Chemotherapy-Induced Diarrhea Is Associated with Changes in the Luminal Environment in the DA Rat

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The microflora of the gastrointestinal tract (GIT) are a complex ecosystem, performing a number of beneficial functions. Irinotecan causes both early and late diarrhea, the latter possibly caused, in part, by changes in the microflora of the GIT. Female DA rats were given atropine subcutaneously, prior to a single 200 mg/kg intraperitoneal dose of irinotecan. Animals were monitored for diarrhea and killed at 30 and 60 mins, 2, 6, 12, 24, 48, and 72 hrs after chemotherapy administration. Control rats received no treatment. Fecal samples and stomach, jejunum, and colon samples were collected and stored at –70°C until required. Standard microbiological culture techniques were used to grow and isolate the flora. Biochemical tests were used to identify the bacteria. The level of growth was noted for relative comparison between time points and graded accordingly. Early diarrhea was observed in the rats from 2–6 hrs after treatment, after which time the diarrhea resolved. Late onset diarrhea was apparent 72 hrs after treatment. Changes were seen in the flora of the stomach, jejunum, colon and feces. The majority of microflora changes were seen 6, 12, and 24 hrs after treatment, with a relative increase or decrease in the presence of bacteria in comparison with control rats. In some rats bacteria were not observed at all time points, and different bacteria not seen in control animals were identified in rats treated with irinotecan. These changes were observed up to 72 hrs after treatment. In conclusion, irinotecan treatment causes changes in the flora of the stomach, jejunum, colon, and feces of rats and is associated with the development of diarrhea. These changes in flora may have systemic effects and in particular may contribute to the development of chemotherapy-induced mucositis. Exp Biol Med 232:96–106, 2007

Key words: irinotecan; chemotherapy; diarrhea; intestinal microflora; electrolytes

Introduction

Mucositis is a major oncological problem caused by the cytotoxic effects of cancer chemotherapy and radiotherapy. Approximately 40% of patients receiving standard dose chemotherapy and 100% of patients receiving high dose chemotherapy and stem cell or bone marrow transplantation exhibit the pain, ulceration, bloating, vomiting and diarrhea associated with mucositis (1–3). An accurate enumeration of patients suffering chemotherapy-induced diarrhea (CID) as a result of treatment has yet to be determined, although general estimates place 10% of patients with advanced cancer as being affected (4). While major progress has been made in recent years in understanding the mechanisms of oral (5–8) and small intestinal mucositis (9–14), the mechanisms of large intestinal mucositis (including CID) remain insufficiently understood. The pathophysiology of diarrhea is complex and likely to involve a number of mechanisms (15, 16). The different types of diarrhea related to cancer and cancer therapy include secretory, osmotic, malabsorptive, exudative and dysmotile (16), although infectious diarrhea, inflammatory diarrhea, and steattorhea may also be related.

Irinotecan hydrochloride (CPT-11) is a relatively new cytotoxic agent used to treat a variety of solid tumors. The primary mechanism of action is to inhibit DNA topoisomerase I (17–20). Irinotecan is converted by hepatic or gastrointestinal carboxylesterases to its active metabolite, SN-38, which is responsible for irreversible DNA damage (21) and has been implicated in the onset of diarrhea. SN-38 is further processed to become SN-38 glucuronide, a less

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toxic form of SN-38 (20). However, SN-38 glucuronide is able to be enzymatically cleaved by β-glucuronidase to return it to the toxic form, SN-38 (20). A number of the intestinal microflora have β-glucuronidase activity and may be responsible in part for the intestinal cytotoxicity of irinotecan (20). Bacterial β-glucuronidase is produced primarily by Enterobacteriaceae (*Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Yersinia* spp, *Citrobacter* spp, *Hafnia* spp, and *Edwardia* spp) and has been reported to be produced by *Flavobacterium* spp, *Bacteroides* spp, *Staphylococcus* spp, *Streptococcus* spp, *Corynebacterium* spp, and *Clostridium* spp (22).

The microflora of the gastrointestinal tract (GIT) is a highly complex ecosystem consisting of both aerobic and anaerobic bacteria (23–25). These microflora have a number of key functions, including protection as well as metabolism of bilirubin, intestinal mucins, pancreatic enzymes, fatty acids, bile acids, cholesterol and steroid hormones (26). Furthermore gastrointestinal bacteria function to process nutrients, regulate intestinal angiogenesis, and work with the immune system (27, 28). Intestinal flora varies between races, sex, age, and diet within humans, and there is some variation between humans and other species (29). Diseased states and/or associated therapeutic regimens can alter the composition of the gastrointestinal microflora. Acute diarrhea causes a profound alteration in bacterial populations of the gut (23). Coliform numbers are increased in the human small intestine, and fecal flora consists of more *Proteus* spp and *Pseudomonas* spp (23). The rapid transit of stools through the human colon during diarrhea results in a decrease in the anaerobic population of the colon. The resolution of the diarrhea is accompanied by the restoration of the normal microflora (23). The aim of this study was to determine if changes in the luminal environment, specifically changes in gasrointestinal flora (especially those genera that are known to produce β-glucuronidase and may interfere with the processing of irinotecan) coincided with subsequent diarrhea in rats treated with irinotecan.

Materials and Methods

Animals. Animals used in this study were female Dark Agouti (DA) rats, weighing between 150 and 170 g. Rats were housed in Perspex cages at a temperature of 22 ± 1°C and subject to a 14:10-hr light:dark cycle. Approval for the use of animals was granted by the Animal Ethics Committees of the Institute of Medical and Veterinary Science (IMVS), and the University of Adelaide, and complied with the National Health and Medical Research Council (Australia) Code of Practice for Animal Care in Research and Teaching (2004). Because of the nature of the diarrhea induced by irinotecan, animals were monitored 4 times daily, and if any animal showed certain criteria (as defined by the Animal Ethics Committees) they were euthanized. These criteria included a dull ruffled coat with accompanying dull and sunken eyes, cold to touch with no spontaneous movement, and a hunched appearance.

Experimental Plan. Eighty-one rats were randomly assigned to groups. For each time point there was 1 group of 6 rats receiving irinotecan and 1 group of 3 control rats receiving no treatment. Rats receiving irinotecan received 0.01 mg/kg subcutaneous atropine (to reduce cholinergic reaction to irinotecan) prior to (within 2 mins) administration of 200 mg/kg intraperitoneal irinotecan. Irinotecan (kindly supplied by Pfizer, Kalamazoo, MI) was administered in a sorbitol/lactic acid buffer (45 mg/ml sorbitol/0.9mg/ml lactic acid, pH 3.4), required for activation of the drug, at time designated 0 hr. Groups of rats were killed using 3% halothane in 100% O_2 anesthesia and cervical dislocation at times 30 and 60 mins, 2, 6, 12, 24, 48, and 72 hrs posttreatment. Immediately prior to anesthesia, fecal samples were aseptically collected by directly collecting the excreted feces immediately as it left the rat in a sterile container in an area cleaned with 70% ethanol. Samples were stored at −70°C. Prior to cervical dislocation, a blood sample was collected *via* cardiac puncture. The GIT (from the pyloric sphincter to the rectum) was dissected out and separated into the small intestine (pyloric sphincter to ileocaecal sphincter) and colon (ascending colon to rectum). The small intestine was flushed with chilled, sterile, distilled water, and 1-cm samples taken at approximately 50% of the length were collected for culture and routine histology. The colon was also flushed with chilled, sterile, distilled water, and contents were collected for electrolyte analysis. Two 1-cm samples of colon, taken at approximately 50% of the length, were collected for culture and histology. The stomach was dissected from the rat and contents emptied and discarded. Two small pieces (1 cm × 0.5 cm) of stomach were collected for culture and histology. All samples collected for culture were stored at −70°C until required, and those for routine histological examination were fixed in 10% neutral buffered formalin.

Diarrhea Assessment. All animals were checked 4 times daily and diarrhea recorded according to previous gradings (8, 12). This was graded as 0, no diarrhea; 1, mild diarrhea (staining of anus); 2, moderate diarrhea (staining over top of legs and lower abdomen); and 3, severe diarrhea (staining over legs and higher abdomen, often with continual anal leakage). All diarrhea assessments were conducted in a blinded fashion by 2 investigators (A.M.S. and R.M.L.).

Electrolyte Analysis. Blood and colonic flush samples were centrifuged (Hereus, Finland) at 3000 rpm for 5 mins. The serum (supernatant) was collected into a fresh tube and analyzed by the Department of Clinical Pathology at the Institute of Medical and Veterinary Science (IMVS), Adelaide, South Australia. Measurements for sodium, potassium, bicarbonate, chloride, and osmolarity were measured from serum samples, and sodium and potassium only were measured from the colonic flush samples.

Histological Examination. Samples of stomach, jejunum, and colon were collected and fixed in 10% neutral buffered formalin for routine histological examination. Fixed samples were processed and embedded in paraffin. Sections of 4-μm thickness were cut and mounted on glass slides. Routine hematoxylin and eosin (H&E) staining was performed. Briefly, the wax was dissolved with xylene and sections rehydrated before staining in Lillie-Mayer's hematoxylin for 10 mins. After differentiating in 1% acid alcohol and blueing in Scott's tap water, sections were counterstained in eosin, then dehydrated, cleared, and mounted, and then examined using light microscopy and reported on by a professional veterinary pathologist.

Culture of Samples. To determine the flora of the DA rat, a variety of selective and nonselective media (Oxoid, Adelaide, Australia) was used in an attempt to identify as many bacteria as possible from the GIT. Media used included horse blood agar (HBA) (30); MacConkey (MAC); HBA + colymycin + nalidixic acid (CNA); MAC + CV; chromogenic *E. coli*/coliform; CIN (31); Raka Ray (32); TCBS (33); xylose-lysine-desoxycholate (XLD) (34); Campylobacter (35); anaerobic (36); anaerobic + nalidixic acid; and anaerobic + nalidixic acid + vancomycin (details shown in Table 1) (37). The mucosal surface of the tissue samples was scraped, and fecal samples were homogenized with a sterile swab and inoculated onto the top third of the plate. A flame-sterilized inoculating loop was used to streak in a zigzag fashion. All plates and broths were incubated at 35–37°C for 24–48 hrs.

The level of growth of each bacterium was graded using a qualitative assessment technique (14, 38) using the following criteria: 0, no growth (NG); 1, very light growth (VLG) (less than 10 colonies); 2, light growth (LG) (growth in the original inoculation zone only); 3, moderate growth (MG) (growth in the first streak line); and 4, heavy growth (HG) (growth in the second streak line or greater). All gradings were conducted in a blinded fashion (A.M.S.). This grading system has been validated in our laboratory using quality control organisms and sample organisms to ensure consistent and valid results.

Each different bacterium present was isolated and subcultured onto horse blood agar plates (Oxoid) for biochemical testing to assist in identification. The first identification step was a Gram stain, followed by a variety of catalase, oxidase (39), coagulase, indole, urease, growth in NaCl, motility (40), oxidation-fermentation (OF) test (41), Lancefield grouping and PYRase (42), depending on Gram stain result. If identification was not possible after biochemical tests were conducted, identification kits were used in which a wider array of biochemical tests was used to identify the bacteria. For anaerobic bacteria the Remel RapID (Oxoid, Adelaide, Australia) kit was used, and for gram-negative aerobic bacteria the Microbact 24E kit was used (43).

Results

Diarrhea. Diarrhea was observed in 23% of treated rats from 2 hrs after treatment (Fig. 1). Mild diarrhea was seen in 23% of experimental rats at 2–6 hrs and at 12 hrs 30% of rats had mild and 5% of rats had moderate diarrhea. This increased to 39% of rats having mild and 12% having moderate diarrhea at 24 hrs. At 48 hrs 20% of rats had mild diarrhea. Late onset diarrhea was apparent at 72 hrs after treatment, with 33% of treated rats having mild diarrhea. No control rats had diarrhea at any time point investigated.

Electrolyte Analysis. Electrolyte levels were altered in rats treated with irinotecan. Serum sodium, chloride, and bicarbonate levels were lower in the experimental rats compared with control rats. The anion gap was higher in experimental rats than control rats, and serum potassium levels were also higher in experimental rats (Fig. 2).

Fecal sodium levels were similar for both experimental and control rats for the early time points. However, between 12–48 hrs there was a peak in fecal sodium levels in experimental rats. Fecal potassium levels between experimental and control rats were similar until 72 hrs, where experimental levels began to increase (Fig. 3). The serum osmolarity levels (Fig. 4) were lower in experimental rats that control rats, with the largest difference at 6–24 hrs.

Histology. Pathological changes caused by irinotecan were seen in the colon, jejunum (Fig. 5), and stomach (not shown). There were no histological changes in control rats or in experimental rats between 30 mins and 2 hrs postchemotherapy. At 6 hrs, changes in the colon included patchy but widely distributed apoptosis of enterocytes, especially in the mid and basal regions of the crypts. Dilated crypt lumina lined with attenuated epithelium and occasional debris in the lumen were observed at 48 hrs and 72 hrs (more severe at 72 hrs) with condensation of the stroma also observed at 72 hrs.

The jejunum showed no histological changes in control rats or in experimental rats between 30 mins and 60 mins postchemotherapy. Between 2 hrs and 72 hrs, patchy widespread apoptosis of basal crypt enterocytes was observed. Extensive damage was observed from 2 hrs. However, the most severe histological changes were seen at 6 hrs following chemotherapy. The severity of changes decreased from 12 hrs to 72 hrs.

The stomach showed no histological changes in control rats, before 2 hrs, and after 24 hrs postchemotherapy. At 2 hrs, patchy but widely distributed apoptosis of individual basal glandular epithelial cells was seen, with slight inflammation. The affected cells were more numerous and superficially disposed at 6 hrs, and at 12 hrs glandular epithelial degeneration was patchy and very limited.

Culture. Changes were seen in the flora of the stomach, jejunum, colon, and feces of rats treated with chemotherapy. The majority of these changes were observed at 6, 12, and 24 hrs after treatment. The organisms identified

Table 1. Materials Used for Culture

Media[a]	Selectivity	Reference
Columbia HBA	Nonselective	Ellner *et al.* 1966
MAC	Gram negative	Murray *et al.* 1999
HBA + CNA	Gram positive	Murray *et al.* 1999
MAC + CV	Gram negative	Murray *et al.* 1999
Chromogenic *Escherichia coli*/coliform	Coliforms	Frampton *et al.* 1988
CIN	*Yersinia* sp	Schiemann 1982
Raka Ray	*Lactobacillus* sp	Saha *et al.* 1974
TCBS	*Vibrio* sp	Davis *et al.* 1981
XLD	*Salmonella* sp and *Shigella* sp	Weissman *et al.* 1975
Campylobacter	*Campylobacter* sp	Patton *et al.* 1981
Anaerobic	Anaerobes	Wren 1980
Anaerobic + Nali	Anaerobes, enhanced Gram positive	Wren 1980
Anaerobic + Nali + Vanc	Anaerobes, enhanced Gram negative	Wren 1980

[a] HBA, horse blood agar; MAC, MacConkey; CNA, colymycin + nalidixic acid; CV, crystal violet; CIN, Yersinia; XLD, xylose-lysine-desoxycholate; Nali, nalidixic acid; Vanc, vancomycin.

were consistent with the expected gastrointestinal microflora population of rats.

Stomach. The stomach mucosal surface of all rats was found to harbor copious numbers of *Lactobacillus* spp, *Enterococcus* spp, and *Staphylococcus* spp. Changes in levels of bacteria following chemotherapy were seen (Fig. 6). *Enterococcus* spp levels peaked at 2–6 hrs. *Peptostreptococcus* spp levels peaked 30–60 mins after the administration of irinotecan. *Serratia* spp decreased early but peaked at 2 hrs. *Staphylococcus* spp fluctuated considerably, with a peak observed at 12 hrs.

Jejunum. The jejunum mucosal surface of all rats was found to accommodate relatively small numbers of organisms compared with the stomach, colon, and feces. The most prominent were *Enterococcus* spp, *Lactobacillus* spp, *Staphylococcus* spp, and *Serratia* spp. Changes were seen in bacterial levels following chemotherapy (Fig. 7). There was a peak at 2 hrs in *Clostridium* spp, *Enterococcus* spp, and *Lactobacillus* spp. There was also a peak in *Serratia* spp at 2 hrs. *Peptostreptococcus* spp levels decreased at 2 hrs. *Bifidobacterium* spp and *Prevotella* spp were detected only between 2–12 hrs, whereas *Pseudomo-*

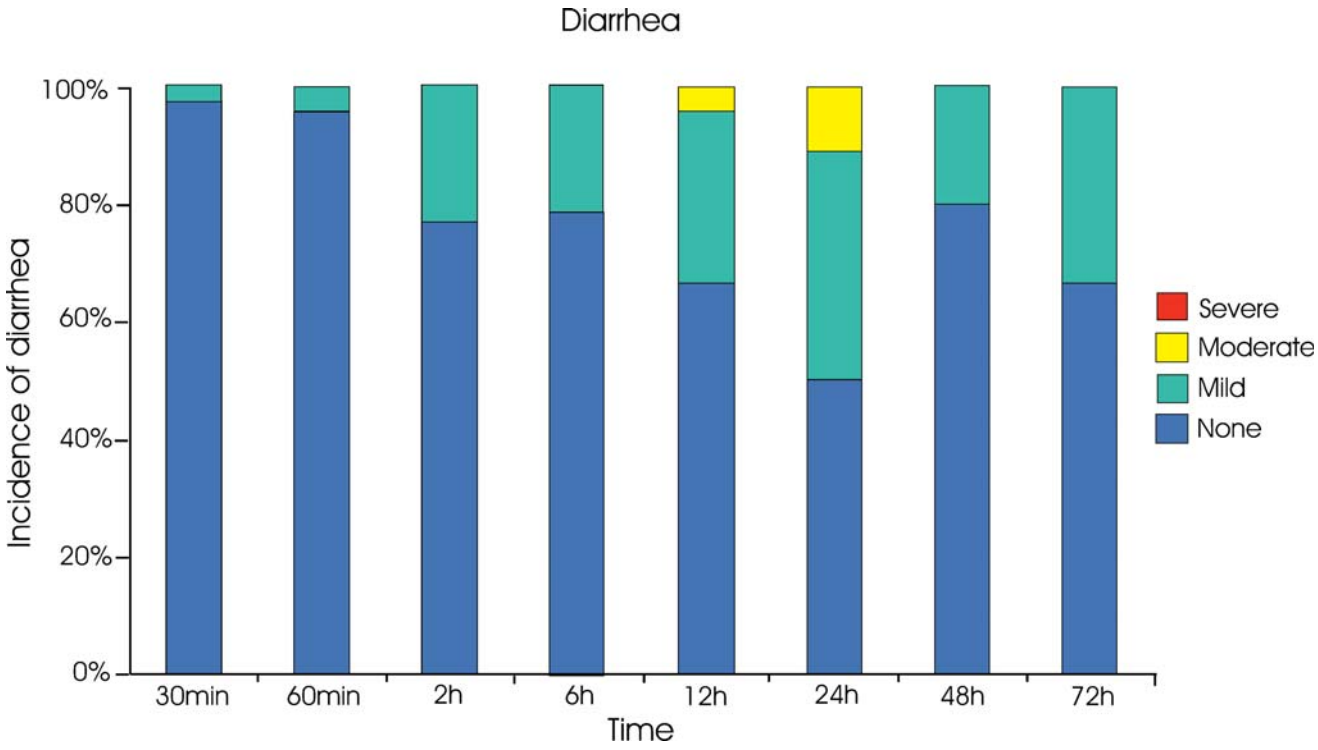


Figure 1. Diarrhea incidence.

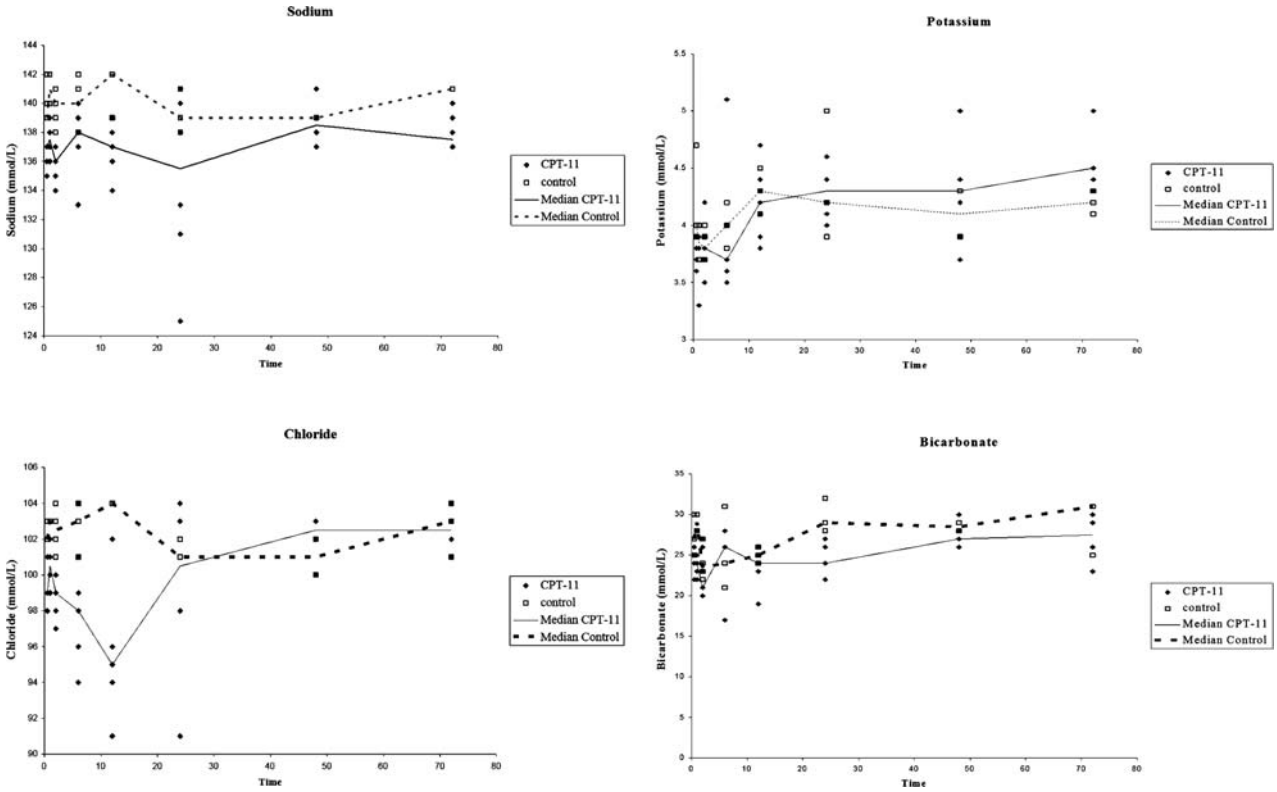


Figure 2. Serum electrolytes. Sodium and chloride levels were reduced early in rats treated with irinotecan (CPT-11). Bicarbonate levels were reduced at later time point after treatment. Potassium level increased after treatment with irinotecan.

nas spp was not detected after 2 hrs, and *Proteus* spp was undetected from 2–48 hrs.

Colon. The mucosal surface of the colon in all rats was found to accommodate a large number of organisms. The most prominent in the control rats were *Enterococcus* spp and *Lactobacillus* spp. Changes were seen in the bacterial levels following chemotherapy (Fig. 8). There was an increase in the levels of *Escherichia* spp between 6–24 hrs. *Clostridium* spp showed an increase at 2 hrs, *Enterococcus* spp increased at 6 hrs, and *Serratia* spp increased between 60 mins–24 hrs. *Staphylococcus* spp increased at 60 mins and again at 48 hrs. *Peptostreptococcus* spp levels increased early, at 30–60 mins. *Veillonella* spp were undetected at 30 mins and 2–6 hrs. *Lactobacillus* spp increased slightly over time. *Bacillus* spp increased at 6 hrs. *Proteus* spp and *Streptococcus* spp were both unable to be detected at 2 hrs.

Feces. In all rats, the bacterial content of the feces was found to be higher than the colon, jejunum, or stomach. The most prominent bacteria were *Lactobacillus* spp,

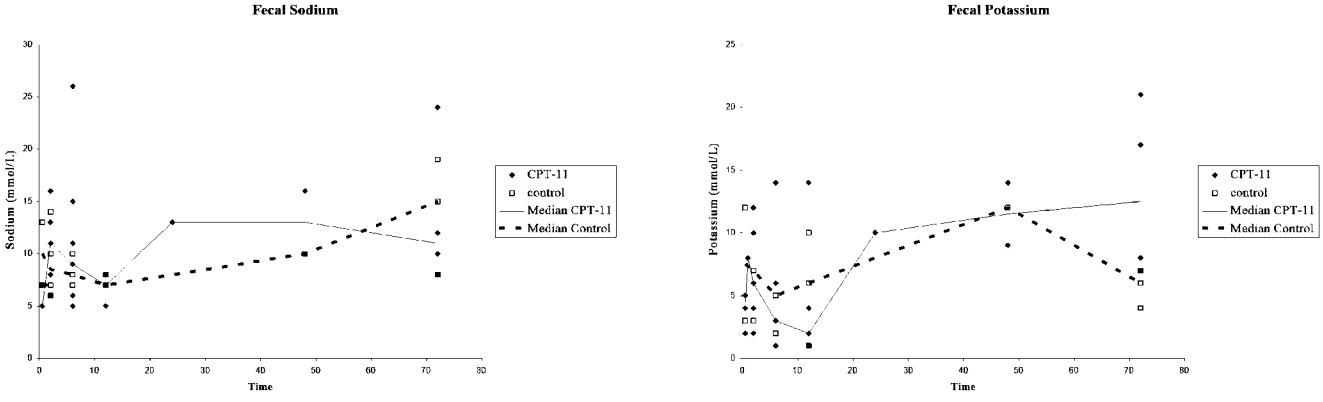


Figure 3. Fecal electrolytes. Sodium levels increased at 24 hrs and 48 hrs in rats treated with irinotecan (CPT-11). Potassium levels remained similar in treated and control rats until 72 hrs, where there was an increase in potassium in treated rats.

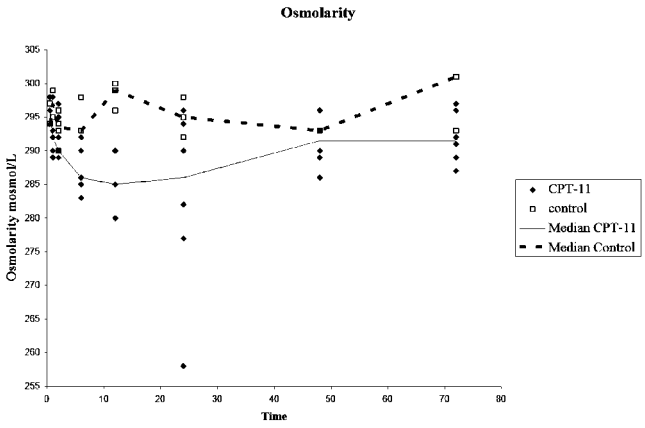


Figure 4. Serum osmolarity levels were lower in rats treated with irinotecan (CPT-11). The largest difference is seen between 6–24 hrs.

Enterococcus spp, *Escherichia* spp, *Proteus* spp, *Serratia* spp, and *Staphylococcus* spp. Changes were detected in the fecal flora following chemotherapy (Fig. 9). *Proteus* spp levels were highest at 24–72 hrs. *Bacillus* spp were undetected at 12 hrs and 72 hrs. *Bifidobacterium* spp were also undetected at 60 mins and 48–72 hrs. *Clostridium* spp were undetected at 60 mins but reached their highest level at 2 hrs. *Peptostreptococcus* spp levels were highest at 30–60 mins. *Escherichia* spp levels fluctuated substantially, and *Lactobacillus* spp levels reached a low point at 30 mins, prior to returning to constant levels for the remaining time points. *Enterobacter* spp reached a small peak at 2 hrs. *Veillonella* spp were unable to be detected following chemotherapy until 12 hrs. *Actinobacillus* spp were detected in control rats but were not able to be detected in any of the

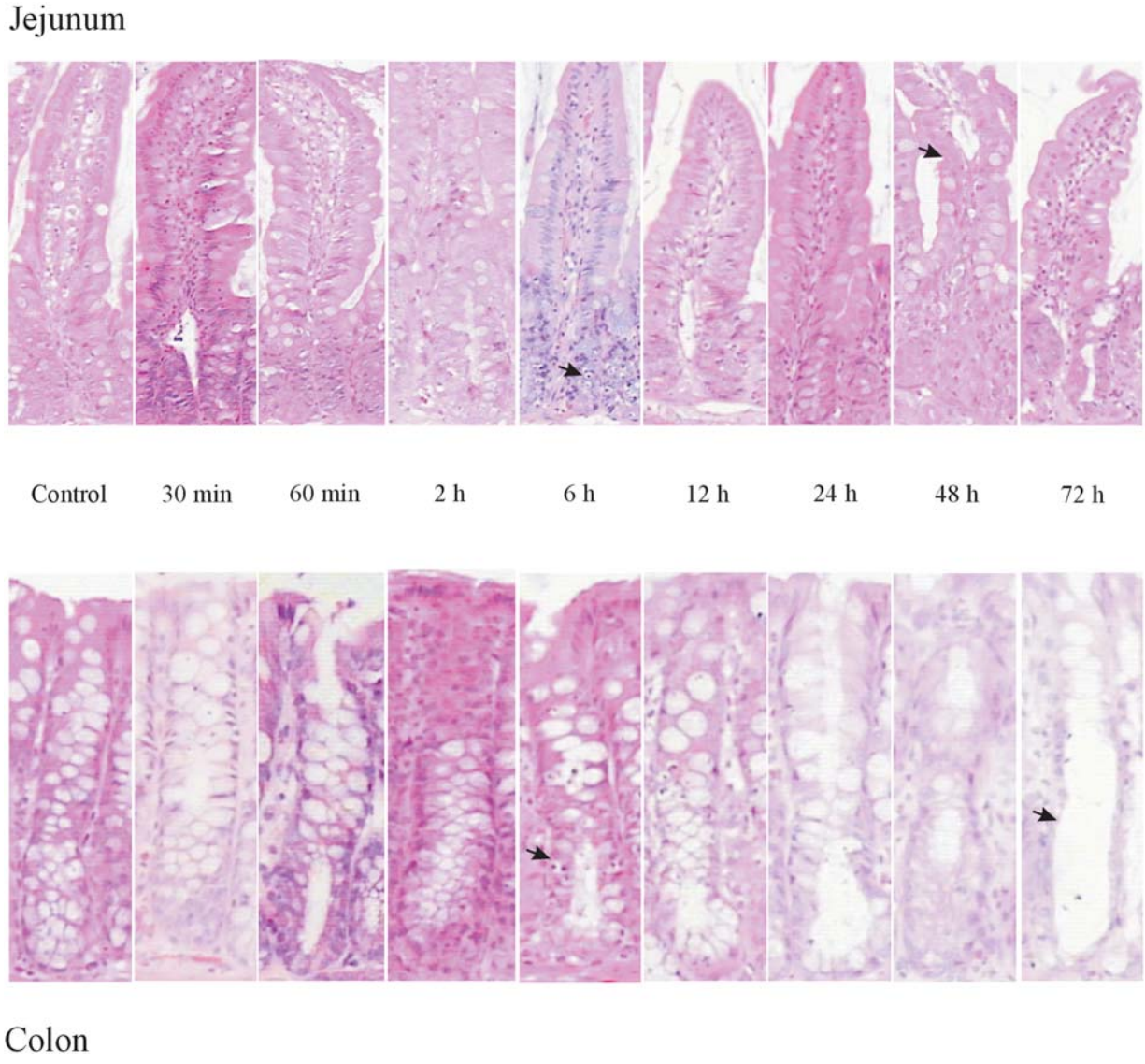


Figure 5. Histopathology. Histopathologic changes are seen in the jejunum and colon following treatment with irinotecan. Changes begin to be evident at 6 hrs after treatment in the jejunum, with apoptosis in the crypt cells. At 48 hrs the villi become blunted, and there is a loss of normal architecture. See arrows. In the colon there are also apoptotic cells at 6 hrs, and by 72 hrs there is complete crypt ablation. See arrows.

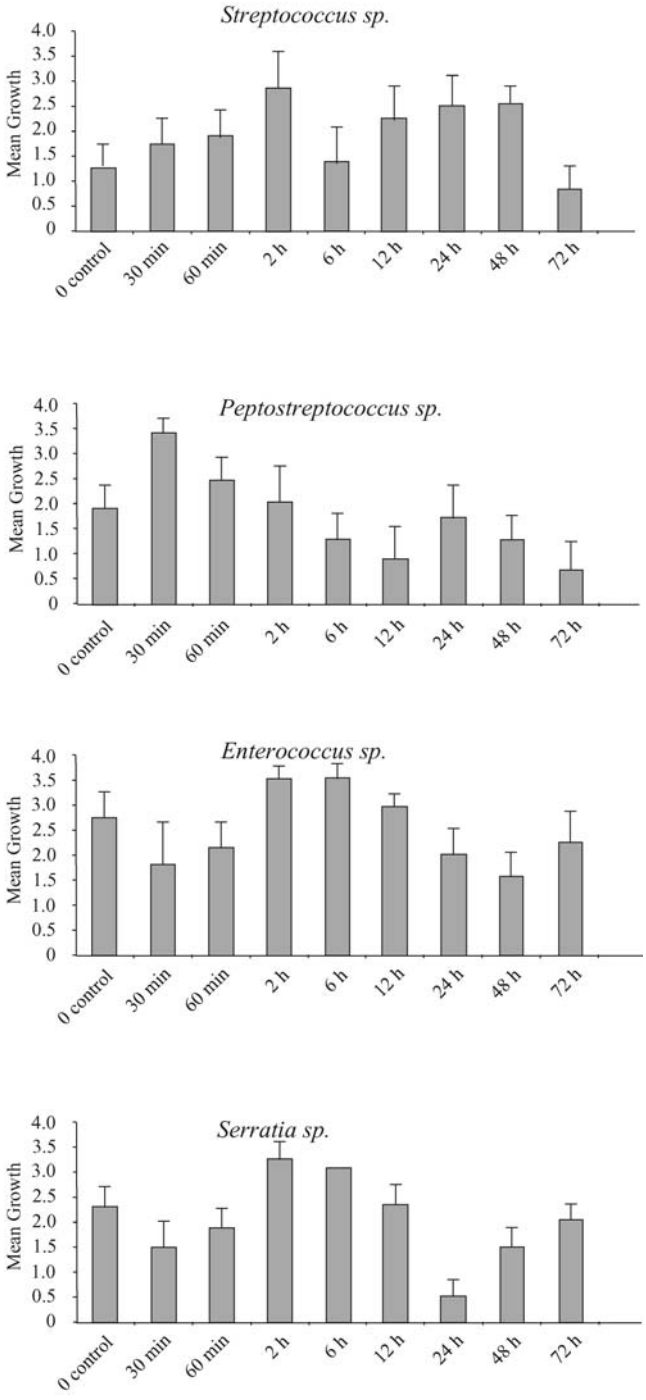


Figure 6. Bacterial changes in the stomach.

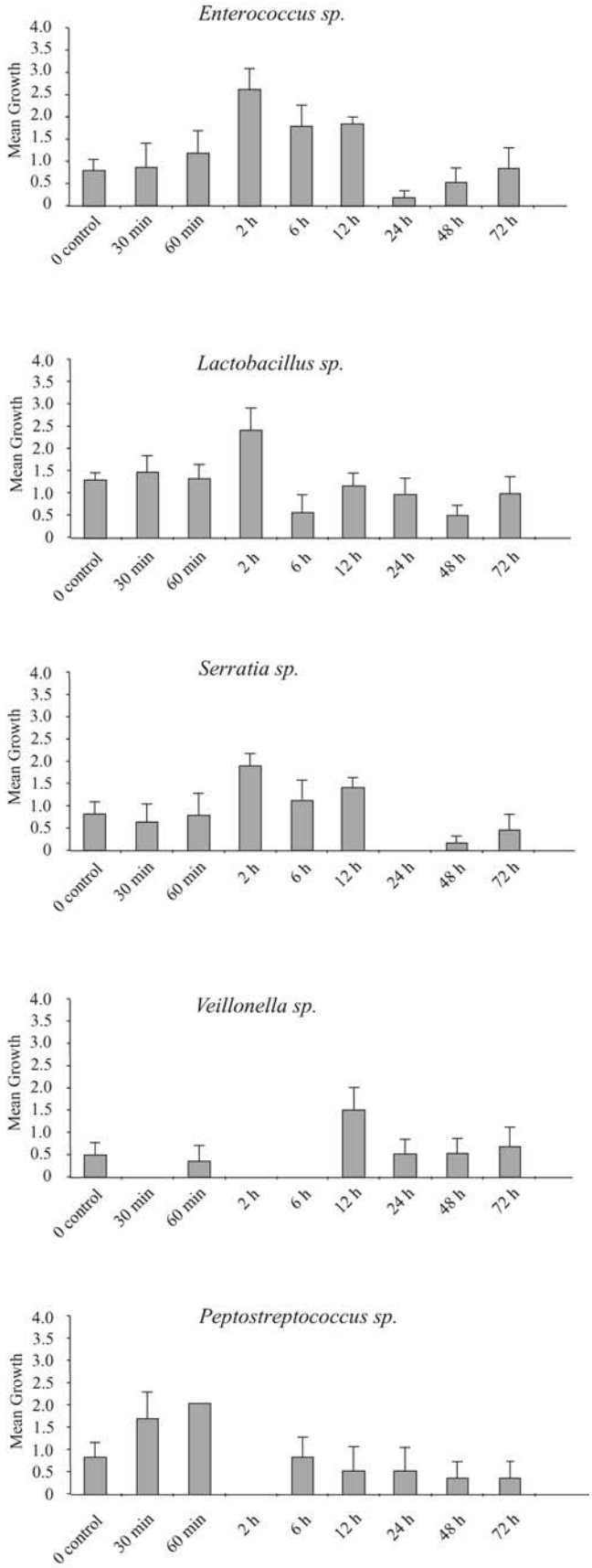
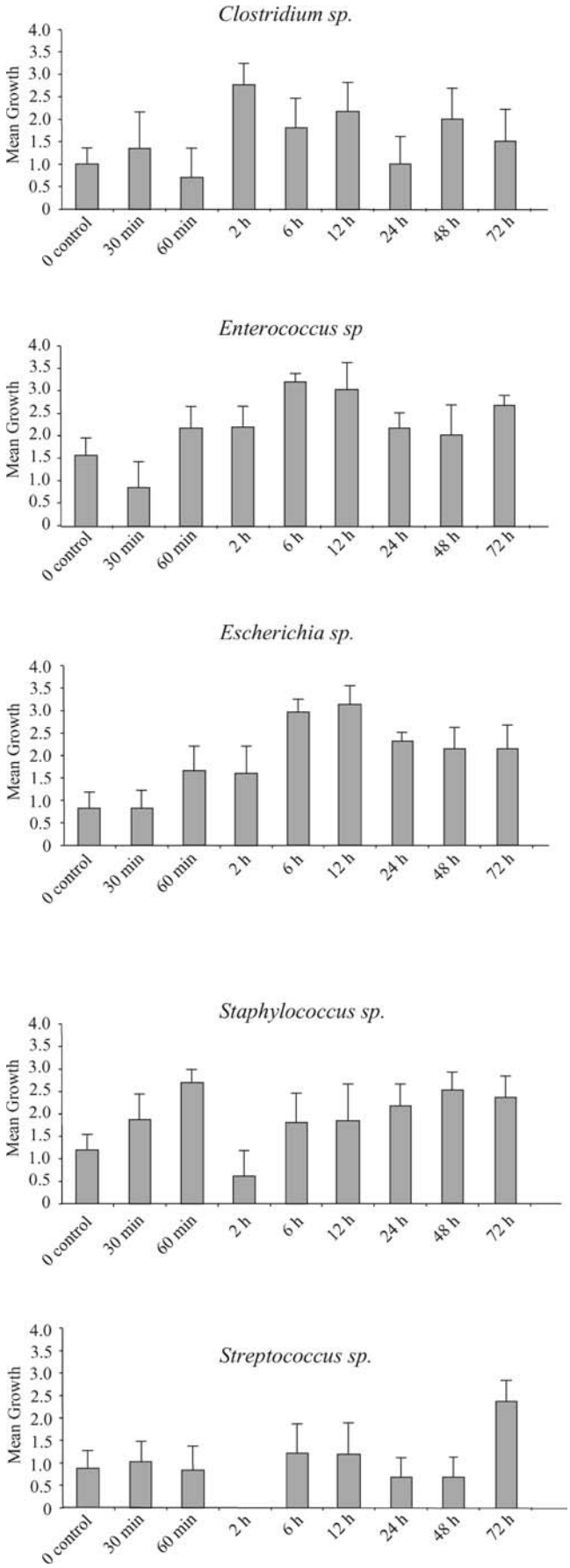


Figure 7. Bacterial changes in the jejunum.

treated rats. Additional data from experiments are available online.

Discussion

Several possible mechanisms have been proposed for the development of irinotecan-induced diarrhea. These range from changes in the architecture and absorption rates (17, 19) of the large intestine, to increases in intestinal β-glucuronidase levels caused by changes in intestinal bacteria



(20). The present study was unique in that it investigated changes in gastrointestinal architecture and correlated these changes with changes in gastrointestinal flora and fecal and serum electrolyte levels. Previous studies have also shown epithelial proliferation to be altered and apoptosis to be increased (8, 12). A key finding of this study was that changes to the gastrointestinal microflora following irinotecan treatment can occur as early as 30 mins after treatment. Previous research has shown that cytotoxic radiotherapy induces p53 (44) within 2 hrs and cytotoxic chemotherapy induces p53 within 40 mins (45). It is therefore not surprising that chemotherapy also alters gastrointestinal microflora rapidly and may be having a direct effect on the bacteria.

The present study showed that changes occur in the composition of the gastrointestinal microflora after treatment with irinotecan. Changes differed between regions of the GIT, with some of the larger changes occurring in bacteria of the colon. This is suggestive of some relationship between these changes and the incidence of diarrhea. It is likely that the changes in bacteria may be a result of the altered ecology of the luminal environment throughout the entire GIT and severe damage to the gastrointestinal architecture. Irinotecan may alter pH levels in the stomach, allowing certain types of bacteria to proliferate or inhibit the growth of others, depending on the bacterial tolerance of acidic conditions (23). Oxygen levels in the lumen of the GIT may also be altered, which may promote or inhibit different bacteria (25). However, there is no evidence at this point in time to indicate which of these events actually occurs first or evidence to confirm which are a direct and/or indirect result of irinotecan.

Bacteria lining the stomach mucosal surface were shown to change over time after chemotherapy, the key changes being an increase in *Enterococcus* spp at 2–6 hrs, an increase in *Peptostreptococcus* spp at 30–60 mins, and an increase in *Serratia* spp at 2 hrs. The microflora bound to the mucosa of the stomach (and protected from the acidic environment) are likely to be increased because of the architecture (and protective functions) of the mucosa being compromised by irinotecan at these time points. Mucosal-bound microflora of the jejunum are likely to be increased because of the decreased motility, architectural damage, and greater overflow of bacteria from the stomach. This is demonstrated by an increase in *Enterococcus* spp, *Lactobacillus* spp, *Clostridium* spp, and *Serratia* spp at 2 hrs (similar to the stomach). These findings correlate with the severe architectural changes observed in the jejunum, especially at 2–12 hrs. Previous studies (also using irinotecan in DA rats) have shown a peak in apoptosis in the jejunum at 6 hrs, as well as villus and crypt hypoplasia in the jejunum at 24 hrs (8, 12).

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Figure 8. Bacterial changes in the colon.

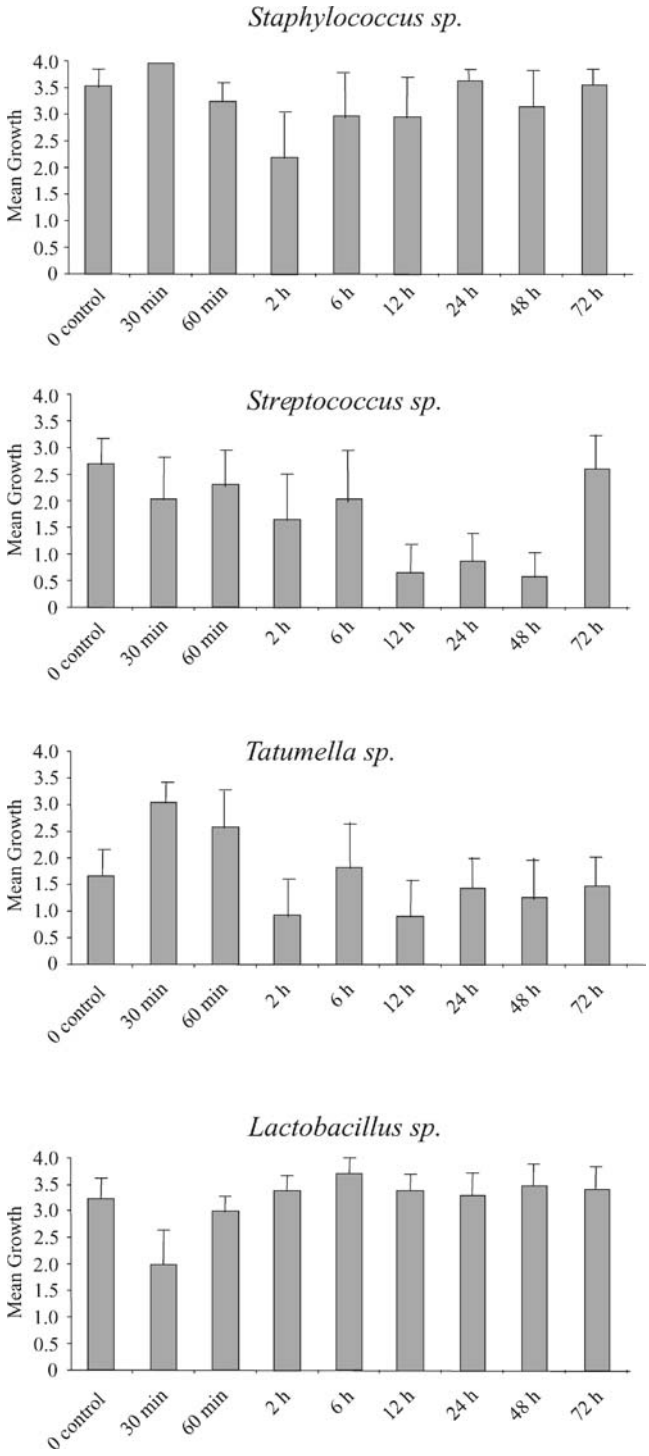

Figure 9. Bacterial changes in the feces.

This study has also demonstrated that a number of the microflora of the colon increase following treatment with irinotecan. Extensive histological changes were observed in the colon at 6 hrs (also time of onset of cholinergic diarrhoea), and from 48–72 hrs (time of onset for diarrhea). This coincides with changes seen in previous studies (8, 12), where irinotecan in DA rats causes a peak in apoptosis in the colon at 6 hrs. The luminal environment in the colon is usually of a reduced nature, allowing the domination of anaerobes (46). An increase in oxygen levels allows the proliferation of aerobic bacteria, in particular those producing β-glucuronidase, including *Escherichia* spp, *Staphylococcus* spp, *Streptococcus* spp, and *Clostridium* spp (20). Lactic acid bacteria such as *Lactobacillus* spp have been suggested to inhibit β-glucuronidase activity (47). *Lactobacillus* spp also increased but only slightly. The changed luminal environment of the GIT results in altered absorption and other functions (48). Changes to absorption are likely to alter the composition of the fecal excrement, including the composition of microflora present. Not all of the fecal microflora producing β-glucuronidase increase after chemotherapy. The information from this study adds to other studies, such as one by Takasuna and colleagues. They suggested the importance of the intestinal microflora in chemotherapy-induced mucositis and characteristic late onset diarrhea of irinotecan, looking at β-glucuronidase activity during the time of diarrhea (20). Our study looks at earlier time points, adding more information to what is known to date.

Changes to the luminal environment, and subsequent changes to absorption, are the likely cause of altered electrolyte levels after chemotherapy treatment. Fecal sodium levels change significantly after irinotecan treatment, as do fecal potassium levels, with both peaking at 24 hrs, when diarrhea incidence is also at its highest level. Water follows the electrolytes into the lumen in an attempt to restore balance, resulting in diarrhea. Serum sodium, chloride, and osmolarity levels are significantly altered. Treated rats have lower sodium levels than control rats for the duration of the experiment; osmolarity levels are also lower, reaching the lowest level at 24 hrs, coinciding with the diarrhea incidence, and chloride levels are significantly lower, reaching the lowest level at 12 hrs, when diarrhea incidence is also high.

In conclusion, irinotecan treatment causes changes in the flora of the stomach, jejunum, colon, and feces of rats, which coincides with the development of diarrhea. These changes in flora may have systemic effects, and in particular may contribute to the development of chemotherapy-induced mucositis. The luminal environment is also altered by irinotecan and as a result may favor different genera of bacteria, allowing them to proliferate. The bacteria producing β-glucuronidase are among those that increase, resulting in SN-38G being converted back to SN-38 at an increased rate, causing significant damage and causing diarrhea. Absorptive function in the intestines is decreased, increasing fecal sodium and electrolyte levels, which may contribute to the irinotecan-induced diarrhea. Further studies are being undertaken to quantify the changes in gastrointestinal microflora and to investigate other consequences of a changed luminal environment.

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