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Anti-inflammatory and antinociceptive activities of indole–imidazolidine derivatives

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) represent a group of approximately 50 different medicines that are widely prescribed for the management of inflammation and that exhibit variable anti-inflammatory, anti-pyretic and analgesic activities. Most NSAIDs also exhibit a shared set of adverse effects, particularly related to gastrointestinal complications; thus, the development of new drugs for the treatment of chronic inflammation and pain continues to be an issue of high interest. Hydantoin and indole derivatives are reported to possess various pharmacological effects, including anti-inflammatory and analgesic activities. Therefore, the aim of this study was to evaluate the potential anti-inflammatory and antinociceptive activities of hybrid molecules containing imidazole and indole nuclei. The anti-inflammatory activities of 5-(1H-Indol-3-yl-methylene)-2-thioxo-imidazolidin-4-one (LPSF/NN-56) and 3-(4-Bromo-benzyl)-5-(1H-indol-3-yl-methy-lene)-2thioxo-imidazolidin-4-one (LPSF/NN-52) were evaluated using air pouch and carrageenan-induced peritonitis models as well as an acetic acid-induced vascular permeability model followed by IL-1β and TNF-α quantification. To evaluate the antinociceptive activities of the compounds, acetic acid-induced nociception, formalin and hot plate tests were also performed. The anti-inflammatory activities of the compounds were evidenced by a reduction in both leukocyte migration and the release of TNF-α and IL-1β in air pouch and peritonitis models. Upon acetic acid-induced nociception, a decrease in the level of abdominal writhing in the groups treated with LPSF/NN-52 (52.1%) or LPSF/NN-56 (63.1%) was observed. However, in the hot plate test, none of the derivatives tested exhibited an inhibition of nociception. These results indicate that the compounds tested exhibited promising anti-inflammatory and antinociceptive activities that likely involved the modulation of the immune system.

1. Introduction

Inflammation is an essential response to tissue injuries that occur due to physical, chemical or biological insults. Both acute and chronic inflammations play essential roles in the restoration of homeostasis [1]. However, the mechanisms by which activated leukocytes combat germs and tumor cells and eliminate tissue debris in areas of inflammation lead to the production of oxidants and/or of cytotoxic cytokines [2,3]. These processes are finely regulated but may occasionally escape control and prolong the inflammatory response, creating a self-perpetuating condition that produces further damage to the injured tissue [4].

The inflammatory cascade is characterized by an immediate initial response, whereupon the detection of antigens and/or damage leads to the recruitment of neutrophils to the site of insult via the action of chemoattractant mediators; this is followed by the subsequent invasion of monocytes/macrophages, which become the primary immune cell type present after 48 h [5,6]. The activation of neutrophils leads to the secretion of cytokines that amplify the inflammatory response, resulting not only in lymphocyte infiltration, but also in vascular changes, edema and enzymatic destruction. Granulocytes migrate into the inflamed sites and promote the generation of arachidonic acid metabolites and leukotrienes by lipoxins [7].

Non-steroidal anti-inflammatory drugs (NSAIDs) represent a group of approximately 50 different medicines that are widely prescribed for the management of pain and exhibit variable anti-inflammatory, anti-pyretic and analgesic activities [8]. However, most NSAIDs also exhibit a shared set of adverse effects, particularly with regard to gastrointestinal complications; thus, the development of new drugs for the treatment of chronic inflammation and pain continues to be an issue of high interest

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[9]. Indeed, most NSAIDs are characterized by deadly gastrointestinal (GI) toxicities. The free –COOH group is responsible for the GI toxicity associated with all traditional NSAIDs [10].

The hydantoins (imidazolidin-2,4-dione) are interesting compounds due to their occurrence in nature, chemical reactivity (with consequent affinity for biomacromolecules) and numerous biological properties that are favorable for drug development. Structural changes in the imidazolidine ring of such compounds yields molecules with variety of pharmacological properties, including anti-inflammatory, antifungal, antibacterial, hypoglycemic, schistosomicides and anti-cancer activities, among others [11,12]. Therefore, the aim of this study was to evaluate the potential anti-inflammatory and antinociceptive activities of hybrid indole–imidazolidines molecules.

2. Materials and methods

2.1. Drugs

5-(1H-Indol-3-yl-methylene)-2-thioxo-imidazolidin-4-one (LPSF/NN-56) and 3-(4-Bromo-benzyl)-5-(1H-indol-3-yl-methylene)-2-thioxo-imidazolidin-4-one LPSF/NN-52) were synthesized and provided by the Núcleo de Pesquisa em Inovação Terapêutica of Universidade Federal de Pernambuco, Brazil. Fentanyl was purchased from Cristália, São Paulo, Brazil. Dexamethasone and diclofenac were purchased from Aché, São Paulo, Brazil. EDTA was purchased from Labtest, São Paulo, Brazil. Mouse TNF-α and IL-1β ELISA kits were purchased from eBioscience, São Paulo, Brazil. Glacial acetic acid was purchased from VETEC, São Paulo, Brazil. Cremophor, carrageenan, Evans blue and indomethacin were purchased from Sigma, USA. All drugs were dissolved in saline, except for LPSF/NN-56 and LPSF/NN-52, which were dissolved in saline containing 5% Cremophor.

2.2. Chemistry

5-(1H-Indol-3-yl-methylene)-2-thioxo-imidazolidin-4-one (LPSF/NN-56) and 3-(4-Bromo-benzyl)-5-(1H-indol-3-yl-methylene)-2-thioxo-imidazolidin-4-one (LPSF/NN-52) were obtained by synthesis as previously described [13] and were authenticated using NMR and IV spectroscopy and mass spectrometry.

2.3. Experimental animals

Male *Mus musculus* mice (N = 6), provided by the animal facilities of UFPE, Recife, Brazil, were used for the evaluation of anti-inflammatory and antinociceptive activities of the compounds. The mice used weighed between 20 and 25 g and were kept in a room with controlled temperature (22 ± 2 °C) and humidity (50–60%) and a 12 h/12 h light/dark cycle. Water and food were made available to the animals without restriction. Before beginning the experiments, animals were acclimated to the laboratory environment for at least 30 min. All animals used for the determination of anti-inflammatory and antinociceptive activities were fasted for 4 h prior to experimentation. The Animal Studies Committee of the Federal University of Pernambuco approved the experimental protocols (number 23076.017928/2010-25). The animals were treated according to the ethical principles of animal experimentation of COBEA (Brazilian College of Animal Experiments) and the norms of the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.4. Experimental procedures

2.4.1. Carrageenan-induced air pouch

The anti-inflammatory activities of the compounds were tested by the formation of air pouches on the dorsal cervical region of mice via a subcutaneous injection of 2.5 mL of sterile air on day 0, followed by a second injection of 2.5 mL of sterile air 3 days later. On day six, the mice received vehicle, indomethacin (10 mg/kg), LPSF/NN-52 or LPSF/NN-56 (3, 10 or 30 mg/kg) orally. The doses were chosen according to the results of a pilot experiment and previous work published by our group using similar compounds [14]. One hour after drug administration, inflammation was induced by injecting 1 mL of carrageenan suspension (1% in saline solution) into the air pouch. After 6 h, the animals were euthanized in a CO_2 chamber, and the pouches were washed with 3 mL of saline solution containing 3 μM of EDTA. A white blood cell count was performed using an ABX Micros 60 hematology analyzer [15]. The average number of leucocytes from the treated groups was compared with the number of leucocytes of the control group, which was defined as 100%. The exudates were centrifuged, and an aliquot of the supernatant was stored at −20 °C for the analysis of cytokine levels.

2.4.2. Carrageenan-induced peritonitis

Carrageenan-induced peritonitis experiments were performed in male Swiss mice as previously described [15,16]. The animals received LPSF/NN-52 and LPSF/NN-56 in 10- and 3-mg/kg doses, respectively. These doses were chosen because they yielded the best activity in the air pouch tests. Indomethacin, at a dose of 10 mg/kg, was used as a positive control and the negative control received vehicle. After 60 min, inflammation was induced via the intraperitoneal application of 0.1 mL/10 g of the phlogistic agent carrageenan (1% in saline). Four hours after the induction of inflammation, the animals were euthanized in a CO_2 chamber, and 2 mL of saline solution containing 3 μM of EDTA was injected into the peritoneal cavity. White blood cell counts were performed using an ABX Micros 60 hematology analyzer. The exudates were centrifuged and an aliquot of the supernatant was stored at −20 °C for the analysis of cytokine levels.

2.4.3. Quantification of IL-1β and TNF-α levels

The exudates collected from the air pouch and peritonitis assays were stored in a freezer at −20 C for the determination of cytokine levels. Quantification of the IL-1β and TNF-α levels in these exudates was performed by sandwich ELISA using kits that were specific for mice according to the manufacturer's instructions (eBioscience, San Diego, California, USA). The lower detection limit of the assays was 10 pg/mL.

2.4.4. Acetic acid-inducted vascular permeability assays

Vascular permeability assays were performed using the technique previously described [17] with minor modifications. The mice were treated orally with LPSF/NN-52 (10 mg/kg), LPSF/NN-56 (3 mg/kg), indomethacin (10 mg/kg) or vehicle. One hour after treatment, under associative anesthesia induced using ketamine/xylazine, Evans blue dye (1%) was injected into the retro-orbital plexus of the animals (0.2 mL/animal). Twenty minutes later, 10 mL/kg body weight of 1% acetic acid in saline was injected intraperitoneally. Twenty minutes after the injection of acetic acid, the mice were sacrificed in a CO_2 chamber. After 5 ml of saline was injected into the abdominal cavity, the washings were collected into test tubes and then centrifuged at 2000 rpm for 10 min. The absorbance of the supernatant was read at 630 nm using an ELISA reader (Thermo plate). The vascular permeability was represented in terms of the absorbance (A630) that leaked into the cavity.

2.4.5. Writhing test

Test animals received LPSF/NN-52 (10 mg/kg), LPSF/NN-56 (3 mg/kg) or diclofenac (30 mg/kg), orally, while the control group received vehicle only. After an hour, 1% acetic acid was injected (0.1 mL/10 g) into the peritoneal cavity of the animals. Ten minutes later, the mice were observed, and the level of writhing during a 20-minute period was recorded. The percentage inhibition of writhing was calculated by comparing the average contortions of the treated

and control groups. This test was performed according to the protocol previously described [18] with modifications.

2.4.6. Formalin test

LPSF/NN-52 (10 mg/kg) and LPSF/NN-56 (3 mg/kg) were orally administered to animals. One group received fentanyl (200 μg/kg, s.c.), while a control group received vehicle only. After 15 min, formalin (20 μL, 2.5%) was injected into the right hind paw of the animals. The time that the animals spent licking their paws was measured in two distinct phases 0–5 min (first phase) and 15–30 min (second phase) after the injection of formalin [19,20].

2.4.7. Hot plate test

The mice were placed on an aluminum plate that was heated to a fixed temperature (55 ± 1 °C). Latency in terms of discomfort reactions (i.e., paw licking or jumping) was determined before and after the administration of drugs. The cut-off time was 20 s. The latency was recorded before and 30, 60, 120 and 180 min following, oral administration of LPSF/NN-52 (10 mg/kg), LPSF/NN-56 (3 mg/kg) or fentanyl (200 μg/Kg, s.c.). The specific behaviors of the animals, and their intensities, were recorded according to the methodology previously described [21].

2.5. Statistical analysis

All results were expressed as mean values ± standard deviation for each experimental group. Statistical analysis between groups was performed by one-way analysis of variance (ANOVA), followed by the Bonferroni test. For a confidence interval of 95%, P values less than 0.05 (P<0.05), as determined using GraphPad Prism software (version 5.0), were considered to be indicative of statistical significance.

3. Results

3.1. Carrageenan-induced air pouch inflammation

The compounds studied exhibited significant anti-inflammatory activities and reduced carrageenan-induced cell migration. LPSF/NN-56 and LPSF/NN-52 were active at doses of 3 mg/kg and 10 mg/kg, respectively, exhibiting anti-inflammatory effects comparable to indomethacin. There was no statistically significant difference between LPSF/NN-52 (10 mg/kg) and LPSF/NN-56 (3 mg/kg), as shown in Table 1.

3.2. Quantification of IL-1β and TNF-α levels in air pouches

Reductions in the levels of TNF-α were observed in animals treated with LPSF/NN-52 (488.32 pg/mL) or LPSF/NN-56 (218.70 pg/mL) in comparison to the control group (1096.47 pg/mL). For comparison, indomethacin reduced the concentration of TNFα to 575.43 pg/mL; thus, LPSF/NN-56 was more effective in reducing TNF-α than indomethacin. Both compounds also reduced the production of IL-

1β relative to untreated control values. LPSF/NN-56 was more active in reducing the production of both cytokines than LPSF/NN-52 (Fig. 1).

3.3. Carrageenan-induced peritonitis

The results obtained in the carrageenan-induced peritonitis test are shown in Table 2. The data revealed that at the doses tested, both LPSF/NN-52 and LPSF/NN-56 caused a significant reduction in the migration of polymorphonuclear leukocytes in comparison to the control group. No statistically significant difference was observed between the LPSF/NN-52 (10 mg/kg) and LPSF/NN-56 (3 mg/kg) treated groups. Indomethacin inhibited cell migration by 57.7%. The statistical analysis revealed that the indole–imidazolidine derivatives evaluated exhibited an inhibitory profile similar to indomethacin.

3.4. Quantification of IL-1β and TNF-α levels in peritonitis

Both LPSF/NN-52 and LPSF/NN-56 promoted a significant decrease in the TNF-α concentration in comparison with the control group. Both compounds also inhibited the production of IL-1 (Fig. 2).

3.5. Acetic acid-induced vascular permeability

The indole–imidazolidine derivatives (LPSF/NN-52 and LPSF/NN-56) evaluated in this study significantly inhibited acetic acid-induced vascular permeability in comparison to the control group. No significant difference was observed between the derivatives, though they both exhibited a lower level of inhibition than indomethacin (Fig. 3).
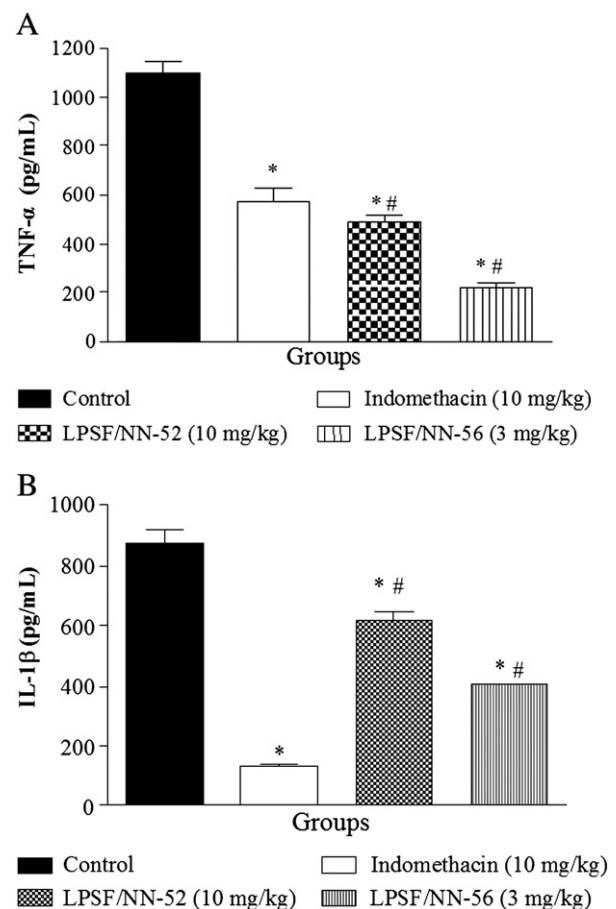

Fig. 1.

Table 1
Effects of LPSF/NN-52 and LPSF/NN-56 on carrageenan-induced air pouch. *P<0.05. Significance was determined with ANOVA one way followed by Bonferroni's post hoc test when compared with control group.

Treated	Dose (mg/kg)	No. of PMNL/mL ($\times 10^6$)	Inhibition %
LPSF/NN-52	3	6.88 ± 1.3*	56.0
	10	4.36 ± 0.7*	72.1
	30	6.24 ± 0.5*	60.1
LPSF/NN-56	3	3.0 ± 0.7*	80.8
	10	5.36 ± 0.6*	65.7
	30	6.70 ± 1.3*	57.2
Indomethacin	10	2.08 ± 0.4*	86.7
Control	–	15.64 ± 1.9	–

Table 2
Effect of LPSF/NN-52 and LPSF/NN-56 on cell migration into peritoneal cavity on carrageenan-induced peritonitis in mice. The animals received LPSF/NN-52 and LPSF/NN-56 in 10- and 3-mg/kg doses, respectively. Data are the mean ± SD of at least six animals. *$P<0.05$. Significance was determined with ANOVA one way followed by Bonferroni's post hoc test when compared with control group.

Treated	Dose (mg/kg)	No. of PMNL/mL ($\times 10^6$)	Inhibition %
LPSF/NN-52	10	4.80 ± 0.7 *	56.1
LPSF/NN-56	3	4.96 ± 0.9*	54.7
Indomethacin	10	4.62 ± 0.3*	57.7
Control	–	10.93 ± 1.6	–

3.6. Writhing test

The antinociceptive activities of LPSF/NN-52 and LPSF/NN-56, as determined using the acetic acid-induced nociception test, are shown in Table 3. Both LPSF/NN-52 (3 mg/kg) and LPSF/NN-56 (10 mg/kg) promoted a statistically significant reduction in the level of writhing compared to the control group. The indole–imidazolidine derivative LSPF/NN-56 exhibited a statistically similar result to standard diclofenac.

3.7. Formalin test

The results shown in Fig. 4 indicate that animals that were treated orally with LPSF/NN-52 or LPSF/NN-56 significantly inhibited formalin-induced pain in comparison to the control group, particularly during the late phase.
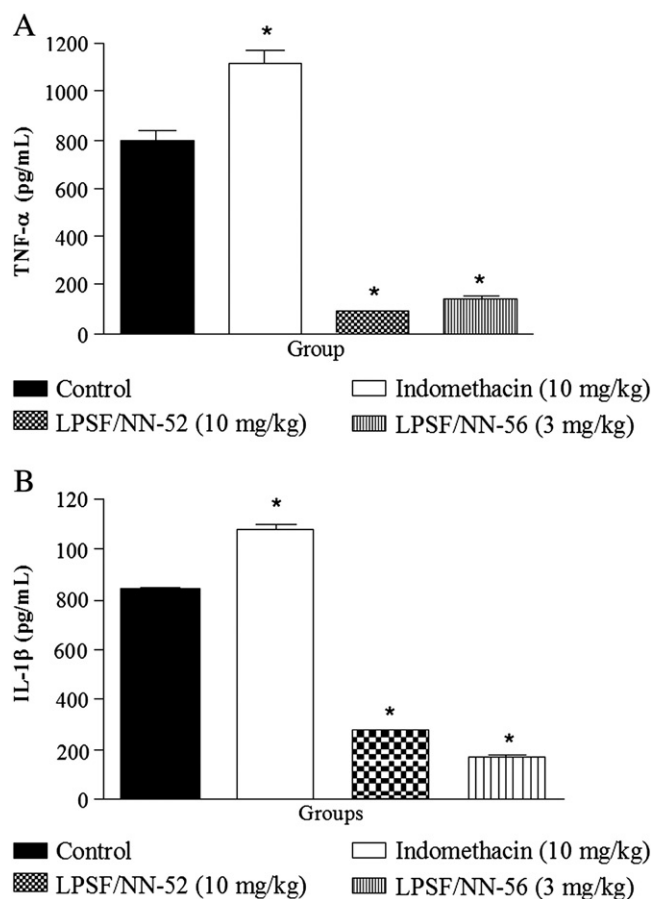
Fig. 2.
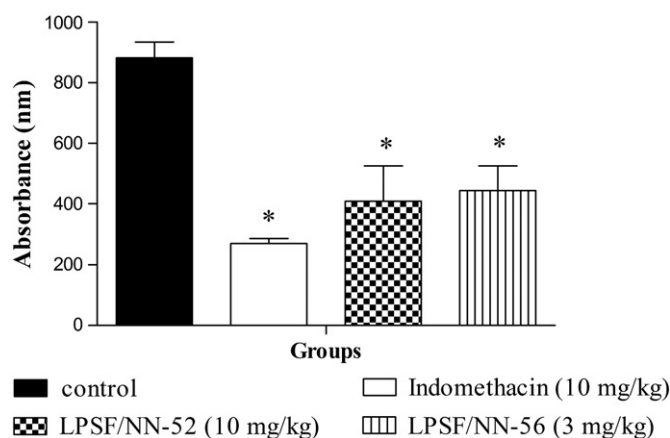
Fig. 3.

3.8. Hot plate test

The compounds tested showed no activity in treated animals during any of the time points observed in the hot plate test in comparison with the control group (Table 4).

4. Discussion

In recent years, molecules containing indole–imidazolidine nuclei have been identified as promising drugs for the treatment of inflammation and pain [15,22]. In this work, we found that LPSF/NN-56 and LPSF/NN-52 derivatives exhibited promising anti-inflammatory and antinociceptive activities.

The subcutaneous air pouch model, an animal model that mimics rheumatoid arthritis, is commonly used as a test for screening anti-arthritic drugs. The air pouch, injected in the back of the animal, forms a membrane with characteristics similar to the inflamed synovium of patients with rheumatoid arthritis [23]. Both LPSF/NN-52 and LPSF/NN-56 exhibited significant anti-inflammatory activity in this assay, suggesting that these substances may be potential anti-arthritic drug candidates. Indomethacin, a drug that was used as a positive control, is a non-steroidal anti-inflammatory indole acetic acid derivative that inhibits the activity of the enzyme cyclooxygenase, decreasing the synthesis of prostaglandins and thromboxane from arachidonic acid [24]. Air pouch experiments performed by Sayar and Melli [25] showed that indomethacin is able to reduce leukocyte migration. According to our results, the compounds that we tested may be characterized by a similar mechanism of action; however, further studies are needed.

The aim of experiments that use the acute peritonitis model is to evaluate leukocyte migration levels by counting the total number of leukocytes that are released during the process of acute inflammation. This process occurs after the inoculation of a phlogistic agent via intraperitoneal administration. The results of such experiments demonstrated that both LPSF/NN-52 and LPSF/NN-56 caused significant

Table 3
Antinociceptive effect of LPSF/NN-52 and LPSF/NN-56 by acetic acid-induced abdominal constriction. The assay procedure was described in the experimental methods. Values are expressed mean ± S.D. The number of animal used for each group was six. *$P<0.05$. Significance was determined with ANOVA one way followed by Bonferroni's post hoc test when compared with control group.

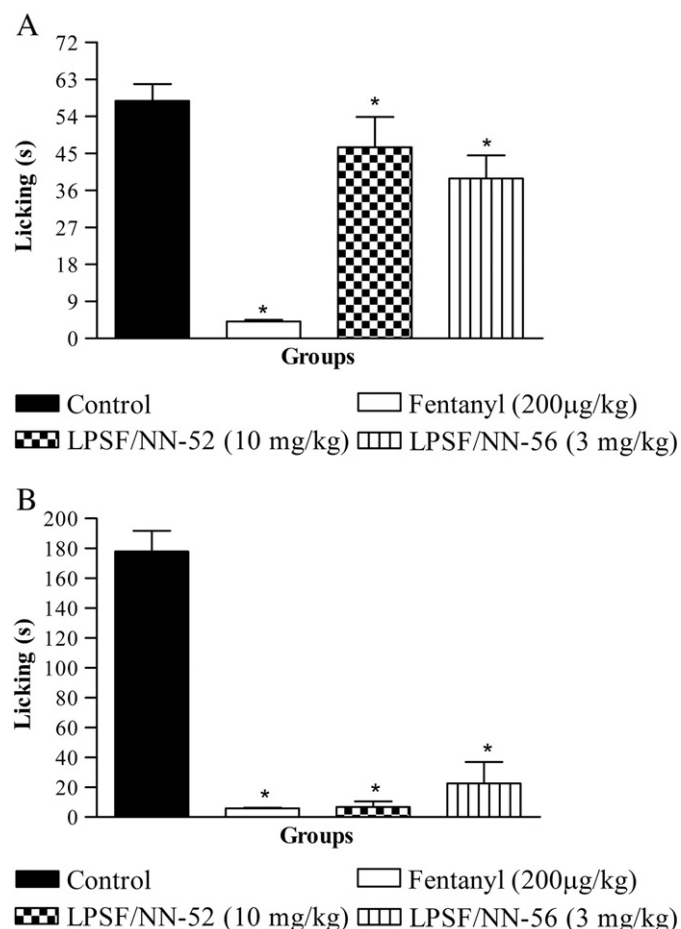| Treated	Dose (mg/kg)	No. writhing (mean ± SD.)	Inhibition %
LPSF/NN-52	10	40.0 ± 3.5*	52.1
LPSF/NN-56	3	30.8 ± 5.9*	63.1
Diclofenac	30	26.8 ± 4.9*	67.9
Control	–	83.5 ± 4.8	–

Fig. 4.

reductions in the level polymorphonuclear leukocyte migration into the peritoneal cavity. Among the models used in experimental studies, the induction of neutrophil migration via the intraperitoneal injection of carrageenan, a substance that stimulates intense chemotactic action, is commonly used in the investigation of acute inflammatory processes [26]. The mechanism of action by which carrageenan induces leukocyte migration may be a synergism between prostaglandins, leukotriene B4 and other chemotactic agents [27]. It is known that non-steroidal anti-inflammatory drugs, such as indomethacin, which inhibit the synthesis of vasodilatory prostaglandins, cause a reduction of blood flow that impairs the migration of polymorphonuclear leukocytes to the sites of inflammation. The results of our tests suggest that the actions of the indole–imidazolidine derivatives may be related to the inhibition of cyclooxygenase. Uchôa et al. [15] evaluated a series of indole derivatives and observed that these substances inhibit COX-1 and, at a lower potency, COX-2.

To assess the action of the compounds under study in cells of the innate immune system we determined the concentrations of TNF-α and IL-1ß in inflammatory exudates obtained through air pouch and peritonitis tests. Our results revealed that both compounds tested decreased the concentrations of TNF-α and IL-1 β in the inflammatory exudates. TNF-α is a cytokine that plays a key role in the innate immune response and is associated with reduced cell migration and exudation [28]. Our results are consistent with a study described by Yu et al. [29] indicating that hydantoin compounds are potent inhibitors of the TNF-α precursor enzyme, making the imidazolidine compounds an attractive target in the search for new anti-inflammatory drugs. IL-1ß promotes the expression of adhesion molecules, leukocyte migration and increased vascular permeability, indicating that it acts as an important pro-inflammatory mediator [30]. The inhibition of neutrophil migration observed in our study may be related to a decrease in IL-1ß and TNF-α levels. These results suggest that the derivatives tested in this study act as modulators of the immune system by decreasing cell migration, exudation and the production of pro-inflammatory cytokines.

Both models of acute inflammation used in this study, despite using the same phlogistic agent, carrageenan, present different cellular characteristics and cytokine profiles. The air pouch model is a valuable tool to determine the effects of new anti-arthritic drugs, since the air pouch lining has been reported to display a histological similarity to synovial membranes [31]. Klenn et al. [32] using the zymosan air pouch model could reliably measure both humoral and cellular parameters, with the only exception of the influx of mononuclear leukocytes which was particularly modest. According to these authors, this lack of monocyte infiltration may be related to the site injected (air pouch vs.

Table 4
Effects of the LPSF/NN-52 and LPSF/NN-56 on the latency time of mice exposed to the hot plate test. Each value represents the mean ± SD of at least six animals. *$P<0.05$. Significance was determined with ANOVA one way followed by Bonferroni's post hoc test when compared with control group.

Groups	(mg/kg)	Latency time (min.)			
		0	60	120	180
LPSF/NN-52	10	9.2 ± 1.8	10.7 ± 0.7	7.12 ± 1.2	8.3 ± 0.5
LPSF/NN-56	3	9.1 ± 0.6	10.6 ± 1.7	11.28 ± 1.7	9.9 ± 0.6
Fentanyl	0.2	9.5 ± 1.7	18.1 ± 0.6*	11.3 ± 0.9*	8.8 ± 1.1
Control	–	9.3 ± 0.5	9.7 ± 1.4	8.8 ± 1.3	8.6 ± 1.5

peritoneal cavity) or to the time points investigated. In addition to the participation of residents in macrophages peritonitis, recent studies suggest that peritoneal CD4(+) T lymphocytes may control recruitment of polymorphonuclear leukocytes (PMN) during peritonitis by an interleukin-17 (IL-17)-dependent mechanism. IL-17 and granulocyte colony-stimulating factor (G-CSF) have been proposed to form an axis regulating PMN transmigration. Studies have shown that the mesothelium-derived G-CSF plays an important role in IL-17A-induced PMN recruitment into the peritoneum [33]. In our study substances tested were more effective in inhibiting IL-1β and TNFα cytokines in the peritonitis, suggesting that these substances are most promising in this type of inflammation. We suggest that LPSF/NN-52 and LPSF/NN-56 derivatives inhibit the PMN recruitment probably by an interleukin-17 (IL-17)-dependent mechanism. However, a direct effect of LPSF/NN-52 and LPSF/NN-56 on the function of each cellular component in the inflammatory response to carrageenan would be needed to determine the cellular basis for the anti-inflammatory effect of these compounds.

To evaluate the action of indole–imidazolidine derivatives in the release of vasoactive amines (e.g., histamine, bradykinin and serotonin) and edema formation, we used an acetic acid-induced vascular permeability test. Both LPSF/NN-52 and LPSF/NN-56 caused a significant inhibition of vascular permeability, with reductions of 53.6% and 49.7%, respectively, compared to control values. Indomethacin derivatives also reduced acetic acid-induced vascular permeability and thus have an effect on the first phase of the inflammatory response by reducing the action of vasoactive amines.

Acetic acid acts by promoting the release endogenous mediators that stimulate the nociceptive neurons. In mice, it promotes an increase in the levels of PGE2, PGF2a, serotonin and histamine and the release of bradykinin and cytokines such as TNF-α and IL-1β [34,35]. This leads to the occurrence of typical behaviors that are characterized by writhing, ataxia and reduced motor activity. Thus, this model allows us to evaluate inflammatory pain and assess whether a molecule has analgesic and anti-inflammatory properties [36]. The results of our tests indicate that the compounds tested promote a significant reduction in the level of writhing of the control group. In these tests, both substances exhibited properties similar to diclofenac, which was used as a positive control.

The induction of painful stimulation via the injection of formalin occurs in two phases, where the first phase (i.e., the first 5 min after injection) is indicative of neurogenic pain and the second phase (i.e., 15 to 30 min after injection) is indicative of inflammatory pain. In these tests, neither LPSF/NN-52 nor LPSF/NN-56 inhibited pain in the first phase and exhibited activity only in the second phase. Drugs that act on the first phase have central action as narcotics, while drugs that act on the second phase affect inflammatory pain [37]. In our study, the inhibition of pain in the second phase of the formalin test suggests a peripheral action similar to NSAID drugs and anti-inflammatory steroids. According to Rosland et al. [38] only inhibition of the second phase of the formalin test is a typical feature of cyclooxygenase inhibitors.

In the evaluation of antinociceptive activity via the hot plate method, our results revealed that at the analysis times tested, the derivatives exhibited no action in comparison to the control treatment. The hot plate test is an animal model used to assess centrally-acting antinociceptive drugs. The behavioral responses exhibited by animals in this test (i.e., paw licking or jumping) occur at a supraspinal level; thus, they are used to investigate the effects of central nociceptives [39]. The results of these experiments complement those obtained in the acetic acid-induced writhing tests, allowing us to conclude that the mechanism of action of the compounds tested is not related to central action, but rather to the ability of the inhibitors to reduce levels of TNF-α and IL-1, as well as the likely inhibition of prostaglandin production.

Based on the data obtained in this study, we conclude that imidazolidine derivatives represent promising anti-inflammatory and antinociceptive drug prototypes that act by inhibiting cell migration

in several models of acute inflammation and plasma exudation. These effects may be related to the modulation of the immune system via reductions in TNF-α and IL-1β concentrations. These compounds act on inflammatory pain and probably have no central effects.

Acknowledgments

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