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Note

Capillary column gas—liquid chromatographic—mass spectrometric assay for 7α-hydroxy-5,11-diketotetranorprostane-1,16-dioic acid, the major human urinary metabolite of prostaglandins E_1 and E_2*

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Prostaglandins (PG's) and their involvement in disease states have been the subject of much study during the past decade. Unraveling their role in inflammation is a particularly active area of research, especially as anti-inflammatory drugs have been shown to be prostaglandin synthetase inhibitors [1]. Significant progress in such areas of biology is often highly dependent upon the development of methods for assay of the involved biochemical agents. Hamberg and Samuelsson [2] have shown that the major human urinary metabolite of PGE_1 and PGE_2 is 7α-hydroxy-5,11-diketotetranorprostane-1,16-dioic acid (PGM), and Hamberg [1] and Seyberth et al. [3] have demonstrated that administration to humans of therapeutic doses of drugs such as indomethacin and aspirin significantly reduces the urinary levels of PGM (presumably reflecting inhibition of PGE_1 and PGE_2 biosynthesis). These authors employed the dimethyl ester-di-([$^2H_3$]methyloxime) of tritium-containing PGM as the internal standard in a selected ion monitoring (SIM) gas—liquid chromatographic—mass spectrometric (GLC—MS) assay. This deuterated derivatized species is introduced into the assay procedure after endogenous PGM has been partially purified and derivatized to its dimethyl ester—dimethyloxime. If possible, an internal standard should be added to a biological specimen at the beginning of an assay procedure. ^2H- and ^3H-labeled PGM has now been prepared by Rosegay and Taub [4]. We wish to report on the use of this doubly-labeled compound as the internal standard in the determination of human urinary PGM levels in patients with rheumatoid arthritis treated

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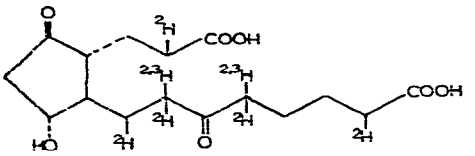
with indomethacin [5]. The procedure is based on those reported earlier [1, 3] with several exceptions, viz. (1) the internal standard is added to the urine prior to the initiation of the assay, and (2) capillary column GLC with splitless injection is employed with electron impact MS. Chemical ionization MS detection in combination with selective stationary phase packed column GLC is also utilized.

EXPERIMENTAL

Isolation procedure

Except for those aspects that involve the internal standard, the isolation procedure is essentially that used by Seyberth et al. [3]. The internal standard was labeled with tritium ($4.0 \cdot 10^5$ dpm/μg) to facilitate the isolation of the metabolite and deuterium (up to 10 ^{2}H atoms per mole).



The electron impact mass spectrum of this compound (as its dimethyl ester—dimethyloxime—TMS ether) shows the M—(90 + 31) fragment ion as base peak; the major species in this characteristic cluster is $^2H_7$, with no $^2H_0$ or $^2H_1$ ions present [6]. The urine sample (20 ml), after addition of 1 μg internal standard and adjustment to pH 4, is charged to an XAD-2 column (15 ml of resin), washed with an excess of water and eluted with ca 50 ml ethanol. After evaporation the residue is derivatized first with diazomethane (0.5 ml ethereal diazomethane) and secondly, with methoxylamine·HCl (15 mg per ml pyridine, overnight at room temperature). The residue after flash evaporation is subjected to reversed-phase partition chromatography on a column prepared by coating 4.5 g of silanized Hyflo-Supercel with 4 ml of stationary phase (lower phase of the equilibrated system 720 ml methanol, 480 ml water, 150 ml chloroform and 50 ml heptane). Radioactive monitoring of the eluate (5-ml cuts; mobile phase is the upper phase from the above-mentioned equilibrated system) is carried out and the 3 or 4 fractions of highest radioactivity content pooled. The residue after evaporation is subjected to thin-layer chromatography (silica gel G) with a developing solvent prepared by equilibrating ethyl acetate, trimethylpentane and water (2:1:2, upper phase). The zone of interest ($R_F \approx 0.50$), located by radioscanning, is eluted with diethyl ether; the diethyl ether solution is reduced to dryness and the residue derivatized with 20 μl of bis-trimethylsilyltrifluoroacetamide—pyridine (2:1) for 0.5 h at room temperature.

Instrumentation

A Finnigan Model 3200 GC—MS instrument is used for the measurements on the derivatized isolate (dimethyl ester—dimethyloxime—TMS ether; endogenous metabolite and internal standard). A 10 m × 0.25 mm SE-30 glass capillary column with splitless injection is used for the electron impact ionization

work; column temperature, 230°; carrier gas (helium) flow-rate, ca. 2 ml/min. Mass spectrometer operating conditions: ionizing potential 70 eV; emission current 0.8 mA; electron multiplier 1800 V. The chemical ionization data are obtained using a 5 ft. 3% OV-17 packed column at 260°; injection port temperature 270°; carrier gas flow-rate 15 ml/min and reagent gas methane (source pressure 1 Torr). Mass spectrometer operating conditions same as above except an ionizing potential of 150 eV. SIM is effected through use of the Finnigan 6110 data system (electron impact) and the Programmable Multiple Ion Monitor (PROMIM) accessory (chemical ionization) monitoring the M—(90 + 31) ions (electron impact) and MH^+ ions (base peak, chemical ionization) arising from the two species of interest. The resulting intensity ratio is then employed to calculate the urinary output of the metabolite per 24 h.

Calculation

$$\text{Amount of PGM per 24 h} = K\frac{I_{365}}{I_{372}} \times \frac{\text{Total 24-h urine volume (ml)}}{20\text{ ml}}$$

or

$$= K\frac{I_{457}}{I_{494}} \times \frac{\text{Total urine volume (ml)}}{20\text{ ml}}$$

where $K = 0.25$ (the fraction of the M—(90 + 31) or MH^+ ion cluster arising from the $^2H_7$ species).

A detection limit of 0.5 ng/ml urine (with a 20-ml urine aliquot) was indicated. Multiple (six) assays of a pooled 24-h urine (11.5 μg per 24 h) gave a coefficient of variation of 10.3%.

RESULTS AND DISCUSSION

Fig. 1 shows the electron impact ion response plots obtained with isolates (equivalent urine volumes) from a patient before administration of indomethacin (left panel) and while on the drug (right panel). With the capillary column at 230°, PGM was found to elute at scan No. 220 (4 min). The *m/e* 365 ion response at scan No. 210 does not interfere with the analysis because of the chromatographic resolution provided by the capillary column. It can be clearly seen that the *m/e* 365 ion response is markedly reduced following the administration of the drug. Glass capillary columns have been used by other workers for improved chromatographic resolution in the measurement of prostaglandins [7, 8] and thromboxane B_2 [9] (see also ref. 11).

To ascertain the effect of indomethacin on the excretion of PGM, three 24-urine collections were made on each patient: during the last days of the "washout period" (control), the "oral indomethacin period", and the "oral and suppository indomethacin period". Aliquots (20 ml) from each of these three one-day collections were added to vials containing 1 μg internal standard and stored at —17° prior to assay. The urine content (μg per 24 h) of PGM in a number of patients treated with indomethacin is shown in Table I. A wide range of control PGM levels was found among the patients, but in each case

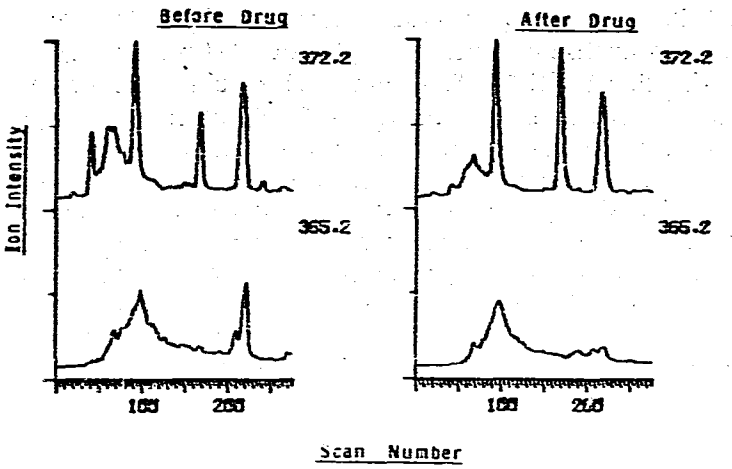


Fig. 1. Electron impact *m/e* 365 and 372 ion response plots obtained with isolates from a patient before administration of indomethacin (left panel) and while on drug (oral plus suppository; right panel).

TABLE I

URINARY OUTPUT (μg/24 h)* OF 7α-HYDROXY-5,11-DIKETOTETRANORPROSTANE-1,16-DIOIC ACID IN PATIENTS WITH RHEUMATOID ARTHRITIS TREATED WITH INDOMETHACIN ORALLY AND ORALLY PLUS SUPPOSITORY

Patient	Sex	Before drug		Oral**		Oral** and suppository***	
		EI§	CI§§	EI	CI	EI	CI
1	M	5.34	5.60	2.76	2.66	2.34	2.41
2	F	2.40	2.68	0.95	0.77	0.81	0.89
3	F	3.73	4.64	1.50	1.59	0.96	1.18
4	M	15.3	15.5	6.20	7.03	4.56	6.42
5	M	24.5	28.0	6.53	9.50	5.00	6.98
6	F	7.92	9.35	3.79	4.35	3.54	4.66
7	F	7.18	7.46	1.08	1.07	3.36	3.26
8	M	16.9	20.1	3.02	4.32	3.73	5.90
9	F	6.63	6.56	1.53	2.09	2.13	3.38
10	F	12.17	13.53	8.68	8.96	2.33	2.83

*Averaged result from two injections.
**25 mg 3 times daily for 3 weeks.
***100 mg each night for 3 weeks.
§ Electron impact.
§§ Chemical ionization.

the urinary output was reduced following administration of indomethacin. These results are similar to the findings of Hamberg [1] and the Vanderbilt group [3, 10] on the effect of oral administration of anti-inflammatory drugs upon urinary levels of PGM.

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