

Effects of Morphine, Fentanyl and Tramadol on Human Immune Response

LIU Zhihen (刘志恒)^{1,2}, GAO Feng (高峰)¹, TIAN Yuke (田玉科)^{1#}

¹ Department of Anesthesiology, Tongji Hospital, Huazhong University of Science and Technology, Wuhan 430030, China

² Department of Anesthesiology, The Second Hospital in Shenzhen, Shenzhen 518035, China

Summary: Morphine has been reported to suppress human immune response. We aimed to observe the effects of morphine, fentanyl and tramadol on NF- κ B and IL-2 from both laboratory and clinical perspective. Jurkat cells were incubated with ten times clinically relevant concentrations of morphine, fentanyl and tramadol before being stimulated with PMA. NF- κ B binding activity and IL-2 levels were measured. In the clinical study, 150 consenting patients were randomized into 3 groups according to the analgesics used in them, namely, group morphine (M), group fentanyl (F) and group tramadol (T). IL-2 was measured preoperatively and 1, 3 and 24 h after operation. Consequently, NF- κ B activation was suppressed by morphine and fentanyl but not by tramadol. IL-2 was significantly decreased by morphine and fentanyl but not by tramadol *in vitro*. In the PCA patients, IL-2 was decreased in group M and increased in group F postoperatively. Whereas in group T, IL-2 was unchanged 1 h after operation but was significantly elevated 3 and 24 h after operation. Our results showed that the inhibition of morphine on IL-2 was most probably related to its suppression on NF- κ B. Fentanyl had different effects on human immune response *in vitro* and *in vivo*. Tramadol may have immune enhancing effect.

Key words: morphine; fentanyl; tramadol; NF- κ B; IL-2

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Morphine, fentanyl and tramadol are widely used in postoperative analgesia. Morphine has been reported to have immunosuppressive effect both *in vitro* and *in vivo*^[1, 2]. In recent years, fentanyl and tramadol are becoming increasingly popular in postoperative patient-controlled analgesia (PCA). Their immuno-modulatory effects are still unclear. Nuclear factor kappa B (NF- κ B) is an important transcription factor, which trans-activates hundreds of immune-related genes and has been named the "central mediator of immune response"^[3]. Therefore, the aim of the present study is to study the effects of morphine, fentanyl and tramadol on NF- κ B and IL-2 in a human T lymphoma cell line, Jurkat cells, and in PCA patients, in order to evaluate the role of these drugs on human immune response and the possible mechanisms of their actions.

1 MATERIALS AND METHODS

1.1 Approval and Consent Obtained

The study was approved by the ethics committee of the Tongji Hospital, affiliated to Huazhong University of Science and Technology (Wuhan, China) and conducted according to the principles of the Helsinki Declaration. An informed, written consent was obtained from each of the clinical patients included in this study.

1.2 Cell Culture and Incubation

Jurkat T cells were incubated in RPMI 1640 complete medium. The cells were diluted into $1.5-2 \times 10^5$ /mL and put in a 37 °C humidified environment containing 5

% CO₂ overnight. Ten times clinically relevant concentrations of morphine (38 mg/mL), fentanyl (20 ng/mL) and tramadol (7.5 mg/mL) were incubated with Jurkat cells for 2 h. One hour before the harvesting of the cells, 0.25 μ g/mL phorbol-12-myristate-13-acetate (PMA) were added to induce NF- κ B activation. The untreated cells were used as negative control and the cells, which were subjected to only PMA, were used as positive control. Then the cells were centrifuged at 1500 r/min for 5 min. The cell pellets were collected for intracellular protein extraction and the supernatant were kept for IL-2 evaluation.

1.3 Total Cell Extracts

The intracellular cell extract were prepared as we had described previously^[4]. The pellet was lysed in 50 mL of ice cold high salt detergent Totex buffer (20 mmol/L Hepes, 350 mmol/L NaCl, 1 mmol/L MgCl₂, 0.5 mmol/L EDTA, 0.1 mmol/L EGTA, 1 % NP-40, 20 % glycerol), with freshly added protease inhibitors: 0.1 % PMSF, 0.5 mmol/L DTT, 1 % aprotinin. The mixture was incubated on ice for 30 min, and finally centrifuged at 13000 r/min for 5 min in a pre-cooled microfuge at 4 °C. The supernatant containing total cell extracts was immediately frozen in liquid nitrogen and stored at -80 °C. The protein concentration of the extracts was measured by using the Bradford-Assay system (New England Biolabs, Germany).

1.4 Electrophoretic Mobility Shift Assay (EMSA)

The NF- κ B oligonucleotide was labeled with γ -[³²P]ATP by T₄ polynucleotide kinase (PNK). The labeled oligonucleotide was desalted using MicroSpin™ G-25 columns. The NF- κ B binding reaction was performed in a volume of 14 mL, containing 20 mg bovine serum albumin, 2 mg poly-deoxy-inosinic-deoxy-cytidy-

LIU Zhiheng, female, born in 1971, M.D., Ph.D.

Corresponding author

lic acid, 2 mL buffer D+(20 mmol/L Hepes, 20 % glycerol, 100 mmol/L KCl, 0.5 mmol/L EDTA, 0.25 % NP-40, 20 mmol/L DTT, 0.1 % PMSF), 4 mL 5×Ficoll buffer (20 % Ficoll-400, 300 mmol/L KCl, 100 mmol/L Hepes, 100 mmol/L DTT, 0.1 % PMSF), 4 mL ddH₂O, 20 mg total cell protein and 1 mL of labeled oligonucleotide at room temperature for 30 min. The electrophoresis was run at 4 % non-denaturing polyacrylamide gels in 0.5×TBE (90 mmol/L Tris-HCl, 90 mmol/L boric acid, 2 mmol/L EDTA) at 230 V for 1.5 to 2 h. The gels were dried with a gel drier at 80 °C for 30 min, then exposed to X-ray films at -80 °C for 5-6 h.

1.5 Clinical Research Protocol

The subjects were 150 patients scheduled for upper abdominal surgery under general anesthesia. Patients with immune disorders, history of drug or alcohol abuse or inability to cooperate with investigation were excluded. The volunteers were induced with midazolam, propofol, fentanyl and vecuronium. Anesthesia was maintained with isoflurane or enflurane. Postoperative analgesic infusion began at skin incision and was infused continuously for 24 h. All patients were randomized into three treatment groups according to the analgesics used in patient controlled analgesia (PCA): group morphine (M), group fentanyl (F) and group tramadol (T). In group M, patients were connected to PCA pumps filling with 0.1 % morphine and 0.01 % droperidol. The pumps were set with a loading dose of 0.075 mL/kg, a bolus of 0.5 mL, background infusion of 1 mL/h, lockout interval of 10 min, and a daily maximal dose of 50 mL. In group F, PCA pumps were filled with 0.002 % fentanyl and 0.01 % droperidol. The pumps were set with a loading dose of 0.1 mL/kg, a bolus of 1 mL, background infusion of 1 mL/h, lockout interval of 5 min, and a daily maximal dose of 100 mL. In group T, PCA pumps were filled with 1% tramadol and 0.01 % droperidol. The pumps were set with a loading dose of 0.15 mL/kg, a bolus of 1 mL, background infusion of 1 mL/h, lockout interval of 5 min, and a daily maximal dose of 60 mL. Fifty µg fentanyl was administered when satisfactory analgesic effect could not be obtained. Venous blood is drawn before operation and 1, 3, 24 h after commencement of analgesic administration. Serum was extracted from the whole blood immediately and stored at -20 °C until analysis. The items recorded included VAS, effective purges, analgesic consumption and MAP, HR, respiratory rate, SpO₂ at each time point. The complication, such as nausea, vomit, pruritus, respiratory depression, hypotension, sedation and extrapyramidal reaction, were also recorded.

1.6 Enzyme Linked Immuno-Sorbent Assay (ELISA)

The IL-2 concentration in 100 mL supernatant obtained previously and in serum of patients was measured using ELISA (the kits from R&D Systems, USA). The ELISA was performed according to the manufacturer's recommendation. Each experiment was repeated five times independently.

1.7 Statistical Analysis

Data were statistically assessed by using an ANOVA with post hoc test. Differences were considered significant at $P < 0.05$. All statistical tests were performed by using SPSS11.0 software package.

2 RESULTS

Effects of the three analgesics on NF- κ B in Jurkat cells are shown in fig. 1. Stimulation of Jurkat cells with PMA could induce NF- κ B DNA binding activity (fig. 1, lane 2). Pretreatment with morphine or fentanyl completely abolished the PMA-induced NF- κ B activation (fig. 1, lanes 3 and 4). NF- κ B activation was not affected by pretreatment with tramadol (fig. 1, lane 5).

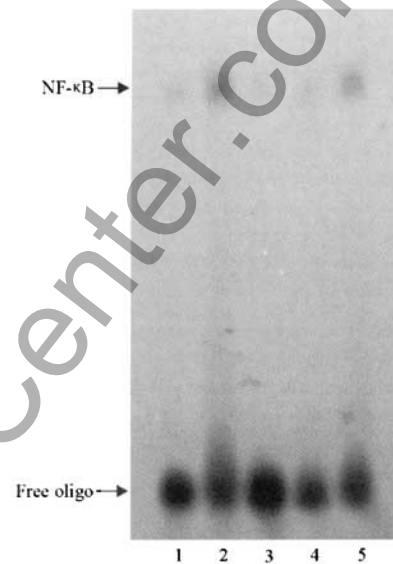


Fig. 1 Morphine and fentanyl inhibited NF- κ B but tramadol did not.

Lane 1, untreated Jurkat cells (negative control); lane 2, PMA (positive control); lane 3, morphine + PMA; lane 4, fentanyl + PMA; lane 5, tramadol + PMA

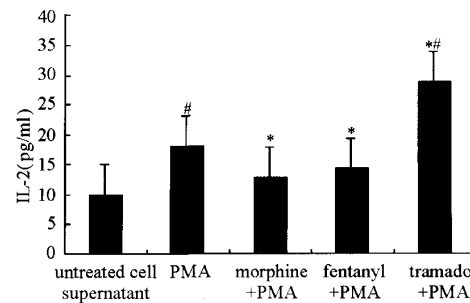


Fig. 2 Effects of morphine, fentanyl and tramadol on IL-2

Lane 1, untreated cell supernatant (negative control); lane 2, PMA (positive control); lane 3, morphine + PMA; lane 4, fentanyl + PMA; lane 5, tramadol + PMA.

$P < 0.05$ compared with negative control, * $P < 0.05$ comparing to PMA group

The changes of IL-2 level in Jurkat cells are described in fig. 2. In untreated cells the IL-2 level was 9.98 ± 2.36 pg/mL. Induction by PMA could increase the IL-2 level to 17.85 ± 5.55 pg/mL. Pretreatment with morphine and fentanyl significantly decreased IL-2 to 12.61 ± 3.78 pg/mL and 14.20 ± 4.91 pg/mL ($P < 0.05$). In contrast, pretreatment with tramadol could increase the secretion of IL-2 to 18.62 ± 5.56 pg/mL ($P < 0.05$).

Patient characteristics were similar among the three groups. No statistical differences were observed in terms of age, gender, weight, the duration of surgery, MAP, HR, RR and SpO₂. No patient needed blood transfusion during or after surgery.

The consumptions of morphine, fentanyl and tramadol were 37.0 ± 8.1 mg, 451.7 ± 113.0 μ g and 534.7 ± 112.9 mg, respectively. Four patients in Group T needed fentanyl (50 μ g) 1 h after operation to achieve a satisfactory analgesic effect. The VAS value and need

for bolus doses were higher in group T than in the other groups, but statistical significance existed between them ($P > 0.05$).

As shown in table 1, serum levels of IL-2 were not different between the three groups before operation. In group M, IL-2 level decreased at 1 h after operation ($P < 0.05$), it was even lower 3 h after operation ($P < 0.01$) and rose after 24 h, but still lower than the pre-anesthesia level ($P < 0.05$). In group F, IL-2 level was increased at all time points after operation ($P < 0.05$). In group T, IL-2 level was not changed 1 h after operation and increased 3 and 24 h after operation ($P < 0.05$).

In group M, one patient suffered from pruritus. The incidence of nausea and vomiting was lower in group F and group T than in group M (data not shown). No other complications, such as respiratory depression, hypotension, sedation and extrapyramidal reactions, were observed in the patients.

Table 1 Changes of IL-2 level of patients in the three groups (pg/ml, $\bar{x} \pm s$)

Group	Preoperation	1 h after operation	3 h after operation	24 h after operation
M	12.28 \pm 8.20	9.61 \pm 7.02*	8.20 \pm 4.41*	9.82 \pm 5.26*
F	11.29 \pm 9.78	16.43 \pm 14.67*	17.23 \pm 8.85*	16.55 \pm 12.93*
T	11.73 \pm 7.11	10.94 \pm 7.56	14.50 \pm 7.90*	17.02 \pm 11.73*

* $P < 0.05$ as compared to preoperation

3 DISCUSSION

Since surgical stress induces immune dysfunction, and postoperative analgesics can be used for a considerable period, the search for analgesic drugs devoid of immunosuppressive effects is of great importance. In the recent years, opioids and opioid peptides have been shown by numerous laboratories to modulate various cytokines or proteins of the immune response, like transcription factors, that might be involved^[5,6]. In the present study, we demonstrated that morphine, fentanyl and tramadol had different effects not only on IL-2, an immunomodulatory and immunotherapeutic cytokine, but also on NF- κ B, a central mediator of human immune response^[3].

A large variety of stimuli were reported to activate NF- κ B. In turn, the transcription factor regulates the expression of inflammatory cytokines, chemokines, immunoreceptors and cell adhesion molecules^[2]. NF- κ B is maintained in an inactive form by sequestration in cytoplasm through interaction with I κ B protein, a group of inhibitory molecules. Activation of NF- κ B requires phosphorylation, ubiquitination and proteolytic degradation of I κ B protein, which then permits translocation of the heterodimer to the nucleus and the subsequent transcriptional activation of target gene^[7]. Many research groups focused on the effects of different medicines on NF- κ B with its signaling pathway so as to evaluate the immunological effect of the drugs^[8].

It has been widely reported that morphine has immunosuppressive effect. Roy *et al* reported that at micromolar concentrations morphine inhibits LPS-induced

synthesis of IL-6 and TNF- α in microphages. Expression of the genes for both these cytokines is dependent on the activation of NF- κ B. They further demonstrated that pretreatment with micromolar morphine led to a significant decrease in NF- κ B activation^[5]. Another group showed that in human neutrophils and monocytes, morphine inhibited NF- κ B nuclear binding activity by a nitric oxide-dependent mechanism^[6]. We based our study on a human T lymphoma cell line, Jurkat cells. From the data above, it may be inferred that in different cell types of human immune system, morphine suppressed transcription factor NF- κ B with its down-stream effects, which may explain the immune-inhibitory function of morphine that has been noted.

In our study, the fentanyl-caused immune alterations *in vitro* seemed to be different from that *in vivo*. Actually this topic is still controversial. *In vitro* fentanyl has been reported to suppress cytokine production by T cells and macrophages, along with the inhibition on T cell cytotoxic activity, B cell proliferation and NK cell activity^[9]. On the contrary, previous studies have also documented an immuno-enhancing effect of fentanyl when used *in vivo*. For example, intravenous fentanyl was reported to increase NK cell cytotoxicity and circulatory CD16(+) lymphocytes in humans^[10]. The causes underlying this difference remains to be studied.

Tramadol is a centrally acting analgesic drug with a dual mechanisms of action: binding to m-opioid receptors and potentiation of monoadrenergic systems^[11]. Many authors reported an enhancing effect of tramadol on the immune activity, both *in vitro* and *in vivo*. For example, after subcutaneous administration tramadol significantly enhanced nature killer activity, IL-2 produc-

tion and splenocyte proliferation in mice^[12]. In postoperative patients who were given tramadol analgesia after abdominal surgery, the NK cell activity was also enhanced^[13]. In the present study, tramadol has been shown to promote IL-2 secretion both *in vitro* and *in vivo*. The NF- κ B activation was not influenced by tramadol. Early studies have provided evidence for heterogeneity of sympathetic nerve system regulation of T- and B-lymphocyte function^[14] and the presence of influence of non-adrenergic pathways on the immune system^[15]. The question of whether the tramadol-elicited IL-2 augmentation is associated with its monoadrenergic effect has not been answered by our study.

The two analgesics, which have shown NF- κ B inhibitory effect, morphine and fentanyl, are both classical opioid receptor agonists. It can be inferred that the classical opioid receptor might be involved in the modulation of NF- κ B, by opioids. However, it has been proved that the NF- κ B inhibition by morphine was not reversed by naloxone^[5]. Although m- and k-opioid receptors are reported to be involved in regulation of lymphoid cell production of antibodies^[16], there is no sufficient evidence to show the relationship between classical opioid receptor and the NF- κ B signaling pathway.

In conclusion, morphine, fentanyl and tramadol showed comparable analgesic activity in postoperative patients. The immunosuppression of morphine, at least partly, through NF- κ B pathway, made it unfavorable for postoperative analgesia. Fentanyl had a different effect on the immune response as seen *in vitro* and *in vivo* studies. Tramadol demonstrated immune enhancement both *in vitro* and *in vivo*. Therefore, tramadol and fentanyl may be preferred to morphine for the treatment of postoperative pain from the immunological point of view.

REFERENCES

- 1 Malik A A, Radhakrishnan N, Reddy K *et al*. Morphine-induced macrophage apoptosis modulates migration of macrophages: use of *in vitro* model of urinary tract infection. *J Endourol*, 2002,16(8):605-10
- 2 Gaveriaux-Ruff C, Matthes H W, Peluso J *et al*. Abolition of morphine-immunosuppression in mice lacking the mu-opioid receptor gene. *Proc Natl Acad Sci U S A*. 1998, 26;95(11):6326-30
- 3 Pahl H L. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene*, 1999, 22:18(49):6853-66
- 4 Loop T, Liu Z, Humar M *et al*. Thiopental inhibits the activation of nuclear factor kappaB. *Anesthesiology*. 2002 May;96(5):1202-13
- 5 Roy S, Cain K J, Chapin R B *et al*. Morphine modulates NF kappa B activation in macrophages. *Biochem Biophys Res Commun*, 1998,245(2):392-6
- 6 Welters I D, Menzebach A, Goumon Y *et al*. Morphine inhibits NF-kappaB nuclear binding in human neutrophils and monocytes by a nitric oxide-dependent mechanism. *Anesthesiology*, 2000,92(6):1677-84
- 7 Ghosh S. Regulation of inducible gene expression by the transcription factor NF-kappaB. *Immunol Res*, 1999, 19 (2-3):183-9
- 8 Yamamoto Y, Gaynor R B. Therapeutic potential of inhibition of the NF-kappaB pathway in the treatment of inflammation and cancer. *J Clin Invest*, 2001,107(2):135-42
- 9 House R V, Thomas P T, Bhargava H N. *In vitro* evaluation of fentanyl and meperidine for immunomodulatory activity. *Immunol Lett*, 1995,46(1-2):117-24
- 10 Yeager M P, Procopio M A, DeLeo J A *et al*. Intravenous fentanyl increases natural killer cell cytotoxicity and circulating CD16(+) lymphocytes in humans. *Anesth Analg*, 2002,94(1):94-9
- 11 Magrini M, Rivolta G, Bolis C *et al*. Analgesic activity of tramadol and pentazocine in postoperative pain. *Int J Clin Pharmacol Res*. 1998;18(2):87-92
- 12 Sacerdote P, Bianchi M, Manfredi B *et al*. Effects of tramadol on immune responses and nociceptive thresholds in mice. *Pain*, 1997,72: 325-330
- 13 Sacerdote P, Bianchi M, Gaspani L *et al*. The effects of tramadol and morphine on immune responses and pain after surgery in cancer patients. *Anesth Analg*, 2000, 90(6):1411-4
- 14 Livnat S, Felten S Y, Carlson S L *et al*. Involvement of peripheral and central catecholamine systems in neural-immune interactions. *J Neuroimmunol*, 1985, 10(1): 5-30
- 15 Madden K S, Moynihan J A, Brenner G J *et al*. Sympathetic nervous system modulation of the immune system. III. Alterations in T and B cell proliferation and differentiation *in vitro* following chemical sympathectomy. *J Neuroimmunol*, 1994,49(1-2):77-87
- 16 Taub D D, Eisenstein T K, Geller E B *et al*. Immunomodulatory activity of mu- and kappa-selective opioid agonists. *Proc Natl Acad Sci U S A*. 1991 Jan 15;88(2):360-4. avio N, Lai M M C. The hepatitis C virus persistence : how to evade the immune system? *J Biosci*, 2003, 28:287-304

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