Elevated Plasma Lipid Peroxide Content Correlates with Rapid Plasma Clearance of All-trans-Retinoic Acid in Patients with Advanced Cancer

Josephia F. Muindi,2 Howard I. Scher, James R. Rigas, Raymond P. Warrell, Jr., and Charles W. Young

Developmental Chemotherapy Service [J. F. M., R. P. W., C. W. Y.], Genitourinary Oncology Service [H. I. S.], Thoracic Oncology Service (J. R. R.), Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York, 10021

ABSTRACT

The addition of lipid hydroperoxides greatly accelerates the rate of oxidative catabolism of all-trans-retinoic acid (RA) in human cell microsomes; hence, hydroperoxides of the arachidonate cascade are particularly active in the microsomal system. We have measured the plasma content of lipid peroxides in cancer patients during the course of therapy with RA, seeking to assess whether a correlation exists between the rate of oxidative catabolism of exogenously administered RA and whole body lipid peroxide levels. The assay used for plasma lipid peroxides is the capacity to react with thiobarbituric acid under specified conditions; the result is expressed as TBARS (thiobarbituric acid reactive substances). RA administration produced its own accelerated clearance RA within 72 h. Patients were considered to have "normal" or "rapid" baseline catabolism of RA if their Day 1 area under RA concentration over time curve was greater or less than 300 ng·h/mL, respectively. The mean plasma TBARS levels were: 12 normal volunteers = 0.14 ± 0.05 µM; 19 "normal" RA catabolizers = 0.27 ± 0.12 µM; and 14 "rapid" catabolizers = 0.82 ± 0.24 µM. P = 0.0008 (rapid catabolizers versus normal volunteers) and 0.05 (rapid catabolizers versus normal catabolizers). Repeat TBARS determinations were made during the course of therapy in 17 patients, all of whom had been converted to "rapid" RA catabolism on therapy. An increase in plasma TBARS levels ≥20% of baseline was observed in 5 of 5 prostate cancer patients and 8 of 12 lung cancer patients treated with continuous RA therapy for 2 and 4 weeks, respectively. These observations support the hypothesis that high levels of lipid peroxides and rapid oxidative catabolism of RA are positively correlated.

INTRODUCTION

The clinical pharmacology of p.o. administered RA3 in APL patients is characterized by a progressive decline in plasma AUCRA with continued daily ingestion of the drug (1, 2); a similar decrease in plasma concentrations of RA with long-term administration of the drug has been reported in pediatric cancer patients (3). These pharmacological observations are of clinical interest because low plasma RA levels in APL patients have been associated with the development of clinical retinoid resistance (2). The progressive decrease in AUCRA during RA therapy appears to be due to accelerated plasma clearance of the drug, deriving from an increase in oxidative catabolism that is produced by exposure to this physiologic retinoid; this catabolic autoinduction response to RA exposure has been demonstrated repeatedly in multiple animal species and cells in culture (4–7). Oxidative catabolism of RA is generally thought to be cytochrome P-450 mediated, although the participation of other fatty acid oxidizing enzyme systems has not been excluded (4, 8). Ketoconazole, a known broad inhibitor of cytochrome P-450 enzyme systems and cellular lipoxygenases, inhibits the metabolism of RA in rats and in cell culture systems (9–11). In like manner, our group has observed that coadministration of ketoconazole increased the observed AUCRA values in lung cancer patients in whom the AUCRA had decreased from baseline values following RA therapy (12). These studies in patients with advanced non-small cell lung cancer also demonstrated much greater interpatient variation in the Day-1 plasma AUCRA than had been observed in patients with APL or prostate cancer. The data in lung cancer patients suggested the existence of two groups of exogenous-RA-naive patients with differing rates of constitutive RA catabolism; hereafter, these two groups will be referred to respectively as "rapid" or "normal" catabolizers. The biochemical basis for RA-induced accelerated oxidative catabolism of RA has not been established; however, several hypotheses have been offered. These have included an increased synthesis of a particular P-450 isof orm with a high affinity for the retinoid (13), or increased synthesis of cofactors which increase the oxidative catabolism of RA. Both cytoplasmic retinoic acid binding protein and lipid hydroperoxides can enhance the oxidation of RA in cell free systems (13–17).

The present communication presents the results of studies examining the possible relevance of whole body lipid peroxide metabolism to rates of oxidative clearance of RA in cancer patients. We have measured the lipid peroxide content of a series of frozen-stored patient plasma samples which had been obtained during the course of several pharmacology studies of RA and correlated the baseline lipid peroxide levels with these patients' respective Day 1 AUCRA results. We have quantitated plasma lipid peroxide concentrations by measuring the concentration of TBARS in plasma (18). This technique is among those used widely in studies of oxidative stress in a variety of populations (19–21). Although the methods used do not establish the nature of the lipid peroxide present in the plasma of these particular individuals, human plasma has been shown to contain measurable quantities of arachidonic acid-derived lipid hydroperoxides generated by cellular lipoxygenases (22).

MATERIALS AND METHODS

Patients and Treatment Protocol. All patients had documented advanced malignancies. The study population consisted of 24 patients with NSCLC, 7 patients with prostate cancer, 1 patient with APL, and 1 patient with plasma cell myeloma. Plasma pharmacokinetic studies were performed on Day-1 following a single p.o. dose of 45 mg/m2 of RA, except for prostate cancer patients who received 50 mg/m2. Plasma samples were collected prior to drug administration and at hourly intervals for 8 h following drug ingestion. All samples were protected from direct light and were stored at −70°C until assay. Plasma samples from 12 normal volunteers were used to establish basal TBARS levels. All samples were assayed within 4 weeks of collection. The available samples also permitted analysis of the effect on plasma TBARS of 2 or 4 weeks of continuous RA therapy; the 2-week and 4-week comparisons were available from pharmacokinetic studies in prostate and lung cancer patients, respectively.

Chemicals. 2-Thiobarbituric acid was obtained from Eastman Kodak Co. (Rochester, NY) while malonaldehyde bis(dimethyl acetal) was from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were obtained from either Fisher Scientific (Fairlawn, NJ) or from T.J. Baker Chemical (Phillipsburg, NJ), and were of the highest grade available.
Assay of Plasma Retinoids. In patients receiving RA therapy plasma retinoids were measured using reverse phase high performance liquid chromatography, previously described (1, 2). The lower limit of detection for this assay is 10 ng/ml.

Assay of Plasma Lipid Peroxides. Plasma lipid peroxides were measured as malondialdehyde using thiobarbituric acid as previously described (18). Briefly, 0.5 ml of plasma was mixed with 1 ml of TBA/TCA/HCl reagent. TBA/TCA/HCl reagent consisted of 15% w/v trichloroacetic acid, 0.375% w/v thiobarbituric acid, and 0.25 N hydrochloric acid. The assay mixture was heated in boiling water for 20 min and then microcentrifuged for 3 min. The absorbance of thiobarbituric acid reactive substance in the clear supernatant was measured at 532 nm using a LKB spectrophotometer from Pharmacia.

A standard curve for quantitation of TBARS was obtained by reacting 0—10 μM of malonaldehyde bis(dimethyl)acetel in 0.5 ml of 0.25 N HCl with TCA/TBA/HCl reagent under similar conditions to those used for the plasma. The results are expressed as μM of TBARS.

Data Analysis. The area under the concentration X time curve over the first 8 h following a single p.o. dose of RA was calculated by the trapezoid approximation method using a PHARMIPCS computer program (23). The same computer program was used for all other statistical calculations.

RESULTS

A progressive decrease in plasma AUC RA has been observed in APL, NSCLC, and plasma cell myeloma patients on continuous p.o. RA therapy. The biochemical basis of these observations is thought to reside in self-induced accelerated RA catabolism. The time course for the onset of accelerated RA catabolism in these patients has, however, not previously been fully documented. Studies in three prostate cancer patients show that self-induced RA catabolism is evident within 72 h of the start of therapy, and may be decreased by 80—90% by Day 7 (Fig. 1).

To study possible relationships between baseline AUC RA and plasma lipid peroxides, we first examined correlations between the cancer patients in the study and plasma obtained from normal volunteers. The mean basal plasma TBARS concentrations for 33 cancer patients and 12 normal volunteers were 0.50 ± 0.60 μM and 0.14 ± 0.11 μM, respectively (P > 0.05). Accordingly, although cancer patients tended to have higher plasma TBARS than did the normal volunteers, supporting a prior report of elevated TBARS levels in cancer patients (21), when the results of all cancer patients versus normal volunteers were compared, the difference was not statistically significant. We then examined these data in relation to the "normal" and "rapid" constitutive RA clearance patterns manifested by the patients, as discussed previously. We have characterized cancer patients receiving exogenous RA as "normal" and "rapid" metabolizers depending on whether their Day-1 AUC RA was greater or less than 300 ng·hr/ml, respectively. The breakpoint of ≤300 ng·hr/ml was chosen because it was unusually low for the APL population (including only 3 of 20 patients) but was a frequent occurrence in patients with advanced NSCL cancer (15 of 31 patients) (12). Furthermore, it is equivalent (over the 8-h time period of the study) to the 1 μM RA concentration routinely used to differentiate cancer cells in vitro.

The pretreatment plasma TBARS concentrations of patients exhibiting normal and rapid RA metabolism, respectively, and normal volunteers are compared in Fig. 2. The mean baseline plasma TBARS level of cancer patients classified as normal metabolizers was 0.25 ± 0.22 μM; this is not statistically different from the mean TBARS level of normal volunteers; however, the 0.82 ± 0.79 μM value for the rapid metabolizers was significantly elevated both in comparison to the volunteer group (P = 0.008) and the "normal" RA metabolizer group (P = 0.05). In Fig. 3 the Day-1 AUC RA levels are presented for the cancer patients that have grouped according to baseline TBARS levels equal to or greater than 0.4 μM; that value is the highest TBARS concentration observed in the normal volunteer plasmas. The inverse relationship between baseline plasma TBARS levels and Day-1 AUC RA is again evident.

To further document the role of lipid peroxides in the oxidative metabolism of RA, we have investigated changes in plasma lipid peroxides occurring in patients on continuous RA therapy. An increase in plasma lipid peroxides in excess of 20% was observed in 5 of 5 prostate cancer patients and 8 of 12 lung cancer patients treated with continuous RA therapy for 2 and 4 weeks, respectively (Fig. 4, A and B).

DISCUSSION

The present observations, taken together with previous reports (1, 2), suggest that patients exhibiting normal RA metabolism become rapid metabolizers within 24—48 h of the start of RA therapy. Ac-
We have previously demonstrated that the addition of physiological lipid hydroperoxides increased the RA-oxidative velocity of human cell microsome preparations by 10–30-fold (13). The most potent lipid hydroperoxides in this system were metabolites of arachidonic acid and linoleic acid that are generated physiologically by cellular lipoxygenases. Based on these findings, we suggested that RA administration might accelerate retinoid oxidation by increasing tissue levels of lipid hydroperoxides, producing in vivo the effect that had been demonstrated in the microsomal model. According to this hypothesis the increase could result from a retinoid-induced activation of the arachidonic acid cascade. This general mechanism might be applicable to hypercatabolism of RA occurring in the absence of prior exposure to exogenous retinoids because the arachidonate cascade can be activated by a broad variety of stimuli, including many that are associated with carcinogenic events (26, 27). It is an additional facet of this proposed mechanism that the actual oxidation step could involve either enzymatic or metal-catalyzed nonenzymatic reactions (13). The nonenzymatic metal-catalyzed RA-oxidation by lipid peroxide in particular could be instantaneous, not requiring induction of additional enzymes beyond those of the AA cascade. Although there are as yet no reported observations on the capacity of exogenously administered retinoic acid to increase the activity of the arachidonate cascade in intact animals or patients, RA exposure in vitro induces both prostaglandin synthase (28) and 5-lipoxygenase activities in HL60 cells in culture.4

Although this study was a retrospective one and the total number of patients examined is modest, the fact that we do observe elevated basal plasma TBARS concentrations in those cancer patients who exhibited low plasma AUC_{RA} levels is supportive of the hypothesis that increased tissue lipid peroxides could contribute to the rapid RA oxidation catabolism by enzymatic (cytochrome P450) and nonenzymatic (metal-mediated) reactions. This conclusion is reinforced by the observed increase in TBARS concentration in plasmas of patients examined sequentially while on continuous RA therapy. Plasma and urinary content of TBARS has been used extensively as an indicator of total body lipid peroxides and, by inference, of oxidative stress (18–22). In this study the assay provided a useful means of testing the lipid peroxide–RA hypercatabolism hypothesis in a quantitative fashion without specifying the lipid species involved; future studies can examine the effects of RA administration on the levels of various eicosanoid metabolites in a prospective manner.

REFERENCES

4 J. Muindi and C. Young, unpublished observations.

Fig. 3. Day 1 AUC_{RA} levels in cancer patients grouped according to baseline TBARS levels less than or greater than 0.4 μM.

Fig. 4. Effect of continuous RA therapy on plasma TBARS content at: (A) 2 weeks (prostate cancer) or (B) 4 weeks (non-small cell lung cancer) of therapy.

Accordingly, the onset of accelerated RA oxidative catabolism is shorter than the 3–7 days required to induce isoforms of cytochrome P450 enzymes in experimental animals with drugs such as phenobarbital (24, 25). Furthermore, our published in vitro studies with human cell microsomes failed to demonstrate a correlation between the degree of cytochrome P450 enrichment and rates of RA oxidative catabolism (13). These observations suggest that other biochemical mechanisms, in addition to cytochrome P450 enzyme induction, may be involved in accelerated RA oxidation in cancer patients.
PLASMA LIPID PEROXIDES AND RETINOIC ACID PHARMACOKINETICS

Elevated Plasma Lipid Peroxide Content Correlates with Rapid Plasma Clearance of All- trans-Retinoic Acid in Patients with Advanced Cancer


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/8/2125

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.