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THEMED SECTION: MEDIATORS AND RECEPTORS IN THE RESOLUTION OF INFLAMMATION

RESEARCH PAPER

Effects of indomethacin-loaded nanocapsules in experimental models of inflammation in rats

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Background and purpose: The effects of systemic treatment with indomethacin-loaded nanocapsules (IndOH-NC) were compared with those of free indomethacin (IndOH) in rat models of acute and chronic oedema.
Experimental approach: The following models of inflammation were employed: carrageenan-induced acute oedema (measured between 30 min and 4 h), sub-chronic oedema induced by complete Freund's adjuvant (CFA) (determined between 2 h and 72 h), and CFA-induced arthritis (oedema measured between 14 and 21 days).
Key results: IndOH or IndOH-NC produced equal inhibition of carrageenan-elicited oedema. However, IndOH-NC was more effective in both the sub-chronic (33 ± 4% inhibition) and the arthritis (35 ± 2% inhibition) model of oedema evoked by CFA, when compared with IndOH (21 ± 2% and 14 ± 3% inhibition respectively) ($P < 0.01$). In the CFA arthritis model, treatment with IndOH-NC markedly inhibited the serum levels of the pro-inflammatory cytokines tumour necrosis factor α and IL-6 (by 83 ± 8% and 84 ± 11% respectively), while the levels of the anti-inflammatory cytokine IL-10 were significantly increased (196 ± 55%). The indices of gastrointestinal damage in IndOH-NC-treated animals were significantly less that those after IndOH treatment (58 ± 16%, 72 ± 6% and 69 ± 2%, for duodenum, jejunum and ileum respectively).
Conclusions and implications: IndOH-NC produced an increased anti-inflammatory efficacy in long-term models of inflammation, allied to an improved gastrointestinal safety. This formulation might represent a promising alternative for treating chronic inflammatory diseases, with reduced undesirable effects.
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Keywords: indomethacin; polymeric nanocapsules; drug delivery; inflammation; gastrointestinal damage

Abbreviations: CFA, complete Freund's adjuvant; COX-1, cyclooxygenase 1; COX-2, cyclooxygenase 2; IL-6, interleukin 6; IL-10, interleukin 10; IndOH-NC, indomethacin-loaded nanocapsules; NC, unloaded nanocapsules; NSAIDs, non-steroidal anti-inflammatory drugs; TNF-α, tumour necrosis factor α

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) represent a group of approximately 50 different medicines widely prescribed for the management of pain, which display variable anti-inflammatory, anti-pyretic and analgesic activities. Their

effects are mainly mediated by the inhibition of cyclooxygenases 1 and 2 (COX-1 and COX-2), with a consequent decrease in the formation of central and peripheral prostanoids (Burian and Geisslinger, 2005). Furthermore, most NSAIDs share a number of adverse effects, especially those related to gastrointestinal complications (Kean and Buchanan, 2005). Patients taking systemic NSAIDs for the treatment of chronic inflammatory diseases (such as rheumatoid arthritis and osteoarthritis) show variable relief of painful symptoms and they have increased risk of developing gastric or duodenal ulcers and bleeding, which might preclude their long-term use (Langford *et al.*, 2006; Fiorucci *et al.*, 2007).

The development of new drugs and/or new formulations for treating chronic inflammatory and painful diseases continues to be an issue of high interest. The pharmacological response to a drug is directly related to its concentration at the required site of action. A non-specific distribution leads to high drug concentration in healthy organs, tissues and cells, leading to toxicity (Soppimath *et al.*, 2001; Couvreur *et al.*, 2002). One method of restricting the drug to the required site is to associate it with a carrier system (Couvreur *et al.*, 2002; Vauthier and Couvreur, 2007). Among the different nanocarrier systems, biodegradable nanoparticles have received considerable attention as potential drug delivery vehicles over the last few years. Polymeric nanoparticles are colloidal structures below 1 μm, which have been designed to encapsulate lipophilic drugs in order to target organs or tissues, to avoid drug degradation, to improve its efficacy or to circumvent the toxicity (Allémann *et al.*, 1998; Pinto-Alphandary *et al.*, 2000; Guterres, 2001; Couvreur *et al.*, 2002; Vila *et al.*, 2002). In this regard, it has been suggested that NSAIDs-loaded nanocapsules might display an increased efficacy, associated with a marked reduction of adverse effects (Guterres *et al.*, 2001; Bansal JoshiBansal *et al.*, 2007). A recent publication from our group reinforced this idea, by showing that indomethacin-loaded nanocapsules (IndOH-NC) were more potent than free indomethacin in decreasing the viability and proliferation of glioma cell lines, without exerting significant cytotoxic effects on normal cells (Bernardi *et al.*, 2008).

In order to provide additional evidence on the effects of alternative delivery systems for NSAIDs, the present study was designed to characterize the effects of systemic treatment with IndOH-NC in rat models of acute or chronic inflammation. Attempts have also been made to determine the gastrointestinal effects of IndOH-NC. Additionally, we have aimed to compare both the anti-inflammatory and the adverse effects of IndOH-NC, with those displayed by free indomethacin in solution.

Methods

Preparation of nanocapsules

Nanocapsules were prepared by interfacial deposition of polymer as previously described (Fessi *et al.*, 1989). At 40°C, indomethacin (0.010 g), poly(ε-caprolactone) (0.100 g), capric/caprylic triglyceride (0.33 mL) and sorbitan monostearate (0.077 g) were dissolved in acetone (27 mL). In a separate flask, polysorbate 80 (0.077 g) was added to 53 mL of water. The organic solution was injected into the aqueous phase under magnetic stirring at room temperature. After 10 min, the acetone was evaporated and the suspensions concentrated under reduced pressure. The final volume was adjusted to 10 mL. Control formulation (unloaded nanocapsules) was prepared by omitting the drug (indomethacin).

Characterization of nanocapsules

After preparation, the pH of the suspensions was determined using a potentiometer (Micronal B-474). Particle size, polydispersity and zeta potential of the suspensions were determined using a Zetasizer®nano-ZS ZEN 3600 model (Malvern, UK). The samples were diluted in water (MilliQ®) (particle size) or in 10 mmol·L^{-1} NaCl aqueous solution (zeta potential). The measurements were made in triplicate. The total concentrations of indomethacin in the formulations were measured by reverse phase high-performance liquid chromatography (HPLC) (Perkin-Elmer S-200, with injector S-200, detector UV-Vis, a guard-column and a Lichrospher 100 RP-18 column of 250 mm, 4 mm and 5 μm; Merck). The mobile phase consisted of acetonitrile/water (70:30, v/v) adjusted to apparent pH 5.0 ± 0.5 with 10% (v/v) acetic acid. Each suspension (100 μL) was treated with acetonitrile (10 mL); the solution was filtered (Millipore 0.45 μm) and injected (20 μL). The HPLC method was previously validated (Pohlmann *et al.*, 2004). Linear calibration curves for indomethacin were obtained in the range of 1–25 μg·mL^{-1} presenting correlation coefficients higher than 0.9992.

Animals

All animal care and experimental procedures used in the present study followed the 'Principles of Laboratory Animal Care' from NIH publication No. 85–23 and were approved by the Ethics Committee of Pontifícia Universidade Católica do Rio Grande do Sul. The number of animals and intensity of noxious stimuli used were the minimum necessary to demonstrate consistent effects of the drug treatment. Male Wistar rats (180–200 g) were obtained from the Central Biotery of the Federal University of Pelotas (Brazil). Animals were housed under conditions of optimum light (12:12 h light–dark cycle), temperature (22 ± 1°C) and humidity (50 to 60%), with food and water provided *ad libitum*. For the experiments, the animals were acclimatized to the laboratory for at least 1 h, and they were used only once in each test.

Carrageenan-induced rat paw oedema-acute protocol

The experiments were conducted according to the method described by Tratsk *et al.* (1997). Under light anaesthesia with oxygen (3%) and isoflurane (2%), the animals received an intradermal (i.d.) injection in the right hindpaw of saline (0.9%) containing carrageenan (300 μg per paw; 100 μL). As a control, the contralateral paw (left paw) received 100 μL of saline. Oedema was measured by means of a plethysmometer (Ugo Basile) at several time points after carrageenan injection (30, 60, 120 and 240 min). Oedema is expressed in mL as the difference between the right and left paws.

In this model, two distinct schedules of treatment have been adopted. In the prophylactic scheme, the animals were

pretreated with IndOH-NC group or indomethacin in solution (solubilized in calcium carbonate 3%) (IndOH group) (both at 1 mg·kg^{-1}, i.p.), 30 min before carrageenan injection. In the therapeutic scheme, the animals received IndOH or IndOH-NC (1 mg·kg^{-1}, i.p.), 60 min after the injection of carrageenan. The control groups received the vehicle solutions: calcium carbonate 3% (control group) or unloaded nanocapsules (NC group) (1 mL·kg^{-1}, i.p.), at the same schedules of administration.

Complete Freund's adjuvant (CFA)-induced rat paw oedema – sub-chronic protocol

The protocol used was similar to that described by Stein *et al*. (1988), with minor modifications. Briefly, isoflurane-anaesthetized animals received an i.d. injection in one hindpaw (right paw) of CFA (1 mg·mL^{-1}; 100 μL; heat-killed and dried *Mycobacterium tuberculosis*, each millilitre of vehicle containing 0.85 mL paraffin oil plus 0.15 mL mannide monooleate), which was suspended in a 1:1 oil/saline emulsion (in a total volume of 200 μL per paw). As a control, the contralateral paw (left paw) received 200 μL of saline. In this model, the animals were treated with IndOH or IndOH-NC (1 mg·kg^{-1}, i.p.), 2 h post-CFA injection, and once a day for 3 days. The control groups received the corresponding vehicle solutions at the same intervals of time. The oedema was measured by using a plethysmometer (Ugo Basile) at several time points following CFA injection (2, 4, 6, 8, 24, 48 and 72 h), and it is expressed in mL as the difference between the right and left paws.

CFA-elicited oedema – arthritis model

The adjuvant-induced arthritis model employed in the present study was similar to that described by Lorton *et al*. (2000), with some modifications. For this purpose, the oedema was induced by CFA injection, as indicated above, and it was assessed daily in a plethysmometer, between days 14 and 21 post-CFA administration. Animals were treated with IndOH or IndOH-NC (1 mg·kg^{-1}, i.p.), twice a day, for 8 days, starting at the 14th day of CFA injection, until the 21st day. Control groups received the respective vehicle solutions.

Determination of cytokine levels in serum

In the arthritis group, the animals were killed on the 21st day by isoflurane inhalation, and blood samples were collected by cardiac puncture. The blood samples were centrifuged at 1300 *g* at 4°C for 10 min. The supernatant was rapidly frozen and stored at –70°C for later measurement of tumour necrosis factor α (TNF-α), interleukin (IL)-6 and IL-10 levels using specific enzyme-linked immunosorbent assay (ELISA) kits, according to the recommendations of the supplier (R&D Systems).

Evaluation of gastrointestinal damage

The occurrence of gastrointestinal lesions was evaluated in the arthritis group, at the end of experiments (21 days after arthritis induction by CFA). For this purpose, rats were killed, and the intestine (duodenum, jejunum and ileum) was slit open opposite the attached mesenteric tissue. The organs were washed with saline and the mucosal surfaces were macroscopically examined according to an arbitrary scale previously reported (Guterres *et al*., 2001). Accordingly, the number and the gravity of erosions were scored on a scale of five grades: grade 0, no lesion; grade 0.5, hemorrhagic point; grade 1, ulcer length <2 mm; grade 2, ulcer length >2 mm; grade 3, lesion with perforation and haemorrhage. Experimental data were obtained by multiplying the score by the number of lesions. The mean scores for each group were calculated and expressed as lesion indexes.

Statistical analysis

The results are presented as the mean ± SEM of 5–8 animals. The statistical significance between groups was assessed by means of one-way analysis of variance (ANOVA) followed by Tukey's test. *P*-values less than 0.05 ($P < 0.05$) were considered significant. The percentages of inhibition between groups IndOH and IndOH-NC were determined in percentage, on the basis of the area under the curve. The statistical significance between groups was assessed by means of unpaired Student's *t*-test. *P*-values less than 0.05 were considered significant.

Results

Physico-chemical characterization of nanocapsule formulations

The nanocapsule formulations were prepared by interfacial deposition of polymer without the need of any subsequent step of purification. IndOH-NC and unloaded nanocapsules presented a macroscopic homogeneous aspect, such as white bluish opalescent liquids. After preparation, the average particle sizes were 240 nm (IndOH-NC) and 226 nm (unloaded nanocapsules). The suspensions showed monomodal size distributions and polydispersity indexes lower than 0.19, indicating narrow size distributions. The pH values were 5.95 (IndOH-NC) and 6.05 (unloaded nanocapsules). The zeta potential values were –6.9 and –7.3 mV respectively. The indomethacin content was 0.991 ± 0.012 mg·mL^{-1} and the encapsulation efficiency was close to 100%.

Carrageenan-induced paw oedema – acute protocol

We firstly examined the effects of IndOH or IndOH-NC treatment, on the oedema induced by carrageenan. The results demonstrated that prophylactic administration of IndOH or IndOH-NC (1 mg·kg^{-1}, i.p., 30 min before carrageenan) markedly inhibited the oedema induced by carrageenan, when compared with the respective control groups, with inhibition of 61 ± 4% and 63 ± 3% respectively (Figure 1). In addition, the oedema elicited by i.d. injection of carrageenan into the rat paw was significantly reduced by the therapeutic administration of IndOH or IndOH-NC (1 mg·kg^{-1}, i.p.) administered 60 min after carrageenan, with inhibition of 31 ± 11% and 44 ± 7% respectively (Figure 2). Comparison of the inhibition observed for IndOH and IndOH-NC did not reveal any significant difference in the effect of the tested formulations

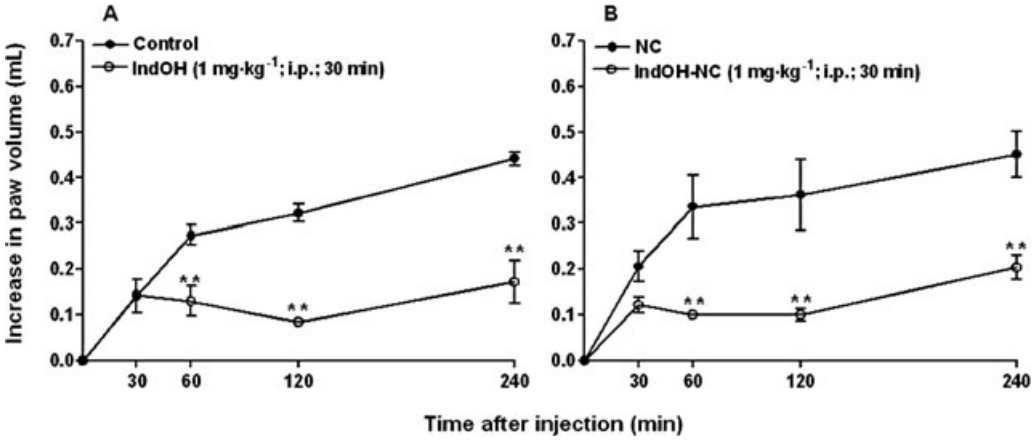

Figure 1 Effect of indomethacin-loaded nanocapsules (IndOH-NC, 1 mg·kg^{-1}, i.p., 30 min before), on rat paw oedema induced by carrageenan (300 μg·paw^{-1}, acute model – prophylactic treatment). (A) Indomethacin in solution (IndOH) and calcium carbonate 3% (Control); (B) Indomethacin-loaded nanocapsules (IndOH-NC) and unloaded nanocapsules (NC). Each point represents the mean of 6–8 animals and vertical lines show the SEM. Asterisks denote the significance levels in comparison to respective control values: $^{**}P < 0.01$.

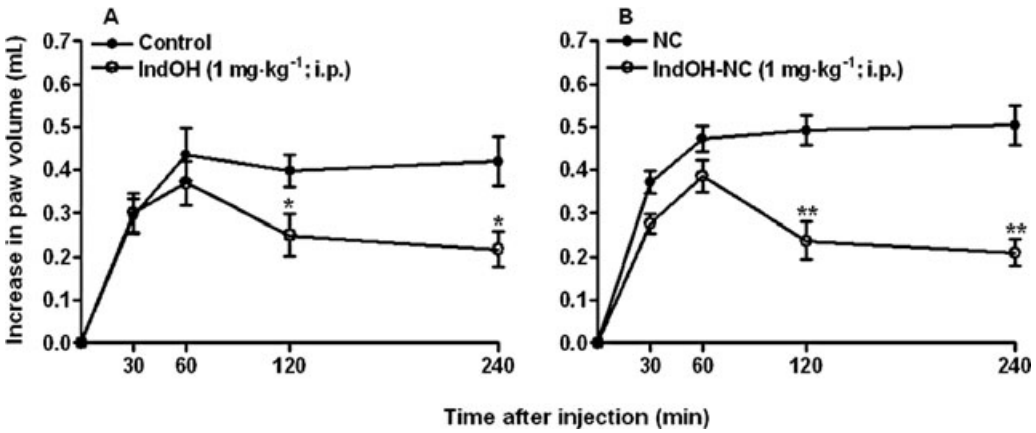

Figure 2 Effect of indomethacin-loaded nanocapsules (IndOH-NC, 1 mg·kg^{-1}, i.p., after 60 min), on rat paw oedema induced by carrageenan (300 mg paw^{-1}, acute model – therapeutic treatment). (A) Indomethacin in solution (IndOH) and calcium carbonate 3% (Control); (B) Indomethacin-loaded nanocapsules (IndOH-NC) and unloaded nanocapsules (NC). Each point represents the mean of 6–8 animals and vertical lines show the SEM. Asterisks denote the significance levels in comparison to respective control values: $^{*}P < 0.05$, $^{**}P < 0.01$.

of indomethacin, in either the prophylactic or therapeutic schedules of treatment ($P > 0.05$) (Figures 1 and 2).

CFA-induced rat paw oedema – sub-chronic protocol

In this experimental set, we compared the effects of IndOH and IndOH-NC in a short period of evaluation following CFA application (until 3 days). The results depicted in Figure 3 show that administration of IndOH or IndOH-NC (1 mg·kg^{-1}, i.p., 2 h after induction of paw oedema, and once a day, for 3 days) significantly reduced the oedema induced by CFA injection, according to assessment in the sub-chronic protocol. The calculated inhibition was 21 ± 2% (IndOH) and 33 ± 4% (IndOH-NC) and these values were significantly different ($P < 0.05$; Figure 3).

CFA-elicited oedema – arthritis model

It is well known that CFA injection into the rat paw evokes a marked and time-related local oedema, which is observed as early as 2 h after and persists for up to 28 days, and presenting signs of systemic alterations. Hence, assessment of CFA-induced oedema in the later phases (after 14 days) is widely adopted as an arthritis model (Lorton *et al.*, 2000). In this study, animals received either IndOH or IndOH-NC (1 mg·kg^{-1}, i.p., twice a day, for 8 days), between the 14th and the 21st day after CFA injection. Both indomethacin formulations were able to significantly reduce the long-term oedema caused by CFA. In these experiments, IndOH-NC exhibited a greater inhibition (35 ± 2%), than IndOH (14 ± 2%) ($P < 0.01$) (Figure 4).

Determination of cytokine levels in serum

The serum of animals in the arthritis group was collected at 21 days after CFA injection, and it was used for determining effects of the different indomethacin formulations on the systemic alterations of cytokine levels. The results demonstrate that the treatment with IndOH-NC (1 mg·kg^{-1}, i.p., twice a day, for 8 days), between the 14th and the 21st day

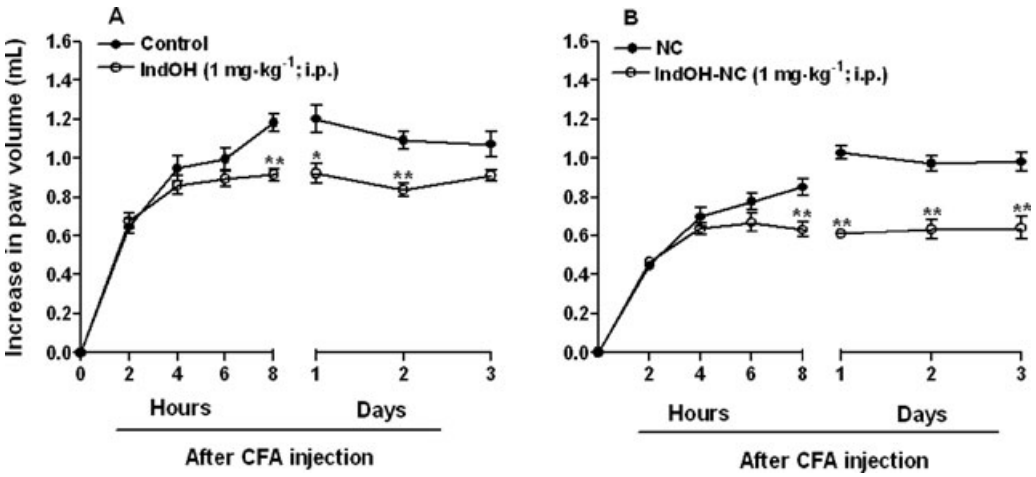

Figure 3 Effect of indomethacin-loaded nanocapsules (IndOH-NC, 1 mg·kg^{-1}, i.p. daily, 2 h after induction of paw oedema by CFA, for 3 days), on rat paw oedema induced by CFA (sub-chronic model). (A) Indomethacin in solution (IndOH) and calcium carbonate 3% (control); (B) Indomethacin-loaded nanocapsules (IndOH-NC) and unloaded nanocapsules (NC). Each point represents the mean of 6–8 animals and vertical lines show the SEM. Asterisks denote the significance levels in comparison to respective control values. *$P < 0.05$, **$P < 0.01$.

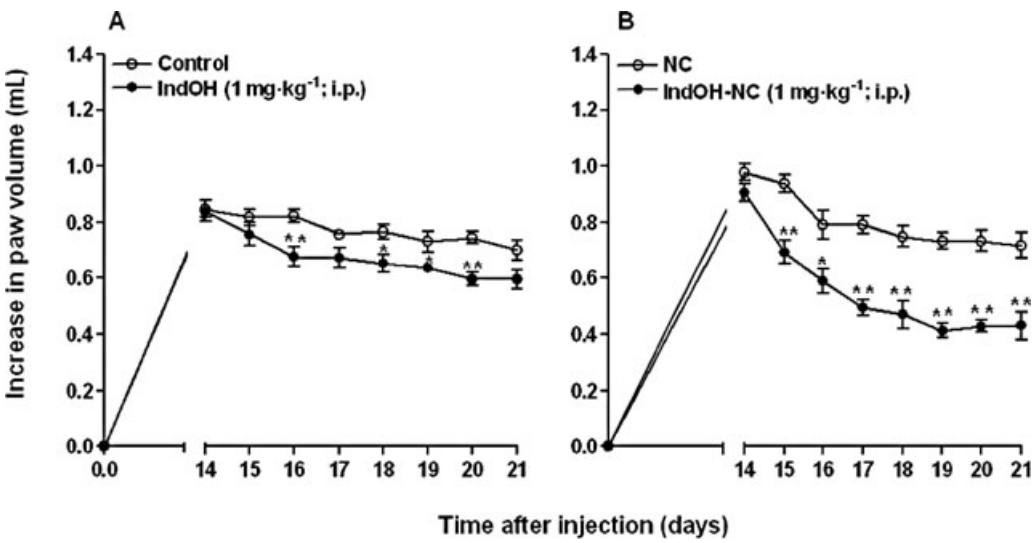

Figure 4 Effect of indomethacin-loaded nanocapsules (IndOH-NC, 1 mg·kg^{-1}, i.p., 14 days after induction of paw oedema, twice a day, for 8 days), on rat paw oedema induced by CFA (arthritis model). (A) Indomethacin in solution (IndOH) and calcium carbonate 3% (control); (B) Indomethacin-loaded nanocapsules (IndOH-NC) and unloaded nanocapsules (NC). Each point represents the mean of 6–8 animals and vertical lines show the SEM. Asterisks denote the significance levels in comparison to respective control values. *$P < 0.05$, **$P < 0.01$.

after CFA injection, produced a striking inhibition in the production of the pro-inflammatory cytokines TNF-α and IL-6 in serum of CFA-injected rats, by 83 ± 8% and 84 ± 11% respectively (Figure 5A and B). Furthermore, the same treatment with IndOH-NC induced a marked increase of the anti-inflammatory cytokine IL-10 by 196 ± 55% (Figure 5C). Conversely, the administration of IndOH (at the same schedule of administration) failed to significantly alter the systemic production of all analysed cytokines (Figure 5).

Evaluation of gastrointestinal damage

This series of experiments was designed to evaluate the gastrointestinal toxicity of IndOH-NC in relation to IndOH, after the long-term administration of both formulations. For the first time, the efficacy and the toxicity were determined using the same groups treated with nanoencapsulated NSAIDs. For this purpose, the intestines in the arthritis group of rats (killed at 21 days) were analysed and the indices of damage were determined separately for duodenum, jejunum and ileum. As shown in the Figure 6, the lesion indices in the animals treated with IndOH-NC were significantly reduced when compared with the IndOH group, by 58 ± 16%, 72 ± 6% and 69 ± 2%, for duodenum, jejunum and ileum respectively. When the total lesion index was calculated (i.e. the total score for the three intestinal regions) the reduction was 68 ± 5% in the IndOH-NC group, compared with the IndOH-treated rats (Figure 6). The animals treated with NC presented a low but significant increase (10 ± 2%) in the total lesion indexes when compared with the control group (Figure 6).

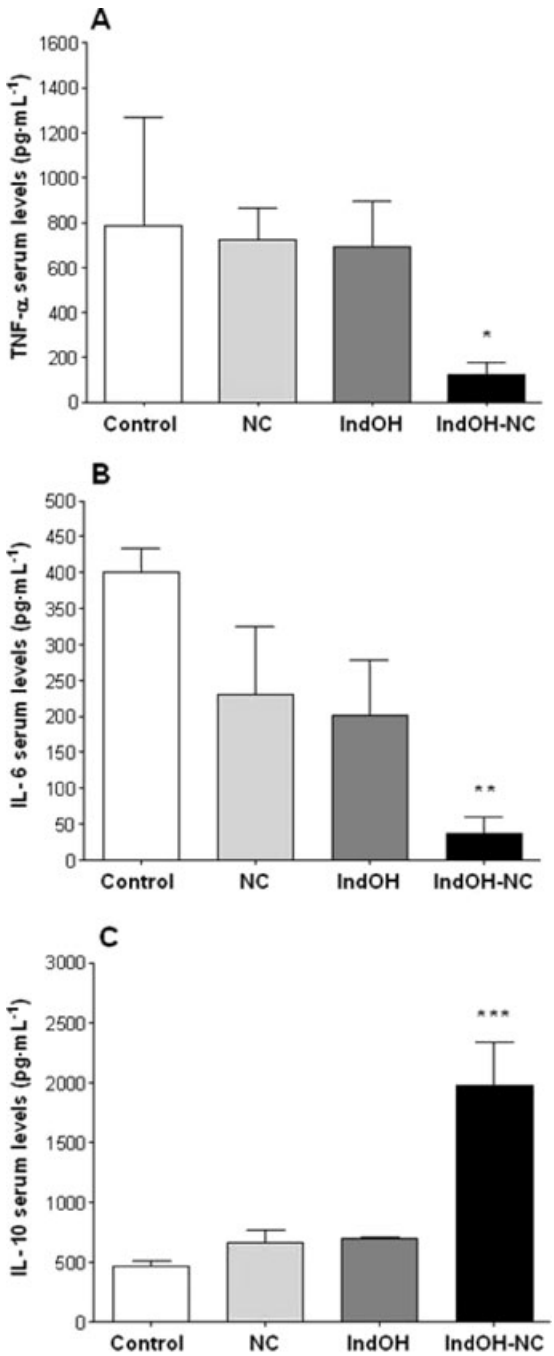

Figure 5 Effect of indomethacin-loaded nanocapsules (IndOH-NC) (1 mg·kg^{-1} IndOH-NC i.p., 14 days after induction of paw oedema, twice a day, for 8 days) on (A) TNF-α and (B) IL-6 and IL-10 levels in serum of animals in the arthritis model. Each point represents the mean of 5–8 animals and vertical lines show the SEM. Significantly different from the control, unloaded nanocapsules (NC) and indomethacin in solution (IndOH) groups for *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Discussion

The present study was conducted to investigate the potential actions of IndOH-NC in experimental models of inflammation in rats. To this end, three classical models of infmallation *in vivo* were employed to evaluate the short and long-term effects of IndOH-NC, in comparison with IndOH: carrageenan-induced acute oedema, CFA-induced sub-chronic inflammation and CFA-induced arthritis. We have also attempted to compare the gastrointestinal toxicity found in rats chronically treated with either IndOH-NC or IndOH.

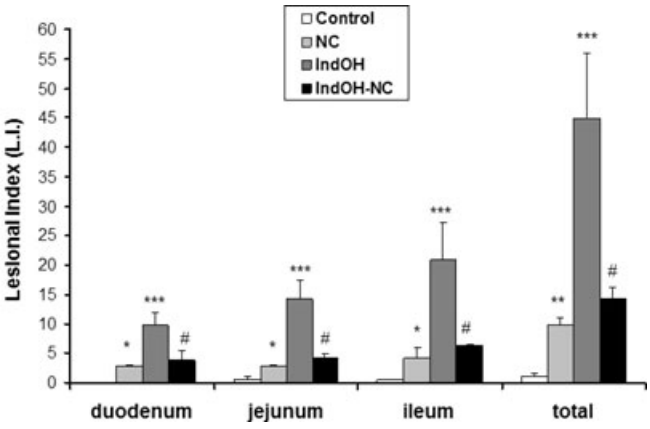

Figure 6 Effect of indomethacin-loaded nanocapsules (IndOH-NC) (1 mg·kg^{-1} IndOH-NC i.p. every 12 h, 14 days after induction of paw oedema, for 8 days) on intestine lesional index (LI) of animals in the arthritis group. Each point represents the mean of 6–8 animals and vertical lines show the SEM. *Significantly different from the control group ($P < 0.05$). **Significantly different from the control group ($P < 0.01$). ***Significantly different from the control and unloaded nanocapsules (NC) groups ($P < 0.001$). #Significantly different from the indomethacin in solution group (IndOH) ($P < 0.001$).

The injection of carrageenan into the rat hindpaw represents a model commonly employed to study acute inflammation and pain. The application of carrageenan causes a rapid formation of oedema, allied to an exacerbated sensitivity to thermal and mechanical stimuli (Rocha *et al.*, 2006). In this regard, carrageenan-induced rat paw oedema is widely used to characterize the mechanisms of action of new anti-inflammatory drugs or formulations, including NSAIDs (Velo *et al.*, 1973; Kawamura *et al.*, 2000; Quintão *et al.*, 2005). We assessed the effects of IndOH-NC in comparison to IndOH, when both formulations were dosed by two distinct schedules of administration, before (prophylactic) or after (therapeutic), the i.d. injection of carrageenan. Our results indicate that IndOH-NC displays an anti-inflammatory efficacy, which is similar to that observed for IndOH, according to assessment in both regimens of treatment. However, no significant difference was observed between the anti-inflammatory efficacy for IndOH-NC and IndOH in the carrageenan acute model of inflammation.

Considering the kinetic properties of polymeric nanocapsules, we decided to investigate whether IndOH-NC might exhibit increased efficacy in long-term models of inflammation. First, we have assessed its effects in the sub-chronic model of inflammation induced by CFA, in which the oedema was measured until 3 days after the application of the inflammatory agent. This experimental set revealed that IndOH-NC presented a significantly higher efficacy in comparison to IndOH. This encouraging result prompted us to test the anti-inflammatory efficacy of IndOH-NC in an experimental model of clinical relevance: CFA-induced arthritis. Repeated treatment with IndOH-NC produced a marked inhibition of

CFA-induced long-term oedema formation (between 14 and 21 days), which was significantly greater than that obtained with IndOH. One plausible explanation for these effects is that, the nanoencapsulation improves drug efficacy and drug bioavailability (Couvreur *et al*., 2002; Schaffazick *et al*., 2003) by providing a more sustained drug release to the inflamed site, according to evaluation in the CFA-arthritis model. It is important to note that the dose of indomethacin used in the present study (1 mg·kg^{-1}) is sub-therapeutic, showing that anti-inflammatory actions of IndOH-NC were noticeably enhanced when compared with the same dose of indomethacin in solution.

It is well known that lowered pH is one of the hallmarks of rheumatoid arthritis (Andersson *et al*., 1999; Levick, 1990). This pH decline may lead a delay in the indomethacin release from the nanocapsules, enhancing its anti-inflammatory effect. Furthermore, plasma protein binding is known to limit indomethacin cellular uptake, by reducing the free fraction of the drug in the circulation (Parepally *et al*., 2006). In this regard, some characteristics of nanoparticulated systems such as the carrier size, the polymer type, as well as their surface features, might induce steric stabilization of nanoparticles, thus inhibiting protein binding and increasing blood circulation time (Brigger *et al*., 2002; Brioschi *et al*., 2007). In this context, a recent publication (Zhang *et al*., 2007) revealed that indomethacin concentrations in plasma were prolonged in the group treated subcutaneously with indomethacin-loaded micelles, compared with indomethacin in aqueous solution. Furthermore, the nanoparticles can accumulate in inflamed tissues due to the greater microvascular permeability in those sites. Additionally in our study, the polymeric nanocapsules were prepared with polysorbate 80, a hydrophilic coating able to delay the protein plasma binding, increasing the particle blood circulation time. Accordingly, all of these factors might well have contributed to the increased efficacy of IndOH-NC observed in the present study.

As reported in the literature, CFA injection can elicit the release of a series of inflammatory mediators, including cytokines. Cytokine production is an important event related to the onset and/or maintenance of inflammatory diseases, such as asthma, arthritis, sepsis and inflammatory bowel disease, among others (Laufer, 2003; Meyer, 2003; Stokkers and Hommes, 2004; Ulloa and Tracey *et al*., 2005; Woodfolk, 2006). Herein, we sought to determine whether the anti-inflammatory effects of IndOH-NC in the CFA-arthritis model, were associated with changes in cytokine generation. Interestingly, our data demonstrate that treatment with IndOH-NC was able to produce a significant decrease of the pro-inflammatory cytokines TNF-α and IL-6, in the serum of arthritic rats. More relevantly, the administration of IndOH-NC also induced a marked increase in the serum levels of the anti-inflammatory cytokine IL-10. Conversely, no significant effect on cytokine production was observed when rats were treated with indomethacin in solution. Thus, on the basis of this series of results, it is possible to infer that increased efficacy of IndOH-NC in comparison to free IndOH, is likely to be related to its ability to alter cytokine production in the inflammatory scenario.

Treatment with NSAIDs has been associated with development of adverse and severe gastrointestinal effects (Asako *et al*., 1992; Tries *et al*., 2002). Accordingly, another important aspect assessed in our study was the gastrointestinal toxicity of IndOH-NC, when dosed in a chronic schedule of administration. Present data clearly demonstrated that animals treated with IndOH-NC showed a significant reduction of intestinal lesion indices, when compared with the animals that received indomethacin in solution. This allows us to suggest that IndOH-NC formulation displayed a lower level of adverse effects than that after IndOH, presenting a desirable, increased gastrointestinal tolerance. Surprisingly, the animals treated with NC also exhibited a significant increase in the intestinal lesion indices when compared with the control group. As previously reported by our group, acute treatment with the NC formulation did not present a significant gastrointestinal toxicity (Guterres *et al*., 2001; Schaffazick *et al*., 2003). It is important to note that in the present study the animals were treated chronically (1 mg·kg^{-1} i.p. every 12 h for 8 days). Therefore, the low gastrointestinal toxicity caused by NC formulations was probably due to this prolonged period of treatment. A relevant point to be discussed is that cytokines might exhibit an important role in mucosal defence (Robinson *et al*., 2008). Therefore, the reduction of TNFα and IL-6 production, associated with the elevation of IL-10 levels might well contribute to the reduced gastrointestinal toxicity observed in the IndOH-NC-treated group.

In summary, the data reported herein clearly demonstrate that polymeric nanocapsules are able to successfully carry indomethacin into the inflammatory sites. Of note, in long-term models of inflammation following CFA injection, IndOH-NC presented an increased anti-inflammatory efficacy, allied to an improved gastrointestinal safety. Thus, the present findings allow us to suggest that IndOH-NC might constitute a relevant and apparently gastrointestinal safe therapeutic alternative for the treatment of chronic inflammatory diseases, such as rheumatoid arthritis with NSAIDs.

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Conflict of interest

None.

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