Species Differences in Sirolimus Stability in Humans, Rabbits, and Rats

ABSTRACT:

Species differences in the in vitro stability of sirolimus was assessed in plasma and whole blood in relation to red blood cell distribution. Fresh blood and plasma samples obtained from humans, rabbits, and rats were aliquoted and spiked with sirolimus. After incubation from 0 to 144 hr in a shaking water bath maintained at 37°C, sirolimus concentrations were quantified by a specific high-performance liquid chromatographic method. Sirolimus was unstable in both plasma and whole blood. Sirolimus degradation half-life in whole blood was 135 hr (vs. 7.2 hr in plasma) in humans, 62 hr (vs. 3.1 hr) in rabbits, and 15 hr (vs. 2.2 hr) in rats. Sirolimus stability is greater in whole blood compared with plasma in proportion to the whole blood/plasma ratio and hematocrit. In vivo instability may account for up to 36% of sirolimus clearance in humans and 13% in rabbits, and this is an important factor in the pharmacokinetics of this drug.

sirolimus (rapamycin, >99%) was obtained as a gift from Dr. Surhen Seghal (Wyeth-Ayerst Research, Princeton, NJ). N-Undecyl-o-toluamide was synthesized by Dr. Walter Conway (Buffalo, NY). All other chemicals were of commercial grade.

Sample Collection. Blood was obtained from healthy drug-free volunteers (arm vein), New Zealand White rabbits (ear artery), and Sprague-Dawley rats (abdominal aorta). Blood was collected on the day of the experiment in heparinized tubes, and part was centrifuged to recover plasma.

Stability Experiments. Blood and plasma were aliquoted in amber vials due to sirolimus light sensitivity. Sirolimus was added at 1000 ng/ml in triplicate, and ethanol concentration was <0.1%. The vials were tightly capped and placed in a shaking water bath set at 37°C. Vials were removed at 0, 1, 2, 4, 6, 8, 24, 48, 72, 96, and 144 hr and stored at −70°C before assayed.

HPLC Assay. Sirolimus was assayed in blood and plasma by a specific, validated reversed phase high-performance liquid chromatographic method (Ferron et al., 1997). In brief, 0.5 ml of diluted or undiluted sample was extracted in foil-wrapped culture tubes. After spiking 1 μg of internal standard (N-undecyl-o-toluamide) and alkalization with 1 ml of 0.1 M sodium carbonate, 10 ml of tert-butyl methyl ether were added. After 30 min of horizontal shaking, the tubes were centrifuged, and the organic phase was transferred into amber scintillation vials and dried under an air stream at room temperature. The residues were reconstituted, transferred into glass inserts held in an amber vial, and centrifuged. The clear supernatant was transferred into clean inserts and injected onto a C18 column maintained at 45°C. The mobile phase was 31% water and 69% methanol, pumped at a 1-ml/min flow, leading to a 30-min run time per sample. The detection was performed at 278 nm. The standard curve was linear from 2.5 to 200 ng with a sensitivity of 1 ng. The interday and intraday coefficients of variation were less than 11.7%. The open ring degradation product, 34-hydroxy sirolimus, did not interfere with sirolimus quantitation.

Data Analysis. The decrease of unchanged sirolimus over time was fitted to eq. 1 using the ADAPT II software (Biomedical Simulations Resource, Los Angeles, CA),

\[
\% \text{ of unchanged sirolimus in WB or PL } = \frac{100 \cdot \exp(-k_{deg,WB} \cdot \text{time})}{1 + k_{deg,WB} \cdot \text{time}}
\]

where \(k_{deg,WB}\) is the in vitro degradation rate constant in whole blood (WB) or plasma (PL). Degradation half-life was obtained as \(\ln 2/k_{deg}\). In vivo, sirolimus pharmacokinetics can be described by a two-compartment model (Ferron et al., 1997; Honcharik et al., 1992). Assuming that degradation occurs either from the central compartment (volume \(V_c\)) or from the peripheral compartment also (\(V_p\)), the calculated in vivo whole blood degradation clearance (\(Cl_{deg,WB}\)) will be as follows:

\[
Cl_{deg,WB} = k_{deg,WB} \cdot V_c \quad \text{or} \quad k_{deg,WB} \cdot V_c + k_{deg,WB} \cdot V_p
\]
Using literature data (Ferron et al., 1997; Honcharik et al., 1992), $Cl_{deg, WB}$ values were compared with in vivo whole blood clearances.

**Results and Discussion**

Sirolimus is unstable in both plasma and whole blood. Concentrations decreased in a mono- or biexponential fashion (fig. 1). Sirolimus plasma degradation half-life was similar in rabbits (3.1 hr) and rats (2.2 hr) but longer in humans (7.2 hr). No differences in plasma or blood pH were found among these species.

Sirolimus stability was greater in whole blood. Indeed, for each time point and species, the per cent of unchanged sirolimus was higher in whole blood than plasma, indicating the role of red blood cells in protecting sirolimus from degradation (fig. 1). Sirolimus whole blood degradation half-life was 135 hr in humans, 62 hr in rabbits, and 15 hr in rats.

Extensive red blood cell distribution may be an important factor explaining the species-related differences observed in sirolimus whole blood stability (Yatscoff et al., 1993; Ferron et al., 1997). In vitro sirolimus blood distribution experiments showed greater whole blood/plasma ratios in humans (9.1–14.3) compared with rabbits (5.5–8.2) and rats (1.5) (Yatscoff et al., 1993, 1995). Figure 2 shows the relationships between plasma and whole blood degradation half-lives and literature values of whole blood/plasma ratios for sirolimus. In addition, differences in hematocrit values are a factor influencing tacrolimus metabolism (Chow et al., 1997). Similar mean hematocrits were obtained in humans (0.47) and rabbits (0.45), but lower values were observed in rats (0.39). Therefore, hematocrit differences may account for part of the species effect on sirolimus degradation.

As the sirolimus degradation product was obtained during in vitro metabolism studies (Streit et al., 1996), instability may be a disposition pathway in vivo. The elimination half-life was 63 hr in humans (Ferron et al., 1997), 15 hr in rabbits (Honcharik et al., 1992), and 9.6 or 30.8 hr in rats (Yatscoff et al., 1995) compared with degradation half-lives of 135, 62, and 15 hr, respectively. Instability may account for 9–36% of the total clearance in humans, depending on whether degradation occurs in the vascular compartment or, as is likely because the process is nonenzymatic, in tissues as well (Ferron et al., 1997). In vivo degradation clearance must be estimated using eq. 2 as it would be difficult or impossible to measure such breakdown rates in vivo in human or animal tissues. The presence of FKBP in tissues may stabilize the drug as occurs in RBC. However, if degradation in tissues occurs at a higher rate than in whole blood, instability may account for more than 36% of the total clearance in humans. Sirolimus instability may account for up to 13% of total clearance in rabbits and 90% in rats.

In conclusion, sirolimus is unstable at 37°C in both whole blood and plasma. Its stability is increased in whole blood in relation to its distribution into the red blood cells, which protect sirolimus from degredation. The increased stability in blood compared with plasma correlates with the whole blood/plasma ratio and hematocrit values. In vivo instability may account for up to 36% of sirolimus clearance in humans and 13% in rabbits.

**References**


