Pharmacokinetics of 9-cis-Retinoic Acid in the Rhesus Monkey

Peter C. Adamson,1 Robert F. Murphy, Karen A. Godwin, Edgar H. Ulm, and Frank M. Balis

Abstract

9-cis-Retinoic acid is a naturally occurring biologically active retinoid capable of binding and transactivating both the retinoic acid receptors and the retinoid X receptors. A study was performed to characterize the pharmacokinetics 9-cis-retinoic acid following i.v. bolus administration in the nonhuman primate. Groups of three animals received i.v. bolus doses of 9-cis-retinoic acid of either 50 or 100 mg/m². Blood and cerebrospinal fluid samples for determination of 9-cis-retinoic acid concentration were obtained prior to and 5, 10, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 360, and 480 min following drug administration. The plasma drug concentration profile of 9-cis-retinoic acid was consistent with a first-order elimination process, with a harmonic mean half-life of 31 min, and a mean clearance of 97 ml/min/m². The pharmacokinetics of 9-cis-retinoic acid were linear over the dose range studied. Plasma concentrations of all-trans-retinoic acid following 9-cis-retinoic acid administration were less than the limit of quantitation (0.1 µM), suggesting that isomerization to all-trans-retinoic acid is not a major metabolic pathway. In contrast to all-trans-retinoic acid, the elimination of 9-cis-retinoic acid did not appear to be capacity limited (saturable). Previous studies in the Rhesus monkey have shown that repeated dosing with all-trans-retinoic acid leads to a reduction of this saturable component of elimination and results in reduced exposure to drug. These studies, in an animal model highly predictive of humans, suggest that declines in plasma concentrations of 9-cis-retinoic acid as a result of its repeat administration at doses up to 100 mg/m² will not occur.

Introduction

Retinoids are a group of naturally occurring compounds and synthetic analogues which are involved in the regulation of growth and differentiation of a wide range of tissues (1). In the clinical setting, topical and oral retinoids have been effective in the treatment of premalignant skin conditions (2–4) and, more recently, oral ATRA2 has been demonstrated to induce complete remissions in a high proportion of patients with acute promyelocytic leukemia (5–7). Retinoids exert their diverse effects through interaction with specific nuclear receptors. The two families of retinoid nuclear receptors that have been described, the retinoic acid receptors (8, 9) and the retinoid X receptors (10, 11), have ligand-binding domains which share only 29% homology. The retinoic acid receptors (α, β, and γ) bind the naturally occurring retinoid ATRA with high affinity, whereas the retinoid X receptors (α, β, and γ) are activated by but do not bind ATRA (12). This multiplicity of receptors and gene pathways may, in part, explain the diverse effects of retinoids on a wide range of cellular processes.

9-cis-RA is a naturally occurring, biologically active retinoid (13). Chemically, it is a geometric isomer of ATRA. It is capable of binding and transactivating both the retinoid X receptors as well as the retinoid acid receptors (6–8). This may, in part, account for its enhanced potency, compared with ATRA, in inhibiting the growth and inducing differentiation of a spectrum of human tumors in vitro (14–17). Another difference between 9-cis-RA and ATRA relates to the binding of these isomers to the CRABPs. CRABPs are cytoplasmic proteins believed to regulate the amount of retinoid reaching and binding nuclear receptors (18, 19). Up-regulation of CRABP could, therefore, diminish the potential effectiveness of retinoids. Studies performed in the Rhesus monkey revealed that, following systemic administration of ATRA, tissue levels of CRABP (measured in skin biopsy specimens) rapidly increased (20). In patients with acute promyelocytic leukemia, the level of CRABP in leukemic blasts at the time of relapse also appears to be elevated (21). In contrast to ATRA, CRABPs do not bind 9-cis-RA (22) and, therefore, 9-cis-RA would not be susceptible to this potential mechanism of retinoid resistance.

Despite their structural similarity, pharmacokinetic studies of 13-cis-RA (isoretinoin) and ATRA demonstrate remarkable differences in the disposition of these two geometric isomers. 13-cis-RA has a prolonged elimination phase with a terminal half-life (t1/2) of 12 to 24 h (23, 24), whereas ATRA is eliminated considerably more rapidly with a t1/2 of approximately 45 min (25–27). In addition, with chronic administration, plasma concentrations of ATRA decline significantly over time (presumably the result of up-regulation of an oxidative, inactivating metabolic pathway; Refs. 25 and 26), a phenomenon not observed with chronic 13-cis-RA dosing (28). Based on these striking differences, it would be difficult to predict the pharmacokinetic behavior of another naturally occurring geometric isomer of ATRA, 9-cis-RA. Since only limited preclinical pharmacokinetic data (in murine models) regarding 9-cis-RA is available (29, 30), we studied the pharmacokinetics of 9-cis-RA following an i.v. bolus in the nonhuman primate model, a model which has previously proven predictive of retinoid pharmacokinetics in humans (20, 31).

Materials and Methods

Animals. Six adult Rhesus monkeys (Macaca mulatta) ranging in weight from 5.6 to 8.6 kg were obtained from Buckshire Corporation (Perkasie, PA). The animals were fed Purina monkey chow and group housed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication 85–23, U.S. Department of Health and Human Services). For pharmacokinetic studies, animals had catheters surgically placed into jugular or femoral veins and attached to an access port implanted s.c. (Vascular Access Port; Access Technology, Skokie IL).

CSF penetration of 9-cis-RA was assessed in three animals with silicone Pudenz catheters surgically placed into the fourth ventricle and attached to a s.c. implanted Ommaya reservoir, as described previously (32).

Drug Formulation and Administration. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO). 9-cis-RA (LGD1057) was kindly provided by Ligand Pharmaceuticals (La Jolla, CA). The N-methylglucamine salt of 9-cis-RA was prepared immediately prior to administration in a room with dimmed lights by a modification of a previously described method (33). One hundred mg of 9-cis-RA were dissolved in 2.5 ml of DMSO, then further diluted with 2.5 ml of propylene glycol, and finally added to 15 ml of a 25% propylene glycol/water solution (v/v%) containing 0.5 g of N-methylglucam-
isomers 13-cis-RA and ATRA. The final solution was filtered through a 0.22 μm filter prior to administration.

Groups of three animals received i.v. doses of 9-cis-RA of 50 and 100 mg/m². Drug was administered over 5 min through a percutaneously placed saphenous vein catheter.

Pharmacokinetics. All samples were obtained in a room with the lights dimmed. Blood samples, drawn through a venous port, and CSF samples, drawn through an Ommaya reservoir, were obtained prior to and 5, 10, 15, 30, 45, 60, 75, 90, 120, 150, 180, 240, 360, and 480 min following drug administration. Plasma was separated from blood by centrifugation (3200 × g for 10 min at 10°C). All samples were wrapped in foil and stored at −20°C until assayed. The AUC from 0 to 8 h was calculated by the trapezoidal method (34). No extrapolation was carried out due to the limited number of data points available at the lower dose to accurately calculate the terminal half-life.

9-cis-Retinoic Acid Assay. The plasma concentration of 9-cis-RA was determined by modification of a previously described HPLC method for ATRA (35). All procedures were performed in a room with the lights dimmed. Briefly, 50 μl of a 5% perchloric acid solution was added to each 500-μl plasma sample and vortexed for 30 s. To this, 500 μl of ethyl acetate was added and vortexed for 60 s. Following centrifugation (10,000 × g for 60 s at 20°C), the organic layer was removed and analyzed by HPLC. The HPLC system included two Waters model 510 pumps (Milford, MA) and a Beckman 5-μm steel ultrachrome column (San Ramon, CA) coupled with a Waters Resolve 5-μm C18 radial compression module. The mobile phase consisted of 95% acetonitrile and 5% ammonium acetate buffer (v/v 1%) at a flow rate of 2.5 ml/min. Eluant was monitored with a Waters 490 multi-wavelength detector at wavelengths of 340 and 360 nm. These conditions provided clear separation of 9-cis-RA from 13-cis-RA and ATRA (Fig. 1). The retention time for 9-cis-RA was approximately 9 min, and its lower limit of detection was 0.03 μM.

Results

The mean plasma concentration-time curves following i.v. bolus administration of 50 and 100 mg/m² of 9-cis-RA to groups of three monkeys is shown in Fig. 2, and the pharmacokinetic parameters derived from these concentrations are shown in Table 1. The plasma elimination of 9-cis-RA was characterized by an exponential decline with a harmonic-mean half-life of approximately 30 min. There was a suggestion of a longer terminal phase at the 100 mg/m² dose level, but this could not be defined accurately. The pharmacokinetics appeared linear over the dose range studied as the AUC doubled from an average (± SD) of 1840 ±310 to 3640 ±1510 for 9-cis-RA was characterized by an exponential decline derived from these concentrations are shown in Table 1. The plasma concentration-time curves following i.v. bolus drug administration. Based on a limit of detection of 0.03 μM, the CSF penetration of 9-cis-RA is less than 0.1%.

Pharmacokinetic parameters of 9-cis-RA in the Rhesus monkey

![Fig. 1. HPLC chromatogram demonstrating separation of 9-cis-RA from its geometric isomers 13-cis-RA and ATRA.](image)

![Fig. 2. Plasma concentration-time curves for groups of three animals receiving i.v. bolus doses of 50 (•) and 100 (○) mg/m² of 9-cis-RA. Points, mean; bars, SD.](image)

![Table 1 Pharmacokinetic parameters of 9-cis-RA in the Rhesus monkey](table)

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Dose (mg/m²)</th>
<th>AUC (μM × min)</th>
<th>Clearance (ml/min/m²)</th>
<th>Vd∞ (l/m²)</th>
<th>t½ (min)</th>
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<td>3440</td>
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<td>5250</td>
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<td>32</td>
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<tr>
<td>Mean (± SD)</td>
<td>97 ± 29</td>
<td>4.8 ± 1.3</td>
<td>31*</td>
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</tr>
</tbody>
</table>

Harmonic mean.

Discussion

Pharmacokinetic studies have demonstrated striking differences between the disposition of the 13-cis- and all-trans- isomers of retinoic acid. However, a more detailed analysis of the pharmacokinetic behavior of retinoids in humans has been limited by the low and variable plasma concentrations achieved following oral administration. Since an i.v. formulation of ATRA was not available for human use, we developed a formulation that could be administered in a nonhuman primate model and demonstrated that ATRA elimination was capacity limited and that this elimination was up-regulated with chronic administration, accounting for the dramatic fall in plasma concentration with daily oral use (20, 31). In addition to the retinoids, this model has also been highly predictive of the pharmacokinetics of other anti-cancer drugs in humans (36–38). Therefore, we studied the pharmacokinetics of 9-cis-RA in this model using an i.v. dosing formulation similar to the one developed for ATRA.

The elimination of 9-cis-RA from plasma was rapid, with a half-life of 31 min. There was a suggestion of a longer terminal phase of
elimination at the higher dose level (Fig. 2) but only when the plasma concentration approached the limit of detection for the assay so that it could not be accurately defined. The calculation of the AUC was thus not extrapolated to infinite time, but this should only result in a small effect on the AUC calculation.

The plasma concentration-time profile of 9-cis-RA demonstrated a rapid terminal exponential elimination phase (Fig. 3; Ref. 31). With repeated administration of ATRA, there was a significant increase in the slope of the plateau phase, suggesting an up-regulation of this saturable elimination pathway (20). In contrast, 9-cis-RA did not display a plateau phase but was apparently eliminated by first-order decay at doses up to 100 mg/m². Because the pharmacokinetic profile of 9-cis-RA is not consistent with a saturable elimination process, this model predicts a reduced likelihood that repeat dosing with 9-cis-RA will produce decreasing AUCs.

The retinoic acid isomers investigated to date are thus remarkable, not only for their range of specificity for nuclear receptors, but for the major differences in drug disposition following systemic dosing. An understanding of these differences will be important as clinical investigations using 9-cis-RA proceed. The short half-life and lack of saturable elimination for 9-cis-RA observed in the Rhesus monkey model suggests that a divided daily dosing schedule of administration would provide increased exposure to drug over time. Human pharmacokinetic studies of this schedule of administration are planned.

References
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