BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 19(2), 248-254 (2011)

Evaluation of Pharmacokinetics of Simvastatin and Its Pharmacologically Active Metabolite from Controlled-Release Tablets of Simvastatin in Rodent and Canine Animal Models

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Abstract

Biotransformation of pharmacologically inactive lactone prodrug simvastatin (SV) into pharmacologically active simvastatin β-hydroxy acid (SVA) exhibits inter-species differences due to variations in amount and activity of esterase enzymes. In this study, we investigated the pharmacokinetics (PK) of SV and its metabolite SVA following oral doses of SV from controlled-release (CR) tablets and immediate-release (IR) tablets in rodent and canine animal models that features different esterase activity. In rat PK study, no SV was detected in plasma for both formulations due to rapid hydrolysis of SV into SVA by plasma esterase. Besides, no significant differences in PK parameters of SV or SVA were observed between both species. In dog PK study, the relative oral bioavailability of CR tablets in terms of SV was 72.3% compared to IR tablets. Regarding formulation differences in dogs, CR tablets exhibited significantly lower C_{max} (p<0.05), and higher T_{max} (p<0.01) and MRT (p<0.01) for both SV and SVA compared to IR tablets. Accordingly, CR tablets of SV with prolonged drug release profiles in both species might be a potential candidate for a more effective delivery of SV with reduced side effects. Besides, similar PK parameters of SV and SVA in both species despite variation in enzyme activities suggested involvement of equally potent biotransformation pathways in these animal species.

Key Words: Simvastatin, biotransformation, pharmacokinetic comparison; Controlled release tablet; Esterase

INTRODUCTION

Simvastatin (SV) is a synthetic lipid lowering agent that has been widely used to treat hypercholesterolemia and has beneficial effects on coronary diseases and mortality rates in patients with hypercholesterolemia (Alberts *et al.*, 1980; Tobert, 1987; Alberts, 1988; Alberts, 1990; Grundy, 1998). However, SV is an inactive lactone prodrug which after administration is primarily metabolized into pharmacologically active metabolite simvastatin β-hydroxy acid (SVA). SVA formed in vivo is a specific and competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase enzyme responsible for conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol (Gotto, 1997; Hebert *et al.*, 1997; Kostner *et al.*, 1989).

Simvastatin immediate release (IR) tablets are commercially available for more than 10 years with standard dose of 20 to 40 mg once a day. However, there have been many commonly reported adverse reactions with IR formulations of statins including upper respiratory tract infections, headache, abdominal pain, constipation, and nausea (Duggan *et al.*, 1989; Bradford *et al.*, 1991; Nishio *et al.*, 2005). It was also reported that higher incidences of adverse events are associated with higher drug concentrations of statins and the most serious adverse events associated with them are hepatotoxicity and myotoxicity (Duggan *et al.*, 1989; Vickers *et al.*, 1990a; Bradford *et al.*, 1991; McClelland *et al.*, 1991). Since, SA has the similar mechanism of action like other statins, higher peak concentrations of SA would be expected to be related with incidence of adverse events. Therefore, formulations of SA with reduced side effects and constant pharmacological activity are of interest.

It was reported that lovastatin IR given as 20 mg twice daily produced a significantly greater reduction in LDL cholesterol

www.biomolther.org

Open Access **DOI: 10.4062/biomolther.2011.19.2.248**

pISSN: 1976-9148 eISSN: 2005-4483

Received Oct 1, 2010 **Revised** Nov 1, 2010 **Accepted** Nov 2, 2010

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levels in patients than 40 mg once daily with reduced side effects (Sun *et al.*, 2002). Subsequently, it was also reported that systemic concentrations of HMG-CoA reductase inhibitors could be minimized and inhibitor efficacy could be enhanced by oral administration of the trimethammonium salt of SVA through an extended release dosage form (Cheng *et al.*, 1993; Curran and Goa, 2003). Besides, SV was more efficiently extracted by the target organ liver than their corresponding β-hydroxy acids with subsequent minimization of systemic burden (Lamson *et al.*, 2002). Based on these reports, it was conceived that controlled release (CR) dosage forms of SV may provide a sustained concentrations and improved safety profile leading to increased patients compliance compared to a conventional IR dosage form. Most importantly, it was suggested that a CR formulation may result in a lower but constant pharmacological availability which might reduce toxic side effects (Davidson *et al.*, 2002; Lukacsko *et al.*, 2004).

Recently, we have developed a CR formulation of SV for effective delivery to the liver in a more sustained manner to provide constant pharmacological availability with reduced side effects. The systemic availability of SV and pharmacological availability of its active metabolite SVA depend on biotransformation of SV by esterases in vivo. Nevertheless, biotransformation of SV in experimental animals exhibits noticeable species differences. In experimental animals, compared to canine plasma, rodent plasma has a huge amount of esterases and hydrolyzes SV rapidly into SVA (Vickers *et al.*, 1990b; Woo *et al.*, 2005). We carried out this study with an objective of investigating the pharmacokinetics (PK) of SV and its metabolite SVA following oral doses of SV from controlled-release (CR) tablets and immediate-release (IR) tablets in rodent and canine animal models that features different esterase activity.

MATERIALS AND METHODS

Materials

Simvastatin (SV), simvastatin β-hydroxy acid (SVA), and lovastatin were kindly obtained from Hanmi Pharmaceutical Company (Seoul, Korea). Ultrapure water MilliQ (Millipore Simplicity 185, Molsheim, France) was used in the analysis of LC-MS/MS. Vitamin-E TPGS, butylated hydroxytoluene, sodium alginate, xanthan gum, locust bean gum, hydroxypropyl methylcellulose, and propylene glycol alginate were of pharmaceutical grade. Other chemicals used in this study were of either reagent or HPLC grade, and used without further purification.

Animals

Male Sprague-Dawley (SD) rats weighing about 275-315 g and male beagle dogs weighing about 6.7-7.8 kg were purchased from Oriental Bio (Seoul, Korea). The rats were kept in plastic cages with free access to standard rat diet (Samyang, Seoul, Korea) and water. Dogs were housed individually with free access to Golden-pet dog diet (Agribrands, Seoul, Korea) and water. All the experimental animals were maintained at a temperature of 22-24°C with relative humidity of 50 ± 10% under 12-hr light-dark cycle.

Rat and dog plasma analysis

The internal standard solution (20 μl, lovastatin 10 ng/ml in 60% acetonitrile) was added to each plasma sample in borosilicate glass tubes followed by the addition of 1 ml of methyl-*t*-butyl ether. The samples were mixed on a vortex mixer for 10 min at 1,400 rpm, followed by centrifugation for 10 min at 3,000 rpm. The supernatant was transferred to a glass tube, evaporated under nitrogen, and reconstituted with 100 μl of mobile phase. An aliquot of 10 μl was injected into LC-MS/MS.

LC-MS/MS assay

HPLC analysis was performed using a Waters Alliance 2795 HPLC system (Waters Corp., Milford, MA, USA). Compounds were separated on an X-terra C18 Column (2.1×10 mm, 3.5 μm, Waters Corp., Milford, MA, USA). The isocratic mobile phase was consisted of acetonitrile and deionization water containing 0.1% formic acid (85:15 v/v). The flow rate of the mobile phase and the column oven temperature were set at 0.2 ml/min and 30°C, respectively.

The HPLC system was coupled to an API 4000 Qtrap mass spectrometer equipped with turbo ion spray ionization source (AB MDS Sciex, Toronto, Canada). The turbo ion spray ionization source was operated in a positive mode. The curtain gas, nebulizer gas, and the turbo gas (nitrogen) pressures were set at 30, 40, and 40 psi, respectively. The turbo gas temperature was set at 450°C, and the ion spray needle voltage was adjusted to 5500 V. The mass spectrometer was operated at a unit resolution for both Q1 and Q3 in the multiple reaction monitoring (MRM) mode with a dwell time of 300 ms in each transition. The transition of the precursors to the product ion was monitored at 419.3 → 199.1 for SV, 437.2 → 302.9 for SVA, 405.2 → 199.2 for LV. The collision energy was set at medium. Data acquisition was preformed with the Analyst 1.4 software (AB MSD Sciex, Toronto, Canada). Calibration curves were prepared by spiking the blank plasma (200 μl) with 20 μl of SV or SVA standard solution at concentrations of 0.2, 0.5, 1, 5, 10, 50, and 100 ng/ml.

Formulations

Newly developed CR tablets of SV were designed for once-a-day oral administration and contained 20 mg of SV, carriers for controlled release, gel hydration accelerators, and other inactive ingredients (Woo *et al.*, 2005). Solid dispersions of SV were prepared by spray drying to increase solubility of the drug using vitamin-E TPGS as a solubilizing agent and butylated hydroxytoluene as a stabilizing agent. A mixture of sodium alginate, xanthan gum, and locust bean gum were used as a carrier for sustained release. A mixture of hydroxypropyl methylcellulose and propylene glycol alginate was used as a gel hydration accelerator. The tablets prepared were capable of maintaining a constant drug level in blood for 24 hours (Woo *et al.*, 2005).

Dissolution test

Dissolution study was carried out according to the USP XXVIII method (Apparatus 2), employing an Erweka DT800 dissolution apparatus (Heusenstamm, Germany). Nine hundred milliliters of the dissolution medium was prepared and used as described in the USP 28. Briefly, pH 7.0 buffer solution containing 0.5% sodium dodecyl sulfate in 0.01 M sodium phosphate was prepared by dissolving 30 g of sodium dodecyl sulfate and 8.28 g of monobasic sodium phosphate in 6,000 ml of water, and pH was adjusted to 7.0 with 50% (w/v) sodium hydroxide solution. The stirring rate was 100 rpm and the temperature of the medium was maintained 37 ± 0.5°C. The dis-

solution was carried out on 6 tablets. At each sampling time (5, 10, 15, 30, 45, 60, 120, and 240 min for IR tablets and 2, 4, 6, 8, 10, 12 and 24 hr for CR tablets), 3 ml of dissolution medium was withdrawn, filtered through a 0.45 μm filter, and analyzed by the above-mentioned analytical method.

PK of SV in rats and dogs

The rats were anaesthetized by intraperitoneal injection of ketamine and xylazine (90:10 mg/kg) and cannulated with a polyethylene (PE) tubing (0.58 mm i.d., 0.96 mm o.d., Natsume, Tokyo, Japan) in the left femoral artery and vein. SV immediate- and controlled-release tablets were orally administered (8 mg/kg dose) to rats (n=7 each). Blood samples were collected prior to and at 0.5, 1, 2, 3, 5, 7, 9, 12, 24, 30, and 36 hr after administration. The oral administration was performed under conscious conditions. Plasma samples were harvested by centrifugation at 1,500 g for 10 min and stored at −20°C until analysis.

Twelve male beagle dogs were weighed and divided into 2 groups with 6 animals in each group. The animals were fasted for 16 hr and weighed before dosing. IR and CR tablets of SV were orally administered (40 mg dose) to dogs (n=6). Blood samples (3 ml) were collected prior to and at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 30, 36, and 48 hr after administration. Plasma samples were harvested by centrifugation at 1,500 g for 10 min and stored at −20°C until analysis.

Data analysis

The plasma concentration profile of SV or SVA as a function of time was analyzed by a non-compartmental method using the nonlinear least squares regression program WinNonlin (Pharsight, Cary, NC, USA). The relative oral bioavailability was calculated by following equation: Relative oral bioavailability=$AUC_{controlled\text{-}release}/AUC_{immediate\text{-}release}$; where, AUC is the area under plasma drug concentration curve from time zero to the last sampling time. Obtained pharmacokinetic parameters were compared by the unpaired t-test. A statistical significance was assumed when the *p*-value was less than 0.05.

RESULTS

Assay validation

In this study, a highly sensitive and simple assay method was developed and validated for the determination of SV and its active metabolite SVA in biological samples using LC-MS/MS. A high linearity was observed over a concentration range from 0.2-100 ng/ml, with correlation coefficients >0.99 for both SV and SVA. The mean slope of the calibration curves used in method validation was 0.3717-0.0181 and 0.0521-0.0033 for SV and SVA, respectively. The intra- and inter-day accuracy for SV ranged from 90.5-106.7% and 86.1-113.7%, respectively, with their respective % CV being less than 11.2% and 14.9%, respectively. The intra- and inter-day accuracy for SVA ranged from 95.7-104.7% and 98.7-106.7%, respectively, with their respective % CV being less than 6.7 and 7.4%, respectively. The LLOQ of this assay was estimated to be 0.2 ng/ml for both SV and SVA. On the basis of these results, this method could be used for the pharmacokinetic analysis of SV IR and CR tablets owing to its good validation results.

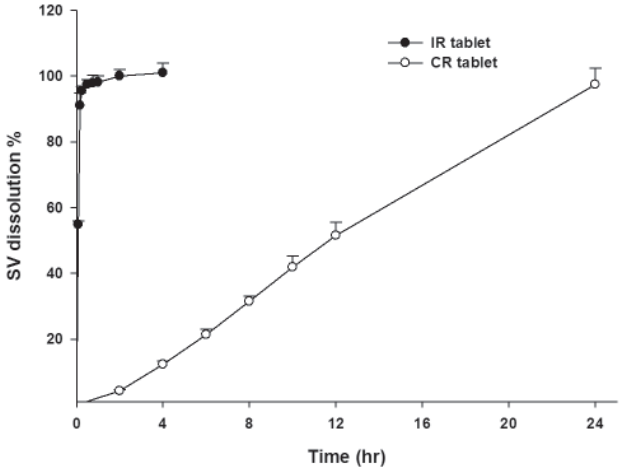

Fig. 1. Mean dissolution profiles of SV from IR and CR tablets (n=6).

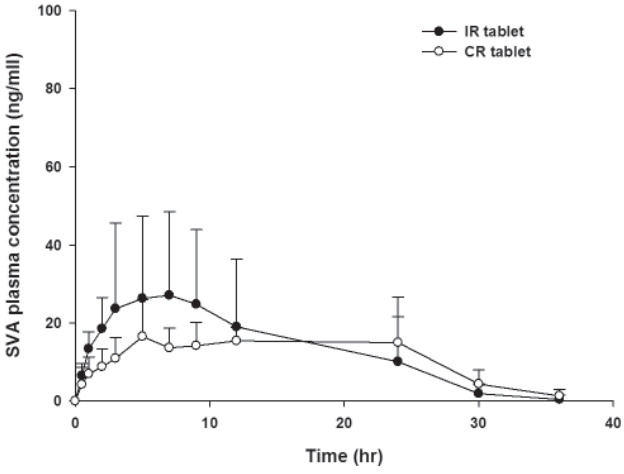

Fig. 2. Mean plasma concentration-time profile of SVA after oral administration of 8 mg/kg dose of SV of IR or CR tablets in male SD rats. Mean ± S.E. (n=7).

Dissolution study

The dissolution profiles of CR and IR tablets in terms of SV mean % dissolved against time obtained is shown in Fig. 1. About 50% of SV was dissolved from IR tablets within 5 min and more than 95% was dissolved within 30 min, showing typical dissolution profiles of IR tablets. In contrast, SV from CR tablets showed sustained release pattern with less than 10% dissolution of SV until 2 hr and about 50% and 90% dissolution in 12 and 24 hr, respectively. Dissolution study demonstrated the ability of CR tablets to deliver SV in a sustained manner of up to 24 hours and was expected to produce similar drug release pattern in the in vivo study.

PK of SV and SVA in rats

In this study, PK profile of SV and SVA following 8 mg/kg oral dose of SV from CR and IR tablets was investigated. There was no SV found in rat plasma following oral administration of both formulations. However, active metabolite SVA was found in rat plasma and quantified using earlier-described

Table 1. Pharmacokinetic parameters of SVA obtained after oral administration of SV at a dose of 8 mg/kg of IR or CR tablets in male SD rats (n=7)

PK parameters	IR	CR
C_{max} (ng/ml)	37.4 ± 22.2	25.0 ± 7.0
T_{max} (hr)	4.0 ± 2.9	14.3 ± 9.2[a]
λ_z (hr^{-1})	0.098 ± 0.086	0.100 ± 0.064
$t_{1/2}$ (hr)	9.9 ± 6.6	8.9 ± 4.3
AUC_{last} (ng.hr/ml)	464.7 ± 359.7	403.6 ± 81.8
MRT_{last} (hr)	10.5 ± 3.7	14.6 ± 3.9
Cl (ml/min/kg)	254.38 ± 95.33	363.86 ± 57.17
V_d (L/kg)	321.38 ± 233.35	279.37 ± 123.78

All values are expressed as mean ± standard deviation. [a]Significant difference (p<0.05) compared to IR tablets. AUC_{last}: area under the concentration-time curve from the time of dosing to last observation, C_{max}: maximum measured plasma concentration, MRT: mean residence time, T_{max}: time of maximum plasma concentration, $t_{1/2}$: half life, λ^z: elimination rate constant.

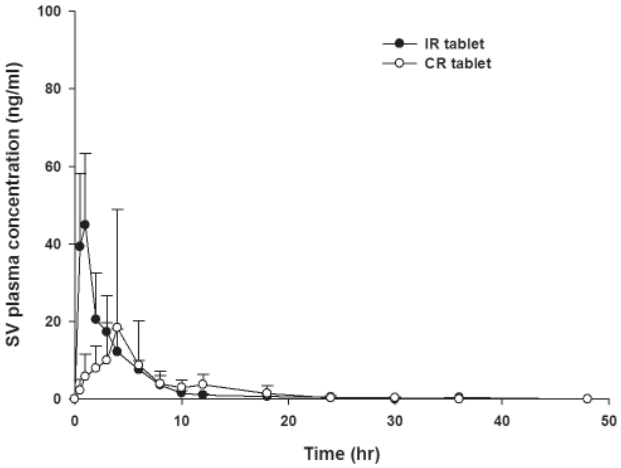

Fig. 3. Mean plasma concentration-time profile of SV after oral administration of 40 mg dose of SV of IR or CR tablets in beagle dogs. Mean ± S.E. (n=6).

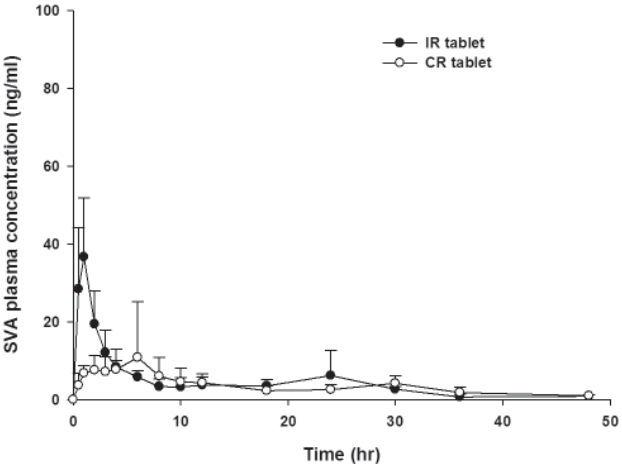

Fig. 4. Mean plasma concentration-time profile of SVA after oral administration of 40 mg dose of SV of IR or CR tablets in beagle dogs. Mean ± S.E. (n=6).

LC-MS/MS method. The time course of mean plasma concentrations of SVA after oral administration of CR and IR tablets at a dose of 8 mg/kg of SV in rats is shown in Fig. 2.

The non-compartmental PK parameters of SVA following 8 mg/kg oral dose of SV from CR and IR tablets in rats are summarized in Table 1. C_{max} of SVA was 37.4 ± 22.2 ng/ml and 25.0 ± 7.0 ng/ml for IR and CR tablets, respectively. There was no significant difference observed between C_{max} of the formulations. However, CR tablets showed significantly longer T_{max} compared to IR tablets (p<0.05) and the mean T_{max} values were 4.0 ± 2.9 hr (ranging between 1.0-7.0 hr) for IR and 14.3 ± 9.2 hr (ranging between 5.0-24.0 hr) for CR tablets. All other PK parameters of SVA (AUC, MRT, $t_{1/2}$, λ_z, Cl, and Vd) were similar and showed no significant differences between CR and IR formulations. The relative oral bioavailability of SVA from CR tablets was 86.9% compared to IR tablets.

PK of SV and SVA in dogs

In dogs, both SV and its active metabolite SVA were quantified and the mean plasma concentration profile of SV and SVA as a function of time obtained after 40 mg oral dose of IR or CR tablets in beagle dogs are shown in Fig. 3 and Fig. 4, respectively. Plasma concentration curve showed typical IR and CR plasma concentration profiles with sharp peak plasma concentration for IR tablets and sustained plasma concentration for CR tablets. The plasma concentration profile was in accordance with the in vitro dissolution profile of both formulations.

The non-compartmental PK parameters of SV and its active metabolite SVA obtained after 40 mg oral dose of SV from IR or CR tablets are summarized in Table 2. Comparison of PK parameters of SV and its active metabolite SVA revealed significantly high MRT for SVA compared to SV for both formulations. The MRT values of SV and SVA were 3.8 ± 1.2 and 11.0 ± 3.9 for IR tablets (p<0.05) and 7.4 ± 2.7 and 15.9 ± 2.2 for CR tablets (p<0.01), respectively. Besides MRT, there were no significant differences in PK parameters such as C_{max}, T_{max}, $t_{1/2}$, or clearance existed between SV and SVA for both formulations.

Comparison of PK parameters of SV from both formulations demonstrated that C_{max} and T_{max} of SV were 50.6 ± 18.5 ng/ml and 0.8 ± 0.3 hr for IR tablets, and 21.9 ± 28.5 ng/ml and 3.7 ± 1.4 hr for CR tablets, respectively. SV from CR tablets exhibited significantly lower C_{max} (p<0.05) and longer T_{max} (p<0.01) compared to IR tablets. The C_{max} and T_{max} of SVA was 36.7 ± 15.1 ng/ml and 1.0 ± 0.01 hr for IR tablets and 15.1 ± 12.3 ng/ml and 3.6 ± 2.2 hr for CR tablets, respectively (Table 2). Although T_{max} of SVA from CR tablets was significantly higher compared to IR tablets (p<0.05), no significant difference was observed with C_{max} of SVA between two formulations. Total systemic exposure (AUC) of SV and SVA were also similar for both formulations with values of 142.0 ± 41.0 and 194.7 ± 56.4 for IR tablets, and 102.7 ± 93.7 ng.hr/ml and 161.3 ± 63.3 ng.hr/ml for CR tablets, respectively (Table 2). The relative oral bioavailability of CR tablets in comparison to IR tablets was 83.8%. The MRT of both SV and SVA was 3.8 ± 1.2 and 11.0 ± 3.9 for IR tablets, and 7.4 ± 2.7 hr and 15.9 ± 2.2 hr for CR tablets, respectively. MRT of both SV and SVA from CR tablets was significantly higher compared to IR tablets

Table 2. Pharmacokinetic parameters of SV and its metabolite SVA obtained after oral administration of SV at 40 mg dose of IR or CR tablets in beagle dogs (n=6)

Parameters	IR Tablets		CR Tablets	
	SV	SVA	SV	SVA
C_{max} (ng/ml)	50.6 ± 18.5	36.7 ± 15.1	21.9 ± 28.5[a,e]	15.1 ± 12.3
T_{max} (hr)	0.8 ± 0.3	1.0 ± 0.0	3.7 ± 1.4[b,e]	3.6 ± 2.2[b,f]
λ_z (hr^{-1})	0.274 ± 0.152	0.117 ± 0.071	0.221 ± 0.215	0.072 ± 0.053
$t_{1/2}$ (hr)	3.2 ± 1.6	7.4 ± 3.2	5.1 ± 3.0	12.5 ± 4.8[a,f]
AUC_{last} (ng.hr/ml)	142.0 ± 41.0	194.7 ± 56.4	102.7 ± 93.7	161.3 ± 63.3
MRT_{last} (hr)	3.8 ± 1.2	11.0 ± 3.9*[a]	7.4 ± 2.7[a,e]	15.9 ± 2.2[b,d,f]
Cl (ml/min/kg)	689.5 ± 206.1	479.5 ± 180.0	1,385.9 ± 842.9	562.8 ± 185.2
V_d (L/kg)	198.6 ± 143.0	306.8 ± 171.0	587.7 ± 531.4	580.3 ± 260.7

All values are expressed as mean ± standard deviation. Values that are significantly different are indicated by [a]p<0.05 and [b]p<0.01, using a t-test, [c]Comparison between SVA and SV of IR tablets, [d]Comparison between SVA and SV of CR tablets, [e]Comparison between SV of IR and CR tablets, [f]Comparison between SVA of IR and CR tablets. AUC_{last}: area under the concentration-time curve from the time of dosing to last observation, C_{max}: maximum measured plasma concentration, CL: clearance, MRT: mean residence time, T_{max}: time of maximum plasma concentration, $t_{1/2}$: half life, Vd volume of distribution, λ_z: elimination rate constant.

(p<0.01). No significant differences in PK parameters of SV and SVA were observed between both formulations.

DISCUSSION

The oral route is the most common route of drug delivery and myriad of drug delivery strategies have been employed to improve oral drug delivery (Wikstrand *et al.*, 2003; Löbenberg *et al.*, 2005). The most common way to prolong drug delivery is to use sustained or extended release dosage forms and aim of such strategies is to increase the availability of the active drug at the site of drug action over a prolonged period of time (Carroll *et al.*, 1990; Shu *et al.*, 2001). A controlled drug delivery system may result in a lower plasma concentration, but it provides a constant pharmacological availability of the drug which might reduce toxic side effects (Thielemann *et al.*, 1996; Halsas *et al.*, 1999; Higaki *et al.*, 2001). This reduced side effect coupled with reduced dose frequency overcome compliance problems with patients, and eventually provides satisfactory clinical management of any disease involved (Wikstrand *et al.*, 2003; Löbenberg *et al.*, 2005). Like other statins, SV has many commonly reported adverse reactions including upper respiratory tract infections, headache, abdominal pain, constipation, and nausea (Duggan *et al.*, 1989; Bradford *et al.*, 1991; Nishio *et al.*, 2005). One of the important adverse effects of simvastatin and the class in general is myopathy or its more severe form rhabdomyolysis. It has also been reported that the adverse events were attributable to the extreme inhibition of the enzyme at higher concentration (Pedersen and Tobert, 2004). So, controlled release dosage forms of simvastatin (SV) are preferred in the management of hypercholesterolemia due to their ability to deliver drug effectively to the liver in a more sustained manner providing constant pharmacological availability with reduced side effects. However, biotransformation of pharmacologically inactive lactone prodrug (SV) into pharmacologically active simvastatin β-hydroxy acid (SVA) exhibits inter-species differences due to variation in amount of esterase activity (Prueksaritanont *et al.*, 2005).

It has been reported previously by Vickers et al that hydrolysis of parent lactone prodrug SV by plasma esterases at physiological temperature was rapid in mouse, rat, and rabbit compared to dogs and humans as the former possess higher plasma esterase activity (Vickers *et al.*, 1990a; 1990b; Prueksaritanont *et al.*, 2005). Therefore, we expected lower concentrations of SV in rat plasma due to abundant presence of esterases that could rapidly biotransform SV into its active metabolite SVA. There was no SV found in rat plasma following oral administration of both IR and CR formulations, suggesting more rapid conversion of parent drug into active metabolite. Absence of SV from both formulations in rat plasma precluded further measurements of SV and its PK profile. However, SVA, active metabolite of SV, was found in rat plasma and was quantified. The PK profile of SVA demonstrated significantly higher T_{max} and slower absorption of CR tablets compared to IR tablets in rats, and this is in compliance with the dissolution profile. Because of virtually unmeasurable levels of SV in rat plasma after oral administration, determination of systemic bioavailability of SV could not be made in this species. Nonetheless, the relative oral bioavailability of SVA from CR tablets was 86.9% compared to SVA from IR tablets. Although, relative bioavailability was a little lower with CR tablets, the concentrations of active metabolite SVA was consistent up to a period of 24 hours with no fluctuations suggesting controlled release and sustained absorption of SV from the GI tract. This is propitious therapeutically, because controlled release of SV is expected to reduce side effects with constant pharmacological availability in plasma (Goldbeter and Claude, 2002; Singh *et al.*, 2010).

Compared to rodents, dog is a more appropriate model for correlation of the physiological disposition of SV in man as both dog and man seemingly lack high plasma esterase compared to rat (Vickers *et al.*, 1990a; Prueksaritanont *et al.*, 2005; Singh *et al.*, 2010). Moreover, dog plasma esterases are not as effective as those of rat in hydrolyzing xenobiotic esters and apparently low levels of lactonase activity in dog blood allow one to make meaningful comparison between the pharmacokinetics of SV and SVA in this animal species (Duggan and Vickers, 1990; Vickers *et al.*, 1990b; Le Couteur *et al.*, 1996). Despite the apparent absence or negligible amount

of esterase in dogs, presence of SVA in vivo suggested involvement of different pathway in the biotransformation of SV. It was reported that in dogs, SV is predominantly converted to its active metabolite SVA by a well-known P450-mediated oxidation in addition to enzymatic hydrolysis by esterases. Besides, P450-mediated oxidation of SV is predominant pathway and is approximately two-fold more rapid than the hydrolysis of SV to SVA in dogs due to relatively faster oxidative metabolism of dog liver microsomes (Martin *et al.*, 2003; Prueksaritanont *et al.*, 2005).

In both species, CR tablets exhibited a significantly lower C_{max} and longer T_{max} compared to IR tablets (p<0.05) which demonstrated typical PK profile of sustained release tablets. This was in compliance with the dissolution profile of both formulations. Besides, significantly higher MRT values of metabolite SVA from both formulations in comparison to SV in dogs suggested prolonged residence of SVA in dogs. Although, HMG-CoA reductase inhibitors have similar potencies, difference in efficacy in vivo can often be related to delivery and residence time of the active drug and/or metabolites to the target, the liver (Vickers *et al.*, 1990a; 1990b; Black *et al.*, 1999; Prueksaritanont *et al.*, 2005). Thus, it might be expected that the enhanced residence would have positive effect on the efficacy of the active metabolite SVA. Regarding formulation differences, the fluctuations in plasma concentration of SV and SVA from CR tablets were smaller compared to IR tablets. It appeared that CR tablets delivered SV in a more sustained fashion, providing smoother plasma concentration profiles and lower maximum plasma concentrations compared with those of IR tablets. Since, peak concentrations of SV is related to the incidence of adverse events, given the smooth plasma concentration coupled with similar AUC values of CR tablets could potentially reduce incidence of such events and could sustain the efficacy of SV at the same time.

In conclusion, although biotransformation of SV involves different pathways in rodents and canines, no significant differences in PK parameters of SVA observed between both species. Regarding formulation differences in dogs, CR tablets exhibited significantly lower C_{max} (p<0.05), higher T_{max} (p<0.01) and MRT (p<0.01) for both prodrug SV and its pharmacologically active metabolite SVA compared to IR tablets. Thus, CR tablets of SV with prolonged drug release profiles might be a potential candidate for a more effective delivery of SV with reduced side effects. Besides, similar PK parameters of SV and SVA in both species suggested involvement of equally potent biotransformation pathways in these animal species tested. Conclusively, this preclinical interspecies PK study in commonly available animal species provide an insight into the behavior of SV from CR and IR formulations and gives a direction for further development process.

ACKNOWLEDGMENTS

This work was supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea government (MEST) (No. R01-2008-000-10244-0).

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