International Immunopharmacology 9 (2009) 968–970

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Comparison of morphine and tramadol effects on phagocytic activity of mice peritoneal phagocytes *in vivo*

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ARTICLE INFO

Article history:
Received 16 February 2008
Received in revised form 16 March 2009
Accepted 3 April 2009

Keywords:
Immune system
Morphine
Phagocyte
Phagocytosis
Tramadol

ABSTRACT

It has been claimed that, in contrast to most opioids, tramadol does not suppress immune functions. We therefore, studied the effects of tramadol in comparison to morphine, on the number of phagocytes and the number of sheep red blood cells (SRBCs) engulfed by each individual cell (phagocytic index) of mouse peritoneal phagocyte.

In an experimental study, 63 BALB/c mice received morphine, tramadol or saline intraperitoneally. On days 3, 5, and 10, the peritoneal phagocytes were incubated with an equal number of SRBCs. The cells were then cytocentrifuged onto gelatin-coated slides and examined microscopically.

Ten days after the start of drug administration, the number of phagocytes and the phagocytic index reduced in morphine group ($P<0.05$), and enhanced in tramadol group ($P<0.05$).

In conclusion, tramadol stimulation of immune system may offer a good alternative to morphine for the treatment of patients in whom immunosuppression might be hazardous or in patients who cannot tolerate the side effects of morphine.

1. Introduction

Opioids are known to have inhibitory effects on humoral and cellular immune responses including decreasing lymphoproliferation, natural killer (NK) activity, macrophage functions, and the production of interferon-γ and interleukin-2 in the rodent [1–3].

The presence of opioid receptors outside the central nervous system is increasingly recognized. These receptors have been identified not only in peripheral nerves but also in immune inflammatory cells [4]. Mu-opioid receptor expression may be altered under conditions like inflammation [5]. Opiates modulate the immune response by interaction with these receptors [6].

The immunosuppression mediated by opiates may explain the increased incidence of infection in opioid abusers [3]. Consistent with these observations, morphine administration has been associated with increased susceptibility of animals or human to bacterial and viral infections [7–9], and with decreased survival in tumor-bearing animals [10,11].

The therapeutic use of morphine might, therefore, be relatively contraindicated in patients in whom immunosuppression may be hazardous, especially in postoperative period, in which the functionality of the immune system is already compromised by stress exposure [12]. Therefore, introduction of an alternative analgesic in these situations would be essential.

Tramadol is a centrally acting, analgesic drug, with a double mechanism of action; it binds with low affinity to Mu-opioid receptors, and activates central monoaminergic pathways inhibiting the neuronal uptake of serotonin and noradrenaline [13,14].

Several reports address the role of macrophages in morphine-induced modulation of immunity [15,16]. It has been suggested that, in contrast to morphine, tramadol does not suppress cellular immune functions, but it increases the activity of the immune system [17].

Therefore, in this study we aimed to compare the effects of tramadol with morphine on the phagocytosis of mice peritoneal phagocytes. The capacity of peritoneal phagocytes to engulf red blood cells was further investigated.

2. Materials and methods

In an experimental study, 63 six-week-old male BALB/c mice (Pasteur Institute, Tehran), with an average weight of 20±2 g, were designated in three groups. Animals were housed at 22±2 °C, with light:dark cycle of about 14:10 h and the interventions were blinded in each stages of experiment for the researcher who carried out the phagocytic study. First group received 20 mg/kg on day 1, 40 mg/kg on day 2, and 60 mg/kg on day 3, of morphine (Darupakhsh Pharmacy Co., Tehran, Iran) intraperitoneally (i.p.) twice a day and 80 mg/kg for following the seven days. Second group treated with the same doses of tramadol (Alborz Pharmacy Co., Tehran, Iran) (In a pilot study tramadol treated mice with equianalgesic doses of morphine underwent seizure, therefore the same doses of morphine were used for tramadol). The

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1567-5769/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.intimp.2009.04.002

third group (control group) received saline (Shahid-Ghazi Pharmacy Co., Tehran, Iran) i.p. in volume of 0.5 ml, as scheduled for the first two groups. Seven mice of each group were sacrificed by cervical dislocation on days 3, 5 and 10. The skin of the abdomen was removed and 5 ml of tissue culture media (TCM) was injected into peritoneal cavity of each mouse. After slow massage the peritoneal fluid was collected using a 5 ml syringe. The collected fluid was centrifuged at 1200 rpm for 5 min. The precipitated cells of each individual mouse were counted under light microscope and suspended at concentration 2×10^6 cells per millimeter in TCM. The collected cells were then incubated with sheep red blood cells (SRBCs) being opsonized with 10% mouse anti-sheep Ig as previously reported [18]. The cell suspensions were gently rotated at 1 rpm and 37 °C for 30 min. Appropriate aliquots of the cell suspensions were harvested, and after two washes with ice-cold HBSS (Hank's Balanced Salt Solution) the cells were cytocentrifuged onto gelatin-coated slides and microscopically examined for phagocytosis of SRBCs. The percentage of phagocytes engulfed SRBCs and the number of erythrocytes ingested per cells (phagocytic index) [19,20], were statistically analysed by one way ANOVA followed by Tukey test for multiple comparisons.

3. Results

Animal characteristics were homogeneous in three groups. Three and five days following morphine or tramadol administration, the number of phagocytes (cells with one or more sheep RBC) was not different compared to control group. However, 10 days after the beginning of drug administration, the number of phagocytes in 100 peritoneal cells in morphine group (22 ± 8; $P<0.05$), and in tramadol group (49 ± 7; $P<0.05$) were significantly different in comparison to control group (32 ± 2), (Table 1).

Experiment was carried out to determine if morphine and tramadol affected the number of SRBCs ingested per cell (phagocytic index). Results showed that the percent of phagocytes engulfed 4, 2–3 and 1 RBC on days 3 and 5 in all three groups were not significantly different, but this index for just day 10 was 12%, 34% and 54%, respectively in morphine treated group ($P<0.001$), 51%, 20% and 29% in tramadol treated group ($P<0.05$), compared to control group which was 20%, 71%, 9%, (Table 2).

4. Discussion

Three and five days after morphine or tramadol administration, the number of phagocytes and the number of erythrocytes engulfed by each individual cell (phagocytic index) were not significantly different compared to saline group. These parameters were reduced in morphine group and increased in tramadol group, 10 days after the beginning of drug administration. Furthermore our results showed a parallel increasing pattern in the number of phagocytes in all three groups, from days 3 to 10. The mechanism involved in this phenomenon is not clearly understood. It could be considered as repeated intra peritoneal injection, which may cause localized inflammation. However, since this phenomenon can be postulated for all three groups, it might not have any effects on our results.

Table 1
The number of phagocytes per 100 peritoneal cells in different groups of mice.

Group	Test day		
	3	5	10
Morphine	11 ± 6	14 ± 3	22 ± 8^a
Tramadol	20 ± 4	23 ± 8	49 ± 7^b
Saline	18 ± 9	19 ± 11	32 ± 2^c

Results are presented as mean phagocytes numbers ± SD for groups of 7 mice. ANOVA and post-hoc Tukey test showed difference between groups; a and c ($p<0.05$), b and c ($p<0.05$) and a and b ($p<0.001$) just for day ten. The differences for days 3 and 5 were not significant.

Table 2
The number of erythrocytes ingested phagocyte (phagocytic index) in three different days.

Group	RBC in Phagocytes	Percentage of phagocytes		
		Test day		
		3	5	10
Morphine	>4	18	21	12^a
	2 and 3	43	43	34
	1	39	36	54
Tramadol	>4	39	28	51^b
	2 and 3	13	37	20
	1	48	35	29
Saline	<4	43	40	20^c
	2 and 3	22	35	71
	1	35	25	9

Results are presented as the mean percent phagocytes for groups of 7 mice. ANOVA and post-hoc Tukey test showed difference between groups; a and c ($P<0.05$), b and c ($P<0.001$) and a and b ($P<0.001$) just for day ten. The differences for days 3 and 5 were not significant.

Additionally, we considered the number of erythrocytes in each cell (phagocytic index) as phagocytic capacity in each group (the more SRBCs in each phagocyte was considered as higher phagocytic capacity). Results showed that the number of phagocytes with 4 or more SRBCs on day 10 in tramadol group was more, and in morphine group was less than saline group. This may mean that sub-acute administration of these two drugs might not affect the immune system. However, chronic usage of morphine may decrease the immune activity but tramadol enhances it. These results are partly in agreement with the results of Sacerdote et al. [21]. In this study 2 h after morphine administration no significant modification of NK activity was observed, indicating no modification of immune system in acute administration of this drug.

In the present study, morphine and tramadol did not have any effect on phagocytosis on the third and fifth days of the experiment. However, on the 10th day, morphine reduced and tramadol enhanced it. The mechanism in which the effects of these compounds appear with delay is not clear. A same phenomenon takes place for some other drugs such as antidepressants. The therapeutic effects of these drugs appear following one or two weeks of drug administration, but their pharmacologic activities can be detected soon after drug usage [22].

It has been shown that tramadol has not had any effect on phagocytes population or the number of latex particles phagocytized (phagocytic index) by polymorphnuclear cells and monocytes. However morphine dose dependently has decreased the monocyte phagocyting index, following incubation with the drug [19]. Difference between the results of the above study and our experiment for tramadol may be due to differences in drug dose, exposure time, methods of the studies or species variations.

Although morphine and tramadol share Mu-opioid mechanisms for their analgesic activities, however, the analgesic effects of tramadol are mediated also via a separate, non opioid mechanism, caused by the inhibition of neuronal uptake of noradrenaline and serotonin [[14,17]. These differences may account for the diverse pharmacodynamic activities of morphine and tramadol on immune functions.

The involvement of the noradrenergic and serotoninergic systems in neural–immune interactions has been studied by using different experimental models. Although both enhancement and reduction of immune responses have been related to the activation of the noradrenergic system [23], the increase of serotoninergic tone has usually been associated with stimulation of NK activity and lymphocyte proliferation [[21,24]. Consistent with these observations, drugs which increase serotoninergic tone, such as D-fenfluramine and fluoxetine, stimulate immune function in rodents [24]. Moreover, in the mouse, the immune effects of tramadol on lymphoproliferation and NK activity were prevented by the administration of the nonspecific serotoninergic antagonist metergoline [21], indicating an

involvement of the serotoninergic system in the immune effects of this drug. Interestingly, tramadol, when added *in vitro* to splenocyte cultures [21], was not able to modulate either proliferation or NK activity, thus eliminating a direct effect of the drug on immune cells. Therefore, it can be suggested that activation of the serotoninergic system might be involved in the immune effects of tramadol.

Compared to most opioids, tramadol has a lower incidence of side effects such as respiratory depression, nausea, vomiting, constipation, and sedation. It may benefit patients with history of peptic ulceration, asthma or opioid dependence and patients who will require long term pain treatment [25].

In conclusion, the immune function is differently affected by morphine and tramadol. Analgesic drugs like tramadol devoid of immunosuppressive effects or with stimulus activity offer a good alternative to morphine for the treatment of patients in whom immunosuppression might be hazardous or in patients who cannot tolerate the side effects of morphine.

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