Introduction

Although cholesterol is used by the body for a variety of functions, increased levels of cholesterol can have adverse affects such as heart attacks, heart disease, and strokes. One of the functions of cholesterol is the production of bile acids that aid in digestion of fats. Most of these bile acids are usually reabsorbed by the body and recycled so cholesterol is not used up in the synthesis of bile acids.

In the intestinal tract, bile acids encounter numerous bacteria. Some of these bacteria convert the primary bile acids chenodeoxycholic acid and cholic acid into lithocholic acid and deoxycholic acid, respectively (1) (see below).

Objectives

- To develop an enzyme assay to monitor the decrease of deoxycholic acid in fecal cultures
- To determine if microbes in the gastrointestinal tract are capable of converting deoxycholic acid to polydeoxycholic acid and other metabolites

Methods

Collection and Processing of Hamster Feces

Hamsters (Syrian Golden) were used as a human model. Hamsters were fed a diet containing dog food, grain, and squirrel food. Feces (less than 24 h old) were collected and added to a 125-ml serum bottle (0.9 g of feces per bottle) containing 50 ml of anaerobic Brain Heart Infusion Broth (DIFCO). Bottles were placed on a shaker and mixed for the contents mixed for 30 min at 500 rpm. After mixing, sterile anionic sodium deoxycholate was added to achieve a final concentration of 0.1 or 1 mM, and fecal cultures were incubated at 37°C. At indicated time intervals, 1-ml samples were removed and frozen for further analysis.

TLC Analysis

Samples (1 ml) of fecal cultures were removed and extracted with 2 ml of chloroform. The lower organic phase was removed and dried under Ar gas at room temperature. Once dry, 100 µl of chloroform was added to resuspend the sample, and 50 µl was spotted onto a TLC plate (silica gel). The spotted plate was placed in a developing tank containing only chloroform. After the solvent had migrated nearly to the top of the plate, the plate was removed, allowed to dry, and placed in a developing tank containing ethyl acetate, cyclohexane, glacial acetic acid, and chloroform (9:2:2:3). After the solvent had migrated nearly to the top of the plate, the plate was removed, sprayed with a charring agent (water, methanol, sulfuric acid, and HCl), and charred at 110°C for 15 minutes so bile acids could be viewed under UV light.

Results

Chenodeoxycholic acid

- A decrease in the amount of deoxycholic acid was detected in fecal cultures incubated for 2 and 4 days (Table 1).
- Bile acids other than deoxycholic acid were also detected in fecal cultures (Figure 4).
- Polydeoxycholic acid was not detected in any of the fecal cultures examined (Figure 4; data not shown).

<p>| Table 1: The disappearance of deoxycholic acid in hamster fecal cultures |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|</p>
<table>
<thead>
<tr>
<th>Incubation (days)</th>
<th>DCBA Concentration</th>
<th>DCBA Decrease</th>
<th>% DCBA Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.100 ± 0.040</td>
<td>0.32</td>
<td>32%</td>
</tr>
<tr>
<td>1</td>
<td>0.087 ± 0.035</td>
<td>0.23</td>
<td>23%</td>
</tr>
<tr>
<td>2</td>
<td>0.080 ± 0.030</td>
<td>0.19</td>
<td>20%</td>
</tr>
<tr>
<td>3</td>
<td>0.073 ± 0.014</td>
<td>0.17</td>
<td>22%</td>
</tr>
<tr>
<td>4</td>
<td>0.069 ± 0.012</td>
<td>0.15</td>
<td>22%</td>
</tr>
</tbody>
</table>

Enzyme and TLC Development

- Deoxycholic acid-metabolizing bacteria were present in hamster feces.
- Based on TLC analysis, the bile acids 5β-cholanic acid-3β, 12a-diol and 5β-cholanic acid-3-one were produced by fecal bacteria via the metabolism of deoxycholic acid.
- Further research needs to be done to discover more sensitive methods for detecting small amounts of polydeoxycholic acid along with the exact pathway for the conversion of deoxycholic acid to polydeoxycholic acid and other bile acid derivatives.
- The role of gastrointestinal microbiota on the host’s health is very important in digestion and could be of further importance if it is found that fecal bacteria are capable of converting bile acids into forms that cannot be reabsorbed by the body, thereby lowering serum cholesterol levels.

References


Summary

- Deoxycholic acid-metabolizing bacteria were present in hamster feces.
- Based on TLC analysis, the bile acids 5β-cholanic acid-3β, 12a-diol and 5β-cholanic acid-3a-ol, 12-one were produced by fecal bacteria via the metabolism of deoxycholic acid.
- Further research needs to be done to discover more sensitive methods for detecting small amounts of polydeoxycholic acid along with the exact pathway for the conversion of deoxycholic acid to polydeoxycholic acid and other bile acid derivatives.
- The role of gastrointestinal microbiota on the host’s health is very important in digestion and could be of further importance if it is found that fecal bacteria are capable of converting bile acids into forms that cannot be reabsorbed by the body, thereby lowering serum cholesterol levels.