IDENTIFICATION OF THE MAJOR URINARY METABOLITES OF 6-KETOPROSTAGLANDIN $F_{1\alpha}$ (6K-$PGF_{1\alpha}$) IN THE RAT

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Received July 18, 1977

Summary: Two main urinary metabolites of 6K-$PGF_{1\alpha}$ were isolated after intravenous injection of this compound into adult male Wistar rats. The structures were identified as: dinor 6K-$PGF_{1\alpha}$ and dinor ω-1-hydroxy 6K-$PGF_{1\alpha}$. The structures of these two novel products were identified by gas chromatography-mass spectrometry of the methyl and ethyl ester derivatives of the O-methyloxime trimethylsilyl derivatives and the methyl ester O-methyloxime t-Butyldimethylsilyl ether derivatives. These results indicate that the main pathway of metabolism of 6K-$PGF_{1\alpha}$ *in vivo* is via β-and ω-oxidation and not via the prostaglandin 15-hydroxydehydrogenase pathway in this species.

Introduction

The 6(9)-oxy cyclase pathway of arachidonic acid metabolism was originally described by Pace-Asciak and Wolfe with the isolation of two bicyclic products whose structures were shown to be: 6(9)-oxy-Δ^{7}-$PGF_{1\alpha}$* and 6(9)-oxy-Δ^{5}-$PGF_{1\alpha}$ (1). That this pathway represented an additional terminal pathway of prostaglandin endoperoxide metabolism was demonstrated later (2). These studies led to the identification of another stable product in this pathway i.e. 6K-$PGF_{1\alpha}$ (3,4). Recently an unstable and biologically active precursor to 6K-$PGF_{1\alpha}$ was isolated whose structure was proven identical to the chemically synthetic product, 6(9)-oxy-Δ^{5}(Z)-$PGF_{1\alpha}$ also known as prostacyclin and now referred to as PGI_2 (5,6).

The relative expression of the diverse terminal pathways of prostaglandin endoperoxide metabolism differs with each organ. The 6(9)-oxy cyclase pathway is the major pathway in the stomach (7), ductus arteriosus (8), arterial blood vessels (9) and the uterus (10); its distribution relative to the other

*PG=prostaglandin; K=keto

ISSN 0006-291X

terminal pathways in several rat organs has recently been described (11).

Because of the ubiquity of this pathway and the occurrence of 6K-$PGF_{1\alpha}$ in rather large amounts (e.g. stomach fundus and arterial blood vessels), we investigated the metabolism of this compound *in vitro* and *in vivo*. Results *in vitro* have appeared elsewhere (12); we report here the isolation and structures of two major urinary products formed after intravenous administration of low specific activity 6K-$PGF_{1\alpha}$ into adult male rats.

Materials and Methods

Tritium-labelled 6K-$PGF_{1\alpha}$ was prepared from 5,6,8,9,11,12,14,15 - octatritiated arachidonic acid (New England Nuclear, specific activity = 72 Curies/ m mole) using microsomal fractions (100,000 x g pellet) of the rat stomach fundus. The preparation and purification of this compound has already been described (7). For the following experiments it was diluted with unlabelled compound to a specific activity 3.36 m Curies/ m mole. Bolus injections (100 µl) of a 2.70 mM solution in saline were given to each rat every 10 minutes for 50 minutes.

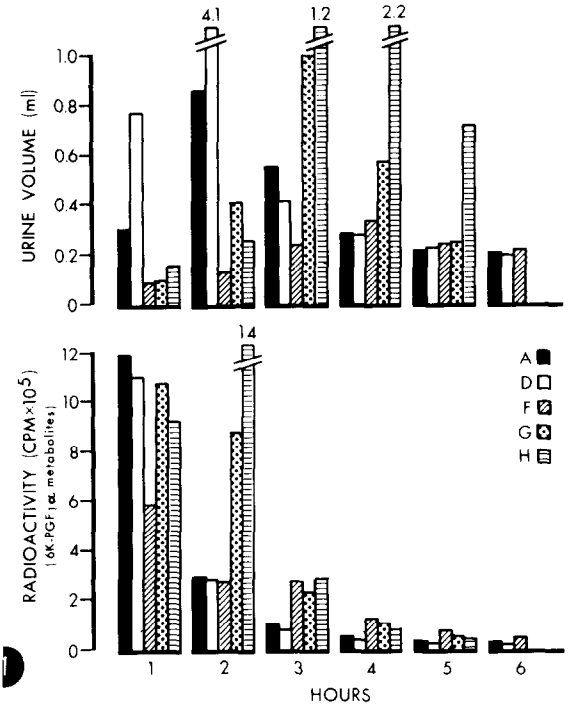
Five adult male Wistar rats (250 - 300 g) were anesthetised with INACTIN (100 mg/kg). Polyethylene catheters were inserted into the carotid artery (for continuous recording of blood pressure during the experiment) and jugular vein (for sample injection). Urine was collected continuously via a cannula inserted into the bladder. Urine volume was estimated by weight; each sample was immediately diluted with twenty volumes of dry and distilled methanol and taken to complete dryness *in vacuo*.

Purification. The methanol extracts were pooled and purified on thick layer chromatography (Silica gel G, 0.5 mm thickness) using as solvent chloroform-methanol-acetic acid-water (90/9/1/0.65 v/v). After development for 2 hours, radioactive products were localised by scanning each plate on a Panax radiochromatogram scanner. Three zones of radioactivity were scraped off i.e. R_fI = 0.43; R_fII = 0.16; R_fIII = 0.25 (R_f $PGF_{1\alpha}$ = 0.14; PGE_1 or 15K-$PGF_{1\alpha}$ = 0.30; 6K-$PGF_{1\alpha}$ = 0 .25). Each zone was eluted with methanol, filtered and taken to dryness.

Derivatives. Methyl esters and ethyl esters were prepared (10 min. 23°) with diazomethane and diazoethane respectively as described previously (4). These were generated freshly from the respective N-methyl and N-ethyl nitrosoguanidines (Aldrich). O-methyloximes were prepared with MOX reagent (Pierce), overnight at 23°. Trimethylsilyl ethers were prepared with TRI SIL Z (Pierce), 5 minutes at 60°. t-Butyldimethylsilyl ethers were prepared by heating the MeMO derivatives with the appropriate silyl chloride in imidazole (Applied Science Labs), 20 min. at 60°. Derivatives were assayed by gas chromatography-mass spectrometry under conditions previously described (4).

Results

Changes in urine volume and total urinary radioactivity in hourly samples after bolus i.v. injections of 6K-$PGF_{1\alpha}$ in five rats are shown in Figure 1.



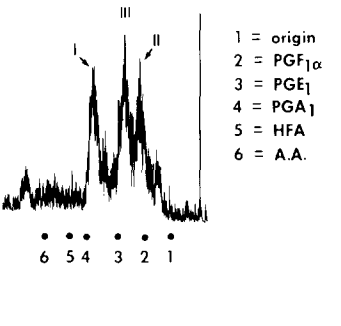


Figure 1. Changes in urine volume and total urinary radioactivity with time following intravenous injection of $^{3}H_{7}$-6K-$PGF_{1\alpha}$ in five rats.

Figure 2. Thin layer radiochromatogram of the combined crude methanol urinary extracts from Rat A (see Fig. 1).

Although considerable variation in urine volume was observed for each animal, the excreted radioactivity was generally highest (except in Rat H) within the first hour and decreased progressively with time. Sampling was continued up to six hours when 30 - 50% of the injected radioactivity was recovered in the urine. The thin layer chromatographic profile of the crude methanol urinary extract (Rat A) relative to authentic standards is shown in Figure 2. Three major radioactive peaks were observed. These were purified, derivatised and analysed by mass spectrometry.

Peak I. The relative retention time of the MeMOTMS* derivative, 23.5

*Me=methyl; Et=ethyl; MO=O-methyloxime; TMS=trimethylsilyl; TBDMS=tertiary butyl dimethylsilyl.

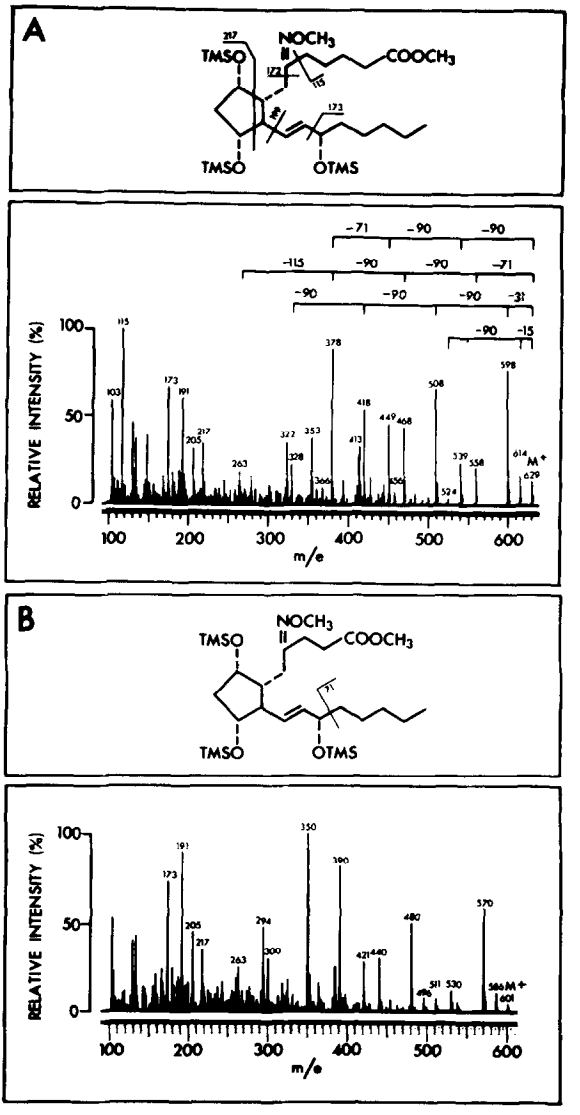


Figure 3 Mass spectra of A) authentic 6K-$PGF_{1\alpha}$ and B) urinary metabolite I as the MeMOTMS derivatives.

carbons, indicated loss of two carbon atoms from the parent compound (retention time 6K-$PGF_{1\alpha}$ = 25.2 carbons). The dinor nature of this product was confirmed by comparison of its mass spectrum (Fig. 3B) with that of starting material (Fig. 3A). Identical fragmentation patterns were observed in the high end of the mass spectrum except that the metabolite (Fig. 3B) displayed fragments 28

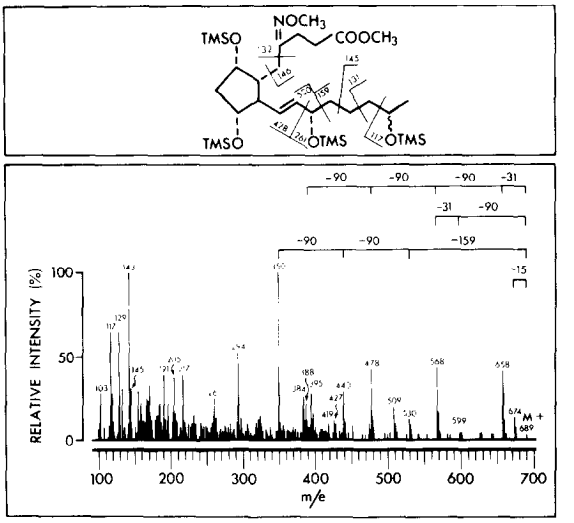


Figure 4. Mass spectrum of the MeMOTMS derivative of urinary metabolite II.

more, the prominent fragment at m/e 115 in 6K-$PGF_{1\alpha}$ was missing from the mass spectrum of the metabolite. The metabolite in peak I is therefore dinor 6K-$PGF_{1\alpha}$. The mass spectra of the EtMOTMS as well as the MeMOTBDMS derivatives were consistent with the proposed structure.

Peak II. This compound was more polar on TLC than 6K-$PGF_{1\alpha}$ indicative of additional oxidation. The MeMOTMS derivative had a retention time of 25.9 carbons. The mass spectrum showed a molecular ion at m/e 689 from which losses of 15 (Me), 31 (MeO) and 90 (TMSOH) were very prominent (Fig. 4). The second most intense peak in the spectrum (m/e 350) could be derived through a loss of 159 i.e. the terminal C_5H_{11} plus two TMS groups. The corresponding fragment at m/e 173 in 6K-$PGF_{1\alpha}$ was shifted in this metabolite to m/e 261 due to an additional TMS group in the terminal portion of the molecule. The high intensity of fragments at m/e 117, 129 ,143 (base peak) and 145 are consistent with the earlier observation of the powerful fragmentation-directing properties of a mass units (2 carbon atoms) lower than those for 6K-$PGF_{1\alpha}$ (Fig. 3A). Further-

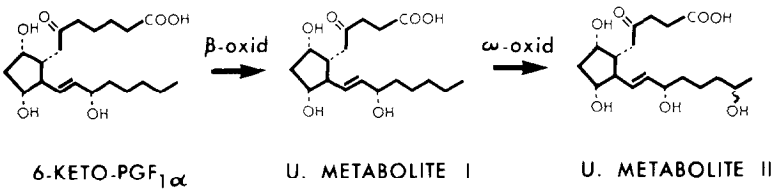


Figure 5. Scheme showing proposed structures of the major urinary metabolites of 6K-$PGF_{1\alpha}$ identified in this study.

hydroxyl group in the ω-1 position (13). The mass spectrum of the EtMOTMS and MeMOTBDMS derivatives supported the proposed structure of material in Peak II as dinor ω-1- hydroxy-6K-$PGF_{1\alpha}$.

Peak III. The mass spectrum of the material in Peak III (MeMOTMS) was indistinguishable from starting material, 6K-$PGF_{1\alpha}$.

Discussion

We have recently shown that 6K-$PGF_{1\alpha}$ is a relatively poor substrate for the rat renal 15-hydroxydehydrogenase *in vitro* (12). This suggested that its metabolism *in vivo* might provide uniquely different products than those reported for the primary prostaglandins and that possibly this compound might be excreted intact in the urine. *In vivo* 6K-$PGF_{1\alpha}$ is metabolised into two major products resulting from β and ω-1 oxidation which are excreted into the urine. The metabolic scheme is shown in Figure 5. We could find no evidence from this study which indicated that oxidation at the ω-position to the corresponding dioic acids also takes place.

It was quite surprising to find a considerable amount of the administered 6K-$PGF_{1\alpha}$ intact in the urine. This suggests that this product might normally go through the intact liver and kidney without metabolism. The unique property of this compound to exist in the cyclic hemi-ketal form might account for its decreased rate of metabolism (4,12).

Acknowledgement

This work was supported by a grant to C.P-A. from the Medical Research Council of Canada (MA-4181). Unlabeled prostaglandins were generously provided by Dr. Udo Axen, the Upjohn Co., Kalamazoo, Michigan. All mass spectra were recorded by Mr. Lajos Marai on a Varian MAT CH-5 coupled gas chromatograph-mass spectrometer, an MRC regional facility at the Best Institute, University of Toronto.

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