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Use of *Bidens pilosa* L. (Asteraceae) and *Curcuma longa* L. (Zingiberaceae) to treat intestinal mucositis in mice: Toxico-pharmacological evaluations

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ABSTRACT

Introduction: Several studies towards the development of an effective treatment for intestinal mucositis have been reported, since this condition represents a major problem in clinical oncology practice due to cytotoxic effects of chemotherapy. However standardized protocols and universally accepted treatment options are yet to be established.
Objectives: Given above, this study evaluated the protective effects of a mucoadhesive formulation containing both *Bidens pilosa* L. (Asteraceae) (BP) and curcuminoids from *Curcuma longa* L. (Zingiberaceae) (CL) on intestinal mucositis induced by 5-fluoruoacil (5-FU) in mice.
Results: As expected, animals only treated with 5-FU (200 mg/kg) showed a significant reduction of 60.3 and 42.4% in villi and crypts size, respectively, when compared to control. On the other hand, the proposed therapeutic/prophylactic treatment with mucoadhesive formulations managed to reduce histopathologic changes in mice bearing mucositis, especially at 125 mg/kg BP + 15 mg/kg CL dose. The formulation promoted an increase of 275.5% and 148.7% for villi and crypts size, respectively. Moreover, chemotherapy-related weight loss was reduced by 7.4% following the treatment. In addition, an increase of 10 and 30.5% in red and white blood cells was observed when compared to 5-FU group. Furthermore, treatments with the mucoadhesive formulation containing BP/CL up modulated Ki-67 and Bcl-2 expression while reduced pro-apoptotic regulator Bax. The formulation also modulated inflammatory response triggered by 5-FU through reduction of 68% of myeloperoxidase activity and a 4-fold increase in anti-inflammatory IL-10 levels. In parallel, the oxidative stress *via* lipid peroxidation was reduced as indicated by decrease of 63% of malondialdehyde concentrations. Additionally, the new formulation presented low acute oral systemic toxicity, being classified in the category 5 (2000 mg/kg < LD_{50} < 5000 mg/kg) of the Globally Harmonized Classification System.
Conclusions: This study showed an interesting potential of the mucoadhesive formulation of BP/CL for the treatment of 5-FU-induced intestinal mucositis. Given the perspectives for the development of a new medicine, clinical studies are in progress to better understand the protective effects of this innovative formulation in treating mucositis.

1. Introduction

Intestinal mucositis represents a major problem in clinical oncology practice due to cytotoxic effect associated with chemotherapy [1]. This pathological condition is correlated with events such as apoptosis, epithelial hypoproliferation, crypts/villi

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size decreasing, inflammatory infiltrate, increased expression of TNF-α, IL-1β and IL-6, with consequent changes in the intestinal absorptive capacity and bacterial colonization [2]. Mucositis is also frequently associated with abdominal pain, diarrhea, vomiting and nausea [3,4]. Additionally, mucositis is the cause of patient longer hospitalization, raising the total cost of the treatment which becomes an economical and public health problem [5]. Although several studies have already been conducted in attempts to treat mucositis, there are still no universally accepted and standardized protocols for the treatment. Substances of natural origin have been recently studied in this context, especially those with anti-inflammatory and anti-oxidant properties such as *Bidens pilosa* L. (Asteraceae) (BP) and *Curcuma longa* L. (Zingiberaceae) (CL).

BP is a plant present in tropical and sub-tropical regions, widely used in folk medicine with an extensive phytochemical constitution [6,7]. In addition to anti-inflammatory and anti-oxidant effects, several pharmacological activities related to BP have been reported such as anti-hypertensive, anti-hyperglycemic, antiulcer, immunosuppressive, hepatoprotective, antileukemic, antimalarial and antibacterial [8,9]. Its efficacy has been recently demonstrated *in vivo* on gastric mucosal injury induced by hydrochloric acid/ethanol in rats. Oral administration of BP exerted a protective effect in these lesions, which probably contributed to the suppression of oxidative stress, prostaglandin production and inflammation [10]. Recently, Ávila et al. [11], demonstrated that a mucoadhesive formulation based on BP reduced intestinal injury in mice caused by 5-fluorouracil, in special the formulation led to a reduction in the local inflammatory infiltrate.

CL and its compounds, especially curcumin, have shown important anti-inflammatory and anti-oxidant properties, and have been widely studied in cancer chemoprevention and suppression of tumor growth [12,13]. CL also showed immunomodulatory capacity, exerting its effects by regulating the expression of several genes and proteins [14]. Moreover, previous study has demonstrated that a formulation containing curcumin, α-tocopherol and sunflower oil was effective in reducing radiation-induced ulceration of the oral mucosa in rats [15]. Furthermore, a pilot study with pediatric patients undergoing chemotherapy has highlighted the promising use of topical curcumin for prevention of oral mucositis [16]. Previous work from our group showed that a mucoadhesive formulation with curcuminoids from CL positively modulated Ki-67 protein in villi and crypts epithelium of mucositis-bearing mice, acting positively on the rate of intestinal cell proliferation [17].

In spite of the wide spectrum of biological effects of the curcumin, its low oral bioavailability and poor water solubility represent a limiting factor for its therapeutic use. These limitations have led to the development of various formulation strategies to improve curcumin effects, such as mucoadhesive products. Mucoadhesion systems are based on polymeric compounds, which have the ability to adhere to the surface of cells, or to the mucus layer that covers the epithelium. These formulations are of great pharmaceutical interest due to their ability in prolonging the residence time of the drug at the absorption site. In addition, they can promote a more intimate contact with the mucosa providing better topical treatment [18,19]. In this regarding, previous work from our group showed that a mucoadhesive formulation of CL also attenuated body weight loss and protected intestinal mucosa from villus shortening and crypt deepening induced by 5-FU [17].

Previous data indicate that BP or CL alone treated intestinal mucositis through different mechanisms, reducing the local inflammatory infiltrate and/or increasing the rate of intestinal cell proliferation, respectively. Based on these findings, this study evaluated the protective effects of a mucoadhesive formulation based on poloxamer 407, a tri-block copolymer with mucoadhesive properties, containing both BP/CL extracts in mice bearing intestinal mucositis induced by 5-fluorouracil (5-FU).

2. Materials and methods

2.1. Chemicals

C. longa (>95% curcuminoid content) and *B. pilosa* glycolic extracts (Ecobidens®) were obtained from GAMMA (São Paulo, SP, Brazil) and CHEMYUNION (Sorocaba, SP, Brazil), respectively. Transcutol HP® (diethylene glycol monoethyl ether) was kindly donated by Gattefossé (Lyon, France). Soluplus® HS15 (Macrogol-15-hydroxystearate) was kindly donated by Pharma Ingredients and Services BASF (São Paulo, SP, Brazil). Polyethylene glycol 400 and sodium azide were acquired from Labsynth (Diadema, SP, Brazil). Butylated hydroxytoluene and 3,3diaminobenzidine (DAB) were obtained from Mapric (São Paulo, SP, Brazil) and Dako (Carpinteria, CA, USA), respectively. 5-Fluorouracil, hexadecyltrimethylammonium bromide, tri-block copolymer poloxamer 407, bovine serum albumin (BSA), EDTA, phenylmethylsulfonyl fluoride (PMSF), kallikrein inhibitor units of aprotinin A, *n*-butanol and *ortho*-dianisidine were purchased from Sigma–Aldrich (St. Louis, MO, USA). ImmunoCruz™ mouse ABC staining systems (sc-2017 and 2018), monoclonal mouse anti-mouse p53 (clone 3H2820), monoclonal mouse anti-human Bcl2 (clone C-2) and polyclonal rabbit anti-mouse Bax (clone P-19) antibodies were acquired from Santa Cruz Biotechnology (Paso Robles, CA, USA); whereas monoclonal mouse anti-human Ki-67 (clone 124) antibody were obtained from Novacastra (Newcastle, UK). Mouse IL-10 BD Cytometric Bead kit was purchased from BD Biosciences (San Diego, CA, USA). Trichloroacetic acid, NaCl, Tween 20, benzethonium chloride, H_2O_2, tris, HCl, methanol and xylene were obtained from Vetec (Rio de Janeiro, RJ, Brazil). Thiobarbituric acid, hematoxylin and eosin stainings were acquired from Merck (Darmstadt, HE, Germany). Xylazine and ketamine were purchased from Syntec (Cotia, SP, Brazil) and König (Santana de Parnaíba, SP, Brazil), respectively.

2.2. Preparation of mucoadhesive and non-mucoadhesive formulations

The mucoadhesive formulation was composed by CL (1%, m/m) and BP (40%, v/v) extracts, tri-block copolymer poloxamer 407 (15%, m/m), Surplus® HS15 (3.2%, m/m), Transcutol HP (10%, v/v), citric acid (to pH 4.5–6.0) and polyethylene glycol 400 as a liquid vehicle. The non-mucoadhesive formulation was prepared without poloxamer, which was replaced by PEG 400.

Formulations were prepared by mixing constituents in a heated reactor (65–70 °C) under mechanical stirring. The pH of the final preparation was adjusted using citric acid. Once prepared, formulations were placed in ambar flasks and stored at room temperature protected from light until use. The total polyphenol content of the BP extract determined by the Folin Ciocalteau method was 88.2 ppm of total polyphenols, which was within the range (20–200 ppm) of the analysis certificate issued by the Ecobidens® manufacturer. The total curcuminoid content of the formulations, based on curcumin levels, was evaluated by UV spectrophotometry (λ = 425 nm).

2.3. Animals

Male Swiss mice (age: 8–10 weeks; weight: 35–40 g) were obtained from the Bioterium at Federal University of Goiás, and all efforts were conducted to ensure the animal welfare. Mice were acclimatized for a week prior to the beginning of experiments and kept under steady conditions with light–dark cycles and controlled temperature, while water and food were provided ad libitum. The experimental protocol (UFG no. 036/2012) was approved by the Research Ethics Committee at this University. At the end of each assay, animals were previously anesthetized by 10 mg/kg of