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# Deconjugation of Bile Acids by Lactobacilli in the Mouse Small Bowel

GERALD W. TANNOCK,[1]* ALBERT TANGERMAN,[2] ANNIE VAN SCHAIK,[2] AND MICHELLE A. McCONNELL[1]

*Department of Microbiology, University of Otago, Dunedin, New Zealand,[1] and Division of Gastrointestinal and Liver Diseases, University Hospital Nijmegen, Nijmegen, The Netherlands[2]*

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**Conjugated and unconjugated bile acid concentrations in the small bowel contents and portal serum samples collected from mice with and without lactobacilli (RLFL and RLF, respectively) as gastrointestinal inhabitants were measured. The major portion of bile acids in the small bowel contents of RLFL mice was unconjugated (67.9%) in contrast to that of RLF animals in which a smaller portion of bile acids was unconjugated (23.5%). This study demonstrated that bile salt hydrolase produced by lactobacilli is active under the conditions prevailing in the proximal small bowels of mice.**

Lactobacilli are numerous in the gastrointestinal tracts of mice, rats, pigs, and chickens, in which at least some lactobacillus strains colonize the surfaces of stratified squamous epithelia lining the proximal regions of the tracts. A layer of lactobacillus cells is present on the epithelial surfaces of the forestomachs of mice and rats, the pars esophageas of pigs, and the crops of fowl (4, 5, 10, 12). Lactobacillus cells shed from these layers inoculate digesta so that lactobacilli are present throughout the gastrointestinal tracts of these animal species (12, 13).

Gastrointestinal strains of lactobacilli have been demonstrated to influence the biochemistry of intestinal milieu of mice (7, 8), including the amount of bile salt hydrolase activity (15). Bile salt hydrolase catalyzes cleavage of the amino acid moiety from the steroid nucleus of conjugated bile acids (deconjugation). Conjugated bile acids enter the small bowel in bile and are important in the emulsification, digestion, and absorption of dietary lipid that occurs in the proximal small bowel (duodenum and jejunum). Unconjugated bile acids are much less efficient in these roles (2). Bile salt hydrolase activity in the mouse intestinal tract is largely due to the production of this enzyme by lactobacilli (15). It has been proposed, therefore, that the presence of bile salt hydrolase-producing lactobacilli in proximal regions of the intestinal tract could adversely affect their animal host; bacterial bile salt hydrolase could decrease the concentration of conjugated bile acids below the level necessary for optimal digestion and absorption of lipid. This could result in slower weight gain by animals (growth depression) than what might be achieved in the absence of lactobacilli (3).

Although lactobacilli are responsible for most of the bile salt hydrolase detected throughout the murine intestinal tract (15), it has not previously been demonstrated that the enzyme deconjugates bile acids in vivo, resulting in an increased concentration of unconjugated bile acids in the intestinal tract. To determine whether bile salt hydrolase activity was of in vivo significance, we compared the bile acid concentrations and the proportions of unconjugated and conjugated bile acids in the small bowel contents and portal blood of mice with and without lactobacilli as gastrointestinal inhabitants.

Reconstituted lactobacillus-free (RLF) mice were derived from our colony and maintained in isolators by gnotobiotic methodology as described previously (14). The animals harbor a gastrointestinal microflora functionally equivalent, on the basis of 26 microflora-associated characteristics, to that of conventional mice, but lactobacilli are absent. Reconstituted lactobacillus-free mice intentionally colonized with lactobacillus microflora (RLFL mice) were derived by inoculating RLF mice with cultures of all three *Lactobacillus* strains constituting the lactobacillus microflora of conventional mice in our facility, *L. delbrueckii* 18 and 21 and *L. fermentum* 20, as described previously (15). Mice harboring these three strains of lactobacilli have increased bile salt hydrolase activity in the intestinal tract compared with that of RLF animals (about 86% more activity in small bowel samples) (15). The RLFL animals used in the study were the progeny of lactobacillus-colonized mice and had therefore been in contact with lactobacilli throughout life (characteristic values for RLFL mice [mean $\log_{10}$ lactobacilli per gram of organ] were as follows: forestomach, 8.5; duodenum, 6.5; jejunum, 7.8; ileum, 8.2; cecum, 8.5).

For the collection of intestinal contents, mice (6 weeks old) were anesthetized with carbon dioxide and killed by cervical dislocation. Some animals were anesthetized with ether, and blood was collected from axillary blood vessels (systemic blood) or the portal vein (portal blood). Mice were then killed by cervical dislocation, and intestinal contents were sampled. Duodenal and jejunal contents were pooled and are referred to hereafter as small bowel contents. Serum expressed from clotted blood samples and small bowel contents were stored at −20°C.

The concentrations of bile acids (unconjugated and conjugated) in serum samples and small bowel contents were determined as follows. Serum (50 to 150 μl) was diluted with 20 ml of 0.5 M phosphate buffer (pH 7.0), and bile acids were extracted with SepPak-C18 cartridges (Waters Associates, Milford, Mass.). Conjugated and unconjugated bile acids were separated with the lipophilic anion exchanger Sephadex LH-20 (Lipidex-DEAP; Packard Instruments, Groningen, The Netherlands). The concentrations of bile acids were then measured enzymatically (3α-hydroxysteroid dehydrogenase) by means of spectrofluorometry (11). Small bowel contents (0.1 to 0.3 g) were extracted twice with 3 ml of methanol. Methanol extracts

* Corresponding author. Mailing address: Department of Microbiology, University of Otago, P.O. Box 56, Dunedin, New Zealand. Fax: (3) 479-8540.

TABLE 1. Percentage of unconjugated bile acids in small bowel contents and serum samples of RLFL mice in relation to coprophagy

| Specimen type[a] | Collar to prevent coprophagy | Total bile acids (± SE)[b] | % Unconjugated bile acids (± SE) |
|---|---|---|---|
| Small bowel | − | 20.8 ± 2.0 | 78.7 ± 4.0 |
| | + | 22.7 ± 3.2 | 83.1 ± 2.9 |
| Systemic serum | − | 22.8 ± 0.6 | 53.4 ± 8.3 |
| | + | 21.5 ± 0.9 | 46.7 ± 2.0 |
| Portal serum | − | 364.4 ± 94.5 | 20.3 ± 7.0 |
| | + | 260.6 ± 56.5 | 18.9 ± 4.2 |

[a] Small bowel samples, five mice per group; serum samples, two to four mice per group.
[b] Data are mean millimoles per kilogram (wet weight) of small bowel contents or mean micromoles per liter of serum.

were pooled, dried under vacuum, and resuspended in 20 ml of 0.5 M phosphate buffer (pH 7.0). Bile acids were then extracted and separated into conjugated and unconjugated fractions, and their concentrations were determined as described above for serum samples. Details of these analyses have been described previously (11). Six serum samples and four small bowel samples were also analyzed by gas chromatography as described previously (9). These results were in good agreement with those obtained by the enzymatic method (data not shown).

A preliminary experiment was carried out to ensure that coprophagy did not influence the concentrations of unconjugated bile acids in small bowel contents and blood. Comparisons between mice that had been maintained in groups in clean cages with paper bedding and without wire grates and had worn (4 days) plastic collars that prevented access to the anus and mice without collars were made. Bile acid concentrations in the small bowel contents of both groups of mice were the same, as were the proportions of unconjugated and conjugated bile acids (Table 1). Systemic serum samples contained 10-fold-less bile acids than did portal serum samples but contained a higher concentration of unconjugated bile acids, reflecting the lower extraction efficiency of the liver for unconjugated molecules (1).

Preliminary analysis of data (Student's $t$ test) showed that the total concentrations and proportions of unconjugated and conjugated bile acids did not differ between males and females of the same murine group. Data from males and females were therefore combined in subsequent analyses. RLF and RLFL mice had similar total concentrations of bile acids in small bowel contents and portal serum samples (Table 2), but the proportion of unconjugated bile acids in the small bowel contents of RLFL mice was greater than that of RLF animals (Table 2) ($P < 0.001$). The proportion of unconjugated bile acids in portal serum samples did not differ, however, between the murine groups (Table 2). We expected the difference in levels of unconjugated and conjugated bile acids in small bowel contents to be reflected in the composition of the portal serum samples since, at least in the case of humans, about 90% of bile acids are absorbed from the small bowel and pass via the portal vein to the liver (2, 6). This difference was not observed, possibly because of the degree and rate of passive absorption of unconjugated bile acids from the intestine that occurs in the jejunum compared with the active transport of conjugated bile acids that occurs in the ileum (1).

TABLE 2. Percentage of unconjugated bile acids in small bowel contents and portal serum samples in relation to colonization of the gastrointestinal tract by lactobacilli

| Specimen type | Mouse group ($n$) | Total bile acids (± SE)[a] | % Unconjugated bile acids (± SE) |
|---|---|---|---|
| Small bowel[b] | RLF (29) | 22.8 ± 1.2 | 23.5 ± 3.4 |
| | RLFL (23) | 22.5 ± 2.5 | 67.9 ± 4.6 |
| Portal serum | RLF (10) | 297.3 ± 33.0 | 13.3 ± 1.7 |
| | RLFL (10) | 387.3 ± 76.7 | 15.5 ± 2.6 |

[a] Data are mean millimoles per kilogram (wet weight) of small bowel contents or mean micromoles per liter of serum.
[b] Combined duodenal and jejunal contents.

Our results have demonstrated that the presence of lactobacilli with bile salt hydrolase activity in the gastrointestinal tracts of mice results in elevated concentrations of unconjugated bile acids in small bowel contents relative to those of lactobacillus-free animals. Bile salt hydrolase of lactobacillus origin is therefore active under the conditions prevailing in the mouse proximal small bowel. Whether this activity influences the digestion and absorption of lipid and the growth rate of animals remains to be investigated.

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