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IDENTIFICATION OF 12-KETO-5,8,10-HEPTADECATRIENOIC ACID AS AN ARACHIDONIC ACID METABOLITE PRODUCED BY HUMAN HL-60 LEUKEMIA CELLS*

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Abstract—An unusual cyclooxygenase-derived metabolite of arachidonic acid has been shown to be produced by *N,N*-dimethylformamide (DMF)-induced, terminally differentiated human HL-60 promyelocytic leukemia cells and to a much lesser extent by untreated cells. Biochemical evidence in conjunction with gas chromatography/mass spectrometry and liquid chromatography/thermospray mass spectrometry analyses indicates that the product is 12-keto-5,8,10-heptadecatrienoic acid (KHT). Both KHT and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT) were produced when arachidonic acid was incubated with cell lysates obtained from differentiated HL-60 granulocytes. Indomethacin and the thromboxane synthetase inhibitor UK-38485 inhibited the production of both metabolites, whereas ethacrynic acid inhibited only the production of KHT. In 100,000 *g* supernatant fractions, obtained from either untreated or differentiated cells, KHT was produced when HHT was used as substrate. The addition of exogenous NAD, but not NADP, to incubations caused a significant increase in the production of KHT coincident with a decrease in the level of HHT. These data suggest that, in both differentiated and undifferentiated HL-60 cells, an NAD-dependent enzyme, apparently 15-prostaglandin dehydrogenase (15-PGDH), is expressed and catalyzes the conversion of HHT to KHT. In differentiated HL-60 cells, this metabolite is produced from arachidonic acid through a multi-enzymatic process involving the activities of cyclooxygenase, thromboxane synthetase and 15-PGDH. The production of KHT from arachidonic acid in undifferentiated HL-60 cells is probably limited, therefore, by the virtual absence of cyclooxygenase activity in these cells.

The human promyelocytic leukemia HL-60 cell line [1] has attracted attention in recent years as an *in vitro* model for studying the regulation of growth and differentiation of human myeloid cells [2, 3]. Treatment of HL-60 promyelocytes with compounds such as dimethyl sulfoxide (Me_2SO)¶, dimethylformamide (DMF) or retinoic acid [4, 5] leads to terminal differentiation along granulocytic lines. Alternatively, exposure of cells to the tumor promoting phorbol ester, PMA, or to the active metabolite of vitamin D_3, $1,25(OH)_2D_3$, induces differentiation to monocyte/macrophage like cells [6, 7]. The differentiated cells display many of the morphological and functional characteristics of their mature myeloid counterparts [2–7].

The metabolism of arachidonic acid by HL-60 cells has been the subject of relatively few investigations. The cyclooxygenase pathway, which gives rise to the prostaglandins, prostacyclin and thromboxane, has been reported to be virtually absent in untreated HL-60 cells, but increases dramatically following Me_2SO or retinoic acid induced granulocytic differentiation [8, 9]. Depending on the inducer used, prostaglandin E_2 (PGE_2) [8] or thromboxane B_2 (TxB_2) and 12-hydroxy-heptadecatrienoic acid (HHT) [9, 10] have been reported to be the major cyclooxygenase metabolites produced by differentiated HL-60 cells. Arachidonic acid has also been shown to be metabolized by the 5-lipoxygenase pathway in mature HL-60 cells [10, 11]. The monohydroxylated eicosanoid, 5-HETE, and the dihydroxy leukotriene, LTB_4, generated by this pathway are consistent with those metabolites produced by normal human peripheral granulocytes [12].

In a study designed to investigate the time course

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¶ Abbreviations: Me_2SO, dimethyl sulfoxide; DMF, *N,N*-dimethylformamide; PMA, phorbol 12-myristate 13-acetate; $1,25(OH)_2D_3$, 1,25-dihydroxy vitamin D_3; PGE_2, prostaglandin E_2; TxA_2, thromboxane A_2; 5-HETE, 5-hydroxyeicosatetraenoic acid; LTB_4, leukotriene B_4; RP-HPLC, reversed-phase high pressure liquid chromatography; GC/MS, gas chromatography/mass spectrometry; LC/MS, liquid chromatography/mass spectrometry; 15-PGDH, 15-prostaglandin dehydrogenase; HHT, 12-hydroxy-5,8,10-heptadecatrienoic acid; KHT, 12-keto-5,8,10-heptadecatrienoic acid; NAD, nicotinamide adenine dinucleotide; $NaBH_4$, sodium borohydride; and AUFS, absorbance units full scale.

for the expression of 5-lipoxygenase activity in DMF-treated HL-60 cells, we reported the detection of an unexpected and atypical arachidonic acid metabolite [11]. This metabolite, which was consistently observed during routine reversed-phase HPLC analysis of lipoxygenase products from differentiated HL-60 cells, was subsequently shown to be of cyclooxygenase origin. On occasion, substantially smaller, yet detectable amounts of the metabolite were also observed in untreated HL-60 cells. The atypical u.v. absorbance spectrum and HPLC elution characteristics of this metabolite, however, indicated that it was not one of the well recognized cyclooxygenase-derived metabolites. In this study we report on the structural identification and the enzymatic production of this unusual metabolite.

MATERIALS AND METHODS

Arachidonic acid was purchased from NuChek-Prep (Elysian, MN). Calcium ionophore A23187 was obtained from Calbiochem (La Jolla, CA). *N,N*-Dimethylformamide (DMF), NAD (grade III), indomethacin and ethacrynic acid were purchased from Sigma (St. Louis, MO). HHT was obtained from Biomol (Philadelphia, PA). The thromboxane synthetase inhibitor, UK-38485, was provided by Dr. P. R. Urquilla, Pfizer (Groton, CT).

Cells. HL-60 cells were derived from peripheral blood leukocytes of a patient with acute promyelocytic leukemia [1] and grown in suspension culture in RPMI 1640 supplemented with 15% fetal bovine serum (heat-inactivated), penicillin (100 units/ml) and streptomycin (100 μg/ml). Cultures were maintained at 37° in a humidified atmosphere of 5.0% CO_2 in air. Cells were maintained in logarithmic growth by seeding at 2–3 × 10^5 cells/ml in fresh medium every 3–4 days. Granulocytic differentiation was induced by treatment of cultures with DMF (0.8%, v/v) for 4 days. For preparation and purification of the arachidonic acid metabolite, cells were harvested by centrifugation at 800 *g*, washed twice with Hanks' balanced salt solution (without calcium and magnesium), and resuspended in Dulbecco's phosphate-buffered saline at a concentration of 2–3 × 10^7 cells/ml. Viability was assessed by trypan blue exclusion and was always >95%. Cell suspensions (10–20 ml) were equilibrated to 37° and the reaction was initiated by the addition of calcium ionophore A23187 (5.0 μM) and arachidonic acid (20 μg/ml). After 5 min the reaction was terminated by the addition of an equal volume of ice-cold methanol, and the pH was adjusted to approximately 3.5 with 2 N formic acid. Cell suspensions were centrifuged in the cold, and the supernatant fraction was removed and brought to 30% methanol by the addition of distilled water. Arachidonic acid metabolites were isolated by solid phase extraction procedures and separated by reversed-phase HPLC as previously described [11]. The unidentified metabolite, peak "A", was collected and subjected to both GC/MS and LC/MS.

Gas chromatography/mass spectrometry. Gas chromatography/mass spectrometry was performed on a Ribermag R10-10C quadrupole mass analyzer. Samples were injected onto a 7 m × 0.25 mm i.d. DB-1 fused silica WCOT capillary column that terminates in the mass spectrometer ion source. Trimethylsilylation of any free hydroxyl moieties was carried out by reacting the sample in a 1:1 mixture with bis-(trimethylsilyl)trifluoroacetamide in pyridine. Keto moieties were converted to methoximines by reaction with methoxylamine·HCl in pyridine. Conditions for gas chromatography were: injector temperature, 280°; interface temperature, 280°; oven temperature program, 150–280° at 10° min; helium flow, 45 cm^3/sec. Conditions for mass spectrometry were: source temperature, 180°; electron energy, 40 eV; filament current, 0.3 mA; trap current, 16 μA.

Liquid chromatography/Thermospray mass spectrometry. Chromatography in the combined LC/MS experiments was carried out using a Beckman 332M liquid chromatograph with a Waters 440 dual wavelength (280 and 254 nm) u.v. absorbance monitor in series between the chromatograph and mass spectrometer. Separations were obtained on a 4.6 × 250 mm Supelcosil LC/18DB column using 0.25 M ammonium acetate buffer (pH 6) in either 65 or 75% methanol (v/v) at a flow rate of 2 ml/min. PGB_2 and HHT were used as bracketing internal standards (see Table 1), and selectivity and efficiency were found similar to the previously reported system

Table 1. Liquid chromatographic*, ultraviolet absorbance ratio† and salient thermospray mass spectrometric‡ data on peak A and related reference compounds

Compound	Retention time (min)	Ultraviolet absorbance ratio	Mol. wt	Prominent ions: *m/z* (%)		
				MH^+	$(MH - 18)^+$	Others
PGB_2	5.1 (4)§	0.39	334	335 (100)	317 (58)	
HHT	11.5 (1)	6.4	280		263 (100)	280 (0.5)
Peak A (AA)‖	9.4 (4)	0.37	278	279 (100)	261 (8)	293 (2), 296 (3)
Peak A (HHT)¶	9.4 (2)	0.36	278	279 (100)	261 (14)	293 (3), 296 (9)

* Separations for all compounds were conducted using a 250 × 4.6 mm Supelcosil LC-18 DB column. Mobile phase was 35% 0.25 M NH_4OAc (pH 6) + 65% MeOH at a flow rate of 2.0 ml/min.

† A_{254}/A_{280}.

‡ Vaporizer exit temperature, 200°; vapor temperature, 278°.

§ Retention times are the average of the number of runs in parentheses.

‖ Peak A obtained from arachidonic acid substrate as described under Materials and Methods.

¶ Peak A obtained from HHT substrate as described under Materials and Methods.

[11]. The liquid chromatograph-mass spectrometer used in this work evolved from an earlier prototype system [13] and except for minor detail is as previously described [14, 15]. The total eluent from the above chromatographic system was directed through the vaporizer to the high pressure ion source. Vaporizer and source temperatures were adjusted to afford a temperature in the vaporizer jet at the point of ion sampling of 278°. A Teknivent 29K data system was used to control the quadrupole mass analyzer and for the acquisition and processing of mass spectrometric data.

Enzymatic production of KHT. HL-60 cells were harvested as above and resuspended in 0.1 M potassium phosphate buffer (pH 7.5) containing 1.0 mM EDTA. Cells were lysed by sonication and then centrifuged at 400 *g* to remove large debris and remaining intact cells. In experiments that utilized arachidonic acid as the substrate, the 400 *g* lysate prepared from DMF-matured cells was used as the enzyme(s) source. In addition, the 100,000 *g* supernatant fractions obtained from both untreated and DMF-matured cells were examined for enzyme activity utilizing HHT as substrate. Lysates (or supernatant fractions) were incubated with appropriate substrate at 37° in the presence or absence of additional NAD (1.0 mM) or NADP (1.0 mM). Reactions were allowed to proceed for the times indicated and terminated by the addition of 2 vol. of ice-cold methanol and sufficient formic acid to reduce the pH to 3.5. After 20 min on ice, the suspensions were centrifuged to remove precipitated proteins, and the metabolites were extracted and chromatographed as described previously.

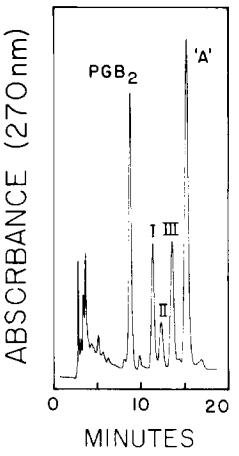

Fig. 1. HPLC analysis of arachidonic acid metabolites from DMF-induced, differentiated HL-60 cells. HL-60 cells were cultured in the presence of DMF (0.8%, v/v) for 4 days, washed, and resuspended in Dulbecco's PBS containing 1.4 mM $CaCl_2$. Approximately 2.5×10^7 cells were incubated with calcium ionophore A23187 (5 μM) and arachidonic acid (33 μM) for 5 min at 37°. The reaction was terminated, and the metabolites were extracted and subjected to RP-HPLC as previously described [11]. Peaks are as follows: PGB_2, internal standard; Peaks I and II, 5*S*,12*R*(*S*)- dihydroxy- 6,8,10,14(E,E,E,Z)- eicosatetraenoic acid; Peak III, 5*S*,12*R*-dihydroxy-6,8,10,14-(Z,E,E,Z)-eicosatetraenoic acid, LTB_4; and peak A, unidentified cyclooxygenase metabolite. Detector sensitivity: 0.02 AUFS.

RESULTS AND DISCUSSION

A typical HPLC chromatogram of the arachidonic acid metabolites produced by calcium ionophore A23187 stimulated, mature HL-60 cells is shown in Fig. 1. In addition to the internal standard, PGB_2, and 5-lipoxygenase derived dihydroxylated eicosanoids (peaks I, II, and III), a cyclooxygenase derived metabolite (peak "A") was detected consistently. Interestingly, this peak was not observed when similar experiments were conducted using normal human peripheral blood granulocytes (data not shown). The u.v. absorbance spectrum [11] of the HL-60 metabolite (λ_{max}; 278 nm) is not typical of cyclooxygenase-derived products with the exception of prostaglandins of the B-series [16]. The elution pattern of peak "A" in the reversed-phase HPLC system also suggested that the compound was less polar than most cyclooxygenase metabolites. The one compound that generally migrates as the least polar of cyclooxygenase metabolites is 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT). This metabolite, typically formed in conjunction with the synthesis of thromboxane A_2 (TxA_2) [17, 18], has been detected as a product of HL-60 cells [10, 11] but was shown to elute immediately after peak "A" in our system [11]. In addition, HHT absorbs maximally in the 235 nm region due to its conjugated diene structure. Although peak "A" demonstrated unique characteristics, a determination of its source and structure could be initially surmised through the integration of certain chemical characteristics of other cyclooxygenase products. The unimodal u.v. absorbance spectrum with a maximum at 278 nm, similar to that of PGB_2, suggested the presence of a conjugated dienone structure, while the HPLC elution of peak "A" suggested that the molecule might be quite similar in size and polarity to HHT. One potential candidate, identified as a metabolite of HHT in a recent report, was 12-keto-5,8,10-heptadecatrienoic acid (KHT) [19]. The reported u.v. maximum (276 nm) and the relative retention times of KHT and HHT under similar RP-HPLC conditions are almost identical to those of peak "A" and HHT in our system. As a preliminary check, peak "A" was biosynthesized, purified, and then subjected to sodium borohydride reduction. The resulting mixture, analyzed by RP-HPLC, showed a loss of peak "A" coupled with the appearance of a peak which had a retention time and u.v. absorbance similar to HHT (Fig. 2). Additional biochemical support for a close relationship between peak "A" and HHT was deduced from earlier experiments using radiolabeled arachidonic acid [11]. In biological systems with the capacity for thromboxane synthesis it is frequently observed that thromboxane B_2 (TxB_2) and HHT are produced in comparable amounts [20]. In HL-60 cells, the yield of TxB_2 was shown to be 5-fold greater than HHT; however, the sum of HHT and peak "A" more closely approximated a 1:1 ratio with TxB_2 (data not shown).

To provide more conclusive proof for the structure of peak "A", the compound was analyzed by GC/

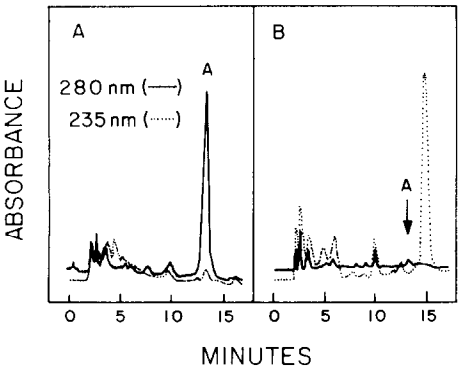

Fig. 2. Effect of $NaBH_4$ reduction on peak A. Peak A was purified by HPLC, evaporated to dryness *in vacuo*, and resuspended in methanolic $NaBH_4$ at 4°. After 40 min, 5 vol. of distilled water was added and the pH adjusted to approximately 4 with dilute sulfuric acid. The mixture was extracted and chromatographed as previously described [11]. HPLC profiles are of peak A before (A) and after (B) $NaBH_4$ reduction.

MS. Following methoximation and trimethylsilylation, the gas chromatogram showed two separate peaks eluting at 5.41 and 5.52 min (data not shown) which displayed ions of high intensity at m/z 379 (M^+), m/z 364 $(M-CH_3)^+$, m/z 348 $(M-OCH_3)^+$, m/z 180, equivalent to $(CH_3(CH_2)_4C(=NOCH_3)(CH=CH)_2)^+$ and two ions, m/z 188 and 149, which are probably products of multiple fragmentation isomeric with $(N\equiv C(CH=CH)_2CH_2CH=CH(CH_2)_3CO^+$ and $(CH_3ON=\overline{C(CH=CH)_2CH_2CH=}\overline{)CH})^+$ respectively (Fig. 3). The two GC peaks most likely represent the syn and anti isomers which would be expected for methoximine derivatives of carbonyl groups. These results are consistent with a structure of trimethylsiloxyl 12-(methoximino)heptadecatriene and in relative agreement with those reported for the methoximine derivative of KHT methyl ester [19, 21].

In addition to GC/MS, the compound was also subjected to analysis by combined liquid chromatography/thermospray mass spectrometry (LC/MS). Strong u.v. absorbance coupled with excellent peak shape and separation on RP-HPLC suggested that the compound would be a good candidate for this type of analysis. The full scan thermospray mass spectrum of peak "A" shown in Fig. 4 shows prominent ions at m/z 279 (MH^+), m/z 261 $(MH-18)^+$, and m/z 302 (MNa^+). Additional ions arising from clusters and fragmentations of clusters were observed at m/z 293 $(MH^+ \ CH_3OH-H_2O)$, m/z 296 (MNH_4^+), m/z 317 ([M − H + Na] NH_4^+) and m/z 323 ([M − H + Na] Na^+). These results indicate a molecular weight of 278 which is consistent with that for KHT.

The production of KHT from arachidonic acid in a biological system could theoretically occur by one of two mechanisms (Fig. 5). The first would involve a heme-catalyzed conversion of the 15-hydroperoxy moiety of prostaglandin G_2 (PGG_2) to a keto function (15-keto-PGG_2) [22] followed by conversion to KHT and malondialdehyde (MDA). This conversion would require that 15-keto-PGG_2 either act as a substrate for thromboxane synthetase or undergo a non-enzymatic breakdown to the observed product. In the former case, the production of some 15-keto-TxB_2 would also be expected. In an HPLC system capable of separating 15-hydroxy from 15-keto prostanoids, only one peak which comigrated with authentic TxB_2 was observed (data not shown). In a nonenzymatic catalysis of 15-keto-PGG_2, products of the more chemically favored oxygen—oxygen bond cleavage (i.e. 15-keto-PGE_2, 15-keto-PGD_2) would be expected in addition to KHT and MDA. These products were not detected. The second mechanism would involve the enzymatic oxidation of the 12-hydroxyl group of HHT. As it has been shown that HHT is an excellent substrate for the NAD-dependent 15-prostaglandin dehydrogenase (15-PGDH; EC 1.1.1.141) purified from porcine kidney [19, 23], experiments were conducted to determine whether a similar mechanism was operable in HL-60 cells.

The effect of exogenous NAD on the relative levels of HHT and KHT produced from arachidonic acid was examined first. Whole cell lysates obtained from DMF-matured HL-60 cells were chosen for this experiment due to the initial requirement for the cyclooxygenase-dependent production of HHT. Cell lysates incubated with arachidonic acid alone produced HHT and, to a lesser extent, KHT (Fig. 6A). Incubations carried out in the presence of added NAD resulted in a large increase in the amount of KHT, concomitant with a decrease in HHT (Fig. 6B). These results suggested that arachidonic acid was first converted by the cyclooxygenase and thromboxane synthetase pathways to HHT and sub-

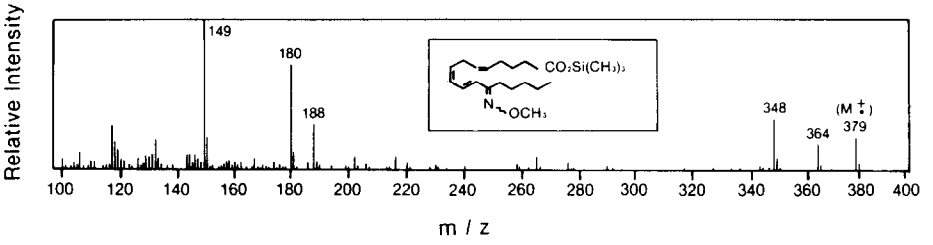

Fig. 3. Mass spectrum of the *O*-methyloxime derivative of the trimethylsilylated ester of peak A. Procedures for the derivatization, gas chromatography and mass spectrometry are described under Materials and Methods.

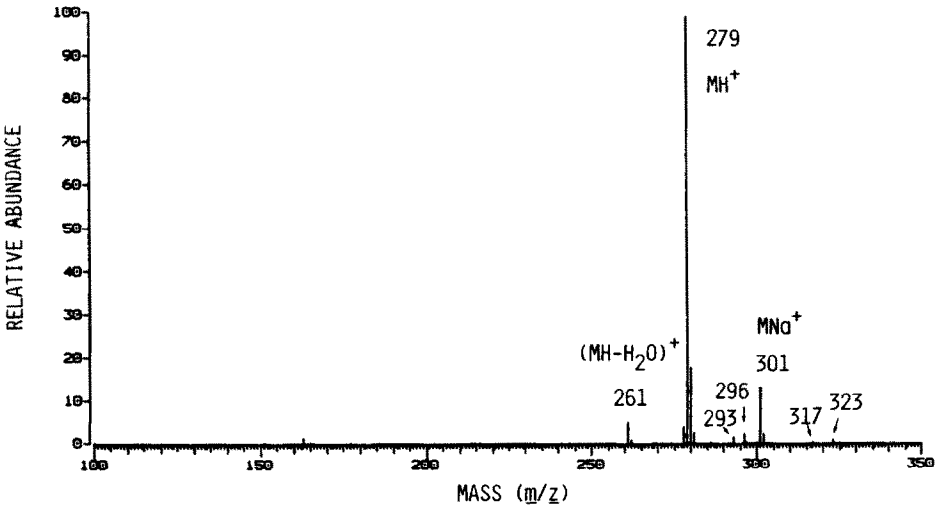

Fig. 4. Thermospray mass spectrum of peak A. Chromatographic and mass spectometric conditions are described under Materials and Methods. HPLC mobile phase was 25% 0.25 M ammonium acetate (pH 6) in 75% methanol.

sequently oxidized via a pyridine nucleotide-dependent dehydrogenase to KHT. The appearance of KHT in incubations lacking additional NAD is most likely due to residual amounts of this cofactor in cell lysates.

Support for the multi-enzymatic production of KHT from arachidonic acid was obtained by utilizing specific inhibitors of the individual enzymes of the proposed pathway (Fig. 7). In the presence of the cyclooxygenase inhibitor, indomethacin (1 μM), HHT and KHT were reduced to non-detectable levels (Fig. 7B). The thromboxane synthetase inhibitor UK-38485 (100 μM) also inhibited the production of both HHT and KHT (Fig. 7C). Ethacrynic acid, which has been reported to be an inhibitor of 15-PGDH [24, 25], inhibited the production of KHT by greater than 50% at a concentration of 30 μM. Coincident with the inhibition of KHT production

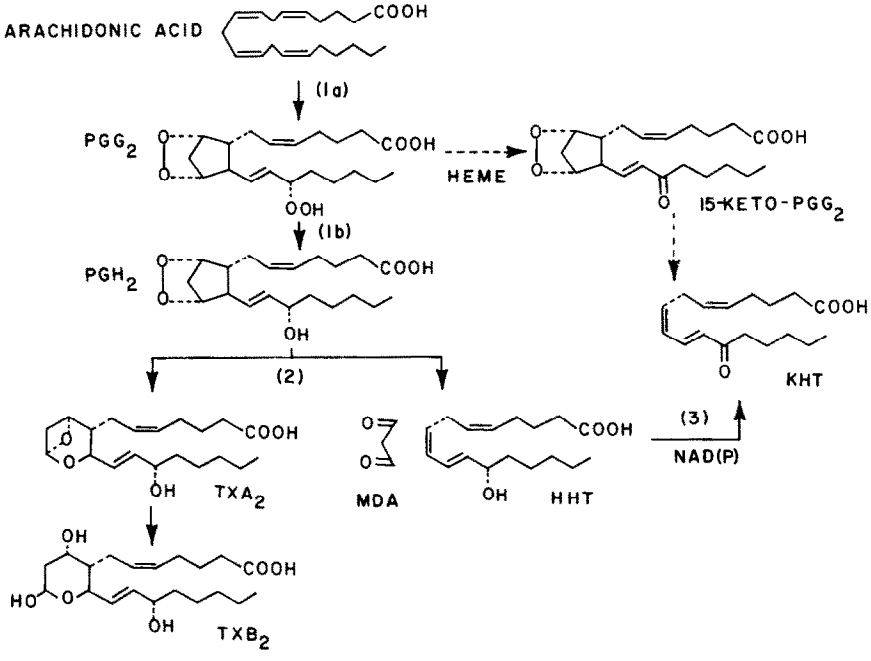

Fig. 5. Proposed pathways for the conversion of arachidonic acid to KHT. The enzymatic steps include (1) PGH synthetase [a] cyclooxygenase, [b] hydroperoxidase, (2) thromboxane synthetase, and (3) 15-prostaglandin dehydrogenase.

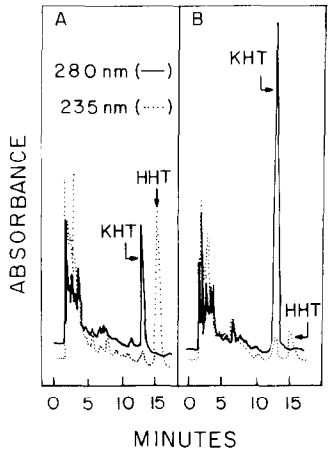

Fig. 6. Production of KHT from arachidonic acid by differentiated HL-60 cell lysates. Cell lysates were prepared from DMF-treated HL-60 cells as described under Materials and Methods. The lysates obtained from approximately 2.5×10^7 cells were incubated with arachidonic acid (30 μM) for 2 min in the absence (A) or presence (B) of exogenous NAD (1 mM). Reactions were terminated, and products were extracted and chromatographed as described. Detector sensitivity: 0.05 AUFS at both 280 and 235 nm.

was a greater than 50% increase in the level of HHT (Fig. 7D).

Since 15-PGDH has been shown to be a soluble (cytosolic) enzyme [26], 100,000 g supernatant fractions were prepared from HL-60 cells and incubated with HHT as substrate. As before, in supernatant fractions obtained from differentiated HL-60 cells, a small amount of KHT was produced in the absence of exogenous NAD; however, in the presence of NAD a dramatic increase in the level of KHT was observed (data not shown). Heat-denatured (boiled) supernatant fractions did not catalyze the reaction. Of particular interest, however, was that supernatant fractions from undifferentiated HL-60 cells were also quite active in converting HHT to KHT. During a 10-min incubation, virtually all of the substrate HHT was converted to KHT in the presence of NAD (Fig. 8B). In this experiment, evidence was also obtained which indicated that the enzyme had a specific requirement for NAD as cofactor. In the presence of NADP (Fig. 8C), the levels of KHT produced from HHT were essentially no different from those produced in control incubations (Fig. 8A).

As confirmation that the metabolite produced from HHT was the same as that produced from arachidonic acid, samples were again analyzed by both GC/MS and LC/MS. As shown in Table 1, the HHT metabolite and the arachidonic acid derived metabolite exhibited essentially identical LC/MS characteristics. In GC/MS, the *O*-methyloxime derivative of the trimethylsilylated ester of the HHT metabolite gave a mass spectrum identical to that produced by the arachidonic acid metabolite (data not shown). These data indicate that the HHT metabolite and the arachidonic acid derived metabolite are indeed the same compound.

In conclusion, the identity of a cyclooxygenase metabolite produced by HL-60 cells has been confirmed as 12-keto-5,8,10-heptadecatrienoic acid. The strict requirement for NAD as cofactor and the inhibition by ethacrynic acid indicate that the enzyme responsible for the conversion of HHT to KHT is

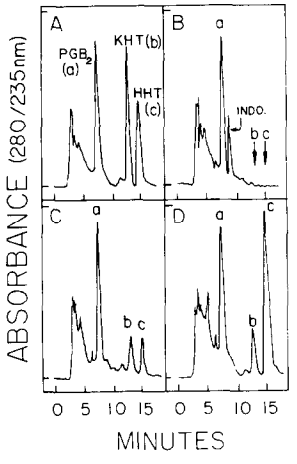

Fig. 7. Effects of selective inhibitors on the production of HHT and KHT. Cell lysates were prepared from DMF-treated HL-60 cells as described under Materials and Methods. The lysates obtained from approximately 2×10^7 cells were preincubated for 10 min at 37° in the presence of (A) no inhibitor, (B) 1 μM indomethacin, (C) 100 μM UK-38485, and (D) 30 μM ethacrynic acid. Arachidonic acid (30 μM) was then added, and after 5 min the reactions were terminated and products extracted and chromatographed as described. PGB_2 (200 ng) was added to the samples as internal standard prior to extraction. PGB_2 and KHT were monitored at 280 nm, and HHT was monitored at 235 nm. Detector sensitivity: 0.02 AUFS at both wavelengths.

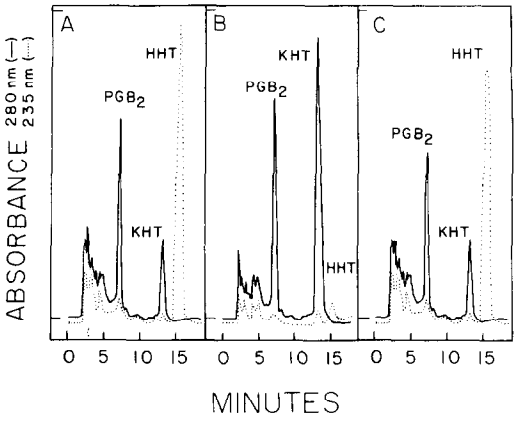

Fig. 8. Production of KHT from HHT by the 100,000 g supernatant fraction of HL-60 cells. High speed supernatant fractions were prepared from undifferentiated (untreated) HL-60 cell lysates as described under Materials and Methods. The 100,000 g supernatant fractions obtained from approximately 3.3×10^7 cells were incubated with HHT (5 μM) for 10 min in the absence (A) and presence of (B) NAD (1 mM) or (C) NADP (1 mM). Reactions were terminated and products extracted and chromatographed as described. PGB_2 (200 ng) was added to the samples as internal standard prior to extraction. Detector sensitivity: 0.02 AUFS for PGB_2 and 0.05 AUFS for all other compounds.

the NAD-dependent 15-prostaglandin dehydrogenase (Type I). Although KHT has been identified previously as a metabolite of HHT via the action of isolated and purified 15-PGDH [19, 23], this study represents the first report of the multi-enzymatic (cyclooxygenase → thromboxane synthetase → 15 PGDH) production of KHT from arachidonic acid in intact cells. A product with similar characteristics has also been detected in chopped lung preparations from sensitized guinea pigs.* While 15-PGDH activity has been observed in guinea pig lungs [27] as well as in a number of other tissues from various species [28, 29], the presence of this enzyme activity in human myeloid cells has not been reported previously. Further support for the presence of 15-PGDH in HL-60 cells has been obtained through preliminary experiments which indicated that PGE_2 was converted to 15-keto-PGE_2 in the presence of NAD.† In view of the role prostaglandins of the E-series are reported to play in the regulation of myelopoiesis [30–32], the expression of 15-PGDH in both the HL-60 promyelocyte and DMF-matured HL-60 granulocyte may have important implications in the pathophysiology of this myeloid leukemia. Studies are currently in progress to further characterize the HL-60 enzyme and to examine its biological significance in the growth and differentiation of these cells.

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