HYPO LIPIDEMIC EFFECT OF A NEW ARYLOXY COMPOUND, S-8527, IN EXPERIMENTAL ANIMALS

Kotaro SUZUKI, Shunji AONO and Hiroshi NAKATANI
Research Department, Pharmaceuticals Division,
Sumitomo Chemical Co. Ltd., Osaka, Japan
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Abstract—The hypolipidemic action of 1,1-bis [4'-1"-carboxy-1"-methylpropoxy]-phenyl cyclohexane (S-8527) was studied in rats, mice and rabbits under various experimental conditions to compare the effects with those of clofibrate. Oral ingestion of S-8527 to normal rats for 7 days lowered serum triglycerides and cholesterol by about 27% at 1 mg/kg and 20% at 3 mg/kg, respectively. The hypolipidemic effect of S-8527 was considered to be twenty to thirty times more potent than that of clofibrate but a hepatomegalic effect of S-8527 was not observed at its effective dose ranging from 1 to 30 mg/kg. S-8527 at 3 mg/kg decreased liver triglyceride concentration by about 20% but the decrease of triglyceride concentration was not observed in clofibrate treated groups. Liver cholesterol concentration was not decreased with any of the doses of S-8527 while clofibrate decreased liver cholesterol concentration by about 20% at 300 mg/kg. However, there were no differences in total content of cholesterol in liver between S-8527 and clofibrate treated groups. With Triton injected rats, a single oral dose of 100 mg/kg of S-8527 depressed the increase of serum triglycerides by about 50% while clofibrate did not. In glycerol-fed rats, S-8527 decreased serum and liver triglycerides more effectively than clofibrate, and in normal mice and cholesterol-fed mice, S-8527 was found to be more active than clofibrate, however the hepatomegalic effect of S-8527 was less than that of clofibrate. Hypolipidemic effects in rabbits were not observed with either S-8527 or clofibrate.

During a course of studies on hypolipidemic activity of various compounds synthesized in our laboratory, we found that a new aryloxy compound, 1,1-bis [4'-1"-carboxy-1"-methylpropoxy]-phenyl cyclohexane (S-8527) caused a marked decrease in serum lipids level at a smaller dose than that required for clofibrate and that there was a less hepatic effect at the effective dose in rats (1, 2). As studies concerning the dose-response relationship in a wider range of doses of S-8527 were not included in the previous report (1), we do so herein.

Recently, several new compounds of this class have been reported to show a lipid-lowering effect in rats and humans at a smaller dose than that required for clofibrate, but one of the general biological features of these compounds is that there is a marked hepatomegalic effect in parallel with the hypolipidemic activity in the rodent (3–7).

In the present study, an elucidation of the dose-response relationship for hypolipidemic activity of S-8527 in normal rats and its effect on hyperlipidemia induced by Triton injection, cholesterol feeding and glycerol loading in rats and mice are given. Hypolipidemic activity of S-8527 in normal mice and rabbits is also investigated.
Animal models and protocols

Normal rats, mice and rabbits: Male Wistar rats weighing 100–160 g were used. S-8527 and clofibrate were suspended in an appropriate amount of 5% gum arabic solution so that the daily dose would be 0.5 ml per 100 g of body weight. The drugs were given to the rats via stomach tube every a.m. for 7 days. Control groups were on an equal volume of vehicle. During the experimental period, the animals were fed on a commercial chow pellet (NIPPON CLEA, CE-2) ad libitum. About 24 hr after the last dose, the rats were anesthetized with ether and blood samples were obtained from the inferior vena cava. After sacrifice, the livers were removed, washed with physiological saline, blotted on filter paper and weighed.

Male mice weighing 13–15 g were fed a commercial diet (NIPPON CLEA, CA-1, powdered form). The test compounds were mixed with the diet and given for 10 days ad libitum. At the end of the feeding period, the animals were sacrificed by decapitation under ether anesthesia and blood samples were collected. The livers were excised. Blood and liver samples from five mice were each pooled for lipids analysis.

Male white rabbits weighing about 2.0 kg were fed 100 g per day of a commercial diet (NIPPON CLEA, CR-1, powdered form). The test compounds were mixed with the diet and given for 4 weeks. At the end of the period, the animals were anesthetized with pentobarbital and blood samples were drawn from the heart. The livers were excised.

Glycerol-fed rats: Male Wistar rats weighing 240–280 g were given 15% glycerol as drinking fluid according to the method of Nikkilä et al. (8) for 7 days and maintained on a commercial chow pellet.

Triton-induced hyperlipidemic rats: Male Wister rats weighing 180–220 g were fasted for 18 hr and then injected with an aqueous solution of Triton WR-1339 (Rohm and Haas Co.) in a dose of 400 mg/kg i.v. as described by Garattini et al. (9). The test compounds were given via stomach tube immediately after the injection of Triton. After 18 hr, blood samples were taken from the inferior vena cava with the animals under ether anesthesia.

Cholesterol-fed mice: Male mice weighing 13–15 g were fed a high cholesterol diet which consisted of 1% of cholesterol, 0.5% of bile extracts, 10% of hydrogenated coconut oil and 88.5% of commercial diet (NIPPON CLEA, CE-2, powdered form). The test compounds were mixed with the diet and given to mice for 10 days ad libitum.

Analytic procedures

Serum cholesterol and triglycerides were determined by a Technicon AutoAnalyzer (10). The liver samples were homogenized with chloroform-methanol (2:1) and total lipids were extracted. The extracts were washed with 0.7% sodium chloride solution according to the method of Folch et al. (11) and used for determination of liver cholesterol and triglycerides.

Test compounds

S-8527 was synthesized in this laboratory and is in the form of a white crystalline
powder. Clofibrate (ethyl p-chlorophenoxyisobutyrate) was obtained from Imperial Chemical Industries Ltd. in England.

RESULTS

Effects on normal rats, mice and rabbits

The percentage changes of serum total cholesterol and triglyceride levels in rats that were on daily oral doses of 1 mg/kg to 100 mg/kg of S-8527 and 30 mg/kg to 300 mg/kg of clofibrate for 7 days are shown in Fig. 1 and Fig. 2. S-8527 decreased serum cholesterol by about 20% at 3 mg/kg and 35% at 100 mg/kg. Clofibrate, on the other hand, lowered serum cholesterol by about 24% at 100 mg/kg and

![Fig. 1. Percentage changes in serum cholesterol concentration in rats after daily oral doses of S-8527 for 7 days. Numbers of rats used were control group, 24; each treated group, 12. See “Methods” for details. *** Significantly different from control (p<0.001).](image)

![Fig. 2. Percentage changes in serum triglyceride concentration in rats after daily oral doses of S-8527 for 7 days. Experimental conditions are as in Fig. 1. * Significantly different from control (p<0.05) ** Significantly different from control (p<0.01) *** Significantly different from control (p<0.001).](image)

![Fig. 3. Percentage changes in liver triglyceride concentration in rats after daily oral doses of S-8527 for 7 days. Experimental conditions are as in Fig. 1. * Significantly different from control (p<0.05) *** Significantly different from control (p<0.001).](image)
27% at 300 mg/kg. S-8527 lowered serum triglycerides by about 27% at 1 mg/kg and 53% at 100 mg/kg. Clofibrate lowered serum triglycerides by about 22% at 100 mg/kg and 39% at 300 mg/kg.

Fig. 3 shows the percentage changes of liver triglyceride levels in rats treated with various doses of S-8527 and clofibrate. S-8527 decreased liver triglyceride level by about 20% at 3 mg/kg and 36% at 100 mg/kg. On the contrary, the decrease of triglycerides was not observed in rats treated with clofibrate at the doses ranging from 30 to 300 mg/kg. Liver total cholesterol was not decreased with doses ranging from 1 to 100 mg/kg of S-8527 for 7 days as shown in Fig. 4. Clofibrate decreased by about 15% at dose of 100 mg/kg and 20% at 300 mg/kg.

Fig. 4. Percentages changes in liver total cholesterol concentration in rats after daily oral doses of S-8527 for 7 days. Experimental conditions are as in Fig. 1.

* Significantly different from control (p<0.05)

*** Significantly different from control (p<0.001)

Fig. 5 shows the changes in liver weight (g/100 g body wt.) in rats after daily oral doses of S-8527 for 7 days. Experimental conditions are as in Fig. 1.

* Significantly different from control (p<0.05)

*** Significantly different from control (p<0.001)

In this study, the average body wt. gain of rats in the control was 57 g for 7 days. Interference with growth of animals did not occur at doses ranging from 1 mg/kg to 100 mg/kg of S-8527 and 30 mg/kg to 300 mg/kg of clofibrate during the experimental period.

Table 1 shows the serum and liver lipids and liver weight in mice fed various concentrations of S-8527 and clofibrate for 10 days. S-8527 lowered serum triglycerides at a concentration of 0.025% by about 40% but liver weight did not change. S-8527 at 0.2% lowered serum triglycerides and liver cholesterol about 58% and 15%, respectively, and increased liver weight by about 26%. Clofibrate decreased serum triglycerides by about 40%, at a dose of 0.1% and 59% at a dose of 0.4%. Increase in liver weight was observed at a dose of 0.1% by about 8% and at a dose of 0.4% by about 55%, respectively.

Table 2 shows the serum and liver lipids in rabbits treated with daily oral doses of 50
mg/animals and 250 mg/animal of S-8527 and 250 mg/animal of clofibrate for 4 weeks. The hypolipidemic effects of S-8527 and clofibrate were not observed. Increase of serum triglycerides was found in S-8527 treated groups. Liver weight was not affected by either S-8527 or clofibrate.

**Effects on glycerol induced hyperlipidemia in rats**

As shown in Table 3, administration of 15% glycerol to rats increased serum triglycerides by about 100%, liver triglycerides by about 30% and liver cholesterol by about 20% when compared with rats on a normal diet. S-8527 decreased serum and liver triglycerides more effectively than clofibrate but did not lower serum and liver cholesterol. Increase of liver weight by S-8527 was less than that of clofibrate.

### Table 1. Effect of S-8527 on serum and liver lipids in mice

| Treatment | Body wt. gain (g/10 days) | Liver wt. (g/100 g body wt.) | Serum lipids TC (mg/100 ml) | Liver lipid TC (mg/100 g) |
|-----------|---------------------------|-------------------------------|-----------------------------|--------------------------|
| Control   | 60                        | 8.05 ± 0.40                   | 6.41 ± 0.12                 | 126.6 ± 3.5              | 98.6 ± 7.9              | 254.5 ± 10.5 |
| S-8527    | 0.025% (30)              | 7.95 ± 0.23                   | 6.14 ± 0.14                 | 122.2 ± 4.7              | 59.3 ± 3.7**            | 256.5 ± 8.9 |
| S-8527    | 0.05% (30)               | 8.16 ± 0.54                   | 6.49 ± 0.10                 | 122.2 ± 6.4              | 61.8 ± 6.6***           | 240.5 ± 4.4 |
| S-8527    | 0.1% (30)                | 7.21 ± 0.57                   | 6.72 ± 0.18                 | 116.5 ± 6.1              | 49.7 ± 3.3**            | 240.5 ± 10.2 |
| S-8527    | 0.2% (30)                | 6.64 ± 0.49                   | 8.10 ± 0.25***             | 114.8 ± 8.2              | 41.8 ± 1.3***           | 215.2 ± 6.7* |
| Clofibrate | 0.1% (30)               | 7.82 ± 0.44                   | 6.93 ± 0.15*               | 132.3 ± 7.9              | 58.0 ± 4.0**            | 234.2 ± 8.9 |
| Clofibrate | 0.2% (30)               | 6.69 ± 0.55                   | 8.26 ± 0.27***             | 136.3 ± 4.4              | 58.5 ± 4.0**            | 243.0 ± 15.9 |
| Clofibrate | 0.4% (30)               | 5.13 ± 0.38***               | 9.87 ± 0.25***             | 134.0 ± 5.8              | 40.7 ± 8.8***           | 210.7 ± 8.5* |

Each value represents the mean ± S.E. of 6-12 pooled samples. Number of animals is shown in parenthesis. TC, total cholesterol. TG, triglycerides.

* Significantly different from control (p<0.05)
** Significantly different from control (p<0.01)
*** Significantly different from control (p<0.001).

### Table 2. Effect of S-8527 on serum and liver lipids in rabbits

| Treatment | Body wt. gain (kg/4 weeks) | Liver wt. (g/100 g body wt.) | Serum lipids TC (mg/100 ml) | Liver lipids TC (mg/100 g) |
|-----------|---------------------------|-------------------------------|-----------------------------|--------------------------|
| Control   | 0.44 ± 0.33              | 3.48 ± 0.27                   | 45.1 ± 6.3                  | 43.9 ± 6.3               | 217.1 ± 9.2             | 207.1 ± 22.2 |
| S-8527    | 50 mg                     | 0.24 ± 0.04**                 | 3.88 ± 0.35                 | 41.4 ± 5.9               | 70.9 ± 9.2*            | 206.8 ± 5.0  |
| S-8527    | 250 mg                    | 0.22 ± 0.06**                 | 3.59 ± 0.21                 | 63.1 ± 13.8              | 80.0 ± 13.1*           | 232.6 ± 10.7 |
| Clofibrate | 250 mg                    | 0.22 ± 0.03**                 | 3.66 ± 0.21                 | 34.0 ± 3.8               | 56.8 ± 5.8             | 211.4 ± 4.5  |

Each value represents the mean ± S.E. of 8 animals. TC, total cholesterol. TG, triglycerides.

* Significantly different from control (p<0.05).
** Significantly different from control (p<0.01).
TABLE 3. Effect of S-8527 on glycerol induced hyperlipidemia in rats

| Treatment          | Body wt. gain (g/7 days) | Liver wt. (g/100 g body wt.) | Serum lipids (mg/100 ml) | Liver lipids (mg/100 g) |
|--------------------|--------------------------|-------------------------------|--------------------------|-------------------------|
| Control            | 25.8 ± 5.8               | 4.56 ± 0.11                  | 61.7 ± 4.0               | 167.5 ± 23.7            | 305.8 ± 17.7            | 728.0 ± 41.6            |
| (15% glycerol)     |                          |                               |                          |                         |                         |                        |
| (6)                |                          |                               |                          |                         |                         |                        |
| S-8527             | 20.8 ± 7.3               | 5.56*** ± 0.16               | 50.0 ± 2.0               | 70.0* ± 5.8             | 367.0 ± 38.4            | 468.0*** ± 70.4         |
| 30 mg/kg (4)       |                          |                               |                          |                         |                         |                        |
| Clofibrate         | -12.0 ± 4.9              | 6.22*** ± 0.24               | 45.0* ± 3.4              | 100.0* ± 11.0           | 235.0** ± 9.0           | 566.8 ± 90.2            |
| 300 mg/kg (6)      |                          |                               |                          |                         |                         |                        |
| Normal diet        | 41.7 ± 3.0               | 4.11** ± 0.08                | 59.2 ± 3.9               | 85.8** ± 7.2            | 255.0* ± 8.2            | 561.5** ± 29.1          |
| (6)                |                          |                               |                          |                         |                         |                        |

Each value represents the mean ± S.E. Number of animals is shown in parenthesis.
TC, total cholesterol. TG, triglycerides.
* Significantly different from control (p<0.05).
** Significantly different from control (p<0.01).
*** Significantly different from control (p<0.001).

Fig. 6. Effect of S-8527 on Triton induced hyperlipidemia in rats. The values represent the mean ± S.E. of 5-6 animals.
TC, total cholesterol. TG, triglycerides.
** Significantly different from control (p<0.01)
*** Significantly different from control (p<0.001)

Effects on Triton induced hyperlipidemia in rats

Injection of Triton produced a marked hyperlipidemia in rats as is shown in Fig. 6. A single oral dose of 100 mg/kg of S-8527 depressed the increase of cholesterol and triglycerides by about 23% and 50%, respectively. Clofibrate at 500 mg/kg inhibited the increase of cholesterol by about 18% but did not inhibit the increase of triglycerides.

Effects on cholesterol-fed mice

Table 4 shows the serum and liver lipids and liver weight in cholesterol-fed mice at various concentrations of S-8527 and clofibrate for 10 days. Hypcholesterolemic action of S-8527 was found at concentration of 0.05% in diet. S-8527 at 0.2% decreased both serum and liver cholesterol and the potency of S-8527 at 0.2% was nearly equal to that of clofibrate at 0.4%. The hepatomegalic action of clofibrate was greater than that of S-8527.
TABLE 4. Effect of S-8527 on serum and liver lipids in cholesterol-fed mice

| Treatment | Body wt. gain (g/10 days) | Liver wt. (g/100 g body wt.) | Serum lipids (mg/100 ml) | Liver lipid TC (mg/100 g) |
|-----------|--------------------------|-----------------------------|-------------------------|-------------------------|
| Control   | (40) 8.93 ± 0.43         | 6.66 ± 0.21                 | 306.0 ± 15.9            | 2260 ± 300              |
| S-8527    | 0.05% (20) 8.80 ± 0.22   | 6.96 ± 0.25                 | 226.0 ± 8.2**           | 1790 ± 490              |
| S-8527    | 0.1 % (20) 8.31 ± 0.36   | 7.61 ± 0.05***             | 209.0 ± 3.8**           | 1240 ± 380              |
| S-8527    | 0.2 % (20) 6.67 ± 0.43** | 8.65 ± 0.30***             | 198.8 ± 7.4**           | 940 ± 260*              |
| Clofibrate| 0.2% (20) 8.41 ± 0.79*   | 8.47 ± 0.38***             | 262.0 ± 32.5            | 1440 ± 340              |
| Clofibrate| 0.4% (20) 6.82 ± 0.45    | 10.90 ± 0.49***            | 244.8 ± 13.9*           | 1200 ± 230*             |

Each value represents the mean ± S.E. of 4-8 pooled samples.  
Number of animals is shown in parenthesis.  
TC, total cholesterol. TG, triglycerides.  
* Significantly different from control (p<0.05).  
** Significantly different from control (p<0.01).  
*** Significantly different from control (p<0.001).

DISCUSSION

The present study shows that S-8527 has a potent hypolipidemic effect in rats and mice under various experimental conditions.

In rats at oral doses of 1-3 mg/kg of S-8527, significant reductions in serum triglycerides and cholesterol were noted and the hypolipidemic effect of S-8527 was dose-dependent. From dose-response curves, S-8527 was considered to be about twenty to thirty times more potent than clofibrate but did not show the hepatomegaly at the dose which lowered serum and liver lipids levels confirming the results of previous reports (1, 2).

The two drugs are different with regard to their effect on the concentration of liver cholesterol in rats. Clofibrate decreased the concentration of cholesterol while S-8527 did not. As clofibrate increased the liver weight, the total content of lipids was compared with that of control. The total content of liver cholesterol was: 23.4 mg with 100 mg/kg of S-8527; 23.9 mg with 300 mg/kg of clofibrate and 22.9 mg in control. Thus, both compounds tended to increase the total content of liver cholesterol. It is possible, therefore, that the lipids lost from the serum pool are shifted to the liver as pointed out by Timms et al. (4).

S-8527 and clofibrate also differ in the effects on liver triglycerides. S-8527 decreased the concentration of liver triglycerides while clofibrate did not. When S-8527 was given to rats, the total content of liver triglycerides was significantly decreased; 28.2 mg per liver with 100 mg/kg of S-8527 and 40.7 mg in control. On the contrary, because of hepatomegaly produced by clofibrate treatment, total content of triglycerides in 300 mg/kg of clofibrate treated group (66.1 mg per liver) was greater than that in control (41.1 mg) as reported by Best et al. (12) and Gould et al. (13). In addition, in Triton injected rats, the lowering effect of serum triglycerides was observed in S-8527 treated groups only. When
S-8527 was administered to rats given glycerol as drinking fluid and to rats a diet containing sucrose as the only source of carbohydrate (1), significant reduction of serum and liver triglycerides was also observed. No measurement of incorporation of radioactive precursor into lipids was made, but the above results suggest that S-8527 inhibits the formation of endogenous triglycerides.

In normal mice and cholesterol-fed mice, the lowering effect of S-8527 in liver and serum lipids was more potent than that of clofibrate.

In rabbits, clofibrate did not affect serum lipids and liver weight confirming the results of Byers et al. (14). Hypolipidemic effect and hepatomegaly of S-8527 were not observed. These results suggest that the lowering effect of aryloxy compounds depends on the difference in lipid metabolism between rats and rabbits (15).

Our present results suggest that in addition to quantitative difference in their activity, S-8527 and clofibrate differ regarding certain mechanisms of hypolipidemic action. Further investigation is underway in this laboratory.

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