Do opioid receptors play a role in the pathogenesis of the inflammatory response in acute pancreatitis?[1]

Os receptores opioides desempenham papel na patogênese da resposta inflamatória na pancreatite aguda?

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ABSTRACT
PURPOSE: To investigate the effect of the opioid blocker naltrexone in the inflammatory response in acute pancreatitis (AP).
METHODS: Acute pancreatitis was induced in anesthetized male Wistar rats by retrograde injection of 2.5% sodium taurocholate diluted in 0.5ml saline into the main pancreatic duct. Animals were randomized to the following experimental groups: Control Group (n=9): animals received an intraperitoneal injection of saline solution (0.5ml), 15 minutes before the induction of AP. Naltrexone Group (n=9): animals received an intraperitoneal injection of naltrexone 0.5ml (15 mg/kg), 15 minutes before induction of AP. Peritoneal levels of TNF-α and serum levels of IL-6 and amylase were determined The volume of the ascitic fluid was also evaluated. Myeloperoxidase (MPO) activities were analyzed in homogenates of pulmonary tissue.
RESULTS: There were no significant differences in the ascitic fluid volume, nor in TNF-α and IL-6 levels in the naltrexone group compared to controls. Treatment with naltrexone did not affect the lung MPO activity compared to control group.
CONCLUSIONS: The opioid receptors don't play an important role in the pathogenesis of the inflammatory response in acute pancreatitis. If opioids affect leukocytes inflammatory signaling, there are no major implications in the pathogenesis of acute pancreatitis.
Key words: Pancreatitis. Physiopathology. Receptors, Opioid. Naltrexone. Rats.

RESUMO
OBJETIVO: Investigar o efeito do bloqueador opióide naltrexone na resposta inflamatória da pancreatite aguda.
METODOS: Pancreatite aguda foi induzida em ratos machos Wistar, através de injeção retrógada de solução de taurocolato de sódio a 2,5% nos ductos pancreáticos. Os animais foram alocados em dois grupos: Grupo controle (n=9) animais receberam 0,5 ml de solução salina intra-peritonial 15 minutos antes da indução da pancreatite aguda e Grupo naltrexone (n=9) animais receberam naltrexone (15mg/kg de peso), em 0,5 ml de volume final por via intraperitoneal, 15 minutos antes da indução da pancreatite aguda. Foram avaliados o volume de ascite, os níveis séricos de amilase e IL-6, assim como TNF-α peritoneal e a atividade da mieloperoxidase (MPO) no tecido pulmonar.
RESULTADOS: Não foram encontradas diferenças significantes nos parâmetros analisados entre o grupo que recebeu solução salina e o que recebeu naltrexone .
CONCLUSÕES: Os receptores opióides não desempenham papel importante na resposta inflamatória sistêmica associada à pancreatite aguda. Se os opioides alteram a sinalização inflamatória nos leucócitos está ação não se reflete na patogênese da pancreatite aguda
Descritores: Pancreatite. Fisiopatologia. Receptores Opióides. Naltrexona. Ratos.

Introduction

The opioid receptor family consists of the µ(MOR), δ(DOR) and κ (KOR) receptors, belonging to the family of seven transmembrane G-nucleotide binding protein-coupled receptors. They are found in the central nervous system and in peripheral neurons.

Immune cells also express opioid binding sites and modulation of immune functions (chemotaxis, cytokine production, degranulation, etc) by opioids have been reported[1]. Studies have also shown that immune cells play a role in the control of inflammatory pain, by the production of endogenous opioid peptides that accumulate in the inflamed tissue. Many substances (corticotrophin releasing factor, cytokines, catecholamines), as well as environmental factors (psychological stress, for example) can liberate these substances. These effects can be aborted by opioid receptor antagonists.

Endogenous opioids derived from immune cells, as α- and β-endorphin, dynorphin A and B, α- and β-neoendorphin and enkephalins produce potent analgesia. These molecules are the natural ligands for MOR, DOR and KOR. In the early phase of inflammation, most endogenous opioids are produced by granulocytes. Later, monocytes become the main source[2]. Exogenous opioids, however, are largely used in medicine to treat pain, in diseases such as acute pancreatitis.

Acute pancreatitis (AP) is an inflammation of varying severity, accounting for 210.000 cases per year in the U.S. or 50 cases per 100.000 inhabitants. It is due to intraparenchymal activation of pancreatic enzymes and is characterized by elevation of serum pancreatic enzymes, including: amylase, lipase, trypsin, cathepsin B, among others[3].

The injury of the pancreatic parenchyma results in leakage and activation of pancreatic enzymes in the interstitium. Activated enzymes also leak to peripancreatic tissues, the peritoneal cavity and the systemic circulation. The inflammatory process results both from local and systemic activation of inflammatory cells, with cytokine production not only in pancreatic tissue and peripancreatic areas, but also in many distant tissues. Tryptolytic pancreatic enzymes into the peritoneal cavity stimulate resident macrophages to produce cytokines that fall into the systemic circulation and contribute to the systemic inflammatory response[4].

During inflammation, numerous mediators are produced by endothelial cells, leukocytes and others, that elicit pain by activation of nocireceptors.

The severity of AP is related to the development of a systemic inflammatory response syndrome (SIRS), due to high cytokine levels, especially interleukin IL-1β, IL-6, IL-8, Tumor Necrosis Factor-α (TNF-α) and NO[5].

It seems obvious that reducing the levels of proinflammatory cytokines could reduce the deleterious effects of AP. Thus, various measures including anti-TNF-α, peritoneal lavage, pentoxifylline and hypertonic saline solution have been studied in order to reduce the systemic inflammatory response and, as a consequence, mortality[6].

Naltrexone hydrochloride, which is a drug used to treat alcoholism, has been studied among the several drugs that theoretically could reduce the inflammatory process in AP. It is an opioid receptor antagonist, a synthetic congener of oxymorphone, differing in its molecular structure, because the methyl group on the nitrogen atom is replaced by the cyclopropylmethyl group. Its beneficial effect on the inflammatory process and on the expression of proinflammatory cytokines has been already described[7,8].

Previous studies have shown a beneficial effect of naloxone, an analogue of naltrexone, in experimental AP, suggesting that endogenous opioid peptides may play an important role in the pathogenesis of AP[9,10]. In fact, Shen *et al*.[10] showed that these drugs may limit the progression of the disease, especially from the edematous to the hemorrhagic form, suggesting that endogenous opioid peptides may be linked to the pathophysiology of AP. These authors, however, did not evaluate the effects of these substances on the systemic inflammatory process.

We hypothesized that opioid receptors could play a role in the pathogenesis of the inflammatory response in acute pancreatitis.

This current study aims to determine the effects of the opioid blocker naltrexone on the inflammatory mediators in an experimental model of AP.

Methods

The experimental protocol was approved by the Ethics Commitee for Animal Research from the Medical School of Sao Paulo University. All animals received care in accordance with the Guide for the Care and Use of Laboratory.

Eighteen adult male Wistar rats weighing 240 to 260g, housed in individual cages in a 12-hour dark light-controlled environment, were used for the experimental protocol. Temperature was kept at 21 to 22ºC and all rats were fed with a standard rat chow and *ad libitum* water.

Reagents

All chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO). TNF-α, IL-6 and IL-10 were assayed with corresponding kits from BioSource International (Camarillo, LA, Calif).

Induction of acute pancreatitis

Surgical anesthesia was induced with ketamine chloride 50 mg/ml (0.2ml/100g body weight) (Ketalar; Parke-Davis, Sao Paulo, Brazil). AP was induced in anesthetized rats by retrograde intraductal injection of 0.5 ml of 2.5% (w/v) sodium taurocholate in 0.9% (w/v) NaCl into the main pancreatic duct for 1 minute at a constant rate using an infusion pump (KD Scientific, Holliston, MA). The proximal part of the hepatic duct was clamped during the injection[11].

Experimental groups

Animals were randomized into the following experimental groups:

Control Group (n=9): animals received an intraperitoneal injection of 0.5ml saline solution, 15 minutes before induction of AP.

Naltrexone Group (n=9): animals received an intraperitoneal injection of 0.5ml naltrexone (15 mg/kg), 15 minutes before induction of AP.

Sample preparations

At two hours after AP induction, animals were anesthetized for blood sampling through cardiac puncture and killed by exsanguination. Peritoneal levels of TNF-α and serum levels of IL-6 and amylase were assayed by a solid-phase sandwich enzyme-linked immune absorbent assay (ELISA). The volume of the ascitic fluid was also evaluated. Myeloperoxidase (MPO) activities were analyzed in homogenates of pulmonary tissue.

Pulmonary myeloperoxidase activity

MPO activity was used as an indicator of neutrophils infiltration in the tissues. Opioid peptides, moreover, are stored in primary granules and are released together with bactericidal enzymes, such as myeloperoxidase. Samples of lung tissue were homogenized with a Polytron homogenizer, using a homogenization buffer containing 0.5% of hexadecyltrimethyl ammonium bromide, 5 mmol/L EDTA and 50 mmol/L phosphate at pH 6.0. Homogenized samples were sonicated and centrifuged (3.000x*g*, 30 minutes) at 4ºC. MPO activity in the supernatant was assayed by measuring the change in A_{460} absorbance, resulting from the decomposition of H_2O_2 in the presence of O-dianisidine. Results were expressed as optical density (OD) at 460 nm[12].

Statistical analysis

The groups were compared using Student's t test and results are presented as mean values ± SEM. The level of p<0.05 was considered statistically significant. The data were analyzed using Graphpad Prism® 4.0 software (San Diego, CA, USA).

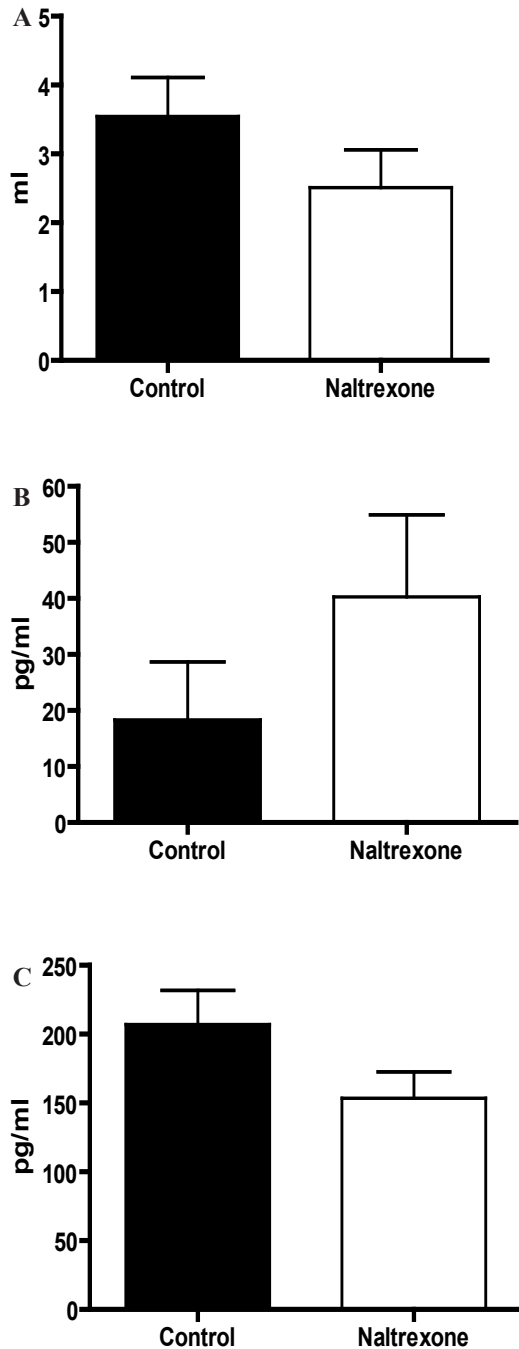**Results**

The elevated serum amylase levels found in the control group were not affected by naltrexone treatment (p=0.140) (Figure 1).
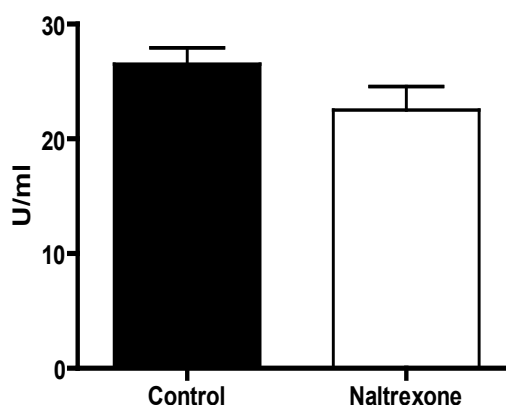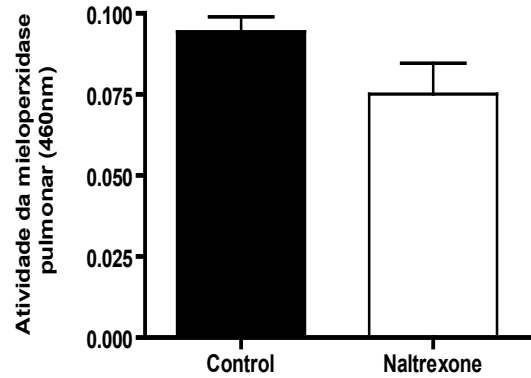

FIGURE 1 - Serum levels of amylase. AP was induced in rats by intraductal injection of 0.5ml of 2.5% taurocholic acid. *Control group* received saline solution administration 15 minutes before AP induction and *Naltrexone group* received naltrexone (15mg/Kg) 15 minutes before AP induction. Data are expressed as mean ± SEM of nine animals per group.

There were no significant differences in the ascitic fluid volume (p=0.208), in TNF-α levels in the ascitic fluid (p=0.234) and in IL-6 serum levels (p=0.107) of the group control, compared to the naltrexone group (Figures 2A, B and C).

FIGURE 3 - Pulmonary myeloperoxidase activity. AP was induced in rats by intraductal injection of 0.5ml of 2.5% taurocholic acid. *Control group* received saline solution administration 15 minutes before AP induction and *Naltrexone group* received naltrexone (15mg/Kg) 15 minutes before AP induction. Data are expressed as mean ± SEM of nine animals per group.

Discussion

Even though in most cases AP is presented in mild or moderate clinical forms, in severe cases morbidity and mortality rates are extremely high. Several studies demonstrated that the severity of the disease is related to the pro-inflammatory cytokines liberated locally and systemically. The possibility of inhibiting the production of cytokines or neutralizing their effects, could minimize the systemic inflammatory response in AP and, therefore, reduce its mortality. Several experimental studies demonstrated that blocking the production of pro-inflammatory cytokines reduces the local and the systemic damage in acute pancreatitis. In fact, it has been demonstrated that several substances were effective in decreasing the pro-inflammatory cytokines in AP, such as pentoxifylline, oxypurinol and hypertonic solution, among others[6].

Opioid receptors have been implicated in the amplification of the inflammatory process and several publications have proposed their implication in the pathogenesis of AP[7-10].

Although opioid receptors alone do not cause inflammation, there is no doubt that they can stimulate lymphocytes and macrophages as well as cytokines production. Indeed, opioid receptors blockade actually reduces inflammation in chemically induced colitis[13]. In fact, naltrexone, an opioid antagonist, is able to block acute endotoxic shock by inhibiting TNFα production[14].

Deep comprehension of the mechanisms that link opioid receptors and immunity are still lacking, but evidence suggests that the interaction of receptors of the central nervous system with the immune system, perhaps via the Hypothalamic-Pituitary-

FIGURE 2 - Ascitic fluid volume and Inflammatory cytokines. AP was induced in rats by intraductal injection of 0.5ml of 2.5% taurocholic acid. *Control group* received saline solution administration 15 minutes before AP induction and *Naltrexone group* received naltrexone (15mg/Kg) 15 minutes before AP induction. Ascitic fluid volume **(A)** Concentration of peritoneal TNF-α **(B)** Serum IL-6 levels **(C)** Data are expressed as mean ± SEM of nine animals per group.

Treatment with naltrexone did not affect lung MPO activity compared to control group (p=0.101) (Figure 3).

Neuroendocrine Axis or by the peripheral nerve play an important role[15].

As already mentioned, Shen *et al.*[10] demonstrated that the opioid receptor antagonist naloxone has a beneficial effect in an experimental model of AP in dogs, blocking the progression of the edematous form to the hemorrhagic form.

In the present study, although we have seen a reduction of the ascitic fluid with the administration of naltrexone, this reduction was not statistically significant (Figure 2A). There was even increased concentration of TNFα in the ascitic fluid with the use of naltrexone, without statistical significance (Figure 2B). The concentration of serum IL-6, although stood at levels somewhat lower in the naltrexone group was not statistically significant as well (Figure 3). The same happened with the serum amylase levels (Figure 1).

Lung myeloperoxidase (MPO) levels also failed to show any difference with the administration of naltrexone. MPO levels assess the systemic action of naltrexone (Figure 3).

There are no clear detrimental effects, thus, of using exogenous opioids to critically ill patients suffering from AP, other than the classic side effects (nauseas, vomiting, diarrhea, etc). Prolonged opioids administration, however, may lead to tolerance. Since some studies suggest that endogenous secretion of opioids may counteract opioids tolerance and peripherally acting opioid lack the central side effects, chemokines receptors agonists are under investigation as a pharmacological tool to stimulate the migration of opioid peptide-containing leucocytes to treat pain. Further studies are necessary to show if endogenous and exogenous opioids significantly increase the pancreatic and peri-pancreatic inflammatory damage and if opioid receptors blockers could decrease pancreatic lesion. If so, these effects have no implications in severe acute pancreatitis where the explosive systemic inflammatory response leads to injury at distant organs and a cytokine storm that is unresponsive to opioid receptors manipulation.

Conclusion

Opioid receptors don't play an important role in the pathogenesis of the systemic inflammatory response in acute pancreatitis.

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