Original research article

Topic application of meloxicam-loaded polymeric nanocapsules as a technological alternative for treatment of the atopic dermatitis in mice

Douglas Mroginski Weber^a, Guilherme Teixeira Voss^a, Renata Leivas de Oliveira^a, Caren A.R. da Fonseca^a, Jaini Paltian^a, K.C. Rodrigues^a, Francine Rodrigues Ianiski^b, R.A. Vaucher^a, Cristiane Luchese^{a,*}, Ethel Antunes Wilhelm^{a,*}

^a Universidade Federal de Pelotas (UFPEL), Centre for Chemical, Pharmaceutical and Food Sciences (CCQFA), Centre of Scientific Chemistry, Biochemistry and Bioprospecting Postgraduate Program (PPGBBio), Pelotas, Brazil

^b Centro Universitário Franciscano, CEP, Nanosciences Postgraduate Program, Santa Maria, Brazil

ARTICLE INFO

Article history:

Received 9 November 2017

Received in revised form 15 February 2018

Accepted 13 March 2018

Available online 20 March 2018

Keywords:

Meloxicam

Nanoparticles

Nanotechnology

2,4-Dinitrochlorobenzene

Non-steroidal anti-inflammatory drugs

ABSTRACT

This study investigated the effect of the topical treatment with meloxicam-loaded nanocapsules (M-NC) on symptoms, inflammatory response and oxidative parameters in an atopic dermatitis (AD) model in BALB/c mice. 2,4-Dinitrochlorobenzene (DNCB) was applied to the dorsal skin on days 1–3 for sensitization. Mice were challenged with DNCB on the ear (on days 14–29) and dorsal skin (on days 14, 17, 20, 23, 26, and 29). Treatments with blank nanocapsules (B-NC), free meloxicam (M-F) or M-NC were applied to the backs of the mice from days 14 to 29. On the day 30, skin severity scores and scratching behaviour were determined. After that, ears and dorsal skin were removed for determination of inflammatory parameters (edema and myeloperoxidase (MPO) activity) and oxidative parameters (thiobarbituric acid reactive species (TBARS) and non-protein thiol (NPSH) levels), respectively. DNCB increased the severity of skin lesions, scratching behaviour, edema and MPO activity of ears and dorsal skin TBARS levels. M-NC reversed skin severity scores, scratching behaviour and inflammatory response induced by DNCB. B-NC and M-F did not have effect in this model. In summary, meloxicam carried by polymeric nanocapsules reversed inflammatory response and ameliorated symptoms in an AD model.

© 2018 Faculty of Health and Social Sciences, University of South Bohemia in Ceske Budejovice.

Published by Elsevier Sp. z o.o. All rights reserved.

Introduction

Atopic dermatitis (AD) is a complex chronic inflammatory skin disease that affects up to 18% of children and up to 5% of adults worldwide, with up to 90% of patients presenting with mild to moderate disease (Bruin Weller et al., 2012; Shaw et al., 2011). This disease is characterized by erythematous and eczematous lesion, intense pruritus, dry and hypersensitivity skin (Bieber et al., 2012; Galli et al., 2008). The patient's skin with AD is very sensitive and has an appearance of chronic redness, after skin will thicken gradually becoming harsh, affecting their appearance and contributing significant psychological, social and quality-of-life burdens to patients

(Berke et al., 2012; Tokura, 2010). In addition, AD is often associated with comorbidities, such as allergic rhinitis and asthma (Bieber, 2010).

Topical treatments are commonly prescribed to improve AD symptoms and reduce inflammation (Carr, 2013). Recommended therapy has been the use of corticosteroids, calcineurin inhibitors, or both (Eichenfield et al., 2014). Although available topical agents are generally well tolerated and despite their efficacy, effectiveness of the their treatments is limited, their applications may cause cutaneous and systemic side effects with long-term use (Eichenfield et al., 2014; Ring et al., 2012). No new treatment has been approved for the AD therapy in the past 15 years. Thus, there is a need for searching of new effective topical treatment options without such safety concerns.

A new alternative treatment of AD is the use of non-steroidal anti-inflammatories. Recently, several studies showed the efficacy and safety of a non-steroidal anti-inflammatory, the crisaborole, for the topical treatment of AD in children and adults (Jarnagin et al., 2016; Paller et al., 2016; Tom et al., 2016; Zane et al., 2016a,b). However, studies using non-steroidal anti-inflammatory drugs

* Author for correspondence: Universidade Federal de Pelotas (UFPEL), Center for Chemical, Pharmaceutical and Food Sciences (CCQFA), Centre of Scientific Chemistry, Biochemistry and Bioprospecting Postgraduate Program (PPGBBio), Campus Capão do Leão, Pelotas, CEP 96010-900, RS, Brazil.

E-mail addresses: cristiane.luchese@ufpel.edu.br (C. Luchese), ethel.wilhelm@ufpel.edu.br (E.A. Wilhelm).

(NSAIDs) on the AD are still scarce. This study provides a new proposal for the treatment of AD using a NSAID, given that AD is an inflammatory disease.

Meloxicam is a highly potent NSAID that selectively inhibits the cyclooxygenase-2 (COX-2), blocking the prostaglandin synthesis and arachidonic acid metabolism (Honma et al., 2014). Its main limitations include poor aqueous solubility, poor incorporation into formulations and poor skin permeation (Jantharaprapap and Stagni, 2007; Lee et al., 2016). In this context, several studies have been carried out in order to improve the percutaneous absorption and bioavailability of meloxicam, avoiding the systemic side effect associated with oral route (Ah et al., 2010; Jantharaprapap and Stagni, 2007; Kasliwal et al., 2008; Lee et al., 2016; Ruiz et al., 2007; Yuan et al., 2007a,b). Importantly, the anti-inflammatory, analgesic and antipyretic properties of the meloxicam were investigated in a variety of studies that evidenced the action of the pharmacological substance in the skin and subcutaneous tissues from studies involving dogs, horses and laboratory animals (Engelhardt et al., 1995; Lees et al., 1991; Viking and Frendin, 2002), as well as in humans (Bayerl et al., 1998). In dermatology, polymeric nanoparticles have been used as an interesting and modern approach to improve drug solubility and efficacy, allowing sustainable distribution within the skin besides reducing dose and frequency of administration (Kumari et al., 2010). However, there is a lack of studies showing the influence of nanoencapsulation of meloxicam in polymeric nanoparticles on its effect in skin diseases, such as AD. Importantly, previous studies from our research group demonstrated that meloxicam-loaded nanocapsules (M-NC) showed higher pharmacological effect than free meloxicam (M-F) in different experimental inflammatory models (Ianski et al., 2012, 2016a,b; Villalba et al., 2014).

Based on the above considerations, the aim of this study was to investigate the M-NC effects on the topical treatment of symptoms, inflammatory response and oxidative stress in an AD model and to compare with those effects observed for M-F.

Materials and methods

Animals

Female adult BALB/c mice (6–8 weeks old) 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 room ($22 \pm 2^\circ\text{C}$). Commercial diet (GUABI, RS, Brazil)

and tap water were supplied ad libitum. We applied strict ethical criteria in our study to minimize pain and suffering and to reduce the number of animals used. Animals were used according to the guidelines of the Local Committee for Care and Use of Laboratory Animals of the Federal University of Pelotas (Pelotas, Brazil), under number 4294-2015. Mice were allowed to acclimatize to the holding room for 2 h before the behavioural procedure. All manipulations were conducted in the light phase.

Drugs

2,4-Dinitrochlorobenzene (DNCB), sorbitan monostearate, polysorbate 80 and poly (ϵ -caprolactone) MW = 80,000 were obtained from Sigma (St. Louis, MO). All other chemicals and solvents used were of analytical or pharmaceutical grade. All reagents were used as received.

Nanocapsules preparation

Suspensions of M-NC were prepared by the method of interfacial deposition of preformed polymer (Fessi et al., 1989), at a concentration of 0.3 mg/ml. Organic phase was composed by meloxicam (0.03 g), poly- ϵ -caprolactone (1.0 g), sorbitan monostearate (0.766 g), Miglyol 810[®] (3.102 g) and acetone (267 ml). Aqueous phase was composed of water (533 ml) and polysorbate 80 0.766 g. Organic phase was added under magnetic stirring into an aqueous phase. Suspensions of blank nanocapsules (B-NC) were prepared using the same protocol of M-NC suspensions, without the presence of drug. Meloxicam was dissolved in sorbitan monostearate, polysorbate 80 and water to obtain the solution of M-F. After preparation of suspensions, polymeric nanoparticles were incorporated into semisolid formulations (Ianski et al., 2012). Hydrogel of M-NC was formed by the dispersion of Carbopol[®] 940 6% (5.0 g), the preservative solution of methyl/propylparaben (1.0 ml), sorbitol (5.0 ml), triethanolamine (pH 7.0) and suspension of M-NC (89 ml). Hydrogel of M-F was prepared with meloxicam (0.0267 g), sorbitan monostearate (0.766 g, polysorbate 80 (0.766 g)) in addition to components previously described for the preparation of M-NC hydrogel, but using Milli-Q[®] water in place of the suspension colloid. Hydrogel of B-NC was prepared using the same protocol of hydrogel of M-NC.

The particle diameter was 247–212 nm for M-NC and blank B-NC, respectively. The polydispersity index was around 0.14 for M-NC and 0.10 for B-NC. The zeta potential was approximately

Fig. 1. Experimental protocol scheme. The dorsal skin of mice was shaved and sensitized with topical 0.5% DNCB on days 1–3. Next, the mice were challenged with 1% DNCB at dorsal skin on days 14, 17, 20, 23, 26, and 29 and the right ear on days 14–29. Mice were treated with a topical application (0.5 g) of free meloxicam (M-F), blank nanocapsules (B-NC) or meloxicam-loaded nanocapsules (M-NC) on days 14–29. Finally, mice were sacrificed on day 30.

–36 mV for M-NC and –35 mV for B-NC. The content of meloxicam nanocoated was 99.9%.

Experimental design

Fig. 1 described experimental protocol of induction and treatment of AD. Mice were randomly divided into five groups ($n = 7$ –8 per group). Mice belonging to group I (control group) were sensitized and challenged with acetone/olive oil (3:1). Animals of group II (DNCB group) were sensitized and challenged with DNCB. Animals belonging to groups III (B-NC + DNCB group), IV (M-F + DNCB group) and V (M-NC + DNCB group) were sensitized and challenged with DNCB and received topical treatment on the dorsal skin with of B-NC, M-F or M-NC, respectively.

AD-like pathology in mice was induced by DNCB as previously described by Chan et al. (2013). Mice were shaved of dorsal hair and DNCB (200 μ l of 0.5 v/v% in acetone/olive oil (3:1 ratio)) was applied to the area on experimental days 1–3 for sensitization. On days 14, 17, 20, 23, 26, and 29 mice were challenged with DNCB (100 μ l of 1 v/v%) on the dorsal skin, while on days 14–29 animals were challenged with DNCB (100 μ l of 1 v/v%) on the ear (right). Treatments with B-NC, M-F or M-NC were applied to the backs of the mice (0.5 g), daily, from days 14 to 29. Control animals received 200 μ l acetone/olive oil (3:1).

Assessment of the severity of skin lesions

On the day 30, the skin severity scores were measured according to Park and Sook (2014). Five signs of skin lesions were described: (1) pruritus/itching, (2) erythema/hemorrhage, (3) edema, (4) excoriation/erosion, and (5) scaling/dryness. The above-mentioned symptoms were graded as follows: 0 (no symptoms), 1 (mild), 2 (moderate), and 3 (severe).

Scratching behaviour

AD-like behavioural changes were investigated using scratching behaviour. Time that mice spent rubbing their nose, ears, and dorsal skin with their hind paws for 20 min was measured and recorded on the day 30 (Kim et al., 2014). The results obtained are expressed in seconds (s).

Inflammatory parameters

Ear edema

On the day 30, after scratching behaviour, mice were sacrificed and both ears were cut at the base and the weight difference between the samples of the control ear (left) and the DNCB-treated ear (right) was established on an analytical balance. The results obtained are represented in weight (mg). Subsequently, ears were used to determine the myeloperoxidase (MPO) activity.

MPO activity

MPO activity was assayed according to the method previously described by Suzuki et al. (1983) with some modifications. The ears were homogenized in PBS (20 mmol/l, pH 7.4) containing ethylenediaminetetraacetic acid (0.1 mmol/l). After the homogenization, samples were centrifuged at 900 rpm at 4 °C for 10 min to yield a low speed supernatant fraction (S_1). Then, S_1 fraction was centrifuged at 20,000 rpm at 4 °C for 15 min to yield a final pellet (P_2). P_2 fraction was resuspended in medium containing potassium phosphate buffer (50 mmol/l, pH 6.0) and hexadecyltrimethyl ammonium bromide (0.5 wt/v%). Samples were finally frozen and thawed three times prior the posterior enzymatic assay. For the MPO activity measurement, an aliquot of resuspended P_2 (100 μ l) was added to a medium containing the medium of resuspension

and N,N,N',N' -tetramethylbenzidine (1.5 mmol/l). The kinetic analysis of MPO was started after addition of hydrogen peroxide (0.01 v/v%), and colour reaction was measured at 655 nm (spectrophotometer U-2001 Hitachi-Japan) at 37 °C. Results are expressed as optic density (OD)/mg protein/min.

Oxidative parameters

Dorsal skin was removed and homogenized in 50 mM Tris-HCl, pH 7.4 (1:10 w/v) to determination of oxidative parameters. The homogenate was centrifuged at 2000 rpm for 10 min, and a S_1 was used for the following determinations: levels of thiobarbituric acid reactive species (TBARS) and non-protein thiol (NPSH).

TBARS levels

TBARS levels, a measure of lipid peroxidation, were determined as described by Ohkawa et al. (1979). An aliquot (200 μ l) of S_1 was incubated with 0.8% thiobarbituric acid, acetic acid buffer pH 3.4 and 8.1% sodium dodecyl sulfate at 95 °C for 2 h. The colour reaction was measured at 532 nm (spectrophotometer U-2001 Hitachi-Japan). TBARS levels were expressed as nmol MDA/mg protein.

NPSH levels

NPSH levels were determined by the method of Ellman (1959). A sample of S_1 was mixed (1:1 v/v) with 10% trichloroacetic acid. The samples were centrifuged at 3000 rpm for 10 min. After the centrifugation, the protein pellet was discarded and free-thiols groups were determined in the clear supernatant. An aliquot of supernatant (200 μ l) was added in 1 M potassium phosphate buffer pH 7.4 and 10 mM 5,5'-dithio-bis (2-nitrobenzoic acid). The colour reaction was measured at 412 nm (spectrophotometer U-2001 Hitachi-Japan). NPSH levels were expressed as μ mol NPSH/g tissue.

Protein quantification

Protein concentration was measured by the method of Bradford (1976), using bovine serum albumin (1 mg/ml) as the standard.

Statistical analysis

Data were expressed as mean $\pm$ standard derivation (SD). Data were analyzed by Graphpad Prism[®] 5 using one-way ANOVA followed by the Newman-Keuls test when appropriated. Values of $p < 0.05$ were considered statistically significant.

Results

Assessment of the severity of skin lesions

Effects of M-NC treatment on severity of skin lesions in mice are demonstrated in Fig. 2a and b [$F_{4,31} = 43.01$, $p < 0.0001$]. One-way ANOVA followed by Newman-Keuls' post-hoc test showed that DNCB significantly increased skin severity scores, when compared with the control group (Fig. 2a, b). M-NC treatment partially reduced (around 51%) the severity of skin lesions induced by DNCB (Fig. 2a, b). In addition, no significant difference in severity of skin lesions was observed after treatments with B-NC or M-F, when compared to DNCB group (Fig. 2a, b).

Scratching behaviour

Effects of M-NC treatment on scratching behaviour are showed in Fig. 3 [$F_{4,31} = 16.22$, $p < 0.0001$]. One-way ANOVA followed by Newman-Keuls' post-hoc test revealed that DNCB-exposed animals increased the scratching time, when compared with the control group (Fig. 3). M-NC treatment partially decreased (around

Fig. 2. Effect of meloxicam-loaded nanocapsules (M-NC), free meloxicam (M-F) or blank nanocapsules (B-NC) on DNCB-induced AD-like symptoms in mice. (a) Images of skin and ear lesions from the groups of mice taken on the last day of the experiment (day 30); (b) Skin lesions scores evaluated on day 30. Data represent the mean $\pm$ standard deviation (SD) (one-way ANOVA followed by the Newman–Keuls' test); (*) $p < 0.05$ compared to the control group, (#) $p < 0.05$ compared to the DNCB group and (&) $p < 0.05$ compared to the M-F.

Fig. 3. Effect of meloxicam-loaded nanocapsules (M-NC), free meloxicam (M-F) or blank nanocapsules (B-NC) on DNCB-induced scratching behaviour. Scratching time was evaluated on day 30. Data represent the mean $\pm$ standard deviation (SD) (one-way ANOVA followed by the Newman–Keuls' test); (*) $p < 0.05$ compared to the control group, (#) $p < 0.05$ compared to the DNCB group and (&) $p < 0.05$ compared to the M-F.

46%) DNCB-induced scratching behaviour, whereas B-NC or M-F treatments were not effective (Fig. 3).

Inflammatory parameters: ear edema and MPO activity

Fig. 4a illustrates the effects of M-NC treatment on ear edema in mice [$F_{4,31} = 22.11$, $p < 0.0001$]. One-way ANOVA followed by Newman–Keuls' post-hoc test demonstrated that DNCB substantially increased right ear swelling, when compared with the control group (Fig. 4a). Topical application of M-NC partially reduced (around 44%) the ear swelling induced by DNCB (Fig. 4a). No significant difference in the ear swelling was observed after treatments with B-NC or M-F, when compared to DNCB group (Fig. 4a).

Results depicted in Fig. 4b show effects of M-NC administration on the ear MPO activity [$F_{4,31} = 28.63$, $p < 0.0001$]. One-way ANOVA followed by Newman–Keuls' post-hoc test revealed that DNCB significantly increased the MPO activity in the mouse ear, when compared to the control group (Fig. 4b). NC-M treatment completely restored the ear MPO activity to the control levels, while B-NC or M-F treatments did not have effect (Fig. 4b).

Fig. 4. Effect of meloxicam-loaded nanocapsules (M-NC), free meloxicam (M-F) or blank nanocapsules (B-NC) on DNCB-induced inflammatory parameters in mice: (a) edema (day 30) and (b) myeloperoxidase (MPO) activity in the ears of mice. Data represent the mean $\pm$ standard deviation (SD) (one-way ANOVA followed by the Newman–Keuls' test); (*) $p < 0.05$ compared to the control group, (#) $p < 0.05$ compared to the DNCB group and (&) $p < 0.05$ compared to the M-F group.

Oxidative parameters: TBARS and NPSH levels

Fig. 5a demonstrates the NC-M effect on dorsal skin TBARS levels of mice [$F_{4,31} = 2.794$, $p < 0.05$]. One-way ANOVA followed by Newman–Keuls' post-hoc test showed that DNCB increased TBARS levels in dorsal skin of mice, when compared with the control group (Fig. 5a). NC-M, M-F or B-NC application did not have effect in reducing the TBARS levels increased by DNCB (Fig. 5a).

As verified in Fig. 5b, statistical analysis demonstrated that NPSH levels of dorsal skin were not altered by DNCB or by NC-M, M-F or B-NC treatments, when compared to the control group [$F_{4,31} = 1.503$, $p > 0.05$].

Discussion

In the present study, we proposed a new therapy for AD using nanotechnology and the meloxicam (a NSAID). Our results confirmed that DNCB induced the AD-like pathology in mice. The most important finding of this study is that topical application of M-NC was effective in attenuate DNCB-induced inflammatory response and subsequently ameliorated AD symptoms in mice. However, B-NC or M-F treatment did not have effect in this model. Therefore, our current results indicate that M-NC could be an alternative for treatment of AD.

Fig. 5. Effect of meloxicam-loaded nanocapsules (M-NC), free meloxicam (M-F) or blank nanocapsules (B-NC) on DNCB-induced oxidative stress parameters in mice: levels of (a) thiobarbituric acid reactive species (TBARS) and (b) non-protein thiol (NPSH) in the dorsal skins of mice. Data represent the mean $\pm$ standard deviation (SD) (one-way ANOVA followed by the Newman–Keuls' test). (*) $p < 0.05$ compared to the control group.

AD is a complex chronic inflammatory skin disease characterized by erythematous and eczematous skin lesions, often intense pruritus, an impairment of the skin-barrier function, increased oxidative stress, and dysfunctional immune system (Bieber et al., 2012; Sivarajani et al., 2013). AD model induced by DNCB is widely used to study this disease, once it mimics the clinical symptoms this pathology (Berke et al., 2012; Fang et al., 2015; Jin et al., 2009; Kim et al., 2014, 2015; Lin et al., 2015; Park and Sook, 2014; Tokura, 2010; Yoon et al., 2015). In this view, the present study confirmed that DNCB caused AD-like symptoms (as evidenced by increase on the severity of skin lesions and scratching behaviour), inflammatory process (as showed by increase in the ear edema and ear MPO activity) and lipid peroxidation (as evidenced by increase of dorsal skin TBARS levels) in BALB/c mice, causing AD-like pathology.

Considering the numerous side effects resulted of long-term use of therapies to AD, there is a great need to develop new and effective therapeutic strategies for AD. In this context, researchers have sought to identify new treatments to AD (Choi et al., 2017a,b; Jiang et al., 2017; Lee et al., 2016; Lin et al., 2017; Voss et al., 2018; Yoon et al., 2015). Here, we demonstrated that meloxicam carried by polymeric nanocapsules topically applied was efficient in the treatment of AD induced by DNCB in mice. In fact, M-NC partially reduced DNCB-induced skin lesion severity, while M-F or B-NC treatments did not have effect in this parameter, indicating

effectiveness of M-NC against AD-like skin damage. Indeed, clinical skin severity scores have been used to evaluate the progression/treatment of AD (Park and Sook, 2014).

Moreover, topical treatment with M-NC partially decreased DNCB-induced scratching behaviour, whereas B-NC or M-F treatments were not effective against the pruritus/itching. Itching is a characteristic symptom of AD and analysis of spontaneous scratching behaviour is useful for evaluating of effectiveness of anti-pruritus/itching (Park and Sook, 2014). The reduction of pruritus/itching is very important for the treatment of AD, given that the act of scratching aggravates the skin lesions and causes more itching ensues. These finding indicated that the treatment with meloxicam into nanocapsules form was effective in attenuating scratching behaviour, reducing the itching, preventing aggravation of the skin lesion in AD.

Another important finding of this study was M-NC treatment reduced the ear edema and ear MPO activity, indicating a reduction severity of inflammation in AD. However, M-F or B-NC treatments did not have effect in these parameters. It is well established that DNCB causes a skin irritation and local inflammation due to increase vascular permeability, developing edema in the ear (Kim et al., 2014; Park and Sook, 2014). Thereby, polymorphonuclear leukocytes migrate to the inflammation local, increasing the MPO activity in the site, given that this enzyme is used as a marker of leukocytes infiltration (Odobasic et al., 2016). Moreover, meloxicam inhibits COX-2 activity, blocking prostaglandins production, arachidonic acid metabolism and cytokine production, reducing the vasodilatation and migration polymorphonuclear leukocytes to the inflammation site (Ah et al., 2010; Mazario et al., 2001). In this context, we can suggest that M-NC treatment could cause a reduction of inflammation by inhibiting the COX-2 activity, reducing the vasodilatation and consequently the edema, and reducing the leukocytes infiltration into the ear tissues that corroborate with the inhibition on the MPO activity.

Oxidative stress has been associated with AD, leading to increase in production of reactive species (Li et al., 2016). In the present study, it was demonstrated an increase in the lipid peroxidation of mouse dorsal skin induced by DNCB, but treatments with M-NC, B-NC or F-M did not have effect in this parameter. A previous study by our group showed the cerebral antioxidant effect of M-NC in a neurodegenerative disease model (Ilaniski et al., 2012). However, here, we can rule out the involvement of M-NC antioxidant effect in the protection against lipid peroxidation in this model of AD.

Taking everything into consideration, results of the present study demonstrated that meloxicam into nanocapsules form showed a better effect in restoring symptoms and inflammatory process in a mouse AD model than M-F. Previously, our group demonstrated that M-NC were more powerful than M-F in several inflammatory models (Ilaniski et al., 2012, 2016a,b; Villalba et al., 2014). Some hypotheses main explain the pharmacological effect more pronounced of M-NC than F-M in AD-like pathology. First, polymeric nanocapsules promoted a sustained release by slowly deliver the meloxicam, prolonging the release of drug. In accordance with the results demonstrated in this study, other authors have reported a superior efficacy of polymeric nanocapsules, when compared to free drug, in different experimental models (Badran et al., 2014; Bernardi et al., 2009a,b, 2010; Ilaniski et al., 2012, 2016a,b; Khachane et al., 2011; Villalba et al., 2014). Second, meloxicam into polymeric nanocapsules contributed to the reduction of skin dryness, reducing the damage caused by DNCB. In accordance, Wissing and Muller (2003) demonstrated that nanopaticles increased the cutaneous hydration degree, reducing transepidermal water loss. Moreover, a clinical symptom of AD is skin dryness and the use of substances that increase the cutaneous hydration are important (Eichenfield et al., 2014).

Based in our results, we recognize that M-NC technology can be used in any inflammatory response in the dermis where an anti-inflammatory treatment is justified (e.g. superficial burn wound or sunburn are indications close at hand, besides trauma). Proving the clinical benefit in these situations is indeed further down the line, but we believe in its potential, thus, more studies are necessary to elucidate these applications. Indeed, previous studies of our research group revealed enhanced anti-inflammatory benefits of M-NC in a mouse pleurisy model, as well as, antinociceptive and antiedematogenic effects in acute models of nociception (Ilaniski et al., 2016b; Villalba et al., 2014).

Although promising results have been obtained here, it is important to note that one of the limitations of this study is the great leap from an experimental inflammatory mice model for the treatment of AD in humans. Indeed, AD is a chronic non-contagious disease with no known causes that has no cure but can be treated. Topical application of hapten in mice, such as DNCB, is commonly used to induce skin contact hypersensitivity and it is widely used as animal model for allergic contact dermatitis. This model mimics the inflammatory process of the skin involved in development and maintenance of AD, however, an inflammatory process is a very broad entity, whereas the allergic background of AD has a more specific mechanism. In addition, no studies on the toxicity of M-NC have been performed in these clinical settings. In this context, further studies are needed to investigate pharmacological applications of M-NC and its toxicity in the skin.

Conclusions

In summary, the data reported herein clearly demonstrated that meloxicam carried by polymeric nanocapsules attenuated inflammatory response and subsequently ameliorated symptoms in a mouse model AD, while F-M treatment did not have effect. Additional investigations will be performed to determine the transdermal penetration of M-NC.

Conflict of interests

All authors declare that not have conflict of interests.

Acknowledgements

We gratefully acknowledge UFPel, FAPERGS (PqG 02/2017), CAPES, CNPq for the financial support. C.L. is recipients of CNPq fellowship.

References

- Ah, Y.C., Choi, J.K., Choi, Y.K., Ki, H.M., Bae, H.A., 2010. A novel transdermal patch incorporating meloxicam: *in vitro* and *in vivo* characterization. *Int. J. Pharm.* 385, 12–19.
- Badran, M.M., Taha, E.I., Tayel, M.M., Al-Suwayeh, S.A., 2014. Ultra-fine self nanoemulsifying drug delivery system for transdermal delivery of meloxicam: dependency on the type of surfactants. *J. Mol. Liq.* 190, 16–22.
- Bayerl, C., Pagung, R., Jung, E.G., 1998. Meloxicam in acute UV dermatitis – a pilot study. *Photodermatol. Photoimmunol. Photomed.* 14, 167–169.
- Berke, R., Singh, A., Guralnick, M., 2012. Atopic dermatitis: an overview. *Am. Fam. Phys.* 86, 35–42.
- Bernardi, A., Braganhol, E., Jäger, E., Figueiró, F., Edelweiss, M.I., Pohlmann, A.R., et al., 2009a. Indomethacin-loaded nanocapsules treatment reduces *in vivo* glioblastoma growth in a rat glioma model. *Cancer Lett.* 281, 53–63.
- Bernardi, A., Zilberstein, A., Jager, E., Campos, M.M., Morrone, F.B., Calixto, J.B., et al., 2009b. Effects of indomethacin-loaded nanocapsules in experimental models of inflammation in rats. *Br. J. Pharmacol.* 158, 1104–1111.
- Bernardi, A., Frozza, R.L., Horn, A.P., Campos, M.M., Calixto, J.B., Salbego, C., et al., 2010. Protective effects of indomethacin-loaded nanocapsules against oxygen-glucose deprivation in organotypic hippocampal slice cultures: Involvement of neuroinflammation. *Neurochem. Int.* 57, 629–636.
- Bieber, T., Cork, M., Reitamo, S., 2012. Atopic dermatitis: a candidate for disease-modifying strategy. *Allergy. Eur. J. Allergy Clin. Immunol.* 67, 969–975.
- Bieber, T., 2010. Atopic dermatitis. *Ann. Dermatol.* 22, 125–137.

- Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principles of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- Bruin Weller, M., Knulst, A., Meijer, Y., Bruijnzeel-Koomen, C., Pasmans, S., 2012. Evaluation of the child with atopic dermatitis. *Clin. Exp. Allergy* 42, 352–362.
- Carr, W., 2013. Topical calcineurin inhibitors for atopic dermatitis: review and treatment recommendations. *Paediatr. Drugs* 15, 303–310.
- Chan, C., Liou, C., Xu, P., Shen, J., Kuo, M., Len, W., et al., 2013. Effect of dehydroepiandrosterone on atopic dermatitis-like skin lesions induced by 1-chloro-2,4-dinitrobenzene in mouse. *J. Dermatol. Sci.* 72, 149–157.
- Choi, J.H., Song, Y.S., Lee, H.J., Kim, G.C., Hong, J.W., 2017a. The topical application of low-temperature argon plasma enhances the anti-inflammatory effect of Jaun-ointment on DNCB-induced NC/Nga mice. *BMC Complement. Altern. Med.* 17, 340.
- Choi, Y.Y., Kim, M.H., Lee, H., Ahn, K.S., Um, J.Y., Lee, S.G., et al., 2017b. *Cynanchum atratum* inhibits the development of atopic dermatitis in 2,4-dinitrochlorobenzene-induced mice. *Biomed. Pharmacother.* 90, 321–327.
- Eichenfield, L.F., Tom, W.L., Berger, T.G., Krol, A., Paller, A.S., Schwarzenberger, K., et al., 2014. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. *J. Am. Acad. Dermatol.* 70, 338–351.
- Ellman, L., 1959. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82, 70–77.
- Engelhardt, G., Homma, D., Schlegel, K., Uetzmann, R., Schnitzler, C., 1995. Anti-inflammatory, analgesic, antipyretic and related properties of meloxicam: a new non-steroidal anti-inflammatory agent with favourable gastrointestinal tolerance. *Inflamm. Res.* 44, 423–433.
- Fang, L., Cheng, C., Hwang, T., Huang, W., Liou, C., Chen, W., Wu, S., 2015. *Danggui buxue* tang inhibits 2,4-dinitrochlorobenzene: induced atopic dermatitis in mice. *Evid. Based Complement. Altern. Med.* 1–10.
- Fessi, H., Puisieux, F., Devissaguet, P., Ammoury, N., Benita, S., 1989. Nanocapsule formation by interfacial polymer deposition following solvent displacement. *Int. J. Pharm.* 55, 1–4.
- Galli, S.J., Tsai, M., Piliponsky, A.M., 2008. The development of allergic inflammation. *Nature* 454, 445–454.
- Honma, S., Shinohara, M., Takahashi, N., Nakamura, K., Hamano, S., Mitazaki, S., et al., 2014. Effect of cyclooxygenase (Cox)-2 inhibition on mouse renal interstitial fibrosis. *Eur. J. Pharmacol.* 740, 578–583.
- Ianiski, F.R., Alves, C.B., Souza, A.C., Pinton, S., Roman, S.S., Rhoden, C.R., et al., 2012. Protective effect of meloxicam-loaded nanocapsules against amyloid- β peptide-induced damage in mice. *Behav. Brain Res.* 230, 100–107.
- Ianiski, F.R., Alves, C.B., Ferreira, C.F., Rech, V.C., Savegnago, L., Wilhelm, E.A., Luchese, C., 2016a. Meloxicam-loaded nanocapsules as an alternative to improve memory decline in an Alzheimer's disease model in mice: involvement of Na⁺, K⁺-ATPase. *Metab. Brain Dis.* 31, 793–802.
- Ianiski, F.R., Silva, F.D., Wilhelm, E.A., Fernandes, R.S., Alves, M.P., Duarte, M., Luchese, C., 2016b. Enhanced anti-inflammatory benefits of meloxicam-loaded lipid-core nanocapsules in a mouse pleurisy model: a comparative study with a free form drug. *J. Appl. Biomed.* 14, 105–112.
- Jantharaprapap, R., Stagni, G., 2007. Effects of penetration enhancers on *in vitro* permeability of meloxicam gels. *Int. J. Pharm.* 343, 26–33.
- Jarnagin, K., Chanda, S., Coronado, D., Ciaravino, V., Zane, L.T., Guttman-Yassky, E., Lebowitz, M.G., 2016. Crisaborole topical ointment, 2%: a nonsteroidal, topical, anti-inflammatory phosphodiesterase 4 inhibitor in clinical development for the treatment of atopic dermatitis. *J. Drugs Dermatol.* 15, 390–396.
- Jiang, X., Lan, Y., Wei, B., Dai, C., Gu, Y., Ma, J., et al., 2017. External application of NF- κ B inhibitor DHMEQ suppresses development of atopic dermatitis-like lesions induced with DNCB/OX in BALB/c mice. *Immunopharmacol. Immunotoxicol.* 39, 157–164.
- Jin, H., He, R., Oyoshi, M., Geha, R.S., 2009. Animal models of atopic dermatitis. *J. Invest. Dermatol.* 129, 31–40.
- Kasliwal, N., Derle, D., Negi, J., Gohil, K., 2008. Effect of permeation enhancers on the release and permeation kinetics of meloxicam gel formulations through rat skin. *J. Pharm. Sci.* 19, 193–199.
- Khachane, P., Date, A.A., Nagarsenker, M.S., 2011. Positively charged polymeric nanoparticles: application in improving therapeutic efficacy of meloxicam after oral administration. *Pharmazie* 66, 334–338.
- Kim, H., Kim, J., Kang, H., Choi, J., Yang, H., Lee, P., et al., 2014. 7,8,4'-trihydroxyisoflavone attenuates DNCB-induced atopic dermatitis-like symptoms in NC/Nga mice. *PLoS One* 9, e104938.
- Kim, C., Kang, M., Lee, Y., Min, W., Kim, Y., Kang, S., et al., 2015. Bathing effects of various seawaters on allergic (atopic) dermatitis – like skin lesions induced by 2,4-dinitrochlorobenzene in hairless mice. *Evid Based Complement. Altern. Med.* 2015, 1–25.
- Kumari, A., Yadav, S.K., Yadav, S.C., 2010. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids Surf. B: Biointerfaces* 75, 1–18.
- Lee, T., Zane, M.H., Hughes, S.S., 2016. Tolerability of crisaborole ointment for application on sensitive skin areas: a randomized, double-blind, vehicle-controlled study in healthy volunteers. *Am. J. Clin. Dermatol.* 17, 519–526.
- Lees, P., Sedgwick, A.D., Higgins, A.J., Pugh, K.E., Busch, U., 1991. Pharmacodynamics and pharmacokinetics of miloxicam in the horse. *Br. Vet. J.* 14, 97–108.
- Li, W., Wu, X., Xu, X., Wang, W., Song, S., Liang, K., et al., 2016. Coenzyme Q10 suppresses TNF- α -induced inflammatory reaction *in vitro* and attenuates severity of dermatitis in mice. *Inflammation* 39, 281–289.
- Lin, G., Gao, S., Cheng, J., Li, Y., Shan, L., Hu, Z., 2015. 1 β -Hydroxylantolactone, a sesquiterpene lactone from *inula japonica*, attenuates atopic dermatitis-like skin lesions induced by 2, 4-dinitrochlorobenzene in the mouse. *Pharm. Biol.* 54, 516–522.
- Lin, L., Zhou, Y., Li, H., Pang, D., Zhang, L., Lu, X., et al., 2017. Polysaccharide extracted from Chinese white wax scale ameliorates 2,4-dinitrochlorobenzene-induced atopic dermatitis-like symptoms in BALB/c mice. *Saudi Pharm. J.* 25, 625–632.
- Mazario, J., Gaitan, G., Herrero, J.F., 2001. Cyclooxygenase-1 vs. cyclooxygenase-2 inhibitors in the induction of antinociception in rodent withdrawal reflexes. *Neuropharmacology* 40, 937–946.
- Odobasic, D., Kitching, A.R., Holdsworth, S.R., 2016. Neutrophil-mediated regulation of innate and adaptive immunity: the role of myeloperoxidase. *J. Immunol. Res.* 2016, 2349817.
- Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95, 351–358.
- Paller, A.S., Tom, W.L., Lebowitz, M.G., Blumenthal, R.L., Boguniewicz, M., Call, R.S., et al., 2016. Efficacy and safety of crisaborole ointment, a novel, nonsteroidal phosphodiesterase 4 (PDE4) inhibitor for the topical treatment of atopic dermatitis (AD) in children and adults. *J. Am. Acad. Dermatol.* 75, 494–503.
- Park, G., Sook, O.H.M., 2014. Inhibitory effects of *juglans mandshurica* leaf on allergic dermatitis-like skin lesions-induced by 2,4-dinitrochlorobenzene in mice. *Exp. Toxicol. Pathol.* 66, 97–101.
- Ring, J., Alomar, A., Bieber, T., Deleuran, M., Fink-Wagner, A., Gelmetti, C., et al., 2012. Guidelines for treatment of atopic eczema (atopic dermatitis) part II. *J. Eur. Acad. Dermatol. Venereol.* 26, 1045–1060.
- Ruiz, M.A., Lopez, J., Benavides, M.M., Dios, J., Gallardo, V., 2007. Rheological behaviour of gels and meloxicam release. *Int. J. Pharm.* 333, 17–23.
- Shaw, T.E., Currie, G.P., Koudelka, C.W., Simpson, E.L., 2011. Eczema prevalence in the United States: data from the 2003 National Survey of Children's Health. *J. Invest. Dermatol.* 131, 67–73.
- Sivaranjani, N., Rao, S.V., Rajeev, G., 2013. Role of reactive oxygen species and antioxidants in atopic dermatitis. *J. Clin. Diagn.* 7, 2683–2685.
- Suzuki, K., Ota, H., Sasagawa, S., Sakatani, T., Fujikura, T., 1983. Assay method for myeloperoxidase in human polymorphonuclear leukocytes. *Anal. Biochem.* 132, 345–352.
- Tokura, Y., 2010. Extrinsic and intrinsic types of atopic dermatitis. *J. Dermatol. Sci.* 58, 1–7.
- Tom, W.L., Van Syoc, M., Chanda, S., Zane, L.T., 2016. Pharmacokinetic profile, safety, and tolerability of crisaborole topical ointment: 2% in adolescents with atopic dermatitis: an open-label phase 2a study. *Am. J. Clin. Dermatol.* 33, 150–159.
- Viking, H., Frendin, J., 2002. Analgesic effect of meloxicam in canine acute dermatitis – a pilot study. *Acta Vet. Scand.* 43, 247–252.
- Villalba, B., Ianiski, F., Wilhelm, E., Fernandes, R., Alves, M., Luchese, C., 2014. Meloxicam-loaded nanocapsules have antinociceptive and anti-dematogenic effects in acute models of nociception. *Life Sci.* 115, 36–43.
- Voss, G.T., Oliveira, R.L., de Souza, J.F., Duarte, L.F.B., Fajardo, A.R., Alves, D., et al., 2018. Therapeutic and technological potential of 7-chloro-4-phenylselanyl quinoline for the treatment of atopic dermatitis-like skin lesions in mice. *Mater. Sci. Eng. C* 84, 90–98. doi:<http://dx.doi.org/10.1016/j.msec.2017.11.026>.
- Wissing, S.A., Müller, R.H., 2003. The influence of solid lipid nanoparticles on skin hydration and viscoelasticity – *in vivo* study. *Eur. J. Pharm. Biopharm.* 56, 67–72.
- Yoon, H., Jang, M., Kim, H., Song, D., Nam, K.-I., Bae, C., et al., 2015. Protective effect of diet supplemented with rice prolamin extract against DNCB-induced atopic dermatitis in Balb/C mice. *BMC Complement. Altern. Med.* 15, 353.
- Yuan, Y., Chen, X., Zhong, D., 2007a. Determination of meloxicam in human plasma by liquid chromatography–tandem mass spectrometry following transdermal administration. *J. Chromatogr.* 852, 650–654.
- Yuan, Y., Li, S., Yu, L., Dang, P., Zhong, D., 2007b. Physicochemical properties and evaluation of microemulsion systems for transdermal delivery of meloxicam. *Chem. Res. Chin. Univ.* 23, 81–86.
- Zane, L.T., Chanda, S., Jarnagin, K., Nelson, D.B., Spelman, L., Gold, L.S., 2016a. Crisaborole and its potential role in treating atopic dermatitis: overview of early clinical studies. *Immunotherapy* 8, 853–866.
- Zane, L.T., Hughes, M.H., Shakib, S., 2016b. Tolerability of crisaborole ointment for application on sensitive skin areas: a randomized, double-blind, vehicle-controlled study in healthy volunteers. *Am. J. Clin. Dermatol.* 17, 519–526.