Nonenzymatic Free Radical-catalyzed Generation of Thromboxane-like Compounds (Isothromboxanes) in Vivo*

(Received for publication, May 30, 1996, and in revised form, July 10, 1996)

Jason D. Morrow, Joseph A. Awad, Aiping Wu, William E. Zackert, Vincent C. Daniel, and L. Jackson Roberts II‡

From the Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tennessee 37232-6602

The isoprostanes (IsoPs) are novel bioactive prostaglandin-like compounds produced in vivo by free radical-catalyzed peroxidation of arachidonyl-containing lipids. Previously, we have identified IsoPs containing F-type and D- and E-type prostane rings that are formed by reduction and rearrangement of IsoP endoperoxide intermediates, respectively. We now explore whether thromboxane B2 (TxB2)-like compounds, termed B2-IsoTxes (B2-IsoTxs), are formed by rearrangement of IsoP endoperoxides. Detection of these compounds was carried out using a stable isotope dilution mass spectrometric assay originally developed for quantification of cyclooxygenase-derived TxB2. Incubations of arachidonic acid with Fe/ADP/ascorbate for 30 min in vitro generated a series of peaks representing putative B2-IsoTx at levels of 62.4 ± 21.0 ng/mg arachidonate. Using various chemical modification and derivatization approaches, it was determined that these compounds contained hemiacetal ring structures and two double bonds, as would be expected for B2-IsoTx. Analysis of the compounds by electron ionization mass spectrometry yielded multiple mass spectra similar to those of TxB2. B2-IsoTxs are also formed esterified to phospholipids; oxidation of arachidonyle-containing phosphatidylcholine in situ followed by hydrolysis resulted in the release of large amounts of these compounds. To explore whether B2-IsoTxs are also formed in vivo, a well-characterized animal model of lipid peroxidation consisting of orogastric administration of CCl4 to rats was used. Levels of B2-IsoTx esterified in lipids in the liver increased 41-fold from 2.5 ± 0.5 to 102 ± 30 ng/g of liver. In addition, circulating levels of free compounds increased from undetectable (<5 pg/ml) to 185 ± 30 pg/ml after CCl4, a 37-fold increase. Thus, we have provided evidence that IsoTxs constitute another novel class of eicosanoids produced in vivo nonenzymatically by free radical-catalyzed lipid peroxidation. These studies thus expand our understanding of products of lipid peroxidation formed in vivo from the free radical-catalyzed peroxidation of arachidonic acid.

Free radical-catalyzed lipid peroxidation has been implicated in the pathogenesis of a wide variety of human disorders (1–4). Nonetheless, much remains to be understood about the mechanisms of oxidant injury in vivo. Previously, we reported the discovery that a series of prostaglandin (PG) F2-like compounds (F2-IsoPs) capable of exerting potent biological activity are produced in vivo in humans as products of the free radical-catalyzed peroxidation of arachidonic acid (5). Formation of these compounds occurs independently of the cyclooxygenase enzyme, which had heretofore been considered obligatory for endogenous prostanoiobiosynthesis. Circulating levels of these compounds increase dramatically in animal models of free radical injury, and quantification of F2-IsoPs has proven to be an important advance in our ability to assess oxidant stress in vivo (5, 6). Formation of F2-IsoPs proceeds through intermediates comprising four positional peroxyl radical isomers, which undergo endocyclization to yield PGG2-like bicycloendoperoxides. These are then reduced to F-ring IsoPs. F2-IsoPs are initially formed in situ from arachidonic acid esterified in phospholipids and are subsequently released preformed by a phospholipase (6, 7). This mechanism of formation is in contradistinction to the formation of cyclooxygenase-derived prostanoiobenzoids in which arachidonic acid esterified in phospholipids must be released prior to oxygenation.

More recently, we reported that IsoPs that are PGD2± and PGE2±-like compounds (D2/E2-IsoPs) also are produced in vivo from rearrangement of isoprostane endoperoxides (8). Like F-ring compounds, they are formed in situ on phospholipids, their formation increases markedly in animal models of oxidant injury, and they exert potent bioactivity. Because TxB can also be formed by nonenzymatic rearrangement of cyclooxygenase-derived PGH2 (9), we explored whether Tx-like compounds can also be generated as rearrangement products of the isoP endoperoxide intermediates. We present evidence that TxB±-like compounds are, in fact, produced both in vitro and in vivo and that they are present both esterified to phospholipids and in the free form. Because these compounds are isomeric to cyclooxygenase-derived TxB, they henceforth will be referred to as B2-IsoTx.

EXPERIMENTAL PROCEDURES

Reagents—Methoxyamine HCl, FeCl3, ascorbate, ADP, pentafluorobenzyl (PFB) bromide, diisopropylethylamine, and Apis mellifera venom phospholipase A2 were obtained from Sigma. Dimethylformamide, undecane, and sodium borohydride were obtained from Aldrich. N,N-Bis(trimethylsilyl)trifluoroacetamide and N-trimethylsilylimidazole were obtained from Supelco (Bellefonte, PA). [7H9]-N,O-Bis(trimethylsilyl)trifluoroacetamide and [7H5]-methoxyamine HCl were purchased from Regis Chemical Co. (Morton Grove, IL). All organic solvents were obtained from Baxter Healthcare (Burdick and Jackson Brand, McGraw Park, IL). C-18 and silica Sep-Paks were purchased from Waters Associates (Milford, MA). TLC was performed on Silica gel.
Analysis of Isothromboxanes—IsoTxs were analyzed by gas chromatography (GC)/negative ion chemical ionization (NICI) mass spectrometry (MS) using a modification of methods described previously for the analysis of TxB2 (10). Briefly, 1.5 ng of [3H]TxB2 internal standard was initially added to a biological fluid and adjusted to pH 3 with 1 N HCl. The sample was applied to a C-18 Sep-Pak cartridge that had been prerinsed with 5 ml of methanol and 5 ml of H2O (pH 3). The cartridge was then washed with 10 ml of H2O (pH 3) followed by 10 ml heptane, and compounds were eluted with 10 ml of ethyl acetate and evaporated to dryness under nitrogen. Compounds were subsequently methoximated by treatment with 250 μl of a 2% solution of aqueous methoxymethanol HCl for 30 min at room temperature. Compounds were extracted with 1 ml of ethyl acetate, and the organic layer was evaporated under nitrogen. Compounds were then converted to a PFB ester by addition of 40 μl of a 10% solution of PFB bromide in acetonitrile and 20 μl of 10% disopropylethylamine in acetonitrile and incubated for 30 min at 37°C. Reagents were dried under nitrogen, and the residue was redissolved in 30 μl of chloroform and 20 μl of methanol and chromatographed on a silica TLC plate to the top in a solvent system of ethyl acetate, decane, which had been stored over a bed of calcium hydride. GC/NICI MS was carried out on a Nermag R10-10C mass spectrometer interfaced with a Digital DEC-PDP computer. GC was performed using a 15-m, 0.25-μm thick DB-1701 fused silica capillary column (J & W Scientific, Folsom, CA). The column temperature was programmed from 190°C to 300°C at 20°C/min. The major ion generated in the NICI mass spectrum of the PFB ester, O-methylxime, and TMS ether derivative of TxB2 (approximately 5 μg), was chromatographed on a separate lane and visualized with 10% phosphomolybdic acid in ethanol by heating. The Rf of the derivatized TxB2 standard in this solvent system was ~0.46. Compounds migrating in the region 1.5 cm above and below the standard were scraped from the TLC plate, extracted with 1 ml of ethyl acetate, and dried under nitrogen.

Following TLC purification, compounds were converted to trimethylsilyl (TMS) ether derivatives by addition of 20 μl of N,O-bistrimethylsilyl trifluoroacetamide and 10 μl of dimethylformamide. The sample was incubated at 37°C for 10 min and then dried under nitrogen. The residue was redissolved for GC/MS analysis in 10 μl of undecane, which had been stored over a bed of calcium hydride. GC/NICI MS was carried out on a Nermag R10-10C mass spectrometer interfaced with a Digital DEC-PDP computer. GC was performed using a 15-m, 0.25-μm thick DB-1701 fused silica capillary column (J & W Scientific, Folsom, CA). The column temperature was programmed from 190°C to 300°C at 20°C/min. The major ion generated in the NICI mass spectrum of the PFB ester, O-methylxime, and TMS ether derivative of TxB2, which would be the same ion generated by IsoTx, was the m/z 614 carboxylate anion M-181 (M-CH2CF3). The corresponding ion generated by the [3H]TxB2 internal standard was m/z 617. Levels of endogenous B2-IsoTx in a biological sample were calculated from the ratio of the area under the m/z 614 chromatographic peaks to the m/z 617 chromatographic peak. In some experiments, compounds were reacted with trimethylsilylimidazole, subjected to catalytic hydrogenation, or reduced with sodium borohydride following TLC purification as described (7). IsoTxs were also analyzed by GC/electron ionization (EI) MS as methyl ester O-methylxime and TMS ether derivatives. Purification and derivatization of compounds for analysis by GC/EI MS were as noted above, except the methyl ester derivatives were formed by treatment of compounds with excess ethereal diazomethane (7).

Analysis of F2- and D2/E2-IsoPs—Purification, derivatization, and analysis of F2-IsoPs and D2/E2-IsoPs by GC/NICI MS were performed as described (6, 7, 8). Quantification of either F2-IsoPs or D2/E2-IsoPs in the present studies differed from previous reports in that the amounts of endogenous IsoPs were determined by comparing the ratios of the area under the chromatographic peaks representing endogenous material to that of the respective standard. Extraction, Purification, and Hydrolysis of Phospholipids—1-Palmitoyl-2-arachidonoylphosphatidylethanolamine oxidized in vitro or lipids from livers of CCl4-treated rats were extracted as described (6, 11). Depending on the experiment, 0.005% butylated hydroxytoluene was added to the lipid extracts during the extraction procedure. The lipid extracts (containing approximately 1 μmol of phospholipid) were hydrolyzed by chemical saponification or by reaction with A. mellifera venom phospholipase A2 (approximately 200 μg) as described (6, 8) and subsequently analyzed for free B2-IsoTx. As a positive control for phospholipase A2 activity, phosphatidylcholine containing 1H-arachidonate in the sn-2 position was added to the incubation mixture, and the percent of radiolabeled arachidonate released was determined as described (6, 8). In all experiments, >95% of esterified 1H-arachidonate was released.

Oxidation of Arachidonic Acid and Arachidonoyl Phosphatidylcholine

![Fig. 1. Analysis of oxidized arachidonic acid for B2-IsoTx by GC/NICI MS. The peak in the m/z 617 ion current chromatogram represents the [3H]TxB2 internal standard. In the m/z 614 ion current chromatogram are a series of peaks consistent with the presence of B2-IsoTx compounds. Levels of compounds are based on a comparison of the ratio of the area under the series of peaks in the m/z 614 chromatogram with the area under the peak in the m/z 617 chromatogram.](http://www.jbc.org/content/218/50/23186/F1.expansion.html)
The finding that large quantities of a series of compounds were formed during oxidation of arachidonic acid in vitro that had TLC and GC/MS properties similar to those of TxB₂ would be consistent with their being B₂-IsoTx. However, additional experimental approaches were used to obtain further evidence that the compounds detected in oxidized arachidonic acid were B₂-IsoTxs. First, no peaks were present when m/z 613 was monitored, indicating that the m/z 614 peaks were not natural isotope peaks of compounds generating an ion of less than 614 Da. When the compounds were analyzed as [²H₉]TMS ether derivatives, the m/z 614 peaks all shifted upward 27 Da, indicating that the compounds have three hydroxyl groups. When the compounds were analyzed as [²H₉]O-methyloxime derivatives, the m/z 614 peaks all shifted upward 3 Da, indicating that they contain one carbonyl group. When the compounds were analyzed following catalytic hydrogenation, there was a disappearance of the m/z 614 peaks and the appearance of new intense peaks 4 Da higher at m/z 618 (Fig. 2). No peaks were detected at m/z 616 or 620. This indicated that all of the compounds contained two double bonds. Collectively, these results indicated that the compounds represented by the m/z 614 peaks contain the same functional groups and the number of double bonds expected for the PFB ester, O-methyloxime, and TMS ester derivative of B₂-IsoTx.

A unique feature of TxB₂ is that it contains a hemiacetal ring, which exists in aqueous solution in an equilibrium between open and closed forms. Thus, powerful evidence that the compounds detected were B₂-IsoTxs would be to demonstrate that these compounds contain a hemiacetal ring. Such evidence can be obtained using different derivatization and chemical modification approaches (15). As shown in Fig. 3A, if putative B₂-IsoTxs are first reacted with methoxyamine, derivatives will be formed in which the hemiacetal ring is open. Subsequent conversion to PFB ester and TMS ether derivatives would be expected to result in a series of compounds with a major fragment ion of 614 Da (M – 181 and M – CH₂C₆F₃) when analyzed by GC/NICI MS. The selected ion monitoring analysis of presumed IsoTx derivatized in this manner has been previously discussed and is shown in Fig. 1. If, on the other hand, as shown in Fig. 3B, the treatment with methoxyamine is omitted, and the compounds are converted to PFB ester and TMS ether derivatives, the hemiacetal ring will remain closed. Derivatives of these compounds would be expected to generate major fragment ions of 585 Da (M – 181) when analyzed by NICI MS. Results using this derivatization approach are shown in Fig. 4A. As is evident, in the upper m/z 585 chromatogram, a series of chromatographic peaks are present that elute at similar retention time to the [²H₉]TxB₂ internal standard represented in the lower m/z 588 chromatogram. Finally, as shown in Fig. 4C, if the carbonyl at C-11 in the open ring form is first reduced with NaBH₄ followed by conversion to PFB ester and TMS ether derivatives, the major M – 181 fragment ion would be generated at 659 Da. Results of this analysis are shown in Fig. 4B. Again, a series of m/z 659 peaks elute from the GC at a retention time similar to this derivative of the TxB₂ internal standard. Collectively, the results of these studies provide additional significant evidence that these compounds contain a hemiacetal ring as does TxB₂.

**Analysis of B₂-IsoTx by EI MS**—To obtain more direct evidence that the compounds detected by NICI MS were B₂-IsoTx, the compounds were analyzed as methyl ester, O-methyloxime, and TMS ether derivatives by EI MS. The results of this analysis yielded a series of compounds eluting over approximately a

---

**Fig. 2.** Catalytic hydrogenation of putative B₂-IsoTx obtained from arachidonic acid oxidized in vitro. A, GC/NICI MS analysis of compounds not subjected to catalytic hydrogenation. Peaks in the m/z 614 chromatogram represent putative B₂-IsoTx, and the peak in the m/z 617 chromatogram represents the [²H₉]TxB₂ internal standard. In addition, no compounds are present in the m/z 618 chromatogram. B, analysis of compounds following hydrogenation. Both the internal standard and m/z 614 peaks in A have shifted up 4 Da following hydrogenation, indicating the presence of two double bonds. The pattern of m/z 618 peaks in B differs somewhat from the pattern of m/z 614 peaks in A. This is likely attributed to different GC characteristics of the compounds in which double bonds have been reduced.

**Fig. 3.** Three different derivatization and reduction approaches used to provide evidence of the presence of a hemiacetal ring in the putative B₂-IsoTx compounds formed from the oxidation of arachidonic acid in vitro. The major fragment ion expected for each derivative when analyzed by NICI MS is shown at the bottom of each pathway.
and TMS ether derivatives. The peak representing this derivative of cyclooxygenase-derived donic acid oxidized in vitro.

The mass spectrum of this derivative of cyclooxygenase-derived donic acid oxidized in vitro. A, analysis of compounds as PFB ester and TMS ether derivatives. In the lower m/z 588 chromatogram is the derivatized [2H3]TxB2 internal standard. In the upper m/z 586 chromatogram are a series of peaks eluting over a 30-s interval. B, analysis of compounds after reduction with NaBH4 followed by conversion to PFB ester and TMS ether derivatives. The peak representing this derivative of the [2H3]TxB2 internal standard is shown in the lower m/z 662 chromatogram. The series of peaks in the upper m/z 659 chromatogram represents presumptive B2-IsoTx compounds. The pattern of peaks in Fig. 1, A and B, likely differs due to different GC characteristics of the various derivatives.

20-s period from the capillary GC column, which yielded mass spectra with characteristics of the EI mass spectrum of TxB2. One of the mass spectra obtained from a major peak is shown in Fig. 5. Other mass spectra obtained from the analysis of the other peaks were similar to that shown in Fig. 5, except that the relative abundance of some of the fragment ions varied or some of the lower molecular weight fragment ions were different. In the mass spectrum shown, there is a prominent ion at m/z 629, representing the molecular ion. Other characteristic ions present are m/z 614 (M – 15, loss of CH3), m/z 598 (M – 31, loss of OCH3), m/z 539 (M – 90, loss of (Me3)3SiO), m/z 524 (loss of 90 + 15), m/z 508 (loss of 90 + 31), m/z 418 (loss of 2 × 90 + 31), m/z 398 (loss of CH3CH = CHCH2COOCH3 + 90), m/z 392 (M – 57 – 2 × 90), loss of CH3(CH2)2CH2 + (2 × 90), m/z 369 (M – 173 – 87, loss of CH3(OH))3Si(CH3)2CH2 + CH2CH2COOCH3, m/z 243, (Me3)3SiO = CHCH2CH = CH(CH2)2COOCH3) and m/z 174 ((Me3)3SiO = CHCH2CH = NOCH3). Of particular interest is the major fragment ion of 243 Da. This ion is not present in the mass spectrum of this derivative of cyclooxygenase-derived TxB2. However, this is an expected ion resulting from a cleavage of the trimethoxysiloxane substituent at C-8, as depicted in the regiosomer shown in Fig. 5 (7, 16). Thus, this EI mass spectral data provide additional confirmatory evidence for the formation of IsoTxs by enzymatic peroxidation of arachidonic acid.

Analysis for the Presence of B2-IsoTx Esterified to Phospholipids in Vivo—Since the above results suggested that IsoTxs could be formed in vitro, we investigated whether these compounds may also be formed in vivo. Previously, we had shown that D2/IsoPs and D2/E2-IsoPs are initially formed in situ from arachidonic acid esterified in tissue phospholipids and subsequently released preformed (6, 8). Therefore, we examined whether IsoTxs are also formed esterified in phospholipids in livers of rats that had been treated with CCl4 to induce lipid peroxidation. To investigate this, lipids were extracted from the livers, subjected to hydrolysis using methanolic potassium hydroxide, and analyzed as free compounds. The results of this analysis are shown in Fig. 6. A series of m/z 614 peaks was present in a pattern very similar to that obtained from analysis of arachidonic acid oxidized in vitro, although the relative abundances of the various peaks differ slightly (cf. Fig. 1). Essentially identical results were obtained when phospholipids were hydrolyzed enzymatically with phospholipase A2 from A. mellifera (data not shown).

Table I compares the amounts of the B2-IsoTxs with D2/E2-IsoPs and F2-IsoPs measured following hydrolysis of lipids from the same livers of both untreated and CCl4-treated rats. The quantities of B2-IsoTx measured following hydrolysis of lipids from livers of CCl4-treated rats were 41-fold higher than those in untreated rats. Levels of free B2-IsoTx measured in lipid extracts that were not subjected to hydrolysis were >1% of the levels measured following hydrolysis (n = 4), suggesting that the compounds detected following saponification were released from an acyl linkage on phospholipids. Pretreatment of animals with indomethacin prior to CCl4 administration with a dosage regimen previously shown to inhibit cyclooxygenase activity by >90% (14) did not affect levels of the compounds measured (p > 0.7, Student’s t test; n = 4), indicating that the cyclooxygenase enzyme is not involved in their formation. Previously we had shown that butylated hydroxytoluene markedly suppresses the formation of F2-IsoPs by autoxidation in vitro (7). The presence of butylated hydroxytoluene (0.005%) in the extraction solution, however, did not affect levels of IsoTx measured (p > 0.6; n = 4), arguing that these compounds are not formed ex vivo by autoxidation during sample processing.

Experiments were then carried out to obtain further evidence of the identity of the compounds represented by the m/z 614 peaks in Fig. 6 as B2-IsoTx. First, analysis of compounds obtained from the hydrolysis of liver extracts as a [2H9]TMS ether derivative resulted in a shift of each of the m/z 614 peaks upwards to 27 Da, indicating the presence of three hydroxyl groups. Analysis as [2H9]O-methylxime derivatives resulted in a shift of the m/z 614 peaks upwards of 3 Da, indicating the presence of one carbonyl group. When the compounds were analyzed following catalytic hydrogenation, there was a disap-
that B2-IsoTxs are formed in plasma at concentrations of 185 pg/ml (n = 5). In summary, these studies support the concept that large amounts of TxB2 were generated from PGH2 if PGH2 does not result in the formation of significant quantities of TxA2, which may, however, be similar to that proposed for the conversion of the cyclooxygenase-derived endoperoxide PGH2 to TxA2 by the enzyme thromboxane synthase (9). Thromboxane synthase is a cytochrome P450 enzyme containing a catalytic iron moiety at its active site. Hecker and Ullrich (9) have proposed that the formation of TxA2 initially involves complexing of the Fe3⁺ in the enzyme active site with the oxygen at C-9 on the endoperoxide PGH2. This is followed by homolytic scission of the endoperoxide bond, leading to formation of an alkoxyl radical. Subsequently, β scission of the C-11—C-12 bond occurs, followed by rearrangement of the molecule to form TxA2, which then rapidly decomposes to TxB2. A similar mechanism might also explain the nonenzymatic formation of B2-IsoTx from the iron-catalyzed peroxidation of arachidonic acid in vitro. Arguing against this mechanism, however, is the observation by Hecker and Ullrich (9) that reaction of inorganic Fe⁺ with PGH2 does not result in the formation of significant quantities of TxB2. In those studies, however, Hecker and Ullrich (9) did find that large amounts of TxB2 were generated from PGH2 if iron was present complexed in a porphyrin such as hemin. Thus, it is possible that the formation of IsoTx in vivo might be catalyzed by porphyrin-containing compounds or Fe-containing enzymes, including Tx synthase. On the other hand, the fact that large amounts of B2-IsoTx can be formed in vitro when
arachidonic acid is oxidized with Fe/ADP/ascorbate would suggest that complexed iron or Fe-containing enzymes are not necessary for the formation of IsoTx.

It should be noted that the quantities of B2-IsoTxs that are formed in vivo are only slightly less than the amounts of D2/E2-IsoPs generated. Since the levels of many of the individual IsoPs in normal human biological fluids are at least an order of magnitude higher than cyclooxygenase-derived prostaglandins, the amounts of IsoTx that are produced in vivo are not trivial. We have previously reported that both F- and D/E-ring IsoPs possess potent biological activities (5, 8, 18–20). Whether IsoTx possess biological activity will be difficult to ascertain. It is reasonable to assume that if IsoTx possess bioactivity, such activity would reside with the TxA-ring compounds rather than the TxB-ring compounds, analogous to cyclooxygenase-derived Txs, of which TxA2 is bioactive but TxB2 is devoid of biological activity. However, because the TxA-ring is extremely unstable, undergoing rapid hydrolysis to form the TxB-ring, isolation of A2-IsoTx for biological testing would be difficult, if not impossible.

It should also be mentioned that there are potentially important biological ramifications associated with the formation of IsoTx esterified in phospholipids. We previously reported that molecular modeling of phospholipids with F2-IsoPs esterified at the sn-2 position revealed them to be extremely distorted molecules (6). Thus, the formation of isoprostane-containing phospholipids in settings of oxidant stress may have deleterious effects on membrane fluidity and integrity, well recognized sequelae of oxidant injury (21). Since we have now discovered that, in addition to F2-IP and D2/E2-IsoPs, IsoTxs are also formed esterified to phospholipids in large quantities, the total quantities of phospholipids containing products of the isoprostane pathway that may be formed in settings of free radical injury are substantially greater than previously thought.

In summary, we report the discovery that IsoTxs are formed in vivo as products of nonenzymatic free radical-catalyzed lipid peroxidation. Analogous to the formation of F2-IsoPs and D2/E2-IsoPs, IsoTxs are formed in situ esterified to phospholipids and subsequently released in free form. Further understanding the biological consequences of the formation of these novel compounds and mechanisms by which they are formed may provide valuable insights into the pathophysiology of oxidant injury.

REFERENCES
1. Halliwell, B., and Gutteridge, J. M. C. (1990) Methods Enzymol. 186, 1–85
2. Southorn, P. A., and Powis, G. (1988) Mayo Clin. Proc. 63, 390–408
3. Ames, B. N. (1983) Science 221, 1256–1264
4. Harman, D. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 7124–7128
5. Morrow, J. D., Hill, K. E., Burk, R. F., Nammour, T. M., Badr, K. F., and Roberts, L. J. I. (1988) Proc. Natl. Acad. Sci. U. S. A. 87, 9383–9387
6. Morrow, J. D., Awad, J. A., Boss, H. J., Blair, I. A., and Roberts, L. J., II (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10721–10725
7. Morrow, J. D., Harris, T. M., and Roberts, L. J. I. (1990) Anal. Biochem. 184, 1–10
8. Morrow, J. D., Minton, T. A., Mukundan, C. R., Campbell, M. D., Zacker, W. E., Daniel, V. C., Badr, K. F., Blair, I. A., and Roberts, L. J., II (1993) J. Biol. Chem. 268, 4317–4326
9. Hecker M., and Ulrich V. (1989) J. Biol. Chem. 264, 141–150
10. Parsons, W. G., and Roberts L. J. (1988) J. Immunol. 141, 2413–2419
11. Radin N. S. (1969) Methods Enzymol. 14, 245–248
12. Longmire A. W., Swift, L. L., Roberts, L. J., Awad, J. A., Burk, R. F., and Morrow, J. D. (1994) Biochem. Pharmacol. 47, 1173–1177
13. Burk R. F., and Lane, J. M. (1979) Toxicol. Appl. Pharmacol. 50, 467–478
14. Jackson E. K. (1989) J. Pharmacol. Exp. Ther. 230, 9–21
15. Roberts, L. J., II, Sweetman, B. J., and Oates, J. A. (1981) J. Biol. Chem. 256, 8384–8393
16. Waugh R. J., and Murphy R. C. (1996) J. Am. Soc. Mass Spectrom. 1, 490–499
17. Morrow, J. D., Awad, J. A., Kato, T., Takahashi, K., Badr, K. F., Roberts, L. J., II, and Burk, R. F. (1992) J. Clin. Invest. 90, 2502–2507
18. Takahashi, K., Nammour, T. K., Fukunaga, M., Ebert, J., Morrow, J. D., Roberts, L. J., II, Hoover, R. L., and Badr, K. F. (1993) J. Clin. Invest. 91, 136–141
19. Banerjee, M., Kang, K. H., Morrow, J. D., Roberts, L. J., and Newman, J. H. Am. J. Physiol. 263, H660–H666
20. Fukunaga, M., Makita, N., Roberts, L. J., II, Morrow, J. D., Takahashi, K., and Badr, K. F. (1993) Am. J. Physiol. 264, C1619–C1624
21. Sevanian A., and Kim, E. (1985) J. Free Radicals Biol. & Med. 1, 263–271
Nonenzymatic Free Radical-catalyzed Generation of Thromboxane-like Compounds (Isothromboxanes) in Vivo

Jason D. Morrow, Joseph A. Awad, Aiping Wu, William E. Zackert, Vincent C. Daniel and L. Jackson Roberts II.

*J. Biol. Chem.* 1996, 271:23185-23190.
doi: 10.1074/jbc.271.38.23185

Access the most updated version of this article at [http://www.jbc.org/content/271/38/23185](http://www.jbc.org/content/271/38/23185)

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 20 references, 9 of which can be accessed free at [http://www.jbc.org/content/271/38/23185.full.html#ref-list-1](http://www.jbc.org/content/271/38/23185.full.html#ref-list-1)