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Short Communication

Simvastatin hydroxy acid fails to attain sufficient CNS tumor exposure to achieve cytotoxic effect: Result of a preclinical cerebral microdialysis study

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Running Title:

Limited CNS tumor penetration of simvastatin hydroxy acid

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Abbreviations:

tECF, tumor extracellular fluid; SVA, simvastatin acid; SV, simvastatin lactone; $K_{p,u}$, tumor to plasma partition coefficient; HTS, high throughput screening; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; BBB, blood brain barrier; AUC, area under concentration time curve; DMPK, drug metabolism pharmacokinetic; LLOQ, lower limit of quantification; MTD, maximum tolerated dose

Abstract

HMG-CoA reductase inhibitors were potent hits against a mouse ependymoma cell line, but their effectiveness against CNS tumors will depend on their ability to cross the blood-brain barrier and attain a sufficient exposure at the tumor. Among HMG-CoA inhibitors that had activity in vitro, we prioritized simvastatin as the lead compound for preclinical pharmacokinetic studies based on its potential for CNS penetration as determined from *in-silico* models. Further we performed systemic plasma disposition and cerebral microdialysis studies of simvastatin (100 mg/kg, PO) in a murine model of ependymoma to characterize plasma and tumor extracellular fluid (tECF) pharmacokinetic properties. The murine dosage of simvastatin (100 mg/kg, oral) was equivalent to the MTD in patients (7.5 mg/kg, PO) based on equivalent plasma exposure of simvastatin acid (SVA) between the two species. Simvastatin (SV) is rapidly metabolized in murine plasma with 15 times lower exposure compared to human plasma. SVA exposure in tECF was <33.8 ± 11.9 µg/L*hr, whereas tumor to plasma partition coefficient of SVA ($K_{p,u}$) was <0.084 ± 0.008. Compared to *in vitro* washout IC_{50}s, we did not achieve sufficient exposure of SVA in tECF to suggest tumor growth inhibition, therefore simvastatin was not carried forward in subsequent preclinical efficacy studies.

Introduction

Ependymomas are rare tumors arising from ependymal tissue of the brain and spinal cord (McGuire et al., 2009). Although the current treatment options for ependymoma of aggressive surgical resection and conformal radiotherapy are effective in selected patients, approximately 40% of patients either fail to respond or relapse from their disease. Moreover, in children less than 3 years of age, the use of radiotherapy is limited by the occurrence of neurocognitive deficits (Merchant et al., 2009; Netson et al., 2012). Thus, effective chemotherapies for ependymoma are sorely needed, and represent an unmet medical need. We have applied a high throughput screening (HTS) approach using various chemical libraries including FDA-approved compounds to find effective chemotherapeutic agents against ependymoma (Mohankumar et al., 2015). In our HTS, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors (e.g., simvastatin, lovastatin, fluvastatin and pitavastatin) were identified as potent and selective cytotoxic agents against a mouse ependymoma cell line.

HMG CoA reductase is an essential enzyme for synthesis of cholesterol via the mevalonate pathway. Interest in the mevalonate pathway as a target for cancer therapy was initially raised when its end product farnesyl isoprenoid was found to be involved in anchoring small GTPase (e.g., RAS and RHO protein) to the cell membrane. This anchorage drives intracellular signal transduction that regulates vital cellular processes such as growth, proliferation, and survival (Casey et al., 1989; Konstantinopoulos et al., 2007). Therefore inhibition of mevalonate pathway is considered a reasonable strategy to hamper tumor growth. Statins, which are inhibitors of the HMG CoA reductase enzyme and sterol synthesis showed tumor inhibitory effect against various human cell

lines including human glioma cell lines (Kikuchi et al., 1997). However, when evaluated in an *in vivo* mouse model of glioblastoma multiforme, simvastatin failed to show tumor inhibitory effects (Bababeygy et al., 2009). Multiple factors could be responsible for failure of chemotherapies against central nervous system (CNS) tumors, but often a primary factor is limited blood-brain barrier permeability (Parrish et al., 2015). Very limited data are available to provide insight into the CNS penetration of statins, and the data available typically provide only a point estimate of total plasma and brain homogenate concentrations (Thelen et al., 2006).

To gain confidence in translating *in vitro* activity results to *in vivo* efficacy studies, it is crucial that distribution of the compound in the target tissue be characterized. We have utilized cerebral microdialysis to assess local drug distribution as part of our standard preclinical drug developmental strategy (Jacus et al., 2014). Objectives of the current study were to prioritize statins for preclinical pharmacokinetic (PK) studies using a physico-chemical property-based *in-silico* brain-to-plasma partition coefficient prediction model, to characterize the plasma PK of simvastatin (SV) and its active metabolite simvastatin acid (SVA), and to determine tumor disposition of SVA using a cerebral microdialysis technique in our murine model.

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Materials and methods

Use of in-silico approach to prioritize compounds

Two different published *in-silico* models were used to prioritize the statins for preclinical studies based on their predicted ability to cross the blood-brain barrier (BBB) (see **Supplement Data** for additional details). The first *in-silico* model predicted logBB (logarithm of ratio between brain and plasma exposure) using compound specific physico-chemical descriptors (Feher et al., 2000). In the second *in-silico* model, we calculated a 'rule of thumb' score using favorable and unfavorable values of compound specific molecular descriptors for CNS penetration. (Pajouhesh and Lenz, 2005; Mensch et al., 2009).

Preclinical pharmacokinetic study

Plasma PK and cerebral microdialysis studies of SV and SVA were performed in CD1 nude mice bearing cortical implants of mouse ependymoma (Mohankumar et al., 2015). Simvastatin (10 mg/mL prepared in 0.5% carboxymethylcellulose) was administered at 100 mg/kg via oral gavage. For the plasma PK study, a serial sacrifice design was used and plasma samples were collected at 0.25, 1.5, 3.5, 6, and 8 hr postdose (n=3 mice per time point) via cardiac puncture for measurement of SV and SVA (see **Supplemental Data** for additional details). For the microdialysis study, a microdialysis probe, precalibrated for recovery, was implanted in the intracerebral murine ependymoma tumor through a preimplanted guide cannula. The microdialysis probe was continuously perfused with aCSF containing 10% w/v β-cyclodextrin (BCD) at a flow rate of 0.5 µL/min. After the probe was allowed to equilibrate for 1 hr, mice were dosed with SV (100 mg/kg, orally) and plasma samples were collected at 0.083,

1.5, and 4.75 hr postdose whereas 1-hr microdialysis fractions were collected up to 6 hr for analysis of SVA (see **Supplemental Data** for additional details).

Pharmacokinetic data analysis

A population PK model was used to derive PK parameters for the plasma disposition of SV and SVA. A drug-metabolite pharmacokinetic (DMPK) model (**Figure 1a**) consisting of a gut and a plasma compartment was fitted to the plasma concentration time data. The apparent mean PK parameters along with their standard error of estimates (SEE) and inter-individual variability (IIV) were estimated using nonlinear mixed effect modeling (NONMEM 7.2, ICON development solutions). First order conditional estimation (FOCE) method with interaction was used to derive population mean parameter estimates and variance terms, whereas SEE were derived using importance sampling method (IMP) with interaction by running the expectation step only (EONLY=1). SVA area under plasma concentration time curve ($AUC_{plasma}^{0\rightarrow t\ hr}$) was estimated by integration of concentration time profile using modeling, whereas area under tECF concentration time curve ($AUC_{tECF}^{0\rightarrow t\ hr}$) was estimated using the trapezoidal method by replacing below LLOQ data with LLOQ as depicted in **Equation I**.

$$AUC_{tECF}^{0\rightarrow t\ hr} = \sum_{i=1}^{t} C_i \times \tau \qquad \textbf{Equation I}$$

Where C_i is the SVA concentration observed in i^{th} dialysate sample collected over 1 hour interval (τ). The extent of SVA distribution in tECF ($K_{p,u}$, tumor to plasma partition coefficient of SVA) was calculated as a ratio of area under unbound tECF to total plasma concentration time profile ($AUC_{tECF}^{0\rightarrow t\ hr} / AUC_{plasma}^{0\rightarrow t\ hr}$). Additional details of the PK data analysis are provided in **Supplemental Data**.

Results and discussion

In-silico approach to prioritize compound

From *in vitro* HTS simvastatin, fluvastatin, lovastatin, and pitavastatin were identified as drugs with antitumor activity against murine ependymoma tumors with *in vitro* 72 hr IC_{50} values below 0.1 µM. To optimize resources and time required for preclinical efficacy studies, it was necessary to establish a method to prioritize which of these drugs would be carried forward into these studies. We chose to prioritize compounds based upon their likelihood for CNS penetration, however, for the statins scarce reliable data on brain distribution is available. Thus, we adapted an alternative approach to assess CNS penetration using *in-silico* models. We used the Feher model and calculated a logBB for simvastatin, lovastatin, fluvastatin, and pitavastatin of -1.120, -1.164, -1.268, and -1.669, respectively. This is compared to our second *in-silico* model which calculated "rule of thumb" scores for the same compounds of 8, 8, 5, and 4, respectively. Based on the results of our *in-silico* model calculations, we ranked the priority of the statins in order of high to low: simvastatin, lovastatin, fluvastatin, and pitavastatin. When Sierra and colleagues measured *in vitro* passive permeability using parallel artificial membrane permeation assay, a similar rank order of the statins was determined (Sierra et al., 2011). Although the results of our *in-silico* approach did not clearly distinguish between the top two statins (i.e., simvastatin and lovastatin), we used other characteristics (i.e., previously published antitumor activity, dosing data in pediatrics) to make the decision to carry simvastatin forward as our lead statin for further preclinical PK studies (Hindler et al., 2006).

Plasma pharmacokinetic study

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After oral absorption, the prodrug SV converts to the active SVA by enzymatic and non-enzymatic means (Vickers et al., 1990). To describe the plasma disposition and quantitate systemic exposures of SV and SVA in CD1 nude mice for comparison with tolerable exposures in human, and to derive a plasma limited sampling model (LSM) for use during cerebral microdialysis studies, we first studied plasma PK of SV (100 mg/kg, oral) and resultant SVA in the murine EPY model using a serial sacrifice design. As shown in **Figure 1b**, plasma SV and SVA concentration-time data were well described using a population based DMPK model (**Figure 1a**). Model predicted population mean parameter estimates ± standard errors were 0.67 ± 0.45 hr^{-1} for absorption constant of SV, 2812 ± 591 L/hr/kg and 281 ± 9.8 L/hr/kg for metabolic clearance of SV (CL_m/F) and systemic clearance of SVA (CL_{SVA}/F), respectively, and 159 ± 1.0 L/kg and 51.4 ± 0.47 L/kg for plasma volume of distribution of SV (V_{SV}/F) and SVA (V_{SVA}/F), respectively. The IIV for K_a, CL_m/F, and CL_{SVA}/F were estimated to be 31%, 84%, and 26%, respectively.

As shown in **Table 1**, we compared model predicted $AUC_{plasma}^{0\rightarrow 12\,hr}$ in our murine model with that in patients studied with high-dosage simvastatin (maximum tolerated dosage - 7.5 mg/kg simvastatin given orally, twice a day) to determine the human equivalent dosage of SV for our murine model (Ahmed et al., 2013). The SVA AUC values observed in our murine model were comparable to those in patients. However, SV AUC values in our murine model were ~15 times lower than patients. This suggests that the metabolism of SV to SVA occurs more rapidly in mouse plasma than in human plasma, which may be due to presence of caboxylesterase enzyme (responsible for metabolism conversion of SV to SVA) in mouse plasma (Bahar et al., 2012). The active

metabolite SVA has been shown to inhibit HMG CoA reductase enzyme of the mevalonate pathway and reduce cholesterol synthesis. Therefore it was suggested that exposure to SVA is associated with its tumor inhibitory activity in several cancers (Gazzerro et al., 2012). In fact, SVA was twice as potent as SV, with respect to cytotoxicity, when applied to our murine EPY cell line *in vitro* (data not shown). Considering the similar exposures of SVA between mice and human patients, 100 mg/kg SV in mice was considered equivalent to the human MTD (7.5 mg/kg) and was used in the subsequent microdialysis study. The optimal plasma sampling time points derived using LSM for SVA for microdialysis experiments were found to be 0.08, 1.5, and 4.75 hr postdose.

Cerebral microdialysis study in tECF

We have used cerebral microdialysis to measure unbound SVA concentration in the tECF of mice with ependymoma tumors. The design of our cerebral microdialysis experiments allows us to collect small volumes of plasma and dialysate; therefore, we were only able to evaluate disposition of SVA during this study. Mean ± standard deviation of plasma PK parameter post hoc estimates from individual microdialysis experiments (n=4) were 0.52 ± 0.30 1/hr for K_a, 2936 ± 313 L/hr/kg for CL_m/F, 12.3 L/kg for V_{sv}/F, 157 L/kg for V_{sva}/F and 238 ± 133 L/hr/kg for CL_{sva}/F. Variance parameters for V_{sv}/F and V_{sva}/F were fixed to zero. The *in vitro* percent recovery of microdialysis probe for SVA was 10.9 ± 4.0%. SVA concentrations in dialysate were corrected for probe recovery to calculate actual SVA concentration in the tECF. Although we used a sensitive (LLOQ = 0.5 ng/mL) LC-MS/MS method to quantify SVA in dialysate samples, over half the samples were below the LLOQ. To depict the SVA tECF disposition, we

used all tECF concentration data obtained during our microdialysis experiments. **Figure 2** shows total plasma and unbound tECF concentration time profile of SVA in four microdialysis experiments. The mean tECF to plasma partition coefficient of SVA ($K_{p,u}$) of SVA estimated by replacing below LLOQ data with LLOQ was 0.084 ± 0.008. Unbound tECF concentration of SVA was below the lowest *in vitro* IC_{50} (0.04 µM ~ 17 µg/L for 72 hr exposure, data not shown) against our mouse ependymoma cell line. Assuming similar *in vitro* and *in vivo* tumor inhibitor potency of SVA against ependymoma and considering the *in vitro* IC_{50} as a minimum concentration cut-off for promoting a compound to further preclinical efficacy studies, simvastatin was unsuccessful in achieving sufficient SVA tECF concentrations at clinically tolerable plasma exposures. Based on these data, simvastatin was not carried forward in our preclinical pipeline. In the literature, simvastatin had a tumor inhibitory effect against several glioma cell lines (Gliemroth et al., 2003). However, when tested *in vivo* at 10 mg/kg oral SV using mouse model of glioblastoma multiforme, simvastatin failed to show tumor growth inhibition (Bababeygy et al., 2009). The disconnect between these two studies could be due to the low concentration of SVA in tECF.

In conclusion, among the four statins with in vitro antitumor activity against mouse ependymoma, we have used an *in-silico* approach to prioritize simvastatin for plasma and tumor microdialysis studies. We have systematically characterized the plasma disposition of simvastatin (SV and SVA) and tECF disposition of SVA, and conclude that the SVA tECF concentration in our mouse model was not sufficient to achieve ependymoma tumor growth inhibition, therefore simvastatin was not pursued further in our preclinical pipeline.

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Authors Contributions:

Participated in research design: Patel, Jacus, Boulos, Freeman, Gilbertson, Stewart

Conducted experiments: Patel, Jacus, Davis, Boulos, Vuppala

Contributed new reagents or analytic tools: Vuppala, Gilbertson, Stewart

Performed data analysis: Patel, Freeman, Stewart

Wrote or contributed to the writing of the manuscript: Patel, Turner, Vuppala, Freeman, Stewart

References

Ahmed TA, Hayslip J, and Leggas M (2013) Pharmacokinetics of high-dose simvastatin in refractory and relapsed chronic lymphocytic leukemia patients. *Cancer Chemother Pharmacol* **72:**1369-1374.

Bababeygy SR, Polevaya NV, Youssef S, Sun A, Xiong A, Prugpichailers T, Veeravagu A, Hou LC, Steinman L, and Tse V (2009) HMG-CoA reductase inhibition causes increased necrosis and apoptosis in an in vivo mouse glioblastoma multiforme model. *Anticancer research* **29:**4901-4908.

Bahar FG, Ohura K, Ogihara T, and Imai T (2012) Species difference of esterase expression and hydrolase activity in plasma. *J Pharm Sci* **101:**3979-3988.

Casey PJ, Solski PA, Der CJ, and Buss JE (1989) p21ras is modified by a farnesyl isoprenoid. *Proceedings of the National Academy of Sciences of the United States of America* **86:**8323-8327.

Feher M, Sourial E, and Schmidt JM (2000) A simple model for the prediction of blood-brain partitioning. *International journal of pharmaceutics* **201:**239-247.

Gazzerro P, Proto MC, Gangemi G, Malfitano AM, Ciaglia E, Pisanti S, Santoro A, Laezza C, and Bifulco M (2012) Pharmacological actions of statins: a critical appraisal in the management of cancer. *Pharmacol Rev* **64:**102-146.

Gliemroth J, Zulewski H, Arnold H, and Terzis AJ (2003) Migration, proliferation, and invasion of human glioma cells following treatment with simvastatin. *Neurosurg Rev* **26:**117-124.

Hindler K, Cleeland CS, Rivera E, and Collard CD (2006) The role of statins in cancer therapy. *Oncologist* **11:**306-315.

Jacus MO, Throm SL, Turner DC, Patel YT, Freeman BB, 3rd, Morfouace M, Boulos N, and Stewart CF (2014) Deriving therapies for children with primary CNS tumors using pharmacokinetic modeling and simulation of cerebral microdialysis data. *Eur J Pharm Sci* **57:**41-47.

Kikuchi T, Nagata Y, and Abe T (1997) In vitro and in vivo antiproliferative effects of simvastatin, an HMG-CoA reductase inhibitor, on human glioma cells. *J Neurooncol* **34:**233-239.

Konstantinopoulos PA, Karamouzis MV, and Papavassiliou AG (2007) Post-translational modifications and regulation of the RAS superfamily of GTPases as anticancer targets. *Nature reviews Drug discovery* **6:**541-555.

McGuire CS, Sainani KL, and Fisher PG (2009) Incidence patterns for ependymoma: a surveillance, epidemiology, and end results study. *Journal of neurosurgery* **110:**725-729.

Mensch J, Oyarzabal J, Mackie C, and Augustijns P (2009) In vivo, in vitro and in silico methods for small molecule transfer across the BBB. *Journal of pharmaceutical sciences* **98:**4429-4468.

Merchant TE, Li C, Xiong X, Kun LE, Boop FA, and Sanford RA (2009) Conformal radiotherapy after surgery for paediatric ependymoma: a prospective study. *Lancet Oncol* **10:**258-266.

Mohankumar KM, Currle DS, White E, Boulos N, Dapper J, Eden C, Nimmervoll B, Thiruvenkatam R, Connelly M, Kranenburg TA, Neale G, Olsen S, Wang YD, Finkelstein D, Wright K, Gupta K, Ellison DW, Thomas AO, and Gilbertson RJ

(2015) An in vivo screen identifies ependymoma oncogenes and tumor-suppressor genes. *Nat Genet* **47:**878-887.

Netson KL, Conklin HM, Wu S, Xiong X, and Merchant TE (2012) A 5-year investigation of children's adaptive functioning following conformal radiation therapy for localized ependymoma. *Int J Radiat Oncol Biol Phys* **84:**217-223 e211.

Pajouhesh H and Lenz GR (2005) Medicinal chemical properties of successful central nervous system drugs. *NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics* **2:**541-553.

Parrish KE, Sarkaria JN, and Elmquist WF (2015) Improving drug delivery to primary and metastatic brain tumors: strategies to overcome the blood-brain barrier. *Clin Pharmacol Ther* **97:**336-346.

Sierra S, Ramos MC, Molina P, Esteo C, Vazquez JA, and Burgos JS (2011) Statins as neuroprotectants: a comparative in vitro study of lipophilicity, blood-brain-barrier penetration, lowering of brain cholesterol, and decrease of neuron cell death. *Journal of Alzheimer's disease : JAD* **23:**307-318.

Thelen KM, Rentsch KM, Gutteck U, Heverin M, Olin M, Andersson U, von Eckardstein A, Bjorkhem I, and Lutjohann D (2006) Brain cholesterol synthesis in mice is affected by high dose of simvastatin but not of pravastatin. *The Journal of pharmacology and experimental therapeutics* **316:**1146-1152.

Vickers S, Duncan CA, Chen IW, Rosegay A, and Duggan DE (1990) Metabolic disposition studies on simvastatin, a cholesterol-lowering prodrug. *Drug metabolism and disposition: the biological fate of chemicals* **18:**138-145.

Footnotes

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Legends for figures

Figure 1: **(a)** Schematic diagram of drug metabolism pharmacokinetic (DMPK) model (K_a - first order absorption constant; V_{sv} and V_{sva} - plasma volume of distribution for SV and SVA, respectively; CL_m - metabolic clearance for SV to SVA metabolism; CL_{sva} - systemic clearance of SVA from plasma compartment) **(b)** Simvastatin (SV) and simvastatin acid (SVA) plasma concentration time profile for full plasma pharmacokinetic study (open circle and open triangle represents observed SV and SVA concentrations, respectively; solid and dotted line represents population mean predicted SV and SVA concentrations, respectively)

Figure 2: Simvastatin acid (SVA) concentration time profile in plasma and tumor ECF for individual microdialysis experiment (open circle represents observed SVA concentrations in plasma, Open triangle and diamond represents SVA concentrations in tumor ECF that are above and below LLOQ, respectively; solid and dashed line represents model predicted concentration in plasma and fractional mean concentration profile in tumor ECF for SVA, respectively; horizontal dotted line represents recovery corrected LLOQ for individual mouse for SVA method for tumor ECF)

Table

Table 1: Comparison of plasma pharmacokinetic parameters for SV and SVA between mouse ependymoma model (100 mg/kg, oral simvastatin) and human patients (Ahmed et al., 2013) (7.5 mg/kg, oral simvastatin)

Parameters	Mice (n=15)* (Mean ± SD)		Human (n=3) (Ahmed et al., 2013) (Mean ± SD)	
	SV	SVA	SV	SVA
AUC_{0-12} (ug/L*hr)	51.9 ± 39.5	362.8 ± 54.2	795.3 ± 753.4	349.3 ± 218.3
C_{max} (ug/L)	28.6 ± 19.0	178.3 ± 36.3	376.7 ± 460.4	109 ± 131.0
T_{max} (hr)	0.23 ± 0.13	0.55 ± 0.14	1.3 ± 0.5	3.3 ± 2.5

*Parameters obtained by simulation

Figure 1

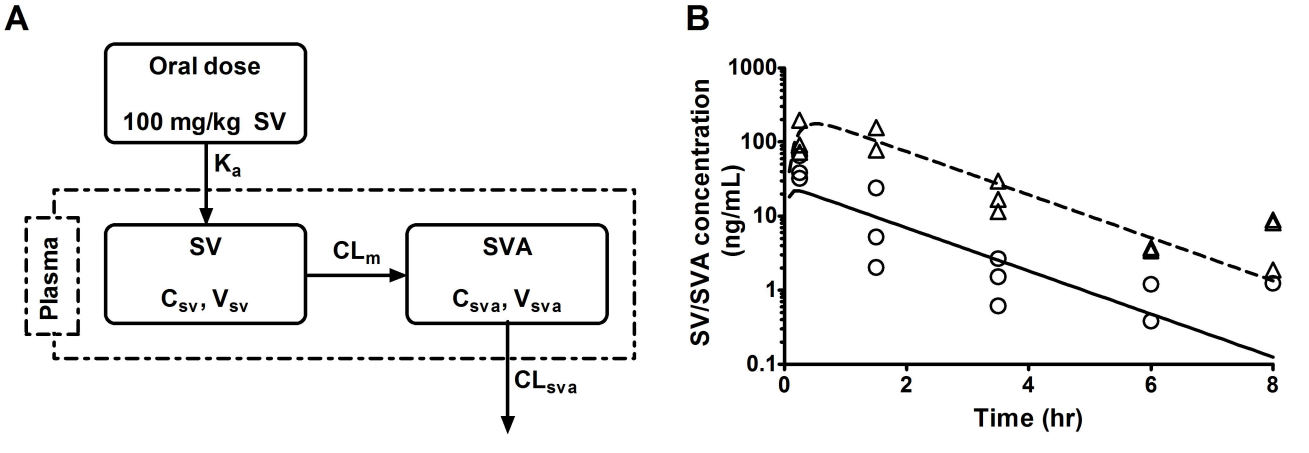
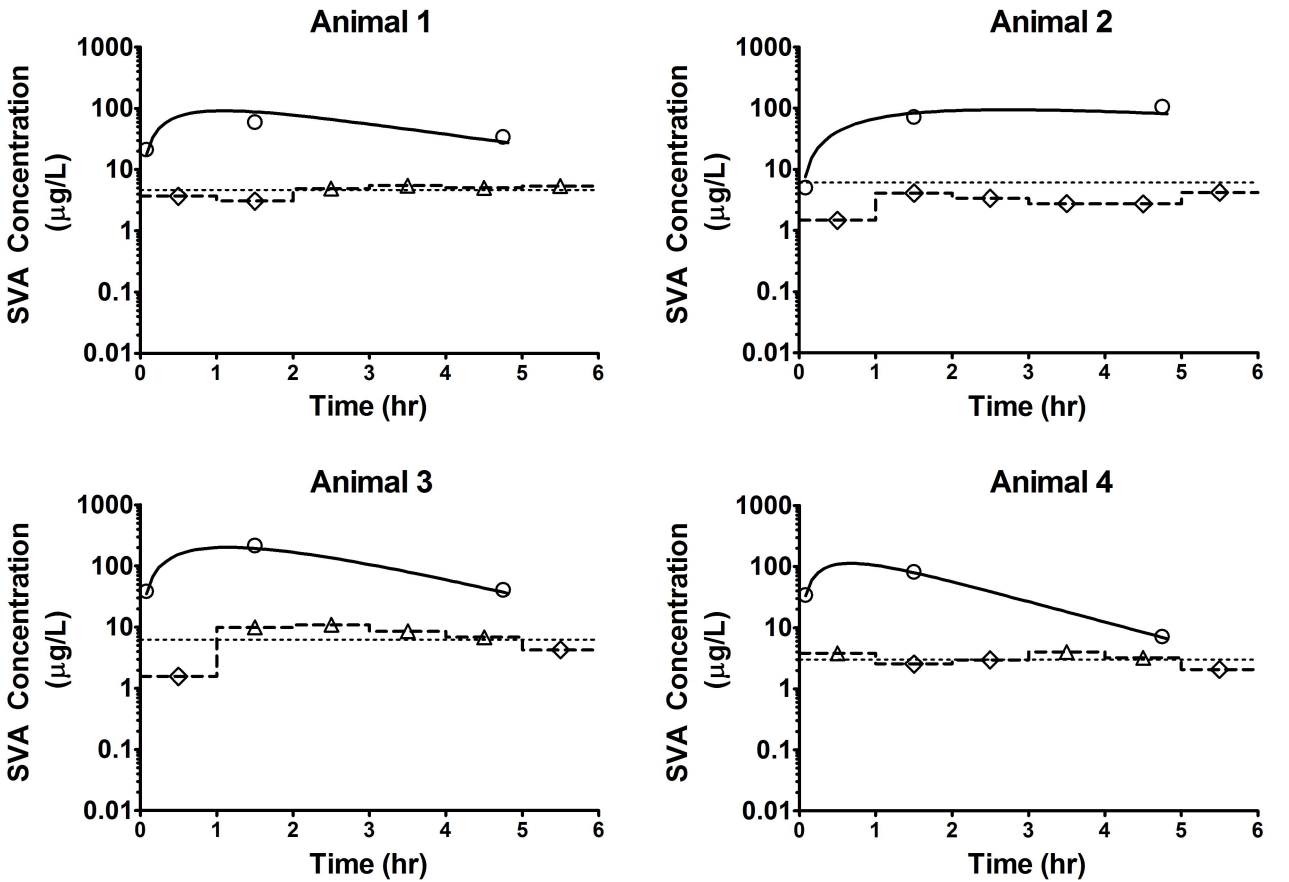

A
Oral dose
100 mg/kg SV
K_a
Plasma
SV
C_{sv}, V_{sv}
CL_m
SVA
C_{sva}, V_{sva}
CL_{sva}
B
SV/SVA concentration (ng/mL)
1000
100
10
1
0.1
0
2
4
6
8
Time (hr)

Figure 2


Animal 1
Animal 2
Animal 3
Animal 4
SVA Concentration (μg/L)
Time (hr)
1000
100
10
1
0.1
0.01
0
1
2
3
4
5
6