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Pharmacokinetics of SD-0542, a Novel Histone Deacetylase Inhibitor, in Rats

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ABSTRACT – This study reports the pharmacokinetics of a novel histone deacetylase inhibitor, SD-0542, in rats after i.v. and oral administration. SD-0542 was injected intravenously at doses of 10, 20, and 40 mg/kg. The terminal elimination half-life ($t_{1/2}$), systemic clearance (Cl), and steady-state volume of distribution (V_{ss}) remained unaltered as a function of dose, with their values ranging from 2.0-2.5 hr, 157.2-214.1 ml/min/kg, and 11.1-17.5 L/kg, respectively, whereas, the initial serum concentration (C_0) and AUC increased linearly as the dose was increased. Renal excretion of SD-0542 was minimal. Oral pharmacokinetic studies were conducted in rats at a dose of 20 mg/kg. The T_{max}, Cl/F, V_z/F, and $t_{1/2}$ were 2.0 hr, 92864 ml/min/kg, 16331 L/kg, and 2.0 hr, respectively. Taken together, SD-0542 showed linear pharmacokinetics over the i.v. bolus dose range studied. SD-0542 was poorly absorbed, with the absolute oral bioavailability of 0.9%.

Key words – SD-0542, Pharmacokinetics, Apicidin A, Bioavailability, Excretion

Histone deacetylases (HDACs) and histone acetyl transferases (HATs) are two types of enzymes with opposing activity involved in determining the state of deacetylation and acetylation of histones. The balance between these two enzymes plays an important role in the modulation of chromatin structure and regulation of cell function, including differentiation, proliferation, and apoptosis.[1] Lately, the therapeutic potential as anti-proliferative or anti-protozoal agents has been shown for several HDAC inhibitors such as suberoylanilide hydroxamic acid (SAHA),[2] n-butylate,[3] depsipeptide,[4] and apicidin.[5] Apicidin [cyclo(*N*-*O*-methyl-L-tryptophanyl-L-isoleucinyl-D-pipecolinyl-L-2-amino-8-oxodecanoyl)], a cyclic tetrapeptide, is a fungal metabolite isolated from the cultures of *Fusarium pallidoroseum*, and exhibits broad spectrum of cell killing activity against members of the Apicomplexan family of protozoa such as *Plasmodium* sp. and *Cryptospridium parvum,* causative agents for malaria and cryptosporidiosis, respectively.[6-7] The antiparasitic activity of apicidin appears to be mediated by the low nanomolar inhibition of Apicomplexan histone deacetylase (HDAC), a key eukaryotic transcription factor essential for chromatin remodeling and the functional regulation of gene transcription.[2,6-7] Apicidin exhibits anti-protozoal activity against *Plasmodium berghei* malaria *in vivo*[6] and anti-proliferative activity against various cancer cell lines.[5,8-9] More recently, apicidin has been reported to possess anti-invasive and anti-angiogenic activities.[10]

A key moiety for the HDAC inhibition activity of apicidin has been reported to be the indole moiety and 8-oxo-2-aminodecanoic (Aoda) side chain.[7] Apicidin A (Figure 1) is an apicidin analogue possessing HDAC inhibition activity comparable with apicidin.[11] SD-0542 (Figure 1) is also an analog of apicidin with modification of Aoda and indole moieties. This study was conducted to characterize the pharmacokinetics of SD-0542 in rats after i.v. and oral administration.

Experimental

Chemicals

SD-0542 and apicidin A were provided by the Kyeonggi Pharmaceutical Research Center at Sungkyunkwan University (Suwon, Korea). HC-toxin, dimethylsulfoxide (DMSO), polyethylene glycol (PEG) 400, ketamine, xylazine, 2-mercaptoethanol, and ammonium acetate were purchased from Sigma (St. Louis, MO). HPLC grade acetonitrile, methanol, and water were purchased from J.T. Baker (Phillipsburg, NJ). Ammonia water and ethanol were obtained from Yakuri Pure Chemicals (Osaka, Japan).

Intravenous Injection Study

Male Sprague-Dawley rats (225-252 g) were purchased from Jeil Co. (Ansung, Korea). The animals were maintained at 23 ± 2°C with a 12 hr of light/dark cycle and relative humidity of 50 ± 10%, and had free access to standard animal diet (Samyang, Seoul, Korea) and water. The rats were anesthetized by i.p. injection of ketamine and xylazine (90:10 mg/kg)

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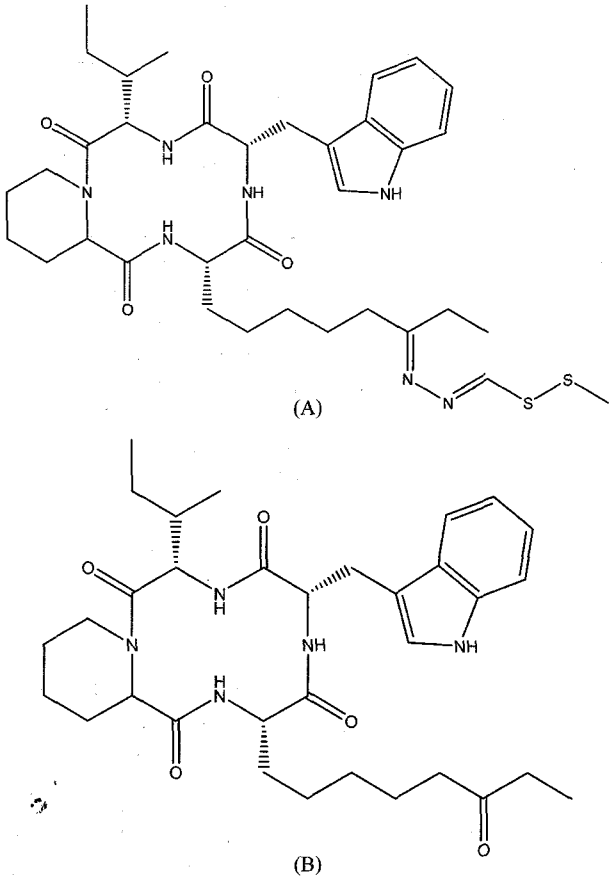

Figure 1–Chemical structures of SD-0542 and apicidin A.

and cannulated with polyethylene (PE) tubing (0.58 mm i.d., 0.96 mm o.d., Natsume, Tokyo, Japan) in the right jugular and left femoral veins. After 2 days of recovery, the rats were injected with SD-0542 into the femoral vein at doses of 10, 20, and 40 mg/kg (n=4 each). Immediately prior to dosing, SD-0542 was dissolved in a DMSO:PEG 400:saline mixture (10:70:20 v/v) (injection volume 0.45-0.50 ml). Blood samples were collected from the jugular vein prior to and 10, 15, 30 min, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 hr following i.v. injection. Serum samples were harvested by centrifugation at 1,500 g for 10 min and stored at -20°C until analysis. Urine samples were also collected until 12 hr post-dosing and kept at -20°C until drug analysis.

Oral Administration Study

The rats (226-234 g) were anesthetized by i.p. injection of ketamine and xylazine (90:10 mg/kg) and cannulated with PE tubing (0.58 mm i.d., 0.96 mm o.d., Natsume, Tokyo, Japan) in the left jugular vein. SD-0542 dissolved in a mixture of DMSO:PEG 400:saline (10:70:20 v/v) was administered by oral gavages (volume 0.45-0.47 ml) at a dose of 20 mg/kg. Venous blood samples were collected before and 10, 15, 30 min, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 hr post-dosing. Serum samples were harvested by centrifugation at 1,500 g for 10 min and stored at -20°C until analysis.

Drug Analysis

Concentrations of SD-0542 and its active metabolite, apicidin A were determined by LC/MS/MS. Briefly, the internal standard solution (100 μl, HC-toxin 5 μg/ml) and 1 mM 2-mercaptoethanol (100 μl) were added to serum samples (100 μl) in borosilicate tubes (Scientific Glass Inc., Rockwood TN) and precipitated by addition of 770 μl of acetonitrile. The samples were mixed on a vortex mixer for 10 sec and centrifugation for 10 min. The supernatant was transferred into a flat-bottomed glass insert (volume 250 μl) and a portion (10 μl) was injected into LC/MS/MS. The HPLC used in the study was a Shimadzu 10Avp system (Kyoto, Japan) consisting of SCL-10Avp system controller, LC-10ADvp pump, SIL-10ADvp auto sampler, CTO-10Avp column oven, and DGU-14A degasser. Compounds were separated on a Hypersil GOLD column (150 × 2.1 mm i.d., 5 μm) (Thermo Electron Co., Bellefonte, PA) with a SecurityGuard column (4 × 2.0 mm i.d., Phenomenex, Torrance, CA). The mobile phase was a mixture of acetonitrile and 10 mM ammonium acetate solution (85:15 v/v). The flow rate of the mobile phase was set at 0.2 ml/min, and the column oven temperature at 40°C. The HPLC system was coupled to an API 2000 triple-quadrupole mass spectrometer equipped with a turbo ion spray ionization (ESI) source (AB MDS Sciex, Toronto, Canada). The ESI source was operated in a positive mode with the curtain, nebulizer, and turbo-gas with nitrogen set at 25, 60, and 60 psi, respectively. The turbo-gas temperature was set at 400°C, and the ion spray needle voltage was adjusted at 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 precursor to product ion was monitored at 698.5 → 84.3 for SD-0542, 594.6 → 84.2 for apicidin A, and 437.4 → 169.0 for HC-toxin. The retention times of SD-0542, apicidin A, and the internal standard were 2.52, 2.50 and 2.49 min, respectively. The standard curves of SD-0542 and apicidin A were linear over the concentration range from 5-5000 ng/ml (r^2=0.9988). The low limit of quantification was 5 ng/ml for both SD-0542 and apicidin A.

Data Analysis

The serum concentration of SD-0542 and apicidin A *vs.* time data was analyzed by a non-compartmental method using the

nonlinear least squares regression program WinNonlin (Pharsight., Cary, NC). The area under the serum concentration versus time curve (AUC) and the area under the first moment curve (AUMC) were calculated using the trapezoidal rule and extrapolation to time infinity. The extrapolation to time infinity was obtained by C_n/λ_z and $t_n C_n/\lambda_z + C_n/\lambda_z^2$ for AUC and AUMC, respectively. The terminal elimination half-life ($t_{1/2}$) was calculated as $0.693/\lambda_z$. The systemic clearance (Cl) and mean residence time (MRT) were calculated as dose/AUC and AUMC/AUC, respectively. The extent of absolute oral bioavailability (F) was estimated as $AUC_{oral}/AUC_{i.v.}$.

Statistical Analysis

Pharmacokinetic parameter values were expressed as the mean ± S.D. A one-way ANOVA was used to test the differences in the pharmacokinetic parameters obtained after i.v. injection of different doses. The statistical significance was set at $p < 0.05$.

Results

The profiles of mean concentration of SD-0542 vs. time after i.v. bolus injection of 10, 20, and 40 mg/kg doses are shown in Figure 2. The serum concentration-time profiles were best described by multi-exponential equations. Pharmacokinetic parameters of SD-0542 are summarized in Table I. Upon i.v. injection of SD-0542 at doses of 10, 20, and 40 mg/kg, mean values for $t_{1/2}$, Cl, and V_{ss} ranged from 2.0-2.5 hr, 157.2-214.1 ml/min/kg, and 11.1-17.5 L/kg, respectively. There were no significant differences in these parameters as a function of administered dose. The serum concentrations of SD-0542 at time zero (C_0) obtained by extrapolation and AUC were linearly increased as the dose was increased. Serum concentrations of apicidin A, an active metabolite of SD-0542, were

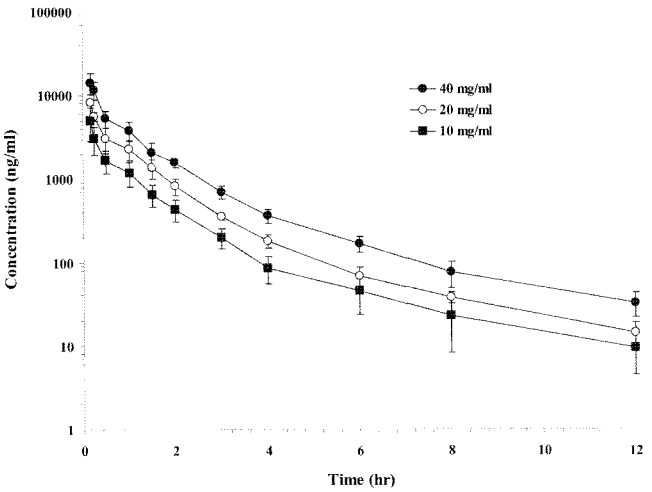

Figure 2–Average serum SD-0542 concentration vs. time curves obtained after i.v. injection of 10, 20, and 40 mg/kg doses in rats (n=4 each).

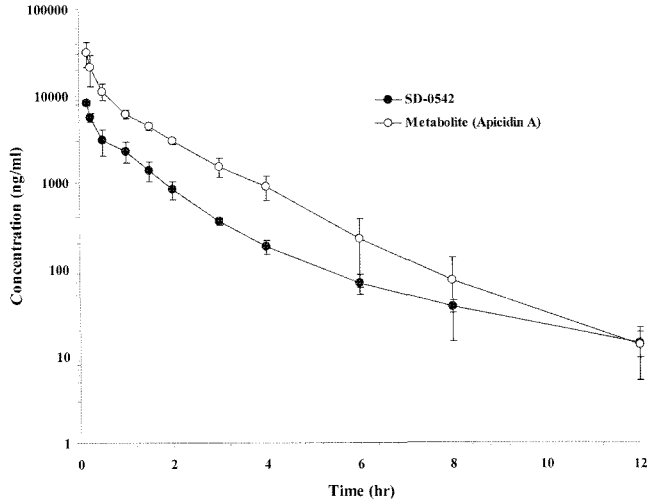

Figure 3–Average serum concentration of SD-0542 and apicidin A vs. time curves obtained after i.v. injection of SD-0542 (20 mg/kg) in rats (n=4).

Table I–*Average Non-compartmental Pharmacokinetic Parameters of SD-0542 Obtained after I.V. Injection of SD-0542 (10, 20 and 40 mg/kg doses) in Rats (mean±S.D.)*

Parameter	10 mg/kg (n = 4)	20 mg/kg (n = 4)	40 mg/kg (n = 4)
Body weight (kg)	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01
$t_{1/2,\lambda z}$ (hr)	2.0 ± 0.7	2.1 ± 0.4	2.5 ± 0.2
C_0 (ng/ml)	13510.9 ± 7411.1	16433.5 ± 4945.3	21740.5 ± 9202.9
AUC (ng·hr/ml)	4683 ± 1300	7991 ± 1227	13668 ± 3256
Cl (ml/min/kg)	157.2 ± 54.4	180.5 ± 30.2	214.1 ± 42.0
V_{ss} (L/kg)	11.1 ± 6.6	12.4 ± 1.7	17.5 ± 4.2
V_z (L/kg)	28.6 ± 15.7	32.3 ± 5.3	45.7 ± 8.8
AUMC (ng·hr^2/ml)	5051 ± 1536	9194 ± 1587	183558 ± 3737
MRT_{iv} (hr)	1.0 ± 0.3	1.1 ± 0.0	1.4 ± 0.1

Table II–*Pharmacokinetic Parameters of SD-0542 and Apicidin A Obtained after I.V. and Oral Administration of SD-0542 (20 mg/kg dose) in Rats (mean±S.D.)*

Parameters	I.V. Injection (n=4)		Oral Administration (n=3)	
	SD-0452	Apicidin A	SD-0452	Apicidin A
$t_{1/2, \lambda z}$ (hr)	2.1 ± 0.4	1.6 ± 0.2	2.0 ± 0.2	2.0 ± 0.3
C_{max} (ng/ml)	16433.5 ± 4945.3	72525.4 ± 17697.8	4.1 ± 2.1	527.6 ± 296.3
T_{max} (hr)	-	-	1.8 ± 0.4	2.7 ± 0.6
Cl/F (ml/min/kg)	42.5 ± 6.6	-	92864.2 ± 31499.4	-
V_z/F (L/kg)	32.3 ± 5.3	-	16331.4 ± 6896.5	-
AUC (ng·hr/ml)	7991 ± 1227	28946 ± 5080	16 ± 5	1713 ± 704
F (%)	-	-	0.9 ± 1.0	-
A_e (ng)[1]	-	18509 ± 5601	-	716 ± 522

[1]Amount excreted unchanged in urine.

also measured after i.v. injection of SD-0542 at a dose of 20 mg/kg dose (n=4) (Figure 3). Throughout the sampling period, serum levels of apicidin A were higher than those of the parent compound. Initial serum concentration (C_0) and AUC of apicidin A were 4.4- and 3.6-folds higher than those of SD-0542 (Table II). The cumulative amount of apicidin A excreted in urine was low (18509 ng), while SD-0542 was not detected in urine. The apparent terminal elimination half-lives were comparable between SD-0542 (2.1 ± 0.4 hr) and apicidin A (1.6 ± 0.2 hr).

The average serum concentration-time profiles of SD-0542 and its active metabolite apicidin A obtained after oral administration of SD-0542 (20 mg/kg dose) are shown in Figure 4. The maximum serum concentration (C_{max}) averaged 4.1 ± 2.1 ng/ml for SD-0542 and 527.6 ± 26.3 ng/ml for apicidin A (Table II). The mean AUC was 16 ± 5 ng/ml for SD-0542 and 1713 ± 704 ng·hr/ml for apicidin A. The cumulative amount of apicidin A excreted in urine was low (716 ng), while SD-0542 was not detectable in urine. The apparent terminal elimination half-lives averaged 2.0 hr for both SD-0542 and apicidin A. The absolute oral bioavailability was extremely low (0.9 ± 1.0%).

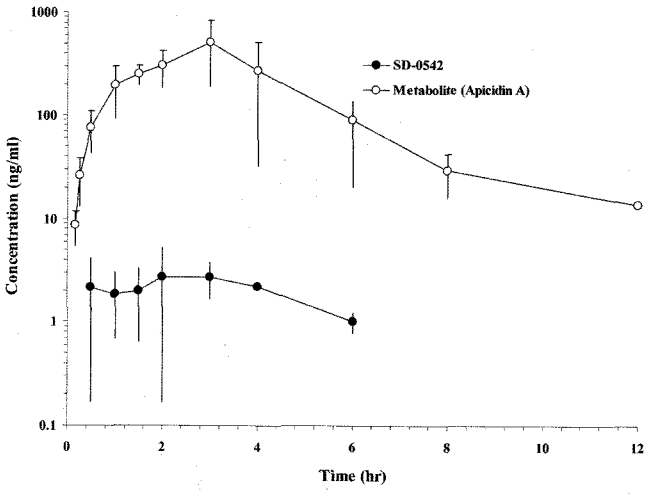

Figure 4–Average serum concentration of SD-0542 and apicidin A vs. time curves obtained after oral administration of SD-0542 (20 mg/kg) in rats (n=3).

Discussion

The pharmacokinetics of SD-0542 were linear over the i.v. dose range from 10-40 mg/kg in rats. This was demonstrated by the fact that $t_{1/2}$, Cl, and V_z remained unaltered and C_0 and AUC increased linearly as a function of dose. SD-0542 exhibited a relatively short $t_{1/2}$ (2.0-2.5 hr) and high Cl (157.2-214.1 ml/min/kg), and was eliminated quickly from the rats. The distribution volume was large, indicating that SD-0542 was extensively distributed to body organs and tissues. SD-0542 was metabolized to apicidin A, the concentration of which exceeding that of SD-0542 throughout the sampling period. The $AUC_{apicidin\ A}/AUC_{SD\text{-}0542}$ ratio was 3.6. To date, limited information is available in literature as to the assay method for and pharmacokinetics of apicidin and its structurally related tetrapeptides.[12-13] Upon i.v. injection (1 mg/kg), apicidin exhibits an elimination half-life of 1.0 ± 0.1 hr, steady-state volume of distribution of 2.6 ± 0.5 L/kg, and systemic clearance of 62.5 ± 11.0 ml/min less than those of SD-0542. The structurally related tetrapeptide, depsipeptide, exhibits greater systemic clearance (425.3 ± 117.7 ml/min) but similar elimination half-life (87.3 ± 57 min) and steady-state volume of distribution (22.3 ± 7.3 L/kg) compared to SD-0542.

SD-0542 was found stable in the dosing vehicle, methanol as well as in rat serum when stored at -20°C, i.e., no degradation occurring for up to 2 hr (unpublished data). At room temperature, however, SD-0542 showed rapid degradation, with % remaining being 80.0 and 70.8% at 0.5 and 1 hr, respectively.

Accordingly, during the course of the study, blood and serum samples were handled to minimize exposure to room temperature.

Upon oral administration, the absolute bioavailability of SD-0542 was extremely low (0.9 ± 1.0%), with high variability. Serum levels of apicidin A were significantly higher than those of the parent compound, with $AUC_{apicidin\ A}/AUC_{SD\text{-}0542}$ ratio being 107.1. This value was greater than that found after i.v. injection (3.6), indicating an extensive hepatic 1st-pass metabolism of SD-0542 to apicidin A. Nonetheless, $AUC_{apicidin\ A}$ after oral administration was less than that after i.v. injection (1713 ± 704 vs. 28946 ± 5080 ng·hr/ml) at a 20 mg/kg dose.

In summary, this study characterized the i.v. and oral pharmacokinetics of SD-0542 in rats. A dose-linearity of pharmacokinetics was observed over an i.v. dose range from 10-40 mg/kg. The oral absorption of SD-0542 was low in rats.

Acknowledgements

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