Biomedicine & Pharmacotherapy 99 (2018) 697–703

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Antioxidant and anti-inflammatory effect of plumieride in dextran sulfate sodium-induced colitis in mice

Thaise Boeing, Priscila de Souza*, Tiago José Bonomini, Luísa Nathália Bolda Mariano, Lincon Bordignon Somensi, Ruth Meri Lucinda, Angela Malheiros, Luísa Mota da Silva, Sérgio Faloni de Andrade

Programa de Pós-Graduação em Ciências Farmacêuticas, Núcleo de Investigações Químico-Farmacêuticas (NIQFAR), Universidade do Vale do Itajaí (UNIVALI), Rua Uruguai, 458, 88302-901 Itajaí, SC, Brazil

ARTICLE INFO

Keywords:
Alamanda
Inflammation
Inflammatory bowel disease
Iridoid
Oxidative stress
Ulcerative colitis

ABSTRACT

This study aimed to investigate the potential effect of plumieride, an iridoid glycoside isolated from *Alamanda cathartica* L. flowers, against dextran sulfate sodium (DSS)-induced colitis in mice. Colitis was induced in female swiss mice by adding DSS 3% to the drinking water. The animals were treated with vehicle (water), 5-aminosalicylic acid (100 mg/kg) or plumieride (10, 30 and 100 mg/kg) once a day, during 7 days. The body weight progression and the disease activity index was evaluated daily. On the eighth day, colons were collected for the measurement of the size, histological, histochemical, biochemical and inflammatory analysis. The cytotoxicity of plumieride on intestinal epithelial cell (IEC-6 cell line) was also evaluated. Plumieride, at dose of 100 mg/kg, significantly attenuated the mice weight loss, showed lower score in the disease activity index, diminished the colon shortening, improved the histological damage and avoided mucosa intestinal mucus depletion when compared with vehicle-treated only group. Moreover, plumieride was able to reduce the amount of colonic lipid hydroperoxides, while augmented reduced glutathione levels and superoxide dismutase activity. Although DSS intake stimulated an increase in myeloperoxidase activity and in tumor necrosis factor content on the colon tissue of the vehicle-treated group, the colons obtained from mice treated with plumieride did not present any of these changes. Taking together, the results of the present study disclose that plumieride exhibited a significant efficacy in attenuating the parameters of experimental ulcerative colitis, which may be mediated by an antioxidant and anti-inflammatory effect.

1. Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) characterized as an immune-mediated chronic intestinal disorder [1,2]. The main pathological feature of UC is a diffuse inflammation involving mucosal changes in the rectum and variable lengths of the proximal colon and more severely of distal colon [3]. Although the etiology of UC is not exactly understood, some factors like environment, intestinal microbiota [4], genetics and abnormal immune response [5] seem to be fundamental to evoke an overproduction of pro-inflammatory mediators, such as the reactive oxygen species, cytokines and neutrophil infiltration, which are recognized as markers in the pathogenesis of colitis [6,1,2].

In clinical practice, patients with UC are usually young adults in productive age, which are affected by several symptoms such as blood in the stool, diarrhea and abdominal pain, contributing to the reduction of their quality of life and increasing the risks of developing colorectal cancer [7]. Unfortunately, the treatments that are used in UC, including 5-aminosalicylic acid (5-ASA), steroids and immunosuppressive drugs (for instance azathioprine and 6-mercaptopurine) only set out to induce and maintain the remission of the disease and even so, 20–40% of the patients do not respond well to these drugs [5]. Recently, anti-TNFα therapies have shown improved response rates in IBD, however the tolerance developed during the treatment and immunogenicity proceed being major concerns [8], which makes essential to continue searching for new therapies with higher effectiveness and lower incidence of side

ABBREVIATIONS: ATR, atropine; CAT, catalase; DAI, Disease Activity Index; DMSO, dimethyl sulfoxide; DSS, dextran sulfate sodium; GSH, reduced glutathione; HE, hematoxylin & eosin; IBD, inflammatory bowel disease; IEC-6, intestinal epithelial cell; LOOH, lipid hydroperoxides; MPO, myeloperoxidase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NV, naïve; PAS, Periodic acid of Schiff; PLU, plumieride; SOD, superoxide dismutase; TNFα, tumor necrosis factor; UC, ulcerative colitis; VEH, vehicle; 5-ASA, 5-aminosalicylic acid

* Corresponding author at: Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade do Vale do Itajaí (UNIVALI), Rua Uruguai, 458, 88302-901 Itajaí, SC, Brazil.
E-mail address: priscilasouza_rc@yahoo.com.br (P. de Souza).

https://doi.org/10.1016/j.biopha.2018.01.142
Received 22 November 2017; Received in revised form 21 December 2017; Accepted 28 January 2018

effects.

Bioactive compounds from natural products, such as those derived from plants, have been studied worldwide to the discovery and the development of new drugs [9]. Iridoids for example, are secondary metabolites found in several medicinal plants species [10,11,2], which have shown been effective in different models of UC in mice [1,2,7].

A potential compound of this class is plumieride, found in *Allamanda cathartica* [10] and also in several species of the genus *Plumeria* [12–15], which has shown some promising biological activities [15,16,12]. Among the properties of this molecule, stands out the antioxidant potential against the peroxidative damage in the liver of rats, especially due its ability to inhibit the membrane lipid peroxidation and to restore the antioxidant system of the organism [15]. Therefore, the potential of iridoids in attenuating inflammatory bowel diseases along with the previous reports of antioxidant potential of the plumieride led us to the main purpose of this study, to investigate the effect of plumieride, an iridoid glycoside isolated from *A. cathartica* flowers, against dextran sulfate sodium (DSS)-induced colitis in mice.

2. Materials and methods

2.1. Chemical characterization of plumieride

Plumieride was isolated from *Allamanda cathartica* flowers extract by column chromatography. The whole process of preparation of the extract, purification, isolation and identification of the plumieride can be accessed in detail in Bonomini et al. [17]. The purity of the compound was assessed by HPLC and NMR analysis as previously reported [17]. The plumieride presented a purity of > 98%.

2.2. Animals

Female swiss mice (25–30 g) were provided by Universidade do Vale do Itajaí (UNIVALI), Itajaí, SC, Brazil. The mice were maintained under standard laboratory conditions (12 h light/dark cycle, temperature of 22 ± 2 °C) with free access to food and water. The experiments were performed under the approval of the institutional animal ethics committee of UNIVALI (certificate number 045/15) and were carried out in accordance with the international standards and following all the ethical guidelines on animal welfare.

2.3. Evaluation of digestive motility

The mice were separated into different groups (n = 6) and were treated with vehicle (VEH: distilled water, 1 mL/kg, p.o.), atropine (ATR: 3 mg/kg, s.c.) or plumieride (PLU: 100 mg/kg, p.o.). Thirty minutes later ATR or one hour after VEH or PLU treatments, the animals received 0.5 mL of a semisolid marker solution (0.05% phenol red plus 1.5% carboxymethyl cellulose). Then, after 20 min, the mice were euthanized and small intestine were removed. The distance traveled by phenol red (A) and the total length of the small intestine (B) were measured to further calculate the digestive motility rate (DM %) through equation $DM = A \div B \times 100$.

2.4. Dextran sulfate sodium (DSS)-induced colitis

Colitis was induced as described by Chassaing et al. [18]. The mice were separated into different groups (n = 6–8) and were orally treated for 7 days with vehicle (VEH: distilled water, 1 mL/kg), 5-aminosalicylic acid (5-ASA: 100 mg/kg) and plumieride (PLU: 10, 30 and 100 mg/kg). Simultaneously, these groups received 3% DSS (Alfa Aesar, Heysham, Lancashire, UK. MW: 40,000) in drinking water, ad libitum, during five days to induce colitis. Further, a noncolitic group (naïve - NV) received water without DSS, and on the 6th and 7th day of experiment, all groups received just water. The animals' body weight, the presence of rectal gross blood, and stool consistency were evaluated daily and used to assign a score and subsequently calculate the Disease Activity Index (DAI) as fully detailed by DA SILVA et al. [19]. After euthanasia in the 8th day of the experiment, liver and spleen of each animal were weighted (g/10 g) and colon length was measured (cm). In addition, representative specimens of the colon (0.5 cm length) were collected, fixed in ALFAC solution (80% alcohol, 15% formaldehyde, and 5% acetic acid), dehydrated, embedded in paraffin wax, sectioned at 5 mm and stained with hematoxylin & eosin (HE) for histological analyzes or stained with Periodic acid of Schiff (PAS) for histochemical analyzes of mucin content. The glycoproteins were quantified using the program Image J® and expressed as pixels/field. The remaining tissue was collected to evaluate oxidative and inflammatory parameters.

2.5. Determination of oxidative and inflammatory parameters

The colonic tissue collected were homogenized with 200 mM potassium phosphate buffer (pH 6.5). The homogenate obtained was used to determine the reduced glutathione (GSH) and lipid hydroperoxides (LOOH) levels. After that, the remaining homogenate was centrifuged at 11,000 rpm for 20 min at 4 °C. The supernatant was used to determine superoxide dismutase (SOD) and catalase (CAT) activity and the pellet was suspended to determine the myeloperoxidase (MPO) activity. Protein concentrations were determined by the Bradford method (Bio-Rad, Hercules, CA, USA) and cytokines content (TNF-??, IL-6 and IL-10) were assessed by enzyme-linked immunosorbent assay (ELISA) using mouse cytokine ELISA kits from BD Biosciences (Franklin Lakes, New Jersey, USA), according to the manufacturer's instructions. For a detailed protocol of these measurements, see Boeing et al. [20].

2.6. Cell viability

Cell viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The IEC-6 (rat small intestine epithelial) cell line were seeded in a 96-well plate at density of 10^6 cells/well. Different concentrations of plumieride (0.1, 1, 10 and 100 µg/ml) or Dulbecco's Modified Eagle Medium (DMEM - control group) or dimethyl sulfoxide (DMSO – control group; 10%) were incubated with the cells for 21 h, then 10 µl MTT (5 mg/ml in PBS) were add in each well. After 3 h of the MTT incubation, the medium was removed and 100 µl of DMSO was added to solubilize the formazan crystals. Finally, each well was homogenized for 5 min and the optical density was read at 570 nm. The viability of the basal group was considered to be 100% and the results were expressed as percentage of cell viability.

2.7. Statistical analysis

The data were expressed as means ± SEM. Statistical analysis were performed using the software GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, USA). Differences between means were determined by one- or two-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. A $p < .05$ was considered significant in all experiments.

3. Results

3.1. Effect of plumieride on the digestive motility rate in mice

Initially, the effect of plumieride, at the highest dose of 100 mg/kg, on digestive motility rate was evaluated in order to determine if this compound could increase this parameter and therefore, cause diarrhea. As shown in the Fig. 1, the mean of the digestive motility rate verified by the distance covered by the semisolid marker solution after 20 min in the VEH group was 50 ± 3.5%. The ATR, a competitive antagonist of muscarinic acetylcholine receptor used as positive control group, significantly decreased the digestive motility rate (23 ± 2.3%)