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ORIGINAL ARTICLE

Treatment with selenium-enriched *Saccharomyces cerevisiae* UFMG A-905 partially ameliorates mucositis induced by 5-fluorouracil in mice

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Abstract

Purpose Gastrointestinal mucositis is a major problem associated with cancer therapy. To minimize these deleterious effects, simultaneous administration of antioxidant components, such as selenium, can be considered. There is a growing interest in the use of yeasts because they are able to convert inorganic selenium into selenomethionine. In the present study, oral administration of *Saccharomyces cerevisiae* UFMG A-905 enriched with selenium was evaluated as an alternative in minimizing the side effects of 5FU-induced mucositis in mice.

Methods Mice body weight, food consumption, faeces consistency and the presence of blood in faeces were assessed daily during experimental mucositis induced by 5-fluorouracil (5FU). Blood was used for intestinal permeability determination, and small intestine for oxidative stress, immunological and histopathological examination.

Results The increased intestinal permeability observed with mucositis induction was partially reverted by *S. cerevisiae* and selenium-enriched yeast. Both treatments were able to reduce myeloperoxidase activity, but only selenium-enriched yeast reduced eosinophil peroxidase activity. CXCL1/KC levels, histopathological tissue damage and oxidative stress (lipid peroxidation and nitrite production) in the small intestine were reduced by both treatments; however, this reduction was always higher when treatment with selenium-enriched yeast was evaluated.

Conclusions Results of the present study showed that the oral administration of *S. cerevisiae* UFMG A-905 protected mice against mucositis induced by 5-FU, and that this effect was potentiated when the yeast was enriched with selenium.

Keywords Probiotics · *Saccharomyces cerevisiae* UFMG A-905 · Selenium · Selenium-enriched yeast · Mucositis · 5-Fluorouracil

Introduction

Mucositis is the most important side effect to cancer therapy such as chemotherapy and/or radiotherapy. It is characterized by an inflammatory response, which affects the gastrointestinal tract [1], altering the intestinal permeability and causing bacterial translocation [2, 3]. Almost all patients undergoing chemotherapy develop mucositis, and this implies in the need of treatment interruption also due to high costs associated with symptom management [1].

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The anti-metabolite agent 5-fluorouracil (5FU) is used in the treatment of many types of cancers, including colorectal and breast cancers. However, its use causes tissue damage through generation of reactive oxygen species (ROS) which precedes the inflammatory process involved in mucositis pathogenesis [4, 5]. Production of ROS can damage lipids, proteins and DNA, followed by an inflammatory response and tissue ulceration [4]. Thus, therapeutic strategies that target ROS production should be very promising. Nowadays, treatment of mucositis is mainly supportive and involves therapy with growth factors (palifermin), anti-inflammatory and antimicrobials agents or analgesics, as well as adjuvants strategies (i.e. glutamine supplementation, cryotherapy and laser therapy). Recently, the use of probiotics has also been recommended as a therapeutic strategy for the prevention of secondary effects of chemo- and radiotherapy in cancer patients [1].

Selenium (Se) is an essential mineral element and micronutrient known for its anti-inflammatory properties due to its presence as selenocysteine in a number of enzymes such as thioredoxin reductases and glutathione peroxidases. These enzymes protect against oxidative stress and are important natural antioxidants essential in many metabolic processes in both humans and animals [6]. Currently, there is an increased interest in yeasts enriched with Se as dietary supplement due to their ability to incorporate inorganic Se and convert it in selenomethionine (SeMet) that is better absorbed by mammals [7]. Moreover, worldwide interest in the use of functional foods containing probiotic microorganisms for health promotion and disease prevention has increased significantly [8]. The majority of probiotic microorganisms are bacteria, and until now there is only one yeast (*Saccharomyces boulardii*) commercialized and used as biotherapeutic agents in human medicine [9]. Previous results obtained in our laboratory showed that *Saccharomyces cerevisiae* strain UFMG A-905, isolated from "cachaça" (a sugar-cane Brazilian distilled alcoholic beverage) production is a potential probiotic for its protective effects against infectious and inflammatory pathologies in animal models [10–18]. Additionally, we have previously demonstrated the beneficial effect of *S. cerevisiae* UFMG A-905 oral administration in a mucositis model induced by irinotecan [10], and its ability to supply bioavailable organic forms of Se [19].

Thus, in the present work, we evaluated the effect of treatment with Se-enriched *S. cerevisiae* UFMG A-905 in a murine model of mucositis induced by 5FU.

Materials and methods

Animals

Female 6–8 weeks-old *Swiss* mice were supplied by the Centre for Animal Care (CEBIO) of the Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil. Animals were maintained in a ventilated animal caging system (Alesco®, Monte Mor, SP, Brazil) with controlled lighting (12 h light–dark cycle), humidity (60–80%) and temperature (22 ± 1 °C). Water and a commercial autoclavable pelleted food (Nuvilab CR1, Nuvital®, Curitiba, PR, Brazil) were sterilized by steam and administered ad libitum [15]. All experimental procedures were carried out according to the standards set forth by the Brazilian National Council for Control of Animal Experimentation (CONCEA). This study was approved under protocol no. 186/2012 by the Ethics Committee on the Use of Animals (CEUA/UFMG).

Yeast and growth conditions

Saccharomyces cerevisiae strain UFMG A-905 belongs to the culture collection of the Laboratory of Biotherapeutic Agents, Federal University of Minas Gerais (Belo Horizonte, MG, Brazil). The yeast was grown overnight at 37 °C, with constant shaking (180 rpm), in YPD (yeast extract 1%, peptone 1%, and dextrose 2%) broth or YPD supplemented with Se (20 mg mL^{-1} Na_2SeO_3) [19]. The culture was then concentrated to obtain 10^9 colony-forming units (CFU) mL^{-1} in saline.

Yeast treatment

Mice pertaining to the experimental group received by gavage a daily dose of 10^8 CFU (0.1 mL of a culture containing 10^9 CFU mL^{-1}) of *S. cerevisiae* UFMG A-905 enriched (or not) with Se resuspended 10 days before mucositis induction as well as after mucositis induction. Control group received sterile saline following the same scheme.

Mucositis induction

Mucositis was induced by a single intraperitoneal (i.p.) injection of 300 mg kg^{-1} of 5FU (Fauldfluor® 50 mg mL^{-1}, Libbs Farmacêutica Ltda, São Paulo, SP, Brazil). Control groups received an i.p. injection of the same volume of sterile saline [20]. Mice were killed 3 days after 5FU injection.

Experimental design

To evaluate the effect of *S. cerevisiae* UFMG A-905 enriched or not with Se, the following six groups were used: (CTL) control not treated and without induced-mucositis; (905) control treated with *S. cerevisiae* UFMG A-905 and without induced-mucositis; (905-Se) control treated with Se-enriched *S. cerevisiae* UFMG A-905 and without induced-mucositis; (5FU) with induced-mucositis; (905 + 5FU) treated with *S. cerevisiae* UFMG A-905 and induced-mucositis; and (905-Se + 5FU) treated with Se-enriched *S.*

cerevisiae UFMG A-905 and induced-mucositis. Animal body weight, food consumption, faeces consistency and the presence of blood in faeces were assessed daily during experimental mucositis. In a first set of experiments (five animals per group), mice were anesthetized 3 days after mucositis induction for intestinal permeability determination after blood collection, and then killed by cervical dislocation for jejunum and ileum removal for histopathological examination. In a second set of experiments, using the same groups described above (but with 15 animals per group), mice were killed on days 1, 2 and 3 (5 animals per day) after mucositis induction. Tissues and blood were collected for determination of myeloperoxidase (MPO, as indicator of neutrophil infiltration) and eosinophil peroxidase (EPO, as indicator of eosinophil infiltration) activities, as well as of production of chemokine CXCL1/KC, nitrite and TBARS (thiobarbituric acid reactive substances).

Determination of intestinal permeability

For the determination of intestinal permeability animals received by oral administration 0.1 mL of DTPA (diethylenetriaminepentaacetic acid) labelled with 18.5 MBq ^{99m}technetium. Four hours after gavage, animals were anesthetized and 500 µL of blood was collected and placed in appropriate tubes for radioactivity determination using an automated gamma counter (Perkin Elmer, Wallac Wizard 1470–020, Cary, NC, USA). Data were expressed as % dose, using the following equation: % dose = [(cpm of blood/cpm of administered dose)] × 100, according to [21].

cpm = counts per minute.

Histological analysis of jejunum

After animal killing, small intestines were isolated and opened along the anti-mesenteric border, and the luminal content was gently removed. Subsequently, tissue was prefixed in Bouin's fixative for 30 min, rolled up to form "*Swiss rolls*", and fixed for an additional 18–20 h in 4% buffered formaldehyde, as previously described [22]. The material was then processed for paraffin embedding and 4-µm sections of each sample were stained with haematoxylin and eosin (HE). Microscopic slides were analyzed by a pathologist and lesions of mucosa and muscular were evaluated using a histopathological grading system described elsewhere [23]. Histopathological scores: normal histological findings (score 0); mucosa: villus blunting, loss of crypt architecture, sparse inflammatory cell infiltration, vacuolization and oedema; normal muscular layer (score 1); mucosa: villus blunting with fattened and vacuolated cells, crypt necrosis, intense inflammatory cell infiltration, vacuolization and oedema; normal muscular layer (score 2); mucosa: villus blunting with fattened and vacuolated cells, crypt necrosis, intense inflammatory cell infiltration, vacuolization and oedema; muscular: oedema, vacuolization, sparse neutrophil infiltration (score 3).

Determination of intestinal myeloperoxidase and eosinophil peroxidase activities

The extent of neutrophil accumulation in the jejunum was measured by determining MPO activity, as previously described [24]. For MPO quantification, 100 mg of jejunum was homogenized in 1.9 mL of PBS and centrifuged at 12,000 *g* for 10 min. The supernatant was discarded, and the cells were lysed. The samples were then centrifuged, the supernatant discarded, and the pellet resuspended in 1.9 mL of 0.5% hexadecyltrimethyl ammonium bromide (HTAB) in PBS, followed by three cycles of freezing in liquid nitrogen, and finally centrifuged at 12,000 *g* at 4 °C for 10 min. The supernatant was used in the enzymatic assay with the addition of an equal amount of substrate (1.5 mM L^{-1} *o*-phenylenediamine and 6.6 mM L^{-1} of H_2O_2 in 0.075 mM L^{-1} Tris–HCl pH 8.0). The reaction was stopped with 50 µL of 1 M H_2SO_4, and the absorbance was read at 492 nm in a microplate spectrophotometer (Spectramax M3, Molecular Devices, LLC., Sunnyvale, CA, USA). For EPO quantification, 100 mg of jejunum was homogenized in 1.9 mL of PBS and centrifuged at 12,000 *g* for 10 min [25]. After centrifugation, supernatant was removed and the pellet was resuspended in 1.9 mL of 0.5% HTAB in PBS saline. The samples were frozen in liquid nitrogen three times and centrifuged at 4 °C and 12,000 *g* for 10 min. Then, 75 µL of supernatant was added to 75 µL of OPD (*o*-phenylenediamine dihydrochloride) diluted in Tris–HCl and H_2O_2 and incubated at 37 °C for 30 min. The reaction was stopped by adding 50 µL of 1 M H_2SO_4 before being read at 492 nm in a microplate spectrophotometer (Spectramax M3). Results were expressed as arbitrary units (AU).

Determination of CXCL1/KC

Chemokine level was determined by ELISA [24] in jejunum samples using commercially available antibodies and according to manufacturer instructions (R&D Systems, Minneapolis, MN, USA). The jejunum tissue (100 mg) was homogenized in PBS (0.4 M NaCl and 10 mM $NaPO_4$) containing anti-proteases (0.1 mM phenylmethylsulphonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA and 20 kallikrein inhibitor units of aprotinin A) and 0.05% Tween 20 (100 mg of tissue mL^{-1} of solution). The samples were centrifuged for 10 min at 3000*g* and the supernatant immediately used for ELISA assays.

Determination of nitrite

The production of NO was determined indirectly by measuring nitrite concentrations based on the Griess reaction [26]. Briefly, 100 µL of intestinal tissue homogenate were incubated with 100 µL of Griess reagent (1% sulphanilamide and 0.1% napthylethylenediamine in 2.5% phosphoric acid) at room temperature for 10 min. Then, absorbance of samples was measured at 540 nm. A standard curve of nitrite concentrations against absorbance, with concentrations ranging from 5 to 240 µM, was used to quantify the concentrations of nitrite in the samples. The results were expressed in µM.

Determination of lipid peroxidation

The measurement of metabolites reactive to thiobarbituric acid was made in microplates [27]. For this, jejunum supernatant (250 µL) was added to 500 µL of solution containing trichloroacetic acid (TCA 15%), thiobarbituric acid (TBA 0.0375%) and hydrochloric acid (HCl 0.25 N). Samples were kept in boiling water bath for 15 min and then placed under running water until cool. Then, 750 µL of butyl alcohol was added, and the tubes vigorously shaken. The samples were centrifuged at 3000 rpm for 10 min at room temperature, and 200 µL of the supernatant was added to a 96-well plate. Absorbance was measured in spectrophotometer at 535 nm and the results were normalized by the protein concentration in the jejunum [28].

Statistical analysis

Results are expressed as means with standard errors for each group. Data were submitted to ANOVA followed by Bonferroni's test (parametric data) or Newman–Keuls test (nonparametric data) using the GraphPad Prism 6.00 software (GraphPad, La Jolla, CA, USA). Results were considered statistically different for at least $p < 0.05$.

Results

Saccharomyces cerevisiae UFMG A-905 enriched (or not) with Se decrease intestinal permeability during 5FU-induced mucositis in mice

Symptoms such as pain, nausea, vomiting, diarrhoea, and loss of appetite lead patients suffering of mucositis to a state of debilitation [29], essentially characterized by weight loss. Figure 1 shows that induction of mucositis with 5FU caused a decrease in weight (− 2.67 g ± 0.9%) and food consumption (− 1.5 g ± 0.02%) when compared to the control group, and both treatments (905 and 905-Se) did not reverse these parameters. On the other hand, the increased intestinal permeability observed with mucositis induction was partially reverted by both treatments ($p < 0.05$) (Fig. 2).

Saccharomyces cerevisiae UFMG A-905 enriched (or not) with Se preserve intestinal architecture during 5FU-induced mucositis in mice

Gastrointestinal mucositis is usually accompanied by epithelial cell loss in the mucosa of the gastrointestinal tract [30]. Histological analysis (Fig. 3) showed that animals with mucositis displayed intestinal lesions with shortened villi containing flattened and vacuolated cells, necrosis of crypts, and inflammatory cell infiltration in mucosa and muscular layer (histological score 3). Mice submitted to mucositis and treated with 905 or 905-Se showed significant recovery of lesions, with partial preservation of villi and crypts, and the presence of sparse inflammatory cell infiltration in

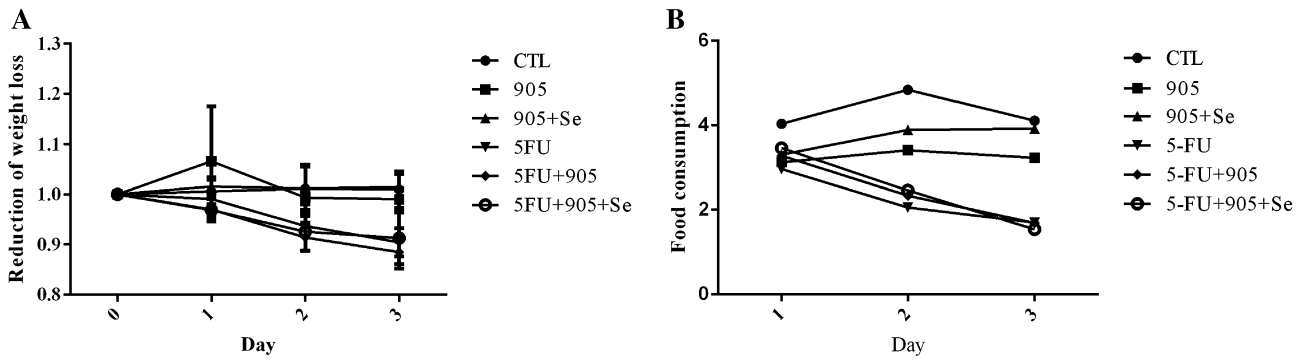

Fig. 1 Evolution of weight loss (**a**) and food consumption (**b**). *CTL* control group, *905* control group treated with yeast, *905-Se* control group treated with yeast enriched with Se, *5FU* experimental group submitted to mucositis induction, *905 + 5FU* experimental group treated with yeast and submitted to mucositis induction, *905-Se + 5FU* experimental group treated with yeast enriched with Se and submitted to mucositis induction. Standard errors represented by vertical bars. ($N = 5$ in each group, $p < 0.05$; ANOVA one-way, followed by Newman–Keuls test)

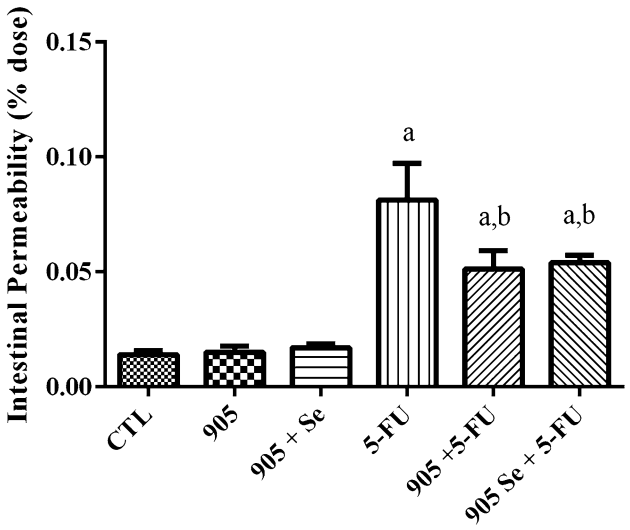

Fig. 2 Intestinal permeability. *CTL* control group, *905* control group treated with yeast, *905-Se* control group treated with yeast enriched with Se, *5FU* experimental group submitted to mucositis induction, *905 + 5FU* experimental group treated with yeast and submitted to mucositis induction, *905-Se + 5FU* experimental group treated with yeast enriched with Se and submitted to mucositis induction. Bars represent average and vertical lines represent standard error. Different letters indicate a statistical difference: (a) indicates significant differences in relation to control groups (CTL, 905, 905-Se); (b) indicates significant differences in relation to mucositis induction by 5FU ($N = 5$ in each group, $p < 0.05$; ANOVA one-way, followed by Newman–Keuls test)

lamina propria (histological score 2). Control mice that only received 905 or 905-Se showed preserved intestinal mucosa histology (histological score 0, as control group) with intact structures, and normal villi and crypts.

Se-enriched *S. cerevisiae* UFMG A-905 reduces inflammatory response in the intestinal tissue during 5FU-induced mucositis in mice

Mucositis is characterized by an inflammation and ulceration of the mucous membranes lining the gastrointestinal tract with infiltration of inflammatory cells, such as neutrophils and eosinophils, which are attracted to the primary site of inflammation by inflammatory cytokines [30]. We observed a significant ($p < 0.05$) increase of neutrophils (2.05 AU) and eosinophils (0.7 AU) infiltration in jejunum 72 h after mucositis induction when compared with the control group (0.6 AU MPO; 0.07 AU EPO). However, only treatment with Se-enriched *S. cerevisiae* UFMG A-905 was able to reduce both neutrophils (1.05 AU MPO, Fig. 4A) and eosinophils (0.16 AU EPO, Fig. 4B) infiltration. This was also associated with reduction of the chemokine CXCL1/KC levels in the jejunum of 905 + 5FU (209 pg/mL) and 905-Se + 5FU (124 pg/mL) groups when compared to animals of the 5FU group (341 pg/mL) (Fig. 5).

Se-enriched *S. cerevisiae* UFMG A-905 reduces nitrite concentration and lipid peroxidation during 5FU-induced mucositis in mice

Since oxidative stress precedes inflammatory response [4], and only the use of Se-enriched *S. cerevisiae* UFMG A-905 is a valid strategy to reduce inflammation in intestinal tissue, we evaluated oxidative response 24, 48 and 72 h after mucositis induction. An increase of nitrite concentration was observed in the jejunum of mice only 24 h after mucositis induction when compared to control group (136 µM). Although *S. cerevisiae* UFMG A-905 was able to reduce this increased concentration of nitrite (to 85 µM), the presence of Se potentiated this effect as demonstrated by a higher reduction of nitrite (32 µM) in mice treated with Se-enriched *S. cerevisiae* UFMG A-905 (Fig. 6A). Regarding to lipid peroxidation, 5FU increased the damage on lipid, essentially 48 h after mucositis induction (3.24 nmol MDA g^{-1} protein), and both treatments reduced these intestinal injury, but with a higher intensity in the 905-Se + 5FU group (0.65 nmol MDA g^{-1} protein) than in the 905 + 5FU (2.15 nmol MDA g^{-1} protein) (Fig. 6B).

Discussion

The importance of screening new probiotic yeasts has been underlined by our group since 2005, and *S. cerevisiae* UFMG A-905, displaying the best probiotic characteristics, was selected among yeasts belonging to different genera either *Saccharomyces* [13] or not [31]. This yeast strain was isolated during the production of "cachaça" and it was shown to be effective against bacterial infections [13–16] also having a protective effect against inflammation, as colitis [17] and mucositis [10], intestinal obstruction [12] and asthma [11] in animal models, as well as ability to reduce aflatoxin production [32], and to convert inorganic Se into organic Se [19].

Alteration of intestinal permeability is an important aspect of mucositis caused by chemotherapy treatment [20]. In the present work, we observed that *S. cerevisiae* UFMG A-905 has the ability to restore normal intestinal permeability and tissue damage when mucositis is induced in mice by 5FU as also observed with the use of other probiotics in mucositis models [10, 33–36]. When Se-enriched yeast is applied to the mucositis model, we also observe a reduction of oxidative stress and decreased inflammatory response. According to the literature, mucositis causes crypt necrosis, loss of normal tissue architecture and neutrophil infiltration [37–39]. We demonstrated, in

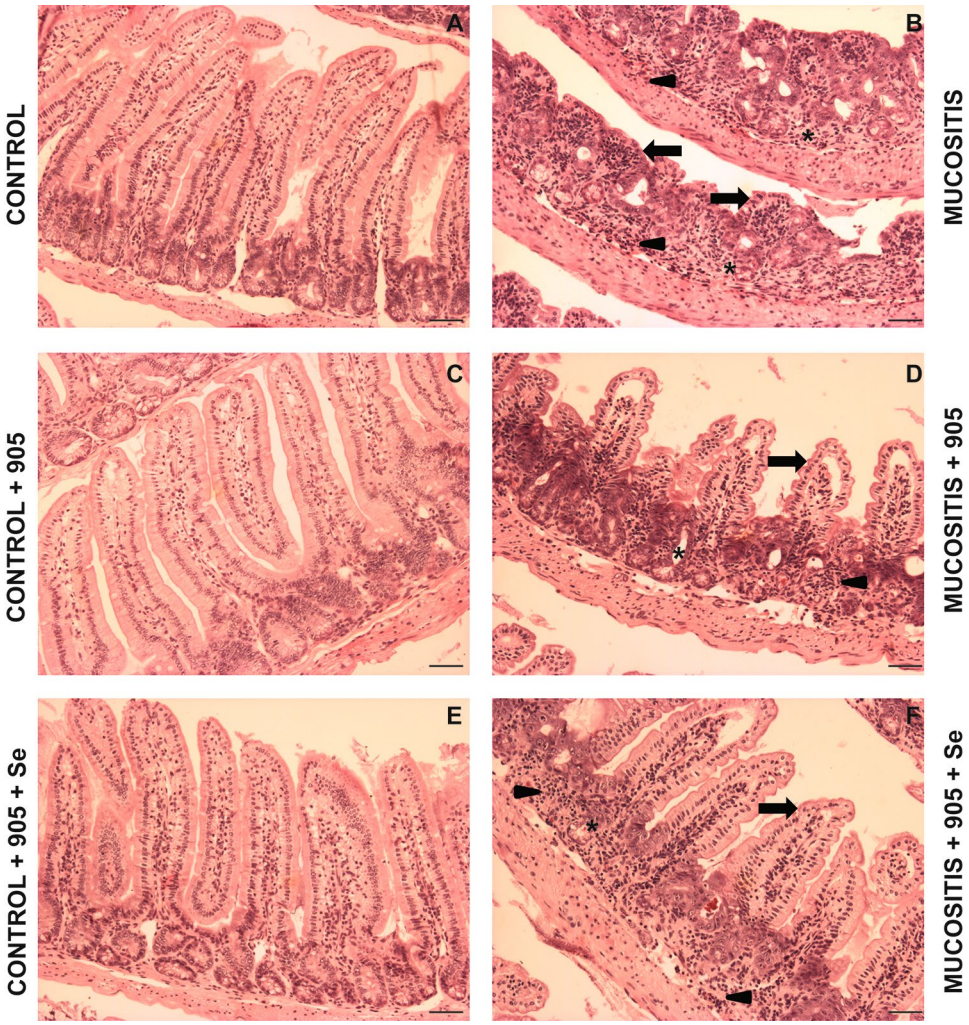

Fig. 3 Histopathological analysis of jejunum. *CTL* control group, *905* control group treated with yeast, *905-Se* control group treated with yeast enriched with Se, *5FU* experimental group submitted to mucositis induction, *905* + *5FU* experimental group treated with yeast and submitted to mucositis induction, *905-Se* + *5FU* experimental group treated with yeast enriched with Se and submitted to mucositis induction. **a**, **c**, **e** Normal histological aspects in mucosa were observed in mice from CTL, 905 + 5FU and 905-Se + 5FU groups. Shortened villi (**b**, arrows), inflammatory cell infiltration in lamina propria and muscular layer (**b**; arrowheads), and necrosis of crypt (**b**, asterisk) were observed in mucositis group. Mice submitted to intestinal mucositis and treated by 905 (**d**) or by 905-Se (**f**) showed partial preservation of the villi (arrows) and crypts (asterisks), presence of inflammatory cell infiltration in lamina propria (arrowheads), but preserved muscular layers. Bar = 50 µm. HE staining

agreement with literature data, that on the third day after 5FU treatment, crypt necrosis, loss of normal tissue architecture and neutrophil infiltration in the jejunum muscular layer can all be detected. When mice were treated with *S. cerevisiae* UFMG A-905, we observed an improvement of these parameters. A similar result was observed in mice after induction of mucositis and treatment with *S. boulardii* [3, 40], as well as in mice after induction of mucositis with irinotecan and treatment with *S. cerevisiae* UFMG A-905 [10]. Smith and colleagues [41] and Whitford and colleagues [36] obtained similar results using *Lactobacillus fermentum* BR11 and *Streptococcus thermophilus* TH-4, respectively. Our group has previously demonstrated that the yeast *S. cerevisiae* UFMG A-905 had the ability to promote intestinal homeostasis in an intestinal inflammatory model of colitis in mice, maintaining epithelial tissue integrity and reduction of intestinal permeability [17]. Infiltration of inflammatory cells was largely associated with destruction of the intestinal mucosa [30]. Others studies also showed that histopathological lesions in mucositis caused by 5FU or irinotecan resulted from the infiltration of neutrophils and eosinophils into the tissue inducing an inflammatory reaction [23, 42]. To confirm the beneficial action of yeast treatment, we also evaluated recruitment of neutrophils and eosinophils during the inflammatory process in the mucositis-induced model. Eosinophils are important in intestinal inflammation because they produce pro-inflammatory cytokines and EPO, which have antibacterial proprieties [43], whereas neutrophil recruitment can increase concentration of cytokines, such as CXCL1/KC, modifying the permeability of the intestinal barrier [44]. These evidences explained why the increase in the levels of neutrophils, eosinophils and CXCL1/KC into the mucosa caused histological damages and increased intestinal permeability. We can note that Se-enriched yeast decreased the high levels of inflammatory markers and consequently reduced the damages on the intestinal mucosa. Similar results for mucositis induced by 5FU were observed by Smith and colleagues [41], Justino and colleagues [3, 33] in animals treated with

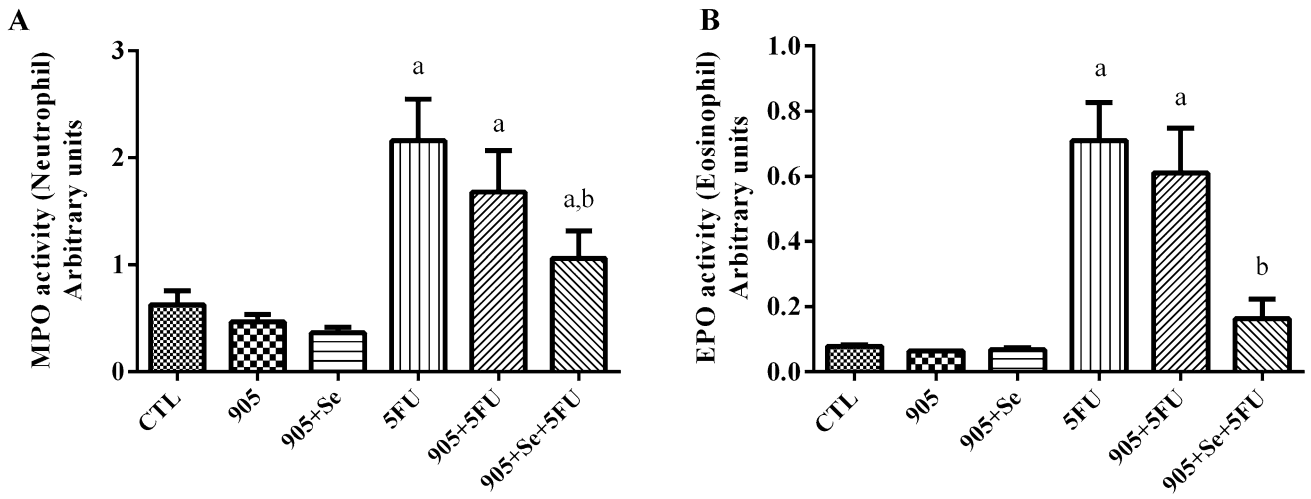

Fig. 4 Evaluation of MPO (**A**) and EPO (**B**) activities in the jejunum. *CTL* control group, *905* control group treated with yeast, *905-Se* control group treated with yeast enriched with Se, *5FU* experimental group submitted to mucositis induction, *905 + 5FU* experimental group treated with yeast and submitted to mucositis induction, *905-Se + 5FU* experimental group treated with yeast enriched with Se and submitted to mucositis induction. Bars represent average and vertical lines represent standard error. Different letters indicate a statistical difference (a) indicate significant differences in relation to control groups (CTL, 905, 905-Se); (b) significant differences in relation to mucositis induction by 5FU ($N = 5$ in each group, $p < 0.05$; ANOVA one-way, followed by Newman–Keuls test)

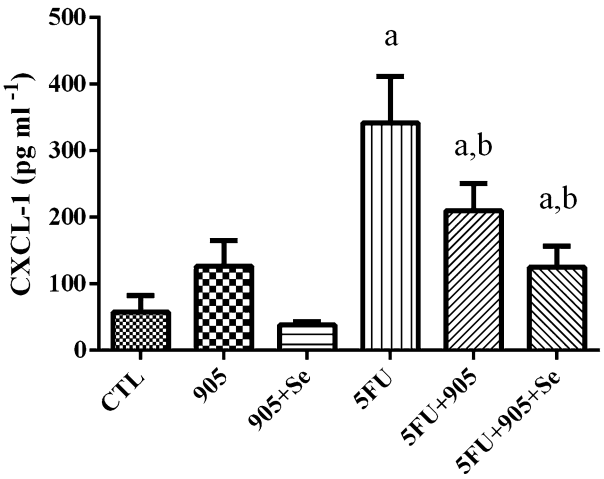

Fig. 5 Concentrations of CXCL1/KC in the jejunum. *CTL* control group, *905* control group treated with yeast, *905-Se* control group treated with yeast enriched with Se, *5FU* experimental group submitted to mucositis induction, *905 + 5FU* experimental group treated with yeast and submitted to mucositis induction, *905-Se + 5FU* experimental group treated with yeast enriched with Se and submitted to mucositis induction. Bars represent average and vertical lines represent standard error. Different letters indicate a statistical difference: (a) indicates significant differences in relation to control groups (CTL, 905, 905-Se) (b) indicates significant differences in relation to mucositis induction by 5FU ($N = 5$ in each group, $p < 0.05$; ANOVA one-way, followed by Newman–Keuls test)

L. fermentum, *S. boulardii* and *Lactobacillusacidophilus*, respectively, and by Maeda et al. [45] treating animals with *N*-acetylcysteine (NAC), an antioxidant compound able to reduce intestinal damage caused by methotrexate, an antitumor drug. Ferreira et al. [46] also demonstrated that the increased recruitment of eosinophils observed in the small intestine with 5FU administration was reduced with the use of butyrate. Therefore, the histopathological lesions observed in mucositis induced by 5FU occurred probably due to infiltration of eosinophils and neutrophils in the mucosa causing an inflammatory reaction. This correlation between cellular recruitment and tissue damages was also observed in previous studies of our group using irinotecan to induce mucositis in mice [10, 42].

Neutrophil recruitment by CXCL1/KC is also related with an increase in iNOS (inducible nitric oxide synthase) activity, which contributes to the inflammatory response in the intestine by activating cascades of mediators and expressions of cytokines and chemokines recruiting neutrophils [47]. In infectious processes, activated cells, such as macrophages, neutrophils and endothelial cells simultaneously secrete NO and reactive oxygen intermediates. The indirect cytotoxic action of NO consists mainly in its reaction with oxygen intermediates, resulting in the production of hydroxyl (HO), a highly reactive radical which increases the toxic action of NO on the NO-producing cells as well as on the adjacent cells [48]. Our results show that induction of mucositis leads to an increase in both CXCL1/KC and NO levels, and that this can be prevented by the simultaneous administration of *S. cerevisiae* UFMG A-905. This protective effect was enhanced when the yeast was enriched with Se. It is well known that Se is capable to counteract the oxidative action of NO, a reactive species involved in the first signalling

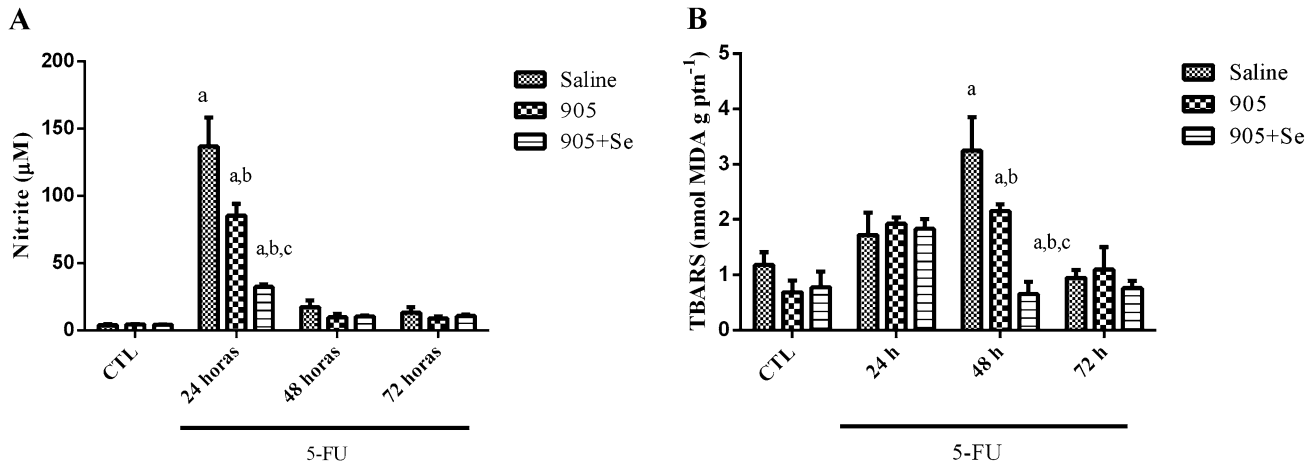

Fig. 6 Nitrite concentration (**A**) and TBARS production (**B**) in the jejunum 24 h, 48 h and 72 h after the mucositis induction with 5FU. *CTL* control group, *905* control group treated with yeast, *905-Se* control group treated with yeast enriched with Se, *5FU* experimental group submitted to mucositis induction, *905* + *5FU* experimental group treated with yeast and submitted to mucositis induction, *905-Se* + *5FU* experimental group treated with yeast enriched with Se and submitted to mucositis induction. Bars represent average and vertical lines represent standard error. Different letters indicate a statistical difference (a) indicates significant differences in relation to control groups (CTL, 905, 905-Se); (b) indicates significant differences in relation to only mucositis induction by 5FU; (c) indicates significant differences in relation to 905 + 5FU ($N = 5$ in each group/day, $p < 0.05$; ANOVA one-way, followed by Newman–Keuls test)

pathways of mucositis [4]. Similar decreases in NO concentration in the jejunum of animals with induced mucositis have been observed using treatments with probiotics [3, 33].

In the present study, decreased lipid peroxidation was also observed after the treatment with *S. cerevisiae* UFMG A-905 enriched or not with Se. Generally, NO acts as a strong inhibitor of the lipid peroxidation chain reaction by scavenging propagatory lipid peroxyl radicals [48]. However, in the presence of superoxide, NO forms peroxynitrite, a powerful oxidant capable of initiating lipid peroxidation and oxidizing lipid-soluble antioxidants. The decrease of lipid peroxidation during the treatment with yeast, and even more with yeast enriched with Se, occurred after the decrease of NO, demonstrating a relationship between these oxidative parameters and the effect of Se in blocking the oxidative action [49].

Treatment with an antioxidant agent is interesting during inflammation since ROS are recognized as important factors in several inflammatory diseases, such as gastric ulcers, colitis [50] and mucositis [4, 51]. The advantage of probiotic therapy (compared to antibiotic treatments) is the absence of some undesirable effects, such as the selection of resistant bacteria, whereas the beneficial mechanisms of these microorganisms are basically the same offered by the indigenous microbiota. Besides this, the use of probiotics can contribute to increase resistance to pathogenic organisms' colonization, immunomodulation and nutritional complementation, reducing the necessity to use antibiotics and anti-inflammatory drugs [13].

In conclusion, results of the present study showed that the oral administration of *S. cerevisiae* UFMG A-905, particularly when enriched with Se, protected mice against pathological consequences associated to mucositis induced by 5FU.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Animal experiments were carried out according to the standards set forth by the Brazilian National Council for Control of Animal Experimentation (CONCEA). This study was approved under Protocol no. 186/2012 by the Ethics Committee on the Use of Animals (CEUA/UFMG).

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