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Bile Acid Metabolism, Bacterial Bowel Flora and Intestinal Function Following Ileal Pouch-Anal Anastomosis in Dogs, with Reference to the Influence of Administration of Ursodeoxycholic Acid

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Imamura, M., Nakajima, H., Takahashi, H., Yamauchi, H. and Seo, G. *Bile Acid Metabolism, Bacterial Bowel Flora and Intestinal Function Following Ileal Pouch-Anal Anastomosis in Dogs, with Reference to the Influence of Administration of Ursodeoxycholic Acid.* Tohoku J. Exp. Med., 2000, **190** (2), 103-117 —— The pathophysiology following a total colectomy with ileal pouch-anal anastomosis (IPAA) has not been sufficiently clarified yet. We investigated bile acid metabolism, bacterial bowel flora and transit of the alimentary tract after IPAA, with reference to administration of ursodeoxycholic acid (UDCA) in dogs undergoing IPAA. Ten adult beagle dogs underwent IPAA at one stage, and were observed for 12 months. UDCA (100 mg/day) was administered orally to five dogs, and the other five did not. In the UDCA(+) group, UDCA replaced other bile acids, especially cholic acid, accounting for 16.5% of gallbladder bile at 12 months after surgery. Both plasma levels and postprandial increase of total bile acids remained unchanged in the UDCA(+) group, but decreased in the UDCA(−) group at 12 months. Fecal excretion of bile acids tended to be smaller in the UDCA(+) group, and the ratio of secondary to primary bile acids was larger in the UDCA(−) group. Almost all the bile acids were in free form in stool, and UDCA constituted 19% in the UDCA(+) group. The transit time of the whole alimentary tract was elongated by administering UDCA, especially at an early period after IPAA. Although both anaerobic and aerobic bacteria decreased after IPAA, the latter decreased more in stool, resulting in an increase in the ratio of total anaerobes/total aerobes, especially in the UDCA(−) group. The decrease in *Bacteroidaceae* and *Lactobacillus* after IPAA was slightly smaller in the UDCA(+) group. Administration of UDCA following IPAA was efficient to induce rapid intestinal adaptation and also to keep the bile acid fraction in the ileal pouch less harmful. ——— IPAA; bile acid metabolism; bacterial bowel flora; intestinal function; UDCA

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In patients with ulcerative colitis undergoing total colectomy and rectal mucosectomy with ileal pouch-anal anastomosis (IPAA), loose frequent stooling is a serious problem. It has been reported that intestinal adaptation, such as colonic transformation of the ileal pouch mucosa and changes in the intestinal bacterial flora, take place with time after surgery (Philipson et al. 1975; Nicholls et al. 1981; Kojima et al. 1982), resulting in improvements in bowel habit and nutritional state. It is known that there are functional inhibition mechanisms in the lower intestine upon the secretion and movement of the upper intestine; that is, the ileal brake (Spiller et al. 1984, 1988) and the colonic brake (Nightingale et al. 1996). It seems that the ileal brake mechanism will play an important role in the absence of the colonic brake, and indeed it has been reported that the ileal brake mechanism remains functional after IPAA (Soper et al. 1990).

After total colectomy, the intestinal bacterial flora changes; that is, both the ratio of anaerobes to aerobes and the total number of bacteria decrease, although they gradually increase with time (Iwagaki et al. 1992). The postoperative changes in microbial flora seem to have a close relation with both the dehydroxylation and deconjugation of bile acids. In normal conditions, conjugated bile acids are actively absorbed in the ileal end, entering the enterohepatic circulation. It is therefore speculated that this mechanism might be disturbed by the morphological and/or functional changes in the mucosa of ileal end, which consists of reservoir in IPAA.

Ursodeoxycholic acid (UDCA) given orally is absorbed efficiently throughout the intestine (Imamura and Yamauchi 1992). Accordingly, it is expected that oral administration of UDCA will compensate the loss of bile acids into the stool, and may affect bile acid metabolism after IPAA.

In the present study, we investigated the effects of oral administration of UDCA on bile acid metabolism, intestinal bacterial flora and intestinal transit time in dogs undergoing IPAA from the viewpoint of intestinal adaptation.

Materials and Methods

Ten adult beagle dogs of both sexes, 12–15 months of age and 7.5–12.0 kg of body weight, were used for the study. Under general anesthesia with pentobarbital sodium (Nembutal®, 25 mg/kg, i.v.), they underwent a total colectomy, mucosal proctectomy with a J-shaped ileal pouch-anal anastomosis at one stage. The procedures of IPAA mimicked those performed on human patients. In brief, the rectal mucosa was first dissected from the dentate line proximally for 6 cm. Then, laparotomy was performed through a midline incision. The ileum was transected at its end, followed by a total colectomy. The rectal mucosa was pulled into the pelvic cavity and removed together with the colon. A J-shaped pouch was constructed using linear stapling devices by doubling the last 24 cm of the ileum on itself. Finally, the ileal pouch was pulled down through the rectal muscle cuff, and anchored to the cuff. After opening the apex of the pouch, an

ileal pouch-anal anastomosis was performed on the dentate line with interrupted sutures of absorbable threads.

After surgery, dogs were randomly divided into two groups; five dogs received UDCA, which was generously supplied by Tokyo Tanabe Pharmaceuticals Co. (Tokyo), (100 mg/day, p.o.) every day for one year; the remaining five did not and served as controls. Daily, each dog consumed 320 g (360 kcal/100 g) of a commercial dog food (ED-1, Sanwa Chemical Lab., Nagoya) consisting mainly of protein (23%), carbohydrate (9%) and fat (5%). The following experiments were performed under appropriate circumstances.

Before, and 2, 6 and 12 months after surgery, a test-meal loading study was performed, following a 24-hour fast except for free access to water, to determine plasma total bile acid levels. During the experiment, dogs were free to move, but did not take either water or food. The test meal (VITA-ONE®, Nippon Pet Food Co., Tokyo) consisted mainly of 42% of protein and 26% of fat. Each dog consumed the meal (20 g/kg body weight) in a few minutes. Blood samples were taken from a foreleg vein, and drawn into heparinized glass tubes at fasting, and 15, 30, 60, 90, 120, 150 and 180 minutes after feeding. Immediately after the experiment, blood samples were centrifuged at 2800 rpm for 15 minutes at 4°C to obtain the plasma. Total bile acid concentrations were measured by an enzymatic method using a bile acid reagent kit (Kyokuto Pharmaceutical Ltd., Tokyo) using the plasma and stool.

Before and 12 months after surgery, analysis of bile acid fractions in the gallbladder bile and stool was performed by high performance liquid chromatography (HPLC) with a detection limit of 0.02 μg/ml. Gallbladder bile was obtained by puncture immediately after laparotomy at each time point. Total fecal excretion of bile acids was also measured at these periods.

Before, and 1, 3, 6 and 12 months after surgery, fecal bacterial flora was examined according to the method previously described by Mitsuoka (1980). Fresh stool was placed immediately into a sterile Petri dish and kept frozen in an anaerobic gas-pack. The procedure was started within 6 hours after collection of fresh stool. Cultivation of both aerobic and anaerobic bacteria was performed at 37°C using the media described in Fig. 1.

At 6 weeks, 6 months and 12 months after surgery, transit time of the alimentary tract (from mouth to anus) was evaluated: that is, the test meal (200 g) mixed with 100 ml of barium sulfate was given to each dog after a 24-hour fast, and the time when barium-containing stool first appeared was measured. This study was performed twice on each dog on different days, and the mean value was used for analysis.

The study protocol was approved by the Research and Ethics Committee, Sendai National Hospital.

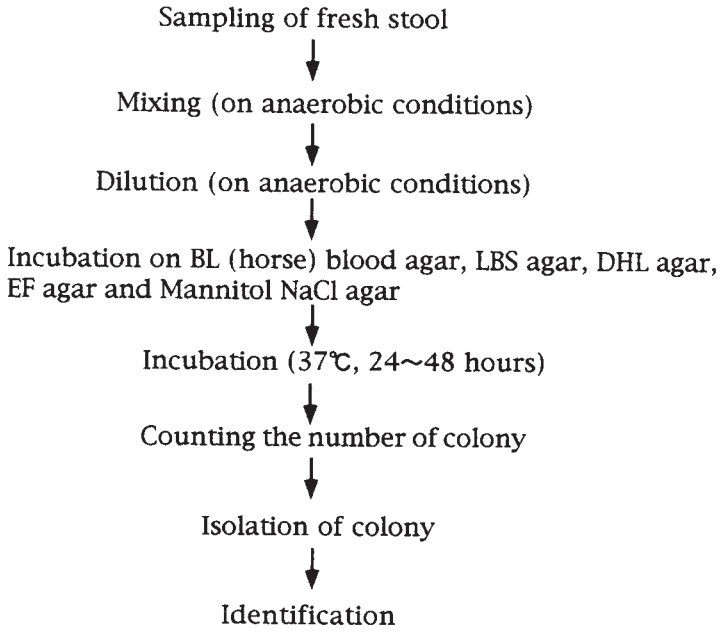

Fig. 1. Examination procedure of intestinal microflora. BL (blood liver) medium, agar for anaerobic bacteria; LBS (Lactobacillus Selective), agar for lactobacilli; DHL (Desoxycholate-Hydrogensulfide-Lactose) medium, agar for enterobacteriaceae; EF (Enterococcus faecalis) medium, agar for enterococci.

Statistical analysis

Data are expressed as mean±standard error of the mean (S.E.M.) or standard deviation (S.D.). The integrated incremental response of total bile acid to the test meal was calculated by the method of Taylor et al. (1978). The statistical difference was assessed by analysis of variance (ANOVA) followed by Fisher's test among three groups, and by Student's t-test between two groups. A p-value of less than 0.05 was considered statistically significant.

Results

Plasma total bile acid levels in response to the test meal

Before surgery, total bile acid concentrations increased promptly after feeding, and reached a plateau at 60 minutes. After surgery, they increased similarly, but at both 2 and 6 months tended to be higher in the UDCA(+) group and lower in the UDCA(−) group than the preoperative levels. At 12 months, total bile acid concentrations in the UDCA(+) group were similar to those in the preoperative group at each time point, but tended to be lower after feeding in the UDCA(−) group.

The incremental response of total bile acid for 3 hours after feeding was slightly higher in UDCA(+) group than in both the preoperative and UDCA(−) groups at both 2 and 6 months. At 12 months, the value in the UDCA(+) group was similar to that in the preoperative group, and in the UDCA(−) group it was slightly lower, though there were no statistical differences (Fig. 2).

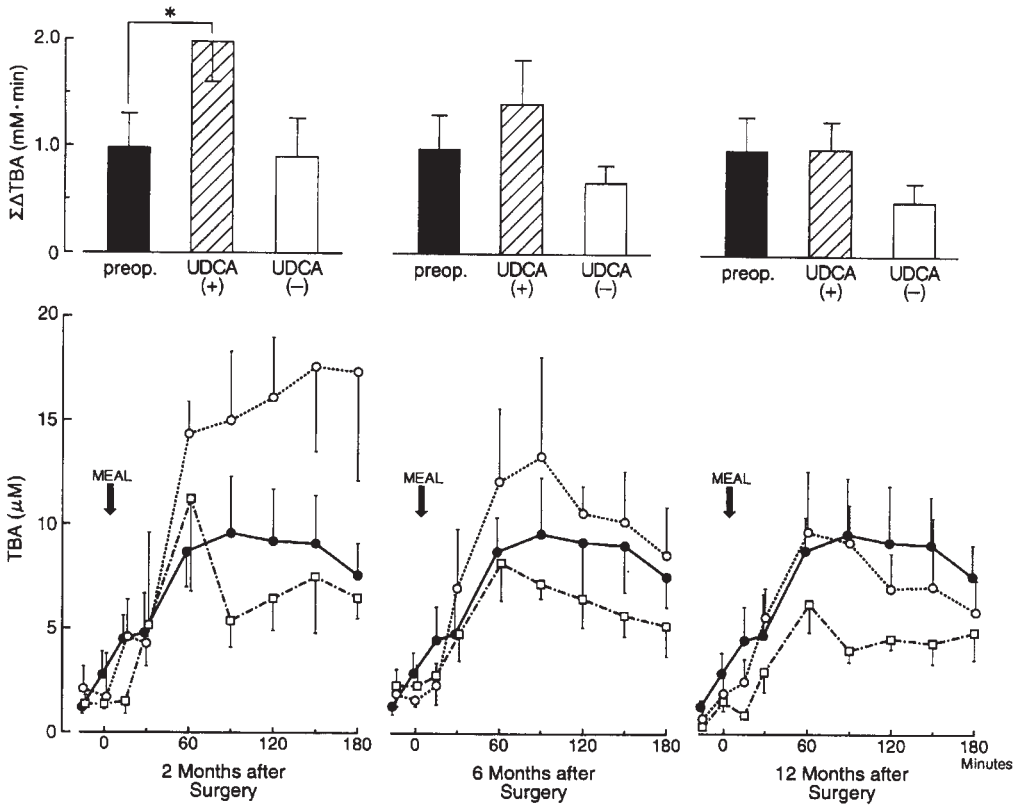

Fig. 2. Plasma total bile acid levels in response to the test meal and postprandial integrated response of total bile acid for 3 hours. Data are expressed in means±S.E.M. $^*p<0.05$.
●——●, preop. ($n=10$); ○·····○, UDCA(+) ($n=5$) and □—·—□, UDCA(−) ($n=5$).

Bile acid composition in bile and stool

In the gallbladder bile of all the three groups, over 98% of bile acids were taurine-conjugated. Before surgery, 69.0±1.0% (mean±S.E.M.) of these were cholic acid (CA), followed by deoxycholic acid (DCA) (21.7±0.9%) and chenodeoxycholic acid (CDCA) (8.7±0.2%). At 12 months after surgery, primary bile acids, especially CA, decreased, and secondary bile acids, especially DCA, increased irrespective of the administration of UDCA. In the UDCA(+) group, UDCA amounted to 16.5±1.0% of taurine-conjugated bile acids (Fig. 3).

Daily fecal excretion of total bile acids was significantly greater at 12 months after surgery in both the UDCA(+) and (−) groups than in the preoperative group. It was greater in the UDCA(−) group than in the UDCA(+) group, although there was no significant statistical difference. Stool volume was 202± 11 g/day (Mean±S.E.M.) before surgery, and, after surgery, 502±49 g/day in the UDCA(+) and 515±51 g/day in the UDCA(−) group. Almost all bile acids in stool was in the free form, and DCA comprised the largest volume both before and after surgery. The ratio of secondary bile acids (DCA+lithocholic acid [LCA]) to primary bile acids (CA+CDCA) was 67.5±49.7 (mean±S.E.M.) (in 2 dogs, primary bile acids were not detected) before surgery, and 245.4±116.2 in the

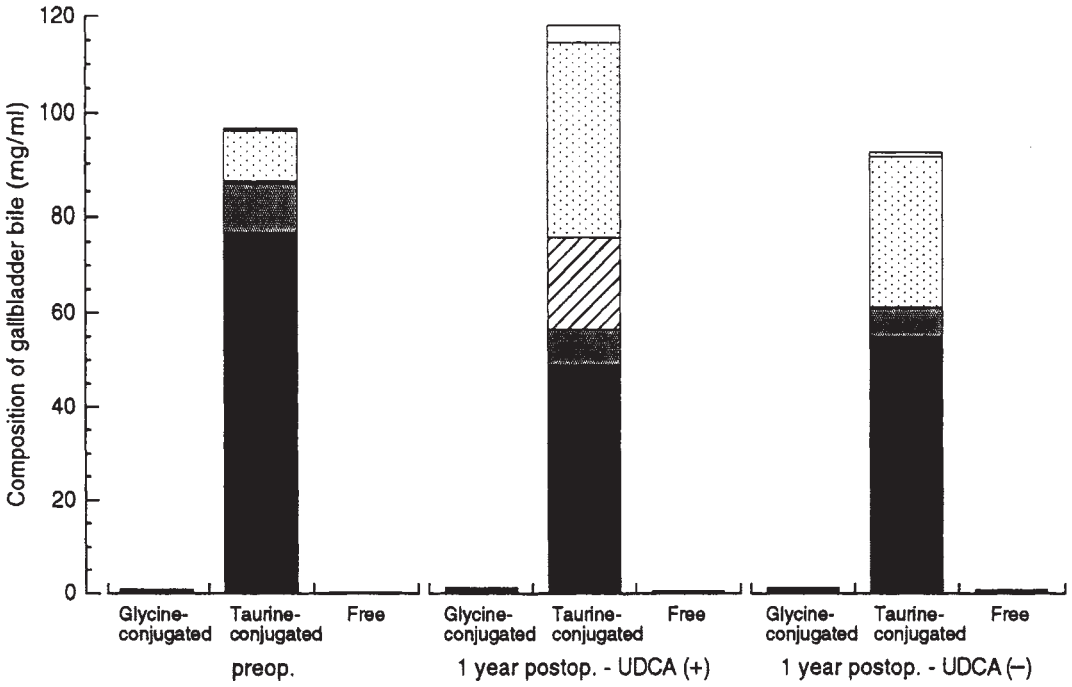

Fig. 3. Composition of bile acids in the gallbladder bile. Each bar shows mean value of preoperative group ($n=10$), and UDCA(+) ($n=5$) and UDCA(−) ($n=5$) groups at 12 months after surgery. ■, CA; ■, CDCA; ▨, UDCA; ⬚, DCA; □, LCA.

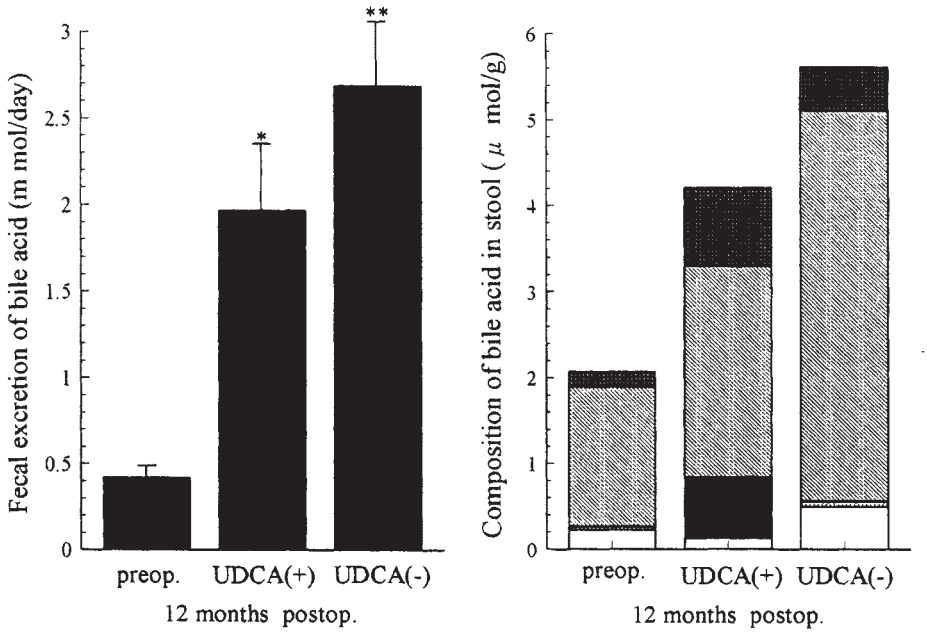

Fig. 4. Bile acid excretion and its composition in stool before (n=6) and at 12 months after surgery ($n=5$ in each group). Fecal excretion is expressed in means±S.E.M. $^*p<0.05$, $^{**}p<0.01$ (compared to preoperative value). Composition of bile acid is shown in mean value. □, CA; ▥, CDCA; ■, UDCA; ▧, DCA; ■, LCA.

UDCA (+) and 129.4±68.6 in the UDCA(−) group after surgery (the difference was not significant among the three groups). In the UDCA(+) group, UDCA constituted 18.9±6.1% of free bile acids (Fig. 4).

Fecal bacterial flora

The fecal bacterial flora was examined in 5 dogs before surgery; in 7 at 1, 3 and 6 months; and in 10 at 12 months after surgery. The numbers of both anaerobes and aerobes decreased after surgery irrespective of the administration of UDCA, with the decrease in total aerobes tending to be larger in the UDCA(−) group, resulting in a greater ratio of total anaerobes to aerobes at 1 and 3 months. At 12 months, however, the numbers of both anaerobes and aerobes as well as the ratio of total anaerobes to aerobes approached those of preoperative values (Table 1, Fig. 5). Before surgery, *Bacteroidaceae* and *Clostridium* were the most dominant flora, but during early period after surgery (at 1 and 3 months) *Bacteroidaceae* and *Eubacterium* were the most dominant. After 6 months, *Bacteroidaceae* and *Clostridium* became dominant, as before surgery. Throughout the postoperative period, the numbers of *Bifidobacterium*, *Clostridium*, *Escherichia coli* and *Staphylococcus* were smaller, and that of *Enterococcus* was larger, than in the preoperative period. Comparing the two groups for changes in each bacterium, the decrease of *Bacteroidaceae* and *Lactobacillus* was smaller in the UDCA(+) group, especially in the early period after surgery (Table 2).

Transit time of the alimentary tract (from mouth to anus)

The transit time of the whole alimentary tract was longer in the UDCA(+) group than in the UDCA(−) group at all three time points, with a significant difference at 6 weeks after surgery. In both groups, the transit time lengthened with time elapsed after surgery (Table 3).

Discussion

Soper et al. (1990) reported that the "ileal brake" still worked after IPAA; that is, infusion of oleic acid into the ileal pouch slowed gastric emptying and small intestinal transit. On the other hand, both morphological and functional changes ("colonization") (LeVeen et al. 1962) occur in the terminal portion of ileum (Philipson et al. 1975; Nicholls et al. 1981; de Silva et al. 1991; Imamura et al. 1999) which constitutes the pouch. Conjugated bile acids are absorbed in the terminal ileum by an active transport mechanism, while unconjugated bile acids are well absorbed throughout the intestine by passive transport (Dietschy 1968; Fujii et al. 1988). We have already shown that UDCA given orally is efficiently absorbed throughout the intestine in dogs undergoing massive small bowel resection(Imamura and Yamauchi 1992). In the present study, postprandial plasma levels as well as the integrated increase of total bile acids in the UDCA(+) group were kept similar to those in the preoperative group at 12 months after surgery, while those in the UDCA(−) group were reduced. It is therefore considered that the bile acid pool decreases after IPAA, and that administration of UDCA compensates to some extent for loss of bile acid pool. In

TABLE 1. *Fecal bacterial flora before and after surgery*[a]

	Preop. ($n=5$)	1 month ($n=7$)	3 months ($n=7$)	6 months ($n=7$)	12 months (Postop.) ($n=10$)
Anaerobes/Aerobes	6.4±3.5	65.2±131.1	43.5±60.3	11.2±13.0	8.9±4.3
Total Anaerobes	10.5±0.3 (100)	10.3±0.2 (100)	10.2±0.4 (100)	9.9±0.8 (100)	10.2±0.3[b] (100)
Total Aerobes	9.5±0.8 (100)	9.1±0.6 (100)	9.6±0.8 (100)	9.0±0.5 (100)	9.3±0.4 (100)
Bacteroidaceae	10.0±0.3 (100)	10.0±0.3 (100)	10.0±0.5 (100)	9.8±0.9 (100)	10.0±0.4 (100)
Bifidobacterium	4.9±3.9 (40)	8.8±0.1 (60)	8.6±0.3 (90)	8.2±0.7 (100)	8.3±0.4 (70)
Lactobacillus	8.8±1.2 (100)	9.1±0.6 (100)	9.2±0.8 (100)	8.7±0.6 (100)	9.3±0.4 (100)
Clostridium	9.9±0.7 (100)	9.1±0.9 (100)	9.1±0.6 (100)	9.3±1.0 (100)	9.3±0.6 (100)
Eubacterium	7.7±3.2 (80)	9.5±0.4 (100)	9.4±0.4 (90)	8.4±0.5 (100)	9.7±0.2 (40)
E. coli	7.9±0.6 (100)	6.6±0.8 (100)	7.5±1.1 (100)	6.4±0.6 (100)	5.8±1.1 (90)**
Proteus mirabilis	7.9±0.6 (100)	6.2±2.5 (60)	4.0±2.0 (30)	4.0±0.1 (30)	4.8±0.6 (80)**
Acinetobacter iwoffi	4.1±2.9 (40)	4.9 (15)	ND (0)	ND (0)	6.0±0.3 (30)
Enterococcus	2.9±1.3 (100)	7.7±0.8 (60)	9.6±1.1 (60)	8.5±0.6 (100)	8.2±0.6 (70)*
Staphylococcus	8.3±1.7 (100)	7.2±0.9 (100)	8.0±1.0 (100)	8.0±0.9 (100)	6.7±0.8 (20)**

[a]Total colectomy and mucosal proctectomy with ileal pouch-anal anastomosis.
[b]Log_{10} (colony-forming units [cfu])/g feces.
Each value represents mean±S.D. of both UDCA (+) and UDCA (−) groups.
* $p<0.05$, ** $p<0.01$ (compared to preop. value). ( ), Detection rate (%)
ND, not detected.

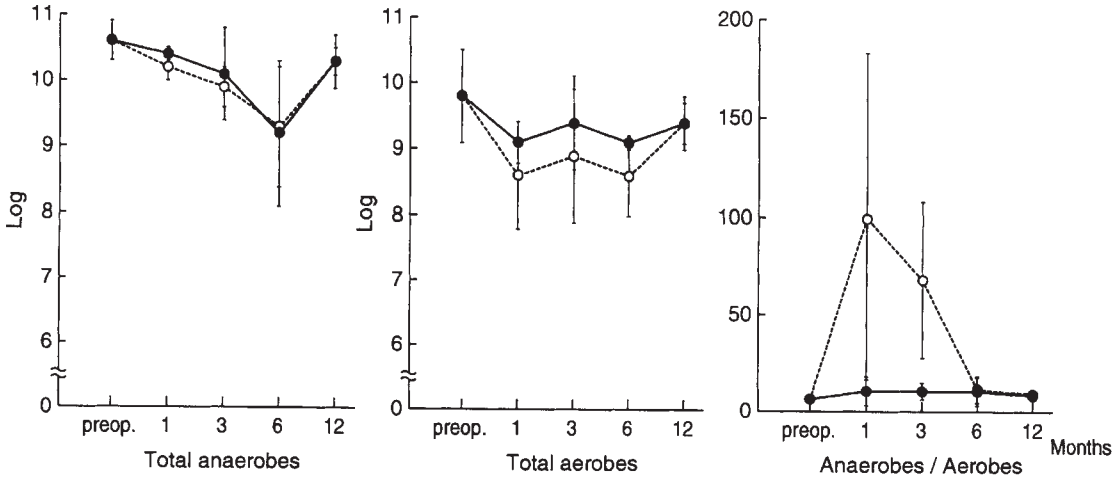

Fig. 5. Total anaerobes and aerobes (means±S.D.), and the ratio of total anaerobes to aerobes (means±S.E.M.). ●—●, UDCA(+) group ($n=4$ at 1, 3 and 6 months, and 5 at 12 months), ○···○, UDCA(−) group ($n=3$ at 1, 3 and 6 months, and 5 at 12 months). Before surgery, $n=5$.

fact, fecal excretion of bile acids tended to be smaller in the UDCA(+) group at 12 months after IPAA. The content of secondary bile acids (DCA+lithocholic acid [LCA]) in both gallbladder bile and stool increased following IPAA, irrespective of administration of UDCA. In addition, most of bile acids in stool were in the free form at 12 months after IPAA. Regarding these points, it is speculated that both dehydroxylation of primary bile acids and deconjugation of bile acids in the small intestine were accelerated after IPAA, probably due in part to changes in the bacterial flora. Furthermore, the mean ratio of conjugated bile acids to total bile acids in stool was larger after surgery (UDCA[+], 0.012; UDCA[−], 0.038 vs. preop., 0.006), indicating that the active transport of conjugated bile acids in the ileal pouch might be disturbed, possibly through morphological and/or functional changes toward the colonic mucosa.

As shown in Fig. 4, the content of DCA in stool was much greater in the UDCA(−) than in the UDCA(+) and preoperative groups. In the UDCA(+) group, UDCA accounted for 19% of fecal bile acids. It is known that secondary bile acids such as DCA damage the gastric mucosa (Harmon et al. 1978; Watanabe et al. 1998), causing reflux gastritis (Gadacz and Zuidema 1978; Stefaniwsky et al. 1985) and also have a close relation with the development of colon cancer (Summerton et al. 1985; Martinrz et al. 1998). It has also been reported that the administration of cholestyramine, which causes a marked increase in the amount of bile salts, enhances the carcinogenic effect in the large intestine (Nigro et al. 1973). In the present study, fecal excretion of bile acids (mostly secondary bile acids) increased prominently after IPAA. Therefore, it is conceivable that the presence of a large amount of secondary bile acids in the ileal pouch could induce neoplastic changes in the pouch mucosa. On the other hand, UDCA has a cytoprotective action on the gastric mucosa (Scarpa et al. 1991) and the liver (Kitani et al. 1985). Therefore, it is speculated that the mucosa of the ileal pouch

TABLE 2. *Fecal bacterial flora before and after surgery*[a] *in each group*

UDCA (−) Group	Preop.[b] ($n=5$)	1 month ($n=3$)	3 months ($n=3$)	6 months ($n=3$)	12 months (Postop.) ($n=5$)
Anaerobes/Aerobes	6.4±3.5	100.2±144.3	68.3±69.8	11.6±11.7	9.3±4.6
Total Anaerobes	10.6±0.3 (100)	10.2±0.2 (100)	9.9±0.3 (100)	9.3±0.9 (100)	10.3±0.2[c] (100)
Total Aerobes	9.8±0.7 (100)	8.6±0.8 (100)	8.9±1.0 (100)	8.6±0.6 (100)	9.4±0.3 (100)
Bacteroidaceae	10.0±0.2 (100)	9.7±0.4 (100)	9.6±0.3 (100)	9.1±1.0 (100)	10.1±0.2 (100)
Bifidobacterium	9.4±0.5 (40)	8.8±0.2 (75)	8.4±0.4 (100)	8.1±0.6 (100)	8.3±0.3 (75)
Lactobacillus	9.3±1.1 (100)	8.5±0.8 (100)	8.5±0.9 (100)	8.3±0.8 (100)	9.4±0.3 (100)
Clostridium	10.2±0.6 (100)	8.6±1.5 (75)	9.0±0.5 (100)	8.6±1.0 (100)	9.5±0.6 (100)
Eubacterium	9.5±0.5 (80)	9.2±0.5 (100)	9.1±0.3 (100)	8.2±0.6 (100)	9.6±0.1 (40)
E. coli	8.3±0.5 (100)	5.7±0.7 (100)	6.6±1.5 (100)	6.1±0.7 (100)	7.1±1.3 (80)
Proteus mirabilis	ND (0)	3.8±3.1 (75)	1.1±2.2 (25)	4.0±0.2 (50)	5.1±0.5 (80)
Acinetobacter iwoffi	ND (0)	1.4±2.9 (25)	ND (0)	ND (0)	6.2 (20)
Enterococcus	4.4±1.2 (100)	7.0±1.0 (75)	8.6±1.4 (50)	7.8±0.6 (100)	7.2 (20)
Staphylococcus	9.7±1.5 (100)	6.4±1.2 (100)	7.6±0.9 (75)	6.8±0.9 (100)	8.2±0.2 (60)

TABLE 2. *Continued*

UDCA (+) Group	Preop.[b] ($n=5$)	1 month ($n=4$)	3 months ($n=4$)	6 months ($n=4$)	12 months (Postop.) ($n=5$)
Anaerobes/Aerobes	6.4±3.5	10.5±14.5	10.5±7.7	10.5±14.5	8.5±4.6
Total Anaerobes	10.6±0.3 (100)	10.4±0.1 (100)	10.1±0.7 (100)	9.2±1.1 (100)	10.3±0.4[c] (100)
Total Aerobes	9.8±0.7 (100)	9.1±0.3 (100)	9.4±0.7 (100)	9.1±0.1 (100)	9.4±0.4 (100)
Bacteroidaceae	10.0±0.2 (100)	10.0±0.3 (100)	9.9±0.7 (100)	9.0±1.2 (100)	10.1±0.5 (100)
Bifidobacterium	9.4±0.5 (40)	8.6 (33)	8.7±0.1 (67)	7.3±0.7 (100)	8.6±0.5 (60)
Lactobacillus	9.3±1.1 (100)	9.1±0.3 (100)	9.0±0.6 (100)	8.6±0.4 (100)	9.4±0.3 (100)
Clostridium	10.2±0.6 (100)	8.4±0.4 (67)	8.5±0.7 (100)	8.3±1.4 (100)	9.6±0.6 (100)
Eubacterium	9.5±0.5 (80)	9.5±0.4 (100)	9.2±0.8 (67)	7.9±0.3 (100)	9.8±0.2 (40)
E. coli	8.3±0.5 (100)	6.5±0.8 (100)	7.1±0.8 (100)	5.9±0.7 (100)	6.3±0.8 (100)
Proteus mirabilis	ND (0)	2.9±2.5 (67)	1.5±2.7 (33)	ND (0)	4.9±0.5 (80)
Acinetobacter iwoffi	ND (0)	ND (0)	ND (0)	ND (0)	5.8±0.2 (40)
Enterococcus	4.4±1.2 (100)	7.6 (33)	9.9 (33)	8.7±0.5 (100)	6.1 (20)
Staphylococcus	9.7±1.5 (100)	7.0±0.5 (100)	6.7±1.1 (100)	7.8±0.8 (100)	8.5±0.7 (80)

[a]Total colectomy and mucosal proctectomy with ileal pouch-anal anastomosis.
[b]Data of preop. are same in both groups.
[c]Log_{10} (colony-forming units [cfu])/g feces.
Each value represents mean±S.D. ( ), Detection rate (%); ND, not detected.

TABLE 3. *Transit time of the alimentary tract (Mouth-Anus)*

Dog group	Time after surgery					
	6 weeks		6 months		12 months	
						(minutes)
UDCA (+) ($n=5$)	108±15	*	118±13	N.S.	126±16	N.S.
UDCA (−) ($n=5$)	79±14		103±23		103±23	

Each value represents mean±S.D. $^*p<0.05$. N.S., not significant.

may be protected from inflammation by UDCA-enriched bile acids. After IPAA, there sometimes happens a serious complication: pouchitis, where the atrophied mucosa of the ileal pouch shows colonic metaplasia (Veress et al. 1995; Stahlberg et al. 1996). Neoplastic transformation of the pouch mucosa sometimes occurs as a result of severe pouchitis (Lofberg et al. 1991; Gullberg et al. 1997). Based upon these reports and the results in the present study, it is therefore probable that administration of UDCA after IPAA will be efficient in terms of not only supplementation of the bile acid pool but also protection of the ileal pouch mucosa from inflammation and even from development of cancer.

In a previous study using dogs undergoing massive small bowel resection, we reported that UDCA given orally slowed the transit of the whole alimentary tract (Imamura and Yamauchi 1992). The present study also showed that UDCA slows transit from the mouth to the ileal pouch, especially in the early period after IPAA. This means that administration of UDCA after IPAA will facilitate the digestion and absorption of intraluminal nutrients by lengthening the contact time between the intestinal contents and the intestinal mucosa. Regarding the mechanism how UDCA slows the intestinal transit, contribution of peptide YY (PYY), which is released from the distal intestine in response to feeding and slows gastrointestinal transit (Wiley et al. 1991; Tohno et al. 1995), was speculated. In our another study (Imamura et al. 1999), it was shown that postprandial levels of plasma PYY as well as the number of PYY-containing cells in the ileal pouch mucosa increased with time after surgery, though they decreased markedly immediately after surgery. It is not yet clarified if UDCA would stimulate the increase of PYY-containing cells in the intestine and also of postprandial plasma PYY levels.

The bacterial flora of fresh stool, which is assumed to reflect that of the ileal pouch, was analysed at several periods after IPAA. Although it was expected that anaerobic bacteria would decrease and aerobes would increase at an early period after IPAA, like fecal bacterial flora following an end-ileostomy (Nasmyth et al. 1989), anaerobes were dominant at as early as one month after surgery. In human studies, Nasmyth et al. (1989) reported that anaerobes were dominant and

the median ratio of anaerobes to aerobes was 100 in stool of patients undergoing ileal-pouch surgery at around 6 months after closure of the diverting ileostomy. O'Connell et al. (1986) reported that fecal bacterial flora from the ileal pouch showed overgrowth of both aerobic and anaerobic bacteria in all patients, compared with values for ileal chyme in healthy controls. These results mean that the bacterial flora of the ileal pouch becomes anaerobes-dominant, like that of the colon, immediately after IPAA. In other words, bacterial colonization seems to occur quickly in the ileal pouch after total colectomy with construction of a pelvic ileal pouch. In the present study, the total number of both anaerobes and aerobes tended to decrease throughout postoperative observation period, and several bacteria such as *Bifidobacterium*, *Clostridium* and *Escherichia coli* remained in reduced numbers even at 12 months after IPAA. We therefore speculate that complete change of the bacterial flora of the ileal pouch to that of the colon will not happen, and that this may be partly related to the occurrence of loose stool after surgery. Of course, the loose consistency of stool has a close relationship with the rapid transit of the alimentary tract and also the limited capacity for water absorption in the "colonized" ileal pouch.

It has been reported that gastric hypersecretion occurs following a total colectomy (Yoshizumi et al. 1996). Accordingly, it is considered that the decrease of intraluminal pH affected the bacterial flora and decreased the number of bacteria in the remaining small intestine including the ileal pouch. In addition, it is well known that the number of bacteria in the intestine decreases when intestinal transit is fast, as in the present study. We therefore suggest that the adaptive capacity of the remaining small intestine is limited in the sence of maintaining the bacterial flora as it was before IPAA, even though the ileal pouch works as a reservoir as early as at one month after its construction. From the results that the decrease of aerobes (such as *Lactobacillus* and *Escherichia coli*) lessened, allowing the ratio of total anaerobes to aerobes to decrease, especially in the early period after IPAA in the group receiving UDCA, it is speculated that administration of UDCA after IPAA appears to be effective in maintaining the intestinal bacterial flora similar to the preoperative state. There may be a relation with the slowing effect of UDCA upon intestinal transit.

In conclusion, the administration of UDCA after IPAA was effective for maintaining postprandial bile acid levels in blood, and in lessening the loss of bile acids in stool as well as in suppressing an increase of harmful secondary bile acids in the ileal pouch. Oral administration of UDCA replaced CA in gallbladder bile. Furthermore, UDCA lengthened the transit time of the whole alimentary tract and suppressed the decrease of aerobic bacteria in stool after IPAA, maintaining the intestinal environment close to the preoperative state.

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