—Regular Articles—

Simvastatin and Atorvastatin Enhance Hypotensive Effect of Diltiazem in Rats

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Effects of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, simvastatin and atorvastatin, on diltiazem-induced hypotension were examined in anaesthetized rats and compared to that of pravastatin. Vehicle, 2 mg/kg/day simvastatin, 2 mg/kg/day atorvastatin, or 4 mg/kg/day pravastatin was administered orally for 4 days. Diltiazem at 3 mg/kg was given orally 2 hours after the final administration of the inhibitors. Arterial blood pressure was measured via a cannula introduced into the left carotid artery, and heart rate was counted from the pulse pressure. In all groups, diltiazem significantly decreased the mean arterial blood pressure without any changes in heart rate. Pretreatment with simvastatin and atorvastatin significantly enhanced the hypotensive effect of diltiazem, while that with pravastatin did not. Heart rate was not modified by pretreatment with the inhibitors. The results indicate that concomitant use of diltiazem with simvastatin or atorvastatin enhances diltiazem-induced hypotension, probably by competitive inhibition of diltiazem metabolism with simvastatin and atorvastatin metabolisms.

Key words——simvastatin; atorvastatin; pravastatin; diltiazem

INTRODUCTION

Cytochrome P450(CYP)3A4 metabolizes several drugs, such as steroid hormones, testosterone;[1] calcium channel blockades, nifedipine and diltiazem;[2,3] 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, lovastatin, simvastatin and atorvastatin;[4—6] benzodiazepines, midazolam;[7,8] and immunosuppressive drugs, cyclosporin.[9] When these drugs are concomitantly used, one drug may competitively inhibit the metabolism of another. This may cause an increase in blood concentration of either drug or both, leading to unexpected effects on the subject ingesting them. There are several reports that the serum concentrations of simvastatin, lovastatin and atorvastatin are increased by itraconazole and grapefruit juice which inhibit CYP3A4.[10—12] However, it is unclear whether pretreatment with HMG-CoA reductase inhibitors influences the pharmacological effect of the drug treated concomitantly. The CYP isoforms in rats are mainly CYP2C11 and CYP3A2, but not CYP3A4 which is a human isoform of CYP.[13] Although diltiazem can be metabolized by CYP3A2,[14] it is uncertain whether a HMG-CoA reductase inhibitor can be metabolized by the same isoforms. Because the rat does not have CYP3A4, the drugs that are metabolized by CYP3A4 in human may be metabolized by CYP3A2. Therefore, we examined the hypotensive effect of diltiazem on rats pretreated with or without HMG-CoA reductase inhibitors. Because both hypercholesterolemia and hypertension are known to be risk factors of ischemic heart disease, these two drugs are often used concomitantly.

METHODS

The investigation conformed to the Guiding Principles for the Care and Use of Experimental Animals in Hokkaido College of Pharmacy (published in 1988; revised in 2001).

Preparation of Animals Male Sprague-Dawley rats, 8-week-old, were used. Either simvastatin (2 mg/kg/day; $n=8$), atorvastatin (2 mg/kg/day; $n=7$), or pravastatin (4 mg/kg/day; $n=8$) was administered by gavage for 4 days. Simvastatin and atorvastatin were dissolved in 0.5% carboxymethyl cellulose (CMC) and pravastatin in distilled water. The same concentration of CMC and distilled water was also given in the vehicle-treated groups; 0.5% CMC ($n=8$) was the vehicle for simvastatin and atorvastatin and distilled water ($n=8$) for pravastatin. On the 4th day, the following procedures were completed within 1 hour after the final administration of vehicle or the HMG-CoA reductase inhibitor;

anaesthetization with sodium pentobarbital (50 mg/kg, *i.p.* and a supplementary dose of about 20 mg/kg, *i.p.*), endotracheal intubation to control respiration using a positive-pressure respirator, and cannulation to the left carotid artery to measure arterial blood pressure. After about 1 hour of stabilization (2 hours after the final administration), diltiazem (3 mg/kg) was orally given by gavage. The arterial blood pressure and heart rate were measured every 3 min by a polygraph (Amplifier 7746, San-ei, Tokyo). We put the rat on a board fixed on its back during the experiment, and raised the head-side end of the board when it was given diltiazem. Because this oral administration of diltiazem caused uncontrollable variations in the blood pressure, we were not able to measure the first 3 min of arterial blood pressure after the drug treatment. Mean arterial blood pressure and heart rate were normalized to the respective values obtained before diltiazem treatment, and are expressed as means±S.E.M.

Statistical Analysis Super ANOVA followed by Dunnett's one-tailed analysis evaluated the significance of differences in the blood pressure and heart rate between groups. Differences were considered statistically significant when $p<0.05$.

RESULTS

Pretreatment with Simvastatin and Atorvastatin Simvastatin and atorvastatin were orally pretreated once a day for 4 days. Mean arterial blood pressures in the vehicle (0.5% CMC)-, simvastatin- and atorvastatin-treated groups just prior to diltiazem treatment were 131±4, 133±3, and 135±6 mmHg, respectively. Heart rates in these groups were 460±14, 461±9, and 454±9 beats/min, respectively. There were no significant differences in the blood pressures or heart rates between groups. Figure 1 shows the influence of vehicle, simvastatin and atorvastatin pretreatments on changes in the mean arterial pressure and heart rate caused by diltiazem treatment. Oral administration of diltiazem at 3 mg/kg significantly decreased the mean arterial pressures in all 3 groups. Pretreatment with simvastatin and atorvastatin significantly potentiated the diltiazem-induced hypotension at several time points. Diltiazem did not change heart rate appreciably, and simvastatin and atorvastatin did not modify the heart rate obtained after diltiazem treatment except for the value at 48 min in the atorvastatin-treated group.

Pretreatment with Pravastatin Changes in the mean arterial pressure and heart rate caused by diltia-

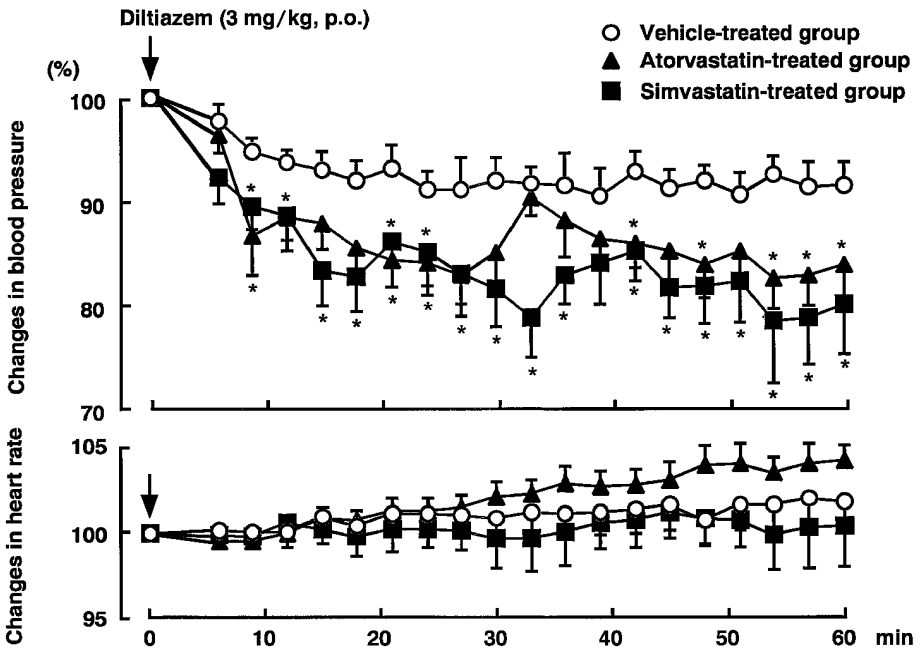

Fig. 1. Effects of Simvastatin and Atorvastatin Pretreatments on Changes in Blood Pressure and Heart Rate induced by Diltiazem

Rats were pretreated with 0.5% CMC as vehicle ($n=8$), 2 mg/kg/day simvastatin ($n=8$), or 2 mg/kg/day atorvastatin ($n=7$) for 4 days. On the 4th day, diltiazem (3 mg/kg) was orally given just 2 hours after the final administration of inhibitors. Values are means with vertical bars indicating S.E.M. *$p<0.05$ compared with the vehicle-treated group at that time.

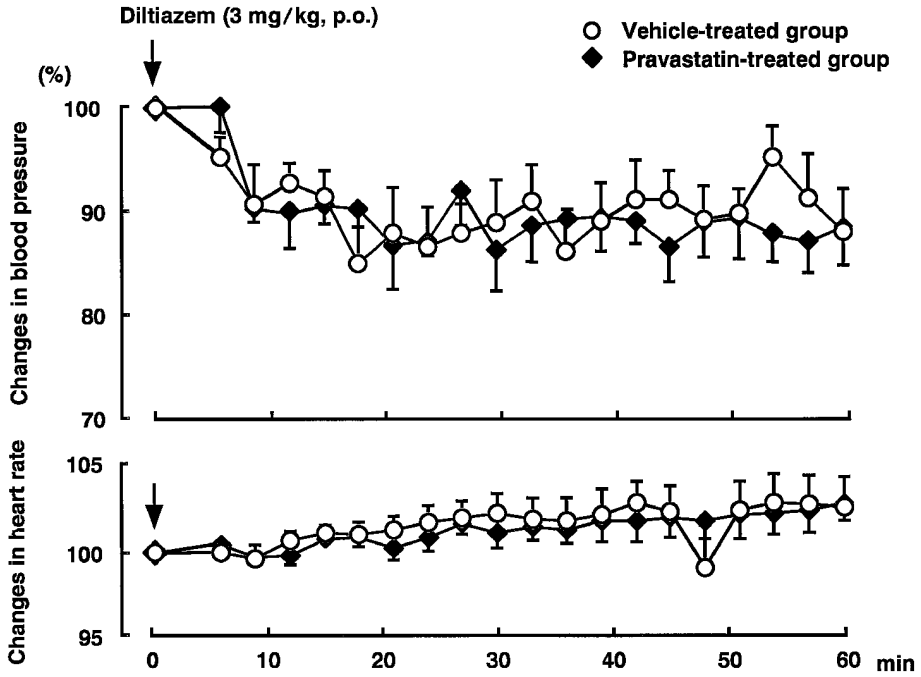

Fig. 2. Effect of Pravastatin Pretreatment on Changes in Blood Pressure and Heart Rate induced by Diltiazem

Distilled water as vehicle ($n=8$) or 4 mg/kg/day pravastatin ($n=8$) was given orally for 4 days. Experimental design is the same as that in Fig. 1. Values are means with vertical bars indicating S.E.M.

zem treatment in the vehicle (distilled water)- and pravastatin-treated groups are shown in Fig. 2; these were 132 ± 4 mmHg and 451 ± 15 beats/min, and 126 ± 7 mmHg and 458 ± 11 beats/min, respectively. The blood pressure and heart rate in the pravastatin-treated group were not significantly different from those in the vehicle-treated group. Diltiazem significantly decreased the mean arterial pressure without any change in heart rate. Pretreatment with pravastatin for 4 days did not affect the changes in mean arterial pressure or heart rate caused by diltiazem treatment.

DISCUSSION

Many investigators have demonstrated the increase in serum concentration of HMG-CoA reductase inhibitors due to interaction with a drug that inhibits CYP3A4. Itraconazole and grapefruit juice markedly increase the serum concentration of simvastatin, lovastatin and atorvastatin.[10—12] These results suggest that the increased HMG-CoA reductase inhibitors increase the incidence of rhabdomyolysis in patients.[15] However, there are few papers that report influences of HMG-CoA reductase inhibitors on pharmacological effects of a drug that is used together with the inhibitor. The present study clearly showed enhancement of the hypotensive effect of diltiazem, when used concomitantly with simvastatin and atorvastatin.

Lypophilic HMG-CoA reductase inhibitors, such as simvastatin, lovastatin, and atorvastatin, are metabolized by CYP3A4 in the liver or small intestine.[4—6] A calcium channel blocker, diltiazem, is also metabolized by CYP3A4.[3] It is possible to consider that the metabolism of diltiazem may be suppressed by a lypophilic HMG-CoA reductase inhibitor, because the two drugs may compete with each other at the CYP3A4. Unfortunately, we did not measure the serum concentration of diltizem in the present study, because we did not have the equipment or technique to do so. Concomitant use of simvastatin or atorvastatin could increase the serum concentration of diltiazem, leading to more potent inhibition of voltage-dependent calcium channels, and then enhance the diltiazem-induced hypotension as compared to the single treatment with diltiazem.

On the other hand, a hydrophilic HMG-CoA reductase inhibitor, pravastatin, is excreted unchanged into urine to a significant extent, and is not much metabolized by CYP3A4.[16] Therefore, the hypotensive effect of diltiazem in the pravastatin-treated group was not different from that in the vehicle-treated group.

There is a difference in CYP isoforms that exist in humans and rats.[13] Rats do not possess CYP3A4, but CYP3A1, 3A2, 3A9, and 3A18 as the CYP3A subfamily.[13] CYP3A2 plays an important role in drug metabolism in rats, and can metabolize diltiazem.[14] However, it is uncertain whether HMG-CoA reductase inhibitors can be metabolized by CYP3A2 in rats. Because the hypotensive effect of diltiazem was potentiated by concomitant use of simvastatin and atorvastatin in the present study, CYP3A2 may be a common enzyme which metabolizes both diltiazem and HMG-CoA reductase inhibitors.

We have demonstrated in previous papers the advantage of hydrophilic HMG-CoA reductase inhibitors over hydrophobic inhibitors. Because a hydrophobic HMG-CoA reductase inhibitor is more membrane permeable, it can inhibit the HMG-CoA reductase in many cells. This causes decreases in the myocardial level of ubiquinone and ATP generation in mitochondria,[17] leading to myocardial contractile dysfunction.[18,19] Hydrophobic HMG-CoA reductase inhibitors also prevent myocardial growth in neonatal piglets through inhibition of intracellular signal transduction.[20] Biosynthesis of ubiquinone and that of isoprenoids required for the signal transduction are regulated by the HMG-CoA reductase.[21] A hydrophilic HMG-CoA reductase inhibitor also is advantageous in clinical use when used with diltiazem.

In conclusion, because concomitant use of diltiazem with hydrophobic HMG-CoA reductase inhibitors enhances its hypotensive effect in rats, a similar drug-drug interaction between them may occur in human, although the isoforms of CYP are different. We should use caution about the dose of diltiazem in patients treated with hydrophobic HMG-CoA reductase inhibitors.

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