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Pharmacokinetics of all-trans retinoic acid (ATRA) in patients with renal cancer concomitantly treated with interferon alpha 2A (IFN)

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Abstract

Eight patients with advanced renal cell cancer were treated in a phase II trial with interferon alpha 2a (9–18 MIU/m^2 subcutaneously) three days a week and all-trans retinoic acid (ATRA) 100 mg/m^2/day orally in three equal fractions 7 h apart. All eight patients underwent pharmacokinetic studies on day 1 of therapy, and six were again studied on day 90. A new simple and rapid HPLC method was developed to measure plasma concentrations of ATRA, with a detection limit of 5 ng/ml at 340 nm. Blood samples were drawn for 7 h following 1/3 oral doses of 100 mg ATRA/m^2. On day 1, peak plasma levels of ATRA (56 ± 37 ng/ml, mean ± SD) were reached after 1–5.5 h. Classically, a significant decrease in both peak plasma levels and the area under the concentration-time curve was observed after 2–6 weeks of ATRA treatment. In our preliminary study, concomitant IFN treatment increased peak plasma levels of ATRA after at least 3 months of treatment in 4 patients and reduced the clearance rate in 4 patients. On day 90, a patient who had not taken INF the previous day and in whom ATRA was undetectable developed a clinical relapse.

Keywords: All-trans retinoic acid; HPLC assay; Interferon alpha 2a; Pharmacokinetics; Renal cell cancer

1. Introduction

All-trans retinoic acid (ATRA) is the physiological metabolite of retinol (vitamin A); human tissues possess the enzymes required for its synthesis. Physiological concentrations of ATRA are 1–3 ng/ml of plasma (De Ruyter et al., 1979; Chiang, 1980).

In plasma, ATRA is bound to serum albumin. It is taken up by the liver and rapidly metabolized into 4-oxo-all-trans retinoic acid and 4-oxo-13-cis retinoic acid (Lucek and Colburn, 1985). The bile appears to be the main elimination pathway.

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ATRA plays an important role in cell proliferation and differentiation, and is used to treat various skin conditions and cancers (Chomienne et al., 1989; Bonhomme et al., 1994; Iakuwa et al., 1989)

Two mechanisms have been forwarded to explain the action of retinoic acid. In the first, all-trans retinoic acid is a co-factor in transfer reactions of a glycosyl radical, whereas in the second, ATRA is involved in gene expression, as in the steroid model. Intracellular binding protein transports all-trans retinoic acid to the cell nucleus, where it can be bound by three nuclear receptors (RAR gamma, RAR B and RAR J). Retinoic acid/nuclear receptor complexes can bind to the activating region of DNA bearing a gene, the transcription of which is then upregulated.

Severals studies have shown that chronic oral administration of ATRA results in a gradual fall in peak plasma concentrations and AUC values, and in an increase in urinary elimination of its metabolite (Lefebvre et al., 1991; Smith et al., 1992). ATRA is catabolized by cytochrome P450-like enzymes that are autoinducible (Muindi et al., 1992; Guiso et al., 1994). Interferon (IFN) depresses the hepatic cytochrome P450 drug-metabolizing system and enhances in vivo ATRA effects (Mannering et al., 1988). In vitro, renal cancer cells show variable sensitivity to the antiproliferative effect of ATRA. ATRA and IFN together possess an enhanced antiproliferative effect on some renal cancer cell lines (Namus et al., 1994).

The aim of this study was to investigate the influence of IFN on ATRA pharmacokinetics in metastatic renal cancer therapy.

2. Materials and methods

2.1. Patients

The pharmacokinetic study protocol was approved by our institutional ethics committee, and the patients gave their written informed consent to participate.

Eight patients with advanced renal cell cancer were treated in a phase II trial with IFN 2a (9–18 MUI/m^2 subcutaneously) 3 days a week, and ATRA 100 mg/m^2/day per os in three equal fractions every 7 h. The patients' mean age was 60 ± 8 years (mean ± S.D.) (range 48–75); there were two women and six men. Mean body weight was 82 ± 4 kg (mean ± SD)

ATRA was formulated in soft-gelatine 10-mg capsules and was supplied by Roche (France). Capsules were administered with meals.

2.1.1. Dosing schedule

On D0 each patient received three oral doses of all-trans retinoic acid with interferon.

2.1.2. Day 1 study

Samples were taken on day 1 at the following times: 0, 0.5, 1, 1.5, 2, 3, 4, 6 and 7 h following 1/3 oral doses of 100 mg/m^2 (33.3 mg/m^2) of ATRA, and on day 2 at 24 h.

2.1.3. Repeated-dose study

Samples were taken on day 90 at the following times: 0, 1, 1.5, 2, 3, 4, 6 and 7 h following 1/3 oral doses of 100 mg/m^2 of ATRA, and on day 91 at 24 h.

2.1.4. Blood sampling

Venous blood was taken from an antecubital vein via an indwelling catheter into heparinized tubes (5 ml/tube).

2.1.5. Sample storage

Plasma was isolated by centrifugation at 5000 rpm for 10 min at 4°C and stored immediately at −20°C until use.

2.2. Assay technique

We developed a new technique for specific and sensitive measurement of ATRA in plasma samples. Metabolites were not measured.

We used a high-performance liquid chromatograph equipped with a Shimadzu LC6A pump (Touzart et Matignon^R) with a flow rate of 2 ml/min, and a UV spectrophotometric detector (Shimadzu SPD6A, Touzart et Matignon^R). The column was a steel Technopack C18-10 μm (300 x 3.9 mm) with an RP8 precolumn (7 μm, 15 x 3.2 mm) (Touzart et Matignon).

The sensitivity of the detector was 0.02 AUFS, and attenuation was set at 0–3. All the reagents were of analytical grade. The mobile phase con-

Table 1
Pharmacokinetics parameters of oral ATRA in 8 patients treated concomitantly with interferon

Number of patients	Cmax (ng/ml)		AUC (μg/h/l)		Cl/F (l/h)		Vd/F(l)	
	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90	Dayl	Day 90
1	45	148	139	268	433	223	1277	2031
2	40	117	156	303	384	198	691	670
3	13	37	33	82	3599	740	9434	1069
4	8	21	319	137	188	437	2305	5851
5	51	ND	40	ND	1498	ND	2840	ND
6	90	—	103	—	579	—	1120	—
7	99	—	180	—	332	—	279	—
8	101	44	169	335	354	178	317	257
Mean ± S.D.	56 ± 37		142 ± 91		921 ± 1156		2283 ± 3029	

ND, not detectable; Cmax, peak plasma concentration; AUC, area under curve;Cl, clearance; Vd/F, apparent distribution volume; F, bioavailability.

sisted of 71% methanol, 12% acetonitrile and 17% 0.1 M ammonium acetate. It was filtered and degassed with ultrasound for 10 min prior to use.

The calibration curve was established by spiking a standard plasma with ATRA at concentrations of 5–3000 ng/ml.

2.2.1. Extraction

Samples were kept in the dark. Plasma (500 μl) was added to ethanol (1 ml) and centrifuged at 5000 rpm for 15 min. The ethanol fraction was injected directly into the loop.

The column was rinsed daily with methanol. Each sample was injected three times and the ATRA concentration was calculated from the integrated surface area under the peaks with reference to standard curves.

2.2.2. Validation of the assay method

The recovery of ATRA from plasma was 97 ± 1% in the concentration range tested (0.005–3 μg/ml). The precision of the assay was 7%, 5% and 6% at concentrations of, respectively, 10, 100 and 300 ng/ml. The retention time was 3.6 min.

2.2.3. Estimation of pharmacokinetic parameters

The following kinetic parameters were determined:

- Tmax (time to peak plasma concentration)
- Cmax (peak plasma concentration)
- Cmin (trough plasma concentration)
- AUC (area under the experimental concentration/time curve)
- $T_{1/2}$ a (absorption half-life)
- $T_{1/2\ b}$ (apparent elimination half-life)
- MRT (mean residence time after a single dose).
- Cl (clearance).
- Vd/F (apparent distribution volume)

Pharmacokinetic parameters were determined by using model-independent methods. The area under the plasma-concentration-time curve (AUC) from administration to the last measurable concentration was derived by the trapezoidal method and extrapolated to infinity. The terminal half life ($T_{1/2\ b}$) was determined by regression analysis.

3. Results

Six patients were studied on both day 1 and day 90. Five (patients no. 1, 2, 4, 5 and 8) received 9 MIU three times a week from day 0 to day 90. The sixth (no. 3) received 18 MIU three times a week from day 0 to day 90. Two other patients (no. 6 and 7) were only studied on day 1, as their cancer progressed and they received 9 MIU IFN 3 times a week. All the patients, except no. 5 and 7, underwent nephrectomy although all had a creatinine clearance in the normal range. Pharmacokinetic parameters are given in Table 1 and Table 2.

Table 2
Pharmacokinetics parameters of oral ATRA in 8 patients treated concomitantly with interferon

Number of patients	Tmax (h)		MRT (h) (AUMC/AUC)		T 1/2 B (h)	
	Day 1	Day 90	Day 1	Day 90	Day 1	Day 90
1	4	2	3.08	9.45	1.88	1.34
2	5	3	3.76	3.47	1.56	1.19
3	3	2	2.65	2.02	1	0.62
4	5.5	4	12.04	13.39	9.76	12.67
5	2	ND	2.02	ND	0.36	ND
6	2.05		1.73		0.52	
7	1		1.58		0.64	
8	1.5	2.75	1.25	1.75	0.62	1
Mean ± S.D.	3.1 ± 1.6		3.7 ± 3.6		2.0 ± 3.2	

ND, no detectable; Tmax, time to peak; MRT, mean residence time; T1/2B, apparent elimination half life.

On day 1 (before administration) ATRA was undetectable in all the patients. Measurable plasma levels were observed 30–60 min after drug ingestion. There was considerable variation among the subjects in the plasma concentration of ATRA, with Tmax values varying between 1 and 5.5 hours and Cmax varying between 8 and 101 ng/ml on day 1 (56 ± 37 ng/ml, mean ± S.D.) and 21 ng/ml to 148 ng/ml on day 90.

On day 1, the mean plasma AUC for the eight patients was 142 ± 91 μg/h/l. The terminal T1/2 averaged 2.0 ± 3.2 h.

The volumes of distribution (Vd/F) were large and variable (279–9434 l). ATRA was no longer detectable in the plasma (< 5 ng/ml) 7 h after administration.

Interferon increased peak plasma levels of ATRA following at least 3 months of treatment in 4 cases, and reduced the clearance rate in 4 patients. Patient no. 5 failed to take IFN 1 day before ATRA and had undetectable ATRA; this was associated with clinical relapse.

The initial ATRA daily dose (180–160 mg/day) was reduced in patients no. 4 and 8, possibly explaining the lower plasma concentration in patient no. 8 on day 90 (El Mansouri et al., 1995). Two kinetic studies were done in patient no. 8 on day 90, the first without concomitant IFN dosing; ATRA was undetectable, as in patient no. 5. The second kinetic study was done after dosing with IFN on the previous day at 21.00 h; in this case ATRA was detected in the serum (Table 1).

These preliminary results showed no relationship between the maximum plasma concentration of ATRA and the response to treatment. However, in the patients with a partial response (no. 1 and 4), the Vd/F ratio increased between day 0 and day 90, except for patient no. 3 (decrease related to a clinical deterioration) and patient no. 2 (unchanged, stable clinical state).

4. Discussion

We have developed a new HPLC technique for simple and rapid measurement of ATRA in plasma. This pharmacokinetic study confirmed the large intersubject differences in kinetics after oral administration of ATRA.

Plasma concentrations of ATRA were relatively low in the eight patients studied, but volumes of distribution were large, indicating good tissue penetration. ATRA disappeared from plasma rapidly. The high (Vd/F) ratio indicates either that ATRA distribution is extensive or that the F value is low (liver first-pass effect) or both. Kinetic studies were done on day 1 and day 90 in six cases; in four of these cases, interferon alpha increased the ATRA Cmax and/or reduced its clearance. In two patients who underwent ATRA kinetic studies on day 90 without concomitant interferon administration, ATRA levels were below the detection limit, while later interferon ad-

ministration restored measurable ATRA values; this suggests that interferon would maintain ATRA plasma levels after 3 months of treatment and increase plasma levels in the others patients. Chronic oral administration of ATRA results in a progressive reduction of the plasma concentration. ATRA induces an increase in its own catabolism by upregulating one or more cytochrome P450 enzymes (Muindi and Young, 1992)

Coadministration of P450 inhibitors (ketoconazole) might also prevent the reduction in plasma ATRA levels. However, these drugs can have side effects (Rigas et al., 1993). Interferon and IFN inducers down-regulate hepatic cytochrome P450 through a pretranslational mechanism involving depression of P450 mRNA levels and a subsequent decrease in P450 synthesis (Cribb and Renton, 1993).

It has been reported that ATRA administered on an intermittent basis allows clearance to be restored to previous values (Adamson et al., 1993). This therapeutic schedule may be associated with lower clinical efficacy and does not always result in increased plasma peak concentrations (Pitot et al., 1994).

Coadministration of IFN with ATRA should increase Cmax and AUC values, and decrease the clearance of ATRA. This would partly explain the enhanced effects of ATRA in vivo.

Our results appear to conflict with those of Smith et al. (1994), but the IFN dosage was lower (3 MIU twice a week) in their study. The inhibitory effect of IFN on P450 cytochrome activity would thus appear to be dose-dependent.

In our clinical trial, three patients with stable disease at 3 months showed a partial response at 6 months. At 3 months, three other patients had stable disease and two had progressed (Paul et al., 1995). If interferon allows plasma ATRA levels to be maintained or increased during chronic ATRA treatment, it would be interesting to determine the interferon dosing schedule (daily?) and dose in order to confirm our results and optimise clinical efficacy.

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