

# Simvastatin-Tacrolimus and Simvastatin-Cyclosporin Interactions in Rats

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**ABSTRACT:** The effect of simvastatin (SV)-tacrolimus (TL) and simvastatin-cyclosporin (CyA) interactions on creatine phosphokinase levels and renal and hepatic function were investigated in rats. Animals were divided into seven groups; (1) SV (150 mg kg<sup>-1</sup> oral) alone, (2) SV + TL (150 mg kg<sup>-1</sup> oral + 0.5 mg kg<sup>-1</sup> intraperitoneal (i.p.)), (3) TL (0.5 mg kg<sup>-1</sup> i.p.) alone, (4) SV + CyA (150 mg kg<sup>-1</sup> oral + 10 mg kg<sup>-1</sup> oral), (5) CyA (10 mg kg<sup>-1</sup> oral) alone, (6) control vehicle for oral administration, and (7) control vehicle for i.p. administration. A marked reduction in body weight and mortality was observed in the (SV + CyA) and (SV + TL) groups. Plasma creatine kinase levels in the (SV), (TL), (SV + CyA) and (SV + TL) groups, 7 days postadministration, were significantly higher compared with those before administration ( $p < 0.05$ ). The plasma urea nitrogen levels in the (TL), (SV + CyA) and (SV + TL) groups after 7 days of administration were significantly higher than those of the controls. In addition, a marked increase in the plasma levels of alanine and aspartate amino transferases were observed in the (SV + CyA) groups. © 1998 John Wiley & Sons, Ltd.

**Key words:** simvastatin; tacrolimus; cyclosporin; interaction; creatine phosphokinase; rats

## Introduction

Simvastatin (SV), a HMG-CoA reductase inhibitor, represents a highly effective therapeutic class of drugs for the treatment of hypercholesterolemia. This agent acts as a competitive inhibitor of the reductase, a rate limiting enzyme in cholesterol biosynthesis [1–3]. However, it is well documented that inhibitors of HMG-CoA reductase cause side-effects such as gastrointestinal disturbances, hepatitis, neuropathy and rhabdomyolysis [1,3–14]. Rhabdomyolysis, which leads to significantly increased plasma creatine phosphokinase (CK) levels and the development of myoglobulinuria and nephrosis, is a serious clinical problem [6–14].

Cyclosporin A (CyA), a potent immunosuppressive agent, is used in the treatment of organ transplantation and psoriasis [15]. It is well known that this drug also causes side-effects such as nephritis and hepatitis [16–18]. The appearance of these side-effects depend on the blood CyA concentration [17]. Previous work has shown that renal transplant recipients have a tendency toward contracting hyperlipidemia [19,20]. Thus, these recipients often receive cholesterol-lowering drugs, such as HMG-CoA reductase inhibitors [21]. Further-

more, the coadministration of CyA with HMG-CoA reductase inhibitors has been reported to increase the frequency of rhabdomyolysis in humans and animals [11,22–24]. By contrast, tacrolimus (TL) is a novel immunosuppressive agent, which is over a 1000 times more immunosuppressive than CyA. Tacrolimus is also used in association with liver and kidney transplantations [25,26].

However, the relationship between the pharmacokinetics of TL and the development of toxicity following coadministration of SV and TL is unclear.

In this paper, the interactions between SV and two immunosuppressive agents were studied. The effects of coadministration of SV with TL or CyA on plasma CK levels, as an indicator of myotonia development, and on renal and hepatic function were investigated in rats.

## Materials and Methods

### Animals

Male Wistar rats (150–165 g) were purchased from Japan Laboratory Animals Co. (Tokyo, Japan). Animals were housed individually in stainless-steel cages and allowed free access to tap water and commercial chow (MF experimental animal chow, Oriental East Co., Tokyo, Japan).

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## Materials

SV and TL were generous gifts from Banyu Pharmaceutical Co. Ltd. (Tokyo, Japan) and Fujisawa Pharmaceutical Co. Ltd. (Osaka, Japan), respectively. CyA (Sandimmun®) was purchased from Sandoz Pharmaceuticals Co. (Tokyo, Japan). Carboxymethyl cellulose sodium salt (CMC), CPK II test Wako, GPT (ALT) test Wako and GOT (AST) test Wako were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Seralizar® for the plasma urea nitrogen assay was obtained from Miles-Sankyo Co. (Tokyo, Japan). IMx Tacrolimus Dynapack and TDx Cyclosporin Dynapack were purchased from Dynabott Co. (Tokyo, Japan). All other reagents were of the analytical grade and commercially available.

## Administration and Preparation of Compounds

SV and CyA were resuspended in a 0.5% CMC aqueous solution, respectively. The solution containing SV or CyA was given orally (p.o.) at a dose of 5 mL kg<sup>-1</sup>. TL was dissolved in ethanol and then resuspended in 0.5% CMC aqueous solution. The final ethanol concentration of the solution was 10% (v/v). This solution was given intraperitoneally (i.p.) at a dose of 1 mL kg<sup>-1</sup>. Animals were divided into seven groups as follows: (1) SV 150 mg kg<sup>-1</sup> p.o. alone (SV); (2) SV 150 mg kg<sup>-1</sup> p.o. + TL 0.5 mg kg<sup>-1</sup> i.p. (SV + TL); (3) TL 0.5 mg kg<sup>-1</sup> i.p. alone (TL); (4) SV 150 mg kg<sup>-1</sup> p.o. + CyA 10 mg kg<sup>-1</sup> p.o. (SV + CyA); (5) CyA 10 mg kg<sup>-1</sup> p.o. alone (CyA); (6) control vehicle for oral administration (control p.o.); and (7) control vehicle for intraperitoneal administration (control i.p.). A 0.5% CMC aqueous solution was used as the control vehicle for oral administration, and a mixture of ethanol and 0.5% CMC aqueous solution (1:9 v/v) was used as the control vehicle for i.p. administration. Each group consisted of eight animals.

Drug administration was performed once a day (11:00–13:00 h) for 7 days. Blood for assay of CK and plasma urea nitrogen was collected from the fundus oculi vein 2 days before administration. Plasma was separated from the blood by centrifugation at 1620 × *g* for 10 min and was stored at –80°C until analysis. On day 7, blood was collected from the caval artery following abdominal surgery under diethylether anesthesia 2 h after the last administration. Blood was collected and divided into two aliquots. The first was used for the determination of CyA and TL concentration, and the other was centrifuged to obtain plasma. The plasma was used for the determination of CK, plasma urea nitrogen, ALT, AST, and SV levels. The plasma and whole blood samples were stored at –80°C.

## SV Assay

SV in plasma was hydrolyzed to simvastatin hydroxy acid (SVA) by adding alkali. SV was quantified as total SVA by a modification of the high-performance liquid chromatographic (HPLC) method of Icona *et al.* [27]: 0.5 mL 0.5 M KOH aqueous solution was added to 0.2 mL plasma and then the mixture was left for 30 min at room temperature to allow complete hydrolysis to SVA. After hydrolysis, 1 mL 0.05M KH<sub>2</sub>PO<sub>4</sub> (pH 7.2) was added to the mixture, and then this was applied to a Bondelut C2 column (1 cc 100 mg<sup>-1</sup>; Varian®, CA, USA) activated successively with 2 vol. water, 2 vol. methanol and 2 vol. water. The column was washed with 2 vol. water, 2 vol. 5% acetonitrile/water, and eluted with 1 mL acetonitrile. The eluate was evaporated to dryness and the residue was dissolved in mobile phase. The chromatographic equipment consisted of the following components: LC-6A liquid chromatography pump (Shimadzu Co., Tokyo, Japan), Nucleosil C18 (25 cm × 5 mm × 7 µm particle size) column and SPD-6AV UV-VIS spectrometric detector (Shimadzu Co., Tokyo, Japan). The mobile phase was a mixture of 60% NH<sub>3</sub>H<sub>2</sub>PO<sub>4</sub> (0.05 M, pH 6.5) and 40% acetonitrile. The flow rate was 1.2 mL min<sup>-1</sup> and the column temperature was maintained at 50°C. Eluted peaks were monitored at a UV wavelength of 238 nm.

## TL and CyA Assay

Whole blood concentrations of TL and CyA were determined by IMx tacrolimus dynapack which utilized a solid phase method based on a competitive EIA method, and TDx cyclosporin sp-dynapack based on a fluorescence polarization immunoassay, respectively [28–30].

## Statistical Analysis

The values are expressed as means ± S.E. Statistical analysis was performed by paired analysis of variance with *p* < 0.05 as the minimal level of significance.

## Results

### Change in Body Weight and Survival

Table 1 summarizes the number of animals surviving after repeated administration in each drug group. Two and three animals in the (SV + TL) and (SV + CyA) groups, respectively, died prior to the 7th day. In the (SV), (TL), (CyA), control (p.o.) and control (i.p.) groups, all animals survived the entire administration period. All animals in the (SV + TL) and (SV + CyA) groups had diarrhea and severe weight loss between the 3rd and 4th days after

Table 1. Number of survivors after repeated administration

Groups	Administration periods (days)						
	1	2	3	4	5	6	7
SV	8/8	8/8	8/8	8/8	8/8	8/8	8/8
SV + TL	8/8	8/8	8/8	8/8	8/8	7/8	6/8
TL	8/8	8/8	8/8	8/8	8/8	8/8	8/8
SV + CyA	8/8	8/8	8/8	7/8	6/8	6/8	5/8
CyA	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Control (p.o.)	8/8	8/8	8/8	8/8	8/8	8/8	8/8
Control (i.p.)	8/8	8/8	8/8	8/8	8/8	8/8	8/8

Number of survivors/total animals.

administration. A significant difference in body weight on the 5, 6 and 7th days was observed for the control and coadministration groups (Figure 1). No significant difference in body weight loss was observed between the control and the (TL), (CyA) or (SV) groups.

### Effect on Plasma CK levels

The plasma CK levels in the (SV), (SV + TL), (TL) and (SV + CyA) groups 7 days after administration were significantly increased compared with those before administration (Figure 2). Although the (SV), (SV + TL), (TL), and (SV + CyA) groups had higher CK levels 7 days after administration compared with the controls (p.o. and i.p.), no significant difference was observed amongst these groups themselves (Figure 2).

### Effect on Renal and Hepatic Function

The plasma urea nitrogen levels in the (SV + TL) and (SV + CyA) groups significantly increased 7 days after administration compared with the levels 2 days before administration (Figure 3). The plasma urea nitrogen levels in the (TL), (SV + TL), and (SV + CyA) groups 7 days after administration were

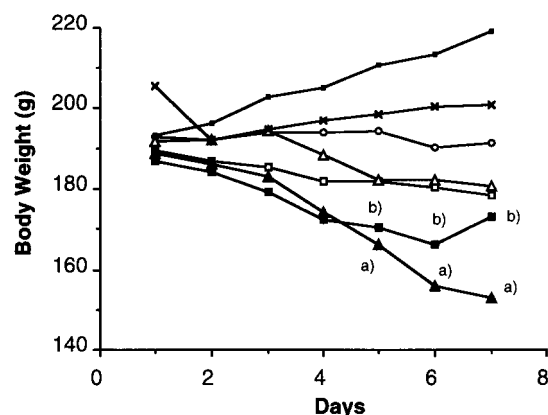


Figure 1. Change in body weight during repeated administration. Each point represents the mean of 5–8 rats: ○, SV; ■, SV + TL; □, TL; ▲, SV + CyA; △, CyA; ×, control (p.o.); ■, control (i.p.). (a)  $p < 0.05$  versus control (p.o.), (b)  $p < 0.05$  versus control (i.p.).

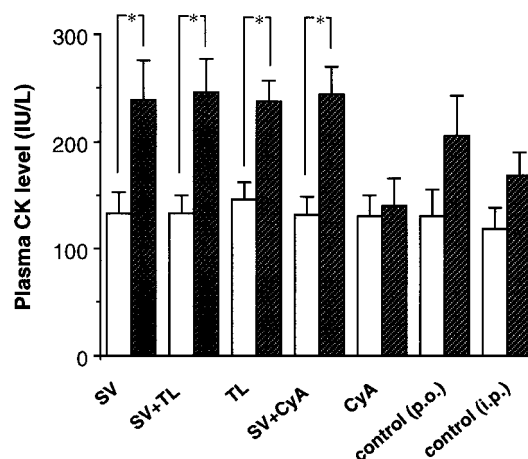


Figure 2. Change in plasma creatine kinase (CK) levels, comparing 2 days before administration and 7 days after administration. Each point represents the mean  $\pm$  S.E. of 5–8 rats. □, 2 days before administration; ■, 7 days after administration, \*  $p < 0.05$

also significantly higher than those in the controls (p.o.). The plasma alanine amino transferase (ALT) and aspartate amino transferase (AST) levels after administration are shown in Figure 4. Both ALT and AST levels in the (SV + CyA) group were markedly higher than those of the controls and other administration groups.

### Plasma and Whole Blood Drug Concentrations

In the (SV + TL) and (SV + CyA) groups, the total SVA plasma concentration was higher than in the (SV) group, but there was no statistically significant difference between the two groups themselves (Figure 5). Although the whole blood concentrations of TL on the 7th day after administration of TL alone was higher than after coadministration with SV, no significant difference was observed between the two

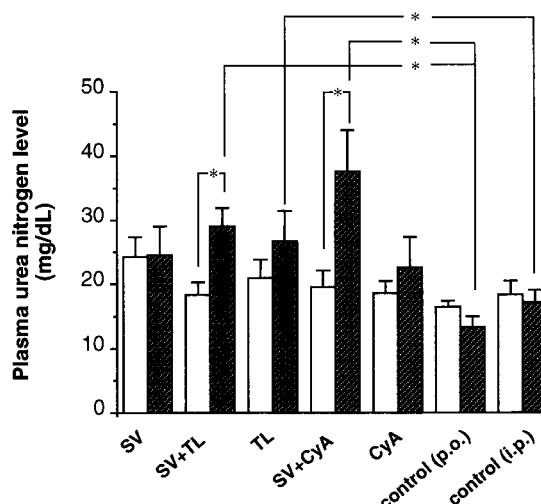


Figure 3. Change in plasma urea nitrogen levels comparing 2 days before administration and 7 days after administration. Each bar represents the mean  $\pm$  S.E. of 5–8 rats. □, 2 days before administration; ■, 7 days after administration; \*  $p < 0.05$

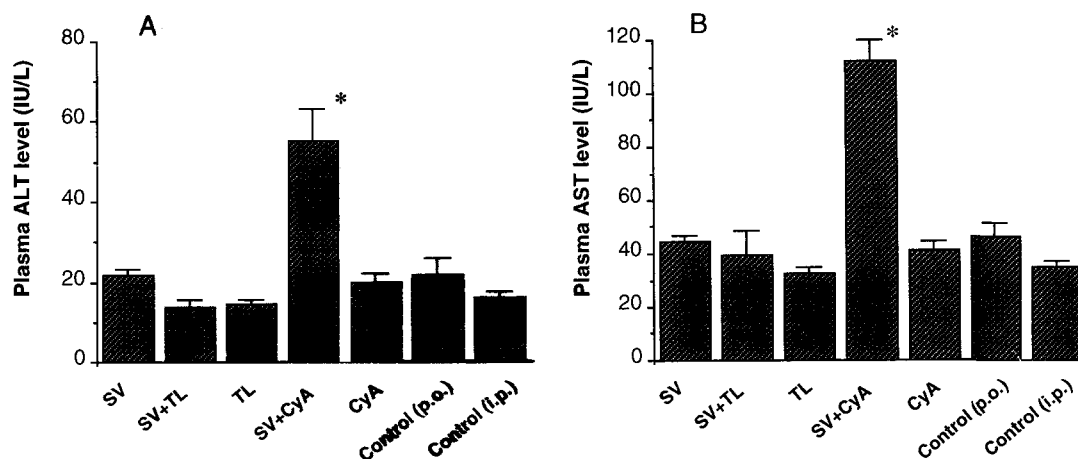


Figure 4. Plasma alanine amino transferase (ALT, panel A) and aspartate amino transferase (AST, panel B) levels 7 days after administration. Each bar represents the mean  $\pm$  S.E. of 5–8 rats. \* $p < 0.05$

groups (Figure 6). Whole blood concentrations of CyA were also not significantly different between the (CyA) and (CyA + SV) groups (Figure 6).

## Discussion

Smith *et al.* [22] reported severe symptoms and an increased mortality were produced following coadministration of simvastatin, with CyA. In our study, a significant reduction in body weight and a high mortality were observed in the (SV + CyA) group. These findings are essentially in agreement with the results of Smith *et al.* [22]. In addition, body weight loss and high mortality were also observed in the (SV + TL) group. These results suggest that coadministration of SV and CyA or TL enhance the toxicity following the administration of these drugs on their own (Table 1, Figure 1).

CK levels, which indicate a myopathic crisis, were used as an indicator of myositis. It has been reported [6–14] that the plasma CK levels in patients with rhabdomyolysis and myopathy induced by HMG-CoA reductase inhibitors is 10–100 times higher than normal levels. In the present study,

although the plasma CK levels in the (SV), (SV + CyA) and (SV + TL) groups increased compared with control groups, no difference was observed among these groups. These results may be due to the administration period being too short to observe any interaction between SV and CyA or TL. The period of drug administration was limited to 7 days because of the dramatic body weight loss and weakness that occurred within a few days of starting drug treatment. However, Smith *et al.* [22] administered drug for 21 days. This discrepancy between our report and that of Smith *et al.* [22], as far as the survival rate is concerned, is unclear. In contrast, a significant increase in plasma CK levels was observed in the (TL) group, suggesting that rhabdomyolysis may be induced by administration of TL alone. More recently, clinical cases of the rhabdomyolysis induced by TL have been reported [31].

As shown in Figure 3, the elevation of plasma urea nitrogen levels by coadministration with SV may be related to an increase in the whole blood concentration of TL and CyA. Several investigators have reported that the development of renal dysfunction by TL or CyA depends on the blood concentration of these drugs [16–18,25,26,32]. The elevation in plasma ALT and AST activity, used as an index of hepatic function, in the (SV + CyA) group may be due to an increase in the blood concentration of CyA and SV following their coadministration, although the degree of increase in blood concentration of these drugs was not significant. On the other hand, TL-induced hepatic toxicity may be weaker than that by CyA [25,26] (Figure 4).

A trend towards an increase in the plasma concentration of SV and blood concentration of CyA and TL was observed after coadministration, but was not significant (Figures 5 and 6). On the other hand, several investigators have reported [22,24,33] that the blood concentrations of CyA were increased by coadministration of HMG-CoA reductase inhibitors. Moreover, Smith *et al.* [22] have

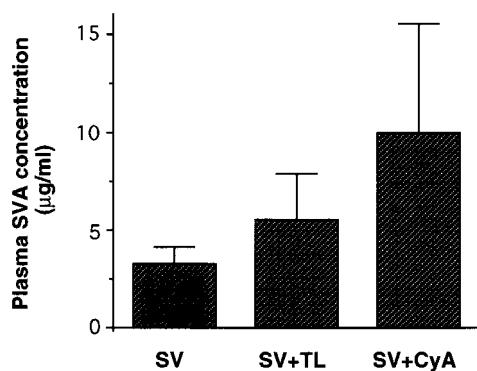


Figure 5. Plasma concentration of simvastatin acid (SVA) 7 days after administration. Each bar represents the mean  $\pm$  S.E. of 5–8 rats

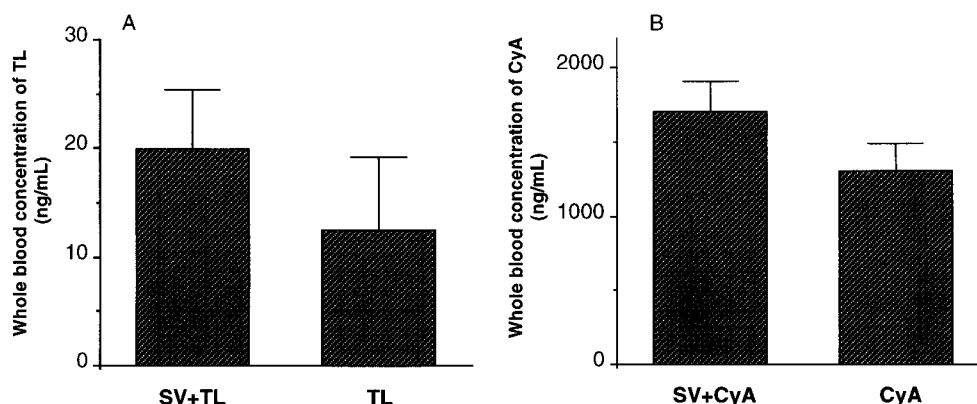


Figure 6. Whole blood concentration of tacrolimus (TL, panel A) and cyclosporin (CyA, panel B) 7 days after administration. Each bar represents the mean  $\pm$  S.E. of 5–8 rats

reported that the area under the plasma concentration time curve (AUC) and maximum plasma concentration for SV in the presence of CyA was 1.5-fold higher compared with those in the absence of CyA. In addition, the muscle SV levels were also increased in the presence of CyA. Arnadottir *et al.* [34] also reported that the AUC<sub>0–24 h</sub> for SV in the presence of CyA was 4-fold higher than in the absence of CyA and, furthermore, it has been reported [23] that CyA altered the disposition of pravastatin. In rats, a total of 80% of an oral dose of simvastatin was excreted in faeces, and 55% in the bile [35]. It has also been reported [22,25,26,35,36] that HMG-CoA reductase inhibitors such as simvastatin and lovastatin are metabolized by the cytochrome P450 3A family in hepatic microsomes, and that both CyA and TL are also metabolized by the same enzyme [15,25,26,37–41]. An explanation for the increased total plasma SV concentrations following coadministration with CyA, may be that CyA inhibits the metabolism of SV [22]. Moreover, Smith *et al.* reported that CyA also inhibits the biliary excretion of SV [22].

In conclusion, the toxicity of SV, TL and CyA may be enhanced by their coadministration. Further, it has been shown that coadministration of SV and CyA or TL may lead to a reduction in renal function and hepatic toxicity.

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