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

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(Received June 16, 1999; accepted October 25, 1999)

This paper is available online at http://www.dmd.org

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–∞ WM/AUC0–∞ 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 AUC0–∞ 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.

Retinoic acids (RAs)¹ 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 (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 multiform 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 retinoids, 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 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; Hultin et al., 1986) and in humans (Colburn et al., 1983; Besner 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, Saint-Quentin en Yvelines, France) monitored by Millennium software (Waters).

Animals. Experiments were performed on 95 male Sprague-Dawley rats (CERJ, Le Genest Saint Isle, France) weighing 200 to 300 g. The rats were 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.

¹ Abbreviations used are: RA, retinoic acid; ATRA, all-trans retinoic acid; WM, white matter; GM, gray matter; AUC, area under the serum or brain concentration versus time curves; DMSO, dimethyl sulfoxide.
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 ...
TABLE 1
Pharmacokinetics of ATRA, 13-cis RA, and fenretinide in the rat serum and brain tissues after i.p. administration at 10 mg/kg

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>ATRA</th>
<th>13-cis RA</th>
<th>Fenretinide</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.6</td>
<td>2.5</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>1.2</td>
<td>4.6</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>2.5</td>
<td>7.5</td>
<td>0.4</td>
</tr>
<tr>
<td>4</td>
<td>4.4</td>
<td>11.9</td>
<td>0.3</td>
</tr>
<tr>
<td>5</td>
<td>6.2</td>
<td>16.9</td>
<td>0.2</td>
</tr>
<tr>
<td>6</td>
<td>8.2</td>
<td>23.7</td>
<td>0.3</td>
</tr>
<tr>
<td>7</td>
<td>10.1</td>
<td>26.5</td>
<td>0.1</td>
</tr>
<tr>
<td>8</td>
<td>12.1</td>
<td>28.6</td>
<td>0.1</td>
</tr>
</tbody>
</table>

The mean concentrations on the WM and GM are significantly different from each other (P < .001). The mean peak concentration for ATRA was 16.9 ± 4.6 µg/ml, whereas the GM contained similar concentration-time curves. The peak serum concentration of ATRA was 16.9 ± 2.9 µg/ml, which was significantly different from the peak concentration in the GM or WM at the tissue peak time. The peak serum concentration for 13-cis RA was 207.5 µg/ml, which was significantly different from the peak concentration in the GM or WM at the tissue peak time. The peak serum concentration for fenretinide was 2.5 ± 1.2 µg/ml, which was not significantly different from the peak concentration in the GM or WM. The mean concentrations at all kinetic times indicated that ATRA, 13-cis RA, and fenretinide were rapidly absorbed and distributed throughout the rat serum and brain tissues. The mean AUC values were determined by the trapezoid rule at ASPET Journals on October 13, 2017 dmd.aspetjournals.org Downloaded from
concentrations of fenretinide. The AUC\textsubscript{cis,WM}/AUC\textsubscript{cis,serum} was greater than 1 for ATRA (1.62) and 13-cis RA (1.76) and less than 1 for fenretinide (0.79). The AUC\textsubscript{cis,WM}/AUC\textsubscript{cis,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\textsubscript{cis,WM}/AUC\textsubscript{cis,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 $C_{\text{max}}$ 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\textsubscript{cis} was 13,740 ± 600 ng/ml 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: $C_{\text{max}}$, 10 mg/liter; $t_{\text{max}}$, 1 h; AUC, 25.9 ± 12.0 mg.h/ml; $t_{1/2}$, 0.72 ± 0.088 h\textsuperscript{-1} or 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 13-cis RA showed a rapid distribution half-life of 1.3 h and a terminal elimination half-life of 24.7 h (Besner et al., 1985) or 17.4 h (Colburn et al., 1983). The apparent plasma $t_{1/2}$ of fenretinide was 27 h (Formelli et al., 1993).

This blood pharmacokinetics determined from the human blood data agree well with parameters calculated for small animals, with a rapid decrease in the blood concentrations of ATRA and 13-cis RA, and a much slower decline in circulating fenretinide. This suggests that the concentrations of retinoids determined in various cerebral tissues of the rat could be representative of the distribution of retinoids in the human brain. The measurement of ATRA, 13-cis RA, and fenretinide concentrations in the WM and GM indicate that the $t_{\text{max}}$ and apparent half-life in WM and GM are quite similar, but the WM, where there are more astrocytes and oligodendrocytes, is more pregant with ATRA and 13-cis RA than the GM. The reverse is true for fenretinide. The movement of ATRA from blood to the WM was very large, possibly involving a conceivable large, rapid penetration of tumor cells in glioma; less 13-cis RA crossed the blood-brain barrier so that the concentrations in the malignant glioma were lower for a shorter time. Fenretinide was taken up by the WM from blood at an intermediate rate with very different kinetics giving low, long-lasting concentrations.

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, long, persistent [especially after repeated dosings (fenretinide)] release of the drug.

**Acknowledgments.** We thank V. Liard for secretarial assistance.

**References**


