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Novel inhibition of *cis*/*trans* retinoic acid interconversion in biological fluids—an accurate method for determination of *trans* and 13-*cis* retinoic acid in biological fluids

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Abstract

All-*trans* retinoic acid (tRA, or tretinoin) can be metabolized through stereoisomerzation to 13-*cis* retinoic acid (13-cRA) in vivo. We have developed a simple, sensitive and accurate method for analyzing tRA and 13-cRA in plasma with the addition of *N*-ethylmaleimide (NEM) and Vitamin C (Vit. C) to prevent the interconversion of *cis*/*trans* retinoic acid. All-*trans* RA, 9-cRA, and 13-cRA were well separated from each other in plasma by using a C18 precolumn and a column with a gradient solvent system of mobile phases A and B at a flow rate of 1.0 ml/min. In addition, thermal stability of tRA and cRA in plasma during the sample preparation under the temperature of 0 and 25 °C were studied. Our results showed that (1) the interconversion ratios (%) (cRA/tRA and tRA/cRA) were decreased with the addition of NEM and Vit. C and the minimum concentrations of NEM and Vit. C to inhibit the interconversion were 50 and 150 μM, respectively, (2) higher concentrations of NEM and Vit. C were required to prevent the interconversion at higher temperature, (3) the precision and accuracy of calibration curve with various concentration of tRA (1–1000 ng/ml) and 13-cRA (5–800 ng/ml) in plasma showed good linearity ($r^2 = 0.9992$ and 0.9994), and between-day errors expressed by coefficient of variation (CV, %) for tRA and 13-cRA which were both less than 5.6%, (4) the mean recovery of the analytes were 78–94% for tRA and 80–92% for 13-cRA at concentration range from 1 to 1000 ng/ml and 5 to 800 ng/ml, respectively, and (5) the limit of quantitation of tRA and 13-cRA were 1 and 5 ng/ml, respectively. In addition, the HPLC method had been successfully applied to the tRA pharmacokinetic study in two hepatoma patients after receiving 45 mg/m^2 per day orally. Thus, our results suggest that the HPLC method for analyzing tRA and 13-cRA in plasma with the addition of NEM and Vit. C is a simple, sensitive and accurate method.

Keywords: Interconversion; Pharmacokinetics; Retinoic acid

1. Introduction

Retinoic acids (RAs) have been widely used for many years for preventing and treating dermatological

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diseases [1,2]. They may also be applied to oncology [3]. Recent studies suggest that all-*trans* retinoic acid (tRA) not only prevent but also treat the development of a variety of cancers in human and animals and of other clinical applications [4–11]. tRA is converted by intracellular isomerases to 9-cRA, or 13-cRA in vivo. The pharmacokinetics and metabolism of tRA are important parameters in determining biological responsiveness and effectiveness. Many difficulties have to be overcome in analyzing this labile compound. tRA is rapidly isomerized and destroyed in the presence of light and oxidants. The thermal instability of tRA further increased the difficulties for accurately analyzing the compound in biological fluids. To study the pharmacokinetics of tRA, further isomerization must be prevented after the sample collection [12–17]. However, no studies of tRA assay [18–20] and pharmacokinetic [21–24] had been studied on the potential isomerization in the biological fluids during sample preparation processes. The aim of this study was to develop a method which not only to prevent oxidation but also to stop the isomerization between *trans* and *cis* retinoic acid (cRA) in the biological fluids, and further to apply this method to a pilot pharmacokinetic study of tRA and 13-cRA in patients with hepatocellular carcinoma.

2. Materials and methods

2.1. Chemicals

The reference standards of tRA, 9-*cis* retinoic acid (9-cRA), 13-*cis* retinoic acid (13-cRA), butylated hydroxytoluene (BHT), and ascorbic acid (HPLC grade, Sigma, USA), *N*-ethylmaleimide (NEM) (Nacalai Tesque, Japan) and the internal standard carbazole (Merck, Germany), methanol, diethyl ether (High-performance liquid chromatography, HPLC grade, Lab-Scan) were all reagent grade and purchased from their respective supplier.

2.2. Equipment and chromatography condition

High-performance liquid chromatographic was performed on a Jasco liquid chromatographic system (Japan), with a model PU-980 pump, a model AS-950 auto-sampler, a model 807-IT integrator, and a model UV-970 UV-Vis detector set at 340 nm. HPLC condition was modified from Vane et al. [18]. Separation was performed on a precolumn (10 μm, Bondapack^{TM}, C18) and Bondapack C18 HPLC column (10 μm, 300 mm × 3.9 mm) with a gradient solvent system of mobile phase A (methanol:0.02 M ammonium acetate) (50:50, v/v) and B (methanol:0.1 M ammonium acetate) (90:10, v/v), at a flow rate of 1.0 ml/min. Acetic acid was used to adjust the pH of the mobile phases A and B to pH 6.65. The solvent program was set at initial solvent composition of 50% B (50% A), linear gradient to 90% B (10% A) from 0 to 10 min. The solvent composition of 90% B (10% A) lasted for 8 min, and then returned to the initial condition (50% B).

2.3. Inhibition of interconversion between tRA and 13-cRA

Because of no 9-cRA was detected after tRA was added to plasma in our preliminary study, the following interconversion studies were focused on tRA and 13-cRA.

2.3.1. Thermal stability

Thermal stability of tRA (500 and 2500 ng/ml) and 13-cRA (800 and 2500 ng/ml) in plasma were studied ($n = 3$) at temperatures 0 and 25 °C during the sample preparation procedure. The sample preparation procedure after addition of inhibitors was described in detail in the Section 2.4. In addition, the temperature combined with the chemical inhibitors was also tested as described in following section.

2.3.2. Chemical inhibitors

To prevent interconversion of tRA and 13-cRA in plasma, various amounts of thiol-blocking agents (e.g. 0.5 mM NEM in this study) and antioxidants (e.g.1.5 mM Vit. C in this study) were added into plasma. In addition, various concentrations of tRA (500 and 2500 ng/ml) and 13-cRA (800 and 2500 ng/ml) were measured. The formation of 13-cRA from tRA and tRA from 13-cRA were calculated by the biotransformed tRA or 13-cRA divided by the original tRA or 13-cRA, respectively.

2.4. Sample preparation

To prevent the photoisomerization of tRA and 13-cRA, all experiments were processed under red

light in an icebox (0 °C). The calibration curves of tRA and 13-cRA were linear in the range of tRA 1–1000 ng/ml and 13-cRA 5–800 ng/ml, respectively. To avoid the interconversion between *trans* and *cis* form in the biological fluid, the antioxidant of sodium ascorbate (1.5 mM, 20 μl) and the thiol-blocking agent of NEM (0.5 mM, 20 μl) were added into 0.2 ml plasma. Samples were then extracted by adding 6 ml diethyl ether with 5% butyl hydroxytoluene (BHT) at 4 °C, and then evaporated under nitrogen gas. One hundred microliters of methanol:acetonitrile (1:1) and 200 μl of mobile phase B solvent were added immediately to the residues and then centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant (200 μl) was taken and then 50 μl was injected into HPLC.

2.5. Assay validation

To assess the precision and accuracy of this assay method, within-day ($n = 6$) and between-day ($n = 6$) calibration curves with various concentrations of (1.0–1000 ng/ml) tRA and (5.0–8000 ng/ml) 13-cRA in the plasma were constructed. Precision and accuracy were determined by back calculation of spiked plasma sample at eight concentrations with respect to a calibration graph prepared each day. The precision was expressed as the within-day and between-day coefficient of variation (CV, %). Accuracy was calculated as the mean deviation of each concentration from the theoretical.

2.6. Pilot pharmacokinetic studies

Two hepatoma patients from a phase I study were included in this study. To investigate the mechanism of clinical RA resistance, we evaluated patients who received tRA as induction of treatment with serial pharmacokinetic studies on the day 1 of treatment and again after the fourth course continuous treatment on day 7. The phase I study was approved by the Investigational Review Board of the Taipei Veterans General Hospital. Before drug administration, a 5 ml blood sample was collected for the individual calibration curve. Samples were collected in a 1.5 ml heparin-rinsed Eppendorf® vial. After oral administration of dose, 45 mg/m^2 of tRA (Tretinoin), serial blood samples were collected at 15, 20, 30, 45, 60, 90 min, and 2, 2.5, 3, 5, 9, 12 h. One milliliter of blood sample was obtained at each time point, then placed into a 1.5 ml Eppendorf® tube and kept at −80 °C until analysis. Heparin was used for continuous sampling. The same procedures were repeated again after the fourth course continuous treatment on day 7.

The plasma concentrations of tRA and 13-cRA were fitted to a non-compartment model. The area under the (first) moment versus time curve (AUMC), mean resident time (MRT), rate constant for elimination phase (β), elimination half-life ($t_{1/2}(\beta)$), volume of distribution (V_d), clearance (CL/F), time for peak plasma RA concentration (T_{max}), and the peak concentration (C_{max}) were read directly from the plasma concentration versus time profile. The area under the concentration-time curve over the first 12 h (AUC_{12}) and time infinity (AUC_∞) following a single oral dose of RA were calculated by the trapezoid rule using an EXCEL computer program. The same computer program was used for all other statistical calculations.

3. Results and discussions

3.1. Inhibition of interconversion between tRA and 13-cRA

3.1.1. Thermal stability

The thermal stability of tRA and 13-cRA in plasma at 25 and 0 °C during the sample preparation and procedures were shown in Fig. 1. In all cases, the percent of interconversions in plasma from tRA to 13-cRA and 13-cRA to tRA were lower at 0 than at 25 °C. At low RA concentration (500 ng/ml tRA and 800 ng/ml 13-cRA), the interconversion from tRA to 13-cRA and 13-cRA to tRA decreased from 19.9±2.32 and 10.51± 1.50% at 25 °C to 6.57±2.17 and 5.92±1.90% at 0 °C, respectively. Similar results were obtained at high RA concentration (2500 ng/ml). The percent of interconversion decreased when the plasma sample was handled at 0 °C. The stability of tRA and 13-cRA with 250, 500, 1000 and 2500 ng/ml in plasma had been studied by our laboratory at −20 °C for 9 days. No interconversion from tRA to 13-cRA and 13-cRA to tRA was observed. Since the maximum tRA and 13-cRA therapeutical concentrations were around 500 ng/ml, the effect of chemical inhibitors on blocking interconversion between tRA and 13-cRA was carried out at low RA concentration.

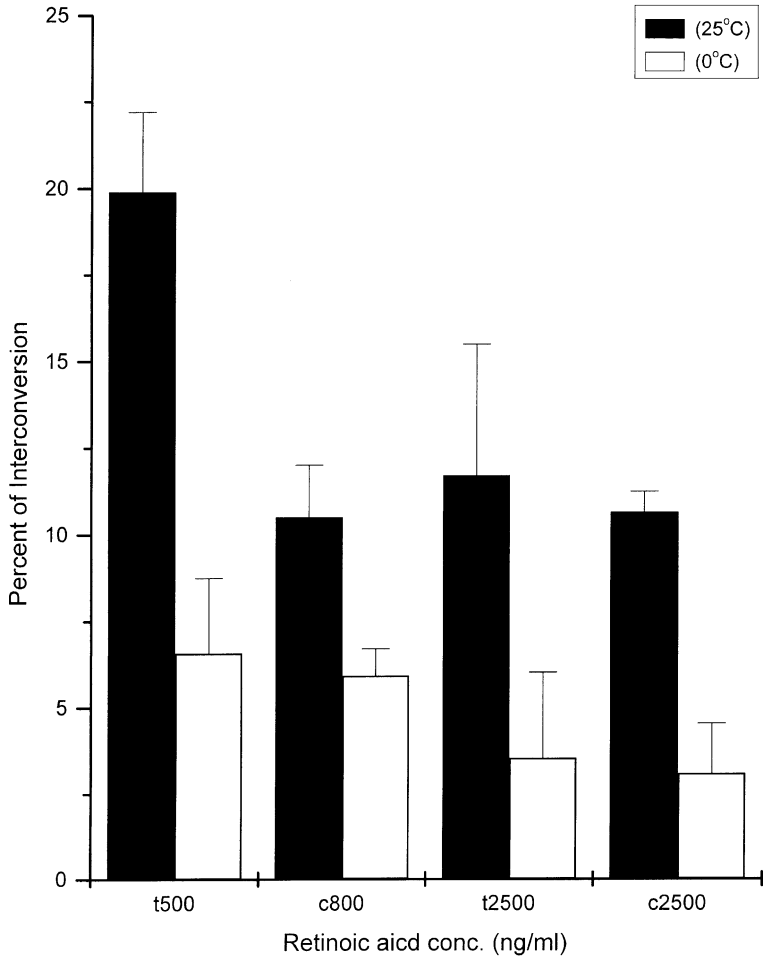

Fig. 1. The thermal stability of tRA (initial concentration 500 and 2500 ng/ml) and 13-cRA (initial concentration 800 and 2500 ng/ml) in plasma (mean ± S.D.) at 25 and 0 °C.

3.1.2. Chemical inhibitors

A thiol-blocking agent (NEM) and an antioxidant agent (Vit. C) were added to block the conversion process of tRA to 13-cRA or 13-cRA to tRA in plasma. The effect of the combination of varies amount of NEM and Vit. C on the interconversion between tRA and 13-cRA in plasma at 25 and 0 °C were shown in the Figs. 2 and 3. In all cases, the percent of interconversion (13-cRA/tRA and tRA/13-cRA) decreased with the increasing inhibitors concentration. Under 25 °C, 19.9 ± 2.32% of 13-cRA was formed from 500 ng/ml tRA in 2 ml of plasma without inhibitors, whereas the interconversion decreased to 3.07 ± 1.7% with adding 50 μmol NEM and 150 μmol Vit. C. However, the interconversion could not be completely blocked with the addition of inhibitors up to 125 μmol of NEM and 375 μmol Vit. C at 25 °C. At 0 °C, the interconversion between tRA and 13-cRA in 2 ml plasma was completed blocked by adding 50 μmol of NEM and 150 μmol Vit. C (which equal to 100 μl of 0.5 mM NEM and 1.5 mM Vit. C). The volume ratio of inhibitors to plasma was at least 1:10 for completely blocking the conversion between tRA and 13-cRA under 0 °C. Therefore, the sample preparation of retinoic acid was carried out at 0 °C with the addition of 20 μl of 0.5 mM NEM and 1.5 mM Vit. C in 200 μl plasma.

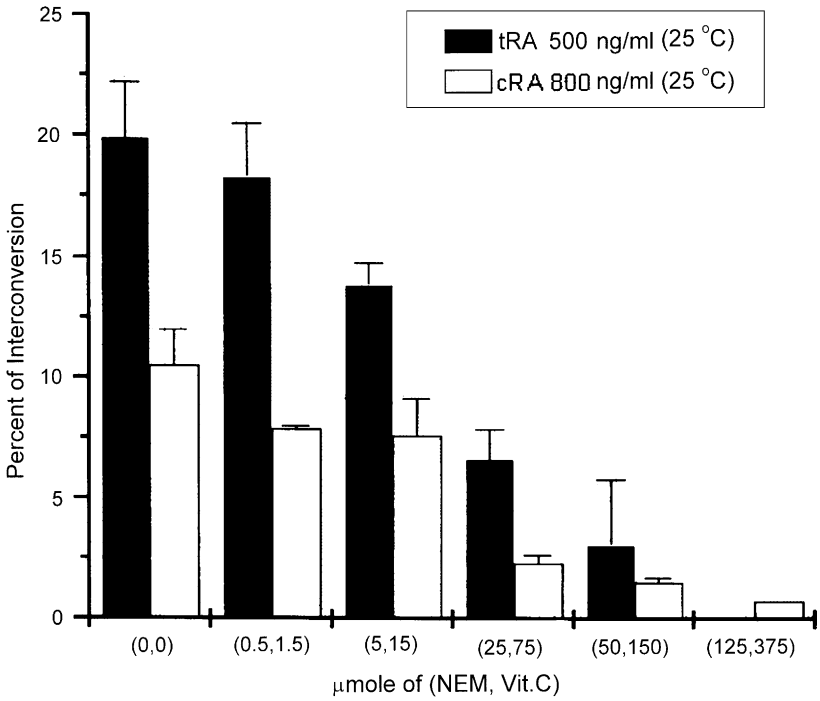

Fig. 2. Percentage of interconversion in plasma (mean ± S.D.) from tRA (initial concentration 500 ng/ml, black column) to 13-cRA and from 13-cRA (initial concentration 800 ng/ml, white column) to tRA at 25 °C with various amounts of chemical inhibitors NEM and Vit. C.

The extraction procedures of tRA in the biological sample used in this study were comparable to most published analytical methods [18–20]. As mentioned earlier, precautions have to be taken during sample preparation to prevent photoisomerization and oxidation of the retinoids. The isomerization of retinoic acid can be mediated by adding NEM. This isomerization in the plasma might probably cause by thiol-containing compound [25]. Vit. C and other compounds such as ethlyenediaminetetraacetate (EDTA) and BHT had been used to prevent oxidation of retinoids in the biological samples before extraction [20]. The need for antioxidants of the sample is dependent on the complexity of the sample extraction processes and has to be investigated by individual analytical method. Therefore, a combination of thiol-blocking agent NEM with antioxidant agent Vit. C was tested in order to block the isomerization from tRA to 13-cRA and 13-cRA to tRA in the biological samples. Our results demonstrated that temperature was an important factor for controlling the degree of isomerization between tRA and 13-cRA during the sample preparation. These processes should be performed as close to 0 °C as possible. In addition, the isomerization in the plasma during the extraction was not blocked in the absence of these chemical inhibitors at 0 °C. Thus, our study showed that the interconversion of RA was abolished by adding a thiol-blocking agent NEM with an antioxidant Vit. C at 0 °C before extraction.

3.2. Analysis of tRA, 9-cRA and 13-cRA by HPLC in the plasma

tRA, 9-cRA, and 13-cRA as well as endogenous materials of plasma were well separated from each other by using HPLC with retention time of 17.3, 16.4 and 15.3 min, respectively. The precision and accuracy of tRA and 13-cRA assay were confirmed by within-day and between-day calibration curves with various concentrations of tRA (1–1000 ng/ml) and cRA (5–800 ng/ml) in plasma (Table 1). For within-day analysis, the coefficients of variation for tRA and 13-cRA were all less than 5.25% for both analytes and the deviation from the expected concen-

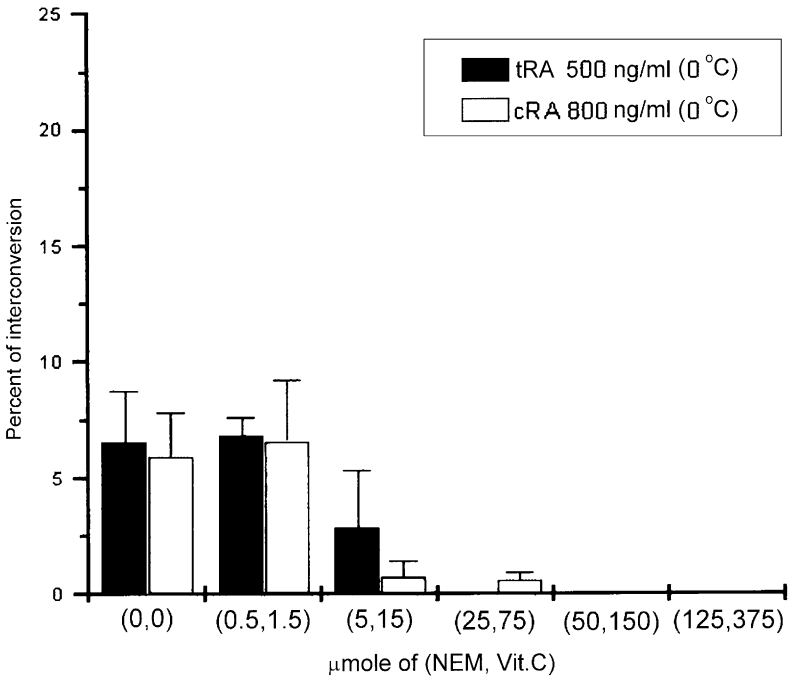

Fig. 3. Percentage of interconversion in plasma (mean ± S.D.) from tRA (initial concentration 500 ng/ml, black column) to 13-cRA and from 13-cRA (initial concentration 800 ng/ml, white column) to tRA at 0 °C with various amounts of chemical inhibitors NEM (0.5 mM) and Vit. C (1.5 mM).

Table 1
Precision and accuracy of all-*trans* retinoic acid (tRA) and 13-*cis* retinoic acid (13-cRA) in plasma by the HPLC method

tRA			13-cRA		
Concentration (ng/ml)	CV (%)	Accuracy (% mean deviation)	Concentration (ng/ml)	CV (%)	Accuracy (% mean deviation)
Within-day ($n = 6$)					
1	3.56	1.91	5	2.61	1.19
5	3.61	1.19	15	5.11	−0.09
10	5.11	−5.92	25	2.49	−5.13
50	2.49	4.79	50	2.67	−0.68
75	2.67	−0.46	100	5.25	7.72
400	5.25	−5.58	400	2.68	1.55
500	2.68	2.37	500	2.92	−3.89
1000	2.92	1.28	800	2.07	−2.36
Between-day ($n = 6$)					
1	8.80	5.60	5	5.57	−2.72
5	7.32	2.36	15	2.16	0.92
10	5.12	−3.02	25	2.50	−5.30
50	1.66	2.41	50	2.66	1.00
75	2.63	0.23	100	4.30	4.90
400	3.03	−0.26	400	2.16	2.46
500	1.84	1.48	500	6.57	0.91
1000	1.31	0.35	800	4.86	−3.49

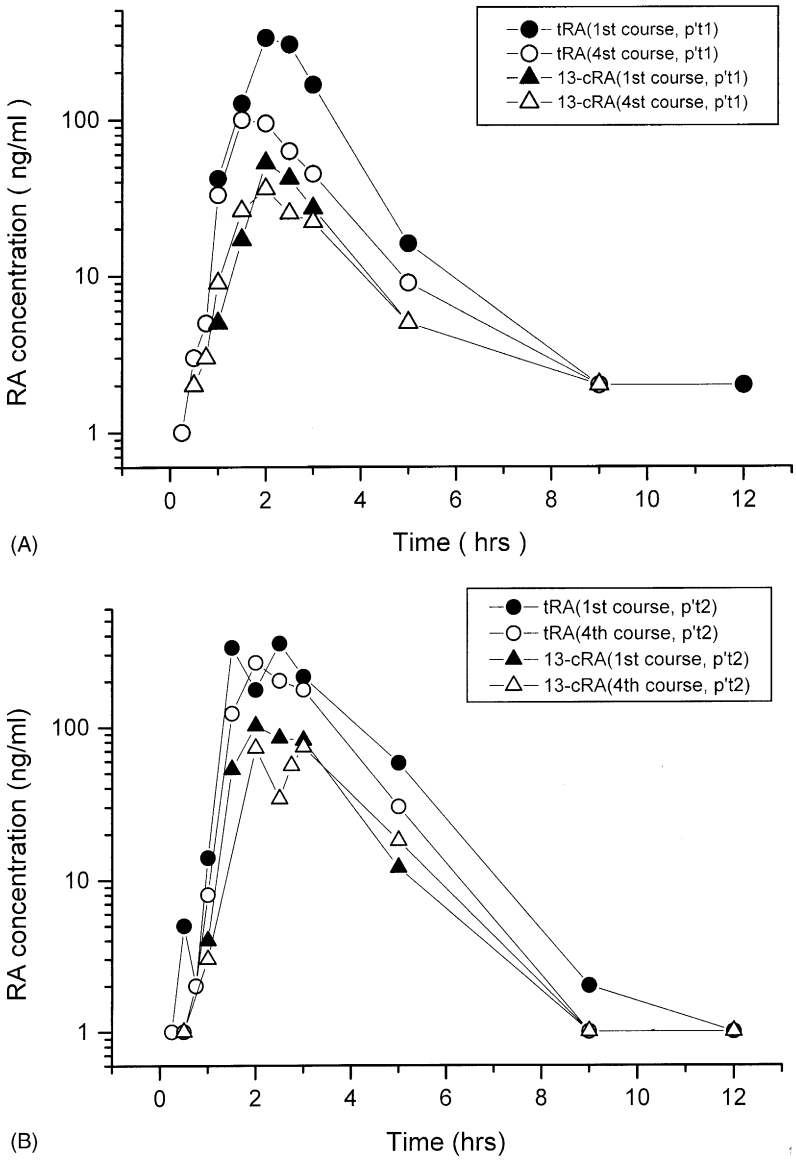

Fig. 4. The plasma concentration of tRA and 13-cRA after oral administration of 45 mg/m^2 per day of tRA in the two hepatocellular carcinoma patients. Both the tRA and 13-cRA concentrations were measured in single dose administration (course 1, day 1) and after continuous treatment (course 4, day 7).

tration, as a measurement of accuracy, ranged from −5.58 to +4.79% for tRA and −5.13 to +7.72% for 13-cRA. For between-day analysis, the coefficients of variation of between-day for tRA and 13-cRA were less than 8.80 and 6.57%, respectively. The respective deviation from the expected concentration ranged from −3.02 to 5.60% for tRA and from −5.30 to 4.90% for 13-cRA. These results indicate that this method is precise and accurate. The recovery was determined by comparing the peak height from unextracted standards with those of extracted standards, across the range of each standard curve. The mean

Table 2
Pharmacokinetic parameters of all-*trans* retinoic acid after oral administration of 45 mg/m^2 all-*trans* retinoic acid in two patients

Pharmacokinetic parameters	Patient 1		Patient 2	
	Course 1 (day 1)	Course 4 (day 7)	Course 1 (day 1)	Course 4 (day 7)
T_{max} (h)	2.0	1.5	2.5	2.0
C_{max} (ng/ml)	331.55	100.46	353.58	265.17
$AUC_{0\rightarrow t}$ (ng h/ml)	677.4	231.4	887.4	612.4
AUC_{∞} (ng h/ml)	681.33	235.20	888.06	612.55
$AUMC_{0\rightarrow t}$ (ng h^2/ml)	1769.27	604.89	2612.8	1757.7
MRT (h)	2.65	2.61	2.94	2.87
β (h^{-1})	0.51	0.52	0.72	0.81
$t_{1/2}(\beta)$ (h)	1.36	1.33	0.97	0.85
V_d/F (l/m^2)	129.50	366.24	70.86	90.39
CL/F (l/h m^2)	66.05	191.32	50.67	73.46

β: rate constants for elimination phase, $t_{1/2}(\beta)$: the half life of drug in the elimination phase, V_d/F: volume of the distribution, AUC_{∞}: area under plasma concentration–time curve to time infinity, CL/F: total plasma clearance, F: fraction of absorption, MRT: mean resident time.

recovery of the analytes was 78–94% for tRA and 80–92% for 13-cRA over the constructed calibration concentration ranges. The limitation of quantitation of tRA and 13-cRA were 1 and 5 ng/ml, respectively.

3.3. Pilot pharmacokinetics

The HPLC method has been successfully applied to the pharmacokinetic study in two hepatoma patients after receiving 45 mg/m^2 per day tRA orally. The plasma concentration-time curves of tRA and 13-cRA after oral administration of tRA were shown in Fig. 4. The corresponding pharmacokinetic parameters of tRA and 13-cRA in hepatoma patients were listed in Tables 2 and 3, respectively. In plasma, tRA is rapidly converted to its major metabolite, 13-cRA. After 2 h ingestion of tRA (70–80 mg) at the first course day 1 treatment, the concentrations of tRA and 13-cRA were rapidly raised, and reached the peak concentration of 342.57 ± 15.7 and 63.37 ± 14.9 ng/ml, respectively.

3.3.1. Pharmacokinetics of tRA after repeatedly oral administration of tRA

In addition to the pharmacokinetic properties of treatment on day 1, the pharmacokinetics of tRA and cRA of the fourth course continuous treatment on day

Table 3
Pharmacokinetic parameters of 13-*cis* retinoic acid after oral administration of 45 mg/m^2 all-*trans* retinoic acid in two patients

Pharmacokinetic parameters	Patient 1		Patient 2	
	Course 1 (day 1)	Course 4 (day 7)	Course 1 (day 1)	Course 4 (day 7)
T_{max} (h)	2.0	2.0	3.0	2.0
C_{max} (ng/ml)	52.7	35.5	73.9	101.6
$AUC_{0\rightarrow t}$ (ng h/ml)	99.2	93.9	236.9	261.5
AUC_{∞} (ng h/ml)	103.3	99.6	238.4	236.9
$AUMC_{0\rightarrow t}$ (ng h^2/ml)	249.4	284.6	768.8	759.9
MRT (h)	0.40	0.33	0.31	0.34
β (h^{-1})	0.85	0.40	0.52	0.63
$t_{1/2}(\beta)$ (h)	0.81	1.74	1.33	1.09
V_d/F (l/m^2)	511.21	1131.62	362.98	268.54
CL/F (l/h m^2)	435.7	451.62	188.75	170.47

β: rate constants for elimination phase, $t_{1/2}(\beta)$: the half life of drug in the elimination phase, V_d/F: volume of the distribution, AUC_{∞}: area under plasma concentration–time curve to time infinity, CL/F: total plasma clearance, F: fraction of absorption, MRT: mean resident time.

7 were also evaluated. Two patients were evaluated on day 1 and day 7 after treatment with a single oral tRA dose of 45 mg/m^2 per day. The plasma tRA concentrations up to 12 h after oral administration of tRA were analyzed. The calculated plasma AUCs for the two patients on day 1 were 677 and 887 ng h/ml, and those on day 7 were 231 and 612 ng h/ml. The AUC of both patients on day 7 were significantly lower than those on day 1. Their C_{max} on day 1 were 331 and 353 ng/ml, and their C_{max} on day 7 (100 and 265 ng/ml) were also significantly lower when compared to those on day 1. The $t_{1/2}(\beta)$ of tRA on day 1 were similar to those on day 7. Similarly, their MRT were 2.65 and 2.61 h at day 1, and 2.94 and 2.87 h at day 7. Their CL/F of tRA increased about two-fold on day 7 (191 and 73 l/h m^2) when compared to those on day 1 (66 and 50 l/h m^2). Their V_d/F of tRA also increased nearly two-fold on day 7 (366 and 90 l/m^2) when compared to those on day 1 (129 and 70 l/m^2).

3.3.2. Pharmacokinetics of 13-cRA after oral administration of tRA

After oral administration of tRA, the major metabolite, 13-cRA, was formed within 0.5 h and reached the peak concentration (52 and 73 ng/ml) at 2–3 h. The plasma AUCs of 13-cRA on day 1 were 99 and 236 ng h/ml and those on day 7 were 93 and 261 ng h/ml in these two patients. The plasma AUCs of 13-cRA were similar after the single dose tRA treatment on day 1 and continuous tRA treatment on day 7. However, the V_d/F of tRA metabolite, 13-cRA, was increased by two-fold (from 511 to 1131 l/m^2) in patient 1, but decreased (from 362 to 268 l/m^2) in patient 2. The CL/F of 13-cRA was similar between the two courses for patient 1 (435 and 451 l/m^2) and patient 2 (188 and 170 l/m^2) at the end. Similar situation was also observed in the MRT, results demonstrated that the MRT decreased from 0.40 to 0.33 h in patient 1, and increased from 0.31 to 0.34 h in patient 2.

4. Conclusion

Our study showed that (1) a simple, sensitive and accurate HPLC method was developed to prevent the interconversion of tRA and 13-cRA in plasma with the addition of converting inhibitors NEM and Vit. C, (2) the minimum concentration of NEM and Vit. C required to inhibit the interconversion were estimated to be 50 and 150 μM, respectively, (3) higher concentrations of inhibitors were required to completely prevent the interconversion at higher temperature, and (4) the HPLC method has successfully been applied to the pilot pharmacokinetic study of tRA and 13-cRA in two patients with hepatocellular carcinoma.

References

[1] C.E. Orfanos, R. Ehlert, H. Gollnick, Drugs 34 (1987) 459.
[2] A.M. Kligman, J. Am. Acad. Dermatol. 39 (1998) S2.
[3] R.A. Chandraratna, Cutis 61 (1998) 40.
[4] L. Degos, C. Chomeienne, M.T. Daniel, Lancet 336 (1990) 1440.
[5] R.P. Warrel, R.P. Frankel Jr., W.H. Miller Jr., N. Engl. J. Med. 324 (1991) 1385.
[6] P. Fenaux, C. Chastang, C. Chomeienne, Lancet 343 (1994) 1033.
[7] P.S. Gill, B.M. Espina, Mougdil, Leukemia 8 (1994) S26.
[8] I. Athanasiadis, M.S. Kies, M. Miller, Clin. Cancer Res. 1 (1995) 973.
[9] D.L. Hill, Annu. Rev. Nutr. 12 (1992) 161.
[10] B. Gilcrest, Lancet 3 (1999).
[11] S. Kang, G.J. Fisher, J. Voorhees, Arch. Dermatol. 133 (1997) 1280.
[12] M.H. Zile, R.C. Inhorn, H.F. DeLuca, J. Biol. Chem. 257 (1982) 3544.
[13] M.E. Cullum, M.H. Zile, J. Biol. Chem. 260 (1985) 10590.
[14] S.W. McClean, M.E. Ruddel, E.G. Gross, J.J. Degiovanna, G.L. Oeck, Clin. Chem. 28 (1982) 693.
[15] P.R. Sundaresan, P.V. Bhat, J. Lipid Res. 23 (1982) 448.
[16] J. Urbach, R.R. Rando, Biochem. J. 299 (1994) 459.
[17] J. Urbach, R.R. Rando, FEBS Lett. 351 (1994) 429.
[18] F.M. Van, J.K. Stoltenborg, C.J. Bugge, J. Chromatogr. 227 (1982) 471.
[19] M.G.M. De Ruyter, A.P. De Leenheer, Anal. Biochem. 98 (1979) 402.
[20] R. Wyss, F. Bucheli, J. Chromatogr. B 700 (1997) 31.
[21] S. El Mansouri, M. Tod, M. Leclerq, O. Petitjean, Drug Metab. Dispos. 23 (1995) 227.
[22] S.S. Stanly, L. Latriano, J. Am. Acad. Dermatol. 39 (1998) S13.
[23] M.B. Regazzi, I. Iacona, C. Gervautti, Clin. Pharmacokinet. 32 (1997) 382.
[24] C.C. Achkar, J.M. Bentel, J.F. Boylan, Drug Metab. Dispos. 22 (1994) 451.
[25] T.W. Shin, Y.F. Shealy, D.L. Strother, D.L. Hill, Drug Metab. Dispos. 14 (1986) 698.