ENHANCED MYOPATHY FOLLOWING ADMINISTRATION OF HYPOLIPIDEMIC AGENTS UNDER URETHANE ANESTHESIA

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The enhanced effect of urethane anesthesia on the serum creatine kinase (CPK) level following administration of hypolipidemic agents was examined to develop a convenient experimental screening method for drug-induced myopathy. After oral administration of a hypolipidemic agent to rats, 25% urethane solution was infused intravenously at a very low rate using a microinfusion pump. Blood samples were collected 7 h after drug administration and the risk of myopathy was evaluated based on the CPK level. When bezafibrate (BF), simvastatin (SV), or pravastatin (PV) (50-500 mg/kg) was orally administered under urethane infusion, enhanced elevation of the serum CPK level was observed dose dependently for BF and SV, but not for PV. The elevation of serum CPK was much higher with BF than with SV or PV. In addition, when SV or PV (50-500 mg/kg) was coadministered with 50 mg/kg of BF, there was a striking increase in the serum CPK level as compared with the drug alone. Without urethane infusion, no significant elevation in serum CPK level was observed even at a high dose of these hypolipidemic agents. These phenomena suggest that the urethane anesthesia enhanced the elevation of the serum CPK level following administration of hypolipidemic agents. We propose that this method is a simple and speedy screening test for drug-induced myopathy.

KEY WORDS urethane; myopathy; bezafibrate; simvastatin; pravastatin

The hypolipidemic agents, bezafibrate (BF), simvastatin (SV), and pravastatin (PV) are widely used in the clinical setting. However, it was reported that myopathy has occurred in patients receiving these drugs. 1-7 Although the incidence of adverse effects is low, they must be considered when administering hypolipidemic agents because they sometimes lead to rhabdomyolysis, followed by severe renal injury. The Ministry of Health and Welfare of Japan has also warned of the occurrence of rhabdomyolysis after therapy with hypolipidemic agents in No. 112 (hypolipidemic agents), No. 119 (PV and SV), and No. 129 (BF) in “information on drug adverse reactions.” 8-10 When developing a new hypolipidemic agent, the incidence of myopathy should be investigated.

The present study was designed to develop a new experimental system for screening for drug-induced myopathy.

MATERIALS AND METHODS

BF and PV were kindly donated by Boehringer Mannheim Co., Ltd., Tokyo, Japan, and Sankyo Co., Ltd., Tokyo, Japan, respectively. SV was extracted from commercial products (Lipovase®) and used in the experiments. Urethane (ethyl carbamate) was purchased from Wako Pure Chemical, Co., Ltd., Osaka, Japan. All other chemicals were of analytical reagent grade. Male Wistar rats, aged 8 weeks old (180-200 g), were used. The rats were not allowed to drink water for 2 days before the experiments. The day before the experiments, the jugular vein was cannulated.11 The rats were fasted overnight after operation and then administered one of BF, SV, PV, SV plus BF, or PV plus BF. Appropriate suspensions of BF or SV were prepared in 0.5% aqueous methylcellulose and PV solution was prepared with distilled water. The drug solution was orally administered to the rats via oral gavage at a dosing volume of 5 ml/kg. Control animals were dosed in a similar fashion with water. After a dosing, the rats were held in Boltzmann cages. One hour after drug administration, 25% urethane was intravenously infused to the rats through the jugular cannula at a constant rate of 5 μl/min for 3 h and afterward infused at a constant rate of 1 μl/min (Chart 1). Under these conditions, the CPK levels in the control rats remained unchanged until 8 h, afterward it began to increase slowly. Fifty μl of blood

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was collected from the jugular vein 7 h after drug administration. The blood samples were used to determine the serum creatine kinase (CPK) level, the skeletal muscle marker enzyme. The enzyme was measured spectrophotometrically using a commercial kit (Dainabot, Co., Ltd., Tokyo, Japan). A similar study was also performed without the injection of urethane.

RESULTS AND DISCUSSION

For this study, BF was chosen as the first model drug for estimating drug-induced myopathy. Figure 1 shows the CPK levels 7 h after an oral single administration of BF to rats with or without urethane infusion. Under urethane infusion, sigmoidal dose-response curves for serum CPK were observed after the administration of BF. The level of serum CPK 7 h following 500 mg/kg of BF increased from 169.45 ± 11.07 IU/l in the controls to 837.58 ± 96.33 IU/l with urethane infusion. However, without urethane infusion, 500 mg/kg of BF was not able to increase the serum CPK level. Regarding the effect of urethane infusion itself on the level of serum CPK, the control level of serum CPK (169.45 ± 11.07 IU/l) was unchanged by urethane infusion until the end of the experiment. These phenomena suggest that the infusion of urethane enhanced the serum level of CPK after BF administration. Similarly, this method was also applied to SV and PV, 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (HMGRLs), to evaluate the incidence of myopathy. A high dose of SV caused a slight elevation in the serum CPK level with urethane infusion, but PV did not increase the serum CPK level under the dose range 50-500 mg/kg even with urethane infusion. Considering the usual dose in humans, CPK elevation was not observed even at 1000 times the usual dose of SV or PV, and this indicates that the incidence of HMGRI-induced myopathy is very low. In contrast, the incidence and severity of BF-induced myopathy was thought to be fairly high, because 10 times the usual dose can cause a significant elevation in the serum CPK level.
Recently, it was reported that patients taking fibrates together with HMGRIs often experience myopathy.\textsuperscript{8,9} Therefore, SV and PV (50-500 mg/kg) were compared for their potential to produce myopathy either alone or in combination with BF. In this study, the BF dose was fixed at 50 mg/kg, because this dose did not cause a change in the serum CPK level. Figure 2 shows serum CPK levels after administration SV or PV in the absence or presence of 50 mg/kg of BF. When SV or PV was coadministered with BF, there was a striking increase in serum CPK levels as compared with SV or PV alone. The interaction between BF and SV or PV appeared to be synergistic. Combination therapy with BF and SV or PV was found to be undesirable.

Judging from the present results, our experimental model using urethane anesthesia might be useful to evaluate drug-induced myopathy. General anesthetics, i.e., halothane, are known to induce malignant hyperthermia, which is characterized by muscle injury, myoglobinuria, high fever, and so on. This illness is thought to be the result of an increase in the intracellular concentration of Ca\textsuperscript{2+}.\textsuperscript{12,13} Urethane also may have action similar to these anesthetics and may be associated with calcium regulation in muscle cells.

Nakahara \textit{et al.} reported that the change in the cytosolic free Ca\textsuperscript{2+} concentration in L6 rat myoblasts stimulated with three kinds of HMGRIs, SV, its acid form, and PV.\textsuperscript{14} SV and its acid form induced an increase in cytosolic free Ca\textsuperscript{2+} followed by cell rupture, although PV induced little or no change in cytosolic free Ca\textsuperscript{2+} and no cell damage resulted. The present results showing that SV gave rise to a more significant increment in serum CPK than did PV under urethane infusion is in line with the results from their report. In addition, BF is also reported to induce an increase in cytosolic free Ca\textsuperscript{2+}.\textsuperscript{15} Considering the urethane effect on the elevation of the serum CPK level after administration of hypolipidemic agents, the intracellular concentration of Ca\textsuperscript{2+} following hypolipidemic agents might be increased several fold by urethane, resulting in increments in the serum CPK level according to their potency in increasing cytosolic free Ca\textsuperscript{2+}.

A variety of hypolipidemic agents has now been developed. We must pay attention to their adverse effects as well as pharmacological effects. The present study proposes a simple and speedy screening test for drug-induced myopathy.

REFERENCES

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