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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)$^{0\rightarrow\infty}$ WM/AUC$^{0\rightarrow\infty}$ 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$^{0\rightarrow\infty}$ was large (25.55 μg ml^{-1} in serum and 57.53 μg ml^{-1} 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 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.

[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.

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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 Millenium 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.

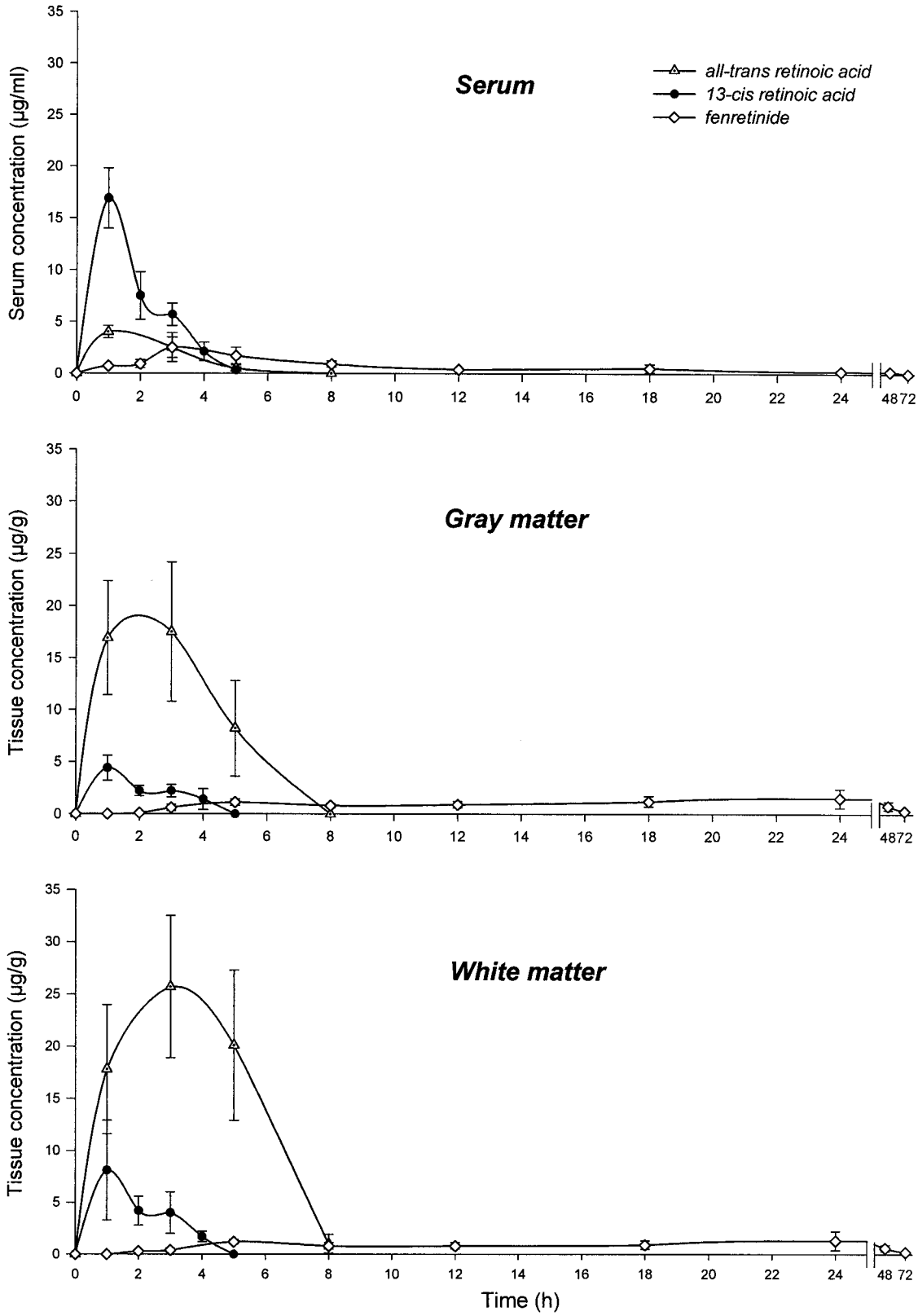

FIG. 1. *Mean serum and cerebral tissue concentration-time profiles of all-trans RA, 13-cis RA, and fenretinide after i.p. injection of rats with the drug (10 mg/kg b.wt).*

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

Drug Administration and Study Design. Rats were give 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

Time (h)	Mean ± S.D. Concentrations									ke	$t_{1/2}$	$AUC^{0\rightarrow\infty}$
	1	2	3	4	5	8	12	18	24	48	72			
	μg/ml or μg/g											h^{-1}	*h*	*μg ml*$^{-1}$ *h*
ATRA														
Serum	4.0 ± 0.6		2.5 ± 1.0		0.5 ± 0.3	<0.03						0.722	0.96	12.25
Gray matter	16.9 ± 5.5		17.5 ± 6.7		8.2 ± 4.6	<0.03						1.321	0.52	80.85
White matter	17.8 ± 6.2		25.7 ± 6.8		20.1 ± 7.2	1.0 ± 0.9						0.677	1.02	131.80
13-*cis* RA														
Serum	16.9 ± 2.9	7.5 ± 2.3	5.7 ± 1.1	2.1 ± 0.9	0.3 ± 0.3							0.934	0.74	32.89
Gray matter	4.4 ± 1.2	2.2 ± 0.5	2.2 ± 0.6	1.4 ± 1.0	<0.03							1.043	0.66	10.20
White matter	8.1 ± 4.8	4.2 ± 1.4	4.0 ± 2.0	1.7 ± 0.5	<0.03							1.210	0.57	18.00
Fenretinide														
Serum	0.7 ± 0.1	0.9 ± 0.4	2.5 ± 1.4		1.7 ± 0.8	0.9 ± 0.3	0.4 ± 0.1	0.5 ± 0.4	0.2 ± 0.1	0.2 ± 0.1	<0.04	0.050	13.76	25.55
Gray matter	<0.04	0.1 ± 0.1	0.6 ± 0.3		1.1 ± 0.3	0.8 ± 0.2	0.9 ± 0.3	1.2 ± 0.5	1.5 ± 0.9	0.8 ± 0.4	0.3 ± 0.1	0.034	20.67	72.37
White matter	<0.04	0.3 ± 0.1	0.4 ± 0.2		1.2 ± 0.2	0.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.4	1.3 ± 0.9	0.6 ± 0.3	0.2 ± 0.1	0.039	17.77	57.53

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 brain samples were 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.

Analysis of Serum and Brain Samples. Concentrations of ATRA, 13-*cis* RA, and fenretinide were measured by HPLC (Wyss, 1990). Spiked standards and deproteinated 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 C_{18}; (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. Terminal half-lives ($t_{1/2}$) 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 $(AUC)_{0\rightarrow\infty}$ values were determined by the trapezoid 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 ke ($ke = 0.693/t_{1/2}$). 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 in 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

concentrations of fenretinide. The $AUC^{0\rightarrow\infty}$ WM/$AUC^{0\rightarrow\infty}$ GM was greater than 1 for ATRA (1.62) and 13-*cis* RA (1.76) and less than 1 for fenretinide (0.79). The $AUC^{0\rightarrow\infty}$ GM/$AUC^{0\rightarrow\infty}$ 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 $AUC^{0\rightarrow\infty}$WM/$AUC^{0\rightarrow\infty}$ 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 ($\leq$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 $\pm$ 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 $AUC^{0\rightarrow\infty}$ was 13,740 $\pm$ 1600 ng.h/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_{max}, 10 mg/liter; t_{max}, 1 h; AUC, 25.9 $\pm$ 12.0 mg.h/liter; $t_{1/2}$, 0.72 $\pm$ 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 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_{max} and apparent half-life in WM and GM are quite similar, but the WM, where there are more astrocytes and oligodendrocytes, is more impregnated 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 low, persistent [especially after repeated dosings (fenretinide)] release of the drug.

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