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Delivery system for mefenamic acid based on the nanocarrier layered double hydroxide: Physicochemical characterization and evaluation of anti-inflammatory and antinociceptive potential

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ABSTRACT

Purpose: The anionic form of the drug mefenamic acid intercalated into the nanocarrier layered double hydroxide (LDH-Mef) was evaluated by anti-inflammatory and antinociceptive assays.
Methods: The LDH-Mef material was characterized by a set of physicochemical techniques, which was supported by Density Functional Theory calculations. The pharmacological effects of LDH-Mef (40 wt% of drug) were evaluated by hemolytic, anti-inflammatory activity and antinociceptive assays.
Results: In vivo assays were conducted for the first time in order to assess the LDH-Mef potential. The hemolytic effects decreased for the intercalated Mef as demonstrated by the higher tolerated hemolytic concentration (1.83 mM) compared to mefenamic acid (MefH), 0.48 mM. Pretreatment of animals with MefH or LDH-Mef reduced carrageenan-, dextran sulfate- and PGE_2-induced paw edema. MefH or LDH-Mef also decrease total leucocytes and neutrophil counts of the peritoneal cavity after inflammation induction with carrageenan. In the nociception model, oral pretreatment with LDH-Mef reduced mechanical hypernociception carrageenan-induced after 3–4 h and also the number of writhings induced by acetic acid.
Conclusions: This work shows the increase of the anti-inflammatory and antinociceptive potential of the drug confined into the LDH, as well as, its hemolytic effect.

1. Introduction

The entrapment of drugs into layered inorganic nanoparticles can provide sustained release minimizing the usual side effects [1]. In addition, the inorganic framework can protect bioactive molecules against degradation processes promoted by light, heat, molecular oxygen etc. and extend their shelf life. Layered double hydroxides (LDHs), also known as hydrotalcite like-compounds, are potential candidates to carry pharmaceutical substances, [2,3,4] since one LDH composition in particular (specifically $[Mg_6Al_2(OH)_{16}]CO_3 \cdot 4H_2O$) is already commercialized as the antacid Talcid™ and also used as excipient [5]. In the 1990s several works have shown the efficacy of Talcid in comparison with some already established antacids like for example Omeprazol [6,7]. So, the low toxicity of LDH after oral administration is well established.

LDHs present positively charged sheets (or layers) in their structures as shown in Fig. 1, A [8]. To maintain the material's electroneutrality it

Abbreviations: LDH-Mef, Layered double hydroxide containing the anionic form (mefenamate anion) of the drug mefenamic acid; LDH, Layered double hydroxide; MefH, Mefenamic acid; Mef, Mefenamate anion; PGE_2, Prostaglandin E2; M^{II} and M^{III}, Divalent and trivalent cations; A^{n-}, An exchangeable anion; Carcinoma A549 and normal L-132, Lung epithelial cells; HeLa, Adenocarcinoma of the cervix; HOS, Osteosarcoma; NSAIDs, Non-steroidal anti-inflammatory drugs; PXRD, Powder X-ray diffraction; CHN, Elemental chemical analyses of C, H, and N; TGA-DTG, Thermal analysis; FT-IR, Fourier transform infrared spectroscopy; FT-Raman, Fourier transform Raman spectroscopy; NaMef, Sodium mefenamate salt; DFT, Density functional theory; LDH-Cl, Mg, Al-LDH material containing chloride anions; TGA-DSC, Thermogravimetric analysis and differential scanning calorimetry; IR, Infrared spectroscopy; Raman, Raman spectroscopy; PBS, Phosphate buffered saline; HTAB, Hexadecyltrimethylammonium bromide; MPO, Myeloperoxidase; Sal, Saline; SEM, Standard error of the mean; ANOVA, Analysis of variance; FTIC, Fluorescein 5′-isothiocyanate dye; Conset, Concentrations for the onset of hemolysis; $PGF_{2\alpha}$, Prostaglandin $F_{2\alpha}$.

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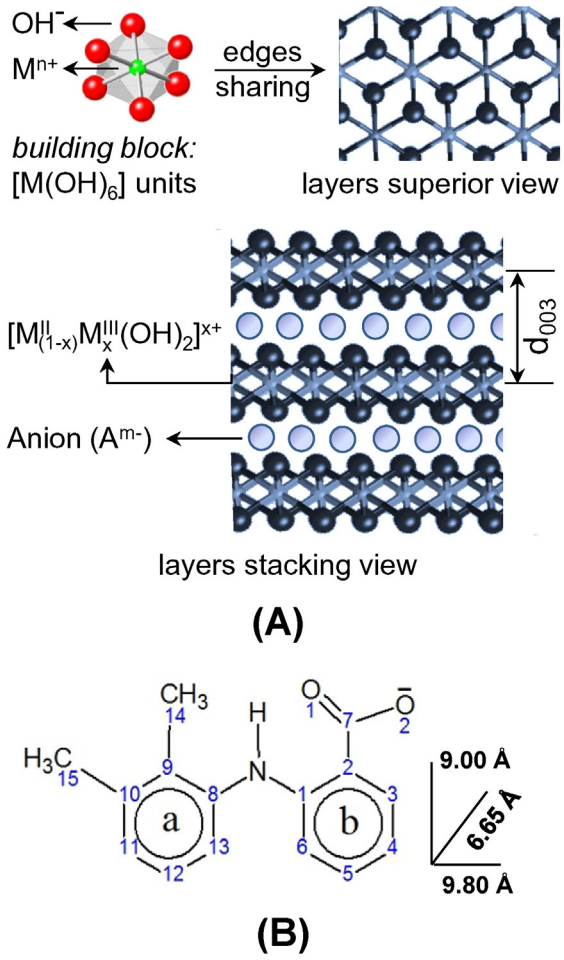

Fig. 1. Schematic representation of (A) layered double hydroxide structure, and (B) mefenamate ion structure (dimensions achieved by DFT calculations).

required the presence of anions that, together with water molecules, promote the LDHs layers stacking. The general chemical formula of this class of inorganic materials is $[M^{II}_{1-x}M^{III}_{x}(OH)_2]^{x+}[A^{n-}]_{x/n}\cdot mH_2O$, where M^{II} and M^{III} are divalent and trivalent cations, and A^{n-} is an exchangeable anion.

In the pharmaceutical and medical context, LDH is a very interesting matrix to be explored as a drug nanocarrier since it is biocompatible and the anionic form of bioactive drugs can be intercalated between its layers [1,9]. Therefore, since the first publication in 2001 by O'Hare group about the intercalation of commercial drugs into LDHs, [10] a growing interest in this layered material for nanotechnological purposes is noticed [1,11,12,13,14].

In vitro and *in vivo* assays have been conducted to evaluate the LDHs' mechanisms of action when administered by enteral (oral), [15] topical, [16] or parenteral (injection) routes [17,18]. For the wide application of LDHs in the pharmaceutical and medical fields, it required the enlightenment of the cytotoxicity of these materials to provide not only information about biological applications, but also to prevent undesirable effects [19]. Choy et al. [20] have shown that these nanoparticles presented low cytotoxicity through tests *in vitro* with cells. The non-steroidal anti-inflammatory drugs (NSAIDs) are suitable candidates for intercalation into LDHs due to the presence of anionic groups in their structure. Moreover, the NSAIDs present limited solubility, low bioavailability and serious gastrointestinal problems [21,22]. A recent review published by Rives et al. [23] reporting studies about anti-inflammatory drugs intercalated into LDHs has pointed out the sustained release of NSAID drugs by *in vitro* assays and also their pharmacological activity by *in vivo* tests.

Mefenamic acid (MefH, Fig. 1B), an NSAID marketed in the US as Ponstel, is indicated for inflammatory diseases and also as an analgesic for the treatment of rheumatoid arthritis, menstrual symptoms and headaches. However, due to its low solubility and side effects, mainly related to gastrointestinal adverse consequences, including bleeding, ulceration or perforation of the stomach or intestines, which can be sometimes fatal, the use of mefenamic acid is compromised [24]. Rives et al.[25,26] have reported the intercalation of mefenamic acid into LDH and studied the release profile in different pH values. The release profile is slower and sustained for the intercalated mefenamate into LDH when compared to the free drug or the physical mixture containing the drug and LDH.

In this paper, we have developed LDH carriers containing magnesium and aluminum in the layers (Mg, Al-LDH) and the anionic form of mefenamic acid (LDH-Mef). LDH-Mef nanoparticles were characterized in detail by chemical analysis, powder X-ray diffraction (PXRD), thermal analysis (TGA-DTG), vibrational spectroscopy (FT-IR and FT-Raman), and particle size and zeta potential measurements. To support the experimental vibrational assignments and discuss the spectral differences between the intercalated mefenamate anion and the free anion, the calculations of vibrational frequencies of sodium mefenamate salt (NaMef) were performed in the framework of the Density Functional Theory (DFT).

In order to assess the cytotoxic effects, hemolytic assays were performed with MefH, LDH-Mef and LDH matrix without drug (*i.e.*, Mg,Al-LDH intercalated with chloride anions). We report here the first studies on the anti-inflammatory and antinociceptive activity on systemically administered LDH-Mef nanoparticles by *in vivo* assays in three different inflammatory disease models, *i.e.* carrageenan, dextran sulfate and PGE_2 induced paw inflammation models, and orally administered LDH-Mef nanoparticles in acetic acid-induced writhings model.

2. Experimental

2.1. Reagents

All reagents used in this work can be found in the Supplementary Material.

2.2. Synthesis of LDH-Mef hybrid material

The inorganic carrier with the drug (LDH-Mef) was isolated in order to evaluate the biological activity of the mefenamate anion entrapped into the LDH framework. Besides it was also prepared as a similar LDH structure but without a bioactive species, *i.e.* a Mg,Al-LDH material containing chloride anions between the layers (abbreviated LDH-Cl).

The LDHs materials (LDH-Mef and LDH-Cl) were synthesized by co-precipitation method as described in the literature [27,28]. The hybrid LDH-Mef sample was obtained using the Mg^{2+}/Al^{3+} molar ratio equal to 2 and mefenamate/Al^{3+} equal to 1. LDH-Cl was obtained using the same conditions but in the absence of the drug.

Initially, the pH of the aqueous solution of 0.1 mol/L of mefenamic acid was adjusted to 10 by addition of 0.2 mol/L of NaOH. Under nitrogen atmosphere (to avoid carbonate ion formation by solubilization of carbon dioxide), a 0.1 mol/L mixed solution of the divalent (Mg^{2+}) and trivalent (Al^{3+}) metal cations (in the chloride form) was added to the mefenamate solution. The pH value was kept at about 10 during the synthesis by the NaOH solution addition. The resulting slurry was aged with vigorous stirring for 1 h under N_2 atmosphere at 25 °C. The solid was separated and washed with deionized water by filtration (to remove ions as sodium and chloride for example). Then the isolated solid was dried under reduced pressured at room temperature.