, an image intensifier was used intermittently to verify the correct positioning of the endoscope and the bone reamer.

In the first stage, the caudal margin of the foramen and the extraforaminal zone were cleared to identify the facet joint. The ascending and descending facet-joint surfaces were excavated and undercut using a bone reamer and a 4-mm endoscopic burr, beginning from the caudal and dorsal areas of the foramen and then moving to the ventral side of the foramen until a decompressed space wide enough to allow the endoscope entry beyond the isthmus of the foramen was created. The decompression of the foramen should be initiated caudally to cranially over

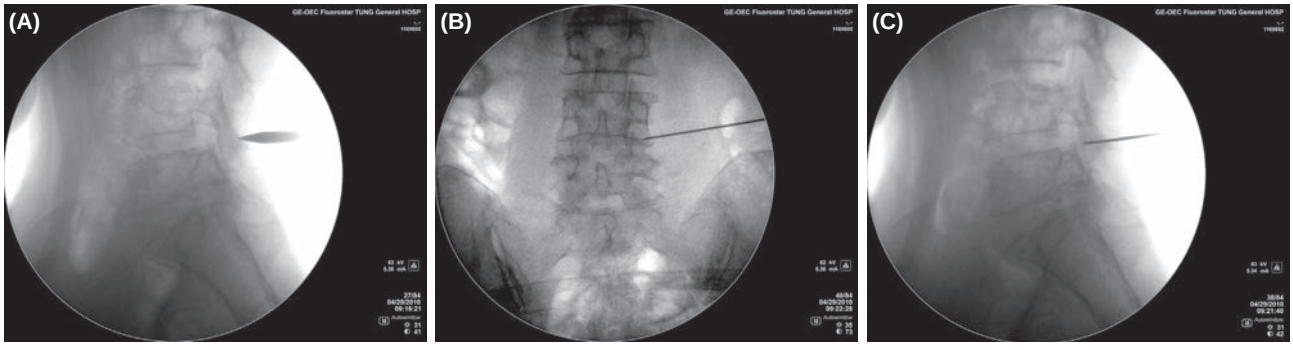

Fig. 1 (A) The entry point must above (posterior) the facet joint which will not injury the abdominal cavity on lateral view. (B) The needle guide position must beween lateral and middle pedicle line on AP view. (C) The needle guide position on the the facet Joint at the junctional point of upper endplate of next vertebral body on Lateral view.

the facet to prevent neural injury. The bone reamer or burr was then advanced through the working cannula until it met the medial pedicular line from the anteroposterior projection and the annulus and epidural space were displayed. In the second stage of surgery, the hard or soft disc protrusions in the epidural or foraminal zones were cleared by laser ablation and micro-punches. Osteophytes along the ascending facet joint and in the superior notch, over the dorsum of the vertebral margin, and over the vertebral shoulder were ablated under endoscopic observation. The superior foraminal ligament was resected with the side-fire laser and micro-punches. In patients with lateral recess stenosis, the decompression was extended caudally to the pedicle of the adjacent vertebra and 2 mm medially beyond the medial pedicular line to undercut the descending facet and ligamentum flavum (Fig. 2).

Finally, the foramen was medially and laterally decompressed to the possible extent until ventral side of the dural sac and traversing nerve root and the junctional area between the dural sac and exiting nerve were visualized (Figs. 3 and 4).

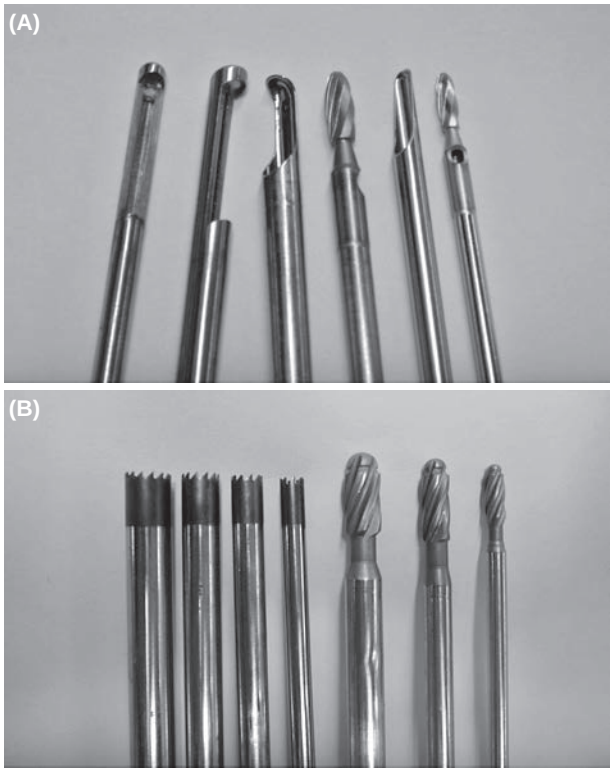

Fig. 2 (A) two type of bone reamer, (B) micropouch and burr with protector.

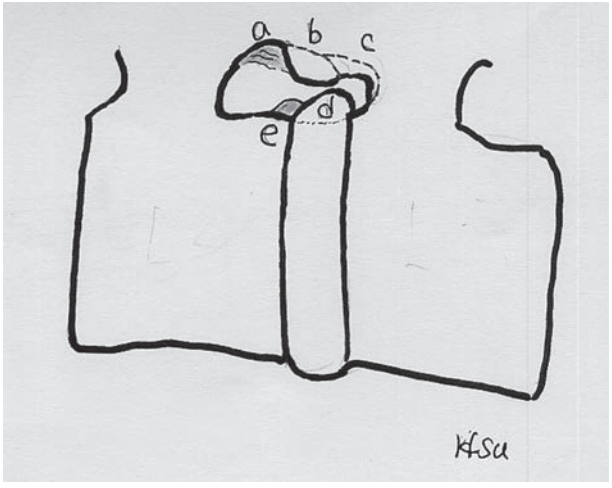

Fig. 3 Foraminal decompression (Foraminoplasty): begin from caudal side of the facet joint (b) then follow clockwise direction around the foramen to manage the pathological entities.
a - Foraminal ligament, b - Hypertrophy facet, c-Pedicle,
d -Disc (hard or soft),e - Shoulder osteophyte

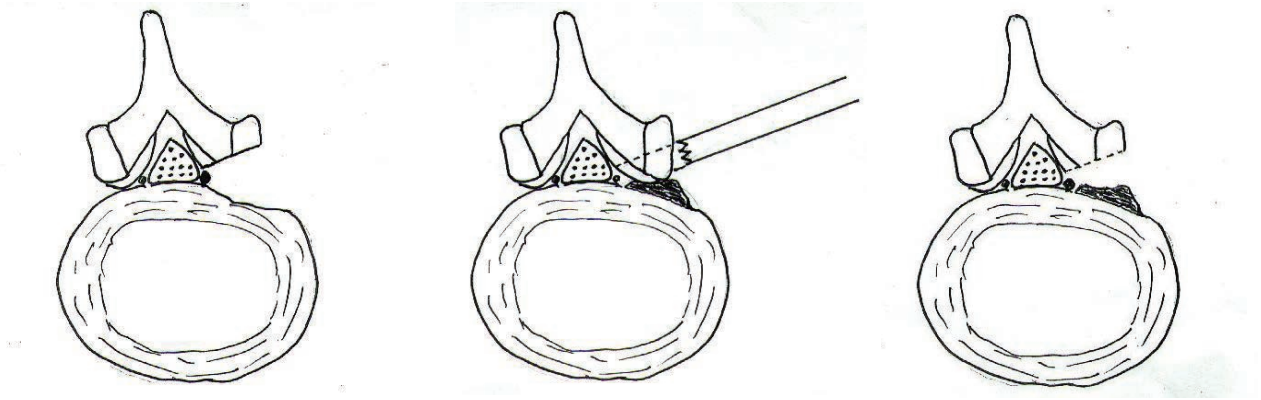

Fig. 4 The surgical procedure. Left: decompress the roof of the foramen with undercutting hyertrophy facet, pedicle and foraminal ligament. Center: decompress the floor of the foramen with removal of osteophytes and disc. Right: lysis and free the traversing and exciting nerve and the foramen was effectively widened

Results

The patients in this study were postoperatively followed for an average period of 30 months (24–42 months). The average operative time was 75 minutes (range, 55–110 minutes) and average hospitalization lasted 1.5 days.

Based on the MacNab scale, the results of patient evaluation are summarized in Table 1. Patients in the foraminal and lateral recess stenosis group showed a less satisfactory result than those in the foraminal stenosis group (65% vs. 83%). The overall success rate was 76%. Six patients were considered to have poor results, including residual back and buttock pain, and the procedure was considered to have failed although there was at least 50% improvement in symptoms as compared with the preoperative state. Three other patients showed poor results, with persistent back and leg pain that remained unchanged from the pre-operative state, and two patients underwent open decompression 4 months after the PEF.

Table 1. summary of post-operative results

	Results (MacNab scale)		
	Foraminal stenosis	Froaminal & lateral recess stenosis	Total
Excellent	12	4	16
Good	8	5	13
Fair	3	3	6
Poor	1	2	3
Satisfaction	20/24(83%)	9/14(64%)	29/38(76%)

The VAS pain scale showed a 4.5-point improvement on average (preoperative VAS: 7.3 to postoperative VAS: 2.8). The Oswestry Disability Index score showed an improvement of over 20% in 30 patients (30/38: 79%) and less than 20% in eight patients (8/38: 21%) at the 2-year follow-up. No major complications or neurovascular injuries were found in any patient. Two patients had transient dysesthesia and received treatment with anti-inflammatory drugs and a short course of steroid therapy. All of the patients had recovered without sequelae at 3 months after the surgery.

Case: An 83-year-old diabetic woman with L45 lateral spinal stenosis, right-sided sciatica, and inability to walk for more than 10 m underwent L45 bilateral PEF and experienced symptom improvement after this procedure. Figures 5–7 show images from this patient.

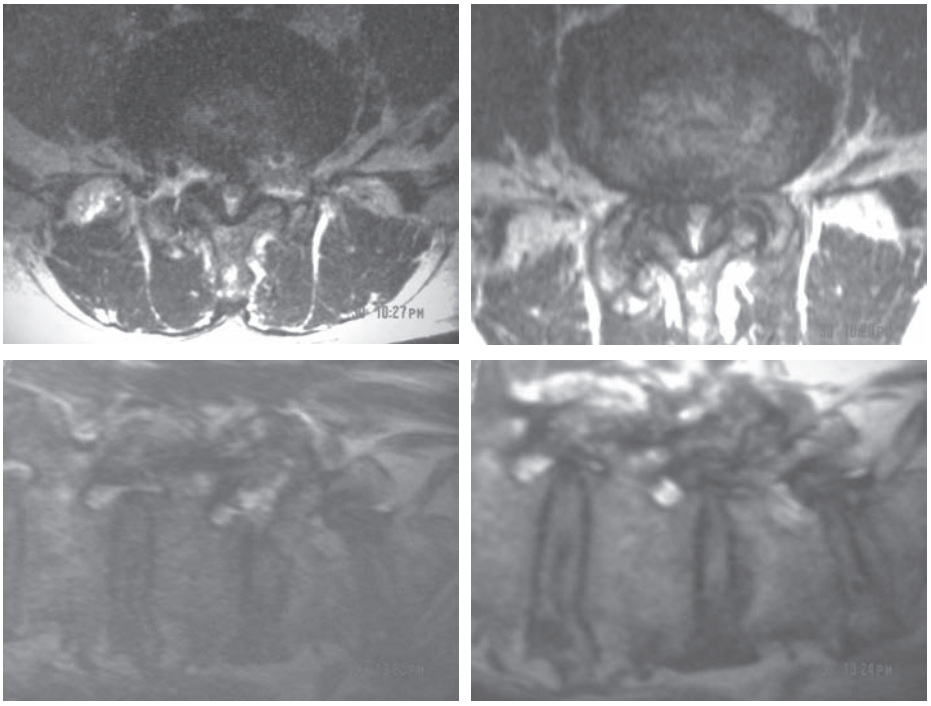

Fig. 5 left upper is pre-operative axial MRI, right upper is post-operative axial MRI, Left lower is pre-operative sagittal MRI, right lower is post-operative sagittal MRI after undercutting the facet joint, partial annulectomy, resect the ligament and osteophytes, the foraminal area is decompressed.

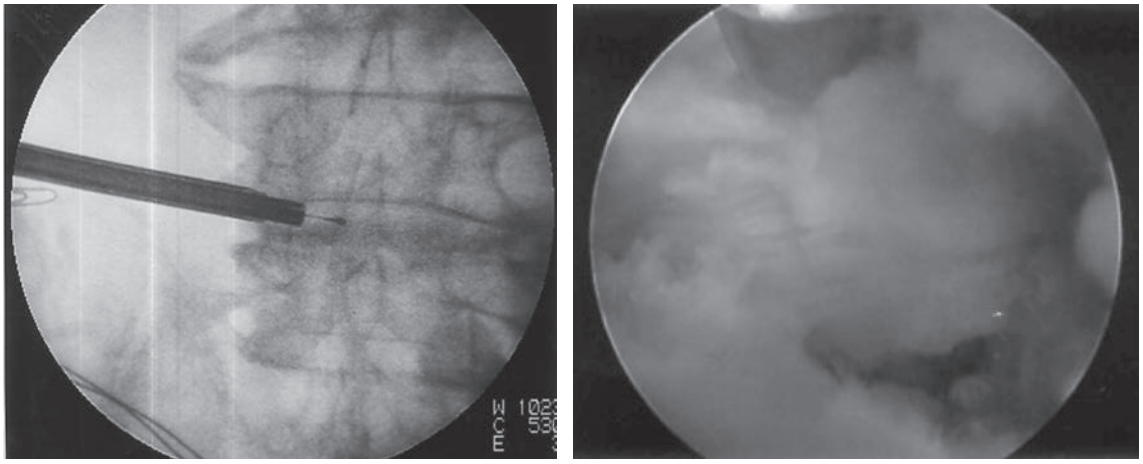

Fig. 6 Decompression must access to the 2mm medial to the medial pedicle line (left) which the ventral side dural sac and traversing root will be seen (right).

Discussion

Despite a heightened awareness of the natural history of degenerative lumbar spinal stenosis, the optimal timing of decompression surgery remains unclear. Little or no improvement in 75% of non-operative patients managed by aggressive conventional therapy was found in a 3-year survivorship analysis[4,5]. Early operative intervention has been previously recommended for the treatment of symptomatic spinal stenosis because the disease always has a progressive course[6]. In a cohort study by Johnsson, patients with a preoperative symptom duration of more than 4 years had a less favorable result at the 5-year follow-up[7]. No differences in the functional outcome for patients with spinal stenosis were found among patients who underwent different types of surgeries, such as decompression only or decompression with fusion with or without instrument, in various studies[8,9]. In 2008, Yoshio reported that degenerative changes in the lumbar spine progressed into disc degeneration, and secondary osteoarthritis of the facet joints was observed in a 15-year cohort study. However, degenerative spondylolisthesis and scoliosis had not progressed in a 70-year-old patient for 15 years. Elderly patients with a lumbar spinal disorder may not require fusion surgery but need treatments based on the natural disease progression in the lumbar spine instead[10]. Therefore, decompression along the foramen is an ideal the treatment of spinal stenosis in elderly patients. However, the complications of general anesthesia are high in elderly patients; moreover, late complications of open surgery have also been reported in these patients. Percutaneous endoscopic transforaminal foraminoplasty is a newer surgical technique that can be performed in the aware state. This technique grants access to the foraminal area through an 8-mm posterolateral incision; the muscle fibers are not severed but are separated with the aid of a beveled working sleeve, thus preventing the denervation of the musculature and scar formation and promoting rapid recovery and the return of function. In the traditional spinal surgeries, there is a compromise of the normal flow of the epidural and neural venous systems following the intraoperative application of the traction

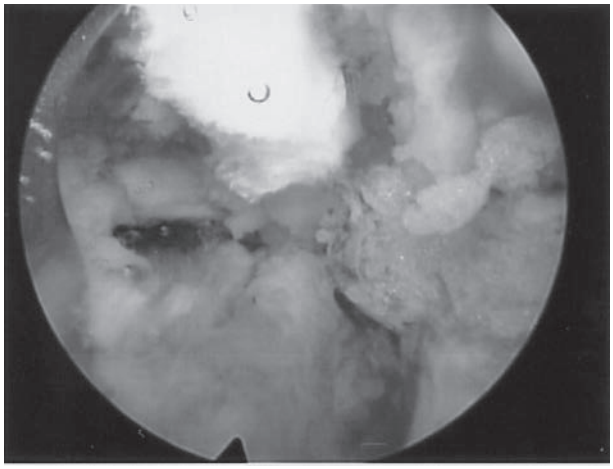

Fig. 7 Decompression cranial to foramen until inspection of the ventral side of the dural sac and traversing nerve root and junctional area between dural sac and exiting nerve.

of the nerve root and the dura, excessive electrocoagulation, and sustained external pressure by osteophytes or bulging discs, which may promote neural venous stasis that can cause chronic edema, fibrosis, hypo-oxygenation of the nerve root, and lead to the development of chronic pain and failed-back syndrome[11-14]. Percutaneous endoscopic transforaminal decompression does not require entry into the spinal canal and, therefore, decreases the risk of the occurrence of these late complications. The following are the principles of foraminoplasty: (1) the enlargement of the foraminal canal by undercutting the hypertrophic facet and the removal of the shoulder and dorsal osteophytes; (2) the removal of hard or soft disc protrusions and thickened ligaments; and (3) the lysis and freeing of the nerve root. Endoscopic foraminoplasty enlarges the foramen to improve the circulation in the spinal canal and also alleviates the symptoms of spinal stenosis. However, a lateral recess bony stenosis, particularly in the horizontal part of the superior facet and medial to the pedicle, is relatively difficult to remove because this part is thicker and harder than the tip of the superior facet. There are technical limitations that are expected to be resolved with the development of newer equipment in the future. In our experience, the percutaneous endoscopic access of the foraminal region of the lumbar spine for treating foraminal stenosis and sciatica is a safe, effective, and minimally invasive procedure. Careful preoperative evaluation and appropriate patient selection is paramount to a satisfactory outcome following arthroscopic foraminal decompression. In addition, patients with severe degenerative spondylosis associated with advanced bony lateral recess stenosis would not benefit from this operative procedure.

Endoscopy revealed that static MR imaging fails to demonstrate the following: (1) the tethering and impaction of the superior facet joint, the tethering to an infolded ligamentum flavum, the facet joint capsule, and the superior foraminal ligament on the nerve; (2) the degree of local scarring and nerves that may have become directly adherent to the disc wall and may have become displaced from their normal path to undergo distortion; and (3) dorsal and shoulder osteophytes that not only encroach upon the foramen but also become tethered to the nerves. In addition, the osteophytes arising from the facet joints, particularly those with significant arthritic changes, can incite local irritation of the exiting nerve within the foramen and the lateral recess. The reduction in neural mobility makes the nerve vulnerable to deformation or irritation from the tension or compression during the different stages and types of spinal movement. Nerve compromise arises at the foramen as the nerve leaves the spinal canal, and the neural distribution matches that normally attributed to segmental disease at a level above the stenosis. This could lead to misdiagnosis and generate incorrect indications for surgical intervention.

Endoscopic foraminoplasty provides a novel method for identifying and treating the source of the pain and sciatica of indeterminate origin, and is a safe and effective method for treating patients with lumbar foraminal stenosis, particularly those who are elderly or medically compromised and those in whom general anesthesia is not recommended. Therefore, for the optimal use of the technique, proficient knowledge of the intervertebral foraminal anatomy and its dynamic status and the pathological mechanisms that mediate back pain and radicular symptoms is required. This technique, which is still in a developmental state, represents an alternative to various open surgical procedures and has a potential for further innovative applications in the future.

Key Points

Percutaneous endoscopic foraminoplasty is a safe and effective method for treating patients with lumbar spinal foraminal stenosis, particularly those who are elderly and medically compromised and those in whom general anesthesia is not recommended.

References

1. Kambin P, Casey K, O' BRIEN E, Zhou L. Transforaminal arthroscopic decompression of lateral recess stenosis. J Neurosurg 1996; 84: 462-7.
2. Rauschning W. Surgical and imaging anatomy of the lumbar spine. In: Proceedings of the 9th international conference of lumbar fusion and stabilisation. Cheshire 1999; 31.
3. Hasegawa T, Mekawa Y, Watnabe R, An H. Morphometric analysis of lumbosacral nerve roots and dorsal nerve root ganglia by magnetic resonance imaging. Spine 1996; 21: 1005-9.
4. Johnsson KE,Rosen I, Uden AThe natural course of lumber spinal stenosis. Clin Orthop 1992; 279: 82-6.
5. Simotas A, Dorey F,Hansraj K, Cammisa F. Nonoperative treatment for lumbar spinal stenosis:clinical and outcome results and a 3-year survivorship analysis. Spine 2000; 25: 197-211.

6. Spivak JM. Degenerative lumbar spinal stenosis. J Bone Joint Surg 1998; 80: 1053-66.
7. Johnsson B, Annertz M, Sjoberg C, Stromqvist B. A prospective and consecutive study of surgically treated lumbar spinal stenosis part II: five year follow-up by independent observer. Spine 1997; 22: 2938-44.
8. Grob D, Humke T, Dvorak J. Degenerative lumbar spinal stenosis. Decompression with and without arthrodesis. J Bone Joint Surg 1995; 77: 1036-41.
9. Mardjetko SM, Connolly PJ, Shott S. Degenerative lumbar spindylolisthesis. Ameta-analysis of literature 1970-1993. Spine 1994; 19: 2257S-65S.
10. Yoshio Enyo, Munehito Yoshida. Do high-aged people with lumbar spinal disorder need instrumentation surgery? The longitudinal study of degenerative change in the lumbar spine. In: Proceedings of the the 8th Pacific and Asian Society of Minimally Invasive Spine Surgery. Bali-Indonesia 2008; 15-18.
11. Bakkum BW, Mestand M. The effects of trans-foraminal ligaments of the sizes of T11 to L5 human intervertebral foramina. J Manipulative Physiol Ther. 1994; 17: 517-22.
12. Hoyland JA, Freemont AJ, Jayson MIV: Intervertebral foramen venous obstruction. Acause of periradicular fibrosis ? Spine 1989; 14: 538-68.
13. Olmarker K, Rydevik B, Holm S: Edema formation in spinal nerve roots induced by experimental graded comprssion. An experimental study on the pig cauda equina with special reference to differences in effects between rapid and slow onset of compression. Spine 1989; 14: 569-73.
14. Parke WW: The significance of venous return impairment in ischemic radiculopathy and myelopathy. Orthop Clin North Am 1991; 22: 213-22.

經皮內視鏡治療腰椎外側狹窄初期報告

徐少克* 郭家孝 陳聖祺

童綜合醫院 骨科部

受文日期：民國 103 年 06 月 26 日；接受刊載：民國 104 年 03 月 11 日

摘要

本文報導了一個初步的結果，經皮內視鏡下椎孔成形術下治療腰椎管外側狹窄的可行性和療效。38 例病患，平均年齡 73 歲（範圍 62-88 歲）被列入本前瞻性研究。入選標準 1. 退行性腰椎間孔狹窄帶或不帶側隱窩狹窄。2. 神經性跛行與坐骨神經痛。3. 病理性的 MR/ CT 確認。4. 至少 3 個月的保守治療無效。所有病患皆在局部感知麻醉狀態下進行經皮內視鏡椎孔成形術。

建立在 MacNab 疼痛評分基礎上，病患同時有椎間孔及側隱窩狹窄組比單純椎間孔狹窄者不滿意（65% 比 83%），總滿意率為 76%。在 VAS 疼痛評分提高 4.5 點的平均值。在兩年隨訪：Oswestry 功能障礙指數得分：30 位患者改善超過 20%（30/38 79%）和 8 位患者改善小於 20%（21% 8/38）。無神經血管損傷併發症產生。只有兩個病人出現暫時性的術後神經性異常疼痛。

經皮內視鏡下椎孔成形術是治療腰椎間孔狹窄的一種安全又有效的方法，特別是對於不適用於全身麻醉的老人和嚴重內科疾病的患者。仔細的術前評估和適當的患者選擇是達成手術滿意極為重要的項目。患者有較嚴重的骨性側隱窩狹窄及嚴重退行性病變不適用於這種手術方式。

關鍵詞：經皮內視鏡、椎孔成形術、椎間孔間狹窄、側隱窩狹窄

* 通訊作者：徐少克醫師 童綜合醫療社團法人童綜合醫院 骨科部 43503 臺中市梧棲區臺灣大道八段 699 號

Case Report

Infantile Hypertrophic Pyloric Stenosis: A Case Report and Literature review

Ssu-Wen Chen[1], Pin-Ho Pan[2,5]*, Peng-Nien Tuan[1], Chien-Hung Kuo[1], Min-Yung Kuo[3], Chiao-Yon Huang[4]

Department of Family Medicine[1], Pediatrics[2], and Pediatric surgery[3], Radiology[4], Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan
Department of Rehabilitation Science[5], Jen-The Junior College of Medicine, Nursing and Management

Received: Aug. 28, 2015; Accepted: Jan. 27, 2016

Abstract

A 2-month-old male infant without relevant disease presented with a 7-week history of intermittent projectile nonbilius vomiting after bottle-feeding of formula. Other symptoms were non-specific. Although feeding was difficult, his body weight gain was good. Abdominal ultrasonography revealed pyloric hypertrophy; this led to a diagnosis of infantile hypertrophic pyloric stenosis (IHPS). He also had a family history of epilepsy, duodenal ulcer, and vesicoureteral reflux; however, the patient himself had none of these diseases and none of his family members had IHPS. Fredet–Ramstedt pyloromyotomy was performed; it resulted in symptom relief. His clinical presentations were mild and body weight gain was normal. There were no peristaltic waves, no obvious pyloric mass palpable without sedation, and no specific findings on a plain abdominal radiograph; this was not consistent with the findings of usual cases in the past.

Key words: Infantile hypertrophic pyloric stenosis, nonbilius vomiting, infant, plain abdominal radiograph, abdominal ultrasound

Introduction

Infantile hypertrophic pyloric stenosis (IHPS) is characterized by immediate postprandial projectile (forceful) nonbilius vomiting and pyloric hypertrophy. Other symptoms and signs include a palpable pyloric "olive," hyperbilirubinemia, electrolyte abnormalities, short intervals between feeds (hungry vomiter), dehydration, peristaltic waves, and failure to thrive[1-4]. IHPS often starts at the age of 2 to 6 weeks but may occur as early as at the age of 1 week or even 5 months[1-3]. The incidence of IHPS is reportedly about 3 cases per 1000 infants[1-3] and 0.39 cases per 1000 living births in Taiwan[4]. Risk factors include male sex, prematurity, bottle feeding, caesarean delivery, first-born children, a parent or sibling with the disease, maternal smoking during pregnancy, and maternal age of less than 20 years[1-6]. We encountered a relatively mild case of IHPS with a unique family history.

*Correspondence to: Pin-Ho Pan, M.D., Department of Pediatric, Tungs' Taichung MetroHarbor Hosptial, No.699, Sec. 8, Taiwan Blvd., Wuqi Dist., Taichung City 43503, Taiwan (R.O.C.)

Case Report

A 2-month-old full-term male infant born to a 28-year-old G6P4A2 nonconsanguineous married woman by caesarean section at gestational week 37, without congenital diseases, metabolic diseases, or dysmorphism presented with a 7-week history of intermittent projectile vomiting after feeding. The symptoms began at the age of 1 week. The vomit was mainly semidigested formula milk and contained no blood or greenish bile. Other symptoms included persistent abdominal distension, decreased urine production, and irritable crying for 1 day. No other gastrointestinal symptoms, such as watery loose stools, were noticed. His body weight was 2700 g (3^{th}–15^{th}

percentile) at birth and 5.2 kg (15^{th}–50^{th} percentile) at the age of 2 months. Although feeding was difficult, his body weight gain was good. The infant had taken several medications, including simethicone, Biotase, and domperidone, which were prescribed at a local medical department, but his symptoms had not improved.

The patient also had a family history of epilepsy, duodenal ulcer, and vesicoureteral reflux. His mother had a history of epilepsy that was treated with lamotrigine at 100–200 mg per day for 9 years. During pregnancy, generalized convulsions were more frequent with conscious loss lasting for 3 min, which occurred approximately 1–2 times per week. His father had a history of duodenal ulcers. One of his brothers had a history of febrile convulsions and recurrent urinary tract infections. His sister had a history of bilateral vesicoureteral reflux with recurrent urinary tract infections. A second brother had a history of seizures with generalized tonic–clonic convulsions and cyanosis, psychomotor retardation, and cystitis with mild left hydronephrosis; he had died of choking and pneumonia.

On physical examination, the patient's vital signs were stable, but he showed dry lips, abdominal distension, and hypoactive bowel sounds. There were no visible peristaltic waves or an obvious palpable pyloric mass at the epigastric area. Laboratory tests showed a white blood cell count of 6,400 cells/mm^3 with 2.5% eosinophils, sodium at 136 meq/L (normal range, 137–150 meq/L), and potassium at 5.6 meq/L (normal range, 3.5–5.3 meq/L). Plain abdominal radiography revealed unremarkable bowel gas patterns (Fig. 1). Abdominal ultrasonography revealed a pyloric muscle thickness of 3.97 mm and length of 20.2 mm; this met the criteria of IHPS (Fig. 2). Besides those abovementioned, there were no negative findings.

Surgical intervention was planned. Under anesthesia, hypertrophic pylorus, a so-called "olive," was palpable on the upper abdomen. Fredet–Ramstedt pyloromyotomy was performed; it included a longitudinal incision of the pylorus from the gastric portion to the vein of Mayo and the separation of the submucosa using a blunt instrument to relieve constriction. After surgery, he recovered well. A week after surgery, the wound healed well and food intake and stool passage were normal.

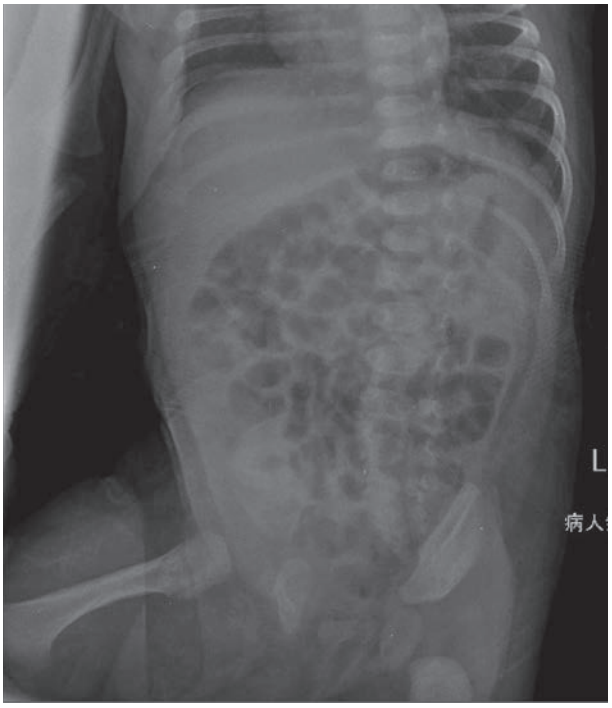

Fig. 1 Abdominal plain radiograph of the patient showed normal bowel gas pattern without a large, dilated stomach.

Discussion

In this case, the symptom of intermittent projectile vomiting after feeding first appeared at the age of 1 week. However, the characteristic "olive" mass was only palpable under anesthesia and no peristaltic wave was observed. Although the patient had signs of dehydration, his body weights gain was good. Taylor et al. reported that the most common presenting symptoms were nonbilius vomiting (99.7%) and

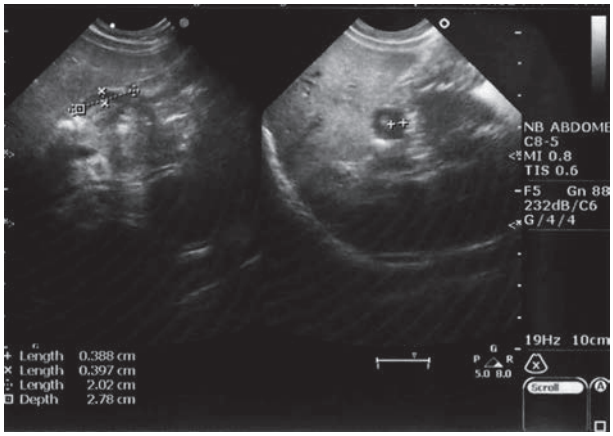

Fig. 2 Abdominal ultrasonography of the patient revealed pyloric muscle thickness 3.97 mm and length 20.2mm, which is compatible to the diagnostic criteria of infantile hypertrophic pyloric stenosis.

failure to thrive or gain weight (46%), although the most common signs were a palpable "olive" (48%), dehydration (32%), and visible peristalsis (25%)[7]. It is obvious that half the total number of IHPS patients may have normal body weight gain or no palpable "olive" mass and majority of the patients have no visible peristalsis.

Imaging studies are indicated for infants with bilius or nonbilius vomiting and no palpable pyloric mass[3]. Although a plain abdominal radiograph may show a "single bobble sign"[8,9], there are no available statistics about the occurrence rate of this sign. In the present case, there was no indication of stomach distension or minimal distal intestinal bowel gas on the radiograph, suggesting pyloric stenosis. In patients with nonbilius vomiting, a differential diagnosis includes gastroenteritis, gastroesophageal reflux, pylorospasm, allergic gastroenteropathy, and antral or pyloric webs[1-3]. These entities may be distinguished by either an upper gastrointestinal series or pyloric ultrasonography[3].

A diagnosis of IHPS is traditionally established by palpation of a pyloric mass[1,2]. However, the rate of diagnosis made solely on the basis of clinical examination has now decreased from 74% to 9%[7]. Two imaging studies that included an upper gastrointestinal series and abdominal ultrasound are commonly used to establish a diagnosis. Ultrasound examination can confirm the diagnosis in majority of the cases[1,2,7]. The criteria with a sensitivity of 95% for diagnosis of IHPS include a pyloric thickness of 3–4 mm, an overall pyloric length of 15–19 mm, and a pyloric diameter of 10–14 mm[1-3]. Abdominal ultrasonography of our patient revealed a pyloric muscle thickness of 3.97 mm and length of 20.2 mm; this met the diagnostic criteria of IHPS (Fig. 2).

Pyloric stenosis is associated with eosinophilic gastroenteritis, which is characterized by recurrent episodes of nonbilius vomiting, malabsorption, abdominal pain associated with an elevated of peripheral eosinophil count, and eosinophilic infiltration of the gastrointestinal tract[1,2]. In this patient, the eosinophil count was in the normal range and there were no signs of malabsorption; thus, an association with eosinophilic gastroenteritis was considered unlikely.

The mechanism of pyloric stenosis remains unknown, but many factors have been implicated, including male sex, prematurity, bottle feeding, caesarean delivery, first-born children, a parent or sibling with the disease, maternal smoking during pregnancy, and maternal age of less than 20 years[1-2,4-6]. Our patient had several risk factors, including male sex, caesarean delivery, and bottle feeding. In addition, there was also a family history of epilepsy, duodenal ulcer, and vesicoureteral reflux. His mother had epilepsy that was treated with lamotrigine at 100–200 mg per day during pregnancy. Although lamotrigine monotherapy has not been associated with an increased risk for major malformations in most available studies[10,11], one study found a dose-dependent increase in malformation rate (2% at <300 mg/day and 4.5% at ≥300 mg/day)[12]. Nevertheless, no association between lamotrigine and IHPS was confirmed[11-13]. Bidair et al. revealed that 6%–33% of IHPS cases were related to anomalies of the central nervous system, gastrointestinal tract, or heart, and a 7-fold increase in urinary tract anomalies, including vesicoureteral reflux[14]. Rogers et al. reported that IHPS was associated with, but not necessarily related to, inherited hyperacidity and duodenal ulcers[15]. The patient's family had diseases associated with IHPS, such as epilepsy, duodenal ulcer, and vesicoureteral reflux; however, the patient himself had none of these diseases and none of his family members had IHPS.

Initial management of pyloric stenosis is directed toward correction of dehydration and electrolyte imbalance[1,3]. After rehydration, there are several options for treatment of IHPS, including pyloromyotomy, endoscopic balloon dilatation, nasoduodenal feedings, and oral or intravenous administration of atropine sulfate. However, surgical management is more time saving and cost effective[1]. Pandya et al. compared open pyloromyotomy and laparoscopic pyloromyotomy and found no difference in the surgical duration, time to full feeding, and length of hospital stay. Although laparoscopic methods are associated with fewer episodes of emesis, fewer doses of analgesia, and less wound complications, the risk of incomplete pyloromyotomy remains[16]. To avoid repeat surgery, the patient underwent open pyloromyotomy and recovered well.

Conclusion

In daily practice, when an infant presents with frequent vomiting, we perform a plain abdominal

radiograph to eliminate ileus or mechanical obstruction. If the image shows normal gastric and bowel gas distribution, we just rely on observation. In the present case, there was no indication of a distended stomach or minimal distal intestinal bowel gas on the radiograph, suggesting pyloric stenosis. However, his symptoms persisted despite medical treatment and change of formula. Finally, IHPS was diagnosed by abdominal ultrasonography. Although the kidney–ureter–bladder radiograph reveals a normal gas pattern, the possibility of IHPS should still be considered, particularly for an infant with frequent nonbilius vomiting unresponsive to medication.

References

1. Anna KH, Chris AL: Hypertrophic Pyloric Stenosis. In: Robert M. Kliegman, Bonita F. Stanton, Joseph W. St Geme III, Nina F. Schor, Richard E. Behrman. Nelson Textbook of Pediatrics, 20th ed. Philadelphia: Elsevier, 2016; 1797-9.
2. Carlos JS, Marilyn JS: Gastrointestinal Tract. In: Marilyn J. Siegel. Pediatric Sonography, 3rd edition. Philadelphia: Lippincott Williams & Wilkins, 2002; 340-3.
3. Frederick A: Pyloric stenosis and congenital anomalies of the stomach and duodenum. In Robert Wyllie, Jeffrey S Hyams. Pediatric Gastrointestinal and Liver disease, 3rd ed. Netherlands: Saunders Elsevier, 2006; 359-72.
4. Leong MM, Chen SCC, Hsieh CS, Chin YY, TS, Shu-Fen Wu, et al. Epidemiological Features of Infantile Hypertrophic Pyloric Stenosis in Taiwanese Children: a Nation-Wide Analysis of Cases during 1997-2007. PLoS ONE 2011; 6: e19404.
5. Camilla Krogh, Robert J. Biggar, Thea ,et al. Bottle-feeding and the risk of pyloric stenosis. Pediatrics 2012; 130: 943-9.
6. Svenningsson A, Svensson T, Akre O, Nordenskjold A. Maternal and pregnancy characteristics and risk of infantile hypertrophic pyloric stenosis. J Pediatr Surg 2014; 49: 1225-31.
7. Nicole D T, Daniel T C, Andrew JAH. Infantile hypertrophic pyloric stenosis: Has anything changed? J Paediatr Child Health 2013; 49: 33-7.
8. Michael Grunebaum: Pediatric Radiological Signs, 1st ed. (vol.1) Lincoln: iUniverse, 2005: 453-8.
9. Rathaus V, Grunebaum M, Ziv N, Kornreich L, Horev G. The bubble sign in the gasless abdomen of the newborn. Pediatr Radiol. 1992; 22: 106-9.
10. Shor S, Koren G, Nulman I. Teratogenicity of lamotrigine. Can Fam Physician. 2007; 53: 1007-9.
11. Tomson T, Battino D, Bonizzoni E, Craig J, Lindhout D, Sabers A, et al. Dose-dependent risk of malformations with antiepileptic drugs: an analysis of data from the EURAP epilepsy and pregnancy registry. Lancet Neurol. 2011; 1: 609-17.
12. Bengt K, Natalia B, Margareta R. The Use of Central Nervous System Active Drugs During Pregnancy. Pharmaceuticals 2013; 6: 1221-86.
13. Matteo Cassina, Arianna Dilaghi, Elena DG, Elena Cesari, Marco DS,et al.ncy outcome in women exposed to antiepileptic drugs: teratogenic role of maternal epilepsy and its pharmacologic treatment. Reproductive Toxicology 2013; 39: 50-7.
14. Bidair M, Kalota SJ, Kaplan GW. Infantile hypertrophic pyloric stenosis and hydronephrosis: is there an association? J Urol 1993; 150: 153-5.
15. Ian M. Rogers. New insights on the pathogenesis of pyloric stenosis of infancy. a review with emphasis on the hyperacidity theory. Open J Pediatr 2012; 2: 97-105.
16. Samir P, Kurt H Pyloric stenosis in pediatric surgery: an evidence-based review. Surg Clin North Am 2012; 92: 527-39.

嬰兒幽門肥厚型狹窄：病例報告及文獻回顧

陳思文[1] 潘品合[2,5*] 段彭年[1] 郭建宏[1] 郭敏勇[3] 黃巧芸[4]

童綜合醫院 家醫科[1] 兒童醫學部[2] 兒童外科[3] 放射科[4]
仁德醫護管理專科學校 復健科[5]

受文日期：民國 104 年 08 月 28 日；接受刊載：民國 105 年 01 月 27 日

摘要

一位二個月大的男嬰，間歇性於瓶餵配方奶後有噴射狀無膽汁嘔吐已七週。 無其他特殊症狀，且體重增加正常。 腹部超音波顯示幽門肥厚，並診斷為幽門肥厚型狹窄。 在家族史方面，有癲癇、十二指腸潰瘍及膀胱輸尿管回流；但病人本身無這些疾病且其他家人並無幽門肥厚病史。 此病人在接受 Fredet - Ramstedt 幽門肌肉切開術後症狀解除。 他的臨床表現較溫和、體重增長正常、理學檢查無明顯蠕動波或摸到橄欖核狀物、腹部 X 光無異常發現 ; 與過去典型表現不同。

關鍵詞：嬰兒幽門肥厚、無膽汁嘔吐、嬰兒、腹部 X 光、腹部超音波

* 通訊作者：潘品合醫師 童綜合醫療社團法人童綜合醫院 兒童醫學部 43503 臺中市梧棲區臺灣大道八段 699 號

Tungs' Medical Journal 10 (2016) 34-38

Case Report

Two viral- and *Pneumocystis Jiroveci*-Related Pneumonia Infections in a Patient with Dermatomyositis: A Case Report

Kuo-Hsi Lin[1], Hau-Hsin Wu[2], Chih-Ming Chen[1], Bei-Rong Su[4], Mao-Wang Ho[3,*]

[1]Division of Infectious Diseases, Department of Medicine, [4]Department of Radiology, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan
[2]Office of Preventive Medicine, Center for Disease Control, Taipei, Taiwan
[3]Division of Infectious Diseases, Department of Medicine, China Medical University Hospital, Taichung, Taiwan

Received: Oct. 15, 2015; Accepted: Jan. 27, 2016

Abstract

Human bocavirus has been reported as an emerging pathogen of the lower respiratory tract, especially in younger children. Human bocavirus rarely causes an infection in adults; however, the significance of its discovery in the respiratory specimens of adult patients is still unclear. In previous reports, human bocavirus DNA was detected in patients with structural lung diseases or hematological malignancies. Herein, we will report on what we believe is the first case of pneumonia secondary to human bocavirus and two other pathogens in a patient with dermatomyositis.

Key words: Bocavirus, Dermatomyositis, Pneumonia

Introduction

Human bocavirus, a recently discovered member of *Parvoviridae*, was first identified in children with respiratory infections in 2005[1]. It was discovered mainly in younger children with a lower respiratory tract infection, especially during the winter season[1,2]. The percentage of children with a lower respiratory tract infection because of human bocavirus ranged from 3.1% to 19%, and most of these children were aged <3 y[1-4]. This virus was also reported to be associated with wheezing in bronchitis or pneumonia in susceptible children, especially if viral loads in the respiratory tract were high[1,3]. However, the significance of human bocavirus in adult patients with pneumonia is still unclear. Some adult cases with structural lung diseases or a compromised immune system have been reported after the detection of human bocavirus DNA in bronchial specimens[5,6]. Herein, we will report on a case with pneumonia secondary to human bocavirus, cytomegalovirus, and *Pneumocystis jiroveci*. To our knowledge, this is the first reported case of pneumonia caused by co-infection with these three pathogens.

*Correspondence to: Mao-Wang Ho, Division of Infectious Diseases, Department of Medicine, China Medical University Hospital, No. 2, Yude Rd., North Dist., Taichung City 404, Taiwan (R.O.C.)

Case Report

A 75-year-old woman with type 2 diabetes mellitus and dermatomyositis presented to the emergency department because of a 1-day fever on November 24, 2014. She was diagnosed with dermatomyositis, presenting with proximal muscle weakness at a local hospital 3 months earlier. She underwent treatment with prednisolone and azathioprine after being diagnosed with dermatomyositis. The treatment course was smooth until 1 week earlier when she developed progressive dyspnea on exertion and fatigue. Her family members noticed that she had fever with chills on the day of presentation at our hospital, and she was sent to the emergency department for admission. She lived with adult family members, and none of her family members had any respiratory tract symptoms prior to her episode of dyspnea. Upon physical examination, she was 155

cm tall and weighed 72 kg. Her vital signs were as follows: blood pressure, 116/70 mmHg; pulse, 93 beats/min; respiration, 24 breaths/min; and body temperature, 37.8ºC. Her level of consciousness was alert. Auscultation of the lungs revealed a crackling noise bilaterally. Blood tests revealed the following: white blood cell count, 8,700/mm^3; segment neutrophil, 93%; lymphocyte, 5%; hemoglobin, 12.9 g/dL; platelet count, 46,000/µL; aspartate aminotransferase, 24 IU/L; alanine aminotransferase, 37 IU/L (normal range, 0-40 IU/L); blood urea nitrogen , 22 mg/dL; creatinine, 0.9 mg/dL; sodium 127 mEq/L; potassium, 4.0 mEq/L; creatinine phosphate kinase, 21 U/L (normal range, 62-287 U/L); and lactic dehydrogenase, 497 IU/L (normal range, 126-252 IU/L). A chest radiograph demonstrated bilateral lung infiltration (Fig. 1a). Moxifloxacin (400 mg once daily) was used to treat her pneumonia. The patient's condition deteriorated while admitted to the ward, and the patient developed respiratory failure the next day. She was transferred to the intensive care unit with non-invasive ventilation because the patient refused intubation. Acyclovir (500 mg every 8 hours) and trimethoprim-sulfamethoxazole (160/800 mg every 8 hours) was prescribed for suspected human simplex virus pneumonitis and *Pneumocystis jiroveci* pneumonia. To perform additional pathogen identification, sputum and serum specimens were sent to a reference laboratory at the Centers of Disease Control of Taiwan. A sputum specimen also sent for detection of *Pneumocystis jiroveci* DNA. Despite antimicrobial treatments, she experienced profound desaturation. After patient approval, the patient was intubated with ventilator support on the third hospital day. Acyclovir was discontinued, and ganciclovir (300 mg once daily) was commenced because of suspicion of cytomegalovirus pneumonia. Upon chest radiography, mild resolution was noticed on the fifth hospital day. However, early in the morning on the sixth hospital day, the patient experienced a sudden cardiac arrest with subcutaneous emphysema. A right-sided tension pneumothorax was revealed with a chest radiograph (Fig. 1b), and a chest tube was inserted during resuscitation. Her hemodynamic status and respiratory condition deteriorated again after resuscitation. Moxifloxacin was discontinued, and imipenem was commenced (500 mg intravenous every 6 hours). Using a multiplex polymerase chain reaction/real-time polymerase chain reaction system to examine the sputum specimen, a report from the reference laboratory at the Taiwanese Centers of Disease Control was positive for cytomegalovirus and human bocavirus DNA. No bacterial pathogen had been isolated from her sputum samples during the entire clinical course. High cytomegalovirus DNA loads (747,050 IU/mL), but not human bocavirus DNA, was also detected in the serum sample. The sputum specimen that was sent for *Pneumocystis jiroveci* DNA detection revealed a positive result. The patient's condition kept deteriorating despite continued

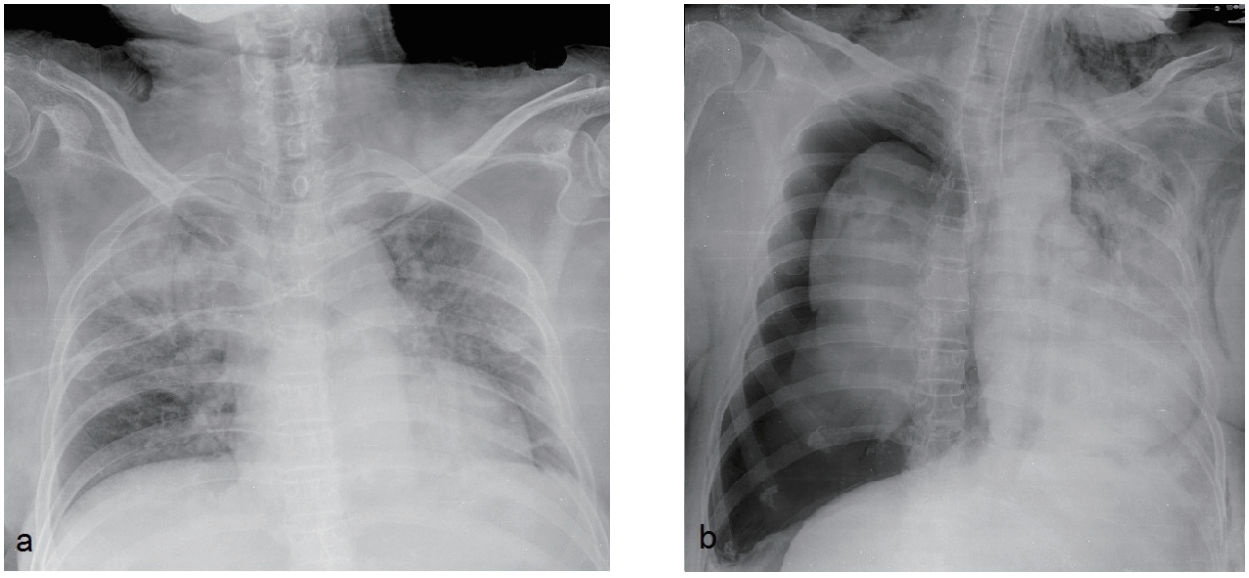

Fig. 1 a. Chest radiography upon admission showing bilateral pulmonary infiltrations. b. Progressive pneumonitis with a right-sided tension pneumothorax

antibiotics and antiviral therapy. She experienced shock that was refractory to treatment, and multiple organ failure developed in the following days. The patient's family members decided to discharge her in critical condition on the 13th hospital day.

Discussion

Since it was first identified in 2005[1], human bocavirus has been frequently isolated from younger children, especially during the winter[1,2]. Co-isolation with other viruses has also been frequently discovered. Viruses are that commonly co-isolated with human bocavirus are respiratory syncytial virus, influenza virus, rhinovirus, and adenovirus[7-9]. A high seroprevalence of anti–human bocavirus antibodies has been reported in adults, implying that most adults have been infected by this virus during childhood[10,11]. However, their isolation in adult respiratory specimens is still rare. Wolfram et al. reported human bocavirus and cytomegalovirus co-isolation in a patient with idiopathic pulmonary fibrosis who experienced respiratory failure. The authors also indicated that human bocavirus might be responsible for the development of idiopathic pulmonary fibrosis[5]. Human bocavirus has also been reported as a cause of pneumonia in children with hematological malignancies. Kupfer et al. reported a case of lymphoma with pneumonia secondary to human bocavirus. Cytomegalovirus DNA was detected in the patient's serum, but not in a bronchoalveolar lavage (BAL) sample from this patient[6]. In our patient, the endobronchial aspirate revealed a positive result for human bocavirus and cytomegalovirus DNA. A high cytomegalovirus DNA load was detected in the patient's serum, confirming the diagnosis of cytomegalovirus disease. Viremia secondary to human bocavirus was not observed in this patient. This is reasonable because human bocavirus viremia occurs most commonly in children aged <2 y[9] and that the prevalence of viremia in adult patients with human bocavirus–associated pneumonia is unclear. Further studies regarding the prevalence and significance of viremia in adults may be necessitated.

Our patient developed pneumothorax during hospitalization, and it led to cardiac arrest eventually. Pneumothorax was frequently seen as a complication in patients with PJP[12]. Patients with bocavirus pneumonia had also been reported having pneumothorax during their treatment course[13,14]. Some of these patients died from pneumothorax related respiratory distress. We treated PJP early but pneumothorax still developed and resulted in tension pneumothorax. It is difficult to know, however, if pneumothorax is due to untreated human bocavirus without pathological studies.

Regarding some factors, we believe that the human bocavirus isolated from our patient's endobronchial aspirate was the causative agent of her pneumonia. First, this patient contracted pneumonia in the winter, when a higher incidence of human bocavirus pneumonia occurs. Second, the patient was administered immunosuppressive therapy for her dermatomyositis, similar to other human bocavirus–infected adult patients. A deficiency in cell-mediated immunity was documented as the main risk factor for the other two pathogens, *P. jiroveci* and cytomegalovirus, co-isolated from a sputum sample. Finally, treatment for the two co-pathogens was initiated early and was maintained throughout the entire treatment course. The patient did not respond to the treatment and died. This fatal pneumonia could be the result of damage secondary to an untreated human bocavirus infection in addition to the other 2 pathogens.

We believe this is the first reported case of pneumonia secondary to human bocavirus, cytomegalovirus, and *P. jiroveci* co-infection in a patient with dermatomyositis. We also provided evidence that human bocavirus is an emerging pathogen of the lower respiratory tract in immunocompromised patients, even if they do not have a structural lung disease or hematological malignancy. Human bocavirus should be considered as a causative agent for refractory pneumonia in patients under immunotherapy.

Reference

1. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proceedings of the National Academy of Sciences of the United States of America 2005; 102: 12891-96.
2. Chuang CY, Kao CL, Huang LM, Huang LM, Lu CY, Shao PL, et al. Human bocavirus as an important cause of respiratory tract infection in Taiwanese children. J Microbial Immunol Infection 2011; 44: 323-7.
3. Allander T, Jartti T, Gupta S, Niesters H. G. M, Lehtinen P, et al. Human bocavirus and acute wheezing in children. Clin Infect Dis 2007; 44: 904-10.

4. Liu WK, Chen DH, Liu Q, Liang HX, Yang ZF, Qin S, et al. Detection of human bocavirus from children and adults with acute respiratory tract illness in Guangzhou, southern China. BMC Infect Dis 2011; 11: 345.
5. Windisch W, Schildgen V, Malecki M, Lenzb J, Brockmannb M, Karagiannidisaet C, et al. Detection of HBoV DNA in idiopathic lung fibrosis, Cologne, Germany. J clini virol 2013; 58: 325-7.
6. Kupfer B, Vehreschild J, Cornely O, Kaiser R, Plum G, Viazov S, et al. Severe pneumonia and human bocavirus in adult. Emerg Infect Dis 2006; 12: 1614-16.
7. Cantais A, Mory O, Pillet S, Verhoevenb PO, Bonneaub J, Patural H, et al. Epidemiology and microbiological investigations of community-acquired pneumonia in children admitted at the emergency department of a university hospital. J clini virol 2014; 60: 402-7.
8. Arnott A, Vong S, Rith S, Naughtin M, Ly S, Guillard B, et al. Human bocavirus amongst an all-ages population hospitalised with acute lower respiratory infections in Cambodia. Influenza and other respiratory viruses 2013; 7: 201-10.
9. Christensen A, Nordbo SA, Krokstad S, Rognlien AG, Dollner H. Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. J clini virol 2010; 49: 158-62.
10. Neske F, Prifert C, Scheiner B, Ewald W, Schubert J, Opitz A, et al. High prevalence of antibodies against polyomavirus WU, polyomavirus KI, and human bocavirus in German blood donors. BMC Infect Dis 2010; 10: 215.
11. Guido M, Zizza A, Bredl S, Lindner J, De Donno A, Quattrocchi M, et al. Seroepidemiology of human bocavirus in Apulia, Italy. Clin Microbiol Infect 2012; 18: E74-6.
12. Chow C, Templeton PA, White CS. Lung cysts associated with Pneumocystis carinii pneumonia: radiographic characteristics, natural history, and complications. AJR Am J Roentgenol 1993; 161: 527-31.
13. Ursic T, Krivec U, Kalan G, Petrovec M. Fatal human bocavirus infection in an 18-month-old child with chronic lung disease of prematurity. Pediatr Infect Dis J 2015; 34: 111-2.
14. Ursic T, Steyer A, Kopriva S, Kalan G, Krivec U, Petrovec M. Human bocavirus as the cause of a life-threatening infection. J Clin Microbiol 2011; 49: 1179-81.

一位皮肌炎病患的兩種病毒及肺囊蟲重複感染的肺炎

林國璽[1] 鄔豪欣[2] 陳志銘[1] 蘇碧榕[4] 何茂旺[3,*]

童綜合醫院 [1]感染科 [4]放射科
[2]衛生福利部疾病管制署預防醫學辦公室
[3]中國醫藥大學附設醫院 感染科

受文日期：民國 104 年 10 月 15 日；接受刊載：民國 105 年 01 月 27 日

摘要

人類博卡病毒（human bocavirus）已被指出是一個新興的下呼吸感染病原，尤其是年紀較輕的兒童身上。然而，人類博卡病毒卻鮮少造成成人的感染。其在成人呼吸道檢體檢出的臨床意義也仍未明。在過去的報告中，人類博卡病毒的ＤＮＡ可在有肺結構性病變及血液惡性腫瘤的病患身上偵測出來。我們將報告人類博卡病毒和其他兩種病原菌在一位皮肌炎病患感炎所造成肺炎的第一例個案。

關鍵詞：博卡病毒、皮肌炎、肺炎

1. Allander T, Tammi MT, Eriksson M, Bjerkner A, Tiveljung-Lindell A, Andersson B. Cloning of a human parvovirus by molecular screening of respiratory tract samples. Proceedings of the National Academy of Sciences of the United States of America 2005;102: 12891-12896.
2. Chuang CY, Kao CL, Huang LM, Huang LM, Lu CY, Shao PL, et al. Human bocavirus as an important cause of respiratory tract infection in Taiwanese children. J Microbial Immunol Infection 2011;44: 323-327.
3. Allander T, Jartti T, Gupta S, Niesters H. G. M, Lehtinen P, et al. Human bocavirus and acute wheezing in children. Clin Infect Dis 2007;44: 904-910.

* 通訊作者：何茂旺醫師 中國醫藥大學附設醫院 感染科 404 臺中市北區育德路 2 號

Case Report

General Anesthesia in a Pediatric Patient with Leigh Syndrome: A Case Report and Literature Review

Ching-Hsin Chang, Chun-Lai Ma, Kuo-Sheng Huang, Liang-Chieh Wang, Yiu-Sion Chen, Kee-Ming Man, Yi-Chun Hung

Department of Anesthesiology[1], Departments of Pharmacy[2], Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan

Received: Nov. 12, 2015; Accepted: Apr. 07, 2016

Abstract

Leigh syndrome is a rare inherited neurodegenerative disorder of infancy and childhood and is characterized by developmental delay of nervous system function and respiratory abnormalities. The latter includes breathing difficulties, aspiration, hypoventilation, and apnea, and it is known that surgery and general anesthesia may cause acute exacerbations with respiratory failure. These patients may also develop episodes of lactic acidosis leading to respiratory failure and death. Owing to the rarity and life-threatening nature of the condition, however, there is no standard protocol for anesthetic management. Based on our own experience of evaluating and performing percutaneous gastrotomy under general anesthesia in a patient with Leigh syndrome, we discuss the attendant dilemmas and solutions and propose a working protocol with an integrated anesthetic plan.

Key words: Leigh syndrome, general anesthesia, anesthetic management

Introduction

Leigh syndrome (LS) is a subacute necrotizing encephalomyelopathy that was first reported in 1951 by Denis Leigh [1]. LS is a severe neurological disorder that typically arises in the first year of life. Typical symptoms are progressive loss of mental and locomotor abilities and death within a couple of years, usually due to respiratory failure. LS is also extremely rare with an estimated prevalence of approximately 1 in 40,000 newborns [2]. Some patients may present with typical symptoms in adulthood or may have symptoms that gradually worsen. Acute exacerbations, particularly with respiratory failure, have been highlighted in the literature, particularly in association with surgery and general anesthesia. However, no effective methods have been developed to prevent or cure these episodic events. In this paper, we present a case of LS undergoing percutaneous gastrostomy under general anesthesia. In addition to sharing our experience, we discuss the attendant dilemmas and solutions, and offer a working protocol with an integrated anesthetic plan.

*Correspondence to: Chun-Lai Ma, M.D., Department of Anesthesiology, Tungs' Taichung MetroHarbor Hospital, No.699, Sec. 8, Taiwan Blvd., Wuqi Dist., Taichung City 43503, Taiwan (R.O.C.)

Case Report

A 1.5-year-old male patient weighing 10 kg was scheduled for percutaneous gastrostomy under general anesthesia. His initial presentation at 6 months old was with failure to thrive, difficulty swallowing, delay in neurological and locomotor development, absence of deep tendon reflexes, hypotonicity, lactic academia, and respiratory symptoms. At that time, brain magnetic resonance imaging showed symmetrical hypodense areas in the lentiform nucleus (globus pallidus), supporting the diagnosis of LS. At the preoperative assessment, the patient was presented to an anesthesiologist under mechanical ventilator support and required an endotracheal tube because of respiratory failure. After transport to

the operating room, routine monitors (non-invasive automatic arterial pressure, three-lead electrocardiography, pulse oximetry, capnography, and esophageal temperature probe) were placed, and all vital signs were within normal limits. Anesthesia was then induced with atropine (0.01 mg/kg) and propofol (1.5 mg/kg).

Neuromuscular blockade was achieved with atracurium (0.5 mg/kg) and maintained with propofol, nitrous oxide, and oxygen. No volatile anesthetics were used. A solution of 0.45% NaCl with 5% dextrose was infused intravenously, and intraoperative mechanical ventilation was provided using a pressure-controlled mode, with the end tidal CO_2 maintained at 30–35 mmHg. During the operation, which lasted 1 hour and was otherwise uneventful, the patient received propofol at 10 mg/kg/hr. After the operation, he was transferred to the pediatric intensive care unit for close observation of his respiration and temperature. The postoperative course was uncomplicated.

Discussion

LS, which typically presents in infancy, is caused by abnormalities of mitochondrial energy generation and is characterized by almost pathognomonic brain changes, particularly in the basal ganglia, thalamus, and brainstem. It is mostly transmitted by autosomal recessive inheritance but can sometimes be transmitted in a mitochondrial pattern [3]. As the illness progresses, hypoventilation and apnea become more apparent, and acute exacerbations with respiratory failure may occur during surgery requiring general anesthesia [4,8]. Therefore, respiratory failure is a common cause of death and postoperative morbidity in patients with LS [4].

Mortalities have been reported in patients with defective pyruvate dehydrogenase complex and various defects of the mitochondrial respiratory chain while under general anesthesia [4,5,6]. The anesthetic agents used in these settings, such as barbiturate or volatile agents, may therefore have adverse effects on patients with LS, including a propensity to cause lactic acidosis and unfavorable metabolic effects [5,7]. Consequently, it is likely that propofol is a better choice because most patients on short-term propofol infusions show no evidence of mitochondrial disturbance [5,9]. Attention to normo-ventilation, acid–base status, and intravenous administration of fluid without lactate, as well as to the avoidance of elective anesthesia in patients with respiratory impairment, have all been recommended in patients with LS; however, practical descriptions of the anesthetic management of patients with LS are lacking [5,6].

Additionally, the use of neuromuscular relaxants in patients with LS has not been studied. We suggest using short acting relaxants (e.g., cis-atracurium, atracurium) titrated to an optimal dose and that neuromuscular function be closely monitored, particularly when repeated doses are indicated [6,7]. The perioperative administration of opioids has not been

Table 1. Anesthetic and perioperative protocol for the management of patients with Leigh syndrome

Possible cardiac and respiratory involvement
• Careful preoperative cardiac and respiratory evaluation (Pre-existing respiratory abnormalities increase the risk of postoperative respiratory failure)
• Pulmonary function testing is suggested
Intraoperative seizures
• Effective preoperative control with antiepileptic therapy
• Normalize serum electrolytes and glucose
• Propofol and midazolam both have antiepileptic effects
• Measure the therapeutic anticonvulsant levels
Lactic acidosis
• Use fluids that contain dextrose during the preoperative fasting period to provide basal glucose requirement
• Check preoperative lactate levels
• Frequent intraoperative serum lactate, pH, and glucose monitoring
• Avoid barbiturates (e.g., thiopental)
• Avoid lactate-containing solutions (e.g., Ringer's lactate)
• There is controversy regarding volatile anesthetic agents compared with propofol
• Avoid opioids with a long duration of action, particularly for short procedures
• Maintain normothermia
• Maintain normocapnia and avoid any hypoxia
• Use sodium bicarbonate for acute exacerbations of acidosis
• Monitor the depth of anesthesia
• Postoperative respiratory monitoring for major procedures
Muscle relaxant considerations
• Use intermediate-acting agents, such as cis-atracurium, atracurium
• Monitor neuromuscular function if repeated doses are used
• Avoid succinylcholine because it may induce an exaggerated hyperkalemic response in myopathic conditions
Malignant hyperthermia
• Although there have been no reported cases after the use of volatile agents, we recommend total intravenous anesthesia

studied in patients with LS, and malignant hyperthermia has never been described.

The major anesthetic concerns related to LS are the substantial risk of respiratory complications, potential for metabolic acidosis, and risk of anesthetic agents inducing adverse metabolic effects. Thus, mechanical ventilation is necessary to prevent from dangerous respiratory acidosis and ensure early action can be taken to compensate for metabolic acidosis if any unexpected events occur during an operation under general anesthesia. For example, an intravenous infusion of sodium bicarbonate may neutralize metabolic acidosis. However, since hypocapnia may inhibit pyruvate carboxylase and worsen the lactic academia, hyperventilation should be avoided [6].

In conclusion, metabolic, neurologic, and respiratory derangements may occur perioperatively in patients with LS. Thus, to avoid complications, following steps need to be taken: anesthetic agents must be chosen with care, blood gases (pH) and lactate should be monitored frequently, normocapnia and normothermia should be maintained throughout, lactate-containing solutions should be avoided, and all patients should be monitored in intensive care with ventilatory support. To clarify this approach, we have integrated the available reports regarding perioperative management and have proposed a protocol for the management of patients with LS requiring surgery under general anesthesia (Table 1).

References

1. Leigh D. Subacute necrotizing encephalomyopathy in an infant. J Neurol Neurosurg Psychiatry. 1951;14: 216-21.
2. Thorburn DR, Rahman S. Mitochondrial DNA-Associated Leigh Syndrome and NARP. Gene Reviews (Internet) 2011.
3. Finsterer J. Leigh and Leigh-like syndrome in children and adults. Pediatr Neurol 2008; 39: 223-35.
4. Grattan-Smith PJ, Shield LK, Hopkins IJ, Collins KJ. Acute respiratory failure precipitated by general anesthesia in Leigh's Syndrome. J Child Neurol. 1990; 5: 137-41.
5. Kocamanoglu IS, Sarihasan E. Anesthetic management of pediatric patient with Leigh syndrome. Rev Bras Anestesiol. 2013; 62: 220-22.
6. Shenkman Z, Krichevski I, Elpeleg ON, Joseph A, Kadari A. Anaesthetic management of a patient with Leigh's syndrome Can J Anaesth 1997; 44: 1091-95.
7. Terkawi AS, Wani TM, Al-Shuaibi KM, Tobias JD. Anesthetic considerations in Leigh disease: case report and literature review 2012; 6: 181-5.
8. Tan AL, Goy R. Anaethetic management of a patients with Leigh's Syndrome with central hypoventilation and obstructive sleep apnoea. Singapore Med J 2013; 54: e250-3.
9. Gozal D,Goldin E, Shafran-Tikva S, Tai D, Wengrower D. Leigh syndrome: anesthetic management in complicated endoscopic procedures. Paediatr Anaesth 2006; 16: 38-42.

嬰幼兒患有 Leigh 症候群之全身麻醉的處置：病例報告及文獻回顧

張錦新　馬振來*　黃國生　王亮傑　陳燿舜　文寄銘　洪宜君

童綜合醫院　麻醉部[1]　藥劑部[2]

受文日期：民國 104 年 08 月 28 日；接受刊載：民國 105 年 01 月 27 日

摘要

Leigh 症候群是一種與粒腺體有關的罕見腦脊髓遺傳疾病，此疾病通常影響在出生 3 個月至 2 歲之間嬰幼兒。臨床上主要症狀是生長發育遲緩，中樞神經系統功能異常和呼吸系統障礙。患者如果需要手術時，所施行的麻醉，可能在麻醉中的施行與麻醉後的照顧，會因為沒有文獻討論，沒有標準流程的指引，而使病患涉入生命危險情況。我們在此報告一名 1.5 歲大患有 Leigh 症候群的幼兒，因胃造瘻而需外科手術。我們想藉此病患在手術施行中所需麻醉進行期間可能遭遇的代謝、呼吸、酸鹼中毒、麻醉葯物選擇、需注意事項做系統性分析與討論，也基於我們的經驗，融合既有文獻建立一標準麻醉處置流程。

關鍵詞：Leigh 症候群、全身麻醉、麻醉處置

* 通訊作者：馬振來醫師　童綜合醫療社團法人童綜合醫院　麻醉部　43503 臺中市梧棲區臺灣大道八段 699 號

Case Report

Anaphylactic Shock During Central Venous Catheter Insertion: A Case Report

Liang-Chieh Wang, Chun-Lai Ma, Yiu-Sion Chen, Kee-Ming Man, Kam-Sun Cheung, Kuo-Sheng Huang*

Department of Anesthesiology, Tung's Taichung MetroHarbor Hospital, Taichung, Taiwan

Received: Nov. 12, 2015; Accepted: Nov. 07, 2016

Abstract

We present a case of anaphylactic shock during central venous catheter insertion. A 78-year-old man with liver cirrhosis, hepatocellular carcinoma, and bone metastasis underwent anesthetic induction for bipolar hemiarthroplasty. He was first anesthetized, but immediately after central venous catheter insertion, he developed shock and cardiovascular collapse that were responsive to defibrillation, resuscitation, and inotropic infusion. He subsequently recovered well and was discharged 6 days later without major sequelae. We excluded major causes for his cardiovascular collapse and conclude that anaphylaxis to chlorhexidine was causative. Although chlorhexidine reduces infection rates because of its superior antiseptic efficacy, a cluster of anaphylactic reactions prompted an alert by the Food and Drug Administration in 1998 regarding its use. We recommend that chlorhexidine-coated central venous catheters be used with caution and that the risk be better emphasized on packaging.

Key words: central venous catheter, chlorhexidine, anaphylactic shock

Background

Central venous catheters (CVCs) are often used in patients undergoing major operations. They not only offer an ideal route for fluid resuscitation but also provide a route for monitoring intravascular fluid status. Chlorhexidine, a bactericidal coating for CVCs, is widely used as a skin disinfectant and in both dental and personal hygiene products. Although anaphylactic reactions to chlorhexidine were once regarded as rare, the more recent literature seems to be challenging this position [1]. Here we report a patient who underwent bipolar hemiarthroplasty and experienced anaphylactic shock during CVC insertion after anesthetic induction.

*Correspondence to: Dr. Liang-Chieh Wang, Department of Anesthesiology, Tung's Taichung MetroHarbor Hospital, No.699, Sec. 8, Taiwan Blvd., Wuqi Dist., Taichung City 43503, Taiwan (R.O.C.)

Case Report

A 78-year-old male patient with liver cirrhosis, hepatocellular carcinoma, and bone metastasis was admitted to our hospital with a peri-prosthetic pathologic right hip fracture. On admission, he was scheduled to undergo bipolar hemiarthroplasty. We traced his operation history, which revealed that he had undergone transurethral resection of the prostate under spinal anesthesia (7 years earlier), high ligation of a left-leg varicose vein under general anesthesia (4 years earlier), transforaminal lumbar interbody fusion (TLIF) under general anesthesia (3 months earlier), and compression hip screw fixation for a right intertrochanteric fracture under general anesthesia (2 months earlier). The same anesthetic agents were used every time he received general anesthesia (lidocaine, fentanyl, thiamylal, rocuronium, and sevoflurane), and we used the same agents during anesthetic induction in the present procedure. Lidocaine (80 mg), fentanyl (100 mcg), thiamylal (300 mg), and rocuronium (40 mg) were administered without

complications, and tracheal intubation was completed smoothly.

No antibiotic was prescribed by the surgeon on this occasion because, during the TLIF operation, cefazolin was considered to have caused skin erythema. However, during that procedure, intravenous injection of cefazolin and CVC insertion (ARROW®Two-Lumen Central Venous Catheterization Set with ARROWgard Blue Catheter, REF CS-25802-E, Czech Republic) occurred concomitantly. Moreover, cefazolin had already been prescribed twice without adverse reaction during the previous operations, but it was the first time that CVC insertion was performed. Despite these factors, the patient was diagnosed as allergic to cefazolin; thus, before anesthetic induction, we overlooked that the patient had undergone CVC insertion for the first time when the previous erythema happened.

In the present surgery, we experienced difficulties gaining peripheral venous access; thus, we opted to insert a CVC. Because the CVC was only sited as a replacement for peripheral venous access, we did not measure the central venous pressure. The patient's vital signs had remained stable for approximately 15 minutes (blood pressure 125/85 mmHg and heart rate 85 beats per minute) after tracheal intubation but before siting the CVC. The CVC insertion was also completed very smoothly and it was fixed in place; however, shortly after insertion, the patient's blood pressure and heart rate both declined. Despite giving 20-mg ephedrine intravenously, the systolic blood pressure dropped to 20 mmHg and the heart rate decreased to zero within 2 minutes. Therefore, epinephrine (1 mg) was given intravenously every 2–3 minutes to a total dose of 4 mg, together with cardiac massage and fluid resuscitation until return of circulation. Electrocardiography then indicated ventricular tachycardia, so a defibrillator was used twice (360 joules each). Thereafter, the heart rate and blood pressure returned to 100 beats per minute and 60/30 mmHg, respectively. The partial pressures of O_2 (PaO_2) and CO_2 ($PaCO_2$) at this time were 423.4 mmHg and 40.9 mmHg, respectively. We also noted skin erythema over his chest, left forearm (Fig. 1), and pelvic area (Fig. 2), so we administered dexamethasone (800 mg) intravenously.

The operation was canceled as a result of these complications, and the patient was sent to intensive care on an intravenous infusion of dopamine at a rate of 10 mcg/kg/min. Echocardiography immediately after arrival revealed that there was no wall motion abnormality, and chest computed tomography also showed no evidence of pulmonary embolism or intracardiac thrombus. However, there was a rise in his cardiac enzymes, which we attributed to use of the defibrillator. The patient regained full consciousness and his blood pressure remained stable, allowing him to be extubated and transferred to a general ward the next day. Although he was discharged 6 days later without major sequelae, he died 4 months later due to cachexia.

Discussion

The differential diagnosis of rapid hemodynamic compromise in this patient indicated either anaphylactic shock, hypovolemic shock, myocardial infarction, pulmonary embolism, or sepsis. The patient's oral mucosa was not dry at any point, and his blood pressure remained stable after anesthetic induction until the insertion of CVC, with no tachycardia when his blood pressure became extremely low; therefore,

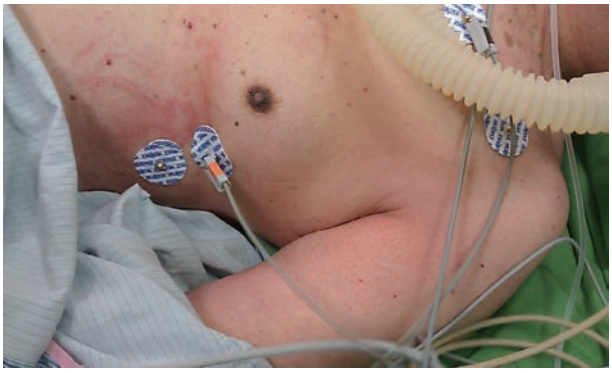

Fig.1 Skin erythema over chest, left forearm.

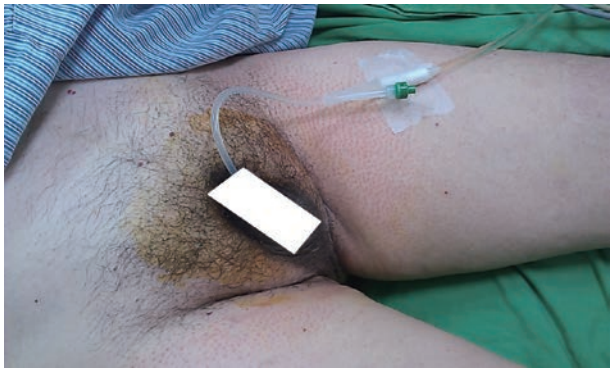

Fig.2 Skin erythema over pelvic area.

hypovolemic shock was excluded early. Although cardiac enzymes were elevated postoperatively, this was explained by the use of an electric defibrillator. Moreover, the echocardiography in intensive care revealed no wall motion abnormalities, indicating that myocardial infarction was unlikely. Pulmonary embolism was also unlikely because the PaO_2 and $PaCO_2$ both increased after resuscitation, and because there was no evidence on chest computed tomography. Finally, the patient was afebrile throughout his hospitalization and all blood cultures were negative, making a septic cause unlikely.

The life-threatening clinical manifestations in this case were consistent with anaphylactic shock due to CVC insertion for the following reasons. First, the clinical features (hypotension and erythema) are typical manifestations of anaphylaxis. Second, epinephrine, corticosteroid, and fluid resuscitation were effective during resuscitation. Third, in this case, the patient had a clear single previous exposure to CVC (a potential sensitizing event) and the cardiovascular collapse occurred immediately after CVC insertion rather than after anesthetic induction. Fourth, the CVC was coated with chlorhexidine, for which there is a warning on the packaging urging extreme caution in patients with known chlorhexidine allergy.

Anaphylaxis is a systemic, potentially fatal, reaction that occurs rapidly after exposure to an offending material. It is an immunoglobulin (Ig)E-mediated reaction to vasoactive substances released by basophils and mast cells. An initial exposure or sensitizing step is required to induce the formation of IgE antibodies directed specifically at the offending substance. This patient had been prescribed cefazolin twice without adverse reactions in 2006 and 2009. Therefore, when he received cefazolin for a third time, with the benefit of hindsight, it should have been considered unlikely to be responsible for the cutaneous erythema experienced during the TLIF procedure, given that CVC was inserted simultaneously for the first time on that occasion.

Acute anaphylactic reactions to chlorhexidine are rare, and the precise incidence is thought to be unknown because many of are likely to go unreported. Over the past 14 years, there have been 14 case reports of anaphylaxis due to chlorhexidine-coated CVCs worldwide. Two cases were in Japan, nine in the UK, one in America, one in Australia, and in New Zealand [2-10]. All cases were limited to the Arrow® product (ARROW®Two-Lumen Central Venous Catheterization Set with ARROWgard Blue Catheter, REF CS-25802-E, Czech Republic), and all patients made a good recovery, with no lasting sequelae. However, an interesting point should be noted. Anaphylaxis typically affects the skin, the respiratory system, the cardiovascular system, and the gastrointestinal system, and is considered present when two or more body systems are affected [11]. Notably, none of the 14 cases reported to date [2-10] have reported signs of bronchospasm. Therefore, bronchospasm may not be a prominent feature of anaphylaxis to chlorhexidine.

Since chlorhexidine was introduced in 1954, it has become a key component in reducing infection rates because of its superior antiseptic efficacy compared with povidone–iodine or alcohol [12]. Its ubiquitous use in countless surgical and interventional procedures and in everyday household products raises the possibility of sensitization in a large proportion of the general population. The first case of anaphylaxis caused by a chlorhexidine-coated CVC was reported in Japan in 1997 [7]. However, a cluster of anaphylactic reactions due to central lines in Japan prompted a withdrawal of chlorhexidine from the 1990s and an alert by the Food and Drug Administration in 1998 [8].

In conclusion, chlorhexidine-coated CVCs should be used with caution, and a careful history for potential allergy should be taken. Moreover, we advocate that the warning mark on the CVC packaging should be made more obvious (i.e., in bold and highlighted) to ensure that clinicians are aware that chlorhexidine-coated CVCs can trigger rare but potentially life-threatening hypersensitivity reactions in susceptible individuals.

References

1. Parkes AW, Harper N, Herwadkar A, Pumphrey R: Anaphylaxis to the chlorhexidine component of Instillagel: a case series. Br J Anaesth.2009; 102: 65-8.
2. Kluger M: Anaphylaxis to chlorhexidine-impregnated central venous catheter. Anaesth Internsive Care. 2003; 31: 697-8.
3. Khoo A, Oziemski P: Chlorhexidine impregnated central venous catheter inducing an anaphylactic shock in the intensive care unit. Heart Lung Circ 2011; 20: 669-70.
4. Guleri A, Kumar A, Morgan RJ, Hartley M, Roberts DH: Anaphylaxis to chlorhexidine-coated central venous catheters: a case series and review of the literature. Surg Infect.2012; 13: 171-4.

5. Jee R, Nel L, Gnanakumaran G, Williams A, Eren E: Four cases of anaphylaxis to chlorhexidine impregnated central venous catheters: a case cluster or the tip of the iceberg? Br J Anaesth. 2009; 103: 614-5.
6. Tommey M: Preoperative chlorhexidine anaphylaxis in a patient scheduled for coronary artery bypass graft: a case report. AANA 2013; 81: 209-14.
7. Oda T, Hamasaki J, Kanda N, Mikami K: Anaphylactic shock induced by an antiseptic-coated central venous catheter. Anesthesiology 1997; 87: 1242-4.
8. Terazawa E, Nagase K, Masue T, Niwa Y, Fukao I, Shimonaka H, et al: Anaphylactic shock associated with a central venous catheter impregnated with chlorhexidine and silver sulfadiazine. Masui 1998; 47: 556-61.
9. Stephen R, Mythen M, Kallis P, Davies DW, Egner W, Rickards A: Two episodes of life-threatening anaphylaxis in the same patient to a chlorhexidine-sulphadiazine-coated central venous catheter. Br J Anaesth 2001; 87: 306-8.
10. Pittaway A, Ford S: Allergy to chlorhexidine-coated central venous catheters revisited. Br J Anaesth. 2002; 88: 304-5.
11. Darouiche RO, Wall MJ Jr, Itani KM, Otterson MF, Webb AL, Carrick MM, et al: Chlorhexidine-alcoholversus povidone-iodine for surgical-site antisepsis. N Engl J Med 2010; 362: 18-26.
12. Lieberman P, Nicklas RA, Oppenheimer J, Kemp SF, Lang DM, Bernstein DI, et al: The diagnosis and management of anaphylaxis practice parameter: 2010 update. J Allergy Clin Immunol.2010; 126: 477-80.

置放中央靜脈導管時發生過敏性休克一個案報告

王亮傑* 馬振來 陳燿舜 文寄銘 張錦新

童綜合醫院 [1]一般外科 [2]腸胃內科 [3]病理科

受文日期：民國 104 年 11 月 12 日；接受刊載：民國 105 年 11 月 07 日

摘要

我們報告一個病例，本預進行右髖雙極半人工關節置換手術，接受麻醉誘導完，施打中央靜脈導管時卻發生過敏性休克。這是一位 78 歲男性病患，有肝硬化與肝癌合併骨轉移，他曾在之前的手術中施打中央靜脈導管時，發生皮膚紅疹，但是當時被解讀為 cefazoline 過敏，即使他更早之前曾經接受過 cefazoline 注射兩次都沒有任何症狀。這次麻醉誘導給予的藥物與之前病患接受全身麻醉時完全一樣，在置放完中央靜脈導管後，病患血壓下降的很快，我們開始心臟按壓，後來在強心劑的給予之下病患血壓回復，病患之後復元的很好並在六天後出院，無產生其他重大併發症。Chlorhexidine 因為有良好的抗菌效果，可以降低感染的機會，但一連串的過敏反應致使 FDA 在 1998 年提出警告；所以包覆 chlorhexidine 的中央靜脈導管應該小心使用。

關鍵詞：中央靜脈導管、chlorhexidine 、過敏性休克

* 通訊作者：王亮傑 童綜合醫療社團法人童綜合醫院 麻醉科 43503 臺中市梧棲區臺灣大道八段 699 號

Tungs' Medical Journal 10 (2016) 48-51

Pathology

Malignant Peritoneal Mesothelioma

Guo-Dong Chen[1], Tzu-Ming Chang[1], Tang-Yi Tsao[2], and Jong-Shiaw Jin[2]

Department of General Surgery[1], and Department of Pathology[2], Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan

Received: Jan. 01, 2015; Accepted: Dec. 03, 2015

Abstract

A 78-year-old woman presented with a 2-month history of abdominal pain, fever, nausea, and vomiting. After admission, abdominal computed tomography showed multiple tumors in the abdominal cavity in close contact with the small bowel loops. She underwent exploratory laparotomy, with intraoperative findings of multiple tumors measuring up to 12 cm over the abdominal and pelvic cavities and tumor adhesion to the small intestine, colon, and omentum. Due to the difficulties associated with debulking surgery, two tumor nodules were removed from the omentum for pathologic examination, including immunohistochemistry, which led to the diagnosis of pathological stage III primary peritoneal mesothelioma (MPM). The patient died 1 month after surgery due to aspiration pneumonia with respiratory failure. MPM has a poor prognosis; it is treated with a combination of systemic chemotherapy, intraperitoneal chemotherapy, cytoreductive surgery, and whole-abdominal irradiation. The median survival time for this type of tumor is consistently less than 1 year.

Key words: peritoneal tumor, mesothelioma, debulking surgery

Pathology Page

A 78-year-old woman presented with a 2-month history of abdominal pain, fever, nausea, and vomiting. After admission, computed tomography scan of the abdomen showed multiple tumors in the abdomen cavity in close contact with the small bowel loops. The patient underwent exploratory laparotomy, with intraoperative findings of multiple tumors measuring up to 12 cm over the abdominal and pelvic cavities and tumor adhesion to the small intestine, colon, and omentum. Due to the difficulties associated with debulking surgery, two tumor nodules were removed from the omentum for pathologic examination.

Grossly, the tumor was solid and gray. Microscopically, the tumor cells were arranged in sheet-like patterns and showed epithelioid (Fig. 1, upper panel) and sarcomatoid (Fig. 1, lower panel) patterns. Immunohistochemistry stains showed diffuse cytoplasmic staining for cytokeratin (Fig. 2, panel A) and the mesothelial cell marker calretinin (Fig. 2, panel B), and were focal positive to the lymphatic endothelial marker D2-40 (Fig. 2, panel C). Gastrointestinal stromal tumor was eliminated by negative staining to Ckit, DOG-1, CD34, and PDGF-alpha. The tumor was diagnosed as primary peritoneal mesothelioma (MPM) with a pathological stage of Stage III (T4, N0, M0). The patient was deceased 1 month after surgery due to aspiration pneumonia with respiratory failure.

MPM is a highly lethal malignancy of the serosal membranes of the peritoneum. The median age at presentation is 53 years. Exposure to asbestos and other mineral fibers is reported to be a risk factor for MPM. The majority of cases of MPM present with diffuse peritoneal involvement, in a highly aggressive form of the cancer. A minority of cases has localized disease. Uncommonly, MPM may metastasize to the abdominal and pelvic lymph nodes, although distant metastases are very uncommon. Lymph node metastases are found in approximately 20–28% of patients.

MPM is characterized macroscopically by hundreds to thousands of individual tumor nodules of varying size and consistency that are usually diffusely disseminated throughout the peritoneal cavity. The

*Correspondence to: Dr. Jong-Shiaw Jin, Department of Pathology, Tungs' Taichung MetroHarbor Hospital, No. 699, Sec. 8, Taiwan Blvd., Wuqi Dist., Taichung 435, Taiwan (R.O.C.)

lesions may range from diffuse subcentimeter, gray, hard nodules to large nodular masses that spread in sheets and coalesce to form plaques and masses, replacing the omentum, circumferentially encasing the bowels, and invading the solid organs, mesentery, and diaphragm. These tumors may have a gelatinous consistency, depending on the hyaluronic acid content; and as they progress, they block peritoneal lymphatics and produce exudative fluid from their surfaces, resulting in ascites.

Histologically, MPM may be classified into three broad histologic subtypes: epithelioid, sarcomatoid, and biphasic (mixed). Epithelioid malignant mesotheliomas are composed of cells that resemble normal mesothelial cells. Architecturally, they form a tubulopapillary or trabecular pattern. Flattened or cuboidal cells with monotonous nuclei line the papilla or tubules; mitotic figures are uncommon. The tumor infiltrates submesothelial connective tissue, fat, and/or muscle. There may be other characteristics, such as signet-ring cell structure or a desmoplastic response, which make this tumor difficult to distinguish from another adenocarcinoma on histologic analysis alone. The sarcomatous pattern, which is less common in the peritoneum as compared with the pleura, is typically composed of tightly packed spindle cells. Malignant osteoid, chondroid, or muscular elements may be present within the tumor. A biphasic tumor

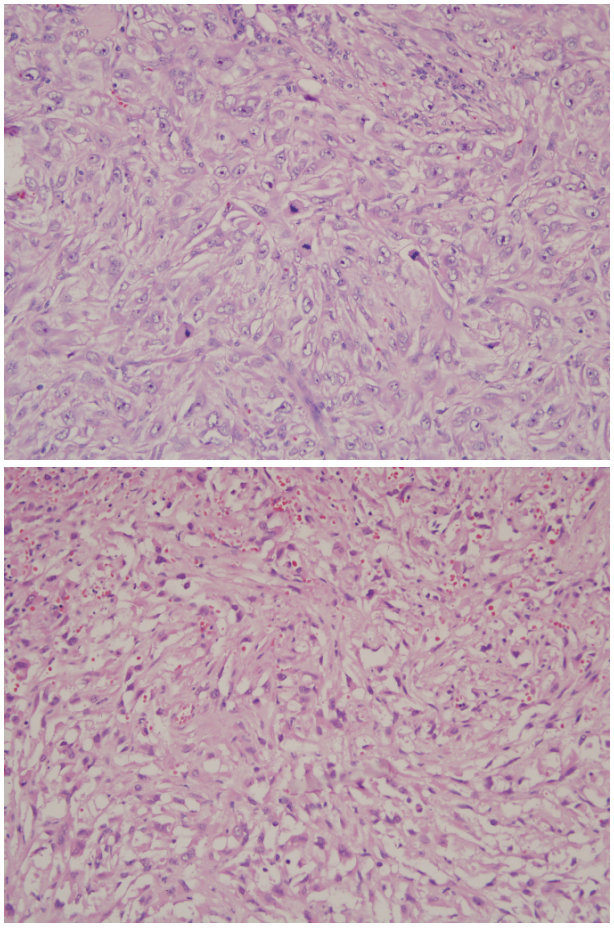

Fig. 1 Histopathology shows tumor cells with epithelioid (upper panel), and sarcomatoid (lower panel) patterns (Heaoxylin-Eosin stain, 400x).

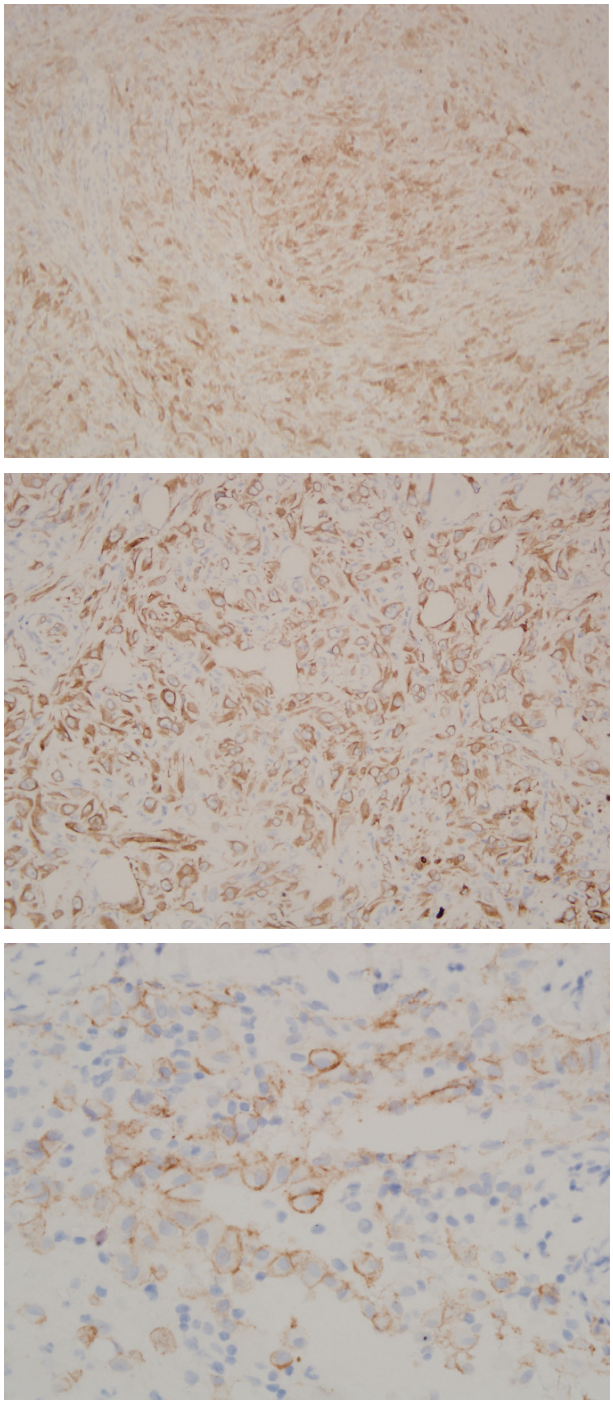

Fig. 2 Immunohistochemistry stains to cytokeratin (panel A), calretinin (panel B), and D2-40 (panel C) show positive cytoplasmic stains in tumor cells (400x).

is defined as one with both epithelioid and sarcomatous components, each contributing more than 10% to the overall histology.

The differential diagnosis of MPM includes gastrointestinal stromal tumor, which shows positive staining to Ckit, CD34, and Dog-1. Primary peritoneal serous carcinoma is mainly arranged in a papillary pattern and shows negative staining to calretinin and D2-40. Leiomyosarcoma shows positive staining to smooth muscle actin, and desmin, and also negative staining to calretinin and D2-40.

Patients with epithelioid-type disease have a mean 5-year survival time of 55 months. In contrast, patients with sarcomatoid-type disease are associated with a worse prognosis, with a mean 5-year survival time of 13 months. MPM is treated with a combination of systemic chemotherapy, intraperitoneal chemotherapy, cytoreductive surgery, and whole-abdominal irradiation. The median survival is consistently less than 1 year. The median survival for untreated patients is approximately 6 months.

References

1. Kaya H, Sezgı C, Tanrıkulu AC. Prognostic factors influencing survival in 35 patients with malignant peritoneal mesothelioma. Neoplasma 2014; 61: 433-8.
2. Lee M, Alexander HR, Burke A. Diffuse mesothelioma of the peritoneum: a pathological study of 64 tumours treated with cytoreductive therapy. Pathology 2013; 45: 464-73.
3. Cao C, Yan TD, Deraco M, Elias D, Glehen O, Levine EA, et al. Importance of gender in diffuse malignant peritoneal mesothelioma. Ann Oncol 2012; 23: 1494-8.
4. Jin S, Cao S, Cao J, Shen J, Hu J, Che D et al. Predictive factors analysis for malignant peritoneal mesothelioma. J Gastrointest Surg. 2015; 19: 319-26.
5. Boffetta P. Epidemiology of peritoneal mesothelioma: a review. Ann Oncol 2007; 18: 985.
6. Rodríguez D, Cheung MC, Housri N, Koniaris LG. Malignant abdominal mesothelioma: defining the role of surgery. J Surg Oncol 2009; 99: 51.
7. Moolgavkar SH, Meza R, Turim J. Pleural and peritoneal mesotheliomas in SEER: age effects and temporal trends, 1973-2005. Cancer Causes Control 2009; 20: 935.
8. Hemminki K, Li X. Time trends and occupational risk factors for peritoneal mesothelioma in Sweden. J Occup Environ Med 2003; 45: 451.
9. Spirtas R, Heineman EF, Bernstein L, et al. Malignant mesothelioma: attributable risk of asbestos exposure. Occup Environ Med 1994; 51: 804.
10. Moran CA, Albores-Saavedra J, Suster S. Primary peritoneal mesotheliomas in children: a clinicopathological and immunohistochemical study of eight cases. Histopathology 2008; 52: 824.
11. Cioffredi LA, Jänne PA, Jackman DM. Treatment of peritoneal mesothelioma in pediatric patients. Pediatr Blood Cancer 2009; 52: 127.

腹膜惡性間皮瘤：病例病理報告

陳國棟[1] 張子明[1] 曹唐義[2] 金忠孝[2]

童綜合醫院 [1]一般外科 [2]病理科

受文日期：民國 104 年 05 月 04 日；接受刊載：民國 104 年 07 月 20 日

摘要

一位 76 歲女性因腹痛、發燒、噁心及嘔吐二個月求診，入院腹部電腦斷層檢查顯示腹腔內多顆腫瘤和小腸連接。腹腔探查顯示腹腔及骨盆腔內多顆腫瘤和小腸、大腸、腹膜相連接，最大的直徑有 12 公分。因手術無法完全去除腫瘤，僅取二顆腫瘤以做病理診斷。病理免疫染色後診斷為腹膜惡性間皮瘤，病理分期為第三期。病患於術後一個月因吸入性肺炎及呼吸衰竭而過世。腹膜惡性間皮瘤的預後極差，一般多以化療、手術切除腫瘤及腹部放射線治療為主，平均存活時間少於一年。

關鍵詞：腹膜腫瘤、間皮瘤、減積手術

* 通訊作者：金忠孝 童綜合醫療社團法人童綜合醫院 解剖病理科 43503 臺中市梧棲區臺灣大道八段 699 號

Tungs' Medical Journal 10 (2016) 52-55

Pathology Page

Intraductal Papillary Mucinous Neoplasm of the Pancreas

Ji-Kuen Yu[1], Lien-Fu Lin[2], and Jong-Shiaw Jin[3,*]

[1]Department of General Surgery, [2]Department of Gastroenterology, and [3]Department of Pathology, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan

Received: Feb. 02, 2015; Accepted: Dec. 03, 2015

Abstract

A 64-year-old man presented with upper abdominal pain and body weight loss of 5 kg over 1 month. Endoscopic ultrasonography of the pancreas showed dilation of the main pancreatic duct with multiple mural nodules (up to 0.5 cm in size) over the pancreatic tail region. The patient received distal pancreatectomy and was diagnosed with intraductal papillary mucinous neoplasm (IPMN) of the main duct, without an invasive component. The main-duct type of IPMN presents with mural nodules and is associated with invasive carcinoma. The branch-duct type of IPMN does not present with mural nodules and is not associated with invasive carcinoma. IPMN without an invasive component carries an excellent prognosis, and these cases have a very low recurrence rate. Due to the one third of IPMNs that are associated with invasive carcinoma, complete surgical resection is recommended.

Key words: pancreatic duct tumor, endoscopic ultrasonography, pancreatectomy

Pathology Page

A 64-year-old man had a history of alcohol-associated pancreatitis on three occasions. In the present case, he presented with upper abdominal pain and body weight loss of 5 kg over the course of 1 month. Laboratory data showed elevation of lipase (563 U/L) and amylase (107 μg/dL). Carcinoembryonic antigen (CEA), cancer antigen (CA)-199, glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) were all within the normal range. After admission, an endoscopic ultrasonography of the pancreas showed dilation of main pancreatic duct with multiple mural nodules (up to 0.5 cm in size) over the pancreatic tail region. The patient underwent distal pancreatectomy and the specimen was sent for pathologic examination.

The gross appearance of the growth showed an irregular tumor (1.5 × 1.2 cm) with multiple foci cysts at the pancreatic tail with mucin content inside the cyst. Four mural nodules (size, 0.2–0.5 cm) were noted in the main pancreatic duct. Microscopically, the intraductal tumor cells were arranged in papillary pattern (Figure 1, upper panel) with intermediate dysplasia of the papillary epithelia (Figure 1, lower panel). Immunohistochemistry stains showed focal staining for a marker of pancreatobiliary epithelia, mucin-1 (Figure 2, upper panel), and strong staining for a marker of intestinal-type epithelia, mucin-2 (Figure 2, lower panel). The tumor was diagnosed as intraductal papillary mucinous neoplasm (IPMN), main-duct type, with intestinal-type epithelia, and intermediate dysplasia of epithelia. No invasive carcinoma was identified. The patient was followed up by computed tomography (CT) of the abdomen, and no tumor recurrence was noted for 2 years.

IPMN is the most common cystic tumor of the pancreas (8–20% of resected specimens), occurring in individuals ranging in age from 25 to 94 years. The differential diagnosis for IPMN includes serous cystic neoplasm, which most often occurs in women and presents as a well-circumscribed, multilocular cyst, occasionally with a central stellate scar and sunburst-type calcification on ultrasonography and CT. Pathology findings associated with serous cystic

*Correspondence to: Dr. Jong-Shiaw Jin, Department of Pathology, Tungs' Taichung MetroHarbor Hospital, No. 699, Sec. 8, Taiwan Blvd., Wuqi Dist., Taichung 435, Taiwan (R.O.C.)

neoplasm include multi-locular cysts with serous lining epithelia. The serous epithelium is rich in glycogen and shows positive periodic acid-Schiff (PAS) staining.

The other differential diagnosis is mucinous cystic neoplasm, which exclusively occurs in women. Endoscopic retrograde cholangiography shows a displacement of the main pancreatic duct, and this tumor does not communicate with the pancreatic duct; a very important finding for differential diagnosis with IPMN. Pathological findings for mucinous cystic neoplasm show well-differentiated columnar epithelia supported by ovarian-like stroma, which shows positive staining to the estrogen and progesterone receptors.

IPMN is clinically classified into the main-duct and branch-duct types. The main-duct type presents with mural nodules, and 37% of affected patients also demonstrate invasive carcinoma. The branch-duct type does not present with mural nodule, and is not associated with invasive carcinoma. Histopathological epithelia may be sub-divided into gastric, intestinal, pancreatobiliary, and oncocytic-type epithelia. The intestinal type of epithelium is associated with invasive carcinoma in 23% cases of IPMN. In contrast, only 2% of IPMN cases with gastric-type epithelium are associated with invasive carcinoma. IPMN without an invasive component carries an excellent prognosis, with 94–100% survival over 5 years and very low recurrence (1.3–8% recurrence rate). In those cases with invasive carcinoma, the 5-year survival is 34–62%. Due to the 35% of IPMN cases that are associated with invasive carcinoma, complete surgical resection is recommended. Post-operative endoscopic ultrasound and CT may be used in the follow up of IPMN.

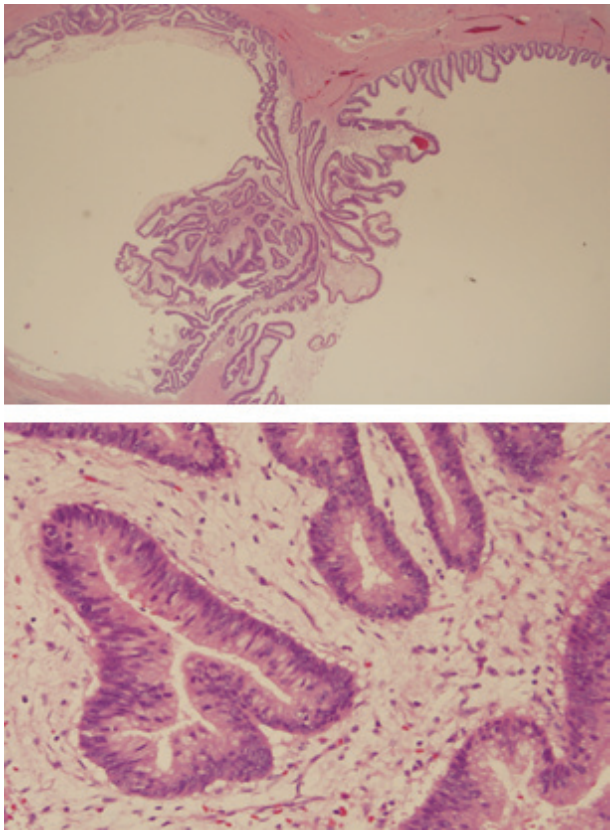

Fig. 1 Histopathology of low magnification shows intraductal papillary tumor within main pancreatic duct (upper panel, hematoxylin-eosin stain, 40x), and high magnification of epithelia shows moderate dysplasia (lower panel. H&E stain, 400x

Reference

1. Kim KW, Park SH, Pyo J, Yoon SH, Byun JH, Lee MG, et al. Imaging features to distinguish malignant and benign branch-duct type intraductal papillary mucinous neoplasms of the pancreas: a meta-analysis. Ann Surg 2014;

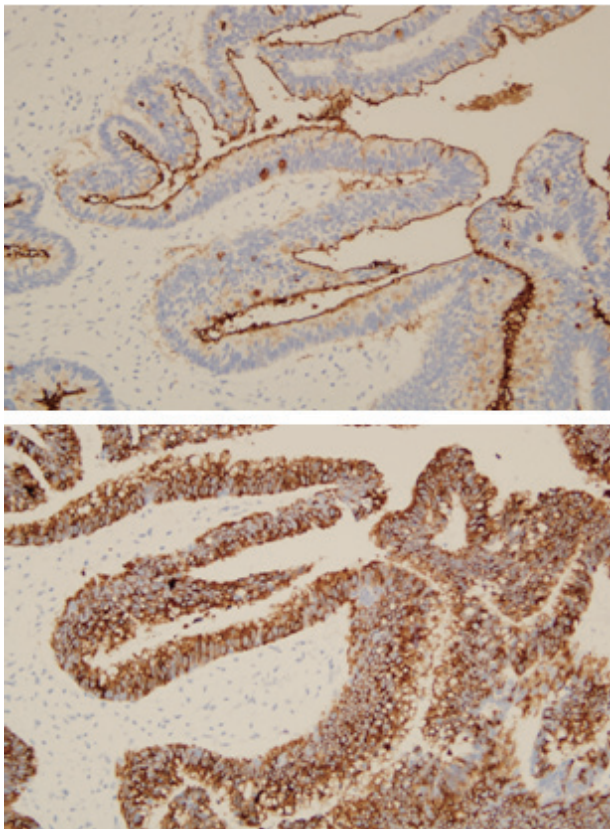

Fig. 2 Immunohistochemistry stains of mucin 1 (upper panel) and mucin 2 (lower panel) in IPMN (400x).

259: 72-81.
2. Hara T, Yamaguchi T, Ishihara T, Tsuyuguchi T, Kondo F, Kato K,et al. Diagnosis and patient management of intraductal papillary-mucinous tumor of the pancreas by using peroral pancreatoscopy and intraductal ultrasonography. Gastroenterology 2002; 122: 34-43.
3. Kim JH, Eun HW, Kim KW, Lee JY, Lee JM, Han JK et al. Intraductal papillary mucinous neoplasms with associated invasive carcinoma of the pancreas: imaging findings and diagnostic performance of MDCT for prediction of prognostic factors. AJR Am J Roentgeno. 2013; 201: 65-572.
4. Alkiviadis E, Thrasyvoulos P, and Emmanouil Z. Endoscopic ultrasound in the diagnosis of pancreatic intraductal papillary mucinous neoplasms. World J Gastroenterol 2014; 20: 7785-93.
5. Ohno E, Hirooka Y, Itoh A, Ishigami M, Katano Y, Ohmiya N et al. Intraductal papillary mucinous neoplasms of the pancreas: differentiation of malignant and benign tumors by endoscopic ultrasound findings of mural nodules. Ann Surg. 2009; 249: 628-34.
6. Stelow EB, Stanley MW, Bardales, RH, Mallery S, Lai R, Linzie BM et al. Intraductal papillary-mucinous neoplasm of the pancreas: the findings and limitations of cytologic samples obtained by endoscopic ultrasound–guided fine-needle aspiration. Am J Clin Pathol 2003; 120: 398-404.

胰管內乳頭狀黏液性腫瘤：病例病理報告

余積琨[1] 林連福[2] 金忠孝[3,*]

童綜合醫院 [1]一般外科 [2]腸胃內科 [3]病理科

受文日期：民國 104 年 02 月 13 日；接受刊載：民國 104 年 12 月 03 日

摘要

一位 64 歲男性最近一個月因上腹痛及體重減輕 5 公斤求診，內視鏡超音波檢查顯示主胰管擴張及有多顆主胰管內腫瘤（最大 0.5 公分）。病患接受部份胰臟切除術後病理診斷為胰管內乳頭狀黏液性腫瘤，主胰管型，沒有發現侵犯性腫瘤。主胰管型腫瘤易伴隨胰管內腫瘤及侵犯性腫瘤；而胰管分支型腫瘤不伴隨胰管內腫瘤及侵犯性腫瘤。沒有侵犯性腫瘤的胰管內乳頭狀黏液性腫瘤預後極好，不易發生腫瘤復發。因有三分之一的病患伴隨侵犯性腫瘤，所以早期手術切除腫瘤有其必要性。

關鍵詞：胰管腫瘤、內視鏡超音波、胰腺切除術

* 通訊作者：金忠孝 童綜合醫療社團法人童綜合醫院 解剖病理科 43503 臺中市梧棲區臺灣大道八段 699 號

Tungs' Medical Journal 10 (2016) 56-58

Image

Perforated Appendicitis: KUB Findings in a Patient with Atypical Presentation

Leo Sun[1], Xi-Shuo Qiu[1]*, Meng-Yu Shi[2]

Department of Pediatrics[1], Radiology[2], Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan

Received: May. 04, 2015; Accepted: Jul. 20, 2015

Abstract

Children with acute appendicitis frequently present with atypical symptoms. Absence of typical symptoms may lead to diagnostic delay and increased risk of perforation. Isolation of perforated appendiceal contents through the peritoneal fat, adjacent intestines, and omentum may result in a localized abscess instead of widespread peritonitis. Careful and thorough history and physical examination are important tools for diagnosing acute appendicitis. CT scan continues to be the gold standard for imaging diagnosis.

Key words: appendicitis, atypical presentation, CT, KUB

Children with acute appendicitis frequently present with atypical symptoms. Periumbilical abdominal pain, nausea and vomiting, and generalized malaise may not always be present. Fever is usually low grade unless perforation has occurred. Localized abdominal tenderness in the right lower quadrant is the single most reliable finding for diagnosis [1]. Absence of these typical symptoms may lead to delayed diagnosis and increased risk of perforation.

A 12-year-old girl without systemic disease visited the emergency department because of low-grade fever, mild abdominal discomfort, and vomiting for 5 days. Physical examination revealed a distended abdomen with mild epigastric tenderness, hypoactive bowel sounds, and a palpable mass over the pelvic region. KUB showed distended small bowel loops and increased density in the pelvic cavity, with upward displacement of the bowels (Fig. 1). She was admitted under the impression of acute gastroenteritis. Abdominal CT scan was ordered due to elevated CRP levels, and it revealed a swollen appendix mimicking a mass lesion (Fig. 2). Surgical appendectomy later confirmed the diagnosis of perforated appendicitis.

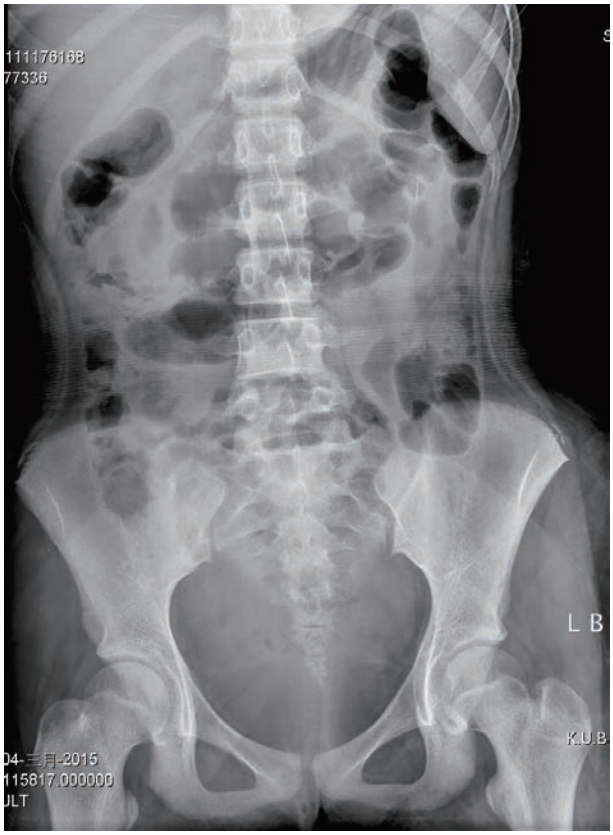

Fig. 1 KUB showed distended bowel loops and increased density in the pelvic cavity.

*Correspondence to: Xi-Shuo Qiu, Department of Pediatrics, Tungs' Taichung MetroHarbor Hospital, No. 699, Sec. 8, Taiwan Blvd., Wuqi District, Taichung City 435, Taiwan (R.O.C.)

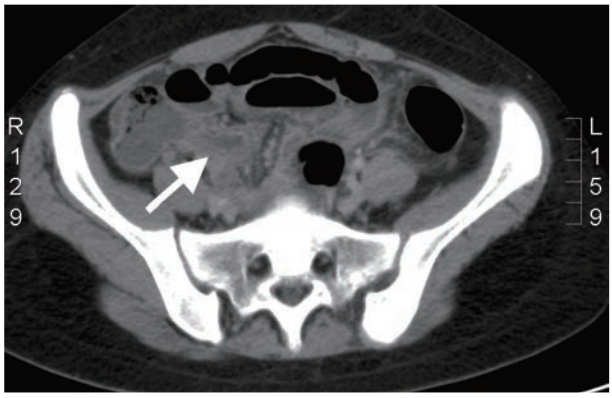

Fig. 2 CT scan revealed swollen appendix smimicking a mass lesion.

Perforated appendicitis without abdominal pain in the right lower quadrant or toxic appearance is uncommon. Isolation of perforated appendiceal contents through the peritoneal fat, adjacent intestines, and omentum may result in a localized abscess instead of widespread peritonitis, leading the patient to appear surprisingly well [2]. Careful and thorough history and physical examination are important tools for diagnosing acute appendicitis. Given that more than half of all cases of appendicitis in children have an atypical presentation, assessment should be primarily focused on the temporal evolution of the illness in relation to the specific presenting signs and symptoms. CT scan continues to be the gold standard for imaging diagnosis.

References

1. Guillerman RP, Brody AS, Kraus SJ. Evidence-based guidelines for pediatric imaging: The example of the child with possible appendicitis. Pediatr Annals 2002; 31: 629.
2. Nance ML, Adamson WT, Hedrick HL. Appendicitis in the very young child: A continuing diagnostic challenge. Pediatri Emerg Care 2000; 16: 160.

破裂性闌尾炎：非典型症狀病人的 KUB 表現

孫若堯[1] 邱希碩[1*] 施孟好[2]

童綜合醫院 兒童醫學部[1] 放射科[2]

受文日期：民國 104 年 05 月 04 日；接受刊載：民國 104 年 07 月 20 日

摘要

兒童急性闌尾炎的臨床症狀常以非典型的型態所表現，因此容易延誤診斷而增加闌尾破裂的風險。有時闌尾破裂後，易被周圍脂肪組織、內臟器官、及網膜所包覆住，可能會造成局部膿瘍病變，無廣泛腹膜炎的症狀產生。急性闌尾炎之主要診斷方式還是依據詳細的病史詢問及身體檢查。電腦斷層則是目前影像學診斷之黃金標準。

關鍵詞：闌尾炎、非典型表現、電腦斷層、腹部 X 光

* 通訊作者：邱希碩 童綜合醫療社團法人童綜合醫院 兒童醫學部 43503 臺中市梧棲區臺灣大道八段 699 號

Instruction to contributors

The Tungs' Medical Journal provides a forum for all fields of medicine, including Editorials, Review Articles, Original Articles, Case Reports, Brief Communications, Images, and Pathology Page. Authors are welcome to submit manuscripts to Tungs' Medical Journal.

Preparing Your Manuscript:

1. Three copies of the manuscript must be submitted as a Word document to the Editor, Tungs' Medical Journal, Tungs' Taichung MetroHarbor Hospital, No.699, Sec.8, Taiwan Blvd., Wuqi Dist., Taichung 43503, Taiwan (R.O.C.) and an electronic copy of the manuscript to E-mail address: Tungs_Journal@ms.sltung.com.tw.
2. The author is responsible for the content of the manuscript. If the content is related to copyright, author needs to obtain the right to use and is legally responsible for it.
3. Please attached the copyright and consent form on submission. All author(s) listed must actually participate in and agree with the conclusion. Upon receiving and completion of printing, the author(s) will receive 20 free copies and compensation. If extra copy is needed, please notify during editing, and this is subjected to charges.
4. The manuscript may be rejected if incompatible with the journal's mission. After acquiring consent from the author(s), the editor may edit the manuscript.
5. For any the manuscript related to "the human specimen for research" or "clinical trial", must follow the guidelines to obtain an IRB approval for the right of participants.
6. For any the manuscript related to the use of animals, it needs to be approval of The Institutional Animal Care and Use Committee to ensure the humane management.

Manuscript format:

1. Editorials are limited to 2000 words, with 150 words of abstract and 7 references.
2. Review articles should provide the reader with a balanced overview of an important and topical subject in the field. This should be limited to 3500 words, with 300 words of abstract and 40 references.
3. Original articles should be presented in the following order: Abstract, Introduction, Materials and Methods, Results, Discussion and Conclusion, Acknowledgements, References, Attachments, Tables, Legends for illustration, and Figures (photographs). This should be limited to 3000 words, with 300 words of abstract and 40 references.
4. Case reports should be arranged by the following sequence: Abstract, Introduction, the Clinical case, Discussion, References, Attachments, Table, Legends for illustration, and Figures. Patients' eyes should be covered for privacy. Diagnosis information or the chart of clinical process should be within 6 months. This should be limited to 1500 words, with 150 words of abstract and 10 references.
5. Brief communications should be concise presentations of preliminary clinical results and technological improvements. This should be exceeded 750 words, 150 words of abstract and 7

references.

6. Images and Pathology page should be limited to 500 words, with 150 words of abstract and 3 references.
7. For other details, please refer to International Steering Committee, for Uniform Requirements for Manuscripts Submitted to Biomedical Journals, please refer to The New England Journal of Medicine 336:309-315,1997.

Specifications for the different article categories

Article Category	Word count limit		No. of references allowed	No. of tables/ figures allowed
	Abstract	Min text*		
Original Articles	≦300	≦3000	≦40	≦5
Case Reports	≦150	≦1500	≦10	≦3
Review Articles	≦300	≦3500	≦60	≦6
Brief Communications	≦150	≦750	≦7	≦1
Images, Pathology Page	≦150	≦500	≦3	≦2
Editorials	≦150	≦2000	≦7	≦1

*Refers to the main body of text only, i.e., does not include article title, abstract, table headings/tables, figure legends and references.

Manuscript preparation:

Manuscript should be double-spaced and numbered pages, and comply with the "uniform requirements for manuscripts submitted to biomedical journals". The first page is the title page, which include title, name of author(s), organization and unit, contact name, phone number, e-mail address and mail address (in both Chinese and English). The second and the third page is for abstract (Chinese content needs to consist with English content) and key words (please include 3 to 5 keywords or phrases in Chinese and English), and should be written in paragraphs following by background and purpose, methods, results and discussion.

Co-corresponding author should mention the contributions on manuscript, such as initiation of research topics, the study design, statistical analysis, interpretation of findings, chapters writing involved, et al.

Please attach two original copies including attachments, charts and legends. Chart should be professional, with only one figure or one table per page, and is arranged in consecutive orders and numbered in Arabic characters. Table should have a title and appropriate interpretation. Picture should be 5" x 7" in size, black and white, glossy and numbered in consecutive orders of appearance.

Reference:

Unpublished articles or abstracts cannot be listed as references, but could be noted as "unpublished observations". Doctoral dissertation or master thesis can be used. Any articles being accepted by magazines but not published yet, please note the name of magazine, year and note "in press".

Original researches, case reports, review articles, communications (includes brief communications), images in clinical medicine, editorial follows the following format:

1. Abbreviations used should follow the format of Index Medicus for all journal titles. When authors are less than 6 people, list all author(s), when more than 6, only list the first 6 followed by “et al.” for the rest.
2. References in the text should be placed where relevant. When a reference article is cited, only the primary author is cited; however, if only two authors are present, both should be listed.
3. Example of references:

Examples of Reference:

1. Periodicals:

   Yang KTA, Chen HD: A semi-automated method for edge detection in the evaluation of left ventricular function using ECG-gated single-photon emission tomography. Eur J Nucl Med 1994;21:1206-11.

2. Monographs:

   Plum F, Posner JB: Diagnosis of Stupor and Coma. 3rd ed. Philadelphia: Davis, 1980:132-3.

3. Monographs with multiple authors:

   Levinsky NG: Fluid and electrolytes. In: Thorn GW, Adams RD, Braunwald E, Isselbacher K, Petersdprf RG eds. Harrison’s Principles of Internal Medicine, 8th ed. New York: Mcgraw-Hill, 1977:364-75.

4. References from web site

   please indicate the title, source, and the retrieving date

   (Accessed Month day, 2016, at http://www.house.gov/xxxx/min/inves_xxx/index_accord.htm.)

Copyright:

童綜合醫學雜誌投稿相關規則

95.9.01 製訂
99.08.17 修訂
100.07.11 修訂
102.07.08 修訂
102.12.27 修訂
103.07.14 修訂
103.12.12 修訂
104.03.13 修訂
104.11.19 修訂

本雜誌刊載與醫學有關之論述，包括原著論文（Original Articles）、病例報告（Case Reports）、綜論（Review Articles）、短論（Communications 、包括 Brief Communications）、影像判讀（Images）、臨床病理討論（Pathology Page）、編著的話（Editorials）等。惠稿請送 43503 臺中市梧棲區臺灣大道八段 699 號童綜合醫學雜誌編審委員會。（E-mail:Tungs_Journal@ms.sltung.com.tw）

壹、投稿前注意事項

1. 惠稿請以英文撰寫，本雜誌接受電子檔投稿或紙本投稿，電子檔投稿請直接將稿件 WORD 檔寄至編審委員會信箱 Tungs_Journal@ms.sltung.com.tw)，紙本投稿需檢附紙本稿件三份及電子檔一份寄至編審委員會（43503 臺中市梧棲區臺灣大道八段 699 號童綜合醫學雜誌編審委員會）。
2. 文件內容需清晰，內容與原稿一致，若複印稿與原稿有差異或遺漏，由作者自行負責。著作中若牽扯到版權所有之內容，作者需取得其使用權，法律責任由作者負責。
3. 投稿同時請附上著作權讓與同意書。所有作者必須實際參與並同意該論述。本院於接受稿件且印刷完成後，將致贈稿酬並贈送 20 份抽印本給通訊作者，如需額外抽印本請於校稿時言明，並酌收成本費用 。第一作者若需抽印本可提出申請，依份數酌收成本費用 。
4. 本刊對於原稿經徵得著者之同意得伸縮或修改之。如不合本刊宗旨者，得退還之。
5. 凡刊載於本雜誌之著作，若涉及「研究用人體檢體採集」及「人體試驗」等情事，應遵守該注意事項，以落實保障受檢人權益。詳文請參考須附上相關審議認可之文件。
6. 論文中如涉及使用脊椎動物進行科學應用計畫者，應檢附該計畫業經所屬機構動物實驗管理小組審議認可之文件，以落實實驗動物之人道管理。

貳、寫作原則

1. 原著論文（Original Articles）按下列順序撰寫：摘要、前言、材料與方法、結果、討論與結論、誌謝、參考文獻、附表、圖片說明、圖片（含照片）。每篇字數 3000 字以內，摘要 300 字以內，參考文獻 40 篇以內。
2. 病例報告（Case Reports）按下列順序撰寫：摘要、前言、病例、討論、參考文獻、附表、圖片說明、附圖、照片。凡病患顏面部位之相片必須遮去眼睛部位，表示尊重隱私。診療資料或臨床經過之圖表，原則上均限六個月以內。每篇字數 1500 字以內，摘要 150 字以內，參考文獻 10 篇以內。
3. 綜論（Review Articles）不必按原著論文格式撰寫，但每篇字數 3500 字以內，摘要 300 字以內，參考文獻 60 篇以內。
4. 短論（Brief Communications），臨床上、技術上的精簡論著，每篇字數 750 字以內，摘要 150 字以內，參考文獻 7 篇以內。
5. 影像判讀（Images）、臨床病理討論（Pathology Page）圖例說明每篇字數 500 字以內，

摘要 150 字以內，參考文獻 3 篇以內。

6. 編者的話（Editorials），每篇字數 2000 字以內，摘要 150 字以內，參考文獻 7 篇以內。
7. 其他細節，請參閱國際指導委員會（International Steering Committee）發表之生物醫學雜誌稿件統一規格（Uniform Requirements for Manuscripts Submitted to Biomedical Journals，見 The New England Journal of Medicine 336:309-315,1997）。
8. 將可接受投稿之稿件種類之摘要字數、字數、參考文獻及圖表相關上限規定，整理於下表：

稿件種類	字數限制		參考文獻	圖 / 表
	摘　　要	內文字數		
原著論文 (Original Article)	≦ 300	≦ 3000	≦ 40	≦ 5
病例報告 (Case Report)	≦ 150	≦ 1500	≦ 10	≦ 3
綜論 (Review Article)	≦ 300	≦ 3500	≦ 60	≦ 6
短論 (Brief Communication)	≦ 150	≦ 750	≦ 7	≦ 1
影像判讀 (Images)、 臨床病理討論 (Pathology Page)	≦ 150	≦ 500	≦ 3	≦ 2
編者的話 (Editorial)	≦ 150	≦ 2000	≦ 7	≦ 1

參、投稿須知

1. 稿件須符合「生物醫學雜誌投稿之統一規定」[1]，請以電腦隔行 double space 書寫，並編頁碼，中文字型以標楷體，英文字型以 Time New Roman 12 號字大小，稿紙之左右緣爲 2.54 公分，上下緣爲 3.17 公分。
2. 第一頁爲標題頁，須列出中文及英文之論文題目、中英文作者姓名、所屬機構及單位之中英文稱號（分屬不同單位，請以阿拉伯數字標出作者與單位）、聯絡人姓名、電話及中英文通訊錄。
3. 第二、三頁爲中文及英文之摘要及關鍵詞（請提供 3 至 5 個關鍵詞或簡短片語），中英文摘要須完全相同，摘要分段撰寫，依序爲背景及目的（Background and purpose）、方法（Methods）、結果（Results）及討論（Discussion）。
4. 相同貢獻作者請加註說明，如研究主題的設定、參與決定研究設計、進行統計分析、詮釋研究結果、以及各章節撰稿等貢獻。
5. 圖表應專業製作，一張紙僅一個附圖或附表，依引用順序以阿拉伯數字標出排列。附表須有標題及說明且不可以照片形式。圖片或照片電子檔（.jpg）必須清晰、分明。附圖須有簡單說明（Legend），並另頁撰寫。光學或電子顯微鏡照片，請註明擴大倍率或比例。

註：[1] 根據「生物醫學雜誌投稿之統一規定」第五版，刊載於 Annals of Internal Medicine 1997;126(1): 36-47.

肆、參考文獻

未經發表之論文或摘要不得列爲參考文獻，但可於本文中說明並註明「未發表」（unpublished observations）。博碩士論文可引用。已被任何雜誌接受刊發但仍未發表之著作，請列出雜誌名稱及年份，並註明「in press」。

原著論文、病例報告、綜論、短論、影像判讀、臨床病理討論、編著的話按下列格式撰寫：

A. 雜誌及期刊

中文例〔作者姓名：題目。雜誌簡稱 年號；卷數：起訖頁數〕

薛玉梅、陳建仁：皮膚砷癌之流行性病學特徵與危險因子。中華衛誌 1996; 15: 1-26。

英文例〔英文原稿中引用的參考文獻，其雜誌或期刊之簡稱應參照 Index Medicus 型式〕

1. Feely J, Wilkinson GR, Wood AJ. Reduction of liver blood flow and propranonol metabolism by cimetidine. N Engl J Med 1981;304:691-6.
2. Kaplan NM. Coronary heart disease risk factors and antihypertensive drug selection. J cardiovasc Pharmacol 1982; 4(suppl 2): 186-365.（引用雜誌附冊時）
3. Tada A, Hisada K, Suzuki T, Kadoya S. Volume measurement of intracranial hematoma by computedtomography. Neurol surg (Tokyo) 1981; 9: 251-6.〔In Japanese: English abstract〕（引用文獻之作者之本文爲非英文，但有英文摘要）。
4. Bhasin S, Storer TW, Berman N, Callegari C, Clecenger B, Phillips J, et al. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 1996; 335: 1-7.（作者超過 6 位時，只須列出前 6 位，其它以「等」(et al) 代替）

＊期刊若有「數位物件識別碼 (digital object identifier, DOI)」，則於文獻末。

B. 單行本：

中文例 [作者姓名：書名，版數（卷數）。發行地；出版公司，年代：引用部份頁數]。

楊志良：生物統計學新論，一版。台北；巨流圖書公司，1984：33-8.

英文例 [英文單行本的書名，除介系詞及連接詞外，第一字母需大寫]

(1) Plum F, Posner JB. Diagnosis of Stupor and Coma. 3rd ed., Philadelphia: Davis, 1980:132-3.

C. 多重作者之單行本：

中文例 [有關文章作者姓名：題目 。編輯者姓名 : 書名 。版數（卷數）。發行地 : 出版公司，年代 ; 引用部份頁數]。

蔣欣欣：護理與健康。顧乃平：護理專業導論。一版。台北 : 匯華出版公司，1991：83-121。

英文例 Levinsky NG: Fluid and electrolytes. In: Thorn GW, Adams RD, Braunwald E, lsselbacher K, Petersdprf RG eds. Harrison’s Principles of Internal Medicine. 8th ed. New York: Mcgraw-Hill, 1977;364-75.

D. 參考文獻引用時，若兩名以下作者請列出姓氏。兩名以上則列出第一名之姓氏，其他以「等」(et al) 代替，並以阿拉伯數字方括弧表示於引用之後。

例：One of the first well documented reports of ECH poisoning with fatality in young children was reported by Miller et al. in 1970[2].

E. 參考文獻引用網路資料請列出文獻名稱及出處以及引用時間

(Accessed Month day, 2016, at http://www.house.gov/xxxx/min/inves_xxx/index_accord.htm.)

伍、著作權

若著作人投稿於本刊經收錄後，同意授權本刊得再授權國家圖書館或其他資料庫業者，進行重製、透過網路提供服務、授權用户下載、列印、瀏覽等行爲。並得爲符合各資料庫之需求，酌作格式之修改。若爲摘譯、譯稿或改寫稿，需附原作者之正本同意書，並附原文影本一份；來稿如涉及版權，概由作者自負文責。

第十卷　第二期　　民國 105 年 07-12 月

童綜合醫學雜誌

編著的話

1　**可信任專業活動（EPAs）測驗在住院醫師訓練的運用**
遲景上

綜　論

5　**抗 PD-1 抗體 : 對晚期非小細胞肺癌的一種新治療的回顧**
鄭聖翰　鄭啟清　陳國書

原　著

12　**蘋果多酚對脂多醣誘導之發炎作用與調節環氧化　、一氧化氮合成　及微管活化磷酸化訊息路徑有關**
李宜臻　李彗禎

21　**經皮內視鏡治療腰椎外側狹窄初期報告**
徐少克*　郭家孝　陳聖祺

病例報告

29　**嬰兒幽門肥厚型狹窄：病例報告及文獻回顧**
陳思文　潘品合　段彭年　郭建宏　郭敏勇　黃巧芸

34　**一位皮肌炎病患的兩種病毒及肺囊蟲重複感染的肺炎**
林國璽　鄔豪欣　陳志銘　蘇碧榕　何茂旺

39　**嬰幼兒患有 Leigh 症候群之全身麻醉的處置：病例報告及文獻回顧**
張錦新　馬振來　黃國生　王亮傑　陳燿舜　文寄銘　洪宜君

43　**置放中央靜脈導管時發生過敏性休克 – 個案報告**
王亮傑　馬振來　陳燿舜　文寄銘　張錦新

臨床病理討論

48　**腹膜惡性間皮瘤：病例病理報告**
陳國棟　張子明　曹唐義　金忠孝

52　**胰管內乳頭狀黏液性腫瘤：病例病理報告**
余積琨　林連福　金忠孝

影像判讀

56　**破裂性闌尾炎：非典型症狀病人的KUB表現**
孫若堯　邱希碩　施孟妤