British Journal of Pharmacology (2000) 131, 851–857

www.nature.com/bjp

Arachidonic acid-mediated cooxidation of all-*trans*-retinoic acid in microsomal fractions from human liver

[1,2]Louise Nadin & *,[1]Michael Murray

[1]School of Physiology and Pharmacology, University of New South Wales, Sydney, NSW 2052, Australia and [2]Storr Liver Unit, Department of Medicine, University of Sydney, Westmead Hospital, Westmead, NSW 2145, Australia

1 The quantitative importance of prostaglandin H synthase (PGHS)-mediated cooxidation of all-*trans*-retinoic acid (ATRA) was evaluated in human liver microsomes ($n=17$) in relation to CYP-dependent ATRA 4-hydroxylation.

2 Observed rates of ATRA cooxidation (4.6–20 pmol mg protein^{-1} min^{-1}) and 4-hydroxylation (8.7–45 pmol mg protein^{-1} min^{-1}) were quantitatively similar and exhibited similar individual variation (4 and 5 fold, respectively).

3 From kinetic studies cooxidation was an efficient process in human hepatic microsomes (V_{max} $K_m^{-1}=0.25$) compared with NADPH- and NADH-mediated 4-hydroxylation by CYP (V_{max} $K_m^{-1}=0.14$ and 0.02, respectively).

4 The capacity of lipid hydroperoxide metabolites of arachidonic acid to mediate ATRA oxidation was established directly, but downstream products (D, E, F and I-series prostaglandins) were inactive.

5 cDNA-expressed CYPs supported ATRA oxidation by lipid hydroperoxides. Whereas CYPs 2C8, 2C9 and 3A4, but not CYPs 1A2 or 2E1, were effective catalysts of the NADPH-mediated reaction, cooxidation supported by 15(S)-hydroperoxyeicosatetraenoic acid was mediated by all five CYPs. The cooxidation reaction in human hepatic microsomes was inhibited by the CYP inhibitor miconazole.

6 These findings indicate that ATRA oxidation is quantitatively significant in human liver. Lipid hydroperoxides generated by intracellular enzymes such as prostaglandin synthase and lipoxygenases are sources of activated oxygen for CYP-mediated deactivation of ATRA to polar products.
British Journal of Pharmacology (2000) **131,** 851–857

Keywords: Cytochrome P450; prostaglandin H synthase; all-*trans*-retinoic acid; human hepatic microsomes; lipid hydroperoxides; retinoid cooxidation; retinoid biotransformation

Abbreviations: APL, acute promyelocytic leukaemia; ATRA, all-*trans*-retinoic acid; CRABP, cellular retinoic acid binding protein; CYP, cytochrome P450; HPETE, hydroperoxyeicosatetraenoic acid; HPLC, high performance liquid chromatography; HPODE, hydroperoxy-(*9Z,11E*)-octadecadienoic acid; PGHS, prostaglandin H synthase; *13Z*-RA, 13-*cis*-retinoic acid; RAR, retinoic acid receptor; RXR, 9-*cis*-retinoic acid receptor

Introduction

Retinoids (or vitamin A derivatives) have biologically important roles in the maintenance of normal vision, immunity and cellular differentiation. All-*trans*-retinoic acid (ATRA) is generated intracellularly by the consecutive dehydrogenations of retinol and retinal (Blomhoff *et al*., 1992) and is the major ligand for the retinoic acid receptors (RARs) that form heterodimers with retinoid X-receptors (RXR) to activate target genes. RARs are members of the nuclear hormone receptor superfamily that also includes receptors for vitamin D_3, thyroid hormone and peroxisome proliferators (Mangelsdorf & Evans, 1995).

The carbon atom at the 4-position of naturally occurring retinoids, such as ATRA, is relatively reactive because it is allylic to the 5,6-double bond of the β-ionone (substituted cyclohexenyl) ring system that is fully conjugated with the side chain double bonds (Figure 1). Thus, carbocations and carbon centred radicals that may be formed at the allylic 4-position are readily stabilized by delocalization. Cytochrome P450 (CYP)-mediated hydroxylation of ATRA at the 4-position is a major biotransformation pathway that precedes elimination (Roberts *et al*., 1979; Leo *et al*., 1989). Prostaglandin H synthase (PGHS) has also been shown to catalyze ATRA biotransformation. Lipid hydroperoxides generated by the cyclo-oxygenase activity of PGHS oxidize retinoids to carbon centred radicals. Subsequent addition of molecular oxygen generates peroxyl radicals that mediate the conversion of further ATRA molecules to the corresponding 5,6-epoxide, 5,8-epoxide and related products (Samokyszyn & Marnett, 1987). Thus, ATRA is cooxidized during the reduction of lipid hydroperoxides to the analogous lipid hydroxides. *In vitro* studies in micellar systems comprising haemin and lipid hydroperoxides have produced similar findings (Samokyszyn *et al*., 1997). To date, however, there is little information on the functional interplay of PGHS and CYP in retinoid biotransformation in tissues.

The present study evaluated the contribution of CYP- and PGHS-dependent ATRA oxidation in human hepatic microsomes. The principal finding to emerge was that ATRA cooxidation by lipid hydroperoxides and CYP-dependent ATRA 4-hydroxylation are quantitatively similar in human hepatic microsomes, although the operative pathway is

*Author for correspondence; E-mail: M.Murray@unsw.edu.au

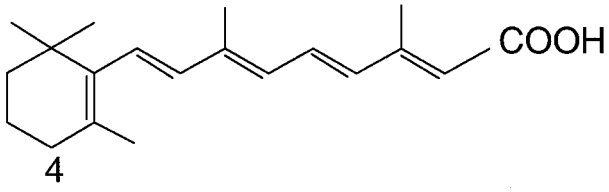

Figure 1 Structure of ATRA; the 4-carbon is indicated.

determined by cofactor availability. The finding that CYP may utilize lipid hydroperoxides generated by enzymes such as PGHS extends the potential role of the mono-oxygenases in retinoid biotransformation. A wider range of CYPs can utilize lipid hydroperoxides in ATRA cooxidation than those that mediate NADPH-dependent 4-hydroxylation. Thus, the relative intracellular availability of NADPH and lipid hydroperoxides determines which CYPs contribute to ATRA biotransformation in human liver.

Methods

Chemicals

[11,13-^{3}H]-ATRA (50–60 Ci mmol^{-1}) was purchased from Amrad Pharmacia Biotech Australia (Melbourne, Vic). Unlabelled ATRA, indomethacin, miconazole, lipid hydroperoxides (5(S)-hydroperoxyeicosatetraenoic acid (5-HPETE), 15(S)-hydroperoxyeicosatetraenoic acid (15-HPETE) and 13(S)-hydroperoxy-(*9Z,11E*)-octadecadienoic acid (13-HPODE)), prostaglandins (D_2, E_2, $F_{2\alpha}$ and I_2) and biochemicals were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Analytical Reagents were from Ajax Chemicals (Auburn, NSW, Australia) and solvents for high performance liquid chromatography (HPLC) were from Rhone Poulenc (Brisbane, QLD, Australia).

Supplies of human liver and preparation of microsomal fractions

Human liver was obtained as the normal margin adjacent to resected hepatic tumours or as the remnants of adult donor livers prepared for orthotopic transplantation of paediatric recipients. Transplantation tissue was obtained through the Australian Liver Transplant Centre (Royal Prince Alfred Hospital, Camperdown, NSW) or the Queensland Liver Transplant Service (Princess Alexandra Hospital, Brisbane). Consent was provided by the donor relatives. Livers were perfused with Viaspan™ solution (NEN-DuPont, Wilmington, DE, U.S.A.) and transported to the laboratory. Samples were frozen on arrival and stored at −70°C until used in the preparation of microsomal fractions.

Liver was thawed and homogenized in potassium phosphate buffer (10 mM, pH 7.4) containing sucrose (0.25 M) and EDTA (1 mM) using a Kinematica polytron (Lucerne, Switzerland). After filtration through nylon gauze (~0.25 mm mesh size), microsomes were isolated by differential ultracentrifugation as described elsewhere (Martini & Murray, 1993). Microsomal protein was estimated by the method of Lowry *et al*. (1951).

Microsomal fractions from human lymphoblastoid cells (AHH-1 TK+/−) that contained cDNA-expressed CYPs 1A2, 2C8, 2C9, 2E1 and 3A4 were obtained from Gentest (Woburn, MA, U.S.A).

Assays of ATRA oxidation in human hepatic microsomes

Microsomal incubations (1.0 ml volume) contained protein (0.25 mg for the NADPH-mediated reaction and 0.2 mg for the arachidonic acid-mediated reaction), [^{3}H]-ATRA (7.5 μM, 1×10^6 d.p.m. incubation^{-1}, except in kinetic experiments where the range was 5–100 μM) in potassium phosphate buffer (0.1 M, pH 7.4). Incubations were run over 45 min for the NADPH- and arachidonic acid-mediated reactions or 5 min for the lipid hydroperoxide-mediated reactions; linearity of product formation was derived in preliminary experiments. Cofactor concentrations were NADPH (1 mM), NADH (1 mM), arachidonic acid (250 μM) or 15-HPETE (30 μM). Inhibitors were added in some experiments in 5 μl dimethylsulphoxide; solvent was added to corresponding controls and did not influence product formation. Reactions were terminated with cold ethanol (0.5 ml containing 125 μg ascorbic acid and 125 μg EDTA). Substrate and products were extracted into ethyl acetate (2 ml) containing 2 mg butylated hydroxyanisole. The organic phase was dried over anhydrous Na_2SO_4 and then removed under N_2.

ATRA oxidation was measured in analogous fashion in microsomes from lymphoblastoid cells that expressed individual CYPs, except that the protein concentration was increased to 2.5 mg ml^{-1} and the incubation time was 60 min. In the case of CYP2C9-containing cellular microsomes, the incubation buffer was Tris-HCl (0.1 M, pH 7.4), whereas the standard phosphate buffer was used with other CYPs. An NADPH-generating system consisting of 3.3 mM glucose 6-phosphate, 0.4 u glucose 6-phosphate dehydrogenase, 1.3 mM NADP and 3.3 mM $MgCl_2$, was used to initiate the NADPH-mediated reactions; 15-HPETE (30 μM) was used in cooxidation reactions.

Separation of ATRA and polar metabolites by HPLC

Following extraction the products of ATRA biotransformation in human hepatic microsomes were separated by reverse phase HPLC, using the procedure of Kochhar *et al*. (1988). Samples were dissolved in methanol (50 μl) and applied to an Ultrasphere C18 column (5 μm, 250 mm × 4.6 mm, Beckman, San Ramon, CA, U.S.A.) attached to a Waters Associates HPLC system (including model 510 pump, model 712 WISP and model 490E programmable detector). The mobile phase consisted of methanol:acetonitrile:0.1 M ammonium acetate, pH 6.8 (73 : 12 : 15). The flow rate was 1 ml min^{-1} and fractions were collected at intervals of 60 s (in NADPH- and NADH-mediated reactions) or 20 s (in arachidonic acid- or 15-HPETE-mediated reactions). ACS II scintillant (Amersham, Australia) was added to eluate fractions and the samples were subjected to β-counting. Elution times for 4-hydroxy ATRA and ATRA in this system were 205 and 600 s, respectively.

Statistics

Unless stated otherwise, data are presented as mean ± s.e.mean of estimates in microsomal fractions from three individual livers. Differences between means of control and treatment groups were detected by the Student's *t*-test. Linear regression analysis was used to correlate microsomal activities.

Results

Arachidonic acid-mediated ATRA cooxidation in human hepatic microsomes

ATRA oxidation was measured in microsomal fractions isolated from 17 individual human liver segments. Information on the recent drug, smoking and alcohol intake histories of the donors is indicated in Table 1; some of these have been reported previously (Sutton *et al*., 1997). Five of the donors were cigarette smokers, three regularly ingested alcohol and several had received drugs, such as dexamethasone, known to modulate CYP function. Whereas the NADPH-dependent metabolism of ATRA yielded only the 4-hydroxy product, the arachidonic acid- and 15-HPETE-mediated reactions yielded several products. Because the relative formation of these cooxidation products varied considerably between individual microsomal fractions, total ATRA oxidation was measured in this study.

Rates of microsomal ATRA cooxidation varied over an approximate 4 fold range (4.6–20 pmol mg protein^{-1} min^{-1}, $n=17$; Figure 2A). By comparison, NADPH-mediated ATRA 4-hydroxylation varied over a 5 fold range (8.7–45 pmol mg protein^{-1} min^{-1}; $n=17$; Figure 2A). Although the variation between subjects was similar there was also no apparent relationship between the pathways of ATRA biotransformation ($r=0.156$; Figure 2B). Exposure of individuals to drugs or chemicals did not appear to influence the observed rates of microsomal ATRA oxidation supported by either NADPH or arachidonic acid.

Arachidonic acid emerged as an alternate cofactor to NADPH for microsomal ATRA biotransformation in human liver. Kinetic studies indicated that the Michaelis constant for the cofactor was 32 ± 6 mM (Figure 3; Table 2). By comparison, the affinities of the reduced pyridine nucleotides NADPH and NADH for enzymes of ATRA biotransformation were lower ($K_m=470\pm10$ and 970 ± 80 μM, respectively; Table 2). V_{max} values for the cofactors varied about 8 fold and the K_m V_{max}^{-1} ratios, a measure of catalytic efficiency, were 0.25, 0.14 and 0.014 for arachidonic acid, NADPH and NADH, respectively. Thus, the cooxidation pathway supported by arachidonic acid was about twice as efficient as the better-described CYP-mediated reaction supported by NADPH.

Role of lipid hydroperoxides in ATRA cooxidation

In the absence of exogenous reduced pyridine nucleotide cofactors, arachidonic acid effectively supported the biotransformation of ATRA to polar products. ATRA cooxidation by a range of endogenous eicosanoid metabolites was evaluated in further studies. As indicated in Figure 4, 5-HPETE and 15-HPETE supported ATRA oxidation (77 and 226 pmol products mg protein^{-1} min^{-1}, respectively). In contrast, the downstream products of the action of cyclo-oxygenase on arachidonic acid, prostaglandins D_2, E_2, $F_{2\alpha}$ and I_2 (prostacyclin), were inactive. 13-HPODE, an analogue of the HPETEs formed by the action of lipoxygenase on (*9Z,11E*)-octadecadienoic acid, also supported ATRA oxidation (114 pmol products mg protein^{-1} min^{-1}). Thus, lipid hydroperoxides formed by the action of cytosolic lipoxygenase enzymes on arachidonic acid and related fatty acids, are able to generate polar products from ATRA.

The possibility that cyclo-oxygenase-mediated lipid peroxide intermediates participate in microsomal ATRA cooxidation was tested. The cyclo-oxygenase inhibitor indomethacin (500 μM) decreased microsomal ATRA cooxidation to 42 ± 16% of the uninhibited activity ($n=3$), whereas the peroxidase inhibitor methimazole (500 μM) was inactive (not shown).

The nature of hydroperoxide-mediated ATRA oxidation was characterized further with 15-HPETE. In microsomal fractions from 10 individual human livers 15-HPETE-mediated ATRA oxidation was extensive and varied over a 4 fold range (450–1800 pmol mg protein^{-1} min^{-1}; Figure 5A). The Michaelis constant for the reaction in human liver microsomes (24 μM; Figure 5B) was similar to that for the NADPH-dependent reaction mediated by CYP (10 μM). The possibility that CYP may participate in this pathway was evaluated using cDNA-expressed human CYPs in lymphoblastoid cell microsomes. Thus, CYPs 2C8, 3A4 and 2C9 were most active in 15-HPETE-mediated ATRA oxidation, but CYPs 1A2 and 2E1 also generated significant quantities of polar products (Table 3). By comparison, CYPs 2C8, 3A4 and 2C9 were major catalysts of NADPH-mediated ATRA 4-hydroxylation, whereas the other two CYPs were inactive. Also in support of a role for CYP in ATRA oxidation by 15-HPETE was the finding that the CYP inhibitor miconazole

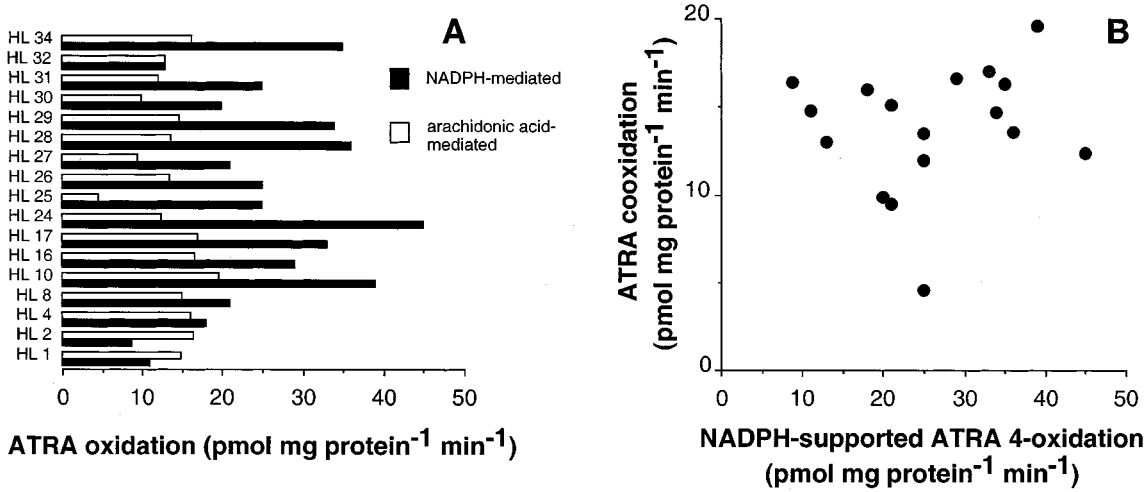

Figure 2 (A) Individual variation in NAPDH-mediated and arachidonic acid-mediated ATRA oxidation in human hepatic microsomes. (B) Plot of the relationship between the activities.

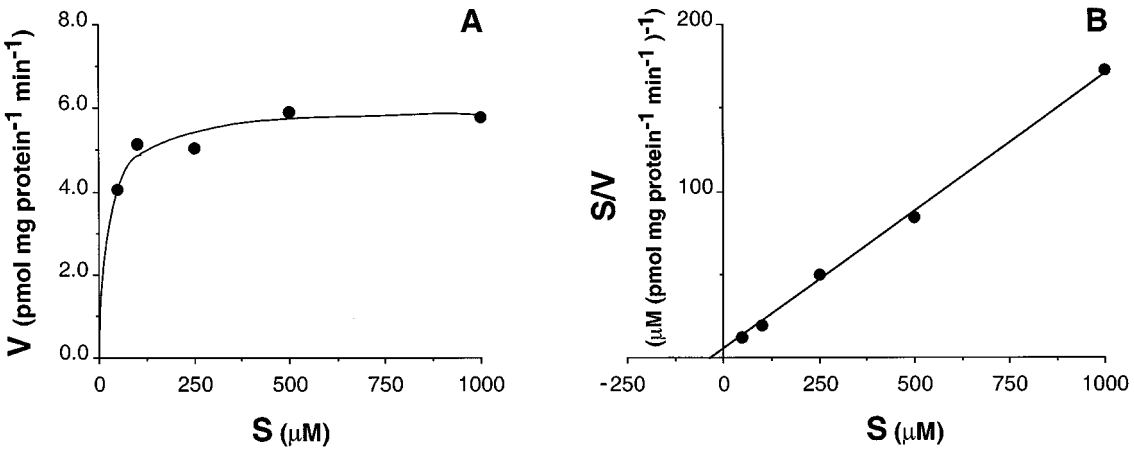

Figure 3 Kinetics of arachidonic acid-mediated ATRA cooxidation in human hepatic microsomes. (A) Representative Michaelis-Menten plot. (B) Hanes-Woolf plot of the data in A.

Table 1 Drug, alcohol and smoking histories of liver donors

Liver	*Drug history*	*Alcohol history**	*Smoking history†*
HL1	ranitidine		
HL2	oxazepam, α-methyldopa		
HL4	none		
HL8	none		
HL10	dopamine, desmopressin		
HL16	dopamine, desmopressin	Yes	
HL17	colloidal bismuth subnitrate, sucralfate		
HL24	flucloxacillin, ceftriaxone		
HL25	enalapril		Yes
HL26	dopamine, imipenem		Yes
HL27	dopamine, desmopressin	Yes	
HL28	unknown		
HL29	simvastatin		
HL30	adrenaline, ranitidine, penicillin		Yes
HL31	dexamethosone, dopamine, desmopressin	Yes	Yes
HL32	prazosin		
HL34	alprazolam, dexamethasone		Yes

*Three or more cigarettes per day; †alcohol 10 g or greater per day.

(250 μM) decreased the activity in human hepatic microsomes to 31±4% of control (data not shown).

Discussion

CYP-dependent 4-hydroxylation in human liver is an established pathway for the termination of the physiological and pharmacological actions of ATRA. Studies undertaken in ram seminal vesicles and with the purified enzyme have also implicated PGHS in the biotransformation of ATRA and its geometric isomer *13Z*-RA (Samokyszyn & Marnett 1987; Samokyszyn *et al*., 1995). The present study examined the contributions of CYP- and PGHS-mediated pathways of ATRA oxidation in human hepatic microsomes. Arachidonic acid-mediated ATRA cooxidation emerged as a quantitatively significant pathway in these fractions, similar in magnitude to NADPH-dependent 4-hydroxylation mediated by CYP. Thus, ATRA cooxidation constitutes an alternative pathway by which ATRA undergoes biotransformation and which may contribute significantly to overall rates of retinoid deactivation.

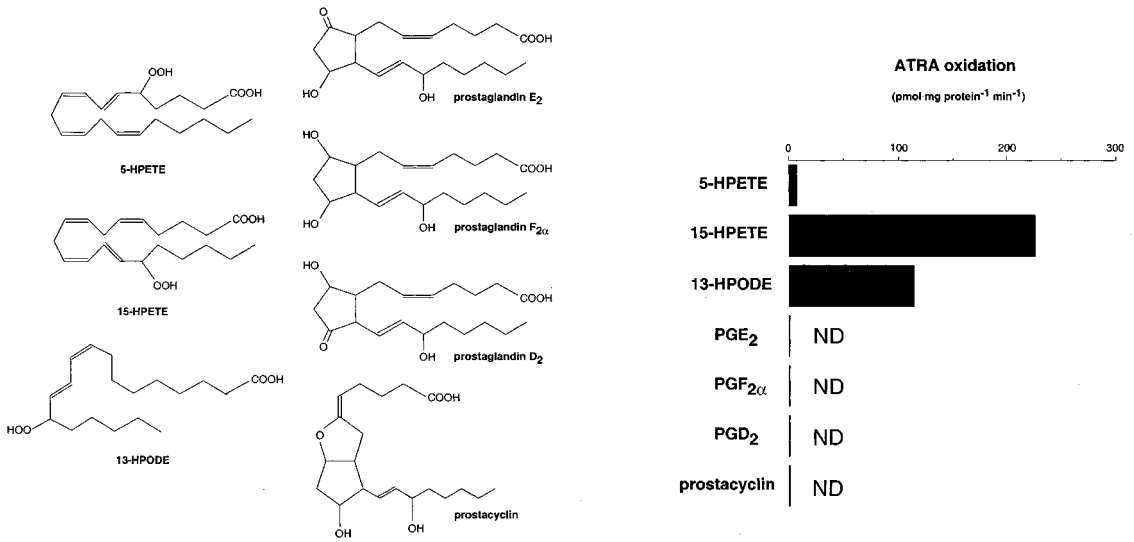

Figure 4 ATRA cooxidation mediated by endogenous eicosanoids in human hepatic microsomes (PG, prostaglandin). ND, not detected.

Like NADPH-mediated ATRA 4-hydroxylation, ATRA cooxidation in human hepatic microsomes exhibited a several fold interindividual variation. However, there was no apparent correlation between the measured rates of product formation by the NADPH- and arachidonic acid-supported pathways, which is consistent with the notion that different enzyme systems are operative. Thus, the relative expression of hepatic CYP and PGHS is a contributory factor in the capacity of individuals to oxidize retinoids.

PGHS has emerged from the present study as a participant in ATRA cooxidation mediated by arachidonic acid; the PGHS inhibitor indomethacin impaired the observed rate of

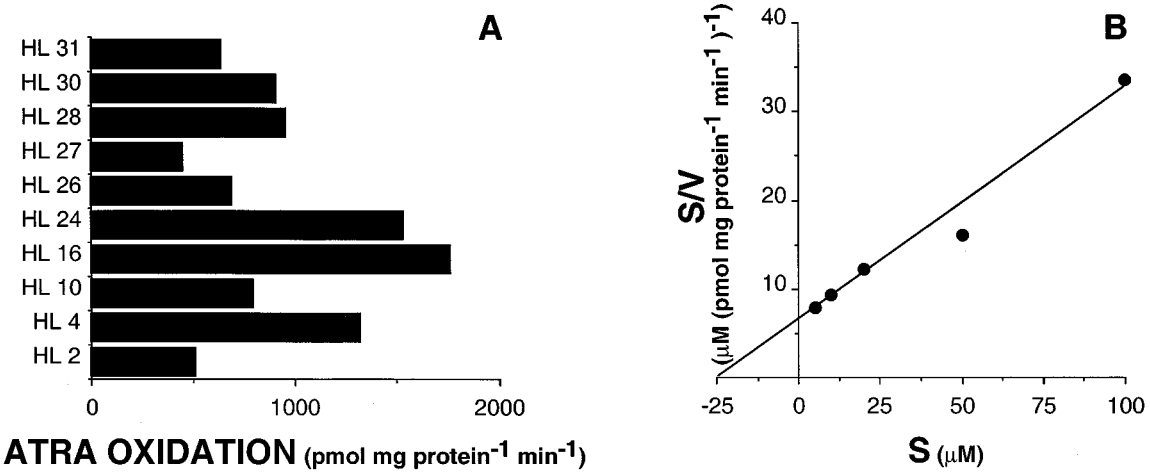

Figure 5 (A) Individual variation in 15-HPETE-mediated ATRA oxidation in human hepatic microsomes. (B) Representative Hanes-Woolf plot of the reaction in microsomes.

Table 2 Kinetic parameters of ATRA biotransformation in human hepatic microsomes mediated by different cofactors

Cofactor	K_m (μM)	V_{max} (pmol mg protein^{-1} min^{-1})	V_{max} K_m^{-1} (pmol mg protein^{-1} min^{-1})
Arachidonic acid	32±7	8±1	0.25
NADPH	470±10	68±2	0.14
NADH	940±80	17±6	0.018

Table 3 NADPH- and 15-HPETE-mediated microsomal ATRA oxidation by cDNA-derived CYPs expressed in human lymphoblastoid cells

CYP	*NADPH-mediated* (pmol product pmol CYP^{-1} h^{-1})	*15-HPETE-mediated* (pmol product pmol CYP^{-1} h^{-1})
1A2	ND*	280
2C8	2.5	1650
2C9	4.7	510
2E1	ND	150
3A4	1.3	380

*ND, Below limit of detection (<0.1 pmol product pmol CYP^{-1} h^{-1}).

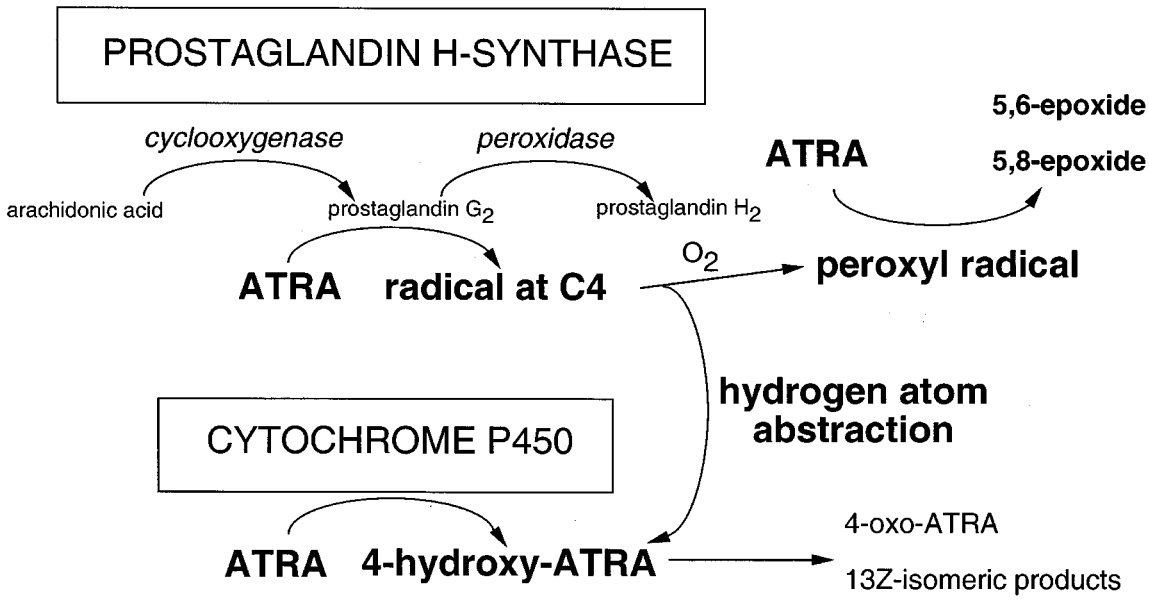

Figure 6 Roles of prostaglandin H-synthase and cytochrome P450 in ATRA oxidation in human hepatic microsomes.

microsomal cooxidation. PGHS is a bifunctional enzyme that possesses cyclo-oxygenase activity, which is responsible for the formation of the endocyclic peroxide prostaglandin G_2, and peroxidase activity, which reduces prostaglandin G_2 to prostaglandin H_2 (Eling *et al*., 1990). A range of lipophilic chemicals, including naturally occurring retinoids, possess readily oxidizable atoms and can act as reducing substrates for the peroxidase activity of PGHS (Markey *et al*., 1987). Thus, ATRA and *13Z*-RA undergo hydrogen atom abstraction from the carbon atom at the 4-position of the β-ionone ring system to generate carbon centred radicals (Samokyszyn *et al*., 1995). The 4-position is relatively reactive because it is a secondary allylic carbon that is fully conjugated with the unsaturated side chain. This radical at C-4 combines with a molecule of oxygen to form peroxyl radicals (Samokyszyn *et al*., 1995). Such radicals are known to generate epoxides, which is consistent with the formation of the 5,6- and 5,8-epoxides from ATRA in micellar systems containing haematin. In hepatic microsomes, however, additional products are formed, including 4-hydroxyATRA, which suggests that the peroxyl radical also undergoes direct reduction by direct abstraction of a hydrogen atom from nearby macromolecules (Figure 6).

Lipid hydroperoxides, such as 15-HPETE, may also provide an activated oxygen atom for the CYP-dependent oxidation of substrates via the peroxide shunt (McMurry & Groves, 1986). This pathway obviates the requirement for CYP catalysis by reduced pyridine cofactors and oxygen activation by electron transfer. Muindi & Young (1993) reported that several lipid peroxides were able to mediate ATRA 4-oxidation in microsomes from lymphoblastoid cells containing cDNA-expressed CYPs. The reaction was not supported in these fractions by arachidonic acid. The present study confirms that lipid hydroperoxides support ATRA oxidation by CYPs and indicates that this pathway is more efficient than that mediated by NADPH.

Significant ATRA cooxidation activity was noted with CYPs 2C8, 2C9 and 3A4, as well as CYPs 1A2 and 2E1. In contrast, ATRA 4-hydroxylation mediated by NADPH was only catalyzed by CYPs 2C8, 2C9 and 3A4. Thus a wider range of CYPs is able to support lipid hydroperoxide-mediated ATRA biotransformation than that mediated by NADPH, which suggests that the haeme and apoprotein regions of the holoenzyme are both important to NADPH-mediated catalysis, whereas the haeme region appears more important in cooxidation. Thus, the direct transfer of an oxygen atom from the lipid peroxide to the CYP haeme (the peroxide shunt), and not the complete CYP electron transport cycle, is the critical factor in cooxidation. The finding that miconazole, which inhibits CYP reactions by binding at the sixth axial position of the haeme moiety, effectively modulated 15-HPETE-dependent ATRA biotransformation is consistent with the involvement of CYP in ATRA cooxidation.

ATRA is effective in the initial phase of treatment of individuals with acute promyelocytic leukaemia (APL) (Castaigne *et al*., 1990). However, longer term administration of ATRA leads to therapeutic failure and the development of retinoid resistance: patients relapse and become unresponsive to further rounds of ATRA therapy (Muindi *et al*., 1992). A progressive decline in the plasma levels of ATRA that are achieved during therapy and enhanced rates of excretion of ATRA metabolites in the urine of treated patients are observed. Thus, the development of retinoid resistance is associated with autoinduction of ATRA catabolism. It appears that CYP26 (Ray *et al*., 1997) and CYPs 3A (Martini & Murray, 1993) are retinoid- and xenobiotic-inducible ATRA 4-hydroxylases present in mammalian tissues. There are also reports of elevated plasma lipoperoxides in patients after ATRA therapy (Muindi *et al*., 1994). From the findings of the present study and those of Muindi & Young (1993), such lipid peroxides support the efficient oxidation of ATRA by CYPs.

Intracellular retinoid binding proteins have been shown to modulate the biotransformation of retinoids and to influence the availability of retinoids for interaction with nuclear RARs (Boylan & Gudas, 1992; Dong *et al*., 1999). Cellular retinoic acid binding protein (CRABP)-I is expressed ubiquitously in cells, whereas CRABP-II exhibits a more restricted distribution (Ross, 1993). Overexpression of CRABP-I in embryonal carcinoma cells has been shown to increase the formation of 4-oxidized metabolites of ATRA; antisense transfection produced the opposite effects (Boylan & Gudas, 1992). Thus, the delivery of ATRA to biotransformation enzymes that mediate 4-hydroxylation, such as CYPs 26 and 2C8, may be enhanced by CRABP-I. Whether this protein also modulates the delivery of ATRA to PGHS in order to influence cooxidation rates remains to be investigated.

In summary, the present study has established that PGHS and CYP contribute similarly to ATRA oxidation in human liver fractions. Thus, PGHS, and possibly other intracellular enzymes that generate hydroperoxides from fatty acids provide the cosubstrates that are utilized by CYPs in retinoid oxidation (Figure 6). It is likely that the cooxidation pathway would predominate under conditions where intracellular concentrations of reduced pyridine nucleotide cofactors are depleted. In this situation a wider range of CYPs are able to contribute to retinoid oxidation than those that are active in the NADPH-mediated pathway.

The support of this project by the Leo and Jenny Leukaemia and Cancer Foundation of Australia is gratefully acknowledged. L. Nadin was the recipient of a postgraduate studentship from the Gastroenterological Society of Australia.

References

BLOMHOFF, R., GREEN, M.H. & NORUM, K.R. (1992). Vitamin A: Physiological and biochemical processing. *Ann. Rev. Nutr.,* **12,** 37–57.

BOYLAN, J.F. & GUDAS, L.J. (1992). The level of CRABP-I expression influences the amounts and types of all-*trans*-retinoic acid metabolites in F9 teratocarcinoma stem cells. *J. Biol. Chem.,* **267,** 21486–21491.

CASTAIGNE, S., CHOMIENNE, C., DANIEL, M.T., BALLERINI, P., BERGER, R., FENAUX, P. & DEGOS, L. (1990). All-*trans*-retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. *Blood,* **76,** 1704–1709.

DONG, D., RUUSKA, S.E., LEVINTHAL, D.J. & NOY, N. (1999). Distinct roles for cellular retinoic acid-binding proteins I and II in regulating signaling by retinoic acid. *J. Biol. Chem.,* **274,** 23695–23698.

ELING, T.E., THOMPSON, D.C., FOUREMAN, G.L., CURTIS, J.F. & HUGHS, M.F. (1990). Prostaglandin H synthase and xenobiotic oxidation. *Annu. Rev. Pharmacol. Toxicol.,* **30,** 1–45.

KOCHHAR, D.M., PENNER, J.D. & SATRE, M.A. (1988). Derivation of retinoic acid and metabolites from a teratogenic dose of retinol (vitamin A) in mice. *Toxicol. Appl. Pharmacol.,* **96,** 429–441.

LEO, M.A., LASKER, J.M, RAUCY, J.L., KIM, C.-I., BLACK, M. & LIEBER, C.S. (1989). Metabolism of retinol and retinoic acid by human liver cytochrome P450 IIC8. *Arch. Biochem. Biophys.,* **269,** 305 – 312.

LOWRY, O.H., ROSEBROUGH, N.T., FARR, A. & RANDALL, R.J. (1951). Protein measurement with Folin phenol reagent. *J. Biol. Chem.,* **193,** 265 – 275.

MANGELSDORF, D.J. & EVANS, R.M. (1995). The RXR heterodimers and orphan receptors. *Cell,* **83,** 841 – 850.

MARKEY, C.M., ALWARD, A., WELLER, P.E. & MARNETT, L.J. (1987). Quantitative studies of hydroperoxide reduction by prostaglandin H synthase. Reducing substrate specificity and the relationship of peroxidase to cyclooxygenase activities. *J. Biol. Chem.,* **262,** 6266 – 6279.

MARTINI, R. & MURRAY, M. (1993). Participation of P450 3A enzymes in rat hepatic microsomal retinoic acid 4-hydroxylation. *Arch. Biochem. Biophys.,* **303,** 57 – 66.

McMURRY, T.J. & GROVES, J.T. (1986). Metalloporphyrin models for cytochrome P-450. In *Cytochrome P-450: Structure, Mechanism and Biochemistry*, ed. Ortiz de Montellano, P.R. pp. 1 – 28 Plenum Press: NY.

MUINDI, J.F., FRANKEL, S.R., MILLER Jr., W.H., JAKUBOWSKI, A., SCHEINBERG, D.A., YOUNG, C.W., DMITROVSKY, E. & WARRELL Jr., R.P. (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 – 303.

MUINDI, J.F., SCHER, H.I., RIGAS, J.R., WARRELL Jr., R.P. & YOUNG, C.W. (1994) Elevated plasma lipid peroxide content correlates with rapid plasma clearance of all-*trans*-retinoic acid in patients with advanced cancer. *Cancer Res.,* **54,** 2125 – 2128.

MUINDI, J.F. & YOUNG, C.W. (1993). Lipid hydroperoxides greatly increase the rate of oxidative catabolism of all-*trans*-retinoic acid by human cell culture microsomes genetically enriched in specific cytochrome P450-isoforms. *Cancer Res.,* **53,** 1226 – 1229.

RAY, W.J., BAIN, G., YAO, M. & GOTTLEIB, D.I. (1997). CYP26, a novel mammalian cytochrome P450, is induced by retinoic acid and defines a new family. *J. Biol. Chem.,* **272,** 18702 – 18708.

ROBERTS, A.B., LAMB, L.C. & SPORN, M.B. (1979). Metabolism of all-*trans*-retinoic acid in hamster liver microsomes: Oxidation of 4-hydroxy to 4-keto-retinoic acid. *Arch. Biochem. Biophys.,* **199,** 374 – 383.

ROSS, A.C. (1993). Overview of retinoid metabolism. *J. Nutr.,* **123,** 346 – 350.

SAMOKYSZYN, V.M., CHEN, T., MADDIPATI, K.R., FRANZ, T.J., LEHMAN, P.A. & LLOYD, R.V. (1995). Free radical oxidation of (*E*)-retinoic acid by prostaglandin H synthase. *Chem. Res. Toxicol.,* **8,** 807 – 815.

SAMOKYSZYN, V.M., FREYALDENHOVEN, M.A., CHANG, H.C., FREEMAN, J.P. & COMPADRE, R.L. (1997). Regiospecificity of peroxyl radical addition to (*E*)-retinoic acid. *Chem. Res. Toxicol.,* **10,** 795 – 801.

SAMOKYSZYN, V.M. & MARNETT, L.J. (1987). Hydroperoxide dependent cooxidation of 13-*cis*-retinoic acid by prostaglandin H synthase. *J. Biol. Chem.,* **262,** 14119 – 14133.

SUTTON, D., NADIN, L., BUTLER, A.M. & MURRAY, M. (1997). Role of CYP3A4 in human hepatic diltiazem *N*-demethylation. Inhibition of CYP3A4 activity by oxidized diltiazem metabolites. *J. Pharmacol. Exp. Ther.,* **282,** 294 – 300.

(Received June 6, 2000
Revised July 4, 2000
Accepted July 4, 2000)