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Polymeric nanocapsules as a technological alternative to reduce the toxicity caused by meloxicam in mice

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ABSTRACT

This study determined whether meloxicam in nanocapsules modifies stomach and liver damage caused by free meloxicam in mice. Male Swiss mice were treated with blank nanocapsules or meloxicam in nanocapsules or free meloxicam (10 mg/kg, intragastrically, daily for five days). On the seventh day, blood was collected to determine biochemical markers (glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, total bilirubin, unconjugated bilirubin, albumin and alkaline phosphatase). Stomachs and livers were removed for histological analysis. There was no significant difference in the biochemical markers in the plasma of mice. Meloxicam in nanocapsules did not have an ulcerogenic potential in the stomach or cause lipid peroxidation in the stomach and liver. Free meloxicam increased the ulcerogenic potential in the stomach and lipid peroxidation in the stomach and liver. Meloxicam in nanocapsules caused less histological changes than free meloxicam. In conclusion, polymeric nanocapsules can represent a technological alternative to reduce the toxicity caused by meloxicam.

1. Introduction

Nanoparticles represent promising drug-delivery systems for the treatment of diseases, since nanocarriers can change physicochemical characteristics of drugs, resulting in differences in pharmacokinetics and pharmacodynamics (Jong and Borm, 2008). Moreover, nanotechnology has been used to reduce toxicity and side effects of drugs (Faraji and Wipf, 2009).

In fact, the main advantage of nanoparticles includes the protection against drug in the gastric mucosa (Guterres et al., 2001; Shahiwala and Misra, 2002; Faraji and Wipf, 2009). Nevertheless, toxicity has also been reduced in other organs, such as the stomach, liver and spleen (Jong and Borm, 2008; Khachane et al., 2011a,b).

Our research group has focused on studying the pharmacological effects of meloxicam-loaded lipid-core nanocapsules (M-NC). In fact, previous studies of our research group demonstrated that M-NC had a superior pharmacological effect compared to free drug in several models, such as antinociceptive and antiedematogenic (Villalba et al., 2014), anti-inflammatory (Ianiski et al., 2016) and neuroprotective (Ianiski et al., 2012) effects. However, the toxicity caused or protection against side effects by these M-NC had not yet been defined.

Meloxicam is a non-steroidal anti-inflammatory drug (NSAID) that has a greater affinity to inhibit the activity of cyclooxygenase (COX)-2 than COX-1 (Yuan et al., 2006; Ah et al., 2010). COX-2 inhibition is related to anti-inflammatory effects of meloxicam, since it reduces prostaglandin synthesis in an inflamed site (Lopez-Garcia and Laird, 1998). However, COX-1 inhibition is related to the side effects of the drug (Mitchell et al., 1993; Engelhardt et al., 1996).

In this context, the objective of this study was to determine whether meloxicam in nanocapsules modifies stomach and liver damage caused by free meloxicam (M-F) in mice, as a new approach to reduce the adverse effects of meloxicam.

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2. Material and methods

2.1. Materials

Sorbitan monostearate, polysorbate 80 and poly (ε-caprolactone) M_W = 80,000 were purchased from Sigma-Aldrich. Meloxicam was obtained from Henrifarma (São Paulo, Brazil) and Miglyol 810® was obtained from Via Farma (São Paulo, Brazil). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.2. Nanocapsules preparation

Suspensions of M-NC were prepared by the method of interfacial deposition of preformed polymer (adapted from Fessi et al., 1988) at a concentration of 0.3 mg/ml. Aqueous phase was composed of water and polysorbate 80, while organic phase was composed of meloxicam, poly-ε-caprolactone, caprylic/capric triglyceride, sorbitan monostearate and acetone. Organic phase was added under magnetic stirring in an aqueous phase. This same nanocapsule preparation protocol was used to obtain suspensions of blank nanocapsules (B-NC), but without the presence of meloxicam. The particle diameter was around 283 and 285 nm for M-NC and B-NC, respectively. The zeta potential was approximately −14.53 mV for M-NC and −16.21 mV for B-NC. The content of nanocoated meloxicam was 99.97%. The polydispersity index was 0.25 for M-NC and 0.26 for B-NC. The preparations were used within a period of 30 days after production.

2.3. Animals

Male adult Swiss mice (20–25 g) were obtained from a local breeding colony. The animals were kept in a separate animal room, on a 12 h light/dark cycle, with light on at 7:00 a.m., and acclimatized (22 ± 2 °C). Commercial diet (GUABI, RS, Brazil) and water were supplied *ad libitum*. Animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Centro Universitário Franciscano, Santa Maria, RS, Brazil, under number 018/2012.

2.4. Experimental protocol

The mice were divided into three groups of ten animals each. Mice belonging to group I received B-NC (33 ml/kg) and served as control group. Animals in group II received M-NC (10 mg/kg). Animals belonging to group III were treated with M-F (10 mg/kg). Treatments were performed intragastrically (i.g.) via gavage, daily for five days. This daily dose administered is the maximum dose achieved due to the drug concentration in the suspension obtained and limited volume of administration. The experimental protocol utilized was similar to Khachane et al. (2011a,b). On the sixth day, mice were deprived of food and received only water. On the seventh day, animals were anesthetized with ketamine/xylazine (80/10 mg/kg) and blood was collected with heparin by cardiac puncture to determine biochemical markers. Moreover, stomach and liver were removed for analysis.

2.5. Body weight gain, relative organ weight and signs of toxicity

The body weight was monitored in the experimental phase and body weight gain was calculated according to formula: Body weight gain = final body weight − initial body weight. In addition, we calculated relative organ weight according to formula: Relative organ weight = organ weight/final body weight of the animal. Animals were monitored for possible signs of toxicity such as tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.

2.6. Biochemical markers

Blood was centrifuged at 900× *g* for 15 min. Plasma was separated for biochemical tests. Hepatic function was evaluated using glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total bilirubin (TBIL), unconjugated bilirubin (UBIL), albumin (ALB) and alkaline phosphatase (ALP). These analyses were performed using a commercial kit (LABTEST, Diagnostica S.A., Minas Gerais, Brazil).

2.7. Ulcerogenic parameters

The stomach was incised along the greater curvature and washed gently with saline 0.9%. The production of hemorrhagic lesions in the glandular portion was observed. Hemorrhagic lesions were evaluated by scores (Khachane et al., 2011a,b): 0.0 − normal (non-bleeding, injuries and latent); 0.5 − latent lesion or generalized hemorrhage; 1.0 − slight lesion (2–3 dotted lines); 2.0 − serious injury (continuous aligned lesion or 5–6 dotted lines); 3.0 − very serious injury (continuous aligned multiple lesions); 4.0 − widespread injury.

2.8. Histological analysis

Stomach and liver morphologies were assessed by light microscopy. Stomachs from individual mice were fixed in bouin for histological evaluation, while livers were collected and fixed in 10% buffered formalin. Then the samples were dehydrated in ethanol, cleared in xylene and embedded in paraffin. Cross-sections, 4 μm thick, were stained with hematoxylin and eosin (HE) before analysis. A histologist blind to the experimental groups analyzed the sections.

2.9. Lipid peroxidation

Stomachs and livers of mice were removed and homogenized in cold 50 mM Tris–HCl, pH 7.4 (1/10, w/v), centrifuged at 900× *g* 4 °C for 10 min. Low-speed supernatant was used for lipid peroxidation assays. Thiobarbituric acid reactive species (TBARS) were used as a measure of lipid peroxidation. TBARS were determined as described by Ohkawa et al. (1979). An aliquot of supernatant (200 μl) was added to the reaction mixture containing: 500 μl thiobarbituric acid (0.8%), 200 μl sodium dodecyl sulfate (SDS) (8.1%), and 500 μl acetic acid (pH 3.4) and incubated at 95 °C for 2 h. The absorbance was measured at 532 nm in a spectrophotometer. Results were reported as nmol malondialdehyde (MDA)/mg protein.

2.10. Protein determination

Protein concentration was measured by the Bradford method (Bradford, 1976), using bovine serum albumin as the standard.

2.11. Statistical analysis

Data are expressed as mean ± standard derivation (S.D.). Statistical analysis was performed using a one-way ANOVA followed by Duncan's test. Values of $p < 0.05$ were considered statistically significant.