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Experimental radiotherapy-induced enteritis: A probiotic interventional study

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OBJECTIVE: Acute radiation of the small intestine causes an immediate and potentially reversible effect on the sensitive regenerative epithelium of the intestinal mucosa while markedly altering the overall intestinal ecosystem. The aim of the present study was test a novel probiotic mixture formulation (Microflorana-F) in an experimental model of acute radiation enteritis with particular interest in endotoxinemia and bacterial translocation.

MATERIALS AND METHODS: Male Wistar rats allocated to three groups were fed for 7 days with: (A) a standard balanced diet; (B) a standard diet with the addition of 1 mL t.i.d. of Microflorana-F and (C) the same probiotic but after heat inactivation. Under ketamine anesthesia, abdominal irradiation was performed at a single dose rate of 20 Gy. Sham-radiated healthy rats served as a control (D). Standard food and active/inactive probiotic supplementation schedule was maintained throughout the study period. When they were killed 14 days later a midline laparotomy and a medium sternotomy was carried out. The mesenteric lymph nodes, whole spleen and liver samples as well as blood, the portal vein and bile samples were cultured. Endotoxinemia was also measured.

RESULTS: Early deaths (1 week) occurred mostly in rats fed standard food or inactivated probiotic. The endotoxin level significantly increased in irradiated rats fed standard food and inactivated probiotic while supplementation with the active form of the probiotic mixture significantly improved such parameters ($P < 0.05$). After radiation injury, mesenteric lymph nodes and portal blood were the samples most frequently yielding bacterial growth. Treatment with only the active form of probiotic significantly reduced the incidence of bacterial contamination in all samples.

CONCLUSIONS: These data suggest that the manipulation of gut ecosystem by biologically effective probiotic preparations might be a worthwhile therapeutic and preventive tool in radiation-induced enteritis.

KEY WORDS: gut ecology, probiotic, radiotherapy-induced enteritis.

INTRODUCTION

During radiation therapy tissues undergo varying degrees of damage which depend upon the radiation dose, the technique employed and the specific sensitivity of the tissue to radiation. Indeed, normal tissue damage is the main dose-limiting factor. Although a number of technical modalities have lowered the complications related to radiation therapy, damage of the gastrointestinal and genito-urinary tract remain a considerable clinical problem encountered in clinical practice.[1] In particular, following irradiation of abdominal or pelvic malignancies, the small bowel is particularly exposed due to its high sensitivity.[2] Although histopathologically the

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radiation-induced gut damage also involves the microvasculature and connective tissue, even in the late changes observed in such conditions, mucosal damage seems to play a very important role in the pathogenesis of clinically observed complications in the gastrointestinal tract.[3] Quite recently, the concept of gut ecology as an integrated system comprising the interplay of several compartments such as gut flora and the mucosal, neurovascular and interstitial tissue network has been pointed out.[4] Indeed, radiation has long been known to bring about alteration of the gut flora and motility as well epithelial permeability.[5,6] There are scanty reports on the benefit of dietary supplementation with *Lactobacillus acidophilus*-fermented milk on radiotherapy-associated diarrhoea.[7] We have recently shown that a novel probiotic was able to significantly reduce endotoxinemia and *Candida* gut translocation while exerting an immuno-stimulatory affect.[8,9] Thus the aim of the present study was test the above probiotic in an experimental model of acute radiation-enteritis with particular interest in endotoxinemia and bacterial translocation.

MATERIALS AND METHODS

Experimental design

Sixty male Wistar rats, 8–12 weeks old and weighing 200–230 g were used in the experiments. The animals were individually housed in aluminum cages in a temperature- and dark/light cycle-controlled vivarium and strict sanitary control was observed. The animals were fed standard food and water ad libitum until the day prior to the study. They were allocated to three groups (of 15 rats each) which were fed for 7 days with: (A) a standard balanced diet; (B) a standard diet with the addition of 1 mL t.i.d. of Microflorana-F (a probiotic preparation containing *L. acidophilus*, *L. helveticus* and *Bifidobacterium* in a ion- and vitamin-enriched medium, Lesmo, Italy) and (C) the same probiotic but after heat inactivation (90°C for 10 min). Anesthesia for irradiation was provided by an intraperitoneal injection of 120 mg/kg ketamine. The irradiation was centered on the abdomen and was performed with a Philips 300 X-ray apparatus operated at 300 kV and 12 mA filtered by 0.5 mm copper and 1 mm aluminum at a target skin distance of 30 cm. The resulting half value layer was 0.8 mm and the dose rate at the level of the abdomen was 2.01 Gy/min using a tube with internal diameter 3 cm. Each treatment was planned and controlled by a planning image on Polaroid film exposed with the treatment X-ray tube but set on small focus and 50 kV. The relative depth dose along the beam's central axis was assessed by thermoluminescence dosimetry in five rats. Lithium fluoride rods with a variation in sensitivity of ± 2% were used. A single dose of 20 Gy was performed. Each cage was examined throughout the day for signs of illness or death. Anesthetized healthy rats undergoing sham radiation and without any further treatment constituted the healthy control group (D). Standard food and active/inactive probiotic supplementation schedule was maintained throughout the study period.

Bacteriological study

When the animals were killed 14 days later, a careful toilette of the anterior chest and the abdominal cavity was obtained. Using a sterile technique, a midline laparotomy and a medium sternotomy was carried out. Mesenteric lymph nodes and whole spleen and liver samples were removed and placed separately in sterile test tubes containing 0.9% sterile saline. The samples were then homogenized and 0.1 mL of tissue homogenate was transferred into trypticase soy broth and thioglycollate broth culture media. Under strict sterile conditions, blood samples were obtained by cardiac puncture by the portal vein and bile samples were collected as well. These specimens were cultured for aerobe and anaerobe bacteria. The isolates were identified by their colonial characteristics, Gram stain morphology and biochemical reactions. Serologic assessment for identifying *Streptococcus* and the API-20E system for identifying *Enterobacteriaceae* were used. The plates were examined after 24 h and 48 h of incubation at 37°C. Cultures growing *diphteroids* or *Staphylococcus epidermidis* were regarded as contaminants and the animals were excluded from the study. In each case 0.1 mL sterile saline was added to tissue homogenizers and processed along with specimens to serve as negative control. Endotoxinemia was measured by following a slightly modified chromogenic method described by Fukui *et al.*[10,11]

Statistical analysis

Significance was established by ANOVA and the level of significance was determined by employing a Duncan's multiple-range test. Data were expressed as means (SD) and a probability value of <0.05 was regarded as indicating that a significant difference existed between experimental groups.

RESULTS

Thermoluminescence dosimetry using a 5.0 Gy test dose showed that a dose of 5.02 ± 0.23 Gy had been effectively delivered to the upper abdomen surface.

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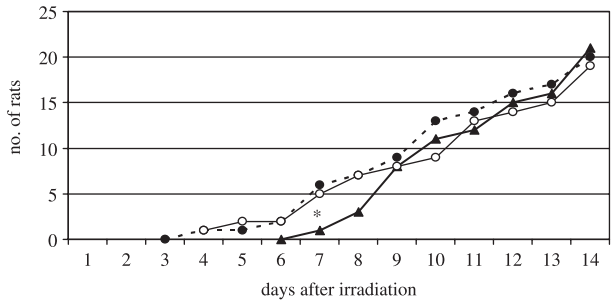

Figure 1. Cumulative death after X-ray irradiation: effect of probiotics. *Probiotic (▲) versus inactivated probiotic (○) control (●). $P < 0.05$.

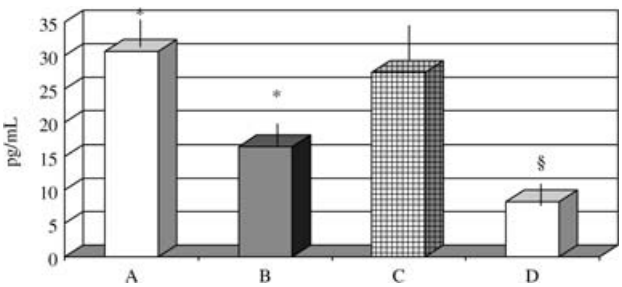

Figure 2. Endotoxinemia in X-ray-irradiated rats: effect of probiotics. A, standard diet; B, standard diet plus probiotic; C, heat inactivated-probiotic; D, sham radiated-healthy rats. *$P < 0.01$ versus healthy control; §$P < 0.05$ versus active probiotic.

Survival study

As compared to the control group, rats treated with the inactivated probiotic or active probiotic mixture showed no significant difference in overall 2-week survival. However, early deaths (1 week) occurred mostly in rats fed standard food or inactivated probiotic (Fig. 1). There was no death in the sham-irradiated rats.

Biochemistry

The endotoxin level significantly increased in irradiated rats fed standard food when compared to healthy control animals (Fig. 2, $P < 0.01$). Unlike the inactivated probiotic, supplementation with the active form of the probiotic mixture significantly improved such parameters ($P < 0.05$).

Bacteriological study

The bacteriological spectrum is shown in Fig. 3. After radiation injury, mesenteric lymph nodes and portal blood were the samples most frequently yielding bacterial growth. Treatment with only the active form of probiotic significantly reduced the incidence of bacterial contamination in all samples ($P < 0.01$ *vs* inactivated probiotic and rats fed with standard food). There was no significant change of the overall aerobic and anaerobic gut flora between the groups of irradiated rats (data not shown). Bacteremia was present in all irradiated rats, both gram-negative and gram-positive organisms being recovered. However, while most cases of rats fed the active form of Microflorana-F (group C.) yielded *Streptococcus*, gram-negative enteric organisms were the predominant blood isolates from untreated and inactivated probiotic-treated rats (Fig. 4). All early deaths were related to gram-negative septicemia and *Pseudomonas* was the only organism recovered from early deaths in untreated or inactive probiotic-treated rats. None of the bacilli contained in the probiotic was recovered from the blood of any rats.

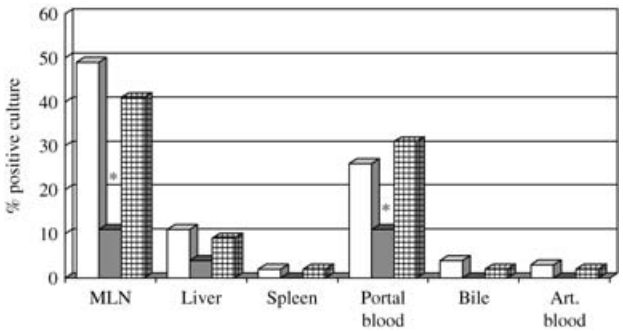

Figure 3. Positive bacterial cultures from tissues tested at the end of experiment: effect of probiotic. □, a standard diet; ■, standard diet plus probiotic; ▦, heat inactivated-probiotic. *Inactivated probiotic versus active formulation. $P < 0.01$.

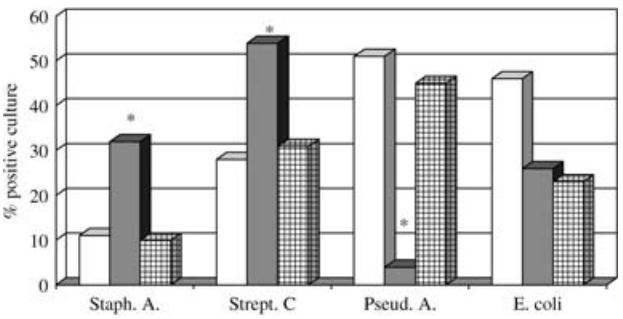

Figure 4. Bacteremia in tissues from irradiated rats tested at the end of experiment: effect of probiotic. □, a standard diet; ■, standard diet plus probiotic; ▦, heat inactivated-probiotic. *Inactivated probiotic versus active formulation: $P < 0.01$.

DISCUSSION

It has been shown that the maintenance of intestinal integrity during radiotherapy or chemotherapy in

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cancer patients positively affects the well-being of patients.[12] Further, otherwise clinically stable patients undergoing radiotherapy may have a subtle small intestinal carbohydrate malabsorption.[13] The intestinal mucosa provides both a mechanical and immunological barrier to the translocation of particles, including antigens and bacteria. However, such a barrier is not complete and a small amount of bacteria can pass from the gastrointestinal tract through the mucosal lamina propria.[14] In critically ill patients such translocation of pathogenic organisms through the intestinal wall into the bloodstream, the peritoneal cavity and abdominal organs is a well-recognized cause of supervening sepsis and life-threatening complications.[15–17] It has long been known that acute radiation to the small intestine causes an immediate and potentially reversible effect on the sensitive regenerative epithelium of the intestinal mucosa[18] while repeated exposure to radiation therapy may lead to a progressive disease through irreversible obliterative vasculitis changes. While the composition of the contents of the small intestine has been shown to influence the development of the acute intestinal radiation syndrome in animals[19] there is a paucity of data on the application of dietary supplementation with probiotics.[7,20] We have recently shown that the novel probiotic we employed in the present study was able to significantly prevent fungal and bacterial translocation in experimental conditions such as acute alcohol intoxication and acute pancreatitis.[8,21] Both these conditions have been shown to be associated with an enhanced permeability of the mucosal gut barrier,[22–24] which is likely to be one of the pathomechanisms of acute radiation damage to the intestine. Accordingly, in the present study we showed that radiation enteritis was associated with a significant bacteremia and endotoxinemia. This picture was significantly reduced, although it did not revert to normal, in rats treated with active probiotic. Further, dietary supplementation with Microflorana-F significantly reduced bacterial colonization in all the samples tested. Although bacteremia was accounted for by either gram-positive and gram-negative organisms, only gram-negative strains were isolated in those rats which suffered early death. It is of interest that all these animals belonged to the groups fed either standard food or heat-inactivated probiotic. Thus, one might postulate that Microflorana-F supplementation, by significantly preventing gram-negative bacteria translocation, as corroborated by the concomitant decrease of endotoxinemia, exerted a significant role in reducing early mortality. Moreover, none of the live bacterial components of the probiotic mixture gave rise to systemic dissemination. This is in accordance with the study of Romanchuk *et al.*[25] who showed a significant survival improvement in total-body irradiated mice when treated with an antimicrobial active on opportunistic microorganisms but that did not affect the level of lactobacteria. Thus, it can be suggested that the probiotic we employed may act by metabolically competing on the ecologic niche with resident pathogens inside the gut lumen and thus performs a potentially positive role on mucosal integrity as well. Indeed, in our previous experimental study in rats with acute pancreatitis, we have shown that employing enemas with nonabsorbable antibiotics and lactulose helped improving the inter-glandular space disruption in the cecal mucosa.[22]

Although the issue of reducing the early and late untoward effects due to radiation therapy is multifaceted and involves not only methodological modalities but also cellular and intracellular variables, as very recently demonstrated,[26,27] from the present study it would appear that the oral administration of selected probiotic preparations might be a worthwhile complementary therapeutic and preventive tool.

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