Effects of γ-Oryzanol and Cycloartenol Ferulic Acid Ester on Cholesterol Diet Induced Hyperlipidemia in Rats

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Abstract—Hypolipidemic effects of γ-oryzanol (OZ) and cycloartenol ferulic acid ester (CAF) on the hyperlipidemia induced by ingestion of a high cholesterol diet (HCD) in male Sprague-Dawley rats were investigated. The test drugs were given orally and intravenously, daily for 12 days with the HCD feeding. The oral administration with OZ and CAF at 100 mg/kg daily for 6 or 12 days did not apparently prevent the hyperlipidemia induced by HCD-feeding. The intravenous administrations with OZ and CAF at 10 mg/kg for 6 days significantly inhibited the increases in serum total cholesterol (TC), phospholipid (PL) and free cholesterol by HCD. OZ and CAF did not inhibit the decreases of TC in high density lipoprotein (HDL-TC) and HDL-PL by HCD. The increases of atherogenic index ([TC-HDL-TC]/[HDL-TC] and [PL-HDL-PL]/[HDL-PL]) with the HCD feeding were reduced by the intravenous administrations of OZ and CAF. Triglyceride, nonesterified fatty acid, lactate dehydrogenase and transaminase (GOT and GPT) markedly decreased below the control level by the intravenous administrations of OZ and CAF for 12 days. These results suggest that the intravenous administrations of OZ and CAF may have accelerated the excretion of lipids in the blood.

The hypolipidemic effect of the usual type of γ-oryzanol has been reported by some workers (1–5). The usual type of γ-oryzanol is a ferulic acid ester of cycloartenol, 24-methylene cycloartenol, cyclobranol and other sterols. We have previously reported the hypolipidemic effect of the usual type of γ-oryzanol as compared to the effect of γ-oryzanol (OZ) with a sterol composition different from that of the usual type in high cholesterol diet (HCD)-fed rats (6). The inhibition of total cholesterol (TC) increase by the administration of OZ was weaker than that of the usual γ-oryzanol, but the inhibition of free cholesterol (FC) and triglyceride (TG) increases and increase of TC in high density lipoprotein (HDL-TC) by OZ were more potent than those of the usual γ-oryzanol, and it may be due to the concentration of cycloartenol as an inclusive ratio of sterols (6). The present study was designed to test this hypothesis, and the effects of cycloartenol ferulic acid ester (CAF) were compared with OZ.

On the other hand, the intestinal absorption of vegetable sterols such as soysterol are markedly lower; therefore, it has been considered that the hypolipidemic mechanism of these sterols was the inhibition of lipid absorption in the intestine (7). Moreover, Gerson et al. (8) have reported that the intraperitoneal administration of β-sitosterol (type of vegetable sterols) increased the lipid excretion from the blood. OZ and CAF, which are types of vegetable sterols, may have the similar effects to β-sitosterol on the lipid metabolism. Therefore, the hypolipidemic effects of the intravenous administration of OZ or CAF on the HCD induced hyperlipidemia in rats were investigated.

Materials and Methods

Animals and drugs: Male Sprague-Dawley strain rats, 5 weeks of age and weighing
about 100 g, were used. OZ and CAF were obtained from Zeria Pharmaceutical Co., Ltd. (Tokyo).

**Methods:** Rats were made hyperlipidemic by a HCD containing 1% cholesterol (6). Control rats were fed commercial food pellets (Nippon Clea Co., Ltd., Tokyo). OZ and CAF were suspended in a vehicle (carboxymethylcellulose 0.06%, propylparaben 0.004%, polyvinylalcohol 0.2%, saline 99.73%) and given by oral administration at 100 mg/kg and by intravenous administration at 10 mg/kg, once a day for 12 days, with HCD feeding. Six rats in each group were sacrificed at the end of the 7th and 13th day. All animals were fasted for 18 hr before sacrifice. Serum was obtained by centrifuging the blood at 2500 rpm for 15 min. Liver lipids were extracted by the method of Bragdon (9).

**Chemical assays:** TC in the serum and liver and HDL-TC in the serum were determined by a colorimetric method (Cholesterol-B Test) (10). Phospholipid (PL) and HDL-PL in the serum were determined enzymatically (Phospholipid-B Test) (11). PL in the liver was determined by a Phospholipids Test Kit based on the method of Yamanishi et al. (12). Free cholesterol (FC) in the serum and TG in the serum and liver were determined enzymatically (Free cholesterol-C Test, Triglyceride-G Test) (13, 14). Nonesterified fatty acid (NEFA) in the serum and liver was determined by a colorimetric method (NEFA Test) (15). HDL-TC and HDL-PL in the serum were separated by the dextran sulfate-magnesium precipitation method (16). Lactate dehydrogenase (LDH) and transaminase (GOT, GPT) were determined by colorimetric methods (LDH-C II Test, Transaminase-C Test) (17, 18). Total protein (TP) and albumin in the serum were determined by the Biuret method (19) and the bromocresole green method (A/G Test) (20). These assays were performed using commercial kits (Wako Pure Chemical Ind. Ltd., Tokyo). TP in the liver was determined by the method of Lowry et al. using bovine serum albumin as a standard (21).

**Statistical analysis:** Results were expressed as the mean±S.D. of 5 to 6 rats. Statistical significances were evaluated by Student’s t-test.

**Results**

**Body weight and liver weight:** Changes in the body weight and liver weight are shown in Fig. 1. Control animals gained weight steadily throughout the experimental period. The animals fed HCD for 6 and 12 days showed an inhibition of body weight gain. The oral and intravenous administrations of OZ and CAF had no effects on these changes by HCD.

**Effects of oral administrations with OZ and CAF on lipids, enzymes and protein in the serum:** The oral administrations of OZ and CAF had no effects on the increases of TC, PL and FC and the decrease of HDL-TC by HCD-feeding (Figs. 1, 2 and 3). The content of HDL-PL decreased by 12 day-administration with OZ to a level below that of the HCD group (Fig. 2). The administration of CAF for 12 days increased the content of NEFA, but LDH activity decreased by 12 day-administrations with OZ and CAF (Fig. 3). The increases of TP and albumin by HCD-fed were not inhibited by the administrations of OZ and CAF (Fig. 4).

**Effects of intravenous administrations with OZ and CAF on lipids, enzymes and protein in the serum:** The administration of OZ for 6 days or CAF for 6 and 12 days inhibited the increase of TC by HCD-feeding. Administration with OZ or CAF for 12 days slightly, but not significantly, inhibited the decrease in HDL-TC by HCD, while the decrease of HDL-PL was not inhibited by the administrations of these drugs (Figs. 1 and 2). The increase in PL content by HCD was inhibited by the administration of OZ for 6 days or CAF for 6 and 12 days (Fig. 2). The atherogenic index was calculated as \([\text{TC-HDL-TC}] / [\text{HDL-TC}](\text{Al-HDL-TC})\) and \([\text{PL-HDL-PL}] / [\text{HDL-PL}](\text{Al-HDL-PL})\). OZ and CAF inhibited the HCD induced increase of the Al-HDL-TC. Furthermore, 6 day-administration of OZ and 12 day-administration of CAF inhibited the increase of the Al-HDL-PL (Fig. 2). The administrations with OZ for 6 days or CAF for 6 and 12 days inhibited the increase of FC by HCD. OZ and CAF showed a marked decrease of TG in comparison to the control and HCD-fed groups (Fig. 3).
The content of NEFA in the administered groups of OZ for 12 days or CAF for 6 and 12 days decreased below that of the HCD-fed group; and 12-day-administration of CAF also decreased the level below the control level (Fig. 3). The administrations of OZ and CAF caused the levels of LDH and GOT to decrease below the levels of these enzymes in the control or HCD-fed group. The administrations with OZ for 6 days or CAF for 6 and 12 days decreased the GPT levels (Fig. 4).

The increase of TP content by HCD-feeding was inhibited by the administration of CAF for 12 days. The administration of OZ for 6 days decreased albumin content below those of the control and HCD-fed group. The increase of albumin content by HCD-feeding was inhibited by the administrations of OZ and CAF for 12 days (Fig. 4).

Lipid and protein contents in the liver:

The intravenous administration with CAF for 12 days slightly, but not significantly, inhibited the increases of TC and TG by HCD. The content of PL decreased by the intravenous administrations of OZ for 6 days or CAF for 6 and 12 days. Furthermore, NEFA content also decreased by the intravenous administration of CAF for 6 days (Fig. 5).

The feeding of HCD and administrations of OZ and CAF did not cause noticeable changes of TP contents in the liver (data not shown).

**Discussion**

In the previous study, we reported that the hypolipidemic effect of OZ may be mainly produced by the CAF among the different sterols in the composition of this compound (6). The present study was designed to demonstrate this inference, and the effect of
Fig. 2. Effects of OZ and CAF on serum lipids and atherogenic index of hyperlipidemia induced by HCD in rats. s-PL, serum phospholipid; s-HDL-PL, PL in serum high density lipoprotein; AI-HDL-TC and AI-HDL-PL, atherogenic index calculated from HDL-TC and HDL-PL. See explanation in Fig. 1.

Fig. 3. Effects of OZ and CAF on serum lipids and LDH of hyperlipidemia induced by HCD in rats. s-FC, serum free cholesterol; s-TG, serum triglyceride; s-NEFA, serum nonesterified fatty acid; LDH, lactate dehydrogenase. See explanation in Fig. 1.
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Fig. 4. Effects of OZ and CAF on transaminase and protein in the serum of rats with hyperlipidemia induced by HCD. GOT and GPT, serum transaminase; s-TP, serum total protein; s-Alb, serum albumin. See explanation in Fig. 1.

Fig. 5. Effects of OZ and CAF on liver lipids of rats with hyperlipidemia induced by HCD. See explanation in Figs. 1, 2 and 3.
OZ was compared with that of CAF. Since
the oral administrations of OZ and CAF at
100 mg/kg did not clearly inhibit the
hyperlipidemia induced by HCD, no com-
parison of the hypolipidemic effects of these
drugs could be made.

On the other hand, it has been reported
that the intraperitoneal administration of β-
sitosterol causes the decrease of cholesterol
content in the blood and liver, and the
mechanism of its hypocholesterolemic effect
is due to the acceleration of cholesterol
oxidation in the liver (8). In the present study,
the intravenous administrations of OZ and
CAF caused a significant inhibition of the
hyperlipidemia in comparison with the oral
administration groups. The excretion of lipids
in the Triton WR-1339 induced hyperlipidemia
in rats was slightly accelerated by the intra-
venous administrations of OZ and CAF (22).
These results demonstrate that OZ and CAF
have an accelerative effect on the lipid
excretions from the blood and liver.

The enzyme activities (LDH, GOT, GPT)
in the serum were not changed by the feeding
of HCD, while there were decreases of
enzyme activities by the intravenous adminis-
trations with OZ and CAF. From these results,
it seemed that the liver injuries by HCD
feeding were enlargement of the liver and
fatty degeneration, and it did not cause
necrosis of the hepatocytes. OZ and CAF did
not improve the liver injuries caused by HCD
feeding according to the histological obser-
vations; therefore, the decreases of enzyme
activities in the serum by the intravenous
administrations of OZ and CAF may not
result from an improvement in the injured
liver, but due to an inhibition of enzyme
release from the hepatocytes.

In summary, similar hypolipidemic effects
on the HCD induced hyperlipidemia were
obtained by the intravenous administrations
of OZ and CAF. Therefore, it was not demon-
strated that the hypolipidemic effect of OZ
may be mainly due to CAF. However, these
results on the intravenous administrations of
OZ and CAF suggest that the reinforcement of
hypolipidemic effects by the oral adminis-
tration of these drugs may be obtained by the
increases of those absorption in the intestine.


