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TETRANOR THROMBOXANE B_2 IS THE PRINCIPAL URINARY CATABOLITE FORMED AFTER I.V. INFUSION OF THROMBOXANE B_2 IN THE RAT

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SUMMARY: We report the isolation and structural elucidation of the major catabolite of Thromboxane B_2 excreted in the urine of male adult Wistar rats into which tritium labeled TxB_2 had been infused intravenously. The identity of this product as tetranor TxB_2 was based on retention times on gas chromatography with radioactivity detection as well as mass spectral data on three derivatives.

INTRODUCTION

Thromboxane B_2 (TxB_2)[1] was recently found to undergo a single β-oxidation reaction *in vivo* in both the cymologous monkey and man resulting in its transformation into dinor-TxB_2, the major product excreted in the urine of these species (1-3). Using this product as an index of the activity of the thromboxane pathway *in vivo* we attempted to measure its levels in rat urine. Surprisingly, we could not detect any of this product by mass spectrometry and suspected that TxB_2 might be transformed into other still unrecognised catabolites. The purpose of this study was to investigate the urinary catabolite profile of tritium labeled TxB_2 infused intravenously into the male adult Wistar rat with the intention of identifying any new major products. We report the isolation and structure of the principal urinary catabolite of TxB_2 in this species.

MATERIALS AND METHODS

Six adult male Wistar rats (250-300g) were anaesthetised with INACTIN (120 mg/kg i.p.). The trachea was cannulated to facilitate breathing and polyethylene catheters were inserted into the jugular vein (for sample infusion) and the bladder (for continuous collection of urine). 5,6,8,9,11,

Thromboxane = Tx; Me = methyl; MO = O-methyloxime; TMS = trimethylsilyl; TBDMS = t-butyldimethylsilyl.

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12,14,15-octatritiated TxB_2 (New England Nuclear, specific activity = 125 Curies/mmole) was diluted with authentic chemosynthetic product (Upjohn Co.) to a specific activity of 100,000 dpm/µg, dissolved in normal saline and infused i.v. at 0.016µg/g/min for 60 minutes. Urine was collected continuously while TxB_2 was being infused and for a subsequent 3 hour period.

Purification. Urine from each experiment was worked up separately. It was diluted with 10 volumes water, acidified to pH3 with 1N HCl and percolated through a short XAD-2 Amberlite column (Rohm & Haas). The column was subsequently washed with 50 ml water and 50 ml acetone. The acetone fraction containing most of the applied radioactivity (>95%) was taken to dryness, resuspended in 1 ml methanol and stored at -20°. The sample was methylated and purified by preparative thin layer chromatography (TLC) using chloroform-methanol-acetic acid-water (90/9/1/0.65 v/v) as developing solvent. One major tritiated zone (R_F = 0.36; R_F of TxB_2 = 0.40) was observed. The silica gel was scraped off and the radioactivity was eluted with methanol. The eluate was purified further on HPLC using a Waters Fatty Acid Analysis column and a developing solvent of acetonitrile-water (33:67 v/v). The metabolite eluted at approximately 8 minutes.

Derivatives: O-methyloximes (MO) were prepared with MOX reagent (Pierce) overnight at 23°. Trideutero methyloximes (d_3-MO) were prepared by reacting the methyl esters with a 2% solution of trideutero methyl hydroxylamine hydrochloride in pyridine (Regis). Trimethylsilyl ethers (TMS) were prepared by treating the Me esters or the MeMO derivatives with TRI SIL Z (Pierce) 5 minutes at 60°. t-Butyl-dimethylsilyl ethers (TBDMS) were prepared by treating the Me or MeMO derivatives with t-butyldimethylchlorosilane in imidazole (Applied Science Labs) 30 minutes at 60°. TBDMS derivatives were extracted into hexane prior to analysis by gas chromatography (GC) or gas chromatography mass spectrometry (GC-MS).

RESULTS

Analysis of urine from six rats infused with tritium labeled TxB_2 by gas chromatography with continuous radioactivity monitoring (GC-MS) revealed the presence of one principal radioactive product whose retention time was shorter than that of TxB_2 (Fig. 1). No tritiated TxB_2 could be detected in any of the urines from the six rats.

GC-retention times of this product (three derivatives) did not provide a clear indication of its possible structure (Table 1). The MeMOTMS and MeMOTBDMS derivatives of this product indicated a separation of only 2.7 and 2.2 carbons respectively from TxB_2. On the other hand the MeTMS derivative showed a difference of 3.9 carbons between the retention time of the urinary catabolite and TxB_2 suggesting possibly that the urinary catabolite might have four carbon atoms less than TxB_2.

The mass spectrum of the MeMOTMS derivative of the urinary catabolite was quite uninformative structurally as its mass spectrum was virtually

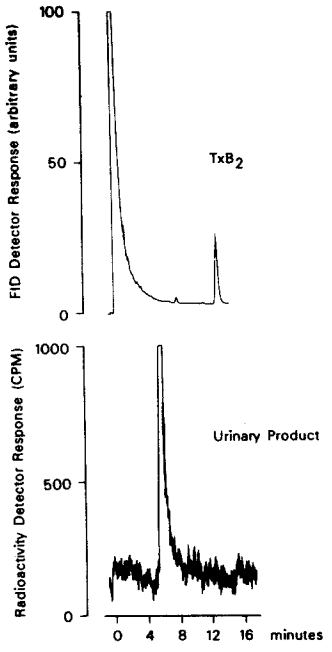

Figure 1. Gas chromatographic analysis of the MeMOTMS derivative of TxB_2 and its principal tritiated urinary catabolite in the rat.

identical in fragmentation pattern and intensities with that of TxB_2 even though the catabolite emerged 2.7 carbons earlier than TxB_2 from the gas chromatographic column (Fig. 2). The reason for this is because the mass

Table I. GC and GC-MS characteristics of TxB_2 and its urinary catabolite

	TxB_2		Urinary Catabolite	
Derivative	R_T* (C-value)	Characteristic Peak** m/z	R_T* (C-value)	Characteristic Peak** m/z
MeTMS	25.0	256	21.1	202
MeMOTMS	24.8	301	22.1	301
Me-d_3MOTMS	24.8	301	22.1	301
MeMOTBDMS	29.2	385 (698)	27.0	385 (644)
Me-d_3MOTBDMS	29.2	385 (701)	27.0	385 (647)

* 3% OV-1 on Gas Chrom Q
** Hewlett-Packard quadrupole GC-MS

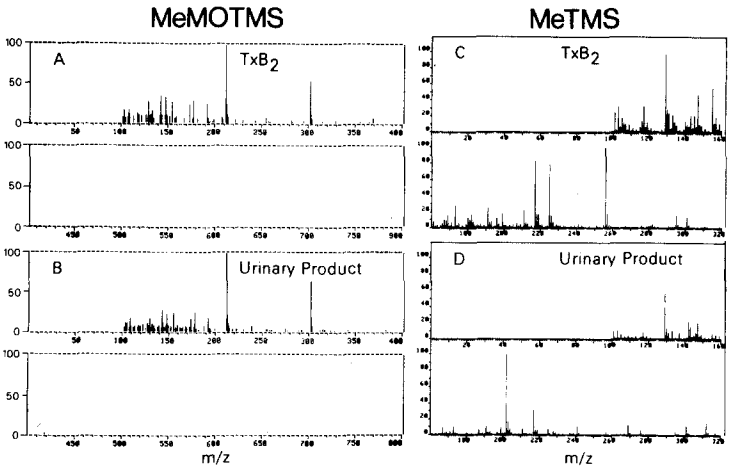

Figure 2. Comparison of partial mass spectra of two derivatives of TxB_2 and the purified urinary catabolite. Mass spectra A,B were recorded on a Hewlett-Packard Model 5992 GC-MS; C and D were recorded on a Model 5985A GC-MS.

spectral fragmentation of TxB_2 is dominated by a cleavage of the C8-C12 bond i.e. m/z 301 and 211 (301-TMSiOH) (4). Confirmation of this interpretation was obtained from the corresponding d_3-MO derivative which also gave prominent peaks at m/z 301 and 211 for both TxB_2 and the urinary catabolite indicating that these peaks lacked the oxime functional group. The MeMOTBDMS derivative was also quite uninformative in general, although some structural information could be derived from the high end of the spectrum since TBDMS derivatives normally show a significant M-57 fragment due to loss of the t-Bu group. While TxB_2 shows an M-57 fragment ion at m/z 698, this fragment was shifted to m/z 644 in the catabolite providing the first positive suggestion that the catabolite was a tetranor derivative of TxB_2. Additional confirmation of these fragments was obtained with the d_3-MO derivative where the corresponding M-57 fragments of both TxB_2 and the urinary catabolite were shifted upscale by 3 mass units (Table 1).

The most striking evidence that the urinary catabolite of TxB_2 was the tetranor derivative was obtained from mass spectral analysis of the MeTMS derivative. Fragmentation of TxB_2 MeTMS is directed towards cleavage of the C8-C12 bond and C9-C10 bond giving a prominent ion at m/z 256 due to the

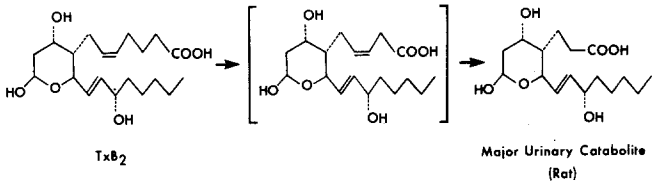

Figure 3. Scheme showing the pathway of catabolism of TxB_2 in the rat. Product in parenthesis is the major product in the human and primate but is undetectable in our study in the rat.

C1-C9 portion of the molecule (5). The corresponding fragment in the urinary catabolite occurs at m/z 202 i.e. 54 mass units less than TxB_2. Thus the urinary catabolite contained C_4H_6 less than TxB_2 in the α portion of the molecule i.e. tetranor TxB_2. These mass spectra are shown in Fig. 2.

DISCUSSION

In this paper we report the identification of a new urinary catabolite of TxB_2 obtained after i.v. infusion of tritiated TxB_2 in the rat. This product is the major tritium-labeled catabolite detected by TLC or GC. Comparison of its mass spectra (three derivatives) with those of TxB_2 indicated that the urinary catabolite is tetranor TxB_2.

It is interesting to note that while the major catabolite of TxB_2 in man and the cymologous monkey appears to be dinor TxB_2 (1-3), we have not detected this product in the study reported here in the rat. Recent studies with $PGF_{2\alpha}$ have indicated that the rat seems to have a very active β-oxidation system excreting C16 and C14 catabolites (6). Thus dinor TxB_2, which should be the first product resulting from β-oxidation *in vivo*, is rapidly transformed further in the rat through an additional β-oxidation step to the tetranor derivative which is the major product excreted (Fig. 3). This is in sharp contrast to the *in vivo* catabolism of 6-keto $PGF_{1\alpha}$ in the rat, which stops at the dinor step (7,8). The facile existence of the latter product in a cyclic 6(9) lactol form could explain its lack of further degradation by the β-oxidation system (7).

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