Chronopharmacology of Probucol in Mice

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ABSTRACT — Mice were maintained under conditions of light from 7 a.m. to 7 p.m. and dark from 7 p.m. to 7 a.m. Probucol was given orally to these animals once daily at 10 a.m. or 10 p.m. for 7 days. Blood samples for serum cholesterol were obtained at 24 hours after the final dosage. Blood samples for plasma probucol were obtained just before and at 3, 6, 12, 24, 48, 72, 96 and 120 hours after the final dosage. The cholesterol lowering effect of the agent at 10 p.m. was greater than that at 10 a.m. Plasma probucol concentrations of the two trials did not differ at any observation point. These data suggest that the effect of probucol varies with its time of administration. This might not be caused by a time-dependent change in plasma probucol concentration.

Keywords: Chronopharmacology, Probucol, Cholesterol, Pharmacokinetics

Probucol is a highly lipophilic agent and is widely prescribed for the treatment of hypercholesterolemia (1). Probucol induces a dose-dependent increase of its plasma concentrations after oral administration and causes a dose-dependent reduction of serum total cholesterol (2, 3).

There is increasing evidence demonstrating that plasma concentrations of lipophilic agents depend on their time of oral administration (4). As probucol is very lipophilic, it is anticipated that plasma probucol concentrations also vary with its time of administration which, in turn, might lead to the time-dependent changes in the effect of the agent. To address this issue, probucol was given orally to mice once a day in the day or night for 7 days. The cholesterol lowering effect of the agent and its plasma concentrations were compared between the day and night trials.

MATERIALS AND METHODS

Male STD: ddY mice (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan) at 10 weeks of age (30–35 g) were maintained for more than 2 weeks under conditions of light from 7 a.m. to 7 p.m. and dark from 7 p.m. to 7 a.m. with free access to food and water.

Experiment 1: Probucol (Daiichi Seiyaku Co., Ltd., Tokyo, Japan) or its vehicle alone was given orally to the first group of mice at 10 a.m. (day trial) and to the second group of animals at 10 p.m. (night trial). Each group of mice received 0.5 ml of olive oil (n = 20) or probucol at 200 (n = 20), 400 (n = 20) or 800 (n = 20) mg/kg in 0.5 ml of olive oil once a day for 7 days. Blood samples at 24 hours after the final dosage were obtained from the abdominal aorta under pentobarbital anesthesia.

Experiment 2: Probucol (800 mg/kg in 0.5 ml of olive oil) was given orally to the first group of mice at 10 a.m. and to the second group of animals at 10 p.m. once a day for 7 days. Blood samples were obtained from the abdominal aorta just before and at 3, 6, 12, 24, 48, 72, 96 and 120 hours after the final dosage of the agent (n = 8 for each sampling point in the day and night trials).

Serum total cholesterol was determined by an autoanalyzer (736, Hitachi, Tokyo, Japan). Plasma probucol concentration was measured by a high performance liquid chromatography (5). Maximum plasma concentration (C_max) and time to maximum concentration (t_max) were calculated directly from the data of plasma concentrations for each group. The area under the plasma concentration-time curve from 0 to 120 hours (AUC_0–120) was determined using the trapezoidal rule. The terminal elimination rate constant (K_{el}) was obtained on the basis of least squares regression analysis. The elimination half-life was calculated as follows: \( t_{1/2} = \ln 2 / K_{el} \)

The results are expressed as the mean ± S.E. Data were analyzed by one-way analysis of variance.
RESULTS

Serum concentrations of total cholesterol were decreased dose-dependently by the repeated dosage of probucol in the day and night trials (Fig. 1). No significant difference was observed in this parameter between the two trials in the vehicle-treated mice. However, following probucol treatment, serum concentrations of total cholesterol in the night trial had a tendency to be lower or were significantly lower than those in the day trial. The change from the control value (% reduction) was shown in Table 1.

Plasma probucol concentrations showed a peak at 12 hours after the final dosage of the agent in the day and night trials and then gradually decreased (Fig. 2). No significant difference was observed in this parameter at any observation point between the day and night trials. Pharmacokinetic parameters for each treatment group are shown in Table 2.

Table 1. Percent (%) reduction in serum total cholesterol following probucol at 10 a.m. or 10 p.m. for 7 days in mice

| Probucol (mg/kg) | Administration time |
|-----------------|---------------------|
|                 | 10 a.m.             | 10 p.m.             |
| 200             | 46.5%               | 54.4%               |
| 400             | 54.3%               | 59.2%               |
| 800             | 55.8%               | 64.0%               |

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\text{% reduction} = \left( \frac{T\text{-chol (vehicle)} - T\text{-chol (probucol)}}{T\text{-chol (vehicle)}} \right) \times 100
\]

T-chol (vehicle) = mean serum cholesterol concentration in mice treated with vehicle alone. T-chol (probucol) = mean serum cholesterol concentration in mice treated with probucol.

Fig. 1. Effect of probucol on serum total cholesterol in mice. Mean ± S.E., n = 20 for each group. Probucol or its vehicle alone was given orally at 10 a.m. (□) or 10 p.m. (□) for 7 days, and blood samples were obtained at 24 hours after the final dosage. vehicle = 0.5 ml of olive oil, once a day. P-200, P-400 and P-800 = Probucol at the dose of 200, 400 and 800 mg/kg in 0.5 ml of olive oil, once a day.

Fig. 2. Plasma probucol concentrations following the repeated dosage of the agent in mice. mean ± S.E., n = 8 for each point. Probucol (800 mg/kg in 0.5 ml of olive oil) was given orally at 10 a.m. (○) or 10 p.m. (●), once a day for 7 days. Blood samples were obtained just before and at 3, 6, 12, 24, 48, 72, 96 and 120 hours after the final dosage of probucol.
Table 2. Pharmacokinetic parameters of probucol following the agent at 10 a.m. or 10 p.m. for 7 days in mice

| Parameter           | Administration time | 10 a.m. | 10 p.m. |
|---------------------|---------------------|---------|---------|
| $C_{\text{max}}$, $\mu$g/ml | 13.3                | 14.3    |
| $t_{\text{max}}$, hr | 12                  | 12      |
| $AUC_{0-120}$, $\mu$g·hr/ml | 966.5              | 902.1   |
| $t_{1/2}$, hr       | 67.9                | 66.6    |

$C_{\text{max}}$ = maximum plasma concentration. $t_{\text{max}}$ = time to maximum concentration. $AUC_{0-120}$ = area under the plasma concentration-time curve from 0 to 120 hours. $t_{1/2}$ = elimination half-life.

DISCUSSION

No significant difference was observed in the control values of serum total cholesterol between the day and night trials in the present study, which is similar to the previous findings in rats (6, 7). The present study has demonstrated that the cholesterol lowering effect of probucol is greater when it is administered at 10 p.m. corresponding to the awake period of mice than when it is administered at 10 a.m. which is their sleep period. This suggests that the pharmacological effect of probucol varies with its time of administration.

The rate of absorption of lipophilic agents following oral administration is believed to be faster at night-time than during daytime in nocturnal rodents (4). As probucol is a highly lipophilic agent, its absorption from the gastrointestinal tract might be faster in the night trial which induces a higher plasma probucol concentration and consequently exerts a greater effect on serum total cholesterol. However, the present finding does not support the hypothesis. Absorption of probucol is relatively slow, and the time to peak plasma concentration ($t_{\text{max}}$) is approximately 24 hours after a single oral administration in human subjects (8). In the present study, the value of $t_{\text{max}}$ after the repeated administration of the agent was 12 hours in the day and night trials in mice. Such a delayed absorption might obscure a potential chronopharmacokinetic phenomenon of probucol.

The mechanisms responsible for the cholesterol lowering effect of probucol are not fully understood, although an increase of hepatic cholesterol 7α-hydroxylase activity (9) which is the rate-limiting enzyme in the conversion of cholesterol to bile acid (10), and an enhancement of biliary cholesterol excretion (9) have been proposed. Remarkable diurnal variations are observed in the activity of this enzyme as well as in the biliary excretion of bile acid and cholesterol with a peak at midnight and a nadir during daytime in nocturnal rodents (11, 12). These indicate that the rate of metabolism and excretion of cholesterol per se is lower during daytime than during night-time in these animals. In the present study, plasma probucol concentrations during daytime were higher after the 10 p.m. dosage than after the 10 a.m. dosage. In addition, the existence of an active metabolite of probucol is not known (8). Therefore, the rate of metabolism and excretion of cholesterol during daytime is speculated to be more enhanced in the night than in the day trials. Thus, although the mechanism is not clear in the present study, we think that the diurnal variations in the activity of hepatic cholesterol 7α-hydroxylase and the biliary excretion of bile acid and cholesterol might contribute to the time-dependent change in the effect of probucol. Further studies are needed to evaluate the mechanism of the chronopharmacological phenomenon of probucol.

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