® Postepy Hig Med Dosw (online), 2018; 72: 52-57
e-ISSN 1732-2693

www.phmd.pl
Review

Received: 11.08.2017
Accepted: 16.10.2017
Published: 16.02.2018

Potential impact of obligatory use of opioids in invasive procedures on interpretation of experimental data

Potencjalny wpływ ustawowego obowiązku stosowania opioidów w inwazyjnych procedurach na zwierzętach na interpretację wyników doświadczeń

Michał Zimecki

Laboratory of Immunobiology, The Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

Summary

Application of opioids as an analgesic drug is a common practice in the prevention of pain in patients and experimental animals in highly invasive procedures. Very recently, new legal regulations were implemented that broaden the application of analgesics in procedures where pain relievers have not been previously obligatory. However, in light of hitherto studies, the application of opioids has adverse effects on the condition of animals in experiments. Harmful effects of opioids include: lower intake of water and food, weight loss, increased mortality, susceptibility to infection by experimental pathogens and chemicals inducing pathological changes. The above listed actions, induced by opioids, may significantly affect interpretation of experimental data. The aim of this article is to review selected studies in animal models, mainly on the application of morphine and buprenorphine, including the mechanism of opioid action. Alternative methods of analgesia, involving other types of pain relievers, such as non-steroid anti-inflammatory drugs, are also described. Since opioids significantly affect the values of investigated parameters, some experimental procedures should be probably modified in order to lower the detrimental effects of this class of pain relievers. In consequence, new protocols would probably consider the application of lower doses of compounds or pathogens required for the induction of defined, experimentally induced disease states. A wider application of analgesics, of a different mechanism of action than opioids, would also be an alternative.

Keywords: **opioids • morphine • buprenorphine • experimental protocols • ethics committee**

GICID: 01.3001.0010.8709
DOI: 10.5604/01.3001.0010.8709
Word count: 4423
Tables: –
Figures: –
References: 57

Author's address: prof. Michał Zimecki, Laboratory of Immunobiology, The Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, ul. R. Weigla 12, 53-114 Wrocław, e-mail: zimecki@iitd.pan.wroc.pl

Introduction

Opioids are commonly used in the pre- and postoperative period of surgery as well as in terminal states of some diseases. Their application is also required in experimental procedures involving vertebrate animals. Recently implemented law regulations (The European Directive 2010/63/EU) recommend obligatory introduction of pain relievers in a broad spectrum of experimental models. Nevertheless, the upper limits of pain, established in this act, may be still a matter of debate [8] and dosages of sedative agents are not decisively determined [26]. More importantly, the use of opioids in animal experimental protocols has profound effects on the course of immunological processes and, consequently, on the interpretation of experimental data [21,46,48]. The aim of this article is to review hitherto reports regarding the effects of opioid application on experimental data and the patient's follow up in clinical practice, mechanism of opioid action and possible use of alternative pain relievers.

Experimental models involving infection with pathogens

Effects of opioids on the susceptibility of mice to various strains of bacteria, their products, viruses and parasites were investigated in a variety of models. BALB/c mice, treated with d- galactosamine and infected with lipopolysaccharide (LPS), undergo a rapid death due to septic shock [27], which can be prevented by naltrexone, the opioid receptor antagonist. The protective effect was reversed by the administration of morphine. However, naltrexone was not effective when mice were infected with staphylococcal enterotoxin B (SEB) or with agonistic anti-Fas antibody. The protective effect of naltrexone was associated with an indirect inhibition of TNF-alpha induced by LPS, but not by SEB. Another interesting study showed differential effects of morphine on the survival of BALB/c mice infected with *Escherichia coli, Shigella flexneri, Listeria monocytogenes, Salmonella enteritidis* and *Yersinia enterocolitica* [3] Morphine increased the susceptibility of mice to *S. enteritidis* and *L. monocytogenes*, but not to other bacteria strains. This effect was particularly strong in the case of *L. monocytogenes*, resulting in 100% mortality, whereas all control mice survived. A study applying *Salmonella enterica* and μ-opiod receptor knockout mice demonstrated that the protective action of naltrexone is definitively dependent on its interaction with μ-opioid receptor [11]. In turn, the studies on *Streptococcus pneumoniae* infection provided some insight into the mechanism of the detrimental action of morphine [55,56]. The authors showed that chronic treatment of mice with morphine delayed neutrophil recruitment into lungs and decreased the levels of proinflammatory cytokines and galectin-3 in the bronchoalveolar lavage fluids and lung tissue [55]. In addition, morphine reduced macrophage inflammatory protein 2 (MIP-2) release by alveolar macrophages stimulated with Streptococcus pneumoniae and NF-kappa B-dependent gene transcription in these cells [56]. The bacterial uptake and killing was also decreased. Mice, inoculated intestinally with *Pseudomonas aeruginosa* and implanted with a morphine pellet for slow opioid release [5], displayed suppression of intestinal mucus, disrupted intestinal epithelium and enhanced mortality. Interestingly, morphine can also transform *P. aeruginosa* to a more virulent phenotype upon direct *in vitro* exposure.

The disruption of the intestinal barrier structure by morphine leads to bacterial translocation to mesenteric lymph nodes [43]. In this phenomenon toll-like receptors (TLR) play a role, since the effect of morphine is significantly reduced in TLR 2 and TLR 4 knockout mice. The damaging effects of morphine on gut barrier were also associated with the alteration of intestinal microflora by the selective growth of Gram-positive pathogenic strains and a decrease in bile-deconjugating strains [7]. Activation of TLR 2 by Gram-positive bacteria in a polymicrobial sepsis model was associated with sustained up-regulation of IL-17A and IL-6 [42]. Nevertheless, not all investigations described the detrimental effects of opioids in experimental bacterial infections. In a model of polymicrobial sepsis, induced by cecal ligation and puncture [29] using tramadol and buprenorphine, no differences in mortality rate between control and opioid-treated mice were registered. One exception was the group treated with a high dose of tramadol, which showed more later deaths than in the group treated with buprenorphine.

Harmful effects of morphine treatment were also observed upon infection with parasites and viruses. Repeated subcutaneous administration of morphine caused 86% mortality of mice infected with avirulent strain of *Toxoplasma gondii* versus 0% in control mice [14]. The effect of neutralizing endogenous opioids by naloxone, an opioid receptor antagonist, on the induction of acquired immunity in herpes simplex virus infected mice was also studied [32]. It was revealed that lymphocyte proliferation, interferon gamma production and delayed type hypersensitivity reaction were higher in the naloxone-treated group. However, levels of the antiviral antibodies were similar in naloxone-treated and control groups.

Surgical procedures and transplantation

The effects of recombinant rat β-endorphin (β-EP) and morphine were tested in a rat model of bone cancer pain [18]. The compounds had a good analgesic effect and β-EP, but not morphine, increased body weight. In terms of immune parameters β-EP increased T cell proliferation, relative quantities of T-cell subsets and NK (natural killer) cytotoxicity, but had no effect on T cell secretion. In contrast, morphine diminished T cell proliferation and the level of T cell subsets. In a model of induced cerebral ischemia in mice buprenorphine and meloxicam (a prostaglandin synthesis inhibitor) were investigated [31]. Such a common side-effect of buprenorphine as decreased food consumption occurred after surgery, but was transient. Buprenorphine did not change the

infarction volume in comparison with that of the control mice. However, treatment with meloxicam demonstrated beneficial effects by a significant reduction of infarct volume. To investigate postoperative analgesics in mice like buprenorphine and flunixin meglumine, radiotelemetry transmitters were surgically implanted [25]. Several activities associated with behavior and food consumption were monitored. Food consumption and body weight were significantly reduced in mice treated with three but not one dose of buprenorphine. The activity of mice was significantly lower in the first 6 h after surgery in the control group as compared to mice with analgesia. In another model of surgery a comparison was conducted between the effects of buprenorphine and karprofen, a non steroid anti-inflammatory drug, and a combination of these drugs [1]. The study monitored food and water intake, body weight, locomotion activity and pain index. Mice treated with buprenorphine showed the highest pain index score in comparison to mice not subjected to surgery. The studied parameter values did not allow us to conclude that all applied combinations of the drugs improved recovery from surgery, as compared to control mice.

The effects of preoperative opioid use were evaluated in several analyses in patients undergoing organ transplantation. In kidney transplant recipients [38,39], high level prescription use before transplantation was clearly associated with an increased risk of mortality and graft loss. The association between opioid use and hospital readmission was analyzed in the case of liver transplantation patients attending a single medical center [52]. The authors found a significantly higher risk of readmission at 30 days and a non-significantly elevated risk at one year after transplantation. In another meta-analysis an association between pretransplant opioid use and graft survival over five years also revealed an increased mortality and graft failure [51] of opioid users.

Very recently (unpublished) we performed alogeneic skin transplants between BALB/c and C57BL/6 mice using for the first time buprenorphine, as required by the local ethics committee. The results indicated that the mean graft survival time in the control mice was significantly shorter, as compared to our previous experiment when no analgesia was applied [45].

Effects of opioids in models of central nervous system and bowel inflammation

The models of experimentally induced neurodegeneration revealed sometimes opposite effects of endogenous and exogenously administered opioids on severity of neuropathology. Mice were pretreated with selective kappa opioid receptor antagonists and given pilocarpine to induce brain damage. It was revealed that the integrity of hippocampal neurons was protected in mice receiving the receptor agonists [49]. In addition, the experimental autoimmune encephalomyelitis (EAE) mouse model [36] revealed an advantageous role of both endogenous and exogenously administered opioid growth factor in diminishing excessive proliferation of T and B lymphocytes in the spleen and inguinal lymph nodes. Nevertheless, treatment with morphine led to neuroinflammation [15].

It is also evident from the literature on colon inflammation that different effects are observed between mouse models where endogenous opioids are stimulated by μ receptor agonists and those derived from clinical studies. Opioid receptors agonists reduced colon inflammation in chemically-induced colitis, and receptor deficient mice were highly susceptible to colon inflammation [47]. A role for Th1 CD4+ and Th17 cells in dextran sulfate (DS) induced colitis was also shown [10]. The cells accumulated in the inflamed gut and expressed a high level of endogenous opioids, thus reducing abdominal pain. Our recent experiment in the model of dextran sulfate induced colitis and use of buprenorphine aroused some concern (unpublished), since 4% DS, causing very low mortality in our previous experiments without analgesia, led to a very high mortality of mice.

Nevertheless, the detrimental effects of opioid use in the alleviation of bowel pain in patients constitute a serious problem [13,34]. To overcome the problem new μ opioid antagonists were developed which selectively block u opioid receptors in the enteric nervous system without penetrating blood-brain barrier. In an alternative approach κ opioid agonists function by modulating nociception in the enteric nervous system without affecting central nervous system side effects.

Effects on immune response, other experimental models, and mechanism of opioid action

The effects on humoral and cellular immune response of several opioids (morphine, fentanyl and methadone) were differential (stimulation or suppression) and also depended on the time of their administration after immunization [21]. Adverse effects of morphine were found in a model of anaphylactic shock when the opioid was injected intravenously or intracerebroventricularly. These effects were reversed by naloxone [2]. A study, involving human peripheral blood mononuclear cells, Jurkat cell line and splenocytes from wild and μ-opioid receptor knockout mice, demonstrated that morphine directs the immune response to Th2 prolife [53]. More importantly, buprenorphine, predominantly used for analgesia in animal experimental protocols, was shown to suppress in rats splenic natural killer cell activity, lymphocyte proliferation and IFN gamma production [12]. Interestingly, morphine does not exclusively act *via* classic opioid receptors [57]. The authors demonstrated that morphine can induce neuroinflammation through TLR 2, a classical innate immune receptor, by binding to myeloid differentiation protein 2. The effects of morphine, buprenorphine and fenantyl, incubated with canine blood, were also investigated with a focus on phagocytic function of neutrophils, respiratory burst,

cytokine production and apoptosis [17]. The respiratory burst was best stimulated by morphine and all opioids stimulated the production of TNF alpha, IL-6 and IL-10 induced by LPS or lipoteichoic acid. The opioids also inhibited the apoptosis of neutrophils. It can be, therefore, concluded that opioids enhanced the inflammatory reaction to bacterial products. In the case anti- Fas--induced hepatitis in mice [33] application of naltrexone reduced liver damage and increased the survival rate. Nevertheless, morphine had no effect on Fas-induced apoptosis in cultured hepatocytes and naltrexone did not modify Fas mRNA expression in the liver. Although neutrophils infiltrated injured livers, the enhancing, apoptotic effect of morphine was preserved even in granulocyte –depleted mice. The authors concluded that in the protective effect of naltrexone a peripheral mechanism operates which does not involve down regulation of Fas mRNA in hepatocytes. Interestingly, deleterious effects of morphine in a breast tumor model in mice, such as increase of tumor weight, metastasis and shortened survival, could be prevented by application of celecoxib, a cyclooxygenase 2 inhibitor [20]. Importantly, the actions of celecoxib did not affect analgesia in this model.

Co-administration of methamphetamine and morphine results in increased toxicity [24]. The toxicity could be significantly attenuated by the administration of N-methyl-D-aspartate receptor (NMDA) antagonists [44]. Interestingly, the lethal effects of combined drug administration could be prevented by immediate cooling of mice. The authors speculated that a reduction in free radical release may play a role in methamphetamine-induced neurotoxicity. In fact, the prooxidant properties of methamphetamine were demonstrated in another study [30]. Interestingly, in a mouse tumor model [54], no major differences between the buprenorphine-treated and control group, in terms of animal discomfort, were found.

Alternative methods of analgesia

Due to adverse or modifying effects of opioids on the studied parameters, analgesics of different mechanism of action, mainly non steroid anti-inflammatory compounds, may be an alternative in animal experiments. When buprenorphine was applied in parallel with indomethacin in evaluation of postsurgical recovery in a model of radiotelemetry implantation, a treatment with indomethacin proved to be more effective regarding levels of mouse activity during their application is more beneficial in comparison to such drugs as light--on periods [9]. In a similar study, ibuprofen-treated mice, but not those treated with buprenorphine, showed significantly higher locomotion activity after surgery and better water intake than the control mice [28]. The adverse symptoms observed after buprenorphine treatment included hyperreactivity, hyperthermia and reduced water and food intake. Buprenorphine was also compared with meloxicam, a non-steroid anti-inflammatory drug from the oxicam group of compounds, in a model of middle cerebral artery occlusion in C57BL/6 mice [31], where the infarct volume was measured as a main consequence of surgery. There was no difference in the infarct volume between saline and buprenorphine-treated mice, but meloxicam significantly reduced infarct volume.

It has to be, however, stressed that significant differences occur in the efficacy of non-steroid analgesic drugs depending on their ability to suppress appropriate isoforms of cyclooxygenase. It appears that only celecoxib is a specific COX-2 inhibitor [16], whereas karprophen [22] and meloxican [19] belong to preferential COX-2 inhibitors (300-500 stronger than COX-1 inhibitors), which is associated with less ulcerogenic and nephrotoxic actions. Thus, their application is more beneficial in comparison to such drugs as flunixin [50], since flunixin and indomethacin inhibit both forms of cyclooxygenase. Moreover, the analgesic potential of flunixin and karprophen is comparable to that of peptidine (agonist of both opioid receptors) [37], but the analgesic efficacy of meloxicam is rather moderate.

Two analgesic drugs, tramadol and gabapentin, alone or in combination, were investigated in tail-flick and hot plate tests [4]. It appeared that the drugs were most effective when used in combination, whereas tramadol alone showed a better antinoceptive effect than gabapentin. Tramadol is presently also classified as an opioid receptor agonist, although its affinity to these receptors is much lower than that of morphine [40]. On the other hand, it inhibits the neuronal uptake of serotonin and norepinephrine, thus providing an explanation for its analgesic property without exerting depressive action on the respiratory system, induction of tolerance or drug dependence. Gabapentin belongs to a group of anticonvulsant drugs, providing relief in neuropathic pain [35]. The drug does not abolish acute pain. The mechanism of its action is probably associated with lowering calcium influx into calcium channels of alpha2-delta type and inhibition of activity of substance P, glutamates and norepinerphine in peripheral nervous system [23].

Anti-inflammatory and analgesic properties were also found in plant extracts of *Holoptelea integrifolia* and *Argyreia speciosa* by applying tail-flick and acetic acid-induced writhing methods [36].

Conclusions

The universal use of analgesic opioids in laboratory practice and clinic has revealed their modifying effects on the course of inflammatory processes and aiding in recovering the ability of the immune system to function. Application of opioids in animal experimental models, for which no analgesia has been not previously required, may lead to a false interpretation of experimental data and also to the increased mortality of animals. Hence, there is a need to elaborate new experimental

protocols, for which no analgesic compounds have been hitherto recommended. Such scientific projects should have particular appreciation and consent from local ethics committees and involve parallel experimental groups of animals receiving and not receiving analgesia. In consequence, new protocols would probably apply lower doses of compounds or pathogens required for the induction of defined, experimentally induced disease states. A wider application of analgesics, of different mechanism of action than opioids, would be an alternative. However, even in such an instance, the effects of alternative analgesics on the course of immunological processes should also be thoroughly investigated.

References

[1] Adamson T.W., Kendall L.V., Goss S., Grayson K., Touma C., Palme R., Chen J.Q., Borowsky AD.: Assessment of carprofen and buprenorphine on recovery of mice after surgical removal of the mammary fat pad. J. Am. Assoc. Lab. Anim. Sci., 2010; 49: 610-616

[2] Amir S.: Morphine exacerbates anaphylactic shock in mice by stimulating central opiate receptors. Neurosci. Lett., 1983; 40: 169-174

[3] Asakura H., Kawamoto K., Igimi S., Yamamoto S., Makino S.: Enhancement of mice susceptibility to infection with *Listeria monocytogenes* by the treatment of morphine. Microbiol. Immunol., 2006; 50: 543-547

[4] Aydin O.N., Ek R.O., Temoçin S., Uğur B., Alaçam B., Şen S.: The antinociceptive effects of systemic administration of tramadol, gabapentin and their combination on mice model of acute pain. Agri., 2012; 24: 49-55

[5] Babrowski T., Holbrook C., Moss J., Gottlieb L., Valuckaite V., Zaborin A., Poroyko V., Liu D.C., Zaborina O., Alverdy J.C.: *Pseudomonas aeruginosa* virulence expression is directly activated by morphine and is capable of causing lethal gut-derived sepsis in mice during chronic morphine administration. Ann. Surg., 2012; 255: 386-393

[6] Banerjee S., Ninkovic J., Meng J., Sharma U., Ma J., Charboneau R., Roy S.: Morphine compromises bronchial epithelial TLR2/IL17R signaling crosstalk, necessary for lung IL17 homeostasis. Sci. Rep., 2015; 5: 11384

[7] Banerjee S., Sindberg G., Wang F., Meng J., Sharma U., Zhang L., Dauer P., Chen C., Dalluge J., Johnson T., Roy S.: Opioid-induced gut microbial disruption and bile dysregulation leads to gut barrier compromise and sustained systemic inflammation. Mucosal Immunol., 2016; 9: 1418-1428

[8] Beauchamp T.L., Morton D.B.: The upper limits of pain and suffering in animal research. Camb. Q. Healthc. Ethics, 2015; 24: 431-447

[9] Blaha M.D., Leon L.R: Effects of indomethacin and buprenorphine analgesia on the postoperative recovery of mice. J. Am. Assoc. Lab. Anim. Sci., 2008; 47: 8-19

[10] Boué J., Basso L., Cenac N., Blanpied C., Rolli-Derkinderen M., Neunlist M., Vergnolle N., Dietrich G.: Endogenous regulation of visceral pain via production of opioids by colitogenic $CD4^+$ T cells in mice. Gastroenterology, 2014; 146: 166-175

[11] Breslow J.M., Feng P., Meissler J.J., Pintar J.E., Gaughan J., Adler M.W., Eisenstein T.K.: Potentiating effect of morphine on oral *Salmonella enterica* serovar *Typhimurium* infection is μ-opioid receptor--dependent. Microb. Pathog., 2010; 49: 330-335

[12] Carrigan K.A., Saurer T.B., Ijames S.G., Lysle D.T.: Buprenorphine produces naltrexone reversible alterations of immune status. Int. Immunopharmacol., 2004; 4: 419-428

[13] Chang H.Y., Lembo A.J.: Opioid-induced bowel dysfunction. Curr. Treat. Options Gastroenterol., 2008; 11: 11-18

[14] Chao C.C., Sharp B.M., Pomeroy C., Filice G.A., Peterson P.K.: Lethality of morphine in mice infected with *Toxoplasma gondii*. J. Pharmacol. Exp. Ther., 1990; 252: 605-609

[15] Charkhpour M., Ghavimi H., Ghanbarzadeh S., Yousefi B., Khorrami A., Mesgari M., Hassanzadeh K.: Protective effect of pioglitazone on morphine-induced neuroinflammation in the rat lumbar spinal cord. J. Biomed. Sci., 2015; 22: 82

[16] Davies N.M., McLachlan A.J., Day R.O., Williams K.M.: Clinical pharmacokinetics and pharmacodynamics of celecoxib. A selective cyclo-oxygenase-2 inhibitor. Clin. Pharmacokinet., 2000; 38: 225-242

[17] Declue A.E., Yu D.H., Prochnow S., Axiak-Bechtel S., Amorim J., Tsuruta K., Donaldson R., Lino G., Monibi F., Honaker A., Dodam J:. Effects of opioids on phagocytic function, oxidative burst capacity, cytokine production and apoptosis in canine leukocytes. Vet. J., 2014; 200: 270-275

[18] Du J.Y., Liang Y., Fang J.F., Jiang Y.L., Shao X.M., He X.F., Fang J.Q.: Effect of systemic injection of heterogenous and homogenous opioids on peripheral cellular immune response in rats with bone cancer pain. A comparative study. Exp. Ther. Med., 2016; 12: 2568-2576

[19] Engelhardt G.: Pharmacology of meloxicam, a new non-steroidal anti-inflammatory drug with an improved safety profile through preferential inhibition of COX-2. Br. J. Rheumatol., 1996; 35 (Suppl. 1): 4-12

[20] Farooqui M., Li Y., Rogers T., Poonawala T., Griffin R.J., Song C.W., Gupta K.: COX-2 inhibitor celecoxib prevents chronic morphine-induced promotion of angiogenesis, tumour growth, metastasis and mortality, without compromising analgesia. Br. J. Cancer, 2007; 97: 1523-1531

[21] Filipczak-Bryniarska I., Nowak B., Sikora E., Nazimek K., Woroń J., Wordliczek J., Bryniarski K.: The influence of opioids on the humoral and cell-mediated immune responses in mice. The role of macrophages. Pharmacol. Rep., 2012; 64: 1200-1215

[22] Fox S.M., Johnston S.A.: Use of carprofen for the treatment of pain and inflammation in dogs. J. Am. Vet. Med. Assoc., 1997; 210: 1493-1498

[23] Gee N.S., Brown J.P., Dissanayake V.U., Offord J., Thurlow R., Woodruff G.N.: The novel anticonvulsant drug, gabapentin (Neurontin), binds to the $\alpha_2\delta$ subunit of a calcium channel. J. Biol. Chem., 1996; 271: 5768-5776

[24] Ginawi O.T., al-Shabanah O.A., Bakheet S.A.: Increased toxicity of methamphetamine in morphine-dependent mice. Gen. Pharmacol., 1997; 28: 727-731

[25] Goecke J.C., Awad H., Lawson J.C., Boivin G.P.: Evaluating postoperative analgesics in mice using telemetry. Comp. Med., 2005; 55: 37-44

[26] Green S.L.: Postoperative analgesics in South African clawed frogs (*Xenopus laevis*) after surgical harvest of oocytes. Comp. Med., 2003; 53: 244-247

[27] Greeneltch K.M., Haudenschild C.C., Keegan A.D., Shi Y.: The opioid antagonist naltrexone blocks acute endotoxic shock by inhibiting tumor necrosis factor-α production. Brain Behav. Immun., 2004; 18: 476-484

[28] Hayes K.E., Raucci J.A.Jr., Gades N.M., Toth L.A.: An evaluation of analgesic regimens for abdominal surgery in mice. Contemp. Top. Lab. Anim. Sci., 2000; 39: 18-23

[29] Hugunin K.M., Fry C., Shuster K., Nemzek J.A.: Effects of tramadol and buprenorphine on select immunologic factors in a cecal ligation and puncture model. Shock, 2010; 34: 250-260

[30] Ito S., Mori T., Kanazawa H., Sawaguchi T.: Differential effects of the ascorbyl and tocopheryl derivative on the methamphetamine--induced toxic behavior and toxicity. Toxicology, 2007; 240: 96-110

[31] Jacobsen K.R., Fauerby N., Raida Z., Kalliokoski O., Hau J., Johansen F.F., Abelson K.S.: Effects of buprenorphine and meloxicam analgesia on induced cerebral ischemia in C57BL/6 male mice. Comp. Med., 2013; 63:105-113

[32] Jamali A., Mahdavi M., Shahabi S., Hassan Z.M., Sabahi F., Javan M., Farsani M.J., Parsania M., Bamdad T.: Naloxone, an opioid receptor antagonist, enhances induction of protective immunity against HSV-1 infection in BALB/c mice. Microb. Pathog., 2007; 43: 217-223

[33] Jaume M., Jacquet S., Cavaillès P., Macé G., Stephan L., Blanpied C., Demur C., Brousset P., Dietrich G.: Opioid receptor blockade reduces Fas-induced hepatitis in mice. Hepatology, 2004; 40: 1136-1143

[34] Kurz A., Sessler D.I.: Opioid-induced bowel dysfunction. Pathophysiology and potential new therapies. Drugs, 2003; 63: 649-671

[35] Laird M.A., Gidal B.E.: Use of gabapentin in the treatment of neuropathic pain. Ann. Pharmacother., 2000; 34: 802-807

[36] Lalan B.K., Hiray R.S., Ghongane B.B.: Evaluation of analgesic and anti-inflammatory activity of extract of *Holoptelea integrifolia* and *Argyreia speciosa* in animal models. J. Clin. Diagn. Res., 2015; 9: FF01-FF04

[37] Lascelles B.D., Cripps P., Mirchandani S., Waterman A.E.: Carprofen as an analgesic for postoperative pain in cats. Dose titration and assessment of efficacy in comparison to pethidine hydrochloride. J. Small Anim. Pract., 1995; 36: 535-541

[38] Lentine K.L., Lam N.N., Xiao H., Tuttle-Newhall J.E., Axelrod D., Brennan D.C., Dharnidharka V.R., Yuan H., Nazzal M., Zheng J., Schnitzler M.A.: Associations of pre-transplant prescription narcotic use with clinical complications after kidney transplantation. Am. J. Nephrol., 2015; 41: 165-176

[39] Lentine K.L., Yuan H., Tuttle-Newhall J.E., Xiao H., Chawa V., Axelrod D., Brennan D.C., Dharnidharka V.R., Beuer C., Schnitzler M.A.: Quantifying prognostic impact of prescription opioid use before kidney transplantation through linked registry and pharmaceutical claims data. Transplantation, 2015; 99: 187-196

[40] Lewis K.S., Han N.H.: Tramadol. A new centrally acting analgesic. Am. J. Health. Syst. Pharm., 1997; 54: 643-652

[41] McLaughlin P.J., McHugh D.P., Magister M.J., Zagon I.S.: Endogenous opioid inhibition of proliferation of T and B cell subpopulations in response to immunization for experimental autoimmune encephalomyelitis. BMC Immunol., 2015; 16: 24

[42] Meng J., Banerjee S., Li D., Sindberg G.M., Wang F., Ma J., Roy S.: Opioid exacerbation of Gram-positive sepsis, induced by gut microbial modulation, is rescued by IL-17A neutralization. Sci. Rep., 2015; 5: 10918

[43] Meng J., Yu H., Ma J., Wang J., Banerjee S., Charboneau R., Barke R.A., Roy S.: Morphine induces bacterial translocation in mice by compromising intestinal barrier function in a TLR-dependent manner. PLoS One, 2013; 8: e54040

[44] Namiki M., Mori T., Sawaguchi T., Ito S., Suzuki T.: Underlying mechanism of combined effect of methamphetamine and morphine on lethality in mice and therapeutic potential of cooling. J. Pharmacol. Sci., 2005; 99: 168-176

[45] Pasikowski P., Goździewicz T., Stefanowicz P., Artym J., Zimecki M., Szewczuk Z.: A novel immunosuppressory peptide originating from the ubiquitin sequence. Peptides, 2011; 32: 2418-2427

[46] Piersma F.E., Daemen M.A., Bogaard A.E., Buurman W.A.: Interference of pain control employing opioids in *in vivo* immunological experiments. Lab. Anim., 1999; 33: 328-333

[47] Philippe D., Dubuquoy L., Groux H., Brun V., Chuoï-Mariot M.T., Gaveriaux-Ruff C., Colombel J.F., Kieffer B.L., Desreumaux P.: Anti-inflammatory properties of the μ opioid receptor support its use in the treatment of colon inflammation. J. Clin. Invest., 2003; 111: 1329-1338

[48] Plein L.M., Rittner H.L.: Opioids and the immune system - friend or foe. Br. J. Pharmacol., 2018 (in print)

[49] Przewłocka B., Machelska H., Lasoń W.: Kappa opioid receptor agonists inhibit the pilocarpine-induced seizures and toxicity in the mouse. Eur. Neuropsychopharmacol., 1994; 4: 527-533

[50] Radi Z.A., Khan N.K.: Effects of cyclooxygenase inhibition on the gastrointestinal tract. Exp. Toxicol. Pathol., 2006; 58: 163-173

[51] Randall H.B., Alhamad T., Schnitzler M.A., Zhang Z., Ford-Glanton S., Axelrod D.A., Segev D.L., Kasiske B.L., Hess G.P., Yuan H., Ouseph R., Lentine K.L.: Survival implications of opioid use before and after liver transplantation. Liver Transpl., 2017; 23: 305-314

[52] Rogal S., Mankaney G., Udawatta V., Good C.B., Chinman M., Zickmund S., Bielefeldt K., Jonassaint N., Jazwinski A., Shaikh O., Hughes C., Humar A., DiMartini A., Fine M.J.: Association between opioid use and readmission following liver transplantation. Clin. Transplant., 2016; 30: 1222-1229

[53] Roy S., Balasubramanian S., Sumandeep S., Charboneau R., Wang J., Melnyk D., Beilman G.J., Vatassery R., Barke R.A.: Morphine directs T cells toward TH2 differentiation. Surgery, 2001; 130: 304-309

[54] van Loo P.L., Everse L.A., Bernsen M.R., Baumans V., Hellebrekers L.J., Kruitwagen C.L., den Otter W.: Analgesics in mice used in cancer research: reduction of discomfort? Lab Anim., 1997; 31: 318-325

[55] Wang J., Barke R.A., Charboneau R., Roy S.: Morphine impairs host innate immune response and increases susceptibility to *Streptococcus pneumoniae* lung infection. J. Immunol., 2005; 174: 426-434

[56] Wang J., Barke R.A., Charboneau R., Schwendener R., Roy S.: Morphine induces defects in early response of alveolar macrophages to *Streptococcus pneumoniae* by modulating TLR9-NF-κB signaling. J. Immunol., 2008; 180: 3594-3600

[57] Wang X., Loram L.C., Ramos K., de Jesus A.J., Thomas J., Cheng K., Reddy A., Somogyi A.A., Hutchinson M.R., Watkins L.R., Yin H.: Morphine activates neuroinflammation in a manner parallel to endotoxin. Proc. Natl. Acad. Sci. USA, 2012; 109: 6325-6330

The author has no potential conflicts of interest to declare.