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In contrast to morphine, buprenorphine enhances macrophage-induced humoral immunity and, as oxycodone, slightly suppresses the effector phase of cell-mediated immune response in mice☆

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ABSTRACT

Background: Opioid receptors are commonly expressed on various immune cells, macrophages especially. Thus, these cells are prone to stimulation with opioids, which seems to be responsible for opioid-induced immunomodulatory effects. While morphine, fentanyl and methadone influence on mouse immune response was recently studied, little is known about the potential immunomodulatory impact of buprenorphine and oxycodone.

Aim: The current research aimed to investigate the influence of buprenorphine and oxycodone on immune responses in mice under homeostatic conditions.

Methods and results: Repeated administration of morphine led to intensification of CHS response in actively sensitized mice, while buprenorphine or oxycodone administration exerted the opposite effect. Further, hapten-conjugated macrophages from mice treated with morphine, when transferred into naive recipients, induced more potent CHS response. The enhanced generation of reactive oxygen intermediates and nitric oxide by macrophages from mice treated with buprenorphine, oxycodone or morphine was also shown, along with increased release of IL-6, TNFα and TGFβ. Treatment with opioids altered expression of antigen phagocytosis and presentation markers. Finally, the inhibitory effect of morphine treatment on induction of humoral immunity by macrophages was demonstrated, while oxycodone failed to influence humoral immune response and buprenorphine actually enhanced B-cell activation.

Conclusions: Current observations confirm that macrophages greatly contribute to immunomodulatory effects of opioids. Studies on immunomodulation by opioids have great importance related to the evaluation of its beneficial and adverse effects on patient condition. Our research showed that oxycodone exerts the weakest immunomodulatory properties, allowing us to assume this drug as safer than morphine during prolonged therapy.

1. Introduction

Pain is the most predominant subjective symptom reported by patients. Whole process of nociception involves stimulation of selected receptors and ion channels not only by neurotransmitters, but also by immune mediators secreted under inflammatory conditions accompanying pain development. These receptors are commonly expressed on various immune cells that, in turn, could be activated to either aggravate or alleviate inflammation-related pain [1]. Finally, immune cells, macrophages especially, produce endogenous opioids, suppressing the nociception process [2]. Simultaneously, macrophages and T lymphocytes express different types of opioid receptors (OpR) [3–6], and thus are prone to stimulation with endogenous and exogenous opioids.

Morphine, the most commonly used analgesic, is a selective agonist of μ-OpR, and buprenorphine is its partial agonist of high affinity and an antagonist of κ-OpR, although, at higher concentrations, also interacting with δ-OpR [7]. Stimulation of δ-OpR is also suspected to be responsible for antinociceptive effect of oxycodone in rodents [8], however this opioid can interact with μ-OpR [8] and κ-OpR [3,4,9].

Abbreviations: CHS, contact hypersensitivity; DPBS, Dulbecco's phosphate-buffered saline; HA, hemagglutination assay; OpR, opioid receptor; PCL, picryl chloride; PFA, hemolytic plaque forming assay; ROIs, reactive oxygen intermediates; SRBC, sheep red blood cells; TNP, trinitrophenol

☆ The authors declare that the manuscript in total, any of its parts or the research were not presented previously.

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Oxycodone is a highly selective, full agonist of human μ-OpR, with low affinity for δ-OpR and κ-OpR [10,11]. Buprenorphine is semisynthetic thebaine derivative acting as partial agonist of μ-OpR, ORL-1 full agonist, and κ-OpR and δ-OpR antagonist. Clinically, buprenorphine facilitates controlling of preoperative pain and is considered as not exacerbating opioid dependence provoked by prolonged overdosing of full μ-OpR agonists [12], which could also be avoided by methadone usage for neuropathic pain treatment, as it is acting through NMDA receptor. Initially tested vaccine against oxycodone abuse, stimulating B-cell generation of antibodies binding addictive drugs in serum to decrease its distribution to the brain [13] may also limit opioid dependence, while susceptibility to opioid treatment may be sex-related, stronger in males, which could result from more active innate immunity in females [14].

Macrophages play a very important role in innate and acquired immunity, as potent phagocytes that can present antigens to activate antigen-specific immune response. Macrophage-secreted cytokines are involved in immune response orchestration and regulation, and cytotoxic macrophages participate as effector cells in delayed-type hypersensitivity response, including its hapten-induced, cutaneous onset, i.e. contact hypersensitivity (CHS) reaction.

Our former research revealed the essential role of macrophages in immunomodulatory effects exerted by morphine, fentanyl and methadone in mice [2]. Generally, treatment of mice with these drugs inhibited humoral immune response, reduced surface expression of molecules involved in antigen phagocytosis and presentation and enhanced generation of reactive oxygen intermediates (ROIs) by macrophages. Further, in contrast to morphine, fentanyl and methadone administration led to suppression of CHS. Treatment with fentanyl decreased secretion of cytokines by macrophages, whereas morphine and methadone caused the opposite effect. The observed effects of morphine administration were partly reversed by co-treatment with naloxone. Apart from previously studied morphine, fentanyl and methadone, the strong opioids recommended and available in Poland for the management of severe pain also include oxycodone and buprenorphine [15]. However, little is known about the potential immunomodulatory impact of buprenorphine and oxycodone on immune system. Therefore, this study aimed to investigate the influence of buprenorphine and oxycodone, compared to morphine, on immune responses in mice under homeostatic conditions, with the special emphasis on possible alterations of immune functions of macrophages.

2. Materials and methods

2.1. Mice

Ten- to 12 week-old male mice (24 ± 3 g) of the inbred CBA strain were subjected to all experiments, that were conducted according to the guidelines of the 1st Local Ethics Committee in Krakow (approval No 123/2013). Animals were from the breeding unit of the Department of Immunology, Faculty of Medicine, Jagiellonian University Medical College, Krakow, Poland and were fed autoclaved food and water ad libitum. Mice were randomly assigned to control or treatment groups consisted of 5–6 animals. The general scheme of experiments is shown in the Fig. 1.

2.2. Opioid drug administration

Morphine sulfate (02DR0910, WZF Polfa S.A., Warsaw, Poland) was administered twice a day in a single dose of 20 mg/kg per mouse (morphine dose was chosen according to the literature [2] and in relation to the average effective dose used clinically), buprenorphine (01AF0512, WZF Polfa S.A., Warsaw, Poland) in a daily dose of 2 mg/kg per mouse was administered once a day, and oxycodone hydrochloride (AB465, Mundipharma Polska Sp. z o.o., Warsaw, Poland) was administered twice a day in a single dose of 20 mg/kg per mouse. The doses of buprenorphine and oxycodone were calculated according to their analgesic potencies in comparison to morphine. Opioid drugs were used as sterile phosphate buffered saline (PBS) solutions for intraperitoneal injections. Control mice were injected with vehicle alone. Donors of macrophages (Fig. 1a), mice actively sensitized with hapten or recipients of effector cells (Fig. 1b), were treated with the proper drug for, respectively, 7 or 6 consecutive days.

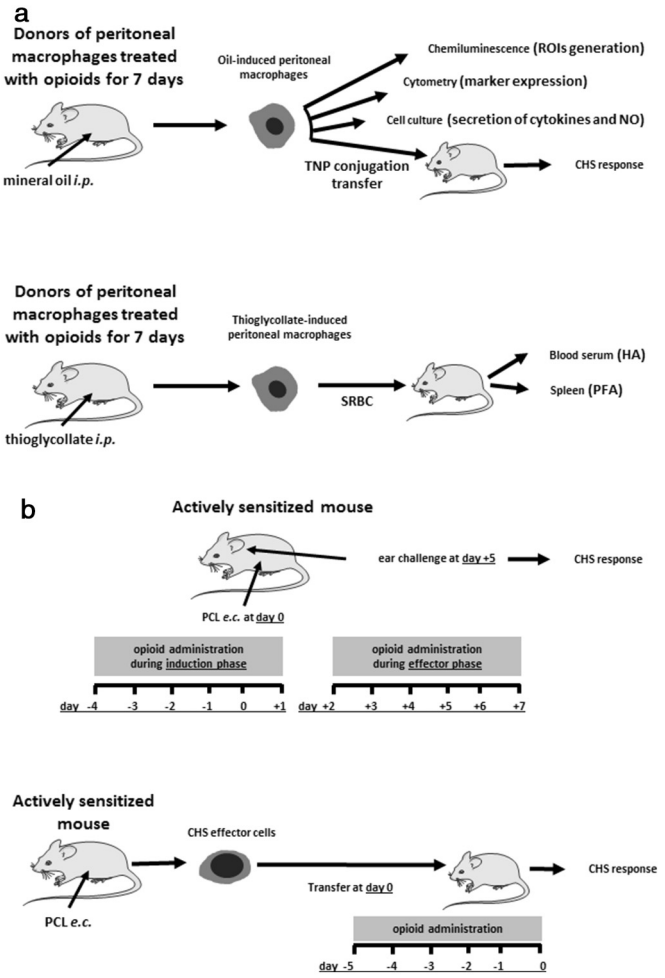

Fig. 1. The general scheme of experiments performed. (A) In each experiment mouse donors of peritoneal macrophages were treated for 7 days with each respective opioid. Oil-induced macrophages (upper panel), harvested from drug-treated mice 5 days after intraperitoneal injection of mineral oil, were tested either for their ability to generate reactive oxygen intermediates (ROIs) in a chemiluminescence assay, expression of surface markers in cytometry or were cultured to assess their secretion of cytokines and nitric oxide. Oil-induced macrophages from opioid-administered donors, after conjugation with trinitrophenyl (TNP) hapten, were also transferred intravenously into naive mice that, 5 days later, were challenged with picryl chloride (PCL) to elicit contact hypersensitivity (CHS) response measured as ear swelling. To assess humoral immune response, thioglycollate-induced macrophages (bottom panel), harvested from opioid-treated donors 5 days after thioglycollate intraperitoneal injection, were pulsed with sheep red blood cells (SRBC) and transferred intraperitoneally into naive recipients. Seven days later blood sera and spleens were collected individually to evaluate, respectively, the titers of specific antibodies in direct hemagglutination assay (HA) and the number of antibody-producing cells in hemolytic plaque forming assay (PFA). (B) Influence of opioids administered for 6 days on active CHS response was tested in both, induction and effector phases of this reaction (upper panel). Treatment with each opioid began either 4 days before (in the induction phase) or 2 days after (in the effector phase) epicutaneous application of PCL for sensitizing mice that were then challenged with PCL to elicit CHS response, measured as ear swelling. Impact of opioids on CHS response was also assessed in intravenous adoptive transfer of CHS effector cells (bottom panel) from actively sensitized, drug-unaffected donors to recipients treated with each opioid for 6 days prior to transfer. Recipient mice were immediately challenged with PCL to elicit CHS response, measured as ear swelling.