Effect of KCD-232, a New Hypolipidemic Compound, on Hyperlipidemia in Experimental Nephrotic Syndrome Rats

Toshiro MOCHIZUKI*, Kodo OKADA*, Kouichi TAKAGI* and Tsutomu IRIKURA*

Introduction
Nephrotic syndrome is characterized by renal damage, proteinuria, hypoproteinemia and edema with marked hypercholesterolemia and hypertriglyceridemia. It has been demonstrated that the cause of hyperlipidemia is attributable to increased hepatic synthesis of lipoprotein1). The experimental nephrotic syndrome can be produced in rats by injection with antikidney serum2) or puromycin aminonucleoside3). It seems to be an excellent model of endogenous hyperlipidemia.

Among many test compounds, 4-(4'-chlorobenzyloxy)benzyl nicotinate (KCD-232) has been found to have hypocholesterolemic and hypotriglyceridemic effects in normal or experimental hyperlipidemic animals4-6).

In this paper, to investigate the hypolipidemic effect of KCD-232, we studied the influence of KCD-232 on serum and liver lipids, hepatic lipid synthesis and post-heparin lipolytic activity in experimental nephrotic syndrome produced by injection with antikidney serum in rats.

Materials and Methods

Materials
[1,14C]sodium acetate (2.8 mCi/m mole) and [carboxyl-14C]triolein (96 mCi/m mole) were purchased from New England Nuclear. KCD-232 and Clofibrate were prepared in our laboratory.

Animals
Male Wistar rats, weighing 230-260 g, were used. They were fed a pelleted stock diet (CE-2, CLEA Japan Inc., Tokyo) and water ad libitum throughout this experiment.

Experimental procedure
Nephrosis was produced in rats by intravenous injection with 1 ml/rat of rabbit anti-rat glomerular basement membrane antiserum (antikidney serum) prepared by the method of Shibata et al.7)

After injection with antikidney serum, KCD-232 and Clofibrate were administered orally once a day at a dose of 100 or 300 mg/kg for 14 days. Control rats received 0.5% CMC (W/V) solution alone.

Rats were starved except for water after the final administration and used for various measurements 4 hr later.

Analysis of serum and liver lipids
Serum and liver lipids were extracted by the method of Folch et al.8) and were measured for cholesterol by Zak's method9), for triglyceride by Van Handel’s method10) and for phospholipid by Chen's method11).

Serum lipoprotein was separated into fractions of high density lipoprotein (HDL) and phosphotungstic acid-precipitable lipoprotein (PTPL) by the phosphotungstic acid-Mg++ precipitable method12).

HDL and PTPL cholesterol levels were determined using a commercial Determiner "555" Kid (KYOWA MEDEX Co., Ltd.).

Other analyses
Urinary samples were collected over 24 hr at 13 days after injection with antikidney serum. Urinary protein was measured by the method of Exton13). Serum protein and albumin concentrations were determined using a commercial A/G-Test Wako Kid (WAKO PURE IND., LTD.).
Measurement of lipid synthesis

Rats were killed by decapitation. The livers were rapidly removed, washed with cold 0.9\% NaCl and prepared in slices.

The incorporation of digitonin-precipitable sterols and fatty acids from [l-14C]acetate in vitro was measured by the method of Endo et al.\(^{14}\)

Measurement of post-heparin lipolytic activity (PHLA)

Rats were anesthetized with sodium pentobarbital. Post-heparin plasma was collected 5 min after injection of heparin sodium (200 IU/kg) (SHIMIZU PHARMACEUTICAL Co., LTD.)

Plasma PHLA was measured by the method of Kuusi et al.\(^{15}\) Selective measurement of PHLA was based on inactivation of lipoprotein lipase (LPL) activity by the elevation of NaCl concentration. Hepatic triglyceride lipase (HTGL) activity was measured with a substrate containing 1 M NaCl and no serum to inactivate LPL activity. LPL activity was calculated by subtracting the HTGL activity from the total PHLA.

Calculation

The statistical analysis was carried out using the Student's t test for paired data.

Results

Hypolipidemic effects

Fourteen days after injection with antikidney serum, rats showed typical signs of nephrotic syndrome. Compared to normal rats, nephrotic rats were observed to have a 24-fold increase in 24-hr urinary protein content and a decrease in the albumin/globulin ratio owing to a marked decrease in the serum albumin concentration.

KCD-232 showed a tendency to reduce the enhanced urinary protein content in nephrosis, but did not modify the albumin/globulin ratio (Table 1).

Serum total cholesterol, triglyceride and phospholipid levels of nephrotic rats were increased, respectively, 5.0 fold, 7.6 fold and 3.8 fold compared to those of normal rats. KCD-232 reduced the elevated serum lipid levels in a dose-dependent manner.

KCD-232 was particularly effective on triglyceride.

By contrast, Clofibrate failed to suppress the elevated triglyceride level (Table 2).

In nephrotic rats, the percentage increase of PTPL cholesterol level (9.5 fold) was larger than that of HDL cholesterol level (3.1 fold), and an elevation of the PTPL/HDL cholesterol ratio was observed. KCD-232 reduced the elevated HDL cholesterol level by 31\% and the PTPL cholesterol level by 51\% at a dose of 100 mg/kg.

Clofibrate showed a significant suppression of elevated HDL cholesterol level, but did not affect PTPL cholesterol level at a dose of 300 mg/kg (Table 2).

Nephrotic rats showed a significant increase of 18\% in relative liver weight; however, no significant alteration was noted in liver lipid levels. Treatment with KCD-232 induced a further increase of relative liver weight, but this increase was less than that of Clofibrate at the same dose. KCD-

<table>
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<th>Table 1</th>
<th>Effects of KCD-232 and Clofibrate on urinary and serum protein concentrations in experimental nephrotic syndrome rats</th>
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<tr>
<td>Group</td>
<td>Compound</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephrotic</td>
<td>Control</td>
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<tr>
<td></td>
<td>KCD-232</td>
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<td></td>
<td>KCD-232</td>
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<td></td>
<td>Clofibrate</td>
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<tr>
<td>Normal</td>
<td>—</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM of 6 rats/group. Figures in parentheses indicate % of Control.

a) p<0.05 to Control.  b) p<0.01 to Control.  c) p<0.001 to Control.
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232 markedly lowered the liver triglyceride level and elevated the liver phospholipid level in a dose-dependent manner (Table 3).

**Lipid synthesis**

Figure 1 shows the effect of KCD-232 on cholesterol and fatty acids synthesis by liver slices incubated with $^{14}$C-acetate.

In nephrotic rats, the incorporation of $^{14}$C-acetate into cholesterol and fatty acids increased 2.2 fold and 2.5 fold, respectively, compared to that of normal rats. By treatment with KCD-232 at a dose of 100 mg/kg, the increased incorporation into cholesterol and fatty acids was significantly suppressed by 71% and 90%, respectively. The actual rate of this incorporation was less than that of normal rats. By contrast, Clofibrate suppressed by 50% the increased incorporation into cholesterol, but did not interfere with the increased incorporation into fatty acids.

**Post-heparin lipolytic activity (PHLA)**

As shown in Figure 2, nephrotic rats showed a significant decrease of 38% in hepatic triglyceride lipase (HTGL) activity; however, a slight decrease in total PHLA was seen.

KCD-232 enhanced the depressed HTGL activity and brought it back toward normal, Clofibrate induced a significant decrease in total PHLA. This decrease mainly depended on the change in lipoprotein lipase activity.

**Discussion**

It is generally agreed that the hyperlipidemia of nephrotic syndrome results from increased hepatic synthesis\(^1\) and impaired removal of lipoproteins\(^16,17\). The experimental nephrotic syndrome appears to provide an excellent model of endogenous hyperlipidemia to evaluate the actions of hypolipidemic drugs.

Therefore, by using experimental nephrotic rats produced by antikidney serum, the hypolipidemic actions of KCD-232 were compared with those of Clofibrate. Our present results also proved that the hepatic synthesis of cholesterol and fatty acids is significantly increased in nephrotic rats. This increased lipogenesis is suggested to be the main cause of the elevated serum lipids of nephrotic rats. To determine the lipoprotein cholesterol distribution, we carried out investigations by the phosphotungstic-Mg$^{++}$ precipitable method. Our preliminary experiment has confirmed that the phosphotungstic acid non-

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Table 3 Effects of KCD-232 and Clofibrate on liver lipids in experimental nephrotic syndrome rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Compound</th>
<th>Dose mg/kg/day</th>
<th>Liver weight % b.w.</th>
<th>Cholesterol mg/g liver</th>
<th>Triglyceride mg/g liver</th>
<th>Phospholipid mg/g liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nephrotic</td>
<td>Control</td>
<td>—</td>
<td>4.64 ± 0.22 (100)</td>
<td>2.80 ± 0.15 (100)</td>
<td>6.19 ± 0.55 (100)</td>
<td>30.87 ± 0.72 (100)</td>
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<td></td>
<td>KCD-232</td>
<td>100</td>
<td>5.08 ± 0.24 (109)</td>
<td>3.03 ± 0.15 (108)</td>
<td>2.92 ± 0.13b (47)</td>
<td>38.38 ± 0.78c (124)</td>
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<tr>
<td></td>
<td>KCD-232</td>
<td>300</td>
<td>5.27 ± 0.26 (114)</td>
<td>2.87 ± 0.08 (103)</td>
<td>2.47 ± 0.12b (40)</td>
<td>41.69 ± 0.89c (135)</td>
</tr>
<tr>
<td></td>
<td>Clofibrate</td>
<td>300</td>
<td>5.87 ± 0.26 (127)</td>
<td>2.38 ± 0.24 (85)</td>
<td>5.09 ± 1.62 (82)</td>
<td>43.33 ± 1.86b (134)</td>
</tr>
<tr>
<td>Normal</td>
<td>—</td>
<td></td>
<td>3.94 ± 0.20 (8)</td>
<td>2.50 ± 0.11 (85)</td>
<td>6.76 ± 0.80 (82)</td>
<td>32.97 ± 0.36a (134)</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM of 6 rats/group. Conditions are described in Table I. Figures in parentheses indicate % of Control.

a) p<0.05 to Control. b) p<0.01 to Control. c) p<0.001 to Control.

Precipitable fraction corresponded to d>1.063 lipoprotein (HDL) prepared by ultracentrifugation from rat serum, and the precipitable fraction-expressed PTPL contained mainly LDL and VLDL. In our present experiment, nephrotic rats showed a significant increase in cholesterol level in both the HDL and PTPL fractions. However, a decreased HDL level has been observed in human nephrosis.

Marsh et al. have described that an increased hepatic synthesis of the HDL fraction in both humans and rats, combined with greater urinary loss in humans, could contribute to those differences.

KCD-232 significantly reduced the elevated HDL and PTPL cholesterol levels. The percentage reduction of the PTPL cholesterol level was larger than that of the HDL cholesterol level.

We have reported that KCD-232, at the employed dose in the present study, showed a tendency to reduce the HDL cholesterol level in normal rats, and rather led an elevation of the decreased
HDL cholesterol level in rats fed a cholesterol diet. The finding that KCD-232 markedly suppressed the increased incorporation of $^{14}$C-acetate into cholesterol by liver slices confirmed that the cholesterol-lowering effect of KCD-232 depends on the inhibition of hepatic cholesterol synthesis.

On the other hand, Clofibrate significantly reduced the elevated HDL cholesterol level, but not the PTPL cholesterol level at all. The reduced level of total serum cholesterol was accompanied by that of HDL cholesterol. Recently Cayen et al. have shown that Clofibrate treatment in normal rats decreased the HDL cholesterol level. These results suggest that the drug actions against cholesterol catabolism of lipoproteins are different between KCD-232 and Clofibrate, although both drugs have an inhibitory activity on hepatic cholesterol synthesis.

In addition to the elevated level of serum cholesterol, nephrotic rats showed a significant increase in the serum triglyceride level.

It can be considered another possibility that this increase may be attributed to an impairment of triglyceride catabolism, together with the increase in hepatic fatty acids synthesis as described above. Therefore, we examined the influence of post-heparin lipolytic activity (PHLA), which plays an important role in the removal of triglyceride-rich lipoproteins.

PHLA consists of at least two triglyceride lipases, namely lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL).

In patients with nephrotic syndrome, different findings have been reported on PHLA. In some studies, it was normal, whereas in other studies it was reported to be low. Mordasini et al. have reported a selective depression of HTGL activity. In our present study, the total PHLA of nephrotic rats was not significantly different from that of normal rats. This result suggests that nephrotic hypertriglyceridemia is presumably caused by increased hepatic triglyceride synthesis, rather than by decreased triglyceride degradation related to PHLA.

Furthermore, the finding that KCD-232 had no effect on total PHLA suggests that the triglyceride-lowering effect of KCD-232 principally depends on the inhibition of hepatic fatty acids synthesis.

Although it has been shown by several investigators that Clofibrate led to a rise in PHLA, Odonkor et al. more recently have reported the different results that the responses of PHLA to a low (5 IU/kg) and a high (500 IU/kg) dose of heparin were different and PHLA after a high dose
of heparin was low in rats fed a high sucrose diet containing 0.25% (W/W) Clofibrate.

Our finding of an observed decrease in PHLA caused by Clofibrate treatment may be explained by the reason that Clofibrate did not suppress the elevated serum triglyceride level in nephrotic rats.

In nephrotic rats, HTGL activity significantly decreased compared to normal rats. The physiological functions of HTGL in lipoprotein metabolism are still unclear. However, it has been reported that HTGL could play a role in the metabolism of HDL and the removal of remnant lipoprotein. Murase et al. have demonstrated that specific inhibition of HTGL caused an accumulation of IDL in the plasma. Although we did not attempt to prove an accumulation of IDL in the present experiment, Marsh et al. have reported that the IDL level markedly increased in nephrotic rats produced by puromycin aminonucleoside. KCD-232 enhanced the depressed HTGL activity and brought it back toward normal.

This finding suggests that the hypolipidemic effect of KCD-232 may be partly due to an increased efficiency of removal of remnant lipoprotein from the circulation, although the mechanism by which KCD-232 increases HTGL activity remains to be elucidated.

In conclusion, KCD-232 markedly reduced the elevated levels of serum lipids in experimental nephrotic syndrome rats. This effect of KCD-232 mainly depended on the inhibition of hepatic lipid synthesis. KCD-232 may have a profile of action in lipid metabolism different from that of Clofibrate.

References
20) Rosenman, R. H. and Byers, S. O.: Lipoprotein
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Summary

The effect of 4-(4'-chlorobenzyloxy) benzyl nicotinate (KCD-232), a new hypolipidemic compound, on serum and liver lipids, hepatic lipid synthesis and post-heparin lipolytic activity (PHLA) was investigated in experimental nephrotic syndrome rats.

Fourteen days after injection with antikidney serum, rats showed a marked increase in serum cholesterol, triglyceride and phospholipid levels, but not in liver lipids level. Cholesterol levels of high density lipoprotein (HDL) and phosphotyrosine acid-precipitable lipoprotein (PTPL) were increased 3.1 fold and 9.5 fold, respectively. KCD-232 significantly reduced the elevated serum lipids and lowered liver triglyceride in dose-dependent manner. The percentage of reduction of the PTPL cholesterol level was larger than that of the HDL cholesterol level. In nephrotic rats, the incorporation of $^{14}$C-acetate into cholesterol and fatty acids by liver slices increased 2.2 fold and 2.5 fold, respectively. This increased incorporation was significantly suppressed by KCD-232 treatment.

Nephrotic rats showed a significant decrease of 38% in hepatic triglyceride lipase (HTGL) activity, but a slight decrease in total PHLA. KCD-232 enhanced the depressed HTGL activity and brought it back toward normal. These effects of KCD-232 on nephrotic hyperlipidemia were markedly different from those of Clofibrate.

Key words: 4-(4'-chlorobenzyloxy) benzyl nicotinate (KCD-232), Clofibrate, Experimental nephrotic syndrome rats, Hypolipidemic effects, Hepatic lipid synthesis, Post-heparin lipolytic activity.