Multi-dose parecoxib provides an immunoprotective effect by balancing T helper 1 (Th1), Th2, Th17 and regulatory T cytokines following laparoscopy in patients with cervical cancer

WENGUANG MA[1,2*], KUN WANG[3*], JONGQIANG DU[4], JUNQI LUAN[2] and GE LOU[1]

[1]Department of Gynecology, The Cancer Hospital, Harbin Medical University, Harbin, Heilongjiang 150081; [2]Department of Gynecology, The First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang 150040; [3]Department of Anesthesiology, The Cancer Hospital, Harbin Medical University, Harbin, Heilongjiang 150081; [4]Department of Anesthesiology, The First Affiliated Hospital, Heilongjiang University of Chinese Medicine, Harbin, Heilongjiang 150040, P.R. China

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Abstract. Analgesic treatment with anti-inflammatory drugs may aid the prevention of postoperative pain and the attenuation of the postoperative immune inflammatory response. The current study presents a randomized, double-blind controlled study, which was performed to investigate the levels of Th1, Th2, Th17 and Treg cytokines, including interleukin (IL)-2, interferon (IFN)-γ, IL-4, IL-10, IL-17, IL-23 and transforming growth factor (TGF)-β in the peripheral blood of patients with cervical cancer following laparoscopy. The effects of perioperative multi-dose parecoxib on postoperative immune function was evaluated. A total of 80 patients with cervical cancer (stage IB/IIA, ASA I-III, aged 18-65 years) that were scheduled for laparoscopy were randomly assigned into either the parecoxib (I; n=40) or control (II; n=40) groups. Group I received 40 mg parecoxib 30 min prior to surgery and then every 12 h subsequent to surgery for 60 h, and group II received normal saline at the corresponding time points. Intravenous tramadol (100 mg) was prescribed for pain relief as required. The mRNA and protein expression levels of cytokines in the peripheral blood were detected by quantitative polymerase chain reaction and ELISA. Pain visual analog scales (VAS) and incidence, analgesic relief, adverse events and the length of hospital stay were recorded. It was demonstrated that the mRNA and protein levels of IL-2, IFN-γ and IL-17 in the two groups were reduced subsequent to surgery, while mRNA and protein expression levels of IL-4, IL-10 and TGF-β were enhanced. Administration of multi-dose parecoxib may diminish the increase in postoperative IL-2, IFN-γ and IL-17 levels, and suppress the excessive production of IL-4, IL-10 and TGF-β. This effect is accompanied by lower VAS scores, pain incidence, postoperative nausea/vomiting and infections. In conclusion, perioperative multi-dose parecoxib was able to alleviate postoperative pain and ameliorate surgery-induced immune suppression by balancing Th1, Th2, Th17 and Treg cytokines following laparoscopy in patients with cervical cancer. The current study provides support to the hypothesis that parecoxib may be a more effective therapeutic strategy than the currently available options, for postoperative pain and immune function management of patients with cancer.

Correspondence to: Professor Ge Lou, Department of Gynecology, The Cancer Hospital, Harbin Medical University, 6 Baojian Road, Harbin, Heilongjiang 150081, P.R. China
E-mail: hydlouge@sina.com

*Contributed equally

Abbreviations: VAS, visual analog scale; COX-2, cyclooxygenase-2; NSAIDs, non-steroidal anti-inflammatory drugs; PGE-2, prostaglandin E-2; CNS, central nervous system

Key words: parecoxib, analgesic, immune suppression, cytokine, cervical cancer

Introduction

Cervical cancer is the second most common malignant disease of the female genital tract, and its incidence increases with advancing age. Patients with cancer exhibit weaker immune surveillance capability and a variety of immunological abnormalities, including the impairment of $CD4^+$ T cell-mediated immunity, cytokine dysregulation in peripheral blood and a lack of dendritic cells at the tissue level (1,2). An impaired immune state is further disrupted by surgical stress, anesthesia and postoperative pain (3). Postoperative immune dysfunction is a problem for patients undergoing surgery for malignant tumors, as it affects the rate of infectious complications, clinical outcomes and the growth of disseminated tumor cells (4-6). Particularly in the elderly with cancer, improved postoperative immunity may result in more favorable long-term oncological results (7,8). Therefore, it is critically important to improve the surgery-induced immune suppression and restore the immune function following surgery in patients with cancer.

The mechanisms underlying immunity depression include the imbalance of immune cells and associated cytokines. Previous studies have focused on defining the cytokine

secretion profiles of $CD4^+$ T cells, including helper T cells type 1 (Th1), Th2, Th17 and Treg, as they are involved in immunological disturbances (9). Th1, Th2, Th17 and Treg cells produce positive or negative effects in the maintenance of normal immune function by secreting various cytokines. It is established that Th1 cells are principal effectors of the cell-mediated immune response, as they secrete IL-2 and IFN-γ, which increase anti-tumor immunity. However, Th2 cells produce the humoral immune response-related cytokines IL-4, IL-5 and IL-10, which are involved in suppressing anti-tumor immunity (10). Th17 cells produce potent pro-inflammatory effects through the production of IL-17, IL-21 and IL-23 (11,12), while TGF-β-producing Treg cells negatively regulate the activation and proliferation of T cells (13). A previous study reported that the imbalance of Th1/Th2 cytokines due to the increased production of IL-10 was closely associated with the weakened immune function of the body (13). The current study indicated that the imbalance of Th17/Treg status may lead to infection, inflammatory response and autoimmune disorders (9). These observations confirmed that alterations to Th1, Th2, Th17 and Treg cell cytokines are potential factors promoting the development of host immune dysfunction, and the fine balance of these cells is crucial for the maintenance of normal immune homeostasis. However, the contribution of these cells to postoperative immunological suppression in patients with cervical cancer remains elusive.

There is strong evidence that effective analgesia is able to relieve surgery-induced immune suppression, increase host resistance to tumor metastasis and improve clinical outcomes (14). Non-steroidal anti-inflammatory drugs (NSAIDs) are used to treat pain, and act by inhibiting the cyclooxygenase enzyme responsible for the release of inflammatory mediators (15). Parecoxib, the first selective cyclooxygenase (COX)-2 inhibitor available for intravenous injection, is able to efficiently and rapidly cross the blood-brain barrier (16,17) and produce peripheral and central antihyperalgesic (18) and immunoregulatory effects (19). An animal and clinical study suggested that the perioperative use of parecoxib significantly enhanced systemic Th1 immune responses by increasing the plasma level of IFN-γ in rat brain tumors (20) and attenuated IL-6 and IL-8 production following surgery for colorectal cancer (21,22). This implies that parecoxib exerts protective effects on surgery-induced immune responses. However, little information is currently available regarding the effects of parecoxib on postoperative immune function in patients with cervical cancer.

Based on the above observations, the present study hypothesized that perioperative multiple-dose parecoxib ameliorates the surgery-induced immunosuppression in patients with cervical cancer by modulating the balance of Th1, Th2, Th17 and Treg cytokines. Therefore, the aim of the present study was to evaluate the mRNA and protein expression levels of Th1, Th2, Th17 and Treg cytokines in the peripheral blood of patients with cervical cancer following laparoscopy, and to investigate the effects of parecoxib on postoperative immune status.

Materials and methods

Patient selection. Between May 2012 and May 2013, a total of 356 patients were diagnosed with cervical cancer in the Cancer Hospital, Harbin Medical University (Heilongjiang, China). Of these, 101 patients met the inclusion criteria, but 21 refused to take part in the study. Finally, 80 patients of ages 18-65 years, with American Society of Anesthesiology (ASA) stages I-III, that were accepted for laparoscopic radical hysterectomy for stages IB and IIA cervical cancer, were evaluated in the current randomized, double-blind controlled trial. The diagnosis of cervical cancer was in accordance with the International Federation of Gynecology and Obstetrics (FIGO) classification. The exclusion criteria were as follows: Severe heart, hepatic or renal disease; coagulopathy; bronchial asthma; a history of chronic pain or regular opioid consumption; morbid obesity (BMI >35 kg/m^2); and any contraindication to NSAIDs. The protocol of the current study was approved by the Ethics Committee of Heilongjiang University of Chinese Medicine (Harbin, China), and written informed consent was obtained from all subjects.

Anesthesia procedures. Phenobarbital sodium (2 mg/kg; (Tianjin Jinyao Amino Acid Co., Ltd., Tianjin, China) was administered intramuscularly 30 min before surgery. Prior to the induction of anesthesia, standard monitors were applied to record non-invasive blood pressure, heart rate and the electrocardiogram. The bispectral index system (BIS) was used to monitor the depth of anesthesia. Anesthesia was induced intravenously with midazolam (0.05 mg/kg; Jiangsu Nhwa Pharmaceutical Co., Ltd., Jiangsu, China), fentanyl (0.03 mg/kg; Yichang Humanwell Pharmaceutical Co., Ltd., Hubei, China) and propofol (2.5-3.0 mg/kg; AstraZeneca UK Limited, Macclesfield, UK), and intubation was performed using vecuronium (0.08 mg/kg; Zhejiang Xianju Pharmaceutical Co., Ltd., Zhejiang, China). The lungs were ventilated mechanically (Aestiva 3000; GE Datex-Ohmeda Instrumentarium Corp., Helsinki, Finland) with a tidal volume of 8-10 ml/kg and ventilator frequency of 12-14 bpm to achieve an end-tidal carbon dioxide tension of 35-45 mm Hg. Anesthesia was maintained with nitrous oxide (60%; Harbin Qing Hua Industrial Gas Co., Heilongjiang, China) and isofluorane (end-tidal concentration 1-3%; Abbott Laboratories, Chicago, IL, USA) to a BIS score of 40-50. To maintain core body temperature normothermia, a circulating warm water blanket (Tianjin Medical Instrument Research Institute, Tianjin, China) was applied. Ringer's acetate (Hunan Kelun Pharmaceutical Co., Ltd., Hunan, China) was administered at a rate of 6-8 ml/kg/h in order to maintain basal fluid requirements. No blood transfusion was given intra-operatively. All patients were operated on by one surgical team, with the same operative protocol under a standardized anesthesia protocol. The surgery was performed as described previously (23) and anesthesia was performed as described previously (24) with small modifications (nitrous oxide concentration adjusted to 60%).

Experimental design. An anesthetist not involved in the data collection performed the randomization with a computer-generated random list with coded sealed envelopes. Patients were randomized into the parecoxib-treated (group I; n=40) or control (group II; n=40) groups. Group I received parecoxib (40 mg; Dynastat, Pfizer, Inc., New York, NY, USA) 30 min prior to surgery and then each 12 h subsequent to surgery until the 60 h time point, while group II received

normal saline as a placebo at the corresponding time points. Intravenous tramadol (100 mg) was prescribed for postoperative pain relief as required.

A syringe containing parecoxib or saline was prepared by a third party and labeled 'study drug'. All patients, surgeons, anesthesiologists and nurses involved in recording postoperative data were blinded to the syringe contents.

Determination of cytokine mRNA expression by quantitative polymerase chain reaction (qPCR). Blood samples were obtained prior to parecoxib or saline administration (basal) and at 24, 48 and 72 h subsequent to surgery. Total RNA was extracted using TRIzol® reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). The concentration of purified RNA was determined by spectrophotometry (SmartSpec Plus; Bio-Rad, Hercules, CA, USA). Reverse transcription (RT) was performed on the total RNA (1 μl) to synthesize cDNA. This was then added into a 20 μl GoldScript one-step RT-PCR kit (Invitrogen Life Technologies), placed in 42˚C water for 60 min and heated in 70˚C water for 15 min to deactivate the reverse transcriptase. The primers were designed and synthesized by the Shanghai Institute of Biochemistry (Shanghai, China). The sequences of the primers used for PCR amplification were as follows: IL-2, forward (F) 5'-GACGCTGGAAATTTCATCAGCA-3' and reverse (R) 5'-GCTCATCATCGAATTGGCACTC-3' (91 bp); IFN-γ, F 5'-AGGCCATCAGCAACAACATAAGTG-3' and R 5'-GACAGCTTTGTGCTGGATCTGTG-3' (140 bp); IL-4, F 5'-TGCACCGAGATGTTTGTACCAGA-3' and R 5'-TTGCGAAGCACCCTGGAAG-3' (92 bp); IL-10, F 5'-CAGACCCACATGCTCCGAGA-3' and R 5'-CAAGGCTTGGCAACCCAAGTA-3' (141 bp); IL-17, F 5'-AACTCATCCATCCCCAGTTG-3' and R 5'-CCGGTTATGGATGTTCAGGT-3' (198 bp); IL-23, F 5'-GCACTGCTGAATGTCCCA-3' and R 5'-CATGCCTAGTGCGTTTGC-3' (311 bp); TGF-β, F 5'-ATGAGCACTGAAAGCATGATC-3' and R 5'-TCACAGGGCAATGATCCCAAAGTAGACCTGCCC-3' (262 bp); GAPDH, F 5'-GGCACAGTCAAGGCTGAGAATG-3' and R 5'-ATGGTGGTGAAGACGCCAGTA-3' (143 bp). PCR amplification was performed on a DNA Engine thermal cycler (96 well alpha unit with hot bonnet; Bio-Rad) using a SYBR Green I (SYBR Green PCR Master Mix; Invitrogen Life Technologies) probe (20 μl DNA per assay). The mRNA expression levels of target genes were normalized against the reference gene GAPDH, and relative mRNA levels of each cytokine were calculated. All samples were run in triplicates for each experiment.

Determination of cytokine protein expression levels by enzyme-linked immunosorbent assay (ELISA). Plasma was separated by centrifugation at 2,000 x g for 15 min at 4˚C and immediately stored in aliquots at 80˚C. The concentrations of cytokines (IL-2, IFN-γ, IL-4, IL-10, IL-17, IL-23 and TGF-β) were determined by ELISA using commercially available kits (Shanghai Senxiong Technology Industry Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Briefly, flat-bottomed 96-well microtiter plates (Sigma-Aldrich, St. Louis, MO, USA) were coated with a monoclonal mouse anti-human anti-cytokine antibody (50 μl/well; 1:100; Senxiong Science and Technology Company, Shanghai, China) diluted in coating buffer (Shanghai Yuanmu Biological Science and Technology Co., Ltd, Shanghai, China) and incubated overnight. The optical density was measured on a plate reader (Bio-Rad) at 490 nm. The results are expressed in pg/ml, and the optical density of the samples was compared to the standard curves.

Variables measured during the study period. Postoperative pain at rest and following movement were assessed by a visual analogue scale (VAS) between 0 (pain free) and 10 (worst possible pain) prior to parecoxib or saline administration (basal) and at 2, 6, 12, 18, 24, 36, 48, 60 and 72 h subsequent to surgery. Mean arterial pressure (MAP) and heart rate (HR) were measured every 6 h for three days after surgery. The immune parameters and pain scores were considered the primary endpoint. Secondary outcomes included the incidence of postoperative pain (visceral, parietal and shoulder pain), analgesic relief, the ocurrence of adverse events and the length of hospital stay.

Sample sizes and statistical analysis. The sample size was calculated based on the preliminary experiment comparing an immune variable (IL-2 protein level) on postoperative day 2 between the two groups and yielded a sample size of n=21 (type I error=0.05 and type II error=0.2) for each group. To accommodate for participants that may not complete the study, 40 patients were enrolled in each group.

The normality of quantitative variables was tested using the Kolmogorov-Smirnov test. Inter-group differences were assessed by Student's t-test. Inter-group comparison of the mean VAS scores at each measurement point was performed with the t-test. Quantitative variables were analyzed using χ^2 or Fisher's exact test. Statistical analyses were performed using SPSS version 13.0 software (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

In the present study, 8 patients were excluded, due to being converted to laparotomy (n=3; 1 from group I, and 2 from group II) and incomplete data collection (n=5; 3 from group I, and 2 from group II). A total of 72 patients (n=36 in each group) were included in the data analysis.

Demographic and surgical information. Demographic and surgical data are presented in Table I. The mean ages of the patients were 52.5±11.8 years in group I and 54.4±13.1 years in group II, which were not statistically different ($P>0.05$). Other features of patients, including weight, ASA physical status, FIGO stage and histology were not significantly different between the two groups ($P>0.05$); duration of surgical procedure did not differ between groups I and II (248.5±65.1 vs. 261.2±57.3 min, respectively; $P>0.05$). Patients in group I and II were similar with respect to blood loss, fluids and lymph node resection ($P>0.05$).

Postoperative pain intensity and incidence. In order to understand the immunoregulatory effect of parecoxib, its effect on postoperative pain following gynecologic laparoscopy was evaluated. Parecoxib is an effective analgesic that acts by inhibiting the synthesis of cyclooxygenase (COX)-2/prostaglandin E (PGE)2

Table I. Demographic and clinical characteristics of patients.

Characteristic	Group I	Group II
Age (years)	52.5±11.8	54.4±13.1
Weight (kg)	63.8±9.9	58.5±7.5
ASA (I/II/III)	4/29/3	7/22/7
FIGO stage (Ib/IIa)	14/22	16/20
Histology		
Squamous	29	32
Adenocarcinoma	6	4
Adenosquamous	1	0
Other	0	0
Duration of surgery (min)	248.5±65.1	261.2±57.3
Blood loss (ml)	299.0±37.0	320.0±48.0
Fluids (ml)	2080.0±350.0	2220.0±430.0
Lymph nodes resected	17.5±2.5	20.5±3.5

Values are expressed as the mean ± standard deviation, or n. Comparisons between the two groups were conducted using the Student's t-test, χ^2 test or Fisher's exact test.

Table II. Incidence of pain subsequent to laparoscopy (n, %).

Group	n	Visceral	Parietal	Shoulder
Group I	36	16 (44.4)[a]	19 (52.8)[a]	5 (13.9)[a]
Group II	36	26 (72.2)	28 (77.8)	13 (36.1)
Total	72	42 (58.3)	35 (65.3)	18 (25.0)

[a]P<0.05 vs. group II. Comparisons of the two groups were performed using the χ^2 test.

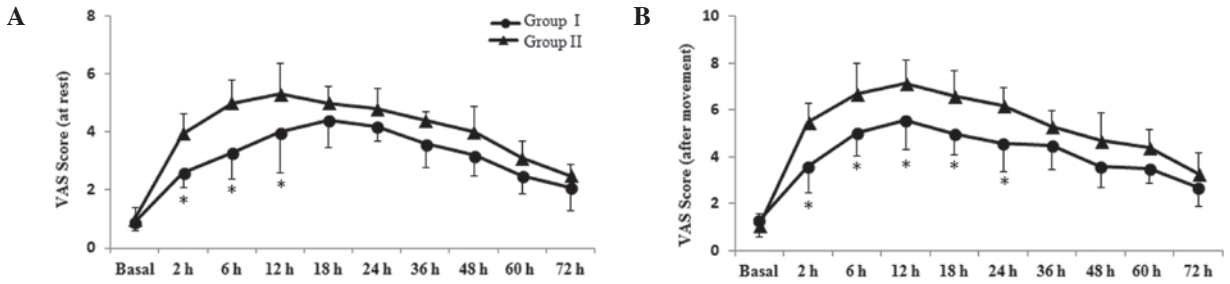

Figure 1. Effects of parecoxib on postoperative pain scores. Postoperative pain (A) at rest and (B) following movement were assessed with a VAS (0, no pain; 10, worst possible pain) prior to parecoxib or saline administration (basal) and at 2, 6, 12, 18, 24, 36, 48, 60 and 72 h subsequent to surgery. Data are presented as the mean ± standard deviation (n=36 in each group). *P<0.05 vs. group II. VAS, visual analog scale.

in the central nervous system. VAS score is a simple and commonly used method for evaluating postoperative pain intensity in studies. Pain scores were assessed using VAS prior to parecoxib or saline administration (basal) and at 2, 6, 12, 18, 24, 36, 48, 60 and 72 h subsequent to sugery; the VAS scores are presented in Fig. 1. Compared with group II, VAS scores at rest for group I were significantly reduced at 2, 6 and 12 h post-surgery (P<0.05; Fig. 1A). After movement, patients in group I experienced reduced pain compared with group II at 2, 6, 12, 18 and 24 h post-surgery (P<0.05; Fig. 1B). The results suggest that parecoxib exerts a stronger analgesic effect following laparoscopy.

Early postoperative pain subsequent to laparoscopy occurs as visceral, parietal and shoulder pain components. The overall incidences of these pain components in the study were 58.3, 65.3 and 25.0%, respectively (Table II). The incidences of visceral, parietal and shoulder pain in group I were lower than those in group II (44.4 vs. 72.2, 52.8 vs. 77.8

Table III. Postoperative adverse events (n, %).

Adverse event	Group I	Group II
Nausea/vomiting	5 (13.8)[a]	12 (33.3)
Sedation	3 (8.3)	2 (5.6)
Headache	1 (2.8)	2 (5.6)
Hypotension	4 (11.1)	3 (8.3)
Hypertension	2 (5.6)	3 (8.3)
Respiratory-related	1 (2.8)	2 (5.6)
Gastrointestinal bleeding	0	0
Cardiovascular-related	1 (2.8)	1 (2.8)
Postoperative infection	2 (5.6)[a]	6 (16.7)

[a]P<0.05 vs. group II. Comparisons of the two groups were performed using the χ^2 or Fisher's exact test.

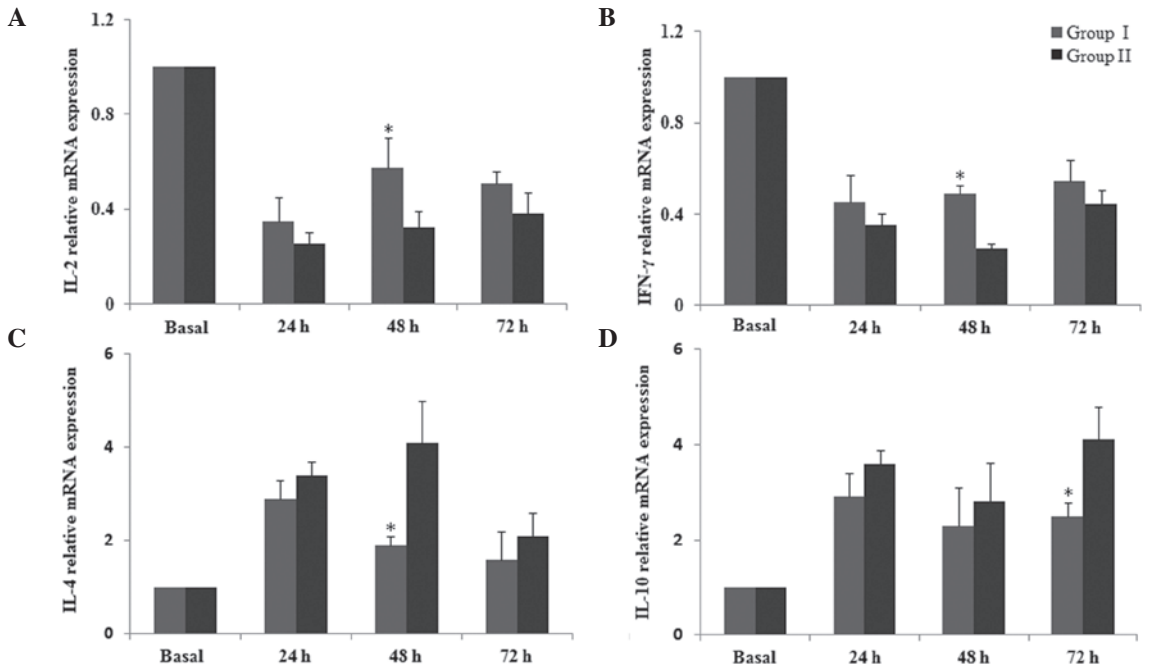

Figure 2. Effects of parecoxib on mRNA expression levels of T helper 1/2 cytokines. Peripheral blood samples were obtained prior to parecoxib or saline administration (basal) and at 24, 48 and 72 h after surgery. Total RNA was extracted for measurements of (A) IL-2, (B) IFN-γ, (C) IL-4 and (D) IL-10 by reverse transcription-quantitative polymerase chain reaction. GAPDH was used as an internal control and relative mRNA expression levels of the cytokines were calculated. All samples were run in triplicate for each experiment. Data are presented as the mean ± standard deviation (n=36 in each group). *P<0.05 vs. group II. IL, interleukin; IFN, interferon.

and 13.9 vs. 36.1%, respectively; P<0.05). This indicates that parecoxib may reduce the incidence of these postoperative pain types following laparoscopy.

Postoperative analgesic relief, length of hospital stay and occurrence of adverse events. The percentage of patients who required postoperative analgesic relief was lower in group I compared with group II (13.9 vs. 69.4%; P<0.01). Neither MAP nor HR differed between the two groups during the 72-h observation period (P>0.05). The length of hospital stay in group I was shorter than group II, but presented no significant difference (7.4±2.2 vs. 9.0±3.1 days; P>0.05). No major complications or adverse events occurred in the two groups. The incidence of postoperative nausea/vomiting and infection was significantly lower in group I than group II (13.8 vs. 33.3% and 5.6 vs. 16.7%; P<0.05); The incidence of complications, including sedation, headache, hypotension, hypertension, respiratory-related, gastrointestinal bleeding and cardiovascular events, during the study period was similar between the two groups (Table III). This suggests that parecoxib is safe and well-tolerated for use in pain control for patients with cervical cancer following laparoscopic surgery.

mRNA expression levels of Th1, Th2, Th17 and *Treg cytokines*. Effective analgesia is able to relieve surgery-induced immune suppression. Recent experiments suggested that parecoxib exerts a stronger antihyperalgesic effect and provides a regulatory effect on the inflammatory immune response. In the

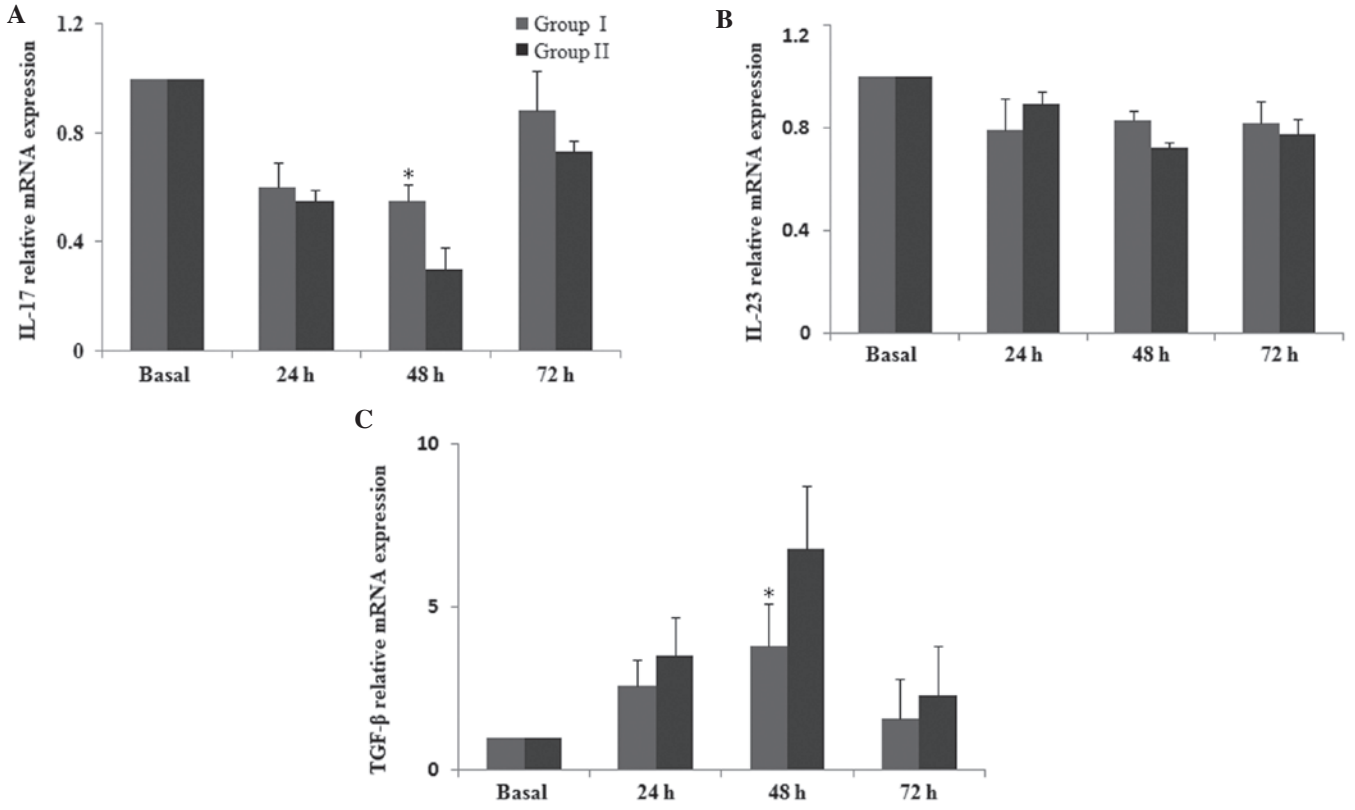

Figure 3. Effects of parecoxib on T helper 17/regulatory T cytokine mRNA expression levels. Peripheral blood samples were obtained prior to parecoxib or saline administration (basal) and 24, 48 and 72 h after surgery. Total RNA was extracted for measurements of (A) IL-17, (B) IL-23 and (C) TGF-β by quantitative polymerase chain reaction. GAPDH was used as an internal control and the relative mRNA expression levels of the cytokines were calculated. All samples were run in triplicate for each experiment. Data are presented as the mean ± standard deviation (n=36 in each group). *P<0.05 vs. group II. IL, interleukin; TGF, transforming growth factor.

present study, the balance of Th1, Th2, Th17 and Treg cells was evaluated to determine the immunoregulatory effects of parecoxib. The mRNA levels of Th1/Th2 cytokines quantified by qPCR are displayed in Fig. 2. The results indicated that the mRNA expression levels of IL-2 and IFN-γ were reduced following surgery, and this reduction was significantly inhibited in group I at postoperative 48 h compared with group II (P<0.05; Fig. 2A and B). As presented in Fig. 2C and D, mRNA expression levels of IL-4 and IL-10 were higher following surgery, and these increases were markedly suppressed at 48 and 72 h, respectively, in group I compared with group II (P<0.05). These findings support the hypothesis that perioperative intravenous administration of multi-dose parecoxib attenuates postoperative immune suppression.

A reduction in the mRNA level of IL-17 subsequent to surgery was observed, which was then attenuated at 48 h, resulting in a higher level of IL-17 in group I compared with that of group II (P<0.05; Fig. 3A). However, the mRNA level of IL-23 during the postoperative 72-h observation period was not significantly altered between the two groups (P>0.05; Fig. 3B). The mRNA level of TGF-β was increased following surgery, and this increase was inhibited at 48 h, with a significantly lower level in group I compared with that of group II (P<0.05; Fig. 3C). These results clearly indicate that parecoxib is able to attenuate the postoperative immune impairment by balancing the expression of Th1, Th2, Th17 and Treg cytokines at the mRNA level.

Protein levels of Th1, Th2, Th17 and *Treg cytokines*. The effects of parecoxib on the protein levels of Th1, Th2, Th17 and Treg cytokines in peripheral blood were also evaluated. Similar to the qPCR result, ELISA analysis indicated a reduction in the protein levels of IL-2 and IFN-γ following surgery, and this reduction was attenuated at 48 h post-surgery in group I compared with group II (P=0.045; Fig. 4A and B). The protein concentration of IL-4 and IL-10 increased following surgery (Fig. 4C and D), and IL-10 production in group I was significantly lower at 72 h subsequent to surgery compared with the levels in group II, (P<0.05), while no difference was identified in the protein level of IL-4 between the two groups (P>0.05). This suggests that parecoxib may be an effective agent for restoring perioperative immune competence.

As indicated by Fig. 5, the protein level of IL-17 was slightly higher at 48 h in group I compared with group II, but the difference was not significant (P>0.05). There was no significant difference in levels of IL-23 during the postoperative 72-h observation period between the two groups (P>0.05). It was observed that the protein level of TGF-β increased subsequent to surgery, however, no significant difference was identified between the two groups (P>0.05). Collectively, these results suggest that parecoxib is able to attenuate the postoperative immune suppression by regulating the expression of Th1, Th2, Th17 and Treg cytokines at the protein level.

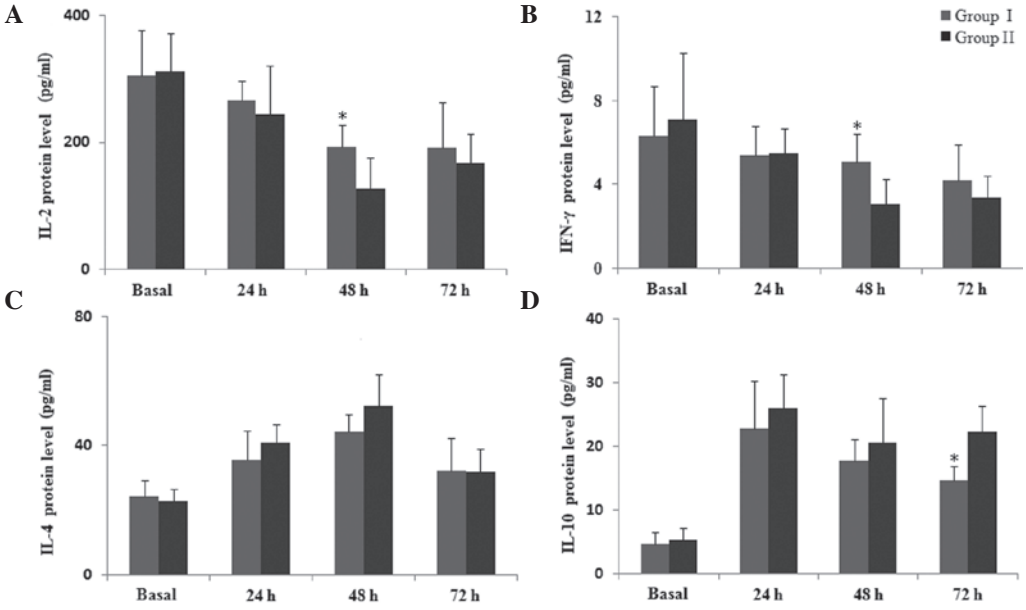

Figure 4. Effects of parecoxib on the protein levels of T helper 1/2 cytokines. Peripheral blood samples were obtained prior to parecoxib or saline administration (basal) and at 24, 48 and 72 h after surgery. Plasma was separated for measurements of (A) IL-2, (B) IFN-γ, (C) IL-4 and (D) IL-10 by ELISA. Data are presented as the mean ± standard deviation (n=36 in each group). *P<0.05 vs. group II. IL, interleukin; IFN, interferon.

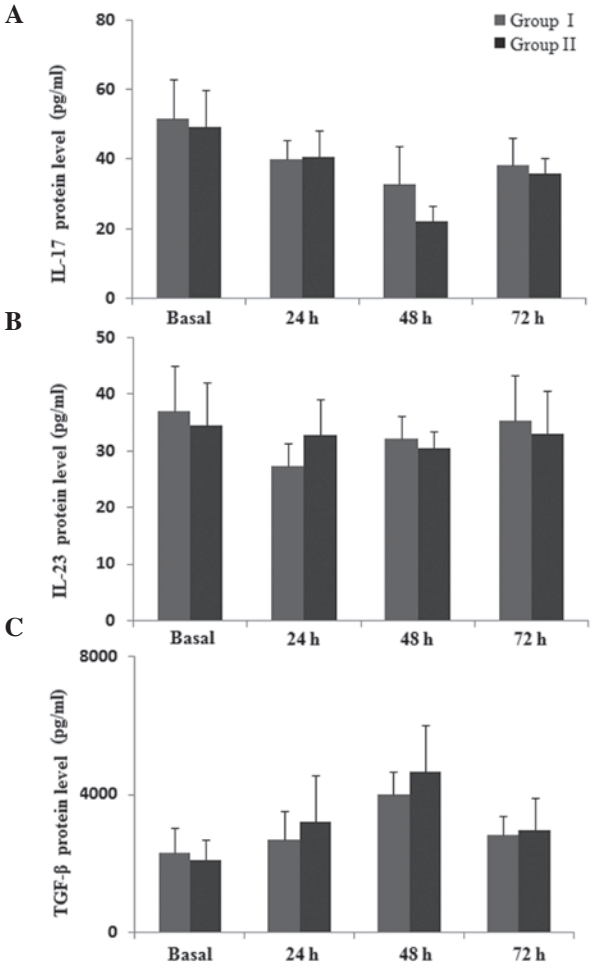

Figure 5. Effects of parecoxib on T helper 17/regulatory T cytokine protein levels. Peripheral blood samples were obtained prior to parecoxib or saline administration (basal) and at 24, 48 and 72 h after surgery. Plasma was separated for measurements of (A) IL-17, (B) IL-23 and (C) TGF-β by ELISA. Data are presented as the mean ± standard deviation (n=36 in each group). *P<0.05 vs. group II. IL, interleukin; TGF, transforming growth factor.

Discussion

The principal findings of the present study demonstrated that the mRNA and protein levels of Th1, Th2, Th17 and Treg cytokines were imbalanced following laparoscopy in the peripheral blood of patients with cervical cancer, with reduced production of IL-2, IFN-γ and IL-17, and an increased expression level of IL-4, IL-10 and TGF-β. Perioperative multiple-dose parecoxib markedly enhanced the expression of IL-2, IFN-γ and IL-17, and suppressed the reduction of IL-4, IL-10 and TGF-β, which contributed to restore the balance of Th1, Th2, Th17 and Treg cytokines, and attenuated surgery-related immune suppression. The findings of the present study also revealed that multiple-dose parecoxib exerted an analgesic effect and resulted in lower VAS scores and pain incidence. These observations indicate that parecoxib, in addition to postoperative pain relief, may ameliorate surgery-induced immune suppression by balancing the expression of Th1, Th2, Th17 and Treg cytokines following laparoscopy in patients with cervical cancer.

Early postoperative pain is the most common complaint following gynecological laparoscopy. It is a multifactorial and complex problem, and includes visceral, parietal and shoulder pain components due to various pain mechanisms (25). Systematic reviews have suggested that COX-2 inhibitors are an effective treatment for acute postprocedural pain (26-28). In the current study, patients experienced mild-to-moderate postoperative pain during the 72-h observation period, and the overall incidences of visceral, parietal and shoulder pain were 58.3, 65.3 and 25.0%, respectively. The data indicated that administration of multi-dose parecoxib (40 mg every 12 h) relieved postoperative pain intensity and incidence, and reduced the requirement for analgesics. These observations are in accordance with results of other studies that indicated that 40 mg parecoxib twice daily ameliorated post-

laparoscopic pain, and attenuated the incidence of visceral and shoulder pain with a reduced requirement for analgesics following gynecological laparoscopy (29,30). In contrast to the traditional opioid drugs, parecoxib exerts its analgesic effect without opioid-induced hyperalgesia. It reduces pain through the inhibition of the COX-2 isoenzyme, which is important in the synthesis of PGE2 and the prevention of the wind-up phenomenon of the CNS. In addition, parecoxib was well-tolerated, as platelets do not contain COX-2 and all synthesis of thromboxane A2 in the platelet is mediated by COX-1. It was demonstrated in the current study that parecoxib reduced postoperative nausea/vomiting and infections, and did not increase gastrointestinal bleeding and cardiovascular-related adverse effects. These findings indicated that multiple-dose parecoxib appears to be effective and safe for use in pain control for patients with cancer following laparoscopic surgery.

Increasing evidence indicates that patients with cervical cancer exhibit an imbalance in the functions of Th1 and Th2 cells, with a Th2-predominant response mode (29), and the Th17/Treg balance has been noted to be disrupted in peripheral blood from patients with cervical cancer (13,32,33). In the present study, in order to improve understanding of the underlying mechanisms of postoperative immune suppression, alterations in the levels of Th1, Th2, Th17 and Treg cytokines following gynecological laparoscopy were observed in patients with cervical cancer. Previous studies have reported that surgical trauma induced a Th1/Th2 balance shift in the form of diminished IL-2 and IFN-γ and increased IL-4 and IL-10 levels, which result in weakened immunity (13,34). However, there is limited information on alterations of Th1, Th2, Th17 and Treg cytokine levels subsequent to gynecological laparoscopy in patients with cervical cancer. In the current study, the results demonstrated that the production levels of IL-2, IFN-γ, IL-17 and IL-23 were markedly reduced following surgery, and the levels of IL-4, IL-10 and TGF-β were significantly increased, implicating surgical trauma in postoperative immune depression, acting by disrupting the balance of Th1, Th2, Th17 and Treg cytokines. These data are consistent with a study by Visser *et al* (35), which demonstrated that the increased expression of IL-10 and TGF-β, and frequencies of Treg cells, influenced the balance between Th17/Treg cells, and promoted the development of immune suppression, implying that the imbalance of Th1, Th2, Th17 and Treg cytokines may be involved in the development of postoperative immune impairment. It provided the understanding that the imbalance of Th1, Th2, Th17 and Treg cytokines may be one of the mechanisms leading to postoperative immune suppression.

A study by Cheng *et al* (36) indicated that patients with cancer undergo a series of neuroendocrine and physiological changes in the body when suffering surgical trauma, stress, postoperative pain and other external noxious stimulation. Additionally, there is a close association and mutual influence between neuroendocrine and immune systems (36). Pain stimulation subsequent to surgery can directly transmit to the CNS and thus stimulate the neuro-immune endocrine system. The simultaneous increase of endogenous catecholamine levels such as corticosteroids and prostaglandins as a result of the stress response is able to suppress cellular immune function and increase the tumor cell transfer probability in the perioperative period (37,38). Therefore, effective analgesia in the perioperative period is able to greatly affect the immune system. Other studies suggested that the cyclooxygenase-2 (COX-2)/PGE2 pathway may be critical in the analgesic and immune inflammatory response (39,40). Much remains elusive regarding the effects of parecoxib on immune function following gynecological laparoscopy in patients with cervical cancer. In the current study, results indicated that multi-dose parecoxib can effectively enhance the production of IL-2, IFN-γ and IL-17, and inhibit the expression of IL-4, IL-10 and TGF-β in patients with cervical cancer. These findings are consistent with those of a previous study, in which parecoxib reduced postoperative pain (41), and another in which it inhibited hippocampal IL-1β and TNF-α expression through downregulation of the COX-2/PGE2 pathway (40). A number of studies have provided data supporting the hypothesis that parecoxib has the ability to enhance the generation of host immunity, by increasing the activity of natural killer cells and plasma IFN-γ levels (42,43). These data indicated that parecoxib exerts protective effects on postoperative immune function by regulating the balance of Th1, Th2, Th17 and Treg cytokines.

Another study indicated that the immune status of the patient may significantly influence the progression and clinical outcome of the cancer. In the present study, the attenuation of postoperative immune suppression by administration of parecoxib may have contributed to the improved postoperative course with reduced postoperative nausea/vomiting and infections. Collectively, the results of the current study further demonstrate that parecoxib can improve the postoperative course, and exert immunomodulatory effects by balancing the mRNA and protein expression levels of Th1, Th2, Th17 and Treg cytokines in the peripheral blood of patients with cervical cancer.

All operations in the present study were performed without blood transfusion in order to avoid the presence of another factor that may increase the surgical inflammatory response. The difference in the immune factors between the two groups is described at the end-point of treatment with the selected analgesic. In the present study, perioperative plasma IL-23 concentration was indicated to be unchanged compared with the pre-operative values, and was unaffected by administration of multi-dose parecoxib, although previous studies have observed increases in IL-23 concentration following cataract surgery (44). However, failure to detect a change in the production of circulating IL-23 at the specific time points does not rule out the possibility that this factor may be released at an earlier time point. This finding may reflect the complex nature of cytokine homeostasis and the potential regulation of the interactions between stress hormones and the immune system.

There were several limitations of the current study. The effects of parecoxib alone were assessed; but comparison of parecoxib and other NSAIDs, such as celecoxib or frubiproten, may aid in the understanding of the specific role of parecoxib in postoperative immune function. Also, the effects were observed for just 72 h subsequent to surgery, as the majority of patients did not request analgesics after this time. A prolonged assessment is required to clarify the long-term immunoregulatory effects of parecoxib. Furthermore, the use of tramadol for the relief of postoperative pain may have intervened in the

assessment of postoperative immune parameters. A previous study reported that intravenous administration of 100 mg tramadol did not influence the immune function following laparoscopy (45). Therefore, in order to be clinically useful, additional studies are required to further elucidate the immunomodulatory effects of parecoxib on the percentages of $CD4^+$ T cell subsets, including Th1, Th2, Th17 and Treg cells.

In conclusion, the current results demonstrated that perioperative multi-dose parecoxib, in addition to its analgesic effect, may ameliorate postoperative immune suppression and improve the clinical course by balancing the expression of Th1, Th2, Th17 and Treg cytokines in the peripheral blood of patients with cervical cancer following laparoscopy. These findings may contribute to an alternative therapeutic regime in the management of postoperative pain and immune responses of patients with cancer.

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