Inhibitory action of gemfibrozil on cholesterol absorption in rat intestine

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Abstract This study was designed to determine whether gemfibrozil inhibits intestinal lipid absorption. Male Sprague-Dawley rats received an oral dose of 30 mg gemfibrozil/kg body weight for 14 days. Mesenteric lymph cannulation was performed, and a lipid infusion containing 40 μmol/h (35.4 mg/h) of radiolabeled triolein and 2.74 μmol/h (1.06 mg/h) of radiolabeled cholesterol with the addition of 1 mg/h of gemfibrozil was infused intraduodenally at a rate of 3 ml/h for 8 h. The lymph was collected, and the radioactivity levels of the lumen and gut mucosa were measured after the infusion. Lymph cholesterol transport was depressed in gemfibrozil-treated rats, in terms of mass measurements as well as radioactivity in a lesser degree. More radioactive cholesterol remained in the proximal portion of the intestinal lumen and mucosa in the treated rats than in the control rats. More radioactive triglycerides also remained in the proximal intestinal lumen of treated rats, although no difference in lymphatic triglyceride transport was observed between the groups. A significant portion of the radioactive cholesterol remained in the lumen in the gemfibrozil-treated rats. Gemfibrozil increased biliary cholesterol excretion. Thus, this study shows that gemfibrozil inhibits cholesterol absorption in rat intestine.—Umeda, Y., Y. Kako, K. Mizutani, Y. Iikura, M. Kawamura, M. Seishima, and H. Hayashi. Inhibitory action of gemfibrozil on cholesterol absorption in rat intestine. J. Lipid Res. 2001. 42: 1214–1219.

Supplementary key words fibrates • mesenteric lymph-fistula • Sprague-Dawley rats • triglyceride • lymph transport • biliary cholesterol

Gemfibrozil is one of the most widely used antihyperlipidemic drugs in the world and has been shown to be effective in preventing coronary heart diseases in hyperlipidemic patients (1), and in patients with a low level of HDL cholesterol and normal or low levels of LDL cholesterol (2). The major pharmacological mechanism of fibrates, including gemfibrozil, in hyperlipidemia is supposed to be a decreased production of VLDL as a result of the decreased synthesis of TG and an increased hydrolysis of TG by the induction of lipoprotein lipase and reduction of apolipoprotein C-III synthesis (3). Recently, Vanhanen and Miettinen (4) performed a sterol balance study in humans and reported that gemfibrozil inhibits cholesterol absorption, resulting in a lower serum cholesterol level. Little information on the effects of fibrates, including gemfibrozil, on intestinal lipid absorption is available. The aim of this study was to determine whether gemfibrozil directly inhibits intestinal lipid absorption by using the mesenteric lymph-fistula rat model. The effect of gemfibrozil on the biliary cholesterol excretion was also assessed.

METHODS

Animals

Male Sprague-Dawley rats (body weight, 250–350 g) were divided into control and experimental groups. The rats in the experimental group received 30 mg/kg body weight of gemfibrozil orally (gavage) each day for 14 days. The oral solution was prepared by sonicating 300 mg of gemfibrozil in 30 ml of 1.5% carboxymethylcellulose solution. Both groups were fed with regular chow.

Surgery of lymph-fistula rat

After an overnight fast, the main mesenteric lymph duct was cannulated with clear vinyl tubing (0.8 mm outer diameter (OD)) according to the method of Bollman et al. (5), as reported previously (6). Silicone tubing (2.2 mm OD) was inserted to a depth of 2 cm down the duodenum and through the fundus of the stomach. The fundal incision was closed by a purse-string suture. Postoperatively, the rats were placed in individual cages with freely available tap water. In addition, a glucose-saline solution (145 mM NaCl, 4 mM KCl, 0.28 M glucose) was infused intraduodenally at a rate of 3 ml/h. The rats were allowed to recover for at least 24 h before the lipid infusion.

Experimental protocol of lipid infusion

On the day after the surgery, the saline-glucose infusion was replaced by a lipid infusion containing 40 μmol/h (35.4 mg/h) of triolein (labeled as glyceryl tri[14C]oleate, 23.0 Bq/mmol),
2.74 μmol/h (1.06 mg/h) of cholesterol (labeled as [17(N)-3H]cholesterol, 338 Bq/nmol), 7.8 μmol/h of egg lecithin, and 57 μmol/h of taurocholate emulsified in a phosphate buffer saline (pH 6.4) in the control group. The lipid emulsion was infused at a rate of 3 ml/h for 8 h. In the experimental rats 1 mg/h of gemfibrozil was added to the lipid emulsion. Lymph was collected before and at hourly intervals throughout the lipid infusion. Lymph lipids were extracted using the method of Folch et al. (7) and separated by thin-layer chromatography. Free and esterified cholesterol was quantified by the o-phthaldialdehyde procedure (8) after saponification of the lipids in separate bands. The lymph TG mass, corrected for free glycerol, was determined by an enzymatic procedure (Boehringer Mannheim kit 450 032) (9). The radioactivity of the lymph was measured during the lipid infusion, and the percentage of lipid transported via the lymph was calculated (hourly amount of infused lipid = 100%).

Collection of mucosal and luminal samples

After the termination of lipid infusion, the rats were anesthetized with ether and were killed by exsanguination. The radioactivity levels in the intestinal lumen and mucosa were determined as reported previously (10). The small intestine was removed and divided into four equal segments. The most proximal intestinal segment was designated as I₁, and the subsequent segments were designated as I₂–I₄. Each segment was rinsed thoroughly using three washes, with each wash containing 3 ml of 10 mM sodium taurocholate in normal saline, and the wash solutions were collected and combined. The stomach and cecum were opened longitudinally and washed in the same manner as the intestine. Any feces that were passed during the infusion study were added to the cecum sample. The samples were then homogenized briefly with a Polytron homogenizer, and three aliquots from each sample were removed. The radioactivity level of these three aliquots was then determined, and the values were averaged.

The remaining intestinal segments were then opened longitudinally and placed in separate Erlenmeyer flasks. Lipids from the intestinal segments were extracted according to the method of Folch et al. (7), and their radioactivity level was determined. The radioactivities of the lipids in the lumen and mucosa were then expressed as a percentage of the total amount of radioactive lipids infused throughout the 8 h.

Biliary cholesterol excretion

The surgery of bile-fistula was performed in another set of control rats, and experimental rats received gemfibrozil for 14 days. The common bile duct was cannulated just below the two hepatic ducts with silicone tubing (outer diameter 0.5 mm) tipped with polyethylene 10 tubing. The rats were recovered in restraint cages with free access to tap water after surgery and bile was collected for 24 h. Cholesterol concentration in bile was measured enzymatically and the average cholesterol excretion was calculated.

Statistical analysis

A repeated measures analysis of the variance test was used to determine whether differences existed between the two groups. The Student’s *t* test for independent means was used where appropriate. Differences with a *P* < 0.05 were considered as significant.

RESULTS

Lymph flow

The fasting lymph flow was 3.72 ± 0.28 ml/h (mean ± SE) in the experimental rats (n = 4) and 3.73 ± 0.58 ml/h in the control rats (n = 4). Lymph flow decreased soon after the beginning of lipid infusion in both groups, and the fall in the experimental group at 1 h and 2 h was significant when compared with the flow rate during fasting (Fig. 1). A similar decrease in lymph flow was observed in other cases immediately after the infusate was changed (11, 12). The lymph flow rate began to increase gradually in both groups thereafter and reached a value of 4.16 ± 0.30 ml/h in the experimental group and 4.18 ± 0.06 ml/h in the control group after 8 h. No statistically significant differences in lymph flow rates were observed between the two groups throughout the entire infusion period.

Lymphatic cholesterol and TG outputs

Lymphatic outputs of the esterified and free forms of cholesterol are shown in Figs. 2 and 3, respectively. The esterified cholesterol output in the fasting lymph was below 0.5 mg/h in both groups (no significant difference between groups). After the beginning of lipid infusion, a difference in esterified cholesterol outputs between the two
groups became prominent after 4 h of infusion, once lymphatic lipid transportation had stabilized. The lymphatic outputs of esterified cholesterol after 8 h of lipid infusion in the experimental and control groups were 1.52 ± 0.14 mg/h and 2.26 ± 0.13 mg/h, respectively. A significant difference \( (P < 0.01) \) in the lymphatic output of esterified cholesterol was observed between the two groups at 4–8 h. The lymphatic output of free cholesterol for the two groups was similar to the pattern observed for esterified cholesterol, but the absolute mass of transported cholesterol in its free form was about one-third the mass of its esterified form. The lymphatic outputs of free cholesterol after 8 h of lipid infusion in the experimental and control groups were 0.25 ± 0.05 mg/h and 0.66 ± 0.07 mg/h, respectively. A significant difference \( (P < 0.02) \) between the two groups was observed at 5–8 h. Although the lymphatic output of free cholesterol after 8 h of lipid infusion was about 1.8 and 3 mg/h for the experimental and control groups, respectively. The reason for this increase is that the chemically measured lipid output represents intestinal absorption of both exogenous and endogenous cholesterol. Although the lymphatic outputs of both esterified and free cholesterol were significantly lower in the experimental group, the lymphatic TG output did not differ between the two groups, as shown in Fig. 4. After the beginning of lipid infusion, the lymphatic TG output increased dramatically and reached a plateau after about 4 h in each group. The TG output at 8 h was 23.73 ± 0.61 mg/h and 22.36 ± 1.81 mg/h for the experimental and control groups, respectively.

Lymphatic outputs of radioactive lipids
The overall output of radioactive cholesterol during the 8 h of lipid infusion was significantly lower in the experimental group \( (P < 0.05) \), and when comparing each hour of lipid infusion, cholesterol transport at 5 and 6 h was significantly lower \( (P < 0.05) \) in the experimental group (Fig. 5). No significant differences in the output of radioactive triolein were observed between the two groups (Fig. 6).
Radioactive lipids in the lumen and mucosa

The overall distribution of radioactive cholesterol in the lumen was significantly different between the two groups (Table 1). The amount of cholesterol remaining in segment I1 of the experimental group was significantly larger than that of the control group, while the amount remaining in segment I3 and the cecum was significantly larger in the control group. The overall distribution of radioactive triolein in the lumen was also significantly different between the two groups (Table 1). The amount of triolein remaining in segment I1 of the experimental group was larger than that of the control group.

The overall distribution of infused cholesterol in the intestinal mucosa was significantly different between the two groups (Table 2). The amount of cholesterol remaining in segment I1 of the experimental group was significantly larger than that of the control group, while the amount remaining in segment I3 was significantly larger in the control group. The data also showed a tendency for a larger amount of triolein to collect in the proximal intestinal mucosa of the experimental group, but the difference between groups was not significant (Table 2).

Total distribution of radioactive lipids

The overall distribution of infused cholesterol in the intestinal mucosa was significantly different between the two groups (Table 2). The amount of cholesterol remaining in segment I1 of the experimental group was significantly larger than that of the control group, while the amount remaining in segment I3 was significantly larger in the control group. The data also showed a tendency for a larger amount of triolein to collect in the proximal intestinal mucosa of the experimental group, but the difference between groups was not significant (Table 2).

Biliary cholesterol excretion

Bile flow and biliary cholesterol excretion in the experimental (n = 4) and control (n = 6) rats are shown in Table 4. Both bile flow and cholesterol excretion were significantly higher in the experimental group than in the control group.

DISCUSSION

In this study, the transport of cholesterol in the lymph was shown to be depressed in gemfibrozil-treated rats. Measurements of cholesterol output (Figs. 2 and 3) and radioactivity (Fig. 5) both support this conclusion. In the former case, the output of both esterified and free forms of cholesterol decreased by the same degree in treated rats when compared with control rats. More exogenous (intraduodenally infused) radioactive cholesterol remained in the most proximal portion of the intestinal lumen (Table 1) and mucosa (Table 2) in the treated rats than in the control rats. Interestingly, radioactive triglycerides also accumulated in the proximal intestinal lumen of experimental rats (Table 1), although no difference in lymphatic triglyceride transport between the gemfibrozil-treated and control rats was observed, either in triglyceride output (Fig. 4) or radioactivity (Fig. 6). By comparing the distribution of radioactive cholesterol in the intestinal lumen, mucosa, and lymph, a significant portion of the cholesterol was found to remain in the lumen of the gemfibrozil-treated rats (Table 3).

Although it was demonstrated that the lymphatic cholesterol transport was depressed by gemfibrozil, it does not necessarily mean that gemfibrozil inhibits the net transfer of cholesterol from the intestinal lumen to the

### Table 1. Distribution of infused radioactive lipids in the lumen of the small intestine

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Group</th>
<th>Stomach</th>
<th>I₁</th>
<th>I₂</th>
<th>I₃</th>
<th>I₄</th>
<th>Cecum</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>Control</td>
<td>0.19 (0.32)</td>
<td>0.50 (0.51)</td>
<td>4.41 (2.65)</td>
<td>6.39 (1.57)</td>
<td>3.43 (1.10)</td>
<td>4.81 (3.23)</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil</td>
<td>0.03 (0.07)</td>
<td>7.68 (3.36)</td>
<td>7.38 (1.98)</td>
<td>4.42 (0.73)</td>
<td>3.90 (2.40)</td>
<td>1.12 (0.87)</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>Control</td>
<td>0.03 (0.03)</td>
<td>0.59 (0.45)</td>
<td>1.04 (1.29)</td>
<td>0.14 (0.09)</td>
<td>0.06 (0.04)</td>
<td>0.08 (0.02)</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil</td>
<td>0.06 (0.05)</td>
<td>7.69 (2.42)</td>
<td>0.36 (0.17)</td>
<td>0.05 (0.05)</td>
<td>0.03 (0.04)</td>
<td>0.08 (0.09)</td>
<td></td>
</tr>
</tbody>
</table>

Values represent the amount of radioactive lipids collected from the mucosa as a percentage of the total infused dose and are expressed as the mean value of 4 rats, with the SD in parentheses. Statistically significant differences between the two groups are noted as a P < 0.05, b P < 0.01, while NS denotes a difference that is not significant.

### Table 2. Distribution of infused radioactive lipids in the small intestine mucosa

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Group</th>
<th>I₁</th>
<th>I₂</th>
<th>I₃</th>
<th>I₄</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>Control</td>
<td>0.43 (0.86)</td>
<td>4.53 (5.31)</td>
<td>5.05 (1.51)</td>
<td>3.14 (2.81)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil</td>
<td>3.11 (1.66)</td>
<td>7.32 (4.30)</td>
<td>2.52 (1.29)</td>
<td>1.69 (1.33)</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>Control</td>
<td>6.17 (6.75)</td>
<td>3.49 (3.56)</td>
<td>0.17 (0.14)</td>
<td>0.06 (0.05)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil</td>
<td>7.86 (1.05)</td>
<td>0.66 (0.17)</td>
<td>0.10 (0.03)</td>
<td>0.11 (0.05)</td>
<td></td>
</tr>
</tbody>
</table>

Values represent the amount of radioactive lipids collected from the mucosa as a percentage of the total infused dose and are expressed as the mean value of 4 rats, with the SD in parentheses. Statistically significant differences between the two groups are noted as a P < 0.05 and b P < 0.01, while NS denotes a difference that is not significant.

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lymphatics by the decreased ratio of the intestinal absorption of cholesterol. As already mentioned in the Results, there are two sources of cholesterol in the intestinal lumen, that is, exogenous and endogenous, and a major part of the latter is bile cholesterol. If the treatment of gemfibrozil suppresses bile cholesterol excretion, it results in the decreased amount of absorbable cholesterol in the lumen. In consequence, lymphatic cholesterol transport will be depressed in the gemfibrozil-treated group even if gemfibrozil does not affect the intestinal absorption ratio of cholesterol. However, this was not the case in our experiment. As shown in Table 4, the rats treated by gemfibrozil in the same manner as the lymph-fistula rats excreted significantly more cholesterol in the bile than the control rats. The increased cholesterol excretion in the bile by gemfibrozil was also reported by other investigators (4, 13, 14). Therefore, because the rats treated by gemfibrozil are supposed to have more absorbable cholesterol in the intestinal lumen than the non-treated rats, the data that lymphatic cholesterol transport was depressed by gemfibrozil indicates that gemfibrozil inhibited the net transfer of cholesterol from the lumen to the lymphatics.

Another question about the data of lymphatic cholesterol transport in our experiment is the discrepancy of the results between mass measurement and radioactivity. While the output of both esterified (Fig. 2) and free (Fig. 3) forms of cholesterol clearly decreased in the gemfibrozil-treated group, the difference of the radioactive cholesterol output between the gemfibrozil-treated group and the control group was relatively small (Fig. 5). Although the overall output of radioactive cholesterol during 8 h of lipid infusion was significantly lower in the experimental group, it was mostly caused by the difference only at 5 and 6 h and a significant difference at 7 and 8 h was not seen. One of the possibilities that may explain this discrepancy is the different dilution rate of the exogenous radioactive cholesterol by endogenous cholesterol in the intestinal lumen between the two groups. If gemfibrozil affected cholesterol excretion in the bile, resulting in a reduced supply of biliary cholesterol to the lumen, the dilution rate of the radioactive cholesterol in the lumen of gemfibrozil-treated rats would be much smaller than that of the control rats. This would explain why the difference shown by the radioactive assay is smaller than that shown by the chemical assay. However, gemfibrozil was shown to increase, but not to decrease, cholesterol excretion in the bile as mentioned above (Table 4).

Another possibility that may explain this discrepancy is related to the argument that biliary cholesterol and dietary cholesterol may be absorbed in a different way because of different physicochemical states of each cholesterol in the intestinal lumen. The former is secreted to the lumen in a bile salt/phospholipid micelle and the latter must be transferred to the micelle from an oil phase before it can be absorbed. Therefore it was claimed that biliary cholesterol is initially absorbed with greater efficiency than is dietary cholesterol (15). But other investigators suggest that the percentage absorption of cholesterol from both endogenous and exogenous is equal because the time it takes to get cholesterol digested, emulsified, formed into micelles, and absorbed is long enough that mixing of cholesterol from endogenous and dietary sources is essentially complete (16). Our data may suggest that gemfibrozil affects absorption of endogenous cholesterol preferentially to exogenous cholesterol in the early time of the absorption when the mixing of endogenous and exogenous cholesterol is not complete. The reason for the discrepancy between the results of the radioactivity and chemical assays remains unknown.

The mechanism of gemfibrozil’s inhibitory action on lymphatic cholesterol transport cannot be confirmed using the results of this experiment. As suggested in Table 3, intracellular processes involved in cholesterol and chylomicron metabolism are probably not interrupted. Instead, the significant increase in the amount of cholesterol that remains within the lumen of gemfibrozil-treated rats suggests that the uptake of cholesterol from the intestinal lumen into the enterocyte might be partially blocked. The optimum luminal environment for cholesterol uptake is probably altered by gemfibrozil, since bile acid output to the duodenum is decreased by gemfibrozil (13, 14) and gemfibrozil itself is strongly hydrophobic, which may disturb the

### TABLE 3. Distribution of radioactive lipids in lymph, mucosa, and lumen

<table>
<thead>
<tr>
<th>Lipid</th>
<th>Group</th>
<th>Lymph</th>
<th>Mucosa</th>
<th>Lumen</th>
<th>ANOVA</th>
<th>Total Recovered*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>Control</td>
<td>48.54 (6.15)</td>
<td>13.15 (2.97)</td>
<td>17.94 (1.13)</td>
<td>5</td>
<td>81.38 (7.53)</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil</td>
<td>42.83 (4.01)</td>
<td>14.64 (5.07)</td>
<td>24.53 (3.72)</td>
<td>3</td>
<td>80.95 (4.33)</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>Control</td>
<td>61.79 (4.40)</td>
<td>9.89 (5.81)</td>
<td>1.94 (1.25)</td>
<td>NS</td>
<td>73.62 (10.21)</td>
</tr>
<tr>
<td></td>
<td>Gemfibrozil</td>
<td>65.23 (3.76)</td>
<td>8.73 (1.06)</td>
<td>8.28 (2.46)</td>
<td>2</td>
<td>82.23 (1.70)</td>
</tr>
</tbody>
</table>

Values represent the amount of radioactive lipids collected from the lymph, mucosa, and lumen as a percentage of the total infused dose and are expressed as the mean of 4 rats, with the SD in parentheses. Statistically significant differences between the two groups are noted as *P < 0.05, while NS denotes a difference that is not significant. *No significant difference (Student’s t-test) in the total amount of recovered radioactivities was observed between the two groups for either lipid.

### TABLE 4. Bile flow and biliary cholesterol excretion

<table>
<thead>
<tr>
<th>Group</th>
<th>Bile flow</th>
<th>Biliary cholesterol excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml/h</td>
<td>mg/h</td>
</tr>
<tr>
<td>Control (n = 6)</td>
<td>0.89 (0.02)</td>
<td>0.55 (0.05)</td>
</tr>
<tr>
<td>Gemfibrozil (n = 4)</td>
<td>1.09* (0.16)</td>
<td>0.82* (0.12)</td>
</tr>
</tbody>
</table>

Values represent the mean with the SD in parentheses. Statistically significant differences between the two groups are noted as *P < 0.05 and **P < 0.01.
absorptive micelle formation. On the other hand, the change in the distribution of lipids within the intestine must also be noted. More lipids, including both cholesterol and triglycerides, remained in the most proximal portion of the lumen (Table 3) and mucosa (Table 2) in gemfibrozil-treated rats. In general, the proximal intestine is more efficient at chylomicron production than the distal intestine (17). In a previous report (10), Bergstedt et al. compared intestinal absorption of glycerol tristearate and glycerol trioleate in rats. They found that the former compound was less efficiently transported in the lymph and that the former compound tended to remain in the distal portion of the intestinal lumen and mucosa. The results of our experiment, in contrast, suggest that cholesterol is not transported more efficiently in the lymph and tends to remain in the proximal region of the intestine in gemfibrozil-treated rats.

In this study, gemfibrozil was shown to directly affect intestinal cholesterol absorption and inhibit the luminal uptake of cholesterol. Although a larger proportion of lipids remained in the proximal intestine of gemfibrozil-treated rats, the relationship between this phenomenon and the mechanism of gemfibrozil’s inhibitory action remains unclear.

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REFERENCES


