Note

Missense Mutation of Abcg5 in Stroke-Prone Spontaneously Hypertensive Rats Does Not Influence Lymphatic Sitosterol Absorption Regardless of the Dose: Comparison with Wistar Rats

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The lymphatic recovery of radiolabeled sitosterol administered in various amounts to the stomach was almost the same between stroke-prone spontaneously hypertensive rats (SHRSPs), a strain having a missense mutation in ATP binding cassette transporter g5 (Abcg5), and Wistar rats, a normal strain. The results suggest that the mutation of Abcg5 in SHRSPs, compared with Wistar rats, did not influence the ability for intestinal sitosterol absorption regardless of the dose.

Key words: ATP binding cassette transporter G5 (ABCG5); ATP binding cassette transporter G8 (ABCG8); intestinal absorption; plant sterol; stroke-prone spontaneously hypertensive rat (SHRSP)

Plant sterols are less absorbable than cholesterol and their deposition in the body is extremely low.1 However, sitosterolemic patients, having various mutations of ATP binding cassette transporter G5 (ABCG5) or ATP binding cassette transporter G8 (ABCG8), deposited plant sterols in the body.2-3) ABCG5 and ABCG8 are expressed in the liver and intestine, and are thought to function as a heterodimer and excrete sterols from enterocytes to the intestinal lumen and from hepatocytes to bile.4) We have previously found that Wistar Kyoto (WKY) rats, spontaneously hypertensive rats (SHRs) and stroke-prone spontaneously hypertensive rats (SHRSPs) deposited plant sterols in the body.5) Since Scoggan et al. and Yu et al. have shown all of these strains to have the same mutation of Abcg5,6,7) it has been thought that the intestinal absorption of plant sterols by these strains would be accelerated by a malfunction of ABCG5 and hence that they would accumulate plant sterols in the body. However, inconsistent results have been obtained in plant sterol absorption studies of rat strains having the mutation of Abcg5. We5) and Batta et al.8) have observed that the intestinal absorption of a trace amount of 3H-sitosterol was higher in rat strains having the mutation of Abcg5 than in normal strains. We have recently observed no difference in the lymphatic absorption of sitosterol and campesterol between SHRSPs and Wistar rats, when 6.7 mg/100 g body weight of campesterol, sitosterol, stigmasterol and sitostanol were each administered to the stomach.9) The lymphatic recovery of sitostanol was significantly higher in SHRSPs than in Wistar rats. However, the difference was very small, because the absorbed amount of sitostanol was much lower than that of sitosterol or campesterol. We therefore thought that the reason why no difference in absorption was apparent in sitosterol and campesterol could have been due to the relatively greater absorbed amounts in that study.9) If the absorbed amounts were relatively low, a difference in absorption might be observed as reported by us5) and Batta et al.8) in studies with a trace amount of 3H-sitosterol given. In the present study, the lymphatic recovery of various amounts of sitosterol administered to the stomach is compared between SHRSPs and Wistar rats.

Four-week-old male SHRSPs (SHRSP/Izm, inbred, SPF; Japan SLC, Shizuoka, Japan) and Wistar rats (Wistar/Kud, outbred, SPF; Kyudo, Fukuoka, Japan) were pair-fed for 4 weeks with an AIN-93G purified diet containing 10% lard. Since rat diets contain a considerable amount of plant sterols, the SHRSPs obtained from the breeder had already deposited plant sterols in the body. To reduce these plant sterols in the body as much as possible, lard was used as the sole dietary fat, because the plant sterol content in lard is extremely low (sterol content in weight percent: cholesterol, 0.0062%; campesterol, 0.00078%; and sitosterol, 0.0013%). The average body weights of the SHRSPs and Wistar rats at surgery were 268 g and 297 g, respectively. The left thoracic lymphatic duct cephalad to the cisterna chili was cannulated. A second indwelling catheter was placed in the stomach for the administration of a test emulsion.9) Three types of test emulsion were prepared. The composition of one emulsion was 67 mg of sodium taurocholate (>97%, Nacalai Tesque, Kyoto, Japan), 17 mg of bovine serum albumin, 67 mg of triolein (Sigma-Japan, Tokyo, Japan), and 122 kBq (65 ng) of...
Table 1. Lymph Flow Rate and Lymphatic Recovery of Sitosterol in SHRSPs and Wistar Rats

<table>
<thead>
<tr>
<th>Dose/100 g of body weight</th>
<th>65 ng</th>
<th>0.33 mg</th>
<th>1.7 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph flow (ml/24h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>75.3 ± 4.3</td>
<td>88.4 ± 3.9</td>
<td>75.8 ± 5.5</td>
</tr>
<tr>
<td>SHRSP</td>
<td>78.1 ± 4.6</td>
<td>76.3 ± 3.3a</td>
<td>72.5 ± 2.9</td>
</tr>
<tr>
<td>Lymphatic absorption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recovery in 24h (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>5.70 ± 0.25</td>
<td>5.65 ± 0.57</td>
<td>5.79 ± 0.27</td>
</tr>
<tr>
<td>SHRSP</td>
<td>5.64 ± 0.47</td>
<td>6.11 ± 0.54</td>
<td>5.59 ± 0.45</td>
</tr>
<tr>
<td>Absorbed mass/100 g of body weight (µg)</td>
<td>(µg)</td>
<td>(µg)</td>
<td></td>
</tr>
<tr>
<td>Wistar</td>
<td>3.61 ± 0.18</td>
<td>18.8 ± 1.9</td>
<td>96.5 ± 4.4</td>
</tr>
<tr>
<td>SHRSP</td>
<td>3.56 ± 0.29</td>
<td>20.4 ± 1.8</td>
<td>93.1 ± 7.5</td>
</tr>
</tbody>
</table>

The animals were pair-fed a diet containing 10% lard for 4 weeks and then subjected to the absorption study. Each data value is the mean ± SE of 8 rats per group.

aSignificant difference between SHRSPs and Wistar rats at p < 0.05.

[22, 23(n)-3H] sitosterol (814 GBq/mmol; Amersham, Buckinghamshire, England) per 1 ml. The other emulsions respectively contained 0.33 mg or 1.7 mg of highly purified sitosterol (98.6%; Tama Biochemical Co., Tokyo, Japan) per 1 ml in addition to 3H-sitosterol. Each test emulsion was administered to the stomach at 1 ml per 100 g body weight, and then lymph was collected for 24 h. The radioactivity in the lymph was measured with a liquid scintillation counter. The animal study was carried out under the guidelines for animal experiments of the Faculty of Agriculture, Graduate School of Kyushu University, and Law 105 and Notification 6 of the Government of Japan. Each data value is expressed as the mean ± SE. Student’s t-test was used for the statistical analysis, a p value of less than 0.05 being considered significant.

There was no significant difference in lymph flow rate between the SHRSPs and Wistar rats, except for the 0.33 mg sitosterol group, in which the SHRSPs showed a lower lymph flow rate than the Wistar rats (Table 1). There was no difference in the lymphatic recovery and absorbed mass of sitosterol between the SHRSPs and Wistar rats at any dose (Table 1). Although we had hypothesized that the intestinal absorption of sitosterol could be accelerated in SHRSPs when the absorbed amount was small, the results suggest that the mutation of Abcg5 in SHRSP rats did not influence the ability for intestinal sitosterol absorption, regardless of the dose, compared with Wistar rats.

We have previously shown that the deposition of sitosterol and campesterol in SHRSPs was considerably higher than that in Wistar rats when a plant sterol diet was fed to the rats.5 It has been thought that a malfunction of ABCG5 and ABCG8 by their mutation resulted in increased plant sterol absorption and hence their deposition in the body.2,3 However, our observations in the present study and in the previous report do not support this. Our results suggest that a missense mutation of Abcg5 in SHRSP rats is not the major reason for the higher deposition of plant sterols.

It has been suggested in research using Niemann-pick C1-like 1 (NPC1L1)-deficient mice and the potential inhibitor of NPC1L1, ezetimibe, that NPC1L1 contributed to about a half of the intestinal incorporation of cholesterol and plant sterols.10,11 We have also suggested that solubility in and affinity for the bile salt micelle of plant sterols were important determinants of its intestinal absorption in rats.12 We estimated that about a half of plant sterols was incorporated into intestinal cells by simple diffusion. There is the possibility that a malfunction of ABCG5 in SHRSPs could be compensated by the function of NPC1L1 and simple diffusion. A further study is necessary to test this.

If the intestinal absorption of sitosterol was the same between SHRSPs and Wistar rats, a difference in biliary secretion through ABCG5/ABCG8 might be an important determinant for the deposition of sitosterol and campesterol in SHRSPs compared with Wistar rats. The biliary secretion of cholesterol almost disappeared in ABCG5/ABCG8-deficient mice,13 suggesting that the ability to excrete plant sterols via ABCG5/ABCG8 in the liver could be more crucial for plant sterol deposition in the body. The biliary secretion of plant sterols in SHRSPs and Wistar rats is now under investigation.

References