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Protective effect of the riboflavin-overproducing strain Lactobacillus plantarum CRL2130 on intestinal mucositis in mice

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ABSTRACT

Objectives: Intestinal mucositis (IM) is a local inflammatory response that causes alterations of the intestinal structure that in turn affect nutrient absorption and a side effect that is commonly associated with cancer treatments. *Lactobacillus plantarum* CRL2130 is a riboflavin-overproducing strain that has previously been shown to provide antiinflammatory properties. The objective of this study was to evaluate the effects of this riboflavin-producing strain in a chemically induced murine mucositis model.
Methods: Mucositis was induced by daily injections of 5-fluororacil (5-FU) after which mice were either given *L. plantarum* CRL2130, CRL725 (strain from which CRL2130 was derived that does not overproduce riboflavin), or commercial riboflavin twice daily during 6 d of chemotherapy agent injections. The effect of the strains and riboflavin was also evaluated in vitro using Caco-2 intestinal cancer cell cultures to determine if they interfere with 5-FU's anticancer activity.
Results: The administration of L. plantarum CRL2130 significantly attenuated the pathologic changes induced by 5-FU in mice such as body weight loss, diarrhea, shortening of villus height, increases in proinflammatory cytokine concentrations, and elevated production of interleukin 10. In vitro assays using Caco-2 cells showed that the effectiveness of 5-FU was not affected by *L. plantarum* CRL2130 and that this strain exerted an inhibitory mechanism against oxidative stress.
Conclusions: These results indicate that the riboflavin-overproducing strain L. plantarum CRL2130 could be useful to prevent mucositis during cancer treatments and would not affect the primary treatment.

Introduction

Mucositis refers to mucosal inflammation and frequently occurs as a side effect of cancer treatments such as chemotherapy and radiation therapy. Fluorouracil (5-FU) is one of the most prescribed chemotherapeutic agents because of its ability to interrupt DNA synthesis and stimulate cell apoptosis [1]. However, one important limitation of 5-FU use is that this drug is not cell-specific because of cytotoxic exertion on both cancerous and other fast-reproducing cells [2]. In this sense, the intestinal epithelium is one of the tissues most sensitive to 5-FU because it is one of the most rapidly renewing tissues in adult mammals. The drug causes the destruction of the intestinal mucosa and especially in the small intestine, which results in intestinal mucositis (IM).

The clinical manifestations of 5-FU-induced mucosal dysfunction include diarrhea, vomiting, pain, weight loss, reduced nutrient absorption and in severe cases increased intestinal permeability with a risk of infections [3,4]. These side effects affect patients' quality of life, which often leads to a discontinuation of chemotherapy and a reduction in cancer treatment efficacy. The available therapeutic measures against mucositis (i.e., protective mucosal coatings, topical antimicrobials, cryotherapy, and antibiotic and analgesic treatments) are focused on the relief of symptoms without decreasing the antineoplastic action of the chemotherapeutics [5]. However, strategies to attenuate chemotherapy-induced IM are limited, which makes research for new effective therapeutics necessary.

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Probiotics have been defined as "microorganisms that when administered in adequate amounts confer health benefits to the host" [6]. In recent years, probiotics have been used to mitigate intestinal mucosal damage in a wide range of gastrointestinal disorders such as inflammatory bowel disease, pouchitis, irritable bowel syndrome, and colorectal cancer [5,7]. In this regard, different beneficial mechanisms have been demonstrated for probiotic lactic-acid bacteria (LAB) including normalization of altered intestinal microbiota [8], improvement of intestinal barrier function [9], prevention of oxidative stress [10], and stimulation of host's immune system [11].

The supply of vitamins has also been suggested as another mechanism by which certain LAB can produce protective effects against these pathologies [12,13]. In recent years, an increasing interest has been reported to select roseoflavin-resistant, spontaneous, riboflavin-overproducing LAB strains, characterize them for their probiotic potential [14,15], and formulate new functional foods [16,17]. A previous study by our group showed that the administration of soymilk that was fermented by the roseoflavin-resistant, riboflavin (B2)-overproducing strain *Lactobacillus plantarum* CRL2130 diminished the severity of inflammation in a 2,4,6-trinitrobenzene sulfonic-acid-induced colitis mouse model [18].

A recent study showed that the oral administration of different riboflavin-producing LAB (as a bacterial suspension, not in a food matrix) was effective to relieve inflammatory symptoms and maintain the mucosal integrity in chemically-induced colitis [19]. These results suggest that riboflavin-producing LAB, in addition to being a source of vitamin, might be an effective therapeutic strategy to promote mucosal protection in other inflammatory pathologies such as IM.

Considering these previous results, the aim of this work was to investigate the effect of the administration of riboflavin-overproducing strain *L. plantarum* CRL2130 in an experimental mouse model of 5-FU-induced IM.

Methods

Preparation of lactic-acid bacteria strains

L. plantarum CRL2130 and CRL725 were obtained from the culture collection of the Centro de Referencia para Lactobacilos (CERELA) in San Miguel de Tucumán, Argentina. *L. plantarum* CRL2130, which is a roseoflavin-resistant mutant from *L. plantarum* CRL725 (originally isolated from sugarcane bagasse), was previously selected due to its riboflavin-overproducing capability [20]. After activation in Man, Rogose, and Sharpe (MRS, Britania, Buenos Aires, Argentina) broth, both strains were inoculated in riboflavin-free culture medium (Riboflavin Assay Medium, Difco, Becton, Dickinson and Co., Sparks, MD) and incubated without agitation for 16 h at 37°C.

For probiotic suspensions, the cells were washed with a sterile saline solution (0.85% m/v NaCl) and resuspended in this solution to obtain suspensions that contained $9 \pm 1 \times 10^8$ colony-forming units/mL.

Animal model and sampling procedure

Animal experiments followed protocols approved by the Animal Protection Committee of CERELA (CRL-BIOT-LT-2016/1 A) in accordance with the current laws of Argentina and international organizations for the use of experimental animals.

This study was conducted using female BALB/c mice (age 6 wk) weighing 20 to 25 g, which were supplied from the inbred-closed colony maintained at CERELA. The mice were kept under a 12 h light/dark cycle at a temperature of 18°C to 20°C and 20% humidity. Animals were given free access to water and food (commercial, riboflavin-free diet, Dyets, Bethlehem, PA) to limit access to the vitamin in a similar way as the host with IM after long periods of chemotherapies.

Twenty-five mice were used and divided into five groups of five animals each The mock group was the control group that received saline i.p injected once a day and orally administered twice a day during 6 d and the other mice were i.p injected with 5-FU (50 mg/kg animal weight) once a day during 6 d and divided into four groups by what was received orally (twice a day for 6 d). The 5-FU + saline group was administered saline and the 5-FU + CRL2130 and 5-FU + CRL725 groups were administered suspensions of *L. plantarum* CRL2130 or CRL725, respectively. The 5-FU + B2 group was administered commercial riboflavin at 4 μg (similar concentration to mice that received administration of *L. plantarum* CRL2130 [18]).

Each mouse was checked once a day to record body weight and stool consistency. Twenty hours after the last 5-FU injection, mice were anesthetized intraperitoneally using a mixture of ketamine hydrocholoride (König Laboratorys, Buenos Aires, Argentina) and xylasine (Bayer, División Sanidad Animal, Buenos Aires, Argentina) and blood was collected by cardiac puncture. The small intestinal contents were recovered by washing with 500 μl of 0.01 M phosphate-buffered saline (PBS; [mM] NaCl [137], KCl [2.7], Na2 HPO4 [10] and KH2 PO4 [1.8], pH 7.4 adjusted with HCl) that contains a complete, mini, ethylenediaminetetraacetic-free, protease-inhibitor cocktail (Roche Molecular Biochemicals, Mannheim, Germany). Samples of jejunum were immersed in formaldehyde (10% v/v in PBS) for further analysis. Figure 1 shows a schematic representation of the experimental protocol with the samples that were obtained for the different assays.

Diarrhea assessment

Diarrhea occurrence was evaluated using a previously described scoring system [4]. A score of 0 indicated a normal stool, 1 indicated slight diarrhea, 2 moderate diarrhea, and 3 watery diarrhea.

Cytokine analysis

Cytokines were measured in serum and intestinal contents. Concentrations of interleukin (IL) 10, IL-17, tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), IL-6, IL-4, and IL-2 were measured using the cytometric bead array mouse inflammation kit (BD Bioscience, San Diego, CA) in accordance with the manufacturer's instructions. The results were expressed as concentration of each cytokine (pg/mL for serum samples and pg/mg of total protein for intestinal samples). The anti-/proinflammatory cytokine ratios (IL-10/IL-17, IL-10/INF- γ, IL-10/TNF-α) were also determined.

Histopathological and morphometric examination

Paraffin-embedded tissues from the jejunum were sectioned (4 μm) and stained with hematoxylin and eosin. For the morphometric examination, tissues were observed in a light microscope (Carl Zeiss- Axio Scope.A1) and AxioVision version 4.8 software was used to measure five intact villi and crypt from each sample (100 X). Values from each group were averaged.

The severity of mucosal inflammation was recorded on the basis of a previously described scoring system [21]. The inflammation was graded from 0 to 3 where 0 indicated normal histologic findings; 1 was villus blunting, loss of crypt architecture, sparse inflammatory cell infiltration, vacuolizatcion and oedema; 2 indicated villus blunting with fattened and vacuolated cells, crypt necrosis, inflammatory cell infiltration, vacuolization and oedema; and 3 was villus blunting with fattened and vacuolated cells, crypt necrosis, intense inflammatory cell infiltration, vacuolization and edema.

In vitro cell cytotoxity assay

Caco-2 human colorectal adenocarcinoma cells (ATCC HTB37, passage 57) were cultured in 25 cm^2 tissue culture flasks that contained Dulbecco's modified Eagle's medium (DMEM; Gibco, Gran Island, NY) supplemented with 10% fetal bovine serum (FBS; NATOCOR, Córdoba, Argentina), penicillin, streptomycin, and amphotericin B (Gibco, Gran Island, NY). The cells were maintained at 37°C in a 5% CO_2 humidified incubator until 70% to 80% cell confluence. After that, cells were seeded in a 96-well plate (5×10^4 cells / mL) for 24 h and incubated in the presence of *L. plantarum* CRL2130, CRL725 (bacteria ratio of $1{:}10^3$ for both cells), or a commercial riboflavin solution at a final concentration of 1.6 μg/mL (concentration produced by the riboflavin-overproducing strain) in DMEM without antibiotic medication. After 24 h, the cells were treated with 5-FU (100 μg/mL) and incubated for 24 h. The viability of the Caco-2 cells was evaluated by MTT (3-[4,5-dymethilthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay. After gentamicin treatment, MTT reagent (Sigma- Aldrich, St. Louis, MO) that was dissolved in PBS at a concentration of 5 mg/mL and sterile-filtered was added to each well (10 μl) and incubated at 37°C for 4 h. The medium was replaced by dimethyl sulfoxide and the absorbance was read at 570 nm in a microplate reader (VersaMax, Molecular Devices, San Jose, CA). The results were expressed as a percentage of viable cells compared with control cells treated with serum-free DMEM only (100% viability).

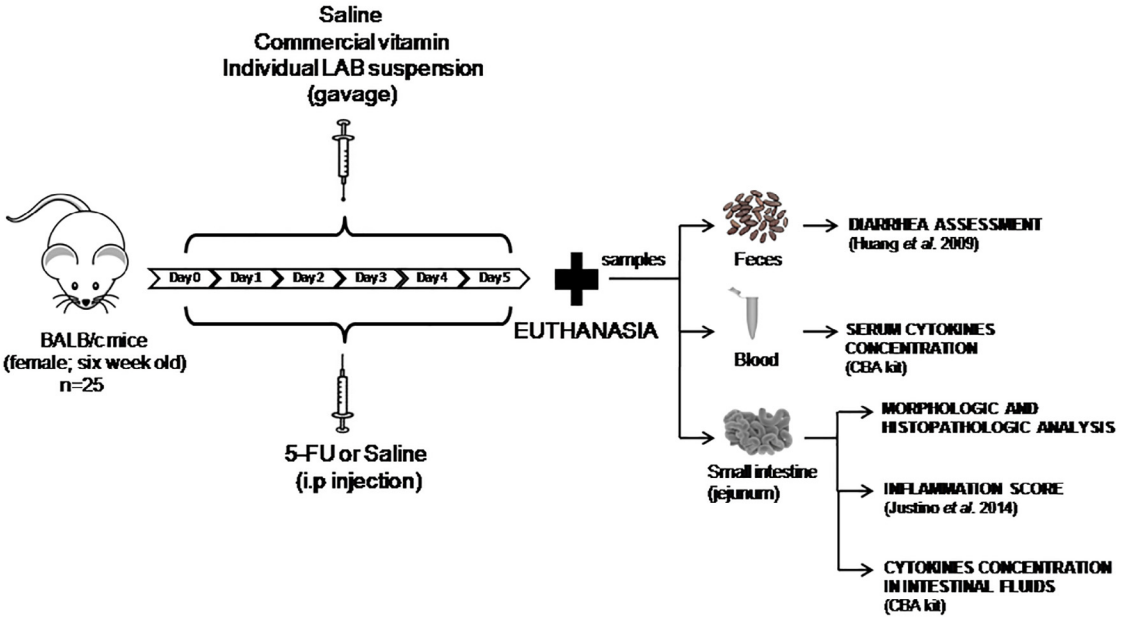

Fig. 1. Scheme of mucositis mouse model and sampling. Mock group received saline. Intestinal mucositis was induced by daily i.p injections of 5-fluororacil during 6 d. Saline, commercial riboflavin, or individual lactic-acid bacteria suspensions of riboflavin-overproducing or control strain were administrated to mice orally twice a day during 6 d. Mice were euthanized and samples of feces, blood, and small intestines were taken for further analysis.

In vitro measurement of intracellular reactive oxygen species

Reactive oxygen species (ROS) production was measured in Caco-2 cells by using 2',7'–dichlorofluorescin diacetate dye (DCFDA, Sigma- Aldrich, St. Louis, MO). In the presence of ROS, DCFDA is oxidized and converted to its fluorescent form.

Cells were seeded in a 24-well plate (2×10^5 cells/mL) and incubated for 48 h at 37°C and 5% CO_2. Subsequently, the medium was replaced with fresh medium-containing *L. plantarum* CRL2130, CRL725 (bacteria ratio of $1{:}10^3$), or commercial riboflavin (1.6 μg / mL). After 24 h of incubation, the cells were washed with PBS and loaded with the DCFDA at a final concentration of 10 μM in DMEM with 2% FBS. The culture was incubated 30 min in the dark at 37° C and 5% CO_2. The dye was removed after 30 min and the cells were oxidized by exposure to 100 μM hydrogen peroxide (H_2O_2) in DMEM for 60 min. The results were read in a fluorescence microplate reader (Biotek Synergy HT, Winooski, VT) and expressed as a percentage of ROS reduction compared with oxidized cells without treatment.

Statistical analysis

Measurement data are presented as the mean ± standard deviation. Data were analyzed using MINITAB 15 software (Minitab, State College, PA). An analysis of variance general linear model followed by Tukey's post hoc test was used for comparison of significance between the groups and differences were considered significant at $P < 0.05$.

Results

Oral administration of riboflavin-overproducing L. plantarum *CRL 2130 prevents body weight loss and diarrhea occurrence induced by 5-FU*

The mice from the mock group showed an average body weight gain of 3.8% (from 24 ± 1 g to 24 ± 1 g) throughout the study. In contrast, the daily administration of 5-FU caused a significant body weight loss in all groups (5-FU + saline, 5-FU + CRL2130, 5-FU + CRL725, and 5-FU + B2), which reached a decrease of 17.4% on average (from 22 ± 4 to 18 ± 3) at the end of the study. The group of mice that received the riboflavin-overproducing strain showed the lowest body weight loss (data not shown).

The diarrhea scores of all groups are shown in Figure 2. The repeated administration of 5-FU to animals caused the onset of diarrhea as shown at the end of the experiment (day 6) for mice from the 5-FU + saline and 5-FU + CRL725 groups compared with the mock group. The lowest diarrhea score was obtained for the 5-FU + CRL2130 group.

Oral administration of riboflavin-overproducing L. plantarum *CRL 2130 prevents proinflammatory cytokine increase induced by 5-FU*

Concentrations of proinflammatory cytokines IL-17, TNF-α, INF-γ, and IL-6 were significantly higher ($P < 0.05$) and IL-10 levels were lower in the serum of mice from the 5-FU + saline group than in the serum samples from the mock group. Similar results were obtained from the 5-FU + CRL725 group. The administration of riboflavin-overproducing strain (5-FU + CRL2130 group) significantly decreased INF-γ levels ($P < 0.05$) and increased the concentration of IL-10 compared with the 5-FU + saline group. Commercial riboflavin treatment (5-FU + B2 group) maintained cytokine levels that were close to those observed for the 5-FU + CRL2130 group but without the significant increase of the IL-10 concentration (Fig. 3A).

Cytokines were also measured in the intestinal contents (Fig. 3B). Treatment with 5-FU increased the levels of all proinflammatory cytokines that were assayed and decreased the levels of IL-10 (5-FU + saline group) in comparison with the mock group. Similar results were obtained for mice that received the control strain (5-FU + CRL725). Mice from the 5-FU + CRL2130 group showed the highest (but not significant different) levels of proinflammatory cytokines with important variations for each mouse and a significant increase ($P < 0.05$) of IL-10 compared with the 5-FU + saline group. Mice from the 5-FU + B2 group showed proinflammatory cytokines levels that were close those observed for the 5-FU + saline group but with a significant increase ($P < 0.05$) for IL-10.

The analysis of anti-/proinflammatory cytokines ratios (IL-10/IL-17, IL-10/INF-γ; IL-10/TNF-α) showed a significant decrease in the 5-FU + saline group compared with the mock group (Figs. 3C

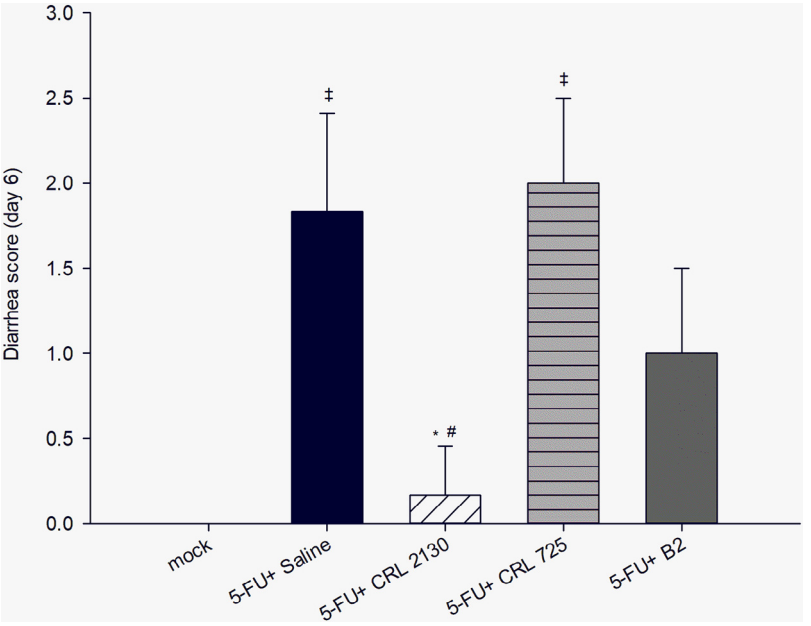

Fig. 2. Effects of riboflavin-overproducing strain on diarrhea induced by 5-fluororacil. Diarrhea occurrence was measured on day 6 using a four-grade scale (0–3). Data are presented as mean ± standard deviation for five animals. ‡ $P < 0.05$ compared with mock group. *$P < 0.05$ compared with 5-fluororacil + saline group. # $P < 0.05$ compared with 5-fluororacil + CRL725 group.

and D). This response was significantly reversed ($P < 0.05$) in the 5-FU + CRL2130 group but not in the 5-FU + CRL725 group both in the serum and intestinal content samples. Similarly, the administration of pure riboflavin (5-FU + B2) resulted in significant increase ($P < 0.05$) of these ratios in both samples.

Oral administration of riboflavin-overproducing L. plantarum *CRL 2130 prevents intestinal mucosal damage induced by 5-FU*

The histologic analyses of jejunum showed intestinal mucosal damages that were associated with 5-FU injections (Figs. 4A and C). Light microscopy revealed a shortening of villus height and crypts that were much deeper (with a significant reduction of villus height/crypt depth ratio) than those observed in mice from the mock group. *L. plantarum* CRL2130 administration increased the villus height/crypt depth ratio compared with those in the 5-FU + saline group. A comparable effect was also observed in the 5-FU + B2 group. No remarkable differences were detected in mice from the 5-FU + CRL725 group.

The administration of 5-FU caused significantly elevated values ($P < 0.05$) of inflammation scores in the small intestine of mice from the 5-FU + saline group compared with the mock group (Figs. 4B and C). Similar inflammation damages were observed in mice from the 5-FU + CRL725 group. Mice from the 5-FU + CRL2130 group significantly decreased ($P < 0.05$) this inflammation score and this effect was comparable with that observed in the 5-FU + B2 group.

Riboflavin-overproducing L. plantarum *CRL 2130 did not interfere in the cytotoxic effect of 5-FU on Caco-2 cells*

L. plantarum CRL2130, CRL725, and the commercial riboflavin had little effect on cell viability on their own (Fig. 5). When cells were exposed to the combination of *L. plantarum* CRL2130 or commercial riboflavin together with 5-FU, cell viability was significantly reduced ($P < 0.05$) to 42.3% and 50.7%, respectively, compared with the control group (68.6% for cells treated only with 5-FU). Treatment with the control strain did not induce any additional effect when compared with 5-FU alone.

Riboflavin-overproducing L. plantarum *CRL 2130 decrease ROS production in Caco-2 cells*

Figure 6 shows the ROS production by treatment. Cells that were incubated with H_2O_2 (H_2O_2 100 μM) showed a significant increase ($P < 0.05$) in ROS generation compared with the control group. An antioxidant effect was not observed with the control strain (H_2O_2 100 μM + CRL725).The riboflavin-overproducing *L. plantarum* CRL2130 (H_2O_2 100 μM + CRL2130) and commercial riboflavin (H_2O_2 100 μM + B2) decreased H_2O_2-stimulated intracellular ROS production and reached values that were similar to those of the controls without H_2O_2.

Discussion

IM as a side effect of chemotherapy or radiation therapy for cancer treatment is under continued investigation to develop new treatment alternatives. The pathogenesis of mucositis is known to comprise of a sequence of events and stages [22,23]. Because proinflammatory cytokines and ROS appear to be key factors during the pathogenesis of IM, agents with antiinflammatory/antioxidant activities may serve for the treatment or prevention of this pathology. In this sense, the antiinflammatory properties of certain probiotics or products that contain these microorganisms have been associated with the reduction of mucositis that is induced during chemotherapy [24,25]. However, the production of antiinflammatory/antioxidant vitamins as a mechanism by which microorganisms could improve the health of hosts that suffer from chemotherapy-induced IM has not been reported.

Riboflavin is widely known to act as an antioxidant and have a potential effect against oxidative stress that is generated during

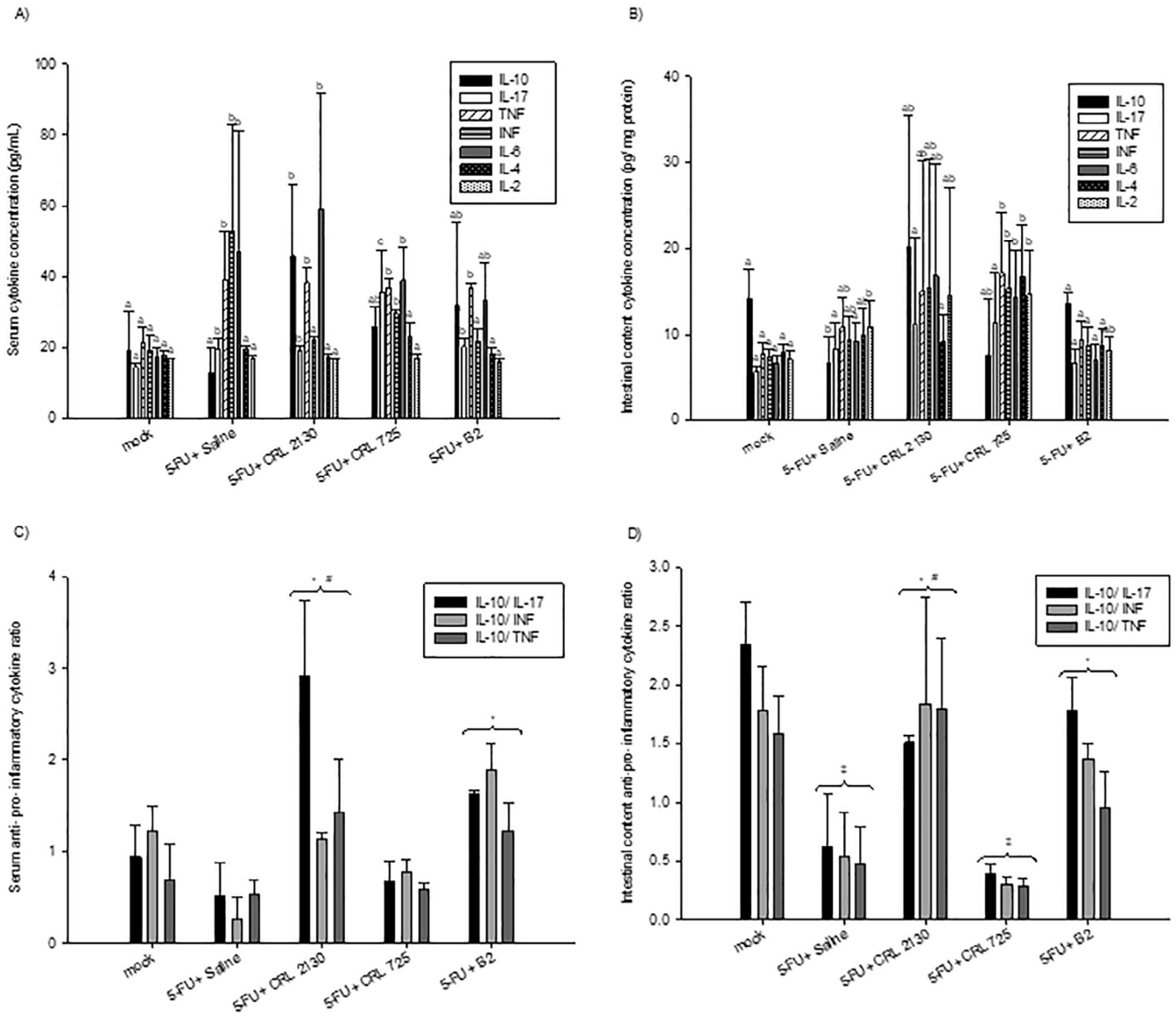

Fig. 3. Effect of riboflavin-overproducing strain and 5-fluorouracil treatment on cytokines levels. Cytokine concentrations were measured by cytometric bead array in A) serum and B) intestinal contents. The ratio between the levels of interleukin 10 and pro-inflammatory cytokines in C) serum and D) intestinal contents were also calculated. The results are presented as mean ± standard deviation for five animals. For each cytokine, different letters (a-c) statistically differ ($P < 0.05$). For the ratios, ‡ $P < 0.05$ compared with the mock group; *$P < 0.05$ compared with the 5-fluorouracil + saline group; and # $P < 0.05$ compared with the 5-fluorouracil + CRL725 group.

the development of various chronic diseases [26]. Besides the antioxidant potential, antiinflammatory actions for riboflavin were reported in experimental models [27,28]. In a recent study, the protective effect of the administration of different riboflavin-producing LAB in mice with TNBS-induced colitis has been demonstrated [29]. Considering these precedents, we hypothesized that the administration of a riboflavin-producing LAB could relieve intestinal damage that is associated with chemotherapy-induced IM. Hence, the administration of *L. plantarum* CRL2130, which is a riboflavin overproducing strain, was evaluated in a model of 5-FU-induced IM.

The administration of daily 5-FU was observed to produce a considerable decrease in body weight and marked diarrhea compared with the control healthy mice in a similar way as reported by other authors [24,25]. Moreover, 5-FU altered the architecture and integrity of the small intestine with diminished villus height/crypt depth ratio, which causes the disruption of the jejunum mucosa with high cellular infiltration in the lamina propria and significantly increases proinflammatory cytokine concentrations (IL-17, TNF-α, INF-γ, and IL-6 in serum and IL-17, TNF-α, IFN-γ, IL-6, IL-4, and IL-2 in intestinal contents) and decreased IL-10 levels. Interestingly, mice injected with 5-FU and given *L. plantarum* CRL2130 were found to have a lower diarrhea score, preserved mucosal architecture with decrease of mucosal inflammation score, and maintenance of the villus and crypt length compared with mice that only received 5-FU. Moreover, the administration of the riboflavin-overproducing strain significantly increased the concentration of IL-10 in serum and intestinal contents, which led to an increase in the ratio of anti-/proinflammatory cytokines in comparison with the 5-FU + saline group.

Our findings suggest that the protective effect of the administration of riboflavin-overproducing strain could be related to

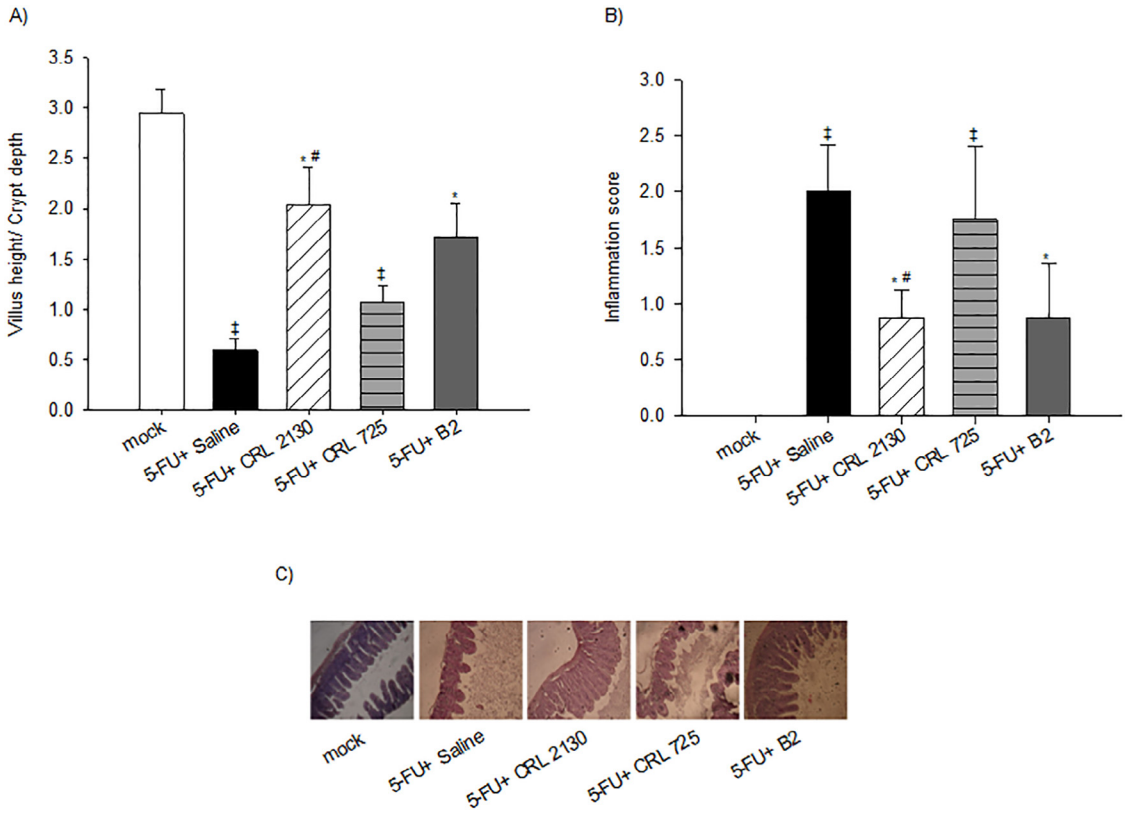

Fig. 4. Effect of riboflavin-overproducing strain on the architecture and inflammation status of the small intestine after treatment with 5-fluororacil. A) Measurement of villus height and crypt depth on histologic sections of jejunum. B) Severity of inflammation graded from 0 to 3 in tissue samples. C) Representative photographs (magnification x50) for each group. Data are expressed as mean ± standard deviation for five mice. ‡ $P < 0.05$ compared with the mock group; * $P < 0.05$ compared with the 5-fluororacil + saline group; and # $P < 0.05$ compared with the 5-fluororacil + CRL725 group.

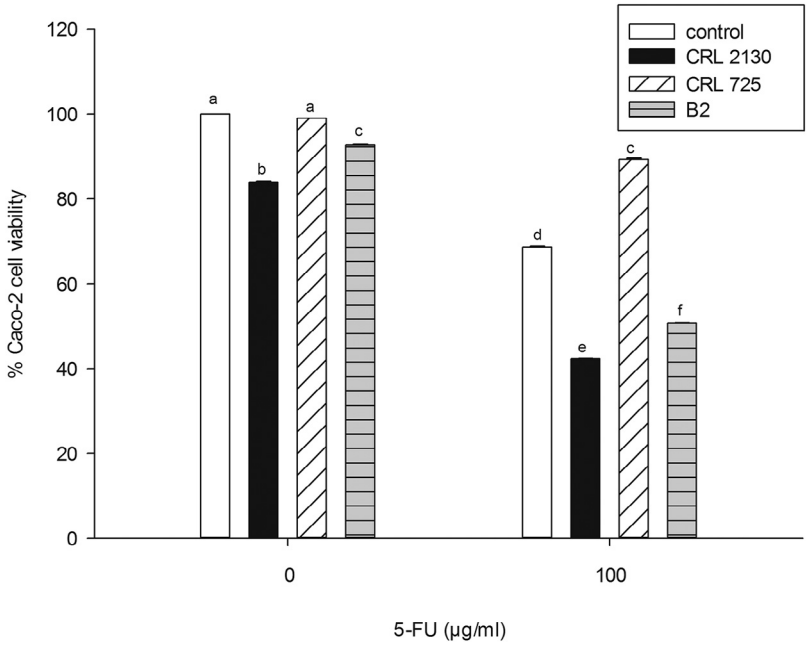

Fig. 5. Cell toxicity assay. Caco-2 cells were incubated with *L. plantarum* CRL2130, CRL725, or commercial riboflavin alone and in combination with 5-fluororacil (100 µg / mL). Data are expressed as percentage of viable cells (100% viability are untreated cells, Dulbecco's modified Eagle's medium controls). Results are mean ± standard deviation from an experiment that was conducted in triplicate. Data with different letters (a-f) are significantly different ($P < 0.05$).

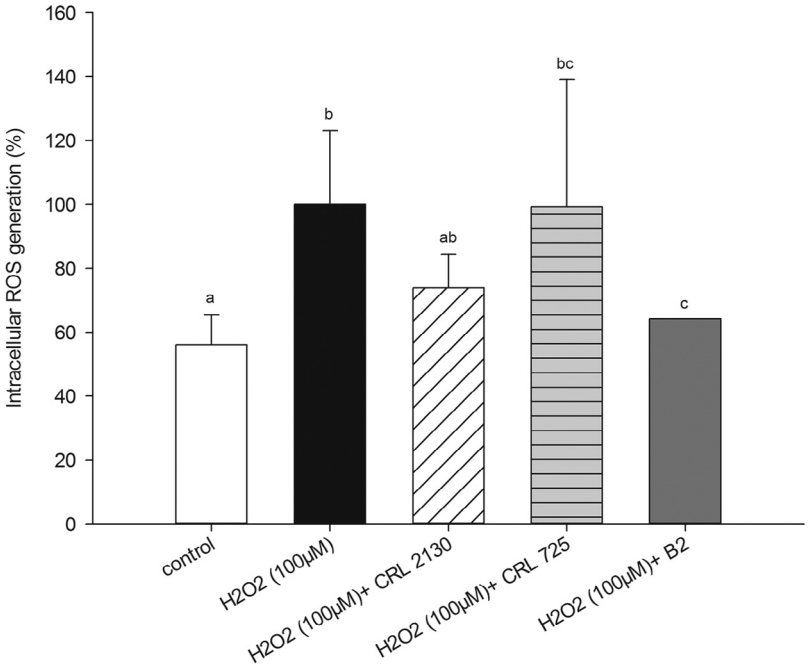

Fig. 6. Reactive oxygen species scavenging capacity of riboflavin-overproducing strain in Caco-2 cells. Caco-2 cells were preincubated with *L. plantarum* CRL 2130, CRL 725, or commercial riboflavin, added 2',7'–dichlorofluorescin diacetate dye and then exposed to hydrogen peroxide. The 2',7'–dichlorofluorescin diacetate dye fluorescence was measured and data are expressed as percentage considering 100% for control cells that were treated only with hydrogen peroxide. The results are mean ± standard deviation from an experiment that was conducted in triplicate. Means without common letter are significantly different ($P < 0.05$).

these increases in the levels of IL-10. The lack of benefits in the group of mice that were injected with 5-FU and received the control strain allowed us to suggest that the effect is due to the production of riboflavin because the only difference between *L. plantarum* CRL2130 and CRL725 is the capacity to produce and release increased amounts of riboflavin [20]. The administration of commercial riboflavin at similar concentrations as produced by the riboflavin-overproducing strain was also found to induce a similar beneficial effect as the bacteria. Currently, some vitamins achieve success in the alleviation of IM in animal models [30,31]. Riboflavin, folate, and multiple vitamin mixture supplements have been previously shown to be efficient in the attenuation cisplatin-induced, intestinal damages-lowering apoptosis indices with a decrease in the oxidative burden in addition to the normalization of the functional integrity of the intestinal mucosa [32].

However, our results showed that the administration of a vitamin-producing probiotic bacterium has the advantage of modulating the immune system of the host (as demonstrated by increased IL-10 in the two samples assayed). Vitamin-producing strains could also protect the vitamin from the harsh conditions of the gastrointestinal tract and allow them to reach the intestines where they could exert their beneficial effects.

In vitro assays were also performed to study whether the riboflavin-overproducing strain could interfere in the chemotherapeutic action of 5-FU. Caco-2 cells that were incubated with *L. plantarum* CRL2130 in presence of 5-FU were observed to significantly reduce cell viability, even more than cells that were incubated with commercial riboflavin. These results suggest that *L. plantarum* CRL2130 did not only prevent damages associated with 5-FU and did not interfere in its cytoxicity but could also improve its chemotherapeutic activity as described for other LAB [20]. Furthermore, the antioxidant effect of the riboflavin-overproducing strain in this study was demonstrated by the scavenging of intracellular ROS that were produced in H_2O_2-stimulated Caco-2 cells. This ROS reduction was not observed when the control strain (*L. plantarum* CRL725) was evaluated so, as explained, by considering the only difference between these two LAB, the suppression of intracellular ROS generation would be due to the antioxidant activity of the vitamin that was produced by the bacterium.

Conclusions

The present work provides evidence that administration of the riboflavin-overproducing strain *L. plantarum* CRL2130 can reduce the severity of 5-FU-induced IM in mice without affecting the effectiveness of 5-FU on colon cancer cells. Therefore, *L. plantarum* CRL2130 could be used as an adjuvant therapy with anticancer chemotherapy. Its effectiveness must now be evaluated in human clinical trials.

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