NONLINEAR PHARMACOKINETICS OF EFAVIRENZ (DMP-266), A POTENT HIV-1 REVERSE TRANSCRIPTASE INHIBITOR, IN RATS AND MONKEYS

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ABSTRACT:
Efavirenz (EFV, Sustiva, Stocrin, DMP-266, L-743,726) is a potent and selective non-nucleoside inhibitor of HIV-1 reverse transcriptase. Pharmacokinetics of EFV was studied in rats and monkeys, the safety assessment species. In rats, after 2 and 5 mg/kg i.v. administrations, the mean CLp, Vdss, and T1/2 were 67 ml/min/kg, 5.0 liters/kg, and 1 h, respectively. EFV was metabolized completely, and the products were excreted almost exclusively via bile. At the higher dose of 15 mg/kg, the CLp was reduced by 36%, implying saturation of metabolism processes. A similar phenomenon occurred in monkeys, where the CLp declined by 60% as the i.v. dose was increased from 5 to 15 mg/kg. After oral dosing, the bioavailability of EFV in rats (10 mg/kg) and monkeys (2 mg/kg) was 16% and 42%, respectively. Higher doses in both species led to disproportionate increases in the AUC and higher Tmax values, suggesting saturation of metabolism and/or prolongation of absorption. The delay in Tmax was more pronounced in monkeys where the plasma concentrations reached plateaus and were sustained for 4 to 20 h. In rats, the prolongation of absorption was due to delayed gastric emptying as demonstrated by >10-fold slower transit of [14C]polyethylene glycol through the stomach of EFV-pretreated animals. The delayed gastric emptying in monkeys also was observed when the animals dosed at 160 mg/kg exhibited emesis, 8 h postdose, which was found to contain a substantial portion of the dose. These results demonstrated that in rats and monkeys, both delayed gastric emptying and saturation of metabolic processes played significant roles in the nonlinear pharmacokinetics of EFV.

Recent advances in the treatment of HIV-1, the causative agent of AIDS, have led to the introduction of several new therapeutic agents; viz., the HIV protease inhibitors indinavir, saquinavir, ritonavir, and nelfinavir, and the HIV reverse transcriptase (RT) inhibitors lamivudine (3TC) and stavudine (d4T), in addition to the previously approved RT inhibitors zidovudine (AZT), zalcitabine (DCC), and didanosine (DDI) (Levy, 1994; Cohen et al., 1996; Erickson and Burt, 1996; Vacca et al., 1994). Some of these agents in mono- and combination therapy, attacking different viral targets, have shown dramatic results in achieving sustained suppression of viral replication (Cohen et al., 1996; Gulick, 1996). The multidrug, single- or multiple-target therapies have revived the development of new and more potent RT inhibitors. In the past, the development of several RT inhibitors was negatively influenced by the rapid emergence of drug-resistant variants of the virus, especially when monotherapy was the means. However, in multidrug therapy, mutations induced by one drug may sensitize the virus to other drugs or may delay emergence of mutants to other drugs. Efavirenz (EFV, structure shown below) is one of the most potent and selective non-nucleoside HIV-1 RT inhibitors described thus far (Young et al., 1995). In vitro, EFV showed a Ki value of 2.9 nM and a CI95 for inhibition of virus in cell culture of 1.5 nM.

It is a potent inhibitor of various mutant viral variants that have emerged. Thus, it has a significant potential for use in HIV therapy. Recent clinical studies have shown that the drug is efficient in arresting the growth of virus in mono- and combination therapy (Fiske et al., 1997a; Ruiz et al., 1997). The studies also have shown a long apparent T1/2 of >40 h for EFV, making once-a-day dosing of patients feasible (Kahn et al., 1997; Fiske et al., 1997b). This report describes the observation of nonlinear pharmacokinetics of EFV, and determination of the underlying causes of that in rats and monkeys, the species used in the safety assessment studies.

Abbreviations used are: RT, reverse transcriptase; EFV, efavirenz, Stocrin, Sustiva, DMP 266, L-743,726, (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one; DMSO, dimethyl sulfoxide; HPLC, high-pressure liquid chromatography.

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Materials and Methods

Chemicals. EFV ([S]-6-chloro-4-(cyclopentylethyl)-1,4-dihydro-4-(trifluoromethyl)-2(H)-3,1-benzoxazin-2-one), [14C]EFV, and L-737,345 were prepared at Merck Research Laboratories. The 14C label was incorporated in the carbonyl group of the benzoxazinone moiety, with specific activity of 38.3 mCi/mmol and a radiochemical purity of >99%. All other chemicals were of either analytical or high-pressure liquid chromatography (HPLC) grade.

Pharmacokinetic Studies in Rats. Groups of male Sprague-Dawley rats (n = 3–5, 250–450 g; Taconic Farms, Germantown, NY) were used to study disposition of EFV after i.v. or p.o. administration. Animals, housed separately in metabolism cages without restraint, were fasted overnight and for 8 h after the dosing and were provided water ad libitum. All animals were cannulated in the right jugular vein for i.v. administration and for blood sampling. For excretion studies, animals were also cannulated in the bile duct and duodenum. The surgical procedure was performed under anesthesia using nembutal (0.1 ml/kg, i.p.), 1 day before the experiment. Bile was recirculated until dosing time. Rats were infused with 0.5 ml of a mixture of 5% glucose/10 mM sodium excretion studies, animals were also cannulated in the bile duct and duodenum. The dosing and were provided water ad libitum. All animals were cannulated in the right jugular vein for i.v. administration and for blood sampling. For excretion studies, animals were also cannulated in the bile duct and duodenum. The surgical procedure was performed under anesthesia using nembutal (0.1 ml/kg, i.p.), 1 day before the experiment. Bile was recirculated until dosing time. Rats were infused with 0.5 ml of a mixture of 5% glucose/10 mM sodium taurocholate/5mM KCl/0.9% NaCl once every hour via a duodenum cannula.

A group of rats (n = 4 or 5) received a bolus dose (2 or 5 mg/kg) in dimethylsulfoxide (DMSO) via the cannula implanted in the right jugular vein. The dosing volume of the DMSO solution was 1 ml/kg. Blood samples were obtained with Heparinized syringes predose and at 5, 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 480, 1440, and 2880 min postdose. Plasma was separated immediately by centrifugation. Another group of rats (n = 5) was dosed at 15 mg/kg EFV, infused i.v. for 5 min at 25 ml/min/0.1 kg, and plasma samples were collected periodically over 52 h.

Three groups of rats (n = 4 or 5, each group) were dosed by gavage at 10, 40, and 160 mg/kg. The dosing solutions were prepared in 0.5% methocel by wet grinding to a particle size of mainly <25 μm. The dosing volume was 5 ml/kg. Blood samples were obtained with Heparinized syringes predose and at 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 480, 1440, and 2880 min postdose. Plasma was separated immediately by centrifugation. Another group of rats (n = 5) was dosed at 15 mg/kg EFV, infused i.v. for 5 min at 25 ml/min/0.1 kg, and plasma samples were collected periodically over 52 h.

Gastric Emptying in Rats. The study design involved four groups of male, fasted rats (n = 3 each). Two groups were dosed orally at 160 mg/kg EFV as an oral suspension in 0.5% methocel (6 ml/kg) in dimethylsulfoxide (DMSO) via the cannula implanted in the right jugular vein. The dosing volume of the DMSO solution was 1 ml/kg. Blood samples were obtained with Heparinized syringes predose and at 5, 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 480, 1440, and 2880 min postdose. Plasma was separated immediately by centrifugation. Another group of rats (n = 5) was dosed at 15 mg/kg EFV, infused i.v. for 5 min at 25 ml/min/0.1 kg, and plasma samples were collected periodically over 52 h.

For the excretion study, rats were dosed i.v. at 5 mg/kg [14C]EFV in 35% ethanol in DMSO. The dosing volume was 1 ml/kg. Bile was collected from 0 to 3 h, 3 to 6 h, 6 to 24 h, and 24 to 48 h. Urine was collected from 0 to 6 h, 6 to 24 h, and 24 to 48 h.

Radioactivity Assay. Aliquots (1 ml) of urine samples were mixed with 15 ml of Beckman Ready-Safe liquid scintillation cocktail and analyzed on a Packard CA1600 counter. The feces were homogenized in water, and 1-ml aliquots of the homogenates were air dried and combusted using a Packard Sample Oxidizer. The resulting carbon dioxide was trapped using Carbosorb/Permafluor (Packard) and analyzed on the liquid scintillation counter.

HPLC-Radioactivity Assay of Urine, Bile, and Feces. Aliquots of urine and bile were chromatographed using System A. The HPLC effluent was mixed (1:3) with Packard Flow-Scint III cocktail and then passed through a Raytest Ramona radioactivity detector (System B). Aliquots of the fecal samples were extracted with 5 volumes of methanol followed by centrifugation. The supernatant was evaporated to dryness under a stream of nitrogen, followed by reconstitution with the mobile phase and analysis by HPLC using System B.

Pharmacokinetic Analysis. Concentration at time zero (C0) from the i.v. data was estimated by back-extrapolation using RSTRIP (Micromath Scientific Software, Salt Lake City, UT). Area under the plasma concentration-time curve, total plasma clearance (CLp), terminal half-life (T1/2), and volume of distribution at steady state (Vdss) were determined by the LAGRAN program (Rocci and Jusko, 1983). Formulae used in the calculation of pharmacokinetic parameters by the LAGRAN program were:

\[
\text{CL}_p = \frac{\text{Dose}_i}{\text{AUC}_{0-\infty}}
\]

\[
\text{Vd}_{ss} = \frac{\text{Dose}_i \times (\text{AUMC}_{0\infty})^{1/2}}{(\text{AUC}_{0\infty})^{3/2}}
\]

where Dosei is the i.v. bolus dose, and AUMC_{0\infty} is the total area under the first moment of the drug concentration curve from time zero to infinity.
Bioavailability (F) was assessed by comparing AUCs after oral and i.v. administrations normalized to the dose. The blood clearance was calculated from CLp, and the whole blood-to-plasma concentration ratio using the equation:

$$CL_p = \frac{CL_m}{C_p/C_m}$$

**Statistical Analysis.** Statistical analyses were performed by the method of analysis of variance (Statview II, Abacus Concept, Berkeley, CA).

**Results**

EFV is a highly lipophilic compound with a log p value of >4 and with aqueous solubility of <20 ng/ml. The compound binds extensively to plasma protein with percentage of unbound values of 0.58, 0.57, and 0.54% for rat, monkey, and human, respectively, at pH 7.4. The plasma protein binding was independent of EFV concentration in the range of 0.72 to 32 μM. The rat, monkey, and human blood-to-plasma partition studies of [14C]EFV at 1.5, 7.9, and 29.7 μM showed that upon incubation with fresh blood for 5, 10, 15, and 30 min the radioactivity equilibrated between erythrocytes and plasma rapidly, within 5 min, and that the ratio was independent of EFV concentration. The blood-to-plasma ratios (mean ± S.D.) for rat, monkey, and human were 0.92 ± 0.04, 0.76 ± 0.04, and 0.74 ± 0.02, respectively.

**Rat Studies.** In rats, EFV was cleared rapidly after an i.v. bolus dose of 2 or 5 mg/kg. The plasma concentration-time profile is shown in Fig. 1. The plasma clearance was 67 ml/min/kg, and the rest of the pharmacokinetic parameters are shown in Table 1. After a higher dose of 15 mg/kg the plasma clearance was reduced to 43 ml/min/kg. The plasma concentration-time profiles in rats after single oral doses at 10, 40, and 160 mg/kg are shown in Fig. 2. After 10 mg/kg EFV dosing, the bioavailability was 16%. At higher doses the AUC values increased in a greater than dose-proportional manner (Table 2); hence, the bioavailabilities at these doses were not calculated. The T_{max} values at 10, 40, and 160 mg/kg were 0.6, 1.5, and 3.5 h, respectively.

After i.v. administration of [14C]EFV at 5 mg/kg to bile duct-cannulated rats, greater than 80% of the radioactivity was excreted in bile over 0 to 3 h. Over 48 h, the recovery of radioactivity in bile was quantitative, with urinary excretion accounting for less than 0.3% of the administered radioactivity. HPLC-radioactivity analysis of the bile showed no unchanged parent compound.

In the gastric-emptying experiment, the amount of the radioactivity (mean ± S.D.) remaining in the stomach of the EFV-pretreated rats at 0.5 h and 3 h after [14C]PEG dosing was 23.5 ± 5.6% and 10.4 ± 8.6% of the dose, respectively. Corresponding values from the control groups were 2.0 ± 0.8% and 0.7 ± 0.2% of the dose (Fig. 3). In the control groups, most of the dosed radioactivity was recovered in the distal intestinal segments.

**Monkey Studies.** In monkeys, after i.v. dosing at 1 or 5 mg/kg, the plasma concentrations declined in a biphasic manner as depicted in Fig. 4. The mean plasma clearance was 11.5 ml/min/kg, and the rest of the pharmacokinetic parameters are shown in Table 1. After higher i.v. dosing at 15 mg/kg, the plasma clearance dropped to 4.6 ml/min/kg.

After oral dosing of the same monkeys at 2 mg/kg, the AUC, C_{max}, T_{max}, and bioavailability were 232 μM-min, 0.5 μM, 2.9 h, and 42%.

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pharmacokinetic parameters (mean ± S.D.) of EFV in rats (n = 3–5) and monkeys (n = 3–8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous dose (mg/kg)</td>
<td>Rat</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>CLm (ml/min/kg)</td>
<td>68.2 ± 10.1</td>
</tr>
<tr>
<td>Vd (liter/kg)</td>
<td>4.4 ± 1.9</td>
</tr>
<tr>
<td>T1/2 (h)</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>AUC0→t (μM · min)</td>
<td>95 ± 15</td>
</tr>
<tr>
<td>Oral dose (mg/kg)</td>
<td>10</td>
</tr>
<tr>
<td>C_{max} (μM)</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>16 ± 5</td>
</tr>
</tbody>
</table>

* Infused over 5 min.
The monkeys were also dosed orally at 10, 40, 80, 120, and 160 mg/kg to determine the highest tolerable dose. At 160 mg/kg, four of four monkeys exhibited emesis, while at 120 mg/kg, three of four had emesis at various times. At 10, 40, 80, and 120 mg/kg, the \( C_{\text{max}} \) values were 3.28, 10.35, 25.43, and 31.64 \( \mu M \), respectively. The plasma concentrations at the higher doses rose rapidly to reach plateaus of about 3, 10, 25, and 32 \( \mu M \) and stayed high from approximately 1.5 to 6, 3 to 24, 8 to 30, and 8 to 30 h for the 10-, 40-, 80-, and 120-mg/kg doses, respectively (Fig. 5). The AUC values at the higher doses increased greater than dose-proportionately (Table 2).

In the excretion study, after i.v. administration at 5 mg/kg \([^{14}\text{C}]\)EFV, the mean radioactivity excreted in urine and feces was 48% and 30%, respectively, over 72 h. HPLC-radioactivity analysis of urine showed no unchanged parent compound. In the feces the parent compound accounted for less than 5% of the dose.

**Discussion**

The plasma concentration-time profiles of EFV after oral dosing to rats were notable in that the concentrations at 40- and 160-mg/kg doses exhibited plateaus of about 4 and 16 \( \mu M \), which were sustained for periods of 1 and 3 h, respectively. A 16-fold increase in the dose caused an AUC increase of about 70-fold (Table 2). These features coupled with the reduced plasma clearance after the high i.v. dose were suggestive of saturation of metabolism and possibly prolongation of absorption (Lin, 1994; Kwan, 1997) of EFV at the higher doses. The possibility of the prolonged absorption of EFV was further explored in rats. \([^{14}\text{C}]\)PEG-4000 was used as a nonabsorbable, non-metabolizable macromolecular probe (Parlesak et al., 1994; Wiklund et al., 1984; Cortot et al., 1975; Krag et al., 1975) for investigating its transit time through the gastrointestinal tract, with and without pretreatment of animals with EFV. The results showed that PEG passed through the stomach rapidly in the control rats, and that most of the dosed radioactivity was found in the distal sections of the intestine. However, there were 10-fold higher amounts of radioactivity still remaining in the stomach at 0.5 and 3 h post-PEG dosing in the EFV-pretreated rats (Fig. 3). Delayed gastric emptying might lead to a slow release of the dose from the stomach, due to changes in peristalsis and migrating motor complex (Gibaldi, 1991; Leesman et al., 1988), in small packets that are subsequently absorbed. This metering effect of stomach and subsequent absorption would lead to sustained, plasma concentrations reaching a plateau. Thus, the dose-dependent, delayed gastric-emptying effect contributed to the nonlinear pharmacokinetics of EFV in rats. Whether EFV caused this effect locally or centrally is not known. Likewise, omeprazole (Rasmussen et al., 1997), morphine (Asai et al., 1997), clarithromycin, and erythromycin (Ohba et al., 1996) are known to delay the gastric emptying, and, in contrast, ranitidine accelerates the emptying (Amir et al., 1996).

In rats, based on the blood-to-plasma ratio of 0.92, the \( CL_p \) value translated to a blood clearance \( (CL_0) \) of 74 ml/min/kg, a value ap-

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**TABLE 2**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Monkey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose, mg/kg</td>
<td>AUC(_{0-\infty}), ( \mu M \cdot \text{min} )</td>
</tr>
<tr>
<td>10</td>
<td>77 ± 24</td>
</tr>
<tr>
<td>40</td>
<td>890 ± 259</td>
</tr>
<tr>
<td>160</td>
<td>5,400 ± 1,900</td>
</tr>
<tr>
<td>80</td>
<td>44,200 ± 17,500</td>
</tr>
<tr>
<td>120*</td>
<td>66,400</td>
</tr>
</tbody>
</table>

* \( N = 1 \).
proaching that of hepatic blood flow. The $V_{ds}$ (4.4 liters/kg) was greater than the total body water (0.6 liters/kg), suggesting that Efavirenz was well distributed outside the vascular system. This observation was consistent with the high lipophilicity of the compound. After i.v. dosing, the parent compound was metabolized completely. These results coupled with the high blood clearance of Efavirenz in rats suggested that the drug may be subject to a high first-pass effect. This would be consistent with the low bioavailability in rats. The plasma clearance after an i.v. dose of 15 mg/kg was slower than that after the lower doses, affirming saturation of metabolic processes. The same phenomenon was demonstrated in monkeys where the plasma clearance, although dose-independent at least up to 5 mg/kg, declined by 60% at the higher dose of 15 mg/kg.

The plasma concentration-time curves were more striking in monkeys than in rats, in that, at higher oral doses the concentrations increased rapidly to plateaus and were sustained for approximately 4 to 20 h. The $T_{max}$ value increased from 2.9 to 26 h when the dose was increased from 2 to 80 mg/kg, suggesting prolongation of absorption. As the dose was increased from 2 to 120 mg/kg, the AUC increased disproportionately by about 280-fold. These data were consistent with saturation of metabolism and/or prolongation of absorption of Efavirenz. The data for the 120-mg/kg dose study are from one animal, as the other three had emesis. In the 160-mg/kg dose group, all monkeys exhibited emesis at various times, one at about 8 h postdose. Analysis of that emesis showed the presence of nearly half of the intact dose, demonstrating that Efavirenz delayed the gastric emptying in monkeys as well. Thus, further exploring the event of emesis in monkeys was fortuitous for the discovery of the delayed gastric-emptying effect in this species. Delayed gastric emptying has been known to alter the absorption profile of drugs such as acetaminophen and cimetidine in humans, showing double-peak phenomena (Clements et al., 1978) or somewhat flattened peak top (Bodemar et al., 1979; Oberle and Amidon, 1987), as is the case for Efavirenz in rats and monkeys. Thus, the nonlinear pharmacokinetics of Efavirenz is attributable to both saturable metabolic processes and the delayed gastric-emptying phenomenon. Since the clinical dose of Efavirenz would be 600 mg q.d., which is $\leq 10$ mg/kg/day, delay in gastric emptying or saturation of metabolism may be insignificant in humans.

Note Added in Proof. Efavirenz has recently been approved by the Food and Drug Administration for DuPont Pharmaceutical Co.

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References