Cancer Chemother Pharmacol (1994) 34: 522–526

Cancer Chemotherapy and Pharmacology

Modulation of all-*trans* retinoic acid pharmacokinetics by liarozole

Vincent A. Miller[1], James R. Rigas[1, 2], Josephia R. F. Muindi[2, 4], William P. Tong[6], Ennapadam Venkatraman[5], Mark G. Kris[1], Raymond P. Warrell, Jr.[2, 3]

[1] Thoracic Oncology Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY 10021, USA
[2] Developmental Chemotherapy Service, Division of Solid Tumor Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY 10021, USA
[3] Leukemia Service, Division of Hematologic Oncology, Department of Medicine, Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY 10021, USA
[4] Clinical Pharmacology Laboratory, Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY 10021, USA
[5] Department of Biostatistics, Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY 10021, USA
[6] Core Laboratory, Memorial Sloan-Kettering Cancer Center, Cornell University Medical College, New York, NY 10021, USA

Received: 24 November 1993/Accepted: 27 January 1994

Abstract. Continuous oral dosing with all-*trans* retinoic acid (RA) is associated with a progressive decrease in plasma drug concentrations that has been linked to relapse and retinoid resistance in patients with acute promyelocytic leukemia (APL). Since oxidation by cytochrome P-450 enzymes is critical in the catabolism of this drug, we evaluated whether pretreatment with an inhibitor of this system, liarozole, could attenuate this phenomenon. A total of 20 patients with solid tumors completed a 4-week course of all-*trans* RA therapy. On days 1, 2, 28, and 29, serial plasma samples were obtained from these patients after ingestion of a single oral dose (45 mg/m^2) of all-*trans* RA. On days 2 and 29, liarozole was given 1 h prior to ingestion of all-*trans* RA at single doses ranging from 75 to 300 mg. The areas under the plasma RA concentration x time curves (AUCs) were then compared in the presence and absence of pretreatment. Following continuous oral treatment, the mean day-28 AUC of all-*trans* RA was significantly lower than the group mean level on day 1 (504 vs 132 ng h^{-1} ml^{-1}; $P = 0.05$). This decline in plasma concentrations on day 28 was partially reversed by liarozole, which increased the mean plasma all-*trans* RA AUC on day 29 to 243 ng h^{-1} ml^{-1} ($P = 0.004$). The lowest dose of liarozole that reliably produced this effect was 300 mg. No enhanced toxicity was associated with liarozole administration. We conclude that liarozole at a dose of 300 mg effectively attenuates the induced decline in all-*trans* RA plasma concentrations that occurs with continuous treatment. This combination may be useful in attenuating or reversing retinoid resistance.

Key words: Retinoic acid – Pharmacokinetics – Liarozole

Introduction

All-*trans* retinoic acid (RA) induces complete remission in a high proportion of patients with acute promyelocytic leukemia (APL) [4, 5, 13, 28]. These findings have prompted broader interest in this retinoid both as a drug for cancer treatment and, potentially, as a means of cancer prevention. In patients with APL, hematologic remissions induced by all-*trans* RA are relatively brief [4, 5, 10, 13] and relapses on therapy are uniformly associated with resistance to further treatment [4, 10].

Recently it has been shown that plasma concentrations of all-*trans* RA progressively decline with continuous dosing [20, 22–24], suggesting that all-*trans* RA might induce its own catabolism. Since all-*trans* RA is catabolized by cytochrome P-450 oxidative enzymes [18, 26], we suggested that the decrease in plasma all-*trans* RA levels might in part result from an increase in the activity of this inducible enzyme system. This hypothesis further suggested that catabolism might be modulated by inhibitors of cytochrome P-450 enzymes. In a recent study we showed that ketoconazole, a broad-spectrum P-450 inhibitor, significantly attenuated the progressive decline in plasma RA concentrations seen with continuous dosing [24]. However, the doses required to observe this effect were occasionally associated with nausea that would have compromised treatment over a prolonged period.

Liarozole {5-[(3-chlorophenyl) (1H-imidazole-1-yl) methyl]-1H-benzimidazole HCl} is a cytochrome P-450 inhibitor more specific than ketoconazole [19] and has exhibited anticancer effects in certain preclinical models [6, 14, 27, 29]. This drug was recently noted to increase endogenous levels of all-*trans* RA [16]. In view of these

This study was supported in part by grant CA-57645 from the National Cancer Institute and by the Bahari Research Fund. One of the authors (J. R. R.) is the recipient of an American Cancer Society Clinical Oncology Career Development Award

Correspondence to: Raymond P. Warrell, Jr., M. D., Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021, USA

activities, we undertook this phase I study to assess whether liarozole could modulate the pharmacokinetics of all-*trans* RA in patients receiving the latter drug on a continuous basis.

Patients and methods

Patients and treatment plan. Patients with solid tumors comprised the group studied. All patients were extensively queried regarding their smoking and alcohol histories, diet, and ingestion of prescription and nonprescription medications. All-*trans* RA was given twice daily at a total dose of 90 mg/m^2 on days 3–27. On days 1, 2, 28, and 29, patients received only a single dose of all-*trans* RA (45 mg/m^2). On days 2 and 29, patients also received liarozole at one of three dose levels (75, 150, and 300 mg, respectively) given after an overnight fast as a single oral dose 1 h prior to all-*trans* RA. After day 29, patients who had stable disease were continued on all-*trans* RA until progression of disease was noted. Signed informed consent was obtained and the study was approved in advance by this center's institutional review board.

Pharmacokinetics study design. Capsules of all-*trans* RA were taken immediately following ingestion of 250 ml of a liquid formula with defined lipid content (Ensure; 8.8 g fat/250 ml). Heparinized blood samples were collected prior to dosing and hourly for up to 8 h. This sampling was conducted on days 1, 2, 28, and 29. All samples were protected from direct light and transported in amber-colored bags.

Assay methods. Plasma retinoid concentrations were measured using reverse-phase high-performance liquid chromatography (HPLC) as previously described [7, 20, 22, 23]. Standard curves for all-*trans* RA were linear at concentrations ranging from 6 to 200 ng/ml.

Pharmacokinetic calculations and statistical analysis. The area under the plasma concentration x time curve (AUC) over the first 8 h following a single oral dose of all-*trans* RA was calculated according to the trapezoidal method [11]. The confidence intervals and the *P* values for the tests were obtained by a bootstrap resampling method [8]. We generated 1000 bootstrap resamples from the individual relative increases (decreases) in the AUC, and the corresponding empirical distribution was used to obtain the appropriate *P* values for the tests.

Results

Patients' characteristics

A total of 26 patients with advanced cancer were evaluated. In all, 22 patients had non-small-cell lung cancer and had either active disease (20 patients) or completely resected disease with an extremely high risk of recurrence (2 patients). The remaining patients had carcinomas of unknown primary (two patients), adenocystic carcinoma (one patient), or medulloblastoma (one patient). Seven patients were inevaluable for complete pharmacokinetic analysis either because their therapy was discontinued due to progression of disease prior to day 28 (six patients) or because pharmacokinetic data were not available (one patient who completed the 4-week study). Of the remaining 20 evaluable patients, 17 had previously used tobacco; however, only 1 had smoked within the preceding 6 months. Ethanol consumption was infrequent; only 4 of 20 patients reported ingesting 1 or more drinks per day. In all, 6 of 20 patients were taking a multivitamin preparation and 6 required daily narcotic analgesics. Altogether, 16 patients had received prior anticancer chemotherapy; the median time from the last cytotoxic treatment was 129 days (range, 21–793 days). Two patients with resected stage IV disease were treated and remained without evidence of disease at 8+ and 10+ months after surgery, respectively.

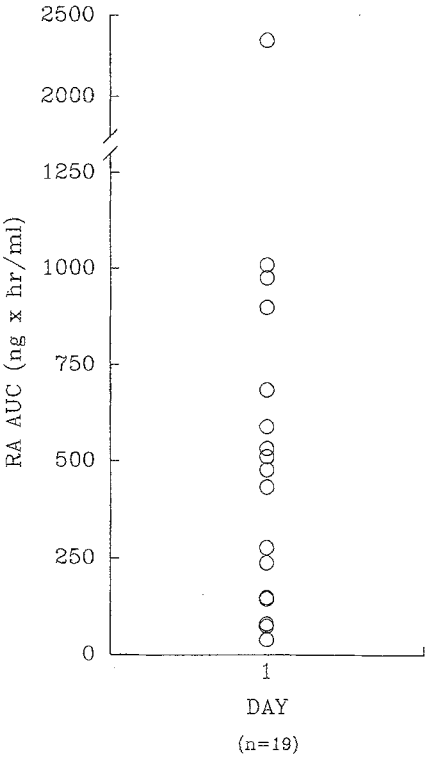

Fig. 1. Plot of individual data points for AUCs after oral administration of all-*trans* RA on the 1st treatment day

No antitumor responses were observed. Of the 18 patients with active cancer, the median time to disease progression was 9.5 weeks (range, 4–28+ weeks). Three patients have continued all-*trans* RA treatment for periods of 6+, 8+, and 8+ months, respectively.

Effects of continuous all-trans RA dosing

Oral administration of all-*trans* RA was characterized by considerable interpatient variability in plasma drug concentrations on day 1 (Fig. 1). With continuous all-*trans* RA treatment, however, the mean plasma AUC for all patients as a group decreased significantly by day 28, similar to that previously described in patients with APL [22]. Continuous administration of all-*trans* RA reduced the mean day-28 AUC by 74%, from the initial day-1 mean value of 504 ± 126 ng h^{-1}/ml^{-1} to 132 ± 28 ng h^{-1} ml^{-1} ($P = 0.05$).

Effects of liarozole

The effects of a single dose of liarozole given prior to all-*trans* RA dosing on days 2 and 29 are depicted in Fig. 2. Pretreatment with any dose of liarozole on day 2 had no

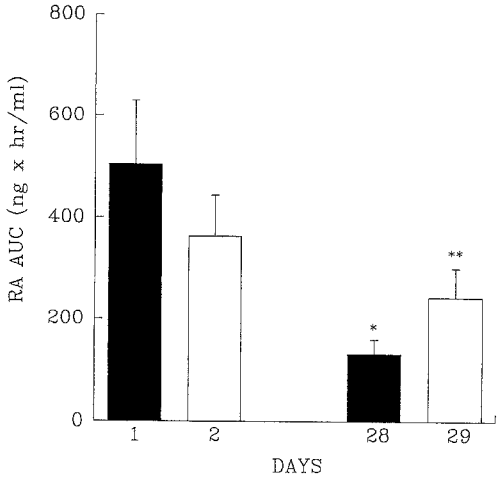

Fig. 2. Effect of continuous all-*trans* RA administration on the AUC (mean ± SEM) in 19 patients. Continuous oral dosing significantly reduced the AUC for all-*trans* RA between day 1 (■) and day 28 (■). Pretreatment with liarozole (□) significantly increased the day-29 AUC for all-*trans* RA as compared with the day-28 value. * $P = 0.05$; ** $P = 0.004$

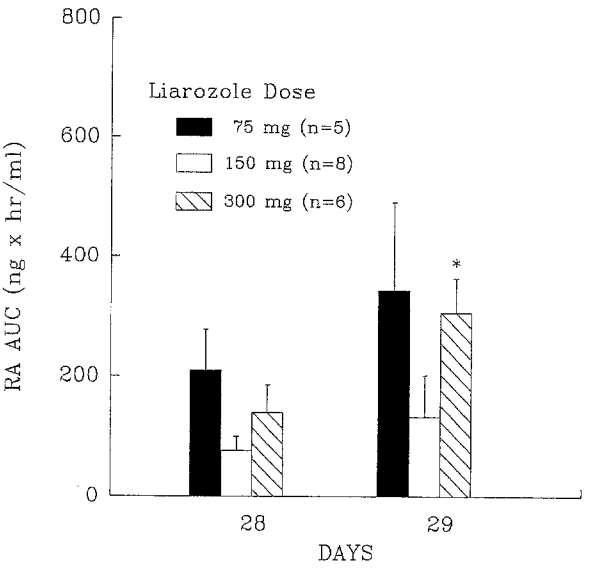

Fig. 3. Dose-response relationship between the day-28 and day-29 all-*trans* RA AUC values (mean ± SEM) for three different doses (75, 150, and 300 mg) of liarozole. Pretreatment with 300 mg of liarozole significantly increased the day-29 AUC for all-*trans* RA. * $P = 0.002$

significant effect on the mean all-*trans* RA AUC for the entire group of patients (363 ng h^{-1} ml^{-1}) as compared with the day-1 value (504 ng h^{-1}/ml^{-1}). There was no difference in liarozole's effect on the day-2 plasma all-*trans* RA AUC relative to the baseline value on day 1. Between day 28 and day 29, liarozole increased the mean plasma AUC for all patients by 84%, from 132±28 ng h^{-1} ml^{-1} on day 28 to 243±57 ng h^{-1} ml^{-1} on day 29 ($P = 0.004$). The effects of three different doses of liarozole (75, 150, and 300 mg) on the day-29 all-*trans* RA AUC are presented in Fig. 3. A single dose of 300 mg of liarozole increased the day-29 AUC in 6 patients by 118% ($P = 0.002$), whereas 150 mg of liarozole increased this value in 8 patients by 73% ($P = 0.60$) and 75 mg of liarozole increased it in 5 patients by 63% ($P = 0.23$).

Adverse reactions

A total of 20 patients completed the 4-week study. Of the six patients who did not complete the study, two died of progressive cancer without evidence of drug toxicity. Among the other four individuals who were taken off study, one patient (a 17-year-old boy with medulloblastoma) developed signs of increased intracranial pressure 1 week after commencing treatment. Although this event was believed to be related to disease progression, a drug-related effect could not be definitively excluded. Headache occurred commonly during the first several days of therapy but resolved despite continued therapy. Most patients experienced cheilitis and dry skin. In some patients, more severe cutaneous reactions occurred as pruritic rashes or scrotal excoriations. Complaints of ear congestion were relatively frequent and resolved upon discontinuation of therapy. Hypertriglyceridemia was common; several patients had triglyceride elevations of >1000 mg/dl. Mild hypercalcemia (not requiring treatment) that reversed with cessation of therapy was observed in several patients. Minor elevations in serum transaminase and alkaline phosphatase levels were also observed, but hyperbilirubinemia did not occur. Five patients required reductions in the dose of all-*trans* RA due to adverse reactions including four cases of dose reduction for mucocutaneous toxicity. The fifth patient, a vegetarian, developed diarrhea that was temporally related to the initiation of RA therapy and improved with reintroduction of dietary fat and concomitant dose reduction. No side effect specifically related to liarozole was observed.

Discussion

All-*trans* RA has engendered considerable clinical interest owing to both its striking activity in APL and its unique effects on cellular differentiation [4, 5, 10, 13, 15, 25, 28]. Recent studies have shown that the clinical pharmacology of all-*trans* RA is markedly different from that of other retinoids, particularly its isomer 13-*cis* RA [2, 3, 12, 16]. After oral therapy, peak plasma concentrations of all-*trans* RA are highly variable, but the plasma half-life is short (about 40 min) and its catabolism is inducible [22, 23]. Our study corroborated these findings and sought to explore the mechanisms of this inducibility and its potential reversal by pretreatment with liarozole.

Ketoconazole, an antifungal agent that inhibits several cytochrome P-450 enzymes, has previously been shown to modulate inducible catabolism of all-*trans* RA [24]. We found that a ketoconazole dose of 400 mg given 1 h before a dose of all-*trans* RA could reliably attenuate this effect. The mechanism of ketoconazole's action in this setting may

be multifactorial and includes inhibition of cytochrome P-450 oxidases responsible for all-*trans* RA catabolism as well as inhibition of cellular lipoxygenases that may act as oxidative cofactors in retinoid breakdown [21]. However, this dose of ketoconazole given chronically can cause nausea and has been associated with hepatotoxicity, hemolytic anemia, leukopenia, thrombocytopenia, and gynecomastia. Ketoconazole has modest activity in patients with advanced prostate cancer, but the usual dose required (400 mg three times per day) has caused adrenal insufficiency.

Liarozole is a novel imidazole derivative that may act as a more specific inhibitor of cytochrome P-450 oxidases. This agent has activity as a single agent in hormone-refractory prostate cancer and in a human breast-cancer cell line (MCF-7) [19, 29]. In a recent phase I study, liarozole exhibited a side-effect profile similar to that of retinoids, including xerosis, rash, nausea and vomiting, and abnormalities in liver-function tests [17]. In clinical trials with prostate cancer, the drug was found to have activity without decreasing adrenal androgen synthesis and with little evidence of a blunted response to adrenocorticotropic hormone (ACTH) stimulation [19]. Liarozole as a single agent has also been shown to increase the plasma concentrations of endogenous all-*trans* RA [6]. These data suggest that liarozole might be more useful than ketoconazole for protracted use in combination therapy.

In the current study, liarozole was incapable of reliably raising plasma AUCs of all-*trans* RA at doses of 75 and 150 mg. However, at the clinically relevant dose of 300 mg, the drug appeared successful in reconstituting a pharmacokinetic profile similar that observed on day 1. Data from a dose-ranging study have also suggested that this oral dose is maximally tolerable [17]. It is possible that the variable response seen with the lower doses of liarozole was related to the relatively small number of patients studied at each of these levels, especially in view of the intrinsic heterogeneity of all-*trans* RA metabolism. In addition, we did not evaluate the effects of liarozole on altering intratumoral levels of all-*trans* RA. In a murine mammary tumor model, pretreatment with liarozole consistently elevated tumor all-*trans* RA concentrations without significantly elevating plasma concentrations [9]. The magnitude of increase in intratumoral all-*trans* RA concentration observed was comparable with that achievable with normal oral all-*trans* RA dosing. Thus, the question as to whether lower doses of liarozole might provide for a more selective all-*trans* RA effect and, perhaps, reduced toxicity should be addressed in future studies. Nevertheless, the liarozole dose we selected (300 mg) has appeared safe and effective for clinical use in the present combination. Conceivably, extended use of the combination may enable a better clinical test of the antitumor effects of all-*trans* RA in diseases other than APL and will perhaps be useful in reversing acquired resistance to this retinoid.

Acknowledgements. The authors thank Hermie Tizon for assistance with the pharmacokinetics studies. All-*trans* RA was kindly supplied by Hoffmann-LaRoche, Nutley, New Jersey. Liarozole was supplied by the Janssen Research Foundation, Piscataway, New Jersey.

References

1. Adamson PC, Boylan JF, Balis FM, et al (1992) Time course of induction of metabolism of all-*trans*-retinoic acid and the up-regulation of cellular retinoic acid-binding protein. Cancer Res 53: 472
2. Brazzell RK, Colburn WA (1982) Pharmacokinetics of the retinoids isotretinoin and etretinate: a comparative review. J Am Acad Dermatol 6: 643
3. Brazzell RK, Vane FM, Ehmann CW, et al (1983) Pharmacology of isotretinoin during repetitive dosing to patients. Eur J Clin Pharmacol 24: 695
4. Castaigne S, Chomienne C, Daniel MT (1990) All-*trans* retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. Blood 76: 1704
5. Chen ZX, Xue YQ, Zhang RI, et al (1991) A clinical and experimental study on all-*trans* retinoic acid-treated acute promyelocytic leukemia patients. Blood 78: 1413
6. DeCoster R, Van Ginckel R, Smets R (1993) Antitumoral, biochemical and histological effects of liarozole fumarate on androgen-dependent and -independent Dunning prostate carcinomas. Proc Am Assoc Cancer Res 34: 109
7. Eckhoff C, Nau H (1990) Identification and quantitation of all-*trans*- and 13-*cis*-retinoic acid and 13-*cis*-4-oxoretinoic acid in human plasma. J Lipid Res 26: 1445
8. Efron B (1982) The jacknife, the bootstrap, and other resampling plans. Soc Indus Appl Math 31–33: 54
9. End DW, Garrabrant TA (1993) Effect of liarozole fumarate (R 85246) on the levels of endogenous retinoic acid in murine solid tumors: comparison to exogenous retinoic acid. Proc Am Assoc Cancer Res 34: 108
10. Frankel SR, Eardley A, Heller G, et al (1994) All-*trans* retinoic acid for treatment of acute promyelocytic leukemia: results of the New York study. Ann Intern Med
11. Gibaldi M, Perrier D (1982) Pharmacokinetics, 2nd edn. Marcel Dekker, New York, p 480
12. Goodman GE, Einspahr JG, Alberts DS, et al (1982) Pharmacokinetics of 13-*cis*-retinoic acid in patients with advanced cancer. Cancer Res 42: 2087
13. Huang ME, Ye YC, Chai JR (1988) Use of all-*trans* retinoic acid in the treatment of acute promyelocytic leukemia. Blood 72: 567
14. Janssen Research Foundation (1991) Investigator's brochure: R 85246. Janssen Research Foundation, Piscataway, New Jersey, p 31
15. Jetten AM, Nervi C, Vollberg TM (1992) Control of squamous differentiation in tracheobronchial and epidermal epithelial cells: role of retinoids. J Natl Cancer Inst Monogr 13: 93
16. Kerr IG, Lippman ME, Jenkins J, et al (1982) Pharmacology of 13-*cis*-retinoic acid in humans. Cancer Res 42: 2069
17. Kreis W, Budman DR, Seidmon EJ, et al (1993) Phase I study of liarozole (R75,251) in primary hormonal failure patients with prostate cancer: a multicenter study. Proc Am Assoc Cancer Res 34: 1205
18. Leo MA, Iidi S, Lieber CS (1984) Retinoic acid metabolism by a system reconstituted with cytochrome P-450. Arch Biochem Biophys 234: 305
19. Mahler C, Verhelst J, Denis L (1993) Ketoconazole and liarozole in the treatment of advanced prostatic cancer. Cancer [Suppl] 71: 1068
20. McPhillips MD, Kalin JR, Hill DL (1987) The pharmacokinetics of all-*trans* retinoic acid and *N*-(2-hydroxyethyl)retinamide in mice as determined with a sensitive and convenient procedure. Drug Metab Dispos 15: 207
21. Muindi JF, Young CW (1993) Lipid hydroperoxides greatly increase the rate of oxidative catabolism of all-*trans* retinoic acid by human cell culture microsomes genetically enriched in specified cytochrome P-450 isoforms. Cancer Res 53: 1226
22. Muindi JRF, Frankel S, Miller WH Jr, et al (1992) Continuous treatment with all-*trans* retinoic acid causes a progressive reduction in plasma drug concentrations: implications for relapse and

retinoid "resistance" in patients with acute promyelocytic leukemia. Blood 79: 299
23. Muindi JRF, Frankel SR, Huselton C, et al (1992) Clinical pharmacology of oral all-*trans*-retinoic acid in patients with acute promyelocytic leukemia. Cancer Res 52: 2138
24. Rigas JR, Francis PA, Muindi JRF, et al (1994) Constitutive variability in the pharmacokinetics of the natural retinoid, all-*trans* retinoic acid, and its modulation by ketoconazole. J Natl Cancer Inst 85: 1921
25. Roberts AB, Sporn MB (1984) Cellular biology and biochemistry of the retinoids. In: Sporn MB, Robert AB, Goodman DS (eds) The retinoids, 2nd edn. Academic Press, New York, p 209
26. Roberts AB, Lamb LC, Sporn MB (1980) Metabolism of all-*trans*-retinoic acid hamster liver microsomes: oxidation of 4-hydroxy- to 4-keto-retinoic acid. Arch Biochem Biophys 199: 374
27. Stearns ME, Fudge K, End D (1993) Antitumoral effects of liarozole fumarate (R85246) on human prostatic PC-3 ML bone tumors in SCID mice. Proc Am Assoc Cancer Res 24: 2255
28. Warrell RP Jr, Frankel SR, Miller WH Jr, et al (1991) Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-*trans* retinoic acid). N Engl J Med 324: 1385
29. Wouters W, Dun J van, et al (1992) Effects of liarozole, a new antitumoral compound, on retinoic acid-induced inhibition of cell growth and on retinoic acid metabolism in MCF-7 human breast cancer cells. Cancer Res 52: 2841