PHARMACOKINETICS OF ALL-TRANS RETINOIC ACID, 13-CIS RETINOIC ACID, AND FENRETINIDE IN PLASMA AND BRAIN OF RAT

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ABSTRACT:

We have measured the pharmacokinetics of three retinoids, all-trans retinoic acid, 13-cis retinoic acid, and fenretinide in rat blood and rat brain [especially white matter (WM) and gray matter (GM)] to help select retinoids for treating human malignant glioma. All-trans retinoic acid permeated well into the WM, giving peak concentration in WM of 25.7 µg/g, 6 to 7 times higher than the peak serum concentration. There was less 13-cis retinoic acid in WM: area under the curve (AUC) 0–24 h WM/AUC 0–24 h serum = 18.00 µg ml⁻¹ h/32.67 µg ml⁻¹ h. The ratio WM/GM was over 1 for these two compounds, but the half-lives were short in the serum and cerebral tissue (0.57–1.02 h). Fenretinide had different pharmacokinetics: the peak concentrations were in serum (1.7 µg/ml) and WM (1.2 µg/ml)—low, but the AUC 0–24 h was large (25.55 µg ml⁻¹ h in serum and 57.53 µg ml⁻¹ h in WM) due to its long elimination half-life (13.78 h in serum and 17.77 h in WM). These findings provide information that may be used to select a retinoid and establish therapeutic regimens that provide optimal efficacy with minimal toxicity.

Retinoids (RAs) have been widely used for many years for preventing and treating dermatological diseases (Orfanos et al., 1987; Kligman, 1998). They may also open up new opportunities in oncology (Chandraratna, 1998). RAs modulate the proliferation, differentiation, and apoptosis of normal and abnormal cells of several cancers in vitro, including the colon (Zheng et al., 1999), prostate (Li and Liang et al., 1999), lung (Weber et al., 1999), and leukemia (Mologni et al., 1999). All-trans retinoic acid (ATRA) and 9-cis RA also influence the morphological differentiation, proliferation, and gene expression of neuroblastoma (Irving et al., 1998) and astrocytoma cells (Dirks et al., 1997). Recurrent malignant cerebral gliomas have been treated with ATRA (Yung et al., 1996; Defer et al., 1997) and 13-cis RA (Kaba et al., 1997).

The survival of patients after resection of a recurrent multifocal glioblastoma remains poor despite advances in imaging, surgical technique, and adjuvant therapies (Barker et al., 1998). As chemotherapy, even the more recent (Chang et al., 1999; Friedman et al., 1999) has little effect on malignant glioma, innovative strategies such as RAs, may be useful as they have both antiproliferative properties and differentiating effects. A preliminary study (Defer et al., 1997) showed a trend to a slowing of disease progression in patients suffering from malignant glioma, with the development of intratumoral calcification. These abnormalities may be partly due to the activation of endothelial tumor tissue-type plasminogen activator production by retinoids, indicating an in vivo action. Controlled efficacy studies are now appropriate. Preclinical pharmacology studies are an important tool for establishing the criteria for selecting the most appropriate molecule. Some blood pharmacokinetics studies of these compounds have already been performed in rodents (Swanson et al., 1980; Wang et al., 1980; Kalin et al., 1981; Hulini et al., 1986) and in humans (Colburn et al., 1983; Besnerr et al., 1985). We believe that the tissue kinetics is also important for treating cerebral intraparenchymal lesions. Few models of glioma have been developed in immunocompetent mice, but they do not have the histological and antigenic characteristics of human gliomas. As there is no reliable rodent model of glial tumor, we used a comparison of the kinetics in white matter (WM) and gray matter (GM), as a predictor of the tumoral kinetics, because glial cells (astrocytes, oligodendroglia) are more concentrated in WM than in GM. Any differences in the behavior of retinoids in the serum and brain compartments may also provide information that can help select a retinoid and establish appropriate therapeutic regimens with optimal efficacy and minimal toxicity. We have, therefore, in rat, compared the pharmacokinetics of three retinoids, ATRA, 13-cis RA, and fenretinide in the blood and brain. The distributions of these three compounds in the brain WM and GM were assessed.

Experimental Procedures

Materials. ATRA, 13-cis RA, and Ro 13-6307 (internal standard) were gifts from F. Hoffman-La Roche SA (Bale, Switzerland). Fenretinide was kindly supplied by Cilag AG (Schaffhausen, Switzerland). Glacial acetic acid, acetonitrile and ammonium acetate (Merck, Darmstadt, Germany), ascorbic acid (Fluka Chimie AG, Bucks, Switzerland), dimethyl sulfoxide (DMSO), and trisodium edetate (Prolabo, Fontenay sous Bois, France) were all of analytical grade.

The HPLC system used was an isocratic pump (model L6000; Merck, Darmstadt, Germany) coupled to a photodiode array detector (model 996; Waters, Millenium software). The HPLC system was housed in groups of five and maintained under standard laboratory conditions (22 ± 1°C, 12-h light/dark cycle, food and water ad libitum) before study.
Drug Administration and Study Design. Rats were given an i.p. injection (10 mg/kg b.wt.) of solution of ATRA, 13-cis RA, or fenretinide (2 mg/ml), all in DMSO and were sacrificed by inhalation of carbon dioxide at 1, 3, 5, and 8 h (ATRA), 1, 2, 3, 4, and 5 h (13-cis RA), or 1, 2, 3, 5, 8, 12, 18, 24, 48, and 72 h (fenretinide) after injection (five animals at each time).

Blood samples (2-3 ml) were taken by cardiac puncture and the blood

Bars represent S.D.; n = 5 at each time.

**Fig. 1.** Mean serum and cerebral tissue concentration-time profiles of all-trans RA, 13-cis RA, and fenretinide after i.p. injection of rats with the drug (10 mg/kg b.wt.).
TABLE 1

<table>
<thead>
<tr>
<th>Substance</th>
<th>Time (h)</th>
<th>Mean ± S.D. Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATRA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>1</td>
<td>4.0 ± 0.6 µg/ml</td>
</tr>
<tr>
<td>Gray matter</td>
<td>2</td>
<td>12.9 ± 5.5 µg/ml</td>
</tr>
<tr>
<td>White matter</td>
<td>3</td>
<td>17.6 ± 6.2 µg/ml</td>
</tr>
<tr>
<td>13-cis RA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>1</td>
<td>16.9 ± 3.9 µg/ml</td>
</tr>
<tr>
<td>Gray matter</td>
<td>2</td>
<td>24.2 ± 7.9 µg/ml</td>
</tr>
<tr>
<td>White matter</td>
<td>3</td>
<td>27.7 ± 8.5 µg/ml</td>
</tr>
<tr>
<td>Fenretinide</td>
<td></td>
<td>0.7 ± 0.1 µg/ml</td>
</tr>
<tr>
<td>Gray matter</td>
<td>2</td>
<td>&lt;&lt;0.04 µg/ml</td>
</tr>
<tr>
<td>White matter</td>
<td>3</td>
<td>&lt;&lt;0.04 µg/ml</td>
</tr>
</tbody>
</table>

Note: Concentrations are expressed in µg/ml or g/g h.

Analysis of Serum and Brain Samples. Concentrations of ATRA, 13-cis RA, and fenretinide were measured by HPLC (Wyss, 1990). Spiked standards and deproteinized rat samples were prepared in foil-lined tubes, working in a darkened room. Serum and tissue homogenate (200 µl) were mixed with 200 µl of acetonitrile, shaken, and centrifuged (4000 rpm for 10 min). An aliquot (50 µl) of the upper phase was injected directly into the HPLC system. The chromatographic conditions were: column, LC ABZ [15 cm × 4.6 mm, i.d.; particle size, 5 µm; reversed phase C18; (Supelco, St. Quentin Fallavier, France)]; mobile phase, 57.5% acetonitrile/25% acetic acid (2% in water)/17.5% methanol [ATRA and 13-cis RA], 80% acetonitrile/20% ammonium acetate (1% in water) (fenretinide); flow rate, 1.3 ml/min (ATRA and 13-cis RA), 1 ml/min (fenretinide); UV detection wavelength, 354 nm.

Standard curves were prepared by adding appropriate amounts (60 ng to 1.8 µg) of ATRA or 13-cis RA in DMSO, or (39 to 780 ng) of fenretinide in DMSO to serum blank samples (200 µl); 30 to 900 ng of ATRA or 13-cis RA, 20 to 390 ng of fenretinide to tissue homogenate blank samples (200 µl). The amount of internal standard Ro 13-6307 was 272 ng for serum standard curves, 136 ng (ATRA or 13-cis RA), and 68 ng (fenretinide) for tissue homogenate standard curves. Standard curves were run every day of determination. Two quality controls (low and high) were tested to estimate the reproducibility, precision, and reliability of the method.

Data Analysis. The serum concentrations of drug are expressed in micrograms of drug per milliliter of serum. The tissue concentrations of drug are expressed in micrograms of drug per gram of wet tissue weight. Results are expressed as means ± S.D. The mean coefficient of variation from five measures at each time gave the interindividual variance. Half terminal lives (t1/2) of ATRA, 13-cis RA, and fenretinide in serum and brain tissues were estimated by least-squares regression analysis of the terminal phase of the concentration-time curves. The area under the serum or brain concentration versus time curves (AUCt,∞) values were determined by the trapezoidal rule during the period of experiment and, when necessary, the infinite part of the curve was calculated as the estimated terminal serum concentration divided by the slope k (k = 0.693/t1/2). The mean concentrations on the WM and GM were compared using ANOVA with a balanced nested design. Data obtained at a given time were compared using a Mann-Whitney rank sum test. A value of P > .02 was considered to be statistically insignificant.

Results

The limits of HPLC quantification were 0.03 µg/ml (ATRA and 13-cis RA), 0.04 µg/ml (fenretinide). The assay accuracy and the between assay variation showed that the differences between the experimental and the theoretical concentrations and the interassay coefficients of variation calculated from quality controls never exceeded 5%, both in serum and tissue homogenate and for the three substances.

The concentration-time profiles of the three retinoids are shown in Fig. 1. The apparent peak serum concentration of ATRA was 4.0 ± 0.6 µg/ml. This concentration was (4–5 times) significantly lower (P < .002) than that in the GM and (6–7 times) significantly lower (P < .002) than that in the WM at the tissue peak time. The peak serum concentration for 13-cis RA was 16.9 ± 2.9 µg/ml, (3–4 times) significantly higher (P < .002) than that in the GM and (P < .01) than that in the WM at the tissue peak time. The peak serum concentration for fenretinide was 2.5 ± 1.4 µg/ml, which was not significantly different from the peak tissue concentration in the GM or the WM. The mean concentrations at all kinetic times indicated that the brain WM took up more ATRA (P < .001) and 13-cis RA (P < .002) than the GM, whereas the WM and GM contained similar vessels were rinsed with 0.9% saline. The brains were removed and kept at 4°C, and WM samples from the corpus callosum and GM samples from the frontal cortex were dissected out. The tissue was weighed [weight (mean ± S.D.) of WM samples: 72.7 ± 32.6 mg; weight of GM samples: 168.8 ± 72.1 mg), homogenized in 500 µl of an aqueous solution containing 0.5 mg/ml each of trisodium edetate and ascorbic acid using light-protected tubes. Blood samples were centrifuged at 4000 rpm. Tubes were stored at −20°C.
concentrations of fenretinide. The AUC$^{cis}$-WM/AUC$^{trans}$ GM was greater than 1 for ATRA (1.62) and 13-cis RA (1.76) and less than 1 for fenretinide (0.79). The AUC$^{cis}$-GM/AUC$^{trans}$ serum was greater than 6 (6.24) for ATRA, greater than 2 (2.83) for fenretinide, and less than 1 (0.31) for 13-cis RA. The AUC$^{0-24h}$-WM/AUC$^{0-24h}$ serum was greater than 10 (10.14) for ATRA, greater than 2 (2.25) for fenretinide, and less than 1 (0.55) for 13-cis RA.

The pharmacokinetic parameters are shown in Table 1. The elimination half-life was longest for fenretinide and shortest (≤1 h) for ATRA and 13-cis RA in all compartments.

**Discussion**

The i.p. injection of rats with ATRA (10 mg/kg) resulted in serum concentrations and pharmacokinetic parameters that were quite consistent with the data from previous studies in small animals. The $C_{max}$ was 4.5 μg/ml at 0.75 h in mice given intragastric ATRA (10 mg/kg) (Kalin et al., 1981). The $t_{1/2}$ was 0.438 ± 0.124 h (Chou et al., 1997) or 0.69 h (Shelley et al., 1982) after an oral dose of 2 or 13.9 mg/kg, respectively; the AUC$^{0-24h}$ was 13,740 ± 660 ng/ml h after an oral dose of 13.9 mg/kg (Shelley et al., 1982). The blood pharmacokinetic data for 13-cis RA were also consistent with the data published by Guchelaar et al. (1992) for rats given an i.p. injection of 2.5 mg of 13-cis RA per 360 g b.wt., as a mixture with polysorbate 80: $C_{max}$, 10 mg/liter; $t_{max}$, 1 h; AUC, 25.9 ± 12.0 mg/liter; $t_{1/2}$, 0.72 ± 0.088 h$^{-1}$ or 1.1 h (calculated from data reported in mice by Wang et al., 1980). The findings were the same for fenretinide: $t_{1/2}$, 12 h (Swanson et al., 1980; Hultin et al., 1986) after i.v. injection (5 mg/kg) in rats.

Only two early papers reported the concentrations of RAs in the total brains of mice. Wang (1980) and Kalin et al. (1981) found higher concentrations of ATRA in the brain than in the serum. Wang (1980) found that the brain concentrations of 13-cis RA were lower than those of the serum.

The pharmacokinetic behavior of orally administered ATRA shows that the drug is rapidly eliminated by humans, with a $t_{1/2}$ of approximately 45 min (Regazzi et al., 1997). The distribution profile of ATRA and 13-cis RA in all compartments.

These findings suggest that the marked difference between the penetrations of ATRA and 13-cis RA could be due to their configurations. The different chemical structure gave fenretinide a different pharmacokinetic profile. Only ATRA and 13-cis RA have been tested in relapsing malignant gliomas to date. Although the correlation between concentration in the target organ and therapeutic efficacy of retinoids has not been established, two therapeutic schedules are possible, one providing high, rapid drug release (ATRA and 13-cis RA), and the other low, persistent [especially after repeated dosings (fenretinide)] release of the drug.

**Acknowledgments**

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**References**


