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Original Research Article

Analgesic effect of flurbiprofen ester and its effect on serum inflammatory factors and B-endorphin expression in rats with incision pain

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Abstract

Purpose: *To study the analgesic effect of flurbiprofen ester in rats with incision pain, and its effect on serum inflammatory factors and β-endorphin expression.*
Methods: *Seventy-five (75) healthy rats with foot contraction threshold induced by basic mechanical stimulation were randomly assigned to control, model control and treatment groups. Flurbiprofen was administered in 3 doses: 5, 10 and 15 mg/kg. Then, 3 mL of ventricular blood was taken from anesthetized rats and the serum levels of tumor necrosis factor-α (TNF- α), interleukin-1 β, interleukin-6 and β-endorphin were measured. The expression of β-endorphin in the spinal cord of rats with lumbar enlargement and ARC was determined.*
Results: *The TNF- α, interleukin-1 β and interleukin-6 concentrations were significantly lower in treatment group than in model rats, and decreased with time and dose ($p < 0.05$). In the treatment group, the level of serum β-endorphin decreased with increase in dose at 1 h, but increased with increase in dose at 5 h and 10 h ($p < 0.05$). The levels of β-endorphin in the spinal cord, was significantly lower in model rats than in control rats ($p < 0.05$).*
Conclusion: *Pre-administration of flurbiprofen ester reduces serum inflammatory factors and upregulates β-endorphin expression in rats with incision pain. Thus, it flurbiprofen exerts analgesic effect.*

Keywords: *Flurbiprofen ester, Incision pain, Rat, Analgesia, Inflammatory factor, β-endorphin*

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INTRODUCTION

Endogenous opioid peptide (EOP) exerts an opioid effect. It is classified into enkephalin, endorphin, dynorphin and nea porphin. Endogenous opioid peptide (EOP) relieves conduction of pain signals through binding to the opioid receptor on the target cell membrane, thereby exerting analgesic effect [1]. The effect of endorphin is the strongest among the members of EOP family, and the distribution of endorphin in the body is very extensive. P-Enkephalin is present in the nervous system, endocrine organs, digestive tract, placenta, and amniotic fluid [2,3]. Under normal conditions, the level of endorphin in the body is low. However, when stress reaction occurs, there is increased release of endorphin, leading to enhancement of

cerebral ischemic brain edema, and inhibition of the frequency and the amplitude of respiratory motion [4,5].

The analgesic effect of endorphin is strong, and it enhances the release of hormones such as auxin, prolactin, somatostatin and glucagon [6]. Surgical wounds cause tissue injury, release of inflammatory factors, inflammatory reactions and hyperalgesia [7]. Flurbiprofen axetil (FA), a cyclooxygenase inhibitor with the effect of anti-inflammatory, antipyretic, anti-rheumatism and analgesic properties, and it is clinically used for treating post-operative and various cancer pain [8]. The present study was carried out to investigate the analgesic effect of FA pre-administration, and its effect on the expression of serum inflammatory factors and endorphin in rats.

EXPERIMENTAL

General information

A total of 75 SPF-grade SD male rats without primary disease were used. The biological license number of experimental animals was SCXK (GUI) 2007-000. The rats were 6.0 - 8.0 weeks old, with mean body weight of 154.11 ± 23.42 g. Approval for the study was received from the ethics committee of our hospital (approval no. 20190216) and the study carried out in line with international guidelines for animal studies [9].

Treatments

Five groups of rats were used (15 rats per group). In control rats, 1 ml fat milk was administered via tail vein injection. After 30 min, the rats were subjected to inhalation of 1 % sevoflurane for 5 min without surgery. Rats in model group were treated with painful plantar incision 30 min after intravenous injection of 1 ml fat milk, and inhalation of 1 % sevoflurane for 5 min. Rats in the low, medium and high treatment groups were injected in the tail vein with flurbiprofen (diluted with fat milk) at levels corresponding to 5, 10 and 15 mg/kg), in that order, for 30 min.

Painful plantar incision

After inhalation of 1 % sevoflurane anesthesia, an incision of about 1cm was made in the proximal plantar of each rat. The skin was cut open, the muscles of the sole were cut lengthwise, and the wound was sewn up after pressing down, without bleeding.

Therapeutic indices

The mechanical stimulation and foot contraction (PMWT) of each rat was measured 1, 5 and 10 h after the operation using von Frey electronic pain detector. The rats were put in a quiet environment, and the mean value of PMWT was obtained for three consecutive tests.

For serum indices, 5 rats in each group were randomly selected at 1, 5 and 10 h after surgery. After abdominal deep anesthesia, 3 mL of ventricular blood was collected from the rats and kept at a temperature of 4 ^{o}C for 20 min. Thereafter, it was centrifuged at 3500 rpm for 5 min, and the serum was refrigerated at -80 ^{o}C to avoid repeated freezing and thawing. Serum TNF-α, IL-1, IL-6, and β-endorphin levels were determined using ELISA.

Rat spinal cord was placed in 4% paraformaldehyde fixative solution and kept in a 4 ^{o}C refrigerator prior to examination. Immunohistochemical staining and image analysis were used to determine the expression level of beta endorphins in the rat spinal cord.

Statistical analysis

Data are presented mean ± SD. Qualitative data are presented as %. Comparison between two groups was performed with *t*-test, while comparison amongst groups was carried out using Chi square test. Ridit test was used to compare grade data. All statistical analyses were done with SPSS20.0 software package. Statistical significance was fixed at $p < 0.05$.

RESULTS

Changes in PMWT level in rats

As shown in Table 1, basic PMWT values of rats were comparable amongst the groups ($p > 0.05$). The PMWT of rats in the model control group was significantly lower than that in the control group at each time point, while PMWT of rats in the treatment group was significantly higher than that in the model control group at each time point ($p < 0.05$).

Changes in serum inflammatory factors

The serum levels of the measured cytokines were higher in model rats than in control at each time point, and increased with time. However, serum cytokine levels were markedly reduced in the treatment groups, relative to model rats, and decreased along with time and dose gain ($p < 0.05$; Table 2, Table 3 and Table 4).

Table 1: Changes in PMWT level at different times after operation (n = 15)

Group	PMWT(g)			
	Baseline	***1h***	***5h***	***10h***
Control	45.16 ± 2.25	44.26 ±1.49	44.83 ±1.29	44.71 ±2.85
Model	44.89 ± 1.13	20.48 ± 1.71[a]	21.48 ± 2.83[a]	22.56 ± 1.85[a]
Low dose	45.26 ± 2.10	28.45 ± 1.56[ab]	27.48 ± 1.73[ab]	30.79 ± 1.65[ab]
Mediu m dose	45.94 ± 2.14	31.40 ± 2.71[abc]	31.05 ± 2.78[abc]	34.56 ± 2.52[abc]
High dose	44.76 ± 1.86	34.52 ± 2.33[abcd]	35.78 ± 2.84[abcd]	40.15 ± 3.10[abcd]

Data are mean ± SD; [a]$p < 0.05$, vs controls; [b]$p < 0.05$, vs model rats, [c]$p < 0.05$, vs low concentration group; [d]$p < 0.05$, vs medium concentration group

Table 2: Changes in serum TNF- α levels at different times after operation (n = 15)

Group	TNF-α (pg/mL)		
	1h	***5h***	***10h***
Control	64.16 ± 10.48	78.18 ± 11.43	85.19 ± 13.76
Model	223.19 ± 21.46[a]	246.79 ± 23.15[a]	276.48 ± 30.41[a]
Low dose	205.17 ± 13.74[ab]	176.48 ± 16.48[ab]	164.73 ± 20.14[ab]
Medium dose	182.18 ± 12.43[abc]	165.48 ± 10.44[abc]	151.43 ± 14.73[abc]
High dose	162.89 ± 9.86[abcd]	130.76 ± 10.86[abcd]	102.75 ± 10.43[abcd]

Data are mean ± SD; [a]$p < 0.05$, vs control; [b]$p < 0.05$, vs model, [c]$p < 0.05$, vs low concentration group; [d]$p < 0.05$, vs medium concentration group

Table 3: Changes in serum IL-6 levels at different times after operation

Group	IL-6 (pg/mL) *1h*	IL-6 (pg/mL) *5h*	IL-6 (pg/mL) *10h*
Control	51.46 ± 10.45	53.48 ± 11.86	56.78 ± 11.49
Model	124.78 ± 18.41[a]	141.76 ± 20.15[a]	168.43 ± 23.48[a]
Low dose	116.48 ± 8.41[ab]	95.46 ± 16.18[ab]	90.15 ± 16.43[ab]
Medium dose	100.16 ± 3.76[abc]	95.15 ± 14.73[abc]	88.45 ± 15.22[abc]
High dose	85.79 ± 2.86[abcd]	72.41 ± 13.11[abcd]	61.74 ± 13.77[abcd]

Data are mean ± SD; [a]$p < 0.05$, vs control; [b]$p < 0.05$, vs model, [c]$p < 0.05$, vs low concentration group; [d]$p < 0.05$, vs medium concentration group

Serum and spinal cord levels of beta-endorphins

As shown in Tables 5 and 6, serum level of beta-endorphins in the model group was significantly different from those in the control group ($p < 0.05$). The serum level of beta-endorphins in the treatment group decreased with increase in dose at 1 h, but increased with increase in dose at 5 and 10 h ($p < 0.05$).

In the spinal cord, the levels of beta-endorphin in the model control group were significantly lower than those in the control group at 5 and 10 h, while beta-endorphin levels were markedly higher in treatment rats than in model rats, and increased with increase in dose ($p < 0.05$).

Table 4: Levels of IL-1 in serum at different time periods after operation (n = 5)

Group	IL-1β (pg/mL) *1h*	IL-1β (pg/mL) *5h*	IL-1β (pg/mL) *10h*
Control	10.45 ± 1.53	12.46 ± 2.73	14.86 ± 3.44
Model	24.76 ± 3.15[a]	28.44 ± 3.46[a]	32.41 ± 4.15[a]
Low dose	23.56 ± 2.74[ab]	20.17 ± 2.15[ab]	17.44 ± 2.81[ab]
Medium dose	21.36 ± 2.16[abc]	18.73 ± 4.51[abc]	16.77 ± 3.46[abc]
High dose	19.21 ± 3.31[abcd]	16.23 ± 2.56[abcd]	12.77 ± 2.56[abcd]

Data are mean ± SD; [a]$p < 0.05$, compared with the control group; [b]$p < 0.05$, compared with the model control group, [c]$p < 0.05$, compared with the low concentration group; [d]$p < 0.05$, compared with the medium concentration group

DISCUSSION

Pain is an unpleasant feeling that accompanies substantive or potential tissue injury. With advances in clinical technology, the mechanism of pain has become one of the areas of interest for medical scholars [10]. Injury to the body induces the release of a large number of inflammatory factors and cytokines which lead to different degrees of inflammatory reactions and production of pain [11]. The NK cells and T lymphocytes are produced by macrophages during stress, and they interact with many receptors in the CNS and promote the synthesis of pain mediators in nerve cells. They also promote the release of inflammatory factors such as IL-1 β and IL-6 [12].

Tumor necrosis factor alpha (TNF-α) is often used as an index for evaluating surgical and early traumatic tissue injuries [13]. Interleukins (ILs) are lymphoid factors which interact with leukocytes and immune cells. They transmit information, regulate the activation of immune cells, and also mediate the proliferation and differentiation of T cells and B cells, which are important in inflammatory response, among which IL-1 β and IL-6 are the most typical.

Table 5: Serum levels of β-endorphin in rats at different times after operation (n = 15)

Group	β-endorphin (pg/mg) *1h*	β-endorphin (pg/mg) *5h*	β-endorphin (pg/mg) *10h*
Control	23.45 ± 5.76	24.89 ± 6.43	26.49 ± 7.81
Model	27.89 ± 5.45[a]	15.49 ± 3.86[a]	13.48 ± 3.41[a]
Low dose	23.49 ± 4.16[ab]	16.46 ± 3.45[a]	21.46 ± 5.22[ab]
Medium dose	20.26 ± 4.29[abc]	23.34 ± 4.56[abc]	26.98 ± 7.58[abc]
High dose	17.98 ± 7.44[abcd]	27.48 ± 5.23[abcd]	27.84 ± 6.48[abcd]

Data are mean ± SD. [a]$p < 0.05$, vs control; [b]$p < 0.05$, vs model, [c]$p < 0.05$, vs low dose group; [d]$p < 0.05$, vs medium concentration group

Table 6: Levels of β-endorphin in spinal cord of rats at different times after operation

Group	β-endorphin (pg/mg) *1h*	β-endorphin (pg/mg) *5h*	β-endorphin (pg/mg) *10h*
Control	0.54 ± 0.12	0.55 ± 0.09	0.53 ± 0.04
Model	0.56 ± 0.13	0.42 ± 0.08[a]	0.38 ± 0.02[a]
Low dose	0.63 ± 0.21[ab]	0.65 ± 0.16[ab]	0.68 ± 0.15[ab]
Medium dose	0.78 ± 0.23[abc]	0.81 ± 0.18[abc]	0.79 ± 0.22[abc]
High dose	0.88 ± 0.25[abcd]	0.89 ± 0.21[abcd]	0.92 ± 0.12[abcd]

Data are mean ± SD. [a]$p < 0.05$, vs control; [b]$p < 0.05$, vs model, [c]$p < 0.05$, vs low dose group; [d]$p < 0.05$, vs medium concentration group

It has been reported that IL-1 β and IL-6 regulate the conduction velocity of pain signal from spinal cord to the brain by acting on pain nerve neurons [14].

In this study, there was no significant difference in the basic value of PMWT among the three groups. The PMWT of the model control group was significantly lower than that of the control group at each time point, while the PMWT of the treatment group was significantly higher than that of the model control group at each time point The serum levels of the measured cytokines were higher in model rats than in control at each time point, and increased with time. However, serum cytokine levels were markedly reduced in the treatment groups, relative to model rats, and decreased along with time and dose gain. These results suggest that FA inhibits hyperalgesia, has obvious analgesic effect, reduces the levels of inflammatory factors, and inhibits inflammation in a dose-dependent fashion.

β-Endorphin, an important inhibitory neurotransmitter in the pain regulation pathway, significantly inhibits the effect of the sensory neurotransmitter substance P. Deficiency of β-endorphin may lead to a certain degree of hyperalgesia [15]. In this study, changes in β-endorphin levels in serum and spinal cord of rats were determined. β-Endorphin levels differed markedly between rats in model and control groups. In the treatment group, the level of serum β-endorphin decreased with increase in FA dose at 1 h, but increased with increase in dose at 5 h and 10 h. In the spinal cord, β-endorphin levels in the model control group were significantly lower than those in the control group at 5 and 10 h, while β-endorphin levels in the treatment group were significantly higher than those in the model control group, and increased with increase in FA dose. Thus, the analgesic effect of FA may be related to increases in β-endorphin level, which is consistent with the report of Luan Yuanhang [16].

CONCLUSION

Pre-administration of FA produces an analgesic effect by lowering serum levels of inflammatory factors, inhibition of inflammatory response, enhancement of anti-inflammatory immune response, reduction of the risk of infection, and upregulation of the expression of β-endorphin.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents. The study was conceived and designed by Nannan Ye; Xiaomin Ye, Xianhua Ye, Nannan Ye collected and analyzed the data; Xiaomin Ye wrote the text. All authors read and approved the manuscript for publication.

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