Simvastatin Hydroxy Acid Fails to Attain Sufficient Central Nervous System Tumor Exposure to Achieve a Cytotoxic Effect: Results of a Preclinical Cerebral Microdialysis Study

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ABSTRACT

3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors were potent hits against a mouse ependymoma cell line, but their effectiveness against central nervous system tumors will depend on their ability to cross the blood–brain barrier and attain a sufficient exposure at the tumor. Among 3-hydroxy-3-methylglutaryl coenzyme A inhibitors that had activity in vitro, we prioritized simvastatin (SV) as the lead compound for preclinical pharmacokinetic studies based on its potential for central nervous system penetration as determined from in silico models. Furthermore, we performed systemic plasma disposition and cerebral microdialysis studies of SV (100 mg/kg, p.o.) in a murine model of ependymoma to characterize plasma and tumor extracellular fluid (tECF) pharmacokinetic properties. The murine dosage of SV (100 mg/kg, p.o.) was equivalent to the maximum tolerated dose in patients (7.5 mg/kg, p.o.) based on equivalent plasma exposure of simvastatin acid (SVA) between the two species. SV is rapidly metabolized in murine plasma with 15 times lower exposure compared with human plasma. SVA exposure in tECF was $<33.8 \pm 11.9 \mu g/l$ per hour, whereas the tumor to plasma partition coefficient of SVA was $<0.084 \pm 0.008$. Compared with in vitro washout IC_{50} values, we did not achieve sufficient exposure of SVA in tECF to suggest tumor growth inhibition; therefore, SV was not carried forward in subsequent preclinical efficacy studies.

Short Communication

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Introduction

Ependymomas are rare tumors arising from ependymal tissue of the brain and spinal cord (McGuire et al., 2009). Although the current ependymoma treatment options 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 younger than age 3 years, 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 U.S. Food and Drug Administration–approved compounds to find effective chemotherapeutic agents against ependymoma (Mohankumar et al., 2015). In our HTS studies, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors, such as simvastatin (SV), 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 involvement in anchoring small GTPase (e.g., RAS and RHO proteins) 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 the 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 effects 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, SV 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 used cerebral microdialysis to assess local drug distribution as part of our standard preclinical drug developmental strategy (Jacus et al., 2014). Objectives of this study were to prioritize statins for preclinical pharmacokinetics (PK) studies using a physicochemical property–based in silico brain-to-plasma partition coefficient prediction model, to characterize the plasma PK of 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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ABBREVIATIONS: AUC, area under concentration time curve; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HTS, high-throughput screening; LLOQ, lower limit of quantification; PK, pharmacokinetics; SV, simvastatin; SVA, simvastatin acid; tECF, tumor extracellular fluid.

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Materials and Methods

Use of an 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 (see the Suppimental Materials and Methods for additional details). The first in silico model predicted the logarithm of the ratio between brain and plasma exposure using compound-specific physicochemical 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 PK 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). SV (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 hours postdose (n = 3 mice per time point) via cardiac puncture for measurement of SV and SVA (see the (Suppimental Materials and Methods for additional details). For the microdialysis study, a microdialysis probe, precalibrated for recovery, was implanted in the intracerebral mouse ependymoma tumor through a preimplanted guide cannula. The microdialysis probe was continuously perfused with artificial cerebrospinal fluid containing 10% (w/v) β-cyclodextrin at a flow rate of 0.5 μL/min. After the probe was allowed to equilibrate for 1 hour, mice were dosed with SV (100 mg/kg, p.o.) and plasma samples were collected at 0.083, 1.5, and 4.75 hours postdose, whereas 1-hour microdialysis fractions were collected up to 6 hours for analysis of SVA (see the (Suppimental Materials and Methods for additional details).

PK Data Analysis. A population PK model was used to derive PK parameters for the plasma disposition of SV and SVA. A drug-metabolite PK model (Fig. 1A) consisting of gut and plasma compartments was fitted to the plasma concentration data. The apparent mean PK parameters, along with their standard error of estimates and interindividual variability, were estimated using nonlinear mixed-effects modeling (NONMEM 7.2; ICON Development Solutions, Baltimore, MD). The first-order conditional estimation method with interaction was used to derive population mean parameter estimates and variance terms, whereas standard errors of estimates were derived using the importance sampling method with interaction by running the expectation step only (EONLY = 1). The SVA area under plasma concentration time curve (AUC_{0→t}^\text{plasma}) was estimated by integration of the concentration time profile using modeling, whereas area under the tumor extracellular fluid (tECF) concentration time curve (AUC_{0→t}^\text{tECF}) was estimated using the trapezoidal method by replacing below lower limit of quantification (LLOQ) data with LLOQ as depicted in eq. 1:

$$AUC_{0→t}^\text{plasma} = \frac{t}{\tau} \sum C_i \times \tau$$

where $C_i$ is the SVA concentration observed in the ith dialysate sample collected over a 1-hour interval (τ). The extent of SVA distribution in the tECF ($K_{\text{pas}}$, the tumor to plasma partition coefficient of SVA) was calculated as a ratio of the area under unbound tECF to total plasma concentration time profile ($AUC_{0→t}^\text{tECF}/AUC_{0→t}^\text{tECF}$). Additional details of the PK data analysis are provided in the (Suppimental Materials and Methods).

Results and Discussion

In Silico Approach to Prioritize Compounds. From in vitro HTS, SV, fluvastatin, lovastatin, and pitavastatin were identified as drugs with antitumor activity against murine ependymoma tumors with in vitro 72-hour 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 was so that the process could be carried forward into these studies. We chose to prioritize compounds based on their likelihood for CNS penetration; however, scarce reliable data on brain distribution are available for the statins. Thus, we adopted an alternative approach to assess CNS penetration using in silico models. We used the Feher model and calculated logarithm of the ratios between brain and plasma exposure for SV, lovastatin, fluvastatin, and pitavastatin of $-1.120$, $-1.164$, $-1.268$, and $-1.669$, respectively. This is compared with 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 the order of high to low: SV, lovastatin, fluvastatin, and pitavastatin. When Sierra et al. (2011) measured in vitro passive permeability using a parallel artificial membrane permeation assay, a similar rank order of the statins was determined. Although the results of our in silico approach did not clearly distinguish between the top two statins (i.e., SV and lovastatin), we used other characteristics (i.e., previously published antitumor activity, dosing data in pediatrics) to make the decision to carry SV forward as our lead statin for the preclinical PK studies (Hindler et al., 2006).

Plasma PK Study. After oral absorption, the prodrug SV converts to the active SVA by enzymatic and nonenzymatic 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 humans, and to derive a plasma limited sampling model for use during cerebral microdialysis studies, we first studied plasma PK of SV (100 mg/kg, p.o.) and resultant SVA in the murine ependymoma model using a serial-sacrifice design. As shown in Fig. 1B, plasma SV and SVA concentration time data were well described using a population-based drug-metabolite PK model (Fig. 1A). The model-predicted population mean ± S.E. parameter estimates were $0.67 ± 0.45$ h$^{-1}$ for absorption constant of SV; $2812 ± 591$ l/h per kg and $281 ± 9.8$ l/h per kg for metabolic clearance of SV (CL_{SV/F}) and systemic clearance of SVA (CL_{SVA/F}, respectively; and $159 ± 10.0$ 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 interindividual variability for K_{pas}, CL_{SV/F}, and CL_{SVA/F} was estimated to be 31%, 84%, and 26%, respectively.

As shown in Table 1, we compared model-predicted AUC_{0→t}^\text{plasma} in our murine model with that in patients studied with high-dosage SV (maximum tolerated dosage of 7.5 mg/kg SV given orally, twice a day) to determine the human equivalent dosage of SV for our murine model.

Fig. 1. (A) Schematic diagram of a drug metabolism PK model. CL_{SV}, metabolic clearance for SV to SVA metabolite; CL_{SVA}, systemic clearance of SVA from the plasma compartment; K_{pas}, first-order absorption constant; V_{SV} and V_{SVA}, plasma volume of distribution for SV and SVA, respectively. (B) SV and SVA plasma concentration time profiles for full plasma PK study. Open circles and open triangles represent observed SV and SVA concentrations, respectively. The solid line and the dotted line represent population mean predicted SV and SVA concentrations, respectively.
The SVA AUC values observed in our murine model were comparable to those in patients. However, SVA AUC values in our murine model were approximately 15 times lower than those in patients. This suggests that the metabolism of SV to SVA occurs more rapidly in mouse plasma than in human plasma, which may be attributable to the presence of carboxylesterase enzymes (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 enzymes 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 ependymoma 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 maximum tolerated dose (7.5 mg/kg) and was used in the subsequent microdialysis study. The optimal plasma sampling time points derived using a limited sampling model for SVA for microdialysis experiments were 0.08, 1.5, and 4.75 hours postdose.

Cerebral Microdialysis Study in tECF. We used cerebral microdialysis to measure the 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. Means (±S.D.) of plasma PK parameter post hoc estimates from individual microdialysis experiments (n = 4) were 0.52 ± 0.30 l/h for $K_{\text{a}}$, 2936 ± 313 l/h per kg for $CL_{\text{m/F}}$, 12.3 l/kg for $V_{SV/F}$, 157 l/kg for $V_{SVA/F}$, and 238 ± 133 l/h per kg for $CL_{SVA/F}$. Variance parameters for $V_{SV/F}$ and $V_{SVA/F}$ were fixed to zero. The in vitro percentage of microdialysis probe recovery for SVA was 10.9% ± 4.0%. SVA concentrations in dialysate were corrected for probe recovery to calculate the actual SVA concentration in the tECF. Although we used a sensitive (LLOQ = 0.5 ng/mL) liquid chromatography–tandem mass spectrometry method to quantify SVA in dialysate samples, over one-half of the samples were below the LLOQ. To depict the SVA tECF disposition, we used all tECF concentration data obtained during our microdialysis experiments. The mean tECF to plasma partition coefficient of SVA ($K_{\text{p,u}}$) estimated by replacing below LLOQ data with the LLOQ was 0.084 ± 0.008. The unbound SVA tECF concentration was below the lowest in vitro IC$_{50}$ (0.04 μM equivalent to 17 μg/l for 72-hour exposure; data not shown) against our ependymoma cell line. Assuming similar in vitro and in vivo tumor inhibitor potency of SVA against ependymoma and considering the in vitro IC$_{50}$ value as a minimum concentration cutoff for promoting a compound to further preclinical efficacy studies, SV was unsuccessful in achieving sufficient SVA tECF concentrations at clinically tolerable plasma exposures. On the basis of these data, SV was not carried forward in our preclinical pipeline.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mice (n = 15)</th>
<th>Humans (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SV</td>
<td>SVA</td>
</tr>
<tr>
<td>AUC$_{0-12}$ (μg/l per hour)</td>
<td>51.9 ± 39.5</td>
<td>362.8 ± 54.2</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (μg/l)</td>
<td>28.6 ± 19.0</td>
<td>178.3 ± 36.3</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.23 ± 0.13</td>
<td>0.55 ± 0.14</td>
</tr>
</tbody>
</table>

*Parameters obtained by simulation. Data are presented as means ± S.D. Human data are from Ahmed et al. (2013).
orally using a mouse model of glioblastoma multiforme, SV 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 SV for plasma and tumor microdialysis studies. We have systematically characterized the plasma disposition of SV and SVA and tECF disposition of SVA, and we conclude that the SVA tECF concentration in our mouse model was not sufficient to achieve ependymoma tumor growth inhibition; therefore, SV was not pursued further in our preclinical pipeline.

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Contributed new reagents or analytic tools: Vuppala, Gilbertson, Stewart.
Performed data analysis: Patel, Freeman, Stewart.
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