Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Effect of tramadol on immune responses and nociceptive thresholds in a rat model of incisional pain*

Yong-min LIU, Sheng-mei ZHU†‡, Kui-rong WANG, Zhi-ying FENG, Qing-lian CHEN
(*Department of Anesthesiology, the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China*)
†E-mail: smzhu20088@yahoo.com.cn

Received Jan. 1, 2008; revision accepted May 10, 2008

Abstract: Objective: To evaluate the effects of tramadol on the proinflammatory responses in a rat model of incisional pain by investigating its effects on nociceptive thresholds and serum interleukin-6 (IL-6) and IL-2 levels. Methods: Forty-two male Sprague-Dawley (SD) rats scheduled for plantar incision were randomly divided into 7 groups (n=6 in each group). Rats in Group 1 receiving general anesthesia with no incision were served as control; At 30 min before skin incision, Groups 2~5 were given 5 ml normal saline or 1, 10, and 20 mg/kg tramadol, respectively, intraperitoneally (i.p.); Group 6 received 10 mg/kg tramadol after operation; Group 7 received 10 mg/kg tramadol before incision, followed by 200 μg/kg naloxone after operation. Mechanical allodynia was measured by electronic von Frey filament to evaluate the nociceptive thresholds 1 h before incision, and 1 h and 2 h after operation. Serum IL-6 and IL-2 levels were measured by enzyme-linked immunosorbent assay (ELISA) 2 h after operation. Results: Mechanical thresholds decreased significantly and serum IL-6 level increased significantly after operation in Group 2 compared with control (P<0.01), and these changes were reversed respectively by tramadol in a dose-dependent manner (P<0.05 and P<0.01, respectively). IL-2 level remained unchanged after operation in Group 2, but decreased in Group 3 (P<0.05), then gradually returned to the normal level in Groups 4 and 5. The intraperitoneally injected tramadol (10 and 20 mg/kg) produced a potent and dose-dependent antinocicptive effect on the lesioned paw. The antinocicptive effects of tramadol were partially antagonized by naloxone (200 μg/kg), suggesting an additional non-opioid mechanism. Conclusion: The results suggest that tramadol could be a good choice for the treatment of pain under the conditions that immunosuppression may be particularly contraindicated.

Key words: Tramadol, Pain, Mechanical allodynia, Interleukin-6 (IL-6), Interleukin-2 (IL-2), Rat
doi:10.1631/jzus.B0820039 **Document code:** A **CLC number:** R614

INTRODUCTION

Opioid analgesics are commonly used for the treatment of both acute (e.g., post-operative) and chronic pain, but some studies argued that they also cause suppression to immune system (Manfredi *et al*., 1993; Clark *et al*., 2007). Tramadol hydrochloride is a centrally acting analgesic with opioid and non-opioid like properties (Raffa *et al*., 1992; Kayser *et al*., 1992).

Cytokines play a pivotal role in coordination and regulation of immune responses (Sacerdote *et al*., 1997; 1999; 2000; Gaspani *et al*., 2002; Niemand *et al*., 2003). Surgical trauma and anesthesia are associated with a complex dysregulation of the immune system and with the activation of both proinflammatory and anti-inflammatory responses (Salo, 1992). Interleukin-1 (IL-1), tumor necrosis factor-α (TNF-α) and IL-6 have local and systemic effects that may limit injury and the spread of infection and provide a suitable environment for tissue healing and repair (Sheeran and Hall, 1997). However, the excessive activity of either proinflammatory or anti-inflammatory cytokines may cause injury to the patient or render the patient immunocompromised (Lin *et al*., 2000). Also, the principal immunologic deficit after trauma and major surgery decreases cell-mediated

‡ Corresponding author
* Project (No.2006A031) supported by the Medical Foundation Insurance of Zhejiang Health Bureau, China

immunity from an impaired natural killer (NK) cell response and T helper 1 (T_{H1}) lymphocyte development, which probably results in preferential T helper 2 (T_{H2}) development (Sheeran and Hall, 1997). IL-2 is crucial to the development of T_{H1} subset of lymphocytes responsible for cell-mediated immunity (Sheeran and Hall, 1997).

It is possible that choice of a drug modulates beneficial immune responses in the perioperative period. It has been demonstrated that tramadol can contribute to beneficial effects on immune functions in patients, namely, induce an improvement of postoperative immunosuppression and increase NK cell activity, lymphocyte proliferation and IL-2 production (Sacerdote *et al*., 1997; 1999; 2000). However, the mechanism of tramadol on modulating cytokine production is unknown.

On considering the dual mechanism of action of tramadol, we considered it of interest to evaluate the potential immunological effects of this drug. Thus, we investigated the antinociceptive effect of tramadol by mechanical stimulation test and evaluated its effects on proinflammatory responses by determining IL-2 and IL-6 production in a rat model of incisional pain. The reversal of the effect of tramadol on the nociceptive response by naloxone was also investigated.

MATERIALS AND METHODS

Animals and laboratory

The experiments were performed on 42 male Sprague-Dawley (SD) rats, weighing 150~250 g. The animals were allowed to habituate to the colony room for 1 week before beginning the experiment. The rats were housed at a constant room temperature of 22 °C with a 12-h alternating light-dark cycle. After the surgical procedure, rats were housed and isolated in a large cage and the floor was covered with sawdust in order to minimize the possibility of painful mechanical stimulation. Chow and water were available ad libitum and the operated incisional animals were able to eat and drink unaided. All protocols were approved by the Medical Faculty Ethnic Committee of Zhejiang University, China.

Experimental procedures

Rats were anesthetized with 300 mg/kg choral hydrate intraperitoneally (i.p.). As described previously (Brennan *et al*., 1996), a 1-cm longitudinal incision was made through skin and fascia of the plantar aspect of the left hind paw including the underlying muscles. The skin was sewed up with two mattress sutures and the wound was covered with iodine.

The rats were randomly divided into 7 groups (n=6 in each group) as shown in Table 1. The control group received a sham operation that consists of anesthesia and sterile preparation of the hind paw without incision. The 5 ml of saline was injected into the rats in Group 2 30 min before skin incision. Groups 3~5 were pretreated by intraperitoneal injection of tramadol (Grünenthal Medicine Co., Ltd., Aachen, Germany) 30 min before plantar incision. Group 6 was treated with 10 mg/kg tramadol postoperatively. In addition, Group 7 was treated with 200 μg/kg naloxone (Fourcicle Medicine Co., Ltd., Beijing, China) immediately after operation with 10 mg/kg tramadol pretreatment. The nociceptive thresholds were then determined at the same time periods.

Table 1 Study groups and drug schedules used for the experimental procedure

Group No.	n	Drug injection (i.p.)
1	6	No incision, baseline (control)
2	6	5 ml saline pretreatment
3	6	1 mg/kg tramadol pretreatment
4	6	10 mg/kg tramadol pretreatment
5	6	20 mg/kg tramadol pretreatment
6	6	10 mg/kg tramadol, postoperatively
7	6	10 mg/kg tramadol pretreatment, 200 μg/kg naloxone, postoperatively

Mechanical allodynia tests

Sensitivity to mechanical stimuli was assessed 1 h before incision, and 1 h and 2 h after operation, by using an electronic von Frey filament (IITC 2390 series electronic von Frey Anesthesiometer, IITC Life Science, Woodland Hills, USA). The rats were placed on a mesh-wire floor within individual plastic boxes, and were allowed to acclimate for 30 min for the first test before operation. The hairless plantar surface of the hind paw was probed by an electronic von Frey probe (ranging from 0.01~58 g). Each monofilament was applied with sufficient force to bend. In the presence of a response (indicated by a brisk withdrawal or flinching), the number of force presented indicated the mechanical pain threshold. Every measurement was performed 5 times on the same hind paw at 30 s intervals, and the response threshold was defined as the lowest force that caused at least 3 withdrawals out of the 5 consecutive applications.

Sample collection

Rats were quickly decapitated to collect trunk blood for assessment of IL-2 and IL-6 levels at 2 h after operation. Rats received anesthesia without incision were used as baseline. All blood samples were centrifuged at 3000 r/min for 10 min, and the separated sera were stored at −80 °C until assay.

Measurement of IL-6 and IL-2 levels by enzyme-linked immunosorbent assay (ELISA)

Serum IL-6 and IL-2 levels were measured by using a polyclonal ELISA kit (RapidBio Lab., Calabasas, California, USA) following the manufacturer's instructions. Briefly, the anti-IL-6 capture polyclonal antibody (pAb) was absorbed on a polystyrene 96-well plate and the IL-6 present in the sample was bound to the antibody coated wells. The biotinylated anti-IL-6 detecting pAb was added to bind the IL-6 captured by the first antibody. After washing, avidin-peroxidase (Sigma, USA) was added to the wells to detect the biotinylated detecting antibody and finally 2,2′-azino-bis(3-ethylbenthiazoline-6-sulfonic acid) (ABTS; Sigma, USA) substrate was added and a colored product was formed in proportion to the amount of IL-6 present in the sample, which was measured at optical density 405 nm (OD_{405}) with an ELISA microplate reader (model 450, Bio-Rad, Chicago, Illinois, USA). A standard curve was generated, and the IL-6 concentration (in pg/ml) of the samples was calculated. The measurement of IL-2 is similar to that of IL-6. All determinations were performed by full-time technical personnel.

Data analysis

Data were presented as mean±*SD*. All the statistical analyses were performed by using values expressed in grams. In the analyses of IL-6 and IL-2 production and analgesic responses, a one-way analysis of variance (ANOVA) was used, followed by Dunnet's test for multiple comparison. $P<0.05$ was considered to be significant.

RESULTS

Behavioral test

Before surgery the mechanical threshold did not show any significant differences between the hind paws of all rats [(49.60±5.1) g vs (48.11±6.2) g, n=42]. The mean threshold in Group 2 was markedly decreased to (16.4±2.46) g 1 h after the surgical procedure and to (3.01±0.7) g 2 h after operation on the incision side ($P<0.001$) compared with the control (Group 1). No significant changes in general behaviors of the animals were observed after injection of tramadol, except for an increase in mechanical threshold.

Pretreatment with 10 and 20 mg/kg tramadol (Groups 4 and 5, respectively) produced an overall significant antinociceptive effect on the lesioned paw early in 1 and 2 h after incision, compared with Group 2 ($P<0.05$ and $P<0.01$, respectively). Administration of 1 mg/kg tramadol before incision did not reverse the mechanical allodynia ($P>0.05$). Administration of 10 mg/kg tramadol immediately after operation (Group 6) also increased the mechanical threshold similar to pretreatment in the same dosage, compared with Group 2 ($P<0.05$). The results of the behavioral test are shown in Fig.1. In Group 7, the reversal of antinociceptive effect of tramadol was confirmed 2 h after operation (Fig.1). This effect was significantly greater than that in Group 2 ($P<0.05$), but less than that in Group 5 ($P<0.05$).

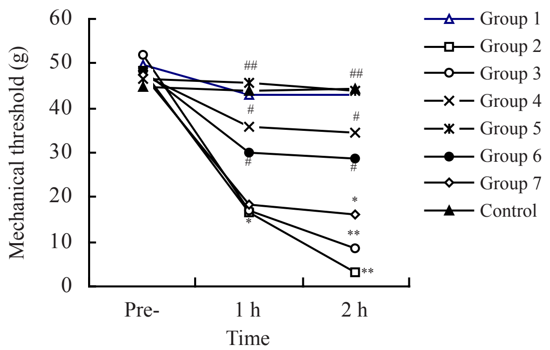

Fig.1 Behavioral tests for different groups

Group 1: no incision, baseline; Group 2: incision group, pretreatment with 5 ml normal saline; Group 3: pretreatment with 1 mg/kg tramadol; Group 4: pretreatment with 10 mg/kg tramadol; Group 5: pretreatment with 20 mg/kg tramadol; Group 6: 10 mg/kg tramadol, postoperatively; Group 7: pretreatment with 10 mg/kg tramadol, plus 200 μg/kg naloxone, postoperatively. Compared with baseline level, $^{*}P<0.05$, $^{**}P<0.01$; Compared with incision group, $^{\#}P<0.05$, $^{\#\#}P<0.01$

Immune responses

The immunostimulatory effect of tramadol was further confirmed by the increases of serum IL-6 and IL-2. Serum IL-6 levels were increased at 2 h after skin incision in Group 2, and reversed by tramadol pretreatment in a dose-dependent manner in Groups 4 and 5 (compared with Group 2, $P<0.05$ and $P<0.01$, respectively) but not in Group 6 (Fig.2). The treatment of tramadol after operation did not decrease the increased levels of IL-6 due to the incision ($P>0.05$). Attenuations of IL-6 production by tramadol pretreatment could not be reversed by naloxone in Group 7 ($P>0.05$).

Compared with baseline levels in Group 1, 2 h after operation the IL-2 levels remained unchanged in Group 2, decreased significantly in Group 3 ($P<0.05$) and then returned to baseline in Groups 4 and 5 ($P>0.05$, Fig.3). IL-2 levels were not elevated after the treatment with tramadol after operation. The effect of tramadol pretreatment on IL-2 was not reversed by naloxone ($P>0.05$).

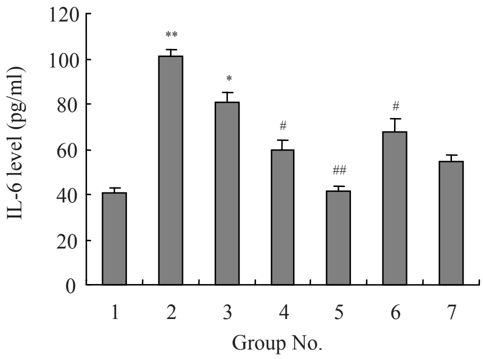

Fig.2 Serum IL-6 levels in different groups
Group 1: no incision, baseline; Group 2: incision group, pretreatment with 5 ml normal saline; Group 3: pretreatment with 1 mg/kg tramadol; Group 4: pretreatment with 10 mg/kg tramadol; Group 5: pretreatment with 20 mg/kg tramadol; Group 6: 10 mg/kg tramadol, postoperatively; Group 7: pretreatment with 10 mg/kg tramadol, plus 200 μg/kg naloxone, postoperatively. Compared with baseline level, $^{*}P<0.05$, $^{**}P<0.01$; Compared with incision group, $^{\#}P<0.05$, $^{\#\#}P<0.01$

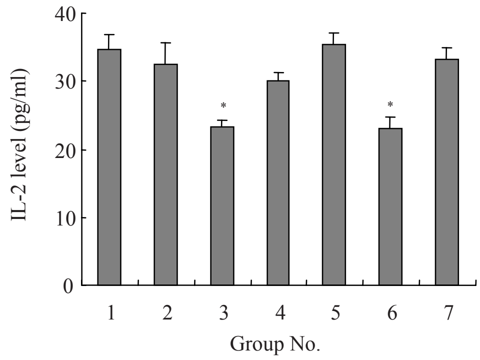

Fig.3 Serum IL-2 levels in different groups
Group 1: no incision, baseline; Group 2: incision group, pretreatment with 5 ml normal saline; Group 3: pretreatment with 1 mg/kg tramadol; Group 4: pretreatment with 10 mg/kg tramadol; Group 5: pretreatment with 20 mg/kg tramadol; Group 6: 10 mg/kg tramadol, postoperatively; Group 7: pretreatment with 10 mg/kg tramadol, plus 200 μg/kg naloxone, postoperatively. Compared with baseline level, $^{*}P<0.05$

DISCUSSION AND CONCLUSION

An experimental pain generated by plantar incision in the hind paw in a rat model may represent a useful model for surgical trauma. It has been clearly shown that allodynia and hyperalgesia to mechanical and thermal stimulations were achieved at maximum severity 2 h after surgery and lasted 2~4 d before spontaneous remission. The selected time point of 2 h postoperatively was found to be adequate for the assessment of analgesic drug properties on incisional pain (Brennan *et al*., 1996).

In the current investigation, using the mechanical threshold to the paw of rat, we clearly demonstrated that tramadol was able to attenuate mechanical pain-related disorders caused by plantar incision in SD rats. The analgesic effect of tramadol results in the activation of both pain inhibiting systems: the opioid and the descending monoaminergic systems. However, the mechanisms of this incisional pain alleviating effect have not been clearly documented. According to Apaydin *et al*.(2000) and Sacerdote *et al*.(1997), three different doses were administered in rats to evaluate the effect of tramadol on nociception and immune systems, and they found that tramadol can produce significant pain relief and suppress proinflammatory cytokine production like IL-6, but has no effect on IL-2 levels.

Evaluation of the opioid component of tramadol on the present incisional pain model

Many studies have indicated that opioids are effective for the treatment of postoperative pain and that incisional pain has been classified as opioid sensitive (St A Stewart and Martin, 2003; Whiteside *et al*., 2004; Clark *et al*., 2007). In the present study, we found that the effect of tramadol was not completely blocked by 200 μg/kg opioid antagonist naloxone, suggesting that tramadol-induced antinociception may be partially mediated via opioid receptors.

Evaluation of the non-opioid component of tramadol on the present incisional pain model

Tramadol, in addition to its affinity to opioid receptors, inhibits the neuronal uptake of 5-HT (5-hydroxytryptamine) and NA (noradrenaline) (Berrocoso *et al*., 2007; Oliva *et al*., 2002; Reimann and Hennies, 1994). Because compounds known to block monoamine uptake potentially have the antinociceptive effects of opioid including tramadol, the antinociceptive potency and profile of tramadol may derive from its combined opioid binding activity and inhibition of monoamine uptake (Sacerdote *et al*., 1997). Tricyclic antidepressants have been also demonstrated to exhibit the modest activity against neuropathic pain after systemic administration due to monoamine reuptake inhibition (Lynch, 2001). There is evidence that, in tramadol, opioid and non-opioid mechanisms act synergistically with respect to analgesia.

Modulation of cytokine production by tramadol

The immune system and different cytokines could be influenced by surgery (Salo, 1992; Hensler *et al*., 1997). In previous studies, it has been shown that the increase in concentration of IL-6 correlates well with operating time, severity of sepsis, and clinical complications after surgery (Damas *et al*., 1992). However, little is known about the IL-2 changes in response to surgery.

IL-6, along with its proinflammatory effects, is a sensitive and early marker of tissue damage, and its magnitude of elevation is directly related to the extent of surgical trauma (Nagahiro *et al*., 2001; Raeburn *et al*., 2002). Yim *et al*.(2000) found that plasma IL-6 was elevated after conventional lobectomy for clinical early-stage lung cancer. The present study shows that IL-6 increased significantly 2 h postoperatively in incision group. This may reflect the proinflammatory activities in response to surgical trauma.

IL-2 is a growth factor for T cells, NK cells, and lymphokine-activated killer cells (Raeburn *et al*., 2002). In normal conditions, immunologic response does not show IL-2 within circulation. Previous studies showed that IL-2 inhibited nociceptive responses of spinal dorsal horn neurons (Song and Zhao, 2000). IL-2 also exerts notable analgesic effect in the peripheral nervous system (Song *et al*., 2002a). The present results also reveal that IL-2 remained unchanged postoperatively in Group 2, suggesting that surgical trauma alone does not affect the IL-2 production.

Tramadol has beneficial effects on immune functions, but has no effect on IL-6 level in clinical cases and the mechanism involved is not well demonstrated. A wide literature has described the suppressive effects of morphine and endogenous opioids on many immune functions due to the activation of central μ-opiate receptors, including lymphocyte proliferation, IL-2 production and NK activity (Manfredi *et al*., 1993; Peterson *et al*., 1993; Panerai *et al*., 1995). The present results suggest that tramadol has some beneficial effects on plantar incisional pain conditions in rats, e.g., suppression of IL-6 production. Tramadol did not interfere with the IL-2 levels, although in low dose (1 mg/kg) it down-regulated the IL-2 production, suggesting that it may not attenuate, to some extent, an impaired immune response in plantar incision and clinically may have a beneficial role in immunomodulation after surgery in cancer patients (Song *et al*., 2002b).

There is no direct evidence that opioids modulate IL-6 response to surgery, but it has been hypothesized that alfentanil acts on opioid receptors, leading to a reduction in intracellular cyclic adenosine monophosphate which is associated with inhibition of IL-6 synthesis (McBride *et al*., 1996). The present results indicate that IL-6 levels were significantly increased 2 h after incision in Group 2 and reversed by tramadol in a dose-dependent manner. The attenuations of IL-6 production by tramadol could not be significantly reversed by naloxone, suggesting that the serum IL-6 response could be influenced significantly by tramadol. It is consistent with the fact that tramadol is a weak μ-opioid agonist (6000-fold less than morphine) (Raffa, 1996).

Postoperative treatment with tramadol made the changes of IL-2 similar to low dose of tramadol pretreatment. The decrease of IL-2 production in Group 3 treated with 1 mg/kg tramadol was due to insufficient pain relief or immunosuppression mediated via μ-receptor. This was confirmed by several experiments

(Sacerdote *et al*., 1997; 1999; Gaspani *et al*., 2002). It showed that IL-2 and morphine exerted similar effects in various aspects by decreasing intracellular adenosine 3',5'-cyclic phosphate (cAMP) content, modulating neuroendocrine activity, suppressing afferent sensory transmission and serving as Ca^{2+} channel blockers (Plata-Salamán and Ffrench-Mullen, 1993; Song *et al*., 2002a). It also revealed that IL-2-induced antinociception is partially mediated by μ-opioid receptors (Song *et al*., 2002b). Therefore, the present findings suggest that the decrease in serum IL-2 with low dose of tramadol in rats partly contributed to the affinity to μ-receptor of tramadol and postoperative depression in cell-mediated immune responses, but with higher dose the modulation of immunity would be obvious.

Naloxone as a μ-opioid receptor antagonist has been found to interfere with some functions of IL-2 (de Sarro and Nisticò, 1990). In the present study, naloxone markedly decreased tramadol-induced antinociception but did not affect the IL-6 and IL-2 productions, suggesting the involvement of μ-opioid receptor in the process of antinociception but not the pro-inflammatory and anti-inflammatory actions. Therefore, it is suggested that some molecules in addition to μ-opioid receptors might be responsible for the antinociceptive effect of tramadol and cytokine production.

It is noteworthy that the antinociceptive effects of tramadol are mediated not only via an opioid mechanism, but also mainly via a separate, non-opioid mechanism, due to the inhibition of neuronal uptake of noradrenaline and serotonin (Kayser *et al*., 1992; Raffa *et al*., 1992). Similarly, drugs which increase serotoninergic tone such as D-fenfluoramine (Clancy and Lorens, 1996) and fluoxetine (El-Nour *et al*., 2007), stimulate immune function in rodents. Also, in vitro, pretreatment with the selective serotonin reuptake inhibitor fluvoxamine, the relatively selective noradrenaline reuptake inhibitor reboxetine, or the non-selective monoaminergic reuptake inhibitor imipramine, significantly inhibited the IFN (interferon)-γ-induced IL-6 and NO production in a dose-dependent manner in microglia (Maes, 2001). In depressed patients, prolonged treatment with antidepressants normalizes the symtomes and reduces the increased serum IL-6 levels (Hashioka *et al*., 2007). Therefore, the activation of serotoninergic system might be involved in the immune effects induced by the administration of tramadol. Further investigation is needed to evaluate the serotonergic mechanism involved in the analgesia of tramadol.

Although the specific mechanism responsible for the effects of IL-6 and IL-2 on pain inhibition is unclear, several factors may contribute to our findings. First, the decrease of IL-2 production treated with low dose of tramadol may be due to its affinity to μ-receptor, while with higher dose the non-opioid mechanism may play a key role in immunomodulation by tramadol. Second, the effects of tramadol on cytokine production may be mainly attributed to the intrinsic immunomodulatory properties of tramadol taken perioperatively, due to the serotonergic descending inhibitory system, including the increase of the serotonergic tone that has been usually associated with stimulation of lymphocyte proliferation, increase of IL-2 production and decrease of IL-6 production, can help to attenuate postoperative immunosuppression (Sacerdote *et al*., 1997; 1999; 2000; Gaspani *et al*., 2002; Wang *et al*., 2003). In short, the antinociceptive mechanism of tramadol combined with the opioid and serotonergic system may play an important role in immune responses.

In conclusion, intraperitoneal administration of tramadol can produce antinociceptive effects on incisional pain in rats. Tramadol was associated with decreased IL-6 and unchanged IL-2 levels, suggesting that it may suppress the inflammation induced by incision and has a beneficial role in the modulation of IL-2 associated with cell-mediated immunity.

References

Apaydin, S., Uyar, M., Karabay, N.U., Erhan, E., Yegul, I., Tuglular, I., 2000. The antinociceptive effect of tramadol on a model of neuropathic pain in rats. *Life Sci*., **66**(17): 1627-1637. [doi:10.1016/S0024-3205(00)00482-3]

Berrocoso, E., de Benito, M.D., Mico, J.A., 2007. Role of serotonin 5-HT1A and opioid receptors in the antiallodynic effect of tramadol in the chronic constriction injury model of neuropathic pain in rats. *Psychopharmacology* (*Berl*.), **193**(1):97-105. [doi:10.1007/s00213-007-0761-8]

Brennan, T.J., Vandermeulen, E.P., Gebhart, G.F., 1996. Characterization of a rat model of incisional pain. *Pain*, **64**(3):493-501. [doi:10.1016/0304-3959(95)01441-1]

Clancy, J., Lorens, S.Jr., 1996. Subchronic and chronic exposure to D-fenfluramine dose-dependently enhances splenic immune functions in young and old male Fischer-344 rats. *Behav. Brain Res.*, **73**(1-2):355-358. [doi:10.1016/0166-4328(96)00114-3]

Clark, J.D., Shi, X., Li, X., Qiao, Y., Liang, D., Angst, M.S., Yeomans, D.C., 2007. Morphine reduces local cytokine expression and neutrophil infiltration after incision. *Mol. Pain*, **3**(1):28. [doi:10.1186/1744-8069-3-28]

Damas, P., Ledoux, D., Nys, M., Vrindts, Y., Groote, D.D., Franchimont, P., Lamy, M., 1992. Cytokine serum level during severe sepsis in human IL-6 as a marker of severity. *Ann. Surg.*, **215**(4):356-362.

de Sarro, G.B., Nisticò, G., 1990. Naloxone antagonizes behavioural and ECoG effects induced by systemic or intracerebral administration of some lymphokines. *Ann. Ist. Super. Sanita*, **26**(1):99-106.

El-Nour, H., Lundeberg, L., Abdel-Magid, N., Lonne-Rahm, S.B., Azmitia, E.C., Nordlind, K., 2007. Serotonergic mechanisms in human allergic contact dermatitis. *Acta Derm. Venereol.*, **87**(5):390-396. [doi:10.2340/00015555-0288]

Gaspani, L., Bianchi, M., Limiroli, E., Panerai, A.E., Sacerdote, P., 2002. The analgesic drug tramadol prevents the effect of surgery on natural killer cell activity and metastatic colonization in rats. *J. Neuroimmunol.*, **129**(1-2):18-24. [doi:10.1016/S0165-5728(02)00165-0]

Hashioka, S., Klegeris, A., Monji, A., Kato, T., Sawada, M., McGeer, P.L., Kanba, S., 2007. Antidepressants inhibit interferon-gamma-induced microglial production of IL-6 and nitric oxide. *Exp. Neurol.*, **206**(1):33-42. [doi:10.1016/j.expneurol.2007.03.022]

Hensler, T., Hecker, H., Heeq, K., Heidecke, C.D., Bartels, H., Barthlen, W., Wagner, H., Siewert, J.R., Holzmann, B., 1997. Distinct mechanisms of immunosuppression as a consequence of major surgery. *Infect. Immun.*, **65**(6):2283-2291.

Kayser, V., Besson, J.M., Guilbaud, G., 1992. Evidence for a noradrenergic component in the antinociceptive effect of the analgesic agent tramadol in an animal model of clinical pain, the arthritic rat. *Eur. J. Pharmacol.*, **224**(1):83-88. [doi:10.1016/0014-2999(92)94822-D]

Lin, E., Calvano, S.E., Lowry, S.F., 2000. Inflammatory cytokines and cell response in surgery. *Surgery*, **127**(2):117-126. [doi:10.1067/msy.2000.101584]

Lynch, M.E., 2001. Antidepressants as analgesics: a review of randomized controlled trials. *J. Psychiatry Neurosci.*, **26**(1):30-36.

Maes, M., 2001. The immunoregulatory effects of antidepressants. *Hum. Psychopharmacol. Clin. Exp.*, **16**(1):95-103. [doi:10.1002/hup.191]

Manfredi, B., Sacerdote, P., Bianchi, M., Locatelli, L., Veljic-Radulovic, J., Panerai, A.E., 1993. Evidence for an opioid inhibitory effect on T cell proliferation. *J. Neuroimmunol.*, **44**(1):43-48. [doi:10.1016/0165-5728(93)90266-2]

McBride, W.T., Armstrong, M.A., McBride, S.J., 1996. Immunomodulation: an important concept in modern anaesthesia. *Anaesthesia*, **51**(5):465-473. [doi:10.1111/j.1365-2044.1996.tb07793.x]

Nagahiro, I., Andou, A., Aoe, M., Sano, Y., Date, H., Shimizu, N., 2001. Pulmonary function, postoperative pain, and serum cytokine level after lobectomy: a comparison of VATS and conventional procedure. *Ann. Thorac. Surg.*, **72**(2):362-365. [doi:10.1016/S0003-4975(01)02804-1]

Niemand, C., Nimmesgern, A., Haan, S., Fischer, P., Schaper, F., Rossaint, R., Heinrich, P.C., Müller-Newen, G., 2003. Activation of STAT3 by IL-6 and IL-10 in primary human macrophages is differentially modulated by suppressor of cytokine signaling 3. *J. Immunol.*, **170**(6):3263-3272.

Oliva, P., Aurilio, C., Massimo, F., Grella, A., Maione, S., Grella, E., Scafuro, M., Rossi, F., Berrino, L., 2002. The antinociceptive effect of tramadol in the formalin test is mediated by the serotonergic component. *Eur. J. Pharmacol.*, **445**(3):179-185. [doi:10.1016/S0014-2999(02)01647-3]

Panerai, A.E., Manfredi, B., Granucci, F., Sacerdote, P., 1995. The beta-endorphin inhibition of mitogen-induced splenocytes proliferation is mediated by central and peripheral paracrine/autocrine effects of the opioid. *J. Neuroimmunol.*, **58**(1):71-76. [doi:10.1016/0165-5728(94)00189-U]

Peterson, P.K., Molitor, T.W., Chao, C.C., 1993. Mechanisms of morphine-induced immunomodulation. *Biochem. Pharmacol.*, **46**(3):343-348. [doi:10.1016/0006-2952(93)90508-T]

Plata-Salamán, C.R., Ffrench-Mullen, J.M., 1993. Interleukin-2 modulates calcium currents in dissociated hippocampal CA1 neurons. *Neuroreport*, **4**(5):579-581. [doi:10.1097/00001756-199305000-00030]

Raeburn, C.D., Sheppard, F., Barsness, K.A., Arya, J., Harken, A.H., 2002. Cytokines for surgeons. *Am. J. Surg.*, **183**(3):268-273. [doi:10.1016/S0002-9610(02)00781-X]

Raffa, R.B., 1996. A novel approach to the pharmacology of analgesics. *Am. J. Med.*, **101**(1A):S40-S46. [doi:10.1016/S0002-9343(96)90034-0]

Raffa, R.B., Friderichs, E., Reimann, W., Shank, R.P., Codd, E.E., Vaught, J.L., 1992. Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. *J. Pharmacol. Exp. Ther.*, **260**(1):275-285.

Reimann, W., Hennies, H.H., 1994. Inhibition of spinal noradrenaline uptake in rats by the centrally acting analgesic tramadol. *Biochem. Pharmacol.*, **47**(12):2289-2293. [doi:10.1016/0006-2952(94)90267-4]

Sacerdote, P., Bianchi, M., Manfredi, B., Panerai, A.E., 1997. Effects of tramadol on immune responses and nociceptive thresholds in mice. *Pain*, **72**(3):325-330. [doi:10.1016/S0304-3959(97)00055-9]

Sacerdote, P., Bianchi, M., Gaspani, L., Panerai, A.E., 1999.

Effects of tramadol and its enantiomers on concanavalin-A-induced proliferation and NK activity of mouse splenocytes: involvement of serotonin. *Int. J. Immunopharmacol.*, **21**(11):727-734. [doi:10.1016/S0192-0561(99)00048-X]

Sacerdote, P., Bianchi, M., Gaspani, L., Manfredi, B., Maucione, A., Terno, G., Ammatuna, M., Panerai, A.E., 2000. The effects of tramadol and morphine on immune responses and pain after surgery in cancer patients. *Anesth. Analg.*, **90**(6):1411-1414. [doi:10.1097/00000539-200006000-00028]

Salo, M., 1992. Effects of anaesthesia and surgery on the immune response. *Acta Anaesthesiol. Scand.*, **36**(3):201-220.

Sheeran, P., Hall, G.M., 1997. Cytokines in anaesthesia. *Br. J. Anaesth.*, **78**(2):201-219.

Song, P., Zhao, Z.Q., 2000. Interleukin 2-induced antinociception partially coupled with μ-receptor. *Cytokine*, **12**(8):1240-1242. [doi:10.1006/cyto.2000.0695]

Song, P., Lie-Cheng, W., Wang, G.D., Zhou, Z., Zhao, Z.Q., 2002a. Interleukin-2 regulates membrane potentials and calcium channels via mu opioid receptors in rat dorsal root ganglion neurons. *Neuropharmacology*, **43**(8):1324-1329. [doi:10.1016/S0028-3908(02)00298-8]

Song, P., Liu, X.Y., Zhao, Z.Q., 2002b. Interleukin-2-induced antinociception in morphine-insensitive rats. *Acta Pharmacol. Sin.*, **23**(11):981-984.

St A Stewart, L., Martin, W.J., 2003. Evaluation of postoperative analgesia in a rat model of incisional pain. *Contemp. Top. Lab. Anim. Sci.*, **42**(1):28-34.

Wang, W., Danielsson, A., Svanberg, E., Lundholm, K., 2003. Lack of effects by tricyclic antidepressant and serotonin inhibitors on anorexia in MCG 101 tumor-bearing mice with eicosanoid-related cachexia. *Nutrition*, **19**(1):47-53. [doi:10.1016/S0899-9007(02)00921-8]

Whiteside, G.T., Harrison, J., Boulet, J., Mark, L., Pearson, M., Gottshall, S., Walker, K., 2004. Pharmacological characterisation of a rat model of incisional pain. *Br. J. Pharmacol.*, **141**(1):85-91. [doi:10.1038/sj.bjp.0705568]

Yim, A.P., Wan, S., Lee, T.W., Arifi, A.A., 2000. VAST lobectomy reduces cytokine responses compared with conventional surgery. *Ann. Thorac. Surg.*, **70**(1):243-247. [doi:10.1016/S0003-4975(00)01258-3]