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ORIGINAL ARTICLE

Chemotaxis of human and rat leukocytes by the delta-selective non-peptidic opioid SNC 80

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ABSTRACT. Opioids like morphine, represent a major source of relief for most chronic moderate to severe nonmalignant pain. However, opioid abuse may lead to infections such as hepatitis and AIDS because opioids have been associated with suppressing various parameters of immune function including antimicrobial resistance, antibody production, monocyte-mediated phagocytosis, and both neutrophil and monocyte chemotaxis. We have previously reported immunopotentiating properties of non-peptidic opioid receptor selective agonists and antagonists. In this study, we evaluated the effects of the non-peptidic delta-opioid receptor agonist (+)-4-((alpha R)-alpha-((2S, 5R)-4-allyl-2, 5-dimethyl-1-piperazinyl)-3-methoxybenzyl)-N, N-diethyl-benzamide (SNC 80) on chemotaxis of rat thymic and human peripheral blood mononuclear cells by using a modified Wilkinson chamber. Cell recruitment is an essential process in acute and chronic inflammatory responses. We observed that SNC 80 at concentrations of 10^{-10},10^{-9},10^{-8},10^{-7}, and 10^{-6} M, significantly ($p < 0.01$) stimulated rat thymic (1.3,1.55,1.58,1.75, and 1.8-fold increases respectively) and human leukocyte (1.13,1.37,1.43,1.7,1.83 fold-increases respectively) chemotaxis (demonstrated by checkerboard assays), compared with untreated control. The effects of SNC 80 on chemotaxis of rat and human leukocytes were antagonized by naloxone, indicating that the modulation of chemotaxis by SNC 80 is via a classic opioid receptor. The development and use of non-peptidic opioids like SNC 80 could have an immediate impact not only as potent analgesics, but in immunoregulation.

Key words: Delta opioid, SNC 80, chemotaxis, leukocytes, rat, human.

RESUMEN. Opioides como la morfina, representan la principal fuente de alivio para la mayor parte de los casos de dolor crónico no maligno, moderado o severo. Sin embargo, el abuso de opioides puede llevar a padecer de infecciones tales como la hepatitis y el SIDA, debido a que estas sustancias se han asociado con la supresión de varios parámetros de función inmune incluyendo a la resistencia antimicrobiana, la producción de anticuerpos, la fagocitosis mediada por monocitos/macrófagos, y la quimiotaxis de neutrófilos y monocitos. Nosotros hemos reportado previamente las propiedades inmunopotenciadoras de agonistas y antagonistas selectivos de receptores de opioides no peptídicos. En el presente estudio, se evaluaron los efectos del agonista opioide no peptídico del tipo delta (+)-4-((alfa R)-alfa-((2S, 5R)-4-alil-2, 5-dimetil-1-piperazinil)-3-metoxibenzil)-N, N-dietil-benzamiae (SNC 80) en la quimiotaxis de linfocitos tímicos de rata y de células mononucleares de sangre periférica humana, utilizando una cámara de Wilkinson modificada. Se observó que el SNC 80 a las concentraciones de 10^{-10},10^{-9},10^{-8},10^{-7}, y 10^{-6} M, estimuló significativamente ($p < 0.01$) la quimiotaxis (demostrado mediante pruebas de tablero) de linfocitos tímicos de rata (1.3,1.55,1.58,1.75, y 1.8 veces de incremento respectivamente) y leucocitos humanos (1.13,1.37,1.43,1.7, y 1.83 veces de incremento respectivamente) comparado con el control sin tratar. Los efectos del SNC 80 sobre la quimiotaxis de leucocitos de rata y humanos se antagonizaron con el uso de naloxona, indicando que la modulación de la quimiotaxis inducida por este opioide es a través de un receptor clásico de opioides. El desarrollo y uso de opioides no peptídicos como el SNC 80 podrían tener un impacto inmediato no sólo como analgésicos potentes, sino en inmunorregulación.

Palabras clave: Opioide delta, SNC 80, quimiotaxis, leucocitos, rata, humano.

INTRODUCTION

Opioids like morphine represent a major source of relief for most chronic moderate to severe nonmalignant pain (Passik & Weinreb, 2000). However, opioid abuse may lead to infections such as hepatitis, tetanus, liver disease, and AIDS because opioids have been associated with suppressing various parameters of immune function including antimicrobial resistance, antibody production, delayed-type hypersensitivity, production of the cytokines IL-1, IL-6 and TNF-alpha, natural killer (NK) cell cytotoxic activity, monocyte-mediated phagocytosis, and both neutrophil and monocyte chemotaxis (Haverkos, 1987; Watson *et al*., 1988; Weber *et al.*, 1991; Arora *et al*., 1990; Rogers *et al*., 2000; Gomez-Flores R & Weber, 1999; Perez-Castrillon *et al*., 1992). Chemotaxis (directional cell movement toward a chemoattractant gradient) requires a cell polarity and the ability of the cell to respond to a directional signal (chemokine) following stimulation of a chemokine receptor (Chung *et al*., 2001). Chemokines are responsible for the recruitment of T and B lymphocytes, monocytes, neutrophils, eosinophils and basophils in acute and chronic inflammatory conditions. They function as G protein-coupled chemotactic factors (Kaplan, 2001).

Opioids, mainly mu and kappa agonists, have been associated with suppression of chemokine-mediated migration

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of monkey (Miyagi *et al*., 2000), rhesus macaque (Liu *et al*., 1992), and human (Perez-Castrillon *et al*., 1992; Hu *et al*., 2000; Hofbauer *et al*., 1998) leukocytes, which depended on binding to opioid receptors (Makman *et al*., 1995). However, chemokine-enhanced human neutrophil chemotaxis was shown for the opioid peptides met-enkephalin and beta-endorphin at lower concentrations, whereas at higher concentrations they were immunosuppressive (Pasnik *et al*., 1999). Interestingly, Rogers *et al*. (2000) showed that opioids and chemokines downregulate their own leukocyte migratory activities by a bidirectional heterologous desensitization of opioid and chemokine receptors (Rogers *et al*., 2000). Similarly, Grimm *et a*l. (1998) showed that met-enkephalin and morphine inhibited IL-8-induced chemotaxis of human neutrophils (Grimm *et al*., 1998). Thus, inhibition of chemokine-induced chemotaxis by opioids may contribute to the immunodeficiencies observed in drug abusers, and may have implications for immunomodulation induced by endogenous neuropeptides (Grimm *et al*., 1998).

SNC 80 ((+)-4-[(alpha R)-alpha-((2S, 5R)-4-allyl-2, 5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N, N-diethylbenzamide) is a non-peptide, highly selective delta-opioid receptor agonist which has been reported not to alter rat NK cell, lymphocyte, and macrophage functions following intracerebroventricular administration (Nowak *et al*., 1998; Bilsky *et al*., 1995). However, *in vitro* and intravenous administration of SNC 80 was shown to increase tumor necrosis factor-alpha and nitric oxide production by rat macrophages (Gomez-Flores & Weber, 2001). In addition, Sharp et al. demonstrated that SNC-80 inhibited the production of p24 antigen, an index of HIV-1 expression (Sharp *et al*., 2001). Some disadvantages to using peptidic opioids include their rapid degradation and their low potential to cross the blood brain barrier, thus limiting their clinical applications (Hambrook *et al*., 1976). In contrast, non-peptide opioid agonists have proven to overcome major disadvantages of peptidic opioids because they are not only highly selective and potent, but also proteolytically stable.

The present study was designed to extend our studies of the effects of SNC 80 on immunoregulation to leukocyte chemotaxis following *in vitro* stimulation. We found that SNC 80 stimulated chemotaxis of resident rat and human leukocytes in a dose-dependent fashion.

MATERIAL AND METHODS

Reagents and culture media. Penicillin-streptomycin solution, L-glutamine, and RPMI 1640 and AIM-V media were obtained from Life Technologies (Grand Island, NY). (+)-4-[(alpha R)-alpha-((2S,5R)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-N,N diethylbenzamide (SNC 80) was synthesized as previously described (Calderon *et al*., 1994).

Animals. Sprague-Dawley male rats (200-220 g) were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN). They were housed in a pathogen free environment, 2-4 animals per cage, fed an *ad libitum* diet of laboratory food pellets and water, and were maintained on a schedule of 12 hours light (0,600-1,800) and 12 hours dark (1,800-0,600) at 21 ± 3 ºC. Animals were euthanized by asphyxiation in 100% CO_2 chamber.

Drug preparation. A stock solution of SNC 80 (1×10^{-3} M) was prepared by dissolving 0.9 mg of this compound in sterile distilled water containing 5% DMSO plus 2.5% 0.1 N HCl, and diluted in pyrogen-free saline solution at appropriate concentrations. The final concentration of solvents in the SNC 80 preparation did not alter cell viability (data not shown) (Kansal *et al*., 1997).

Cell preparation. Thymus was immediately removed after rat death. A single-cell suspension was prepared by disrupting this organ in RPMI 1,640 medium. Cell suspension was washed twice with this medium, and suspended in AIM-V medium. Since serum has been reported to activate leukocytes (Chen *et al*., 1994) and to induce leukocyte chemotaxis (Wilkinson & McKay, 1971), the culture medium was changed at this step to the serum-free medium AIM-V, which has been observed to support leukocyte culture (Kaldjian *et al*., 1992). Human mononuclear leukocytes were isolated from peripheral blood of healthy donors using Ficoll-Hypaque gradient (Sigma Chemical Co., St. Louis, MO), then the mononuclear cells were recovered and washed twice with RPMI-1640, and suspended in AIM-V medium.

Chemotaxis assay. Chemotaxis was determined according to Wilkinson (Wilkinson, 1975; Gomez-Flores *et al*., 1990) using 8-µm pore size filters (Millipore Corporation, Bedford, MA). The cell-free lower compartment of each chamber contained the different concentrations of SNC 80, AIM-V medium alone (negative control), and casein (3 mg/ml) (positive control) in a final volume of 5 ml. The upper compartment of each chamber contained 0.2 ml of 2×10^6 cells/ml rat or human leukocytes. Chambers were incubated for 3 hr at 37°C. After incubation chambers were disassembled and filters were fixed in ethanol, stained with hematoxylin, processed in the usual manner (Wilkinson, 1975; Gomez-Flores *et al*., 1990) and examined with light microscopy. For quantitative access, the lower surface counting method was used, and the total number of migrating cells per filter was counted. Averages of at least triplicate determinations in each experiment were recorded. Data were plotted as the result of placing 1×10^6 cells/syringe in the upper compartment.

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Checkerboard Assays. To distinguish leukocyte chemotaxis from chemokinesis induced by SNC 80, checkerboard assays were performed according to Zigmond and Hirsch (Zigmond & Hirsch, 1973). In brief, SNC 80 was placed alone in the lower compartment (positive gradient of chemotactic factor), in both compartments (absence of gradient), or in only the upper compartment (negative gradient) of a chemotaxis chamber. The lower compartment contained only SNC 80 diluted at 10^{-7}M or AIM-V medium, and the upper compartment contained 2 X 10^6 rat or human leukocytes per ml, in AIM-V medium or in test material. Other leukocyte chemotaxis-stimulating concentrations of SNC 80 were also tested, providing similar response trends to those observed with 10^{-7} M (data not shown).

Opioid receptor antagonism test. Rat thymus and human leukocytes were adjusted to 2 x 10^6 cells/ml and placed in the upper compartments of the chemotaxis chamber. They were incubated with naloxone or SNC 80 at 10^{-7}M each, and SNC 80 in combination with naloxone (10^{-7} M) which were placed in the lower compartment. The cultures were then processed according to the chemotaxis assay described above. % Chemotaxis stimulation represents the increase of SNC 80 and/or naloxone-mediated chemotaxis compared to untreated control. Naloxone at 10^{-9} M was also tested against 10^{-7} M of SNC 80, resulting in similar results from those observed with 10^{-7} M naloxone (data not shown).

***Viability assay*.** Cell viability was assessed by the MTT reduction assay as described elsewhere (Gomez-Flores and Weber, 2001). In brief, resident, SNC80 or naloxone-treated rat thymic lymphocytes (100 µl of 2 x 10^6 cells/ml) were cultured in AIM-V medium for 24 hours. Next, lymphocyte cultures were incubated with MTT (0.5 mg/ml, final concentration) for 1.5 h at 37ºC. Formazan crystals were then solubilized by using lysing buffer, after which optical densities were measured at 570 nm (Gomez-Flores et al., 1995).

Statistical analysis. The results were expressed as mean ± SEM of the response of 3 separate rat thymuses to each treatment (SNC 80 and/or naloxone groups) (3 replicate determinations per treatment from a representative experiment. All experiments were repeated at least three times with similar results. Level of significance was assessed by Student's *t* test.

RESULTS

Effect of SNC 80 on chemotaxis of rat thymic leukocytes. As shown in Figure 1, *in vitro* treatment with SNC 80 significantly ($p < 0.01$) stimulated migration of rat thymic leukocytes (1.2- to 1.7-fold increases at concentrations ranging from 10^{-10} to 10^{-6} M respectively). A peak response was observed at 10^{-7} M since it was not significantly different ($p = 0.147$) from that at 10^{-6} M; concentrations higher than 10^{-6} M were toxic for the cells (Gomez-Flores and Weber, 2001), and were not utilized. SNC 80 stimulated a chemotactic response the treatments "absence of gradient" and "negative gradient" failed to induce leukocyte migration in response to SNC 80 (Fig. 2).

Effect of SNC 80 on chemotaxis of human leukocytes. In vitro treatment with SNC 80 significantly ($p < 0.01$) stimulated migrating response of human leukocytes (1.34- to 1.8-fold increases at concentrations ranging from 10^{-9} to 10^{-6} M respectively) (Fig. 3). This response was chemotactic and directed since the treatments "absence of gradient" and "negative gradient" failed to induce leukocyte migration in response to SNC 80 (Fig. 4).

Effect of naloxone on SNC-mediated rat and human leukocyte chemotactic activity. Treatment with naloxone completely suppressed ($p < 0.01$) chemotactic activity of SNC 80 towards rat and human leukocytes (Fig. 5). Naloxone alone did not significantly alter migratory responses of rat and human leukocytes in AIM-V medium (Fig. 5). Incubating the cells with naloxone in the upper chamber abrogated the migrating response or rat and human leukocytes towards SNC 80 (data not shown).

***Viability assay*.** SNC 80 and naloxone at concentrations ranging from 10^{-8} M to 10^{-6} M did not alter viability of rat lymphocytes and human peripheral blood mononuclear cells (Fig. 6).

DISCUSSION

Chemokines are not only inducible mediators of inflammation, but also are involved in modulation of angiogenesis, lymphocyte maturation, and leukocyte physiological traffic and homing (Taub & Oppenheim, 1994). They stimulate the recruitment of immunocompetent cells to inflammatory sites. Their inhibition may negatively affect the inflammatory process, while their activation might promote it and enhance wound healing and tissue repair (Taub & Oppenheim, 1994). *In vivo* chemotaxis depends on a cascade of events leading to the recruitment of leukocytes to sites of pathological damage. This includes production of chemotactic factors by leukocytes (stimulation of chemotaxis by bacterial products may be also involved), activation of leukocyte adhesion molecules, and extravasation to the inflammation site. Significant alteration or deficiency in any of these processes, will negatively affect the final outcome of an homeostatic response which leads to the elimination of the inflammation-inducing agent. In this regard, there is a congenital disorder named *leukocyte adhesion deficiency* (LAD) caused by the absence or critically reduced cell surface expression of leukocyte integrin molecules which are required for the normal processes of leukocyte adherence and chemo-

taxis; this deficiency causes life-threatening bacterial and fungal infections (Kuijpers *et al*., 1997). There are other abnormalities in which neutrophil chemotaxis is impaired, these include familial Mediterranean fever; psoriasis vulgaris, Behcet's syndrome and Sweet's syndrome (Matzner, 1987). In addition, neutrophils from HIV-infected patients have abnormalities in chemotaxis, phagocytosis, and bacterial killing (Pitrak, 1999).

Opioids are usually utilized to treat enduring severe pain in patients with life-limiting illness such as cancer, congestive heart failure, chronic obstructive pulmonary disease, and other organ system failure as the disease progresses (Pacl, 2001). However, some of the side effects of opioids include depression/affective and anxiety/panic disorders, drug abuse, alcohol abuse, and immunomodulation (Ader *et al*., 1985). In this regard, it has been shown the direct effect of selective opioids on leukocytes to enhance, suppress, or have no effect on *in vitro* parameters of immune function (Gomez-Flores & Weber, 1999). Opioids can modulate immune function indirectly by activating opioid receptors within the nervous system which stimulates the activity of neuroendocrine axes or neurotransmission pathways. However, direct immunomodulation results from the effects of opioids on cells of the immune system. This requires the expression of membrane opioid receptors in these cells. Opioid agonists have been demonstrated to bind to μ, κ, and δ opioid receptor types on the surface of lymphocytes, monocytes, and NK cells. Binding and activation of these receptors by opioid agonists has been observed to alter resistance to a variety of infectious agents, including the human immunodeficiency virus (HIV) (Gomez-Flores & Weber, 1999), and augment cancer development, as reported in several studies showing an increase of metastasis in different models of tumor growth (Ishikawa *et al*., 1993). Opioids are also involved in altering leukocyte proliferation and differentiation, cytotoxicity, cytokine and antibody production, phagocytosis, chemotaxis, and signal transduction pathways (Rogers *et al*., 2000; Gomez-Flores & Weber, 1999). Modulation of the inflammatory response appears to be a target of these compounds, including effects on lymphocyte and NK cell function, phagocytic activity, as well as the response of leukocytes to various chemoattractant molecules (Gomez-Flores & Weber, 1999; Perez-Castrillon *et al*., 1992; Liu *et al*., 1992; Hu *et al*., 2000; Hofbauer *et al*., 1998).

SNC 80 has been proven to be a potent opioid analgesic acting at the δ-opioid receptor (DOR), in both rats and Rhesus monkeys (Bilsky et al., 1995; Negus et al., 1998). SNC 80 has also been reported to possess immunomodulatory activity after in vitro and intravenous administration particularly by increasing tumor necrosis factor-alpha and nitric oxide production by rat macrophages (Gomez-Flores & Weber, 2001). Stimulation of leukocyte chemotaxis by SNC 80, as shown in the present study, might be considered an additional evidence of the immunomodulatory properties of this opioid, since chemotaxis is a critical step in the process of inflammation. Furthermore, we have recently observed in an *in vivo* murine tumor model, that administration of SNC 80 was associated with reduction in tumor volume and weight, and increase in mouse survival (data not published), which might be associated with stimulation of an inflammatory process leading to recruitment of leukocyte to the tumor site, and consequently an improved defense against the development of this tumor. In the present study, we showed that *in vitro* exposure to SNC 80 stimulated rat and human leukocyte migratory response in a dose-dependent fashion (we have used the general term "leukocytes" because it was not possible for us to define the specific type of migrating cells, particularly for human cells, under light microscopy and typical staining procedures, although most of these cells have a diameter between 6 and 8 microns, and certainly most of thymic cells were T lymphocytes). This response was chemotactic (directed migration) rather than chemocinetic (random migration), and was antagonized by naloxone (a specific opioid receptor antagonist), confirming that SNC 80-induced chemotaxis depended on opioid receptors on leukocytes. SNC80 and naloxone wer observed no to alter rat and human leukocyte viability (Fig. 6). The observed chemotactic response of lymphocytes toward SNC 80 appeared to be dependent on the concentration of the drug (Figs. 1 and 3). However, a peak response was noticed at 10^{-7} M for both human and rat leukocytes. As mentioned before, we did not include data on lymphocyte migrating response to SNC 80 at concentrations higher than 10^{-6} M since they were toxic for the cells (Gomez-Flores and Weber, 2001). It is common for others to report a bell-shaped type chemotaxis when using opioids and leukocytes (Rogers *et al*., 2000; Szabo *et al*., 2003). In this regard, the response drop in the curve is explained as saturability of chemokine receptors on leukocytes which desensitizes them to the action of the chemokines (Rogers *et al*., 2000), but it may be due, although not evidenced, to cell toxicity as well. In contrast, others have shown a "typical" dose-response curve by stimulating human peripheral mononuclear cells, lymphocytes and monocytes with antithrombin (Kaneider *et al*., 2002). We have noticed differences in the migrating response of human *vs* rat leukocytes (Figs. 1 and 3), and observed about 50% less migrating response of human cells towards SNC80. This may be due to the relative purity of the different populations utilized, thymic cells (mostly T lymphocytes) *vs* human peripheral blood mononuclear cells (T and B lymphocytes, monocytes, and neutrophils), which then may suggest that T lymphocytes are the primary target of the opioid.

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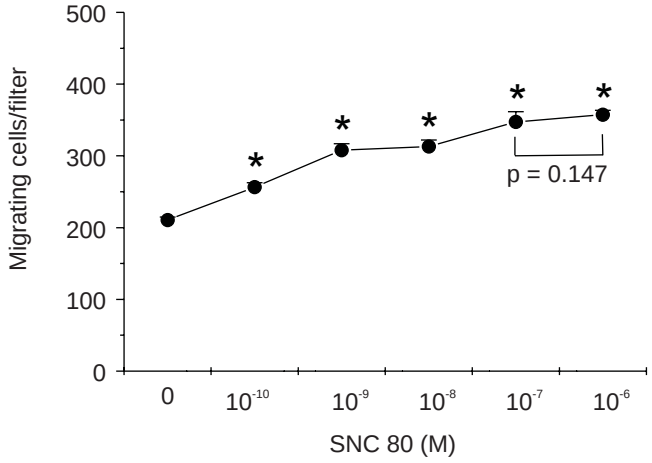

Figure 1. *Rat thymic leukocyte chemotaxis induced by SNC 80. The cell-free lower compartment of each chamber contained SNC 80 and the upper compartment contained 2 X 10^6 rat leukocytes per ml. Chemotaxis was then determined as described in the text. Data represent mean ± SEM of triplicates from a representative experiment. *p < 0.01 compared to AIM-V medium untreated control (213 ± 7 migrating cells per 1 X 10^6 cells/ml). Leukocyte migrating response to casein (positive control) was 402 ± 35 migrating cells per 1 X 10^6 cells/ml.*

We showed that resident leukocytes were highly stimulated to migrate by SNC 80. This is relevant because it has been shown that activated, but not resident lymphocytes, respond by locomotion and chemotaxis to cytokine attractants including IL-15 and IL-2 and several chemokines (Wilkinson *et al*., 1997). It is known that chemotaxis constitutes the first line of defense in the immune system, and chemokines function as chemoattractants for pro-inflammatory cells recruiting them from the blood to sites of infection. It has been shown, however, that chemokine-induced chemotaxis of monkey leukocytes was inhibited by mu-opioid agonists and reverted by naloxone (Choi et al., 1999). We have previously reported the immunomodulatory effects of *in vitro* and intravenous administration of SNC 80 to increase tumor necrosis factor-alpha and nitric oxide production by rat macrophages (Gomez-Flores and Weber, 2001), increase expression of TNF-α and IL-8 mRNA signals following stimulation of whole human peripheral blood mononuclear cells, and stimulate rat lymphoproliferation in a time- and dose-dependent fashion (unpublished observations). Activated macrophages secrete several substances such as TNF-α, that are directly involved in tissue inflammation, tumor cell killing and antimicrobial activity (Klimp *et al*., 2002; Kamada *et al*., 2000; Gomez-Flores *et al*., 1997). Stimulation of leukocyte

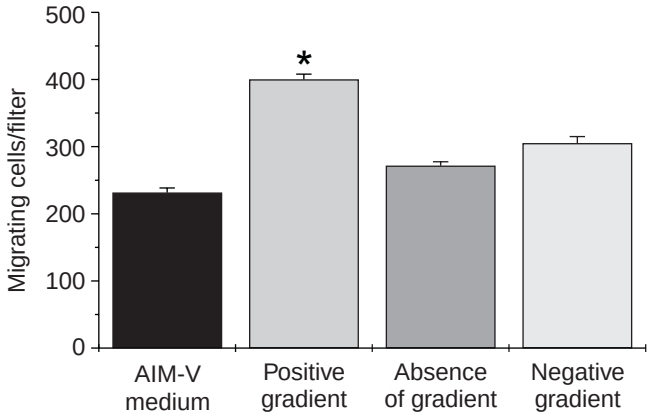

Figure 2. *Rat thymic leukocyte chemotaxis checkerboard assay. SNC 80 was placed alone in the lower compartment (positive gradient), in both compartments (absence of gradient), or in only the upper compartment (negative gradient). The lower compartment contained only SNC 80 diluted at 10^{-7} M or AIM-V, and the upper compartment contained 2 X 10^6 rat thymic leukocytes per ml, in AIM-V medium or in SNC 80. Chemotaxis was then determined as explained in the text. Data represent mean ± SEM of triplicates from a representative experiment. *p < 0.01 compared to AIM-V medium untreated control (213 ± 7 migrating cells per 1 X 10^6 cells/ml). Leukocyte migrating response to casein (positive control) was 402 ± 35 migrating cells per 1 X 10^6 cells/ml.*

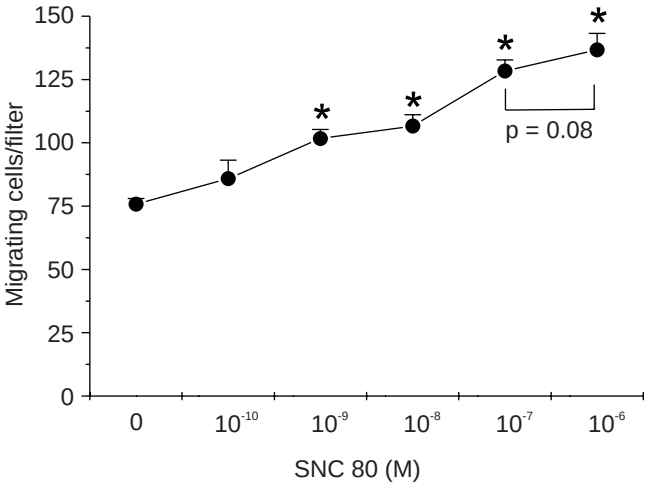

Figure 3. *Human peripheral blood leukocyte chemotaxis induced by SNC 80. The cell-free lower compartment of each chamber contained SNC 80 and the upper compartment contained 2 X 10^6 human leukocytes per ml. Chemotaxis was then performed as described in the text. Data represent mean ± SEM of triplicates from a representative experiment. *P < 0.01 compared to AIM-V medium untreated control (75 ± 4 migrating cells per 1 X 10^6 cells/ml). Leukocyte migrating response to casein (positive control) was 138 ± 12 migrating cells per 1 X 10^6 cells/ml.*

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chemotaxis by SNC 80, as shown in the present study, might be considered an additional evidence of the immunomodulatory properties of this opioid, since chemotaxis is a critical step in the process of inflammation. Finally, we have recently observed in an *in vivo* murine tumor model, that administration of SNC 80 was associated with reduction in tumor volume and weight, and increase in mouse survival (unpublished data) which might be associated with stimulation of an inflammatory process leading to recruitment of leukocyte to the tumor site and consequently an improved defense against the development of this tumor. In addition to antinociceptive properties (Bilsky et al., 1995; Calderon et al., 1994) and non-immunosuppressive effects (Nowak et al., 1998), or even immunopotentiating activity (Gomez-Flores and Weber, 2001), SNC 80 has been reported to inhibit the production of p24 antigen (an index of HIV-1 expression) in human DOR-transfected T cells, which may indicate that SNC 80 possesses adjuvant activity to anti HIV-1 standard therapy (Sharp et al., 1998).

Of interest, bi-directional heterologous desensitization of opioid and chemokine receptors, as shown by Rogers *et al*. (Rogers *et al*., 2000) and Grimm *et al*. (Grimm *et al*., 1998), and more recently by Szabo *et al*. (2002), may have a role in the immunodeficiencies observed in drug abusers, and particularly relevant for the immunomodulatory effects

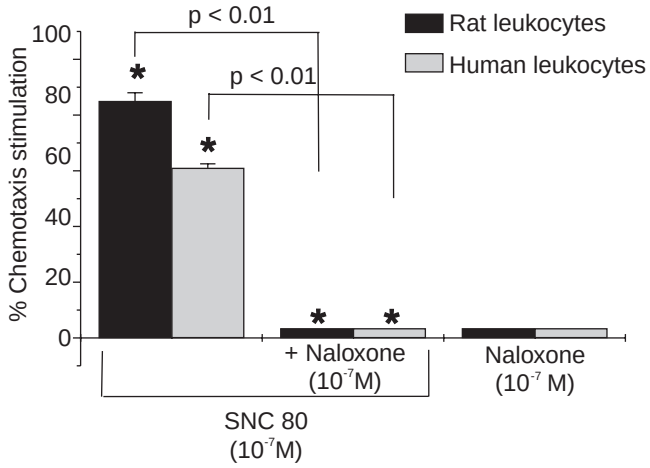

Figure 5. *Suppression of rat and human leukocyte chemotaxis by naloxone. Rat thymus and human peripheral blood leukocytes were adjusted to 2 x 10^6 cells/ml and placed in the upper compartments of the chemotaxis chamber. They were incubated with naloxone or SNC 80, and SNC 80 in combination with naloxone which were placed in the lower compartment. The cultures were then processed according to the chemotaxis assay described above. % Chemotaxis stimulation represents the increase of SNC 80 and/or naloxone-mediated chemotaxis compared to untreated control. Data represent mean ± SEM of triplicates from a representative experiment. *P < 0.01 compared to AIM-V untreated control (rat and human leukocyte migrating responses to AIM-V medium control were 213 ± 7 and 75 ± 4 per 1 X 10^6 cells/ml respectively, whereas their respective migrating response to casein (positive control) was 402 ± 35 and 138 ± 12 migrating cells per 1 X 10^6 cells/ml).*

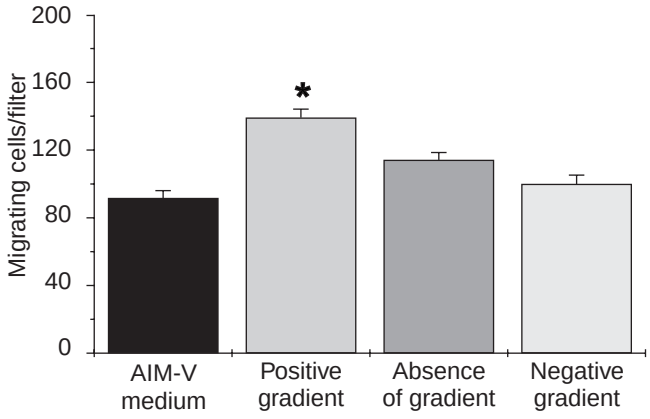

Figure 4. *Human leukocyte chemotaxis checkerboard assay. SNC 80 was placed alone in the lower compartment (positive gradient), in both compartments (absence of gradient), or in only the upper compartment (negative gradient). The lower compartment contained only SNC 80 diluted at 10^{-7}M or AIM-V, and the upper compartment contained 2 X 10^6 human leukocytes per ml, in AIM-V medium or in SNC 80. Chemotaxis was then performed as detailed in the text. Data represent mean ± SEM of triplicates from a representative experiment. *P < 0.01 compared to AIM-V medium untreated control (75 ± 4 migrating cells per 1 X 10^6 cells/ml). Leukocyte migrating response to casein (positive control) was 138 ± 12 migrating cells per 1 X 10^6 cells/ml.*

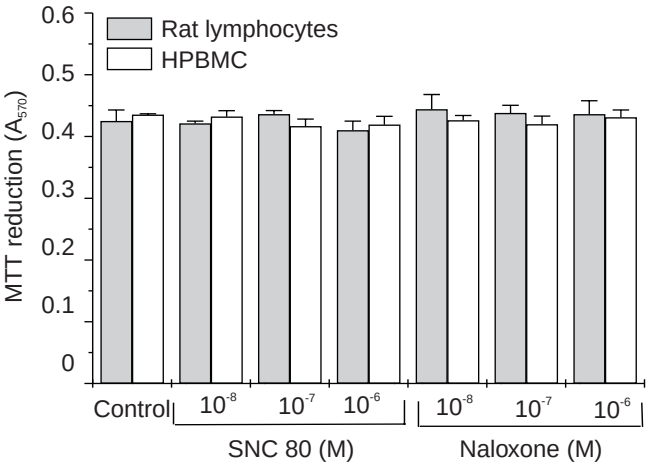

Figure 6. *Effect of SNC 80 and naloxone on viability of rat and human leukocytes. Rat thymic lymphocytes and human peripheral blood leukocytes were adjusted to 2 x 10^6 cells/ml and incubated in the presence or absence of SNC80 or naloxone in AIM-V medium for 24 hours, after which lymphocyte cultures were incubated with MTT (0.5 mg/ml, final concentration) for 1.5 h at 37°C. Optical densities were then measured at 570 nm. Data represent mean ± SEM of triplicates from a representative experiment.*

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of endogenous neuropeptides (Sharp *et al*., 2001). Szabo *et al*., (2002) demonstrated that the chemotactic activities of mu- and delta-opioid receptors were desensitized after activating the chemokine receptors CCR5, CCR2, CCR7, and CXCR4, probabley via a unique pathway involving calcium-independent PKC isotypes (Zhang et al., 2003). We have recently observed that SNC 80 did not alter or desensitize human or rat leukocyte chemotaxis towards casein (data not shown); however, we have not tested other chemotactic agents.

SNC 80 could then be potentially utilized in many different clinical situations where immunosuppression is undesirable as shown for μ-selective ligands such as morphine, which was recently observed to attenuate leukocyte rolling and sticking in both arterioles and venules via nitric oxide production, thus limiting the inflammation process (Lysle *et al*., 1993; Ni *et al*., 2000). The inhibitory effect of morphine on immune functions might increase susceptibility of opioid addicts to develop infections and cancer. Because of their effects on immune function, μ-opioid agonists are not optimal for management of moderate to severe pain following a variety of surgical procedures, cancer, and other related traumatisms. In contrast, the delta opioid SNC 80 may be useful as an immunopotentiating agent in the clinics, thus making SNC 80 potentially suitable in treating not only pain, but also ameliorating the immune status of immunocompromised individuals.

Knowledge of how opioids produce direct effects on the immune system may allow the discovery, design and synthesis of new opioids that have specific immunoregulatory properties. Future research should provide a clearer understanding of the cellular and molecular targets of opioid action within the immune system, as well as intracellular signaling activity. The development of highly selective, site-specific designer drugs may enhance opioid function, and suppress negative effects on immune function and drug dependence. Finally, developing analgesics selective for δ-opioid receptors may become potentially relevant in the clinics for the immunocompromised individual.

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