In vitro study of the cytotoxic and genotoxic effects of indomethacin-loaded Eudragit® L 100 nanocapsules

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ABSTRACT. Indomethacin is a non-steroidal anti-inflammatory agent included in one of the most commonly used drug classes worldwide. The use of this drug results in certain side effects, including gastrointestinal complications. Therefore, there exists a need to develop better methods for the delivery of such drugs into the body, such as those employing nanoparticles. The aim of the present study was to evaluate the cytotoxic and genotoxic effects of indomethacin-loaded Eudragit® L 100 nanocapsules (NI; based on methacrylic acid and methyl methacrylate) on cells unable (lymphocytes) and able to metabolize drugs (HepG2 cells), using comet and cytokinesis-block

micronucleus (CBMN) assays *in vitro*. Cells were exposed to NI at concentrations of 5, 10, 50, 125, 250, and 500 µg/mL. The comet assay showed that NI induced no significant DNA damage in either cell type at any of the concentrations tested. The CBMN test confirmed these results; however, the highest concentration of 500 µg/mL resulted in a small but statistically significant clastogenic/aneugenic effect in HepG2 cells. These findings should encourage the development of new investigations of this nanomaterial as a delivery vehicle for anti-inflammatory drugs, such as indomethacin.

Key words: Comet assay; Micronucleus test; Eudragit L 100; MTT assay; Drug delivery

INTRODUCTION

Indomethacin is a non-steroidal anti-inflammatory drug for the relief of pain and inflammation, and is commonly used by rheumatoid arthritis, osteoarthritis, osteitis deformans, bursitis, tendonitis, and neuralgia sufferers. Recently, indomethacin/3-isobutyl-1-methylxanthine administration was shown to efficiently and safely promote neurogenic differentiation of bone marrow mesenchymal stem cells, suggesting a promising treatment for nervous system diseases (Mu et al., 2015). Indomethacin acts by inhibiting both cyclooxygenase-1 (COX-1) and -2 (COX-2; Yoshikawa et al., 1993; Beck et al., 2000). COX-2 is known to be associated with inflammation and related diseases, whereas COX-1 is involved in homeostasis. Decreased production of prostaglandins (PGs), especially PGE_2, results in reduced gastric mucosal protection, leading to injuries of the gastrointestinal tract and potential cardiovascular problems, renal toxicity, high blood pressure, and fluid retention. PGE_2 derives from the activity of COX-1, and is one of the most abundant PGs in the body under physiological conditions, being involved in the immune response, blood pressure, and gastrointestinal mucosal integrity (Jones, 2001; Ricciotti and FitzGerald, 2011; Bacchi et al., 2012). PGs are the end products of arachidonic acid (AA) release from the cell membrane mediated by phospholipases in response to stimuli. Free AA is then converted to PGs via COX and lipoxygenase (Vane et al., 1998; Smith et al., 2000; Bacchi et al., 2012).

Nanoparticles demonstrate great potential as delivery vehicles for anti-inflammatory drugs. As many nanoparticle types are available for this purpose, it is necessary to examine each to identify the most viable (Kadam et al., 2012; Sharma et al., 2012; Lim et al., 2013). Since the oral route is the most widely used and the easiest for patients to adhere to, it represents the optimal nanoparticle/drug administration approach, improving effectiveness and reducing the dose needed, consequently diminishing side effects (Yun et al., 2013).

Previous studies have shown that anti-inflammatory drugs used in conjunction with nanoparticle drug delivery vehicles show a greater reduction in inflammation than when used alone (Prasad et al., 2013). For example, indomethacin-loaded nanocapsules produce an increased anti-inflammatory effect in long-term models of inflammation, allied to improved gastrointestinal safety (Bernardi et al., 2009). Nanoparticles envelop drugs, enabling the bio-distribution of water-insoluble compounds, resulting in increased half-life and pharmacokinetics. Moreover, treatment is more effective since drugs are directed to particular target areas, allowing the use of smaller total doses (Uskoković, 2013).

Eudragits®, well-known pharmaceutical excipients, have been widely used in various sustained- and controlled-release formulations (Ibrahim et al., 2010; Jose et al., 2011). Eudragit L 100 (methacrylic acid:methyl methacrylate, 1:1) is an enteric pH-dependent copolymer soluble above pH 6.0, and is commonly used together with other Eudragit L nanomaterial in the preparation of enteric solid dosage forms as an effective coating skeleton (Lee et al., 2012; Hao et al., 2013).

Bearing these factors in mind, and considering that little is known of the side effects of indomethacin-loaded Eudragit L 100 nanocapsules, this study aimed to evaluate the cytotoxicity and genotoxicity of indomethacin nanoencapsulated with Eudragit L 100. An *in vitro* protocol was used employing two human cell types, one lacking (lymphocytes) and the other possessing (HepG2 cells) the ability to metabolize drugs, with cytogenetic characteristics being assessed by two recognized methods, the comet assay and the micronucleus (MN) test.

MATERIAL AND METHODS

Chemical compounds

Nanoencapsulated indomethacin (NI) was diluted in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) for use with HepG2 cells and Roswell Park Memorial Institute 1640 medium (RPMI; Gibco) for lymphocytes. Methyl methanesulfonate (MMS; CAS No. 66-27-3; Sigma-Aldrich, St. Louis, MO, USA) and benzo[*a*]pyrene (Sigma-Aldrich) were used as positive controls, due to their DNA damage-inducing effects, recognizable in comet and MN assays. The other main reagents used in this study were obtained from the following suppliers: trypsin (Sigma-Aldrich), phytohemagglutinin (PHA; Sigma-Aldrich), cytochalasin B (Sigma-Aldrich), normal-melting point (NMP) agarose (Invitrogen, Waltham, MA, USA), low-melting point (LMP) agarose (Invitrogen), fetal bovine serum (FBS; Gibco), ethylenediaminetetraacetic acid (EDTA; Merck, Kenilworth, NJ, USA), Triton X-100 (J.T. Baker, Center Valley, PA, USA), trypan blue (Sigma-Aldrich), thiazolyl blue tetrazolium bromide (MTT; Sigma-Aldrich), ethidium bromide (Sigma-Aldrich), dimethyl sulfoxide (DMSO; Sigma-Aldrich), Giemsa stain (Synth, Diadema, SP, Brazil), and Histopaque-1077 (Sigma-Aldrich).

Nanoparticle preparation

The NI used in the present study was prepared using a 1:10 formulation of indomethacin:Eudragit L 100, the latter being an anionic copolymer based on methacrylic acid and methyl methacrylate. An oil/water-based method was used for nanoencapsulation, employing polyethylene glycol and polysorbate 80 (Dupeyrón et al., 2013). Drug nanoparticles were prepared by placing 20 mL alcoholic polymer solution, together with indomethacin and a hydrophilic polymer (40 mg), in 120 mL acidic aqueous phase containing the surfactant, while stirring. The NI was then centrifuged and collected. This method and all related details are described in Dupeyrón et al. (2013).

Cell types

Human peripheral blood lymphocytes (PBL) collected from three (two men and one woman) healthy, non-smoking volunteers aged from 18 to 27 years, and a human hepatoma

cell line (HepG2) expressing phase 1 and 2 drug-metabolizing enzymes (Hewitt and Hewitt, 2004) were used in the present experiments. At the time of donation, peripheral blood donors provided written informed consent for the use of their samples in this study. The Human Ethics Committee of the Universidade Estadual Paulista (UNESP), Marília town, Brazil, approved the present study on August 6, 2014 (protocol 0952/2014).

HepG2 cells were obtained from the American Type Culture Collection (Rockville, MD, USA; ATCC No. HB-8065). PBL were cultured in RPMI medium at 37°C in a 5% CO_2 atmosphere at 95% relative humidity. HepG2 cells were grown in DMEM supplemented with 10% FBS and antibiotics (Gibco) at 37°C, in a humidified incubator containing an atmosphere of 5% CO_2 in air.

Cytotoxicity analysis

Trypan blue staining was used as a cell viability test, and was performed as described by Panda et al. (2012). This test assesses damage to cell membranes. Cells with a disrupted plasma membrane are colored blue when exposed to this stain, whereas those with intact membranes remain unstained. Aliquots of 2 x 10^5 cells were placed on 24-well culture plates in 2 mL RPMI medium per well, in a 37°C humidified incubator with a 5% CO_2 in air atmosphere, and exposed to NI for 24 h at concentrations of 5, 10, 50, 125, 250, 500, and 1250 µg/mL. Viability tests for both cell types were carried out according to Strober (1997). A freshly prepared solution of 10 µL 0.05% trypan blue in distilled water was mixed with 10 µL cell suspensions for 20 min, spread on a microscope slide, and covered with a coverslip. Culture medium itself was used as negative control, and Triton X-100 diluted in medium lacking FBS was used as a positive control. Non-viable cells appeared blue. At least 100 cells were counted per group, and tests were conducted using biological duplicates with three technical replicates.

The MTT test of cytotoxicity was used for HepG2 cells. This assay is used to evaluate cellular survival, proliferation, and activation, and is based on the capacity of succinate dehydrogenase within the mitochondria of viable cells to convert the tetrazolium salt MTT to insoluble, purple formazan. The formazan product accumulates inside living cells, their membranes being permeable to this compound (Fotakis and Timbrell, 2006). The MTT assay was performed on 96-well plates, according to the protocol described by Mosmann (1983), with some modifications. In each well, 1 x 10^5 cells were seeded in a total volume of 100 µL medium supplemented with FBS, except for the blank control wells, which contained medium lacking FBS. The plates were then incubated for 24 h, and the medium replaced with fresh culture medium (lacking FBS), to a final volume of 200 µL. Positive and negative controls consisting of 1% Triton X-100 in culture medium (lacking FBS), and pure culture medium (also lacking FBS), respectively, were included in order to validate the experiments. To the other wells, NI at concentrations of 5, 10, 50, 125, 250, 500, and 1250 µg/mL was added, and the plates were incubated in a 37°C humidified incubator in 5% CO_2 in air for 24 h. After this period, the medium was replaced with 150 µL MTT diluted in phosphate-buffered saline (PBS) at a concentration of 1 x 10^{-6} mg/mL, with which cells were incubated for 4 h. The MTT solution was subsequently discarded, and 200 µL DMSO was added to each well. The plates were then analyzed with a spectrophotometric microplate reader using a 540-nm filter (Epoch; Biotek, Winooski, VT, USA). Statistical analyses were performed with GraphPad Prism 5.02 (GraphPad Software, Inc., La Jolla, CA, USA), and comprised analysis of variance (ANOVA) and Tukey comparison tests ($P < 0.05$ being considered significant).

Comet assay

The comet assay was performed as described by Tice et al. (2000). Both cell types were used in this protocol. First, slides were prepared by adding a thin, uniform layer of NMP agarose. Cells (2 x 10^5) were added to a 24-well plate and, in the case of HepG2 cells, left for 24 h to attach. NI concentrations of 5, 10, 50, 125, 250, and 500 µg/mL were used, and controls were included. These concentrations were selected due to the absence of toxicity observed in the trypan blue exclusion test. The positive control consisted of 75 µM MMS, and the negative control comprised the drug carrier diluted in the same culture medium used for the corresponding cells. After 4 h of treatment, cells were removed and suspended in 120 µL LMP agarose on the previously prepared microscope slides. Cells were then left for 1 h in a container of cold lysis solution consisting of 89 mL of a stock solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH set to 10.0 with ~8 g solid NaOH, 890 mL distilled water and 1% sodium lauryl sarcosine), plus 1 mL Triton X-100 (Merck) and 10 mL DMSO (Merck), for lysis to occur. The slides were transferred to the anode end of an electrophoresis box and covered with an alkaline buffer consisting of 300 mM NaOH and 1 mM EDTA, pH <13, where they were left for 20 min for DNA denaturation to take place. After this period, electrophoresis was performed in a 4°C ice bath for 20 min at 25 V and 300 mA (0.722 V/cm). The slides were then submerged for 15 min in a neutralization buffer comprising 0.4 M Tris-HCl, pH 7.5, dried at room temperature, and fixed in 100% ethyl alcohol for 10 min. The slides were dried and stored overnight, before being rinsed in distilled water, coated with 30 µL 1X ethidium bromide-staining solution, and covered with a coverslip. Slides were evaluated immediately at 400X magnification using a fluorescence microscope (Olympus, Tokyo, Japan) with a 515-560-nm excitation filter and a 590-nm barrier filter. All experiments were performed three times, with samples in duplicate.

The extent and distribution of DNA damage indicated by the single cell gel electrophoresis assay was ascertained by examining at least 100 randomly selected and non-overlapping cells (50 cells per coded slide) per culture well, in a blind manner. These cells were scored visually according to tail size into the following four classes: class 0, no tail; class 1, tail shorter than the diameter of the head (nucleus); class 2, tail length one- to two-times greater than the diameter of the head; and class 3, tail length more than twice the diameter of the head. Comets with no heads, in which almost all the DNA was present in the tail, or those with a wide tail were excluded from the evaluation as these probably represented dead cells (Hartmann and Speit, 1997). The total score for 100 comets, ranging from 0 (no damage) to 300 (severe damage), was obtained by multiplying the number of cells in each class by the damage class number, and summing these values.

Cytokinesis-block MN (CBMN) assay

The CBMN assay was performed according to the protocol described by Fenech (2000). Both cell types were used in this test. Experiments were carried out on PBL from two donors, in duplicate, and on HepG2 cells, in triplicate. The cultured cells were kept in a 25-cm^2 flask and various treatment concentrations (5, 10, 50, 125, 250, and 500 µg/mL) and controls were added. The doses were selected based on their not inducing toxicity in the trypan blue exclusion test. For the positive controls, 150 µM MMS and 2 µM benzo[*a*]pyrene were used for PBL and HepG2 cells, respectively.

Whole blood samples (0.4 mL) were added to 5 mL culture medium supplemented with 10% FBS. PHA was added to each culture flask at 10 µL/mL, and PBL were incubated in a 37°C humidified incubator in 5% CO_2 in air for 72 h. Forty-four hours after starting the cultures, the PBL were exposed to different concentrations (5, 10, 50, 125, 250, and 500 µg/mL) of NI. Four hours after addition of the test compound, 6 µg/mL cytochalasin B was added to each flask. The cells were harvested by centrifugation for 5 min at 850 *g*, and pellets were resuspended in a chilled hypotonic solution of 0.075 M KCl for 5 min. Subsequently, cells were washed once with 5 mL cold 3:1 methanol:acetic acid solution (v/v), and the fixation procedure was applied three times. Formaldehyde (1%) was added to the final fixative, in order to preserve the cytoplasm. Cell suspensions were placed on slides and stained with 5% Giemsa dye in PBS, pH 6.8, for 5 min.

HepG2 cells were cultured for one complete cell cycle (24 h) and treated for 24 h with the compound of interest before the MN test. The cells were subsequently washed, harvested, and incubated with cytochalasin B for 28 h. After this period, the same procedure as that described above for PBL was followed.

In accordance with the standard criteria (Fenech, 2000), MN analysis was performed on coded slides by scoring 1000 binucleated cells for each culture flask. An optical microscope (Primo Star; Zeiss, Oberkochen, Germany) was used at 100X magnification. As a measure of cytotoxicity, the nuclear division index (NDI) was calculated as follows: NDI = (M1 + 2[M2] + 3[M3] + 4[M4]) / N, where M1-M4 represent the number of cells out of 500 (N) in each culture with one to four nuclei.

Statistical analysis

After checking for normal distribution, data from the comet and MN assays were subjected to one-way ANOVA and the Tukey multiple comparison test using GraphPad Prism 5.02. P values <0.05 were considered to represent significantly different results.

RESULTS

The number of viable cells present in cell suspensions was determined using the trypan blue exclusion test. PBL and HepG2 cells were tested with the same NI concentrations (5, 10, 50, 125, 250, 500, and 1250 µg/mL). For both cell types (Figure 1A and B), only the administration of 1250 µg/mL resulted in viability scores <80%. Cytotoxic evaluation of NI by MTT assay using HepG2 cells showed similar effects (Figure 2). It was not possible to test higher concentrations due to the limited ability to dilute the indomethacin in the vehicle used. For genotoxicity and mutagenicity experiments, concentrations resulting in cell viability higher than 80% were used.

Results of the genotoxic assessment of NI in human PBL and HepG2 cells are shown in Figure 3. As expected, the MMS-positive control caused an increase in the total number of damaged cells of both types, confirming the sensitivity of the comet assay to detect genotoxicity. In both groups, NI treatment led to no significant changes in the total number of damaged cells, with scores at all six tested concentrations being compared to the negative control. In human PBL, comet assay scores ranged from 16.50 ± 8.50 to 38.17 ± 11.43, while in HepG2 cells, these values varied between 7.66 ± 2.30 and 44 ± 23.07, indicating that NI does not induce genotoxic effects.

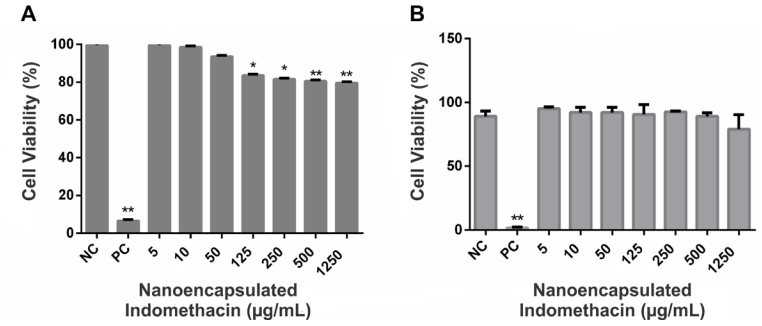

Figure 1. Effect of indomethacin-loaded Eudragit nanocapsules on cell viability of **A.** human lymphocytes and **B.** HepG2 cells based on the trypan blue test. NC = negative control; PC = positive control. *Statistically different in comparison to the negative control ($P < 0.01$). **Statistically different in comparison to the negative control ($P < 0.001$).

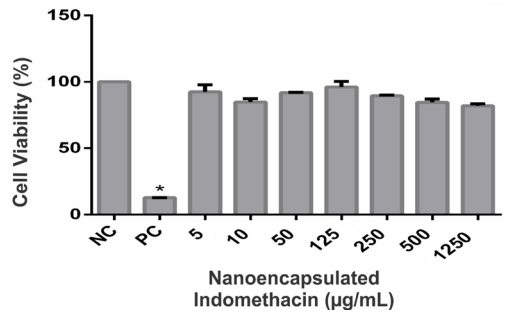

Figure 2. Cytotoxicity based on the colorimetric thiazolyl blue tetrazolium bromide assay in HepG2 cells exposed to different concentrations of indomethacin-loaded Eudragit nanocapsules. NC = negative control; PC = positive control. *Statistically different compared to the negative control ($P < 0.001$).

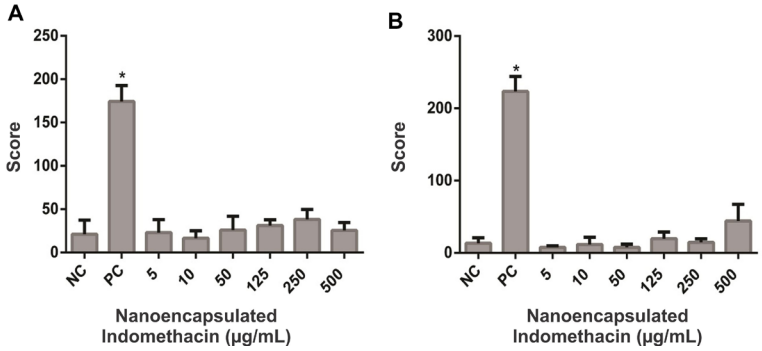

Figure 3. Effect of indomethacin-loaded Eudragit nanocapsules on **A.** human lymphocytes and **B.** HepG2 cells assessed by comet assay. NC = negative control; PC = positive control. *Statistically different compared to the negative control ($P < 0.001$).

The MN data recorded as a measure of the clastogenic/aneugenic impact of NI on each cell type are shown in Tables 1 and 2. The results were similar to those observed using

the comet assay. The CBMN assay demonstrated no marked alterations in the number of micronucleated cells in either cell line. However, administration of 500 µg/mL NI resulted in a statistically significant increase in the frequency of micronucleated HepG2 cells, indicating clastogenic/aneugenic effects at this concentration. NI exerted no significant influence on cell division as measured by the NDI.

Table 1. Micronucleus frequency and nuclear division index in human lymphocytes treated with indomethacin-loaded Eudragit nanocapsules.

Test substance	Treatment		Binucleated cells with MN (2000 cells scored)		NDI per 1000 cells
	Period (h)	Concentration (µg/mL)	N	%	(Mean ± SD)
Negative control	24	0	2	0.10	2.023 ± 0.007
MMS (positive control)	24	150[a]	35*	1.75	2.535 ± 0.114
NI	24	5	0	0.00	2.567 ± 0.00
NI	24	10	3	0.15	2.293 ± 0.834
NI	24	50	2	0.10	2.469 ± 0.120
NI	24	125	3	0.15	2.279 ± 0.162
NI	24	250	4	0.20	2.352 ± 0.076
NI	24	500	4	0.20	2.156 ± 0.234

MN = micronucleus, NDI = nuclear division index, SD = standard deviation, MMS = methyl methanesulfonate, NI = nanoencapsulated indomethacin. [a]Concentration in µM. *Significantly different from the negative control ($P < 0.001$).

Table 2. Micronucleus frequency and nuclear division index in HepG2 cells treated with indomethacin-loaded Eudragit nanocapsules.

Test substance	Treatment		Binucleated cells with MN (3000 cells scored)		NDI per 1500 cells
	Period (h)	Concentration (µg/mL)	N	%	(Mean ± SD)
Negative control	24	0	4	0.13	1.673 ± 0.063
Benzo[*a*]pyrene (positive control)	24	2[a]	65**	2.16	1.657 ± 0.183
NI	24	5	4	0.13	1.543 ± 0.140
NI	24	10	3	0.10	1.622 ± 0.169
NI	24	50	4	0.13	1.688 ± 0.110
NI	24	125	9	0.30	1.627 ± 0.231
NI	24	250	10	0.33	1.753 ± 0.268
NI	24	500	18*	0.60	1.761 ± 0.167

MN = micronucleus, NDI = nuclear division index, SD = standard deviation, NI = nanoencapsulated indomethacin. [a]Concentration in µM. *Significantly different from the negative control ($P < 0.05$). **Significantly different from the negative control ($P < 0.001$).

DISCUSSION

In vitro studies are widely utilized for the screening and grading of chemicals, and constitute important tools to enhance our understanding of their hazardous effects and predict the consequences of human exposure (Broadhead and Combes, 2001).

Indomethacin is principally known for its anti-inflammatory effects, being widely used in the treatment of fever and inflammation in patients with osteoarthritis, rheumatoid arthritis, osteitis deformans, bursitis, tendonitis, and headache (Baber et al., 1980; Summ et al., 2010). However, side effects resulting from the use of this type of medication are a concern. For instance, indomethacin is known to cause gastritis and gastric ulcers, especially in the elderly and patients with a history of ulcerative diseases (Hernández-Díaz and Rodríguez, 2000;

Bacchi et al., 2012). Therefore, the development of novel alternative drug delivery systems that reduce such side effects is required.

In this study, considering the great potential of drug-enveloping nanoparticles to mitigate certain side effects, the cytotoxicity and genotoxicity of NI was assessed using two different cell lines: lymphocytes from human peripheral blood (unable to metabolize drugs), and human hepatoma HepG2 cells (drug-metabolizing cells). The loss of membrane integrity, which occurs late in apoptosis and early in necrosis, can be detected by cellular uptake of trypan blue. This method allows observers to distinguish between cells with intact and disrupted membranes. The former exclude trypan blue, whereas cells in the late stages of apoptosis and those undergoing necrosis take up the dye, giving a blue appearance (Cook and Mitchell, 1989). Here, we used the trypan blue assay to test seven NI concentrations. Only the highest tested concentration (1250 µg/mL) was found to be cytotoxic for lymphocytes, with cell viability rates less than 80% for both cell types. A very similar enteric nanoparticle to that analyzed in the present study was tested in a previous investigation in combination with omeprazole (OME). An MTT assay of the *in vitro* cytotoxic activity of nanocoated OME at concentrations ranging from 50 to 800 µg/mL showed that Caco-2 cell viability decreases as nanoparticle concentration increases (Hao et al., 2013).

Determination of the potential of NI to cause DNA damage was another objective of the present study. The comet assay is one of the most important and widely applied *in vitro* methods in genotoxicology and DNA damage studies (Mosmann, 1983). Moreover, this test is among the most commonly used to assess nanoparticle genotoxicity (Vandghanooni and Eskandani, 2011). This assay allows the basic evaluation of genetic material with double- and single-strand breaks, which appears visually as a "comet" with a distinct head consisting of intact DNA, and a tail including damaged or broken DNA fragments (Collins et al., 2008). Our comet assay showed that none of the tested NI concentrations resulted in significant DNA damage in either of the cell types analyzed.

The third aspect investigated in this study was the capacity of NI to cause chromosome damage. The CBMN cytogenetic assay detects chromosomal aberrations in cells previously exposed to the test agent. It is based on the evaluation of an increase in the frequency of binucleated cells with MN (Hartmann and Speit, 1997). In our study, CBMN analysis revealed no statistically significant differences in the number of micronucleated lymphocytes between the control and treatment groups, comprising six NI concentrations. Kalf et al. (1989) showed that in rat bone marrow, indomethacin can prevent the myelotoxic effects of benzene, significantly decreasing the number of MN observed as a result. However, when administered alone, the frequency of MN was not seen to significantly increase, corroborating our results obtained with human lymphocytes. On the other hand, our CBMN assay of HepG2 cells demonstrated that the highest concentration of NI tested (500 µg/mL) caused a small increase in micronucleated cells, indicating a clastogenic/aneugenic effect at this concentration.

A previous *in vivo* analysis revealed that indomethacin exerts genotoxic effects at a dose equivalent to 75% of the LD_{50} (36 mg/kg body weight). However, at lower doses such as 12 and 24 mg/kg, significant genotoxicity was not observed in treated mice (Devi and Polasa, 1987). In addition, other studies have shown that low-dose indomethacin can protect mouse cells against the genotoxicity of benzene (Pirozzi et al., 1989).

Bernardi et al. (2009) investigated the effects of systemic treatment with indomethacin-loaded poly(D,L-lactide) polymer and compared them with those of free indomethacin. NI was found to result in increased anti-inflammatory efficacy in long-term models of inflammation in

rats, as well as improved gastrointestinal safety. This formulation might represent a promising alternative for the treatment of chronic inflammatory diseases with reduced undesirable effects; therefore, its biosafety must be evaluated.

Our literature review failed to identify any previous studies involving an evaluation of the genotoxicity of Eudragit L 100 nanoparticles. However, Shah et al. (2013) assessed the cyto- and genotoxicity of variously composed nanocarriers, including those of nanoparticulate polyethylene glycol polymers, as present in Eudragit L 100. Using Chinese hamster ovary CHO-K1 cells and MN tests in *in vitro* experiments, the authors found that neutral nanocarriers (based on electric charge), such as those composed of polyethylene glycol, do not induce MN formation. Nevertheless, only very low nanoparticle concentrations were analyzed in their study, compared with the present study.

Hao et al. (2013) analyzed the *in vitro* cytotoxic activity of OME nanoencapsulated with Eudragit L 100-55 (methacrylic acid:ethyl acrylate copolymer, 1:1, type A), an enteric nanomaterial very similar to Eudragit L 100, reporting the internalization of nanoparticles by Caco-2 cells after a 4-h incubation. The authors also observed that the viability of these cells decreased linearly with decreasing Eudragit L 100-55 concentration, indicating that the enteric nanoparticle's toxicity derived from OME. Empty nanoparticles exhibited insignificant levels of cytotoxicity, suggesting that the nanomaterial studied can be used as a safe drug delivery system. Considering the similarity between the nanomaterial used by this team and that employed in the present study, we can hypothesize that the genotoxic effect described here in response to the highest NI concentration must have been caused by increasing indomethacin levels in cells, as observed in *in vivo* genotoxicity studies involving administration of indomethacin alone (Devi and Polasa, 1987). This hypothesis needs to be tested in further studies, since in the present study we had the study limitations, where was not possible to include as experimental controls indomethacin without the nanoparticle and nanoparticles lacking indomethacin.

In conclusion, under the experimental conditions used in the present study using *in vitro* comet and MN assays, we observed that indomethacin-loaded Eudragit L 100 nanocapsules are not genotoxic to leukocytes and HepG2 cells. Considering the gastroprotective potential of Eudragit nanoparticles, these results encourage the study of their use as a delivery mechanism for anti-inflammatory drugs such as indomethacin, and other medications causing gastrointestinal injuries.

Conflicts of interest

The authors declare no conflict of interest.

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