Metabolism of Leukotriene B₄ to Dihydro and Dihydro-oxo Products by Porcine Leukocytes*

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Porcine leukocytes contain a novel pathway for the metabolism of leukotriene B₄ (LTB₄) which results in reduction of the conjugated triene chromophore to a conjugated diene. These cells converted LTB₄ to two major metabolites, both of which exhibited maximal absorbance at 230 nm in their UV spectra. These products were purified by high pressure liquid chromatography and identified as 10,11-dihydro-LTB₄ and 10,11-dihydro-12-oxo-LTB₄ on the basis of the mass spectra of various derivatives. The position of the double bond of LTB₄, which had been reduced was established by cleaving the remaining double bonds of 10,11-dihydro-LTB₄ with ozone followed by oxidation or reduction of the resulting ozonide and products by mass spectrometry. Experiments with deuterium-labeled substrate indicated that LTB₄ could be directly converted to 10,11-dihydro-LTB₄ without the prior oxidation of either of its hydroxyl groups, as is required for the formation of dihydro metabolites of prostaglandins. Incubation of porcine leukocytes with 10,11-dihydro-LTB₄ and 10,11-dihydro-12-oxo-LTB₄ indicated that these two products can be interconverted and are in equilibrium with one another. The dihydro-oxo metabolite can therefore be formed from 10,11-dihydro-LTB₄, although we have not ruled out the possibility that it is also produced via 12-oxo-LTB₄, which could be a transitory intermediate. These results indicate that porcine leukocytes contain a novel reductase/dehydrogenase pathway distinct from the pathway responsible for the metabolism of prostaglandins. This pathway is also different from the pathway in human polymorphonuclear leukocytes which converts 6-trans-isomers of LTB₄ to dihydro products, since the latter pathway involves 5-oxo intermediates and results in a shift in the positions of the remaining double bonds.

Stimulation of polymorphonuclear leukocytes (PMNL) with various agents results in the release of arachidonic acid and its conversion to products of the 5-lipoxygenase pathway, including 5-hydroxy-6,8,11,14-eicosatetraenoic acid, leukotriene B₄ (LTB₄), 6-trans-LTB₄, and 12-epi-6-trans-LTB₄. LTB₄ is an important mediator in inflammatory reactions. It is a potent chemotactic agent for PMNL, a potent agonist for platelets, and a potent mediator of asthma. It is also a potent mediator of inflammation and its conversion to products of the 5-lipoxygenase pathway, including 5-hydroxy-6,8,11,14-eicosatetraenoic acid, leukotriene B₄ (LTB₄), 6-trans-LTB₄, and 12-epi-6-trans-LTB₄. LTB₄ is an important mediator in inflammatory reactions. It is a potent chemotactic agent for PMNL (2, 3) and stimulates thromboxane release (14).

We have recently identified a second pathway for the metabolism of LTB₄ and related compounds in PMNL which results in the reduction of one of the three conjugated double bonds of the triene system to give a conjugated diene. LTB₄ is metabolized to such a product by rat PMNL, along with a large number of other metabolites, including 19-hydroxy-LTB₄ (15). On the basis of the UV spectrum of the dihydro product formed by rat PMNL, one of the double bonds between the 5- and 12-hydroxyl groups of LTB₄ appeared to have been reduced, but the location of the remaining double bonds was not determined. Neither was the mechanism for the formation of this product investigated. Although we have not identified dihydro metabolites of LTB₄, incubation of human PMNL with LTB₄, we found that these cells convert 6-trans-isomers of LTB₄ to their dihydro products (16, 17). It is possible that ω-oxidation of LTB₄, is too rapid in human PMNL to permit reduction to these products, since 20-hydroxy-LTB₄ is not a substrate for the reductase pathway by PMNL from this species. Alternatively, the reductase pathways in rat and human PMNL may be different from one another, and it is possible that LTB₄ is a substrate only for the rat enzyme.

Unlike human and rat PMNL, we found that porcine PMNL possess very little ω-oxidation activity with respect to LTB₄. PMNL from this species should therefore be a very good model to investigate the metabolism of LTB₄, and related substances by the reductase pathway without the complication of concomitant metabolism by ω-oxidation. We report here that the 10,11-double bond of LTB₄ is reduced by porcine leukocytes to give 10,11-dihydro-LTB₄, followed by oxidation of the 12-hydroxyl group to yield 10,11-dihydro-12-oxo-LTB₄.

MATERIALS AND METHODS

Preparation of Substrates—Unlabeled LTB₄ was prepared by incubation of arachidonic acid (NuChek Prep, Inc.) and the divalent

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†The abbreviations used are: PMNL, polymorphonuclear leukocytes; LT, leukotriene; HETE, hydroxyeicosatetraenoic acid; ODS, octadecylsil; RP-HPLC, reversed-phase high pressure liquid chromatography; GC-MS, gas chromatography-mass spectrometry; tR, retention time; MSTFA, N-methyl-N-(trimethylsilyl)trifluoroacetamide; PG, prostaglandin.
cation inophore, A23187 (10 μM, Calbiochem), with porcine PMNL (18), which had been preincubated with 5,8,11,14-eicosatetraenoic acid (kindly supplied by Dr. J. R. Paulsrud of Hoffman-La Roche) in order to inhibit 12-lipoxygenase (19). Under these conditions, the major dihydroxy products formed were LTB₄, and its two 6-trans-isomers. LTB₄ was purified and quantitated by reversed-phase high pressure liquid chromatography (RP-HPLC) as described previously (17) and was identical with synthetic LTB₄, kindly provided by Dr. J. R. Paulsrud of Hoffman-La Roche. Under these conditions, the substrate was [5,6,8,9,11,12,14,15-²H₄]LTB₄, which was prepared as described previously (17). [1-⁴C]LTB₄ and [5,6,8,9,11,12,14,15-²H₄]LTB₄ were synthesized by incubation of [1-⁴C]arachidonic acid or [5,6,8,9,11,12,14,15-²H₄]arachidonic acid (Du Pont-New England Nuclear), respectively, with human PMNL (9).

Preparation of Porcine Leukocytes—Leukocytes were prepared (9) by treatment of blood with Dextran T-500 (Pharmacia LKB Biotechnology Inc.) to remove most of the red blood cells. Red blood cells remaining in the pellet were lysed by treatment with 0.135 M NH₄Cl. After washing with 0.15 M NaCl, the cells were resuspended in Dulbecco’s phosphate-buffered saline, containing 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 8.1 mM Na₂HPO₄, 0.5 mM MgCl₂, and 0.9 mM CaCl₂. This crude preparation of leukocytes, including both polymorphonuclear leukocytes (PMNL) and mononuclear cells, was used for all the experiments described in this manuscript.

Incubation Conditions—Porcine leukocytes (10⁶ cells/ml) were incubated with LTB₄, (2 μM) for 60 min at 37 °C. The incubations were terminated by centrifugation at 4 °C at 400 × g for 10 min. The pellet was then washed by recentrifugation in an amount of ethanol sufficient to give a final concentration of 15% when combined with the first supernatant.

Purification of Metabolites—The incubation medium was extracted using cartridges (Waters C₁₈ Sep-Paks) of ODS silica (9, 20). After loading the sample onto the cartridge, it was washed with 15% ethanol in water (20 ml), water (20 ml), and petroleum ether (20 ml). Eicosanoids were then eluted with redistilled methyl formate (10 ml). The material in the methyl formate fraction was purified by RP-HPLC using a Waters solvent delivery system, a Raytest Ramona 5-LS radioactivity monitor, and a Waters model 490 UV detector. The stationary phase was a Waters Novapak C₁₈ column (4 μm particle size; 3.9 × 150 mm), which was eluted with gradients formed from water/acetic acid (100:0.05) and acetonitrile/acetic acid (100:0.05) as indicated in the text. The flow rate was 2 ml/min.

Derivatization Reactions—Products purified by RP-HPLC were methylated with diazomethane and converted to their trimethylsilyl ether derivatives by treatment with N-methyl-N-trimethylsilyltri-fluoroacetamide (MSTFA) (30 min, 23 °C).

In some cases, products were converted to methoxylamine derivatives prior to methylation by treatment with methoxylamine hydrochloride (1 mg) in pyridine (0.1 ml) overnight at room temperature. These methoxylamine derivatives were purified by RP-HPLC.

Compounds dissolved in methanol (0.3 ml) were hydrogenated with H₂ in the presence of PtO₂ for 90 s at room temperature. The mixtures were filtered through an open column of silicic acid (0.5 g), which was washed with MeOH (2 ml). After evaporation of the methanol, the products were purified by RP-HPLC.

Ozonolysis was performed by treatment of either dihydro-LTB₄ or its methyl ester derivative with ozone for 2 min at -20 °C. The resulting ozonides were either subjected to oxidation with hydrogen peroxide (30%; 50 μl) in the presence of acetic acid (200 μl) (16 h at 45 °C) (21) or were reduced with sodium borohydride (0.5 mg in 0.1 ml of 1 N NaOH) for 60 min. The solution was diluted with water and acidified, and the products were extracted with methyl formate.

Gas Chromatography-Mass Spectrometry (GC-MS) —Electron impact GC-MS was performed on a VG ZAB instrument located in the Biomedical Mass Spectrometry Unit of McGill University. The stationary phase was a column (20 m × 0.32 mm) of DB-1 (J and W Scientific, Inc.).

RESULTS

Metabolism of LTB₄ by Porcine Leukocytes—LTB₄ (2 μM) was incubated with porcine leukocytes at 37 °C for 60 min, and the products were separated by RP-HPLC (Fig. 1). LTB₄ was almost completely converted to two products which had longer retention times (tₚ). Both of these products absorbed light in the UV region at 235 nm but not at 280 nm, suggesting that one of the three conjugated double bonds of the substrate had been reduced. The retention time (87 min) of product B-1 was similar to that of a conjugated diene metabolite of LTB₄, which we had previously isolated from rat PMNL and partially characterized (15). The second product (tₚ = 98 min) had a retention time similar to a less polar unidentified product which we had observed in incubations with rat PMNL (15). Unlike human and rat PMNL, only small amounts of polar metabolites, presumably ω-oxidation products, were formed by porcine leukocytes (Fig. 1). Although all of the experiments reported here were conducted with a crude fraction of porcine leukocytes, containing PMNL, monocytes, and lymphocytes, experiments with leukocyte fractions purified by centrifugation on Ficoll-Paque indicated that nearly all the LTB₄-metabolizing activity resided in the PMNL fraction (data not shown).

Metabolites B-1 and B-2 had very similar UV spectra, with single absorption maxima at 230 nm. This indicates that in each case one of the double bonds of the triene chromophore had been reduced, resulting in the formation of a conjugated diene.

Identification of Metabolite B-1 (Dihydro-LTB₄)—Unlabeled and deuterium-labeled metabolite B-1 were prepared by incubation of LTB₄, and [5,6,8,9,11,12,14,15-²H₄]LTB₄, respectively, with porcine leukocytes. The mass spectrum of the methyl ester, trimethylsilyl ether derivative of unlabeled metabolite B-1, which has a C value of 23.4, is shown in Fig. 2. The mass spectrum of the corresponding derivative of deuterium-labeled B-1 (see Fig. 6, below) is similar to that shown in Fig. 2, except that each of the ions is displaced to a higher mass, depending on the number of deuterium atoms present. The number of deuterium atoms (d) in each of the fragment ions of deuterium-labeled B-1 is indicated below in brackets.
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after the m/z value of the corresponding ion in the mass spectrum of unlabeled B-1. Fragment ions in the mass spectrum of the methyl ester, trimethylsilyl ether derivative of unlabeled B-1, observed at m/z 496 (M + 8 atoms of deuterium (d) in the mass spectrum of the corresponding derivative of deuterium-labeled B-1), 481 (M - 15; 6d), 465 (M - 31), 406 (M - 90; 8d), 395 (Cᵩ to C₂₋ 8d), 391 (M - 15-90; 8d), 385 (Cᵩ to C₂₋ 6d), 375 (M - 31-90; 8d), 361 (M - 2 × 90; 6d), 360 (395-90; 8d), 295 (385-90; 6d); 269 (Cᵩ to C₂₋ 4d), 265, 255 (Cᵩ to C₀₋ 4d), 225 (Cᵩ to C₂₋ 2d), 213 (C₁₂ to C₀₋ 3d), 205 (385 - 2 × 90; 6d); 203 (Cᵩ to C₁₋ 1d), 181 (5d), 129, 119, and 105. In some cases, there was partial loss of deuterium from one of the positions of the deuterium-labeled compound, giving rise to a pair of ions in its mass spectrum. The number shown above always corresponds to the more intense of these two ions. In all cases, the mass spectrum of the deuterated compound supports the compositions assigned to the fragment ions. These mass spectra clearly indicate that metabolite B-1 is a dihydro metabolite of LTB₄, in which one of the three conjugated double bonds has been reduced. The mass spectrum of dihydro-LTB₄ is clearly different from that of the corresponding derivative of 6,11-dihydro-12-epi-6-trans-LTB₄ (6,12-dihydroxy-7,9,14-eicosatrienoic acid), which we previously identified after incubation of 12-epi-6-trans-LTB₄, with human PMNL (17). Since the mass spectra of the parent compounds, LTB₄ and 12-epi-6-trans-LTB₄, are virtually identical, this suggests that the two double bonds between carbons 5 and 12 may be in different positions in the dihydro metabolites of these two compounds. The ion at m/z 229 (231 in the deuterium-labeled compound) in the mass spectrum of dihydro-LTB₄ would suggest that a double bond is present between carbons 6 and 7, and therefore that metabolite B-1 is identical with 5,12-dihydroxy-6,8,14-eicosatrienoic acid.

The mass spectrum of the methyl ester, trimethylsilyl ether derivative of hydrogenated metabolite B-1, is identical with that of the corresponding derivative of LTB₄, confirming that the hydroxyl groups were still present in the 5- and 12-positions.

The methyl ester of [1⁻¹⁴C]metabolite B-1 was subjected to oxidative ozonolysis, and the products were methylated and separated by RP-HPLC using a gradient between 10 and 30% acetonitrile in water over 30 min. The major peak of radioactive material (tₘₙ, 7 min) had a retention time identical with that of the major radioactive product formed after ozonolysis of the methyl ester of 5-hydroxy-6,8,11,14-[¹⁴C]eicosatetraenoic acid. The mass spectrum of the trimethylsilyl ether derivative of the radioactive ozonolysis product (C value, 11.2) derived from metabolite B-1 had major fragment ions at m/z 247 (M - 15), 231 (M - 31), 203 (base peak; Me₃SiO⁺=CH(CH₂)₃-CO₂Me), 187, 171, 159, 155, 145, 129, and 119 (203-90). This mass spectrum is virtually identical with that of the methyl ester, trimethylsilyl ether derivative of α-hydroxyadipic acid, indicating that one of the double bonds of B-1 is between carbons 6 and 7 (Fig. 3). This was confirmed by reductive ozonolysis of unmethylated metabolite B-1. In this case, the ozonolysis product was reduced with sodium borohydride, followed by treatment with MSTFA, leading to the formation of the trimethylsilyl ether, ether derivative of 5,6-dihydroxyhexanoic acid (Fig. 3). The latter product (C value, 13.1), which was also formed after treatment of 5-hydroxy-6,8,11,14-eicosatetraenoic acid in an identical manner, had a mass spectrum with major ions at m/z 349 (M - 15), 261 (Me₃SiO⁺=CH(CH₂)₃-CO₂SiMe₃), 171 (261-90), 147, and 129.

Identification of Metabolite B-2 (Dihydro-oxo-LTB₄)—The second metabolite (B-2) formed from LTB₄ by porcine leukocytes was a conjugated diene which was less polar than 10,11-dihydro-LTB₄, suggesting that one of the two hydroxyl groups could have been oxidized to an oxo group. Reaction of metabolite B-2 with methoxyamine, followed by methylation with diazomethane, gave two products (tₘₙ, 57 and 60 min), which were separated by RP-HPLC using a gradient between 36 and 70% acetonitrile over 120 min. These products were converted to their trimethylsilyl ether derivatives and analyzed by GC-MS. The mass spectrum of the derivative with a tₘₙ of 60 min, which was the more abundant of the two products, had a C value of 23.8 and a mass spectrum (Fig. 4A) with fragment ions at m/z 451 (M, 7d), 436 (M - 15; 7d), 420 (M - 31; 7d), 350 (Cᵩ to C₀; 7d), 330 (M - 31-90; 7d), 309 (340-31; loss of 31 from Cᵩ to C₀; 5d), 268, 220, 208 (base peak, loss of 31 from Cᵩ to C₀; 5d), 268 (Cᵩ to C₀; 1d), 181 (5d), 167 (3d), 146 (5d), 129, 119 (4d), and 105 (4d). The numbers of deuterium atoms in the corresponding ions from deuterium-labeled B-2 are shown above in brackets. The mass spectrum of the O-methyloxime derivative with the shorter retention time (57 min) had a C value of 22.8 and a mass spectrum similar to that shown in Fig. 4A, except that the ions at m/z 350, 330, and 167 were less intense, whereas those at m/z 268 and 181 were relatively more intense. These mass spectra indicate that metabolite B-2 is a 20-carbon fatty acid containing three double bonds, one hydroxyl group, and one oxo group. The ion at m/z 203 would suggest that the hydroxyl

![Fig. 2. Mass spectrum of the trimethylsilyl ether derivative of the methyl ester of the dihydro metabolite (B-1) of LTB₄.](image1)

![Fig. 3. Formation of ozonolysis products from dihydro-LTB₄ (B-1). The methyl ester of metabolite B-1 was reacted with ozone, followed by oxidation of the resulting oxime with hydrogen peroxide, methylation with diazomethane, and formation of the trimethylsilyl ether derivative by addition of MSTFA. Alternatively, the oxime of underivatized metabolite B-1 was reduced with sodium borohydride, and the products were converted to their trimethylsilyl ether, ether derivatives.](image2)
group is at position 5, but this is not very conclusive because it is a relatively weak ion.

More conclusive evidence concerning the position of the hydroxyl and oxo groups in metabolite B-2 was obtained from the mass spectra of its methyl ester, trimethylsilyl ether derivatives, before and after hydrogenation. The mass spectrum of the trimethylsilyl ether derivative of hydrogenated B-2 is shown in Fig. 4B, which has a C value of 23.7, indicated that the oxo group of this compound was converted to an enol during the trimethylsilylation procedure. Major ions were observed at m/z 494 (M), 479 (M – 15), 463 (M – 31), 404 (M – 90), 389 (C1 to C20), 389 (M – 15), 397 (M – 31), 381 (M – 47), 327 (C6 to C20), 203 (base peak, C1 to C15), 171, 141 (C12 to C20), 129, and 113. The intense ions at m/z 203 and 327 clearly indicate that the hydroxyl group of metabolite B-2 is in the 5-position, whereas the ion at m/z 141 is consistent with an oxo group in the 12-position. The oxo group of the hydrogenated substance also partially enolized during the trimethylsilylation procedure, but to a much lesser extent than the nonhydrogenated product (data not shown).

Assuming that the double bonds in metabolites B-1 and B-2 are in identical positions (this would seem reasonable, since these compounds are interconvertible; see below), metabolite B-2 would be 10,11-dihydro-12-oxo-LTB4 (i.e. 5-hydroxy-12-oxo-6,8,14-eicosatrienoic acid).

Mechanism for the Formation of LTB4 Metabolites—10,11-Dihydro-LTB4 could be formed either by the direct reduction of LTB4 or could require prior activation of the triene system by oxidation of the 12-hydroxyl group to an oxo group (Fig. 5). 10,11-Dihydro-12-oxo-LTB4 could be formed either by oxidation of 11,12-dihydro-LTB4 by a dehydrogenase, or by reduction of 12-oxo-LTB4, which could be a transitory intermediate in the reaction (Fig. 5). In order to determine whether LTB4 could be reduced directly, [5,6,8,9,11,12,14,15-2H]LTB4 was incubated with porcine leukocytes, and the resulting dihydro metabolite was analyzed by GC-MS (Fig. 6). It would seem reasonable to assume that any loss of deuterium from the fragment corresponding to C12-C20 (m/z 215, 2d; m/z 216, 3d) would be due to loss from the 12-position, since the

![Fig. 4. Mass spectra of various derivatives of dihydro-oxo-LTB4 (metabolite B-2). A, the methyl ester, trimethylsilyl ether. O-methylxime derivative of B-2; B, the methyl ester, trimethylsilyl ether derivative of the enol isomer of B-2; and C, the methyl ester, trimethylsilyl ether derivative of hydrogenated B-2.](image)

![Fig. 5. Possible mechanisms for the formation of dihydro metabolites of LTB4. Both the direct reduction of the substrate by a reductase and the indirect reduction, via oxo intermediates, are shown. When the substrate was [5,6,8,9,11,12,14,15-2H]LTB4, deuterium atoms were present in the 5- and 12-positions of the substrate. Both of these deuterium atoms would be retained if the substrate was reduced directly, but one would be lost if there were 5-oxo or 12-oxo intermediates.](image)
deuterium atoms present in the 14- and 15-positions are not affected by the reaction and are unlikely to be lost. On this basis, it would appear that about 65% of the deuterium atoms in the 12-position of the substrate were retained (Fig. 6). The ratio of the intensities of the ions at m/z 203 to 204 indicates that at least 90% of the deuterium at carbon-5 was retained. The ratios of the intensities of the ions at m/z 488 (7d) to 489 (8d) (M − 15) and 390 (5d) to 391 (6d) (C; to C52) are consistent with the loss of about one-third of the deuterium atoms from the 12-position of the substrate, with very little loss from the 5-position (Fig. 6). These results suggest that the principal pathway for the formation of 10,11-dihydro-LTB4 is via the direct reduction of LTB4, by a reductase.

The partial loss of deuterium from the 12-position of 10,11-dihydro-LTB4, suggests that a 12-oxo compound is formed during the reaction and is converted to dihydro-LTB4. To test this hypothesis more directly, tritium-labeled 10,11-dihydro-LTB4 was incubated separately with porcine leukocytes, and the reaction products were analyzed by HPLC (Fig. 7). The results clearly indicate that these two products are interconverted. The initial reaction appears to favor the formation of 10,11-dihydro-LTB4, since after incubation with this substrate, equilibrium was reached after about 20 min with about 15% of the substrate being converted to the 12-oxo product (Fig. 7A, inset). On the other hand, the conversion of 10,11-dihydro-12-oxo-LTB4 to 10,11-dihydro-LTB4 was somewhat slower, but had not reached equilibrium by 60 min, at which time about 26% of the substrate had been converted to dihydro-LTB4 (Fig. 7B, inset). These results suggest that the loss of deuterium from the 12-position that we observed in the above experiments could be due to interconversion of the dihydro and dihydro-oxo metabolites of LTB4. Thus, 10,11-dihydro-12-oxo-LTB4 could have arisen exclusively from 10,11-dihydro-LTB4, although the possibility that it is also formed from 12-oxo-LTB4 cannot be ruled out.

**DISCUSSION**

Unlike human and rat PMNL, porcine PMNL exhibit little LTB4 ω-hydroxylase activity. Instead, the major pathway for the metabolism of LTB4 by these cells is reduction of its 10,11-double bond to give 10,11-dihydro-LTB4. The 12-hydroxyl group of the latter substance can then undergo oxidation to give 10,11-dihydro-12-oxo-LTB4. Analogous products are formed during the metabolism of prostaglandins by var-

![Fig. 6. Mass spectrum of deuterium-labeled 10,11-dihydro-LTB4. Porcine leukocytes were incubated with [5,6,8,9,11,12,14,15-3H]arachidonic acid for 60 min at 37°C, and the products were purified by HPLC. Deuterium-labeled 10,11-dihydro-LTB4 was converted to the trimethylsilyl ether derivative of its methyl ester prior to analysis by GC-MS.](image)

![Fig. 7. Interconversion of 10,11-dihydro-LTB4 and 10,11-dihydro-12-oxo-LTB4.](image)
possible that the former product could also be synthesized from 12-oxo-LTB₄. However, we have not isolated the latter substance and do not have any direct evidence for its formation in these reactions. Thus, it would appear that the mechanism for the formation of dihydro metabolites of LTB₄ is quite different from that for the conversion of prostaglandins to their dihydro metabolites.

The reductase pathway which metabolizes LTB₄ in porcine leukocytes would also appear to be distinct from a similar pathway which we recently found to metabolize 6-trans-isomers of LTB₄ in human PMNL (17). In the latter case, only dihydro- and ω-oxidized dihydro metabolites were isolated; no dihydro-oxo products were identified, although we now have preliminary evidence for their existence. However, experiments with substrates labeled with deuterium in the 5- and 12-positions indicated that the 5-position of the dihydro product from 12-epi-6-trans-LTB₄ contained practically no deuterium, whereas no deuterium was lost from the 12-position of the substrate. This suggested that oxidation of the 5-hydroxyl group was a prerequisite for reduction of 6-trans-isomers of LTB₄ by human PMNL. Another difference between the two pathways is that in the human, the conjugated double bonds appear to shift from the 6-, 8-, and 10-positions in the triene substrate to the 7- and 9-positions in the diene product. Porcine leukocytes, on the other hand, reduce the 10,11-double bond of LTB₄ directly, without any shift in the positions of the double bonds.

We recently found that rat PMNL convert LTB₄ to a series of products in which the conjugated triene chromophore of the substrate appeared to have been reduced to a conjugated diene structure (15). These experiments were rather difficult to interpret, however, because the products formed by rat PMNL were extensively metabolized by ω-oxidation. Consequently, although a dihydro isomer of LTB₄ was tentatively identified, its precise structure or its mechanism of formation could not be determined (15). More recent experiments suggest, however, that the reductase pathway of metabolism of LTB₄ in rat PMNL is quite similar to that in porcine leukocytes.

Metabolism of LTB₄ by the reductase pathway may be important for the metabolism of this substance by cells which do not possess a high degree of LTB₄ ω-hydroxylase activity, as is the case with porcine and rat leukocytes. Kaever et al. (24) have recently reported that rat macrophages, T-lymphocytes, mesangial cells, and fibroblasts convert LTB₄ to a dihydro metabolite, which was not completely characterized. These workers have recently shown that the potency of this metabolite of LTB₄ on human PMNL was considerably lower than that of LTB₄ itself (25). It is not clear whether the dihydro metabolite isolated by Kaever et al. is identical with 10,11-dihydro-LTB₄, which was isolated in the present study. It is therefore possible that the latter compound, as well as 10,11-dihydro-12-oxo-LTB₄, could be biologically active. A 10,11-dihydro derivative of 12-hydroxy-5,8,11,14-eicosatetraenoic acid (12-HETE), which was formed after incubation of bovine corneal microsomes with arachidonic acid, was found to have potent proinflammatory properties (26). We have found that porcine leukocytes convert 12-HETE to a similar product by the reductase pathway. We are currently investigating the biological activities of products formed by this pathway in leukocytes.

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