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Note

Quantitative determination of 6-keto prostaglandin $F_{1\alpha}$ in biological fluids by capillary gas chromatography—chemical ionization mass spectrometry

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Prostaglandin I_2 (PGI_2) was shown by Moncada et al. [1] to be a labile substance generated by arterial walls. The prostaglandin is a potent inhibitor of platelet aggregation and plays an important role in the processes of inflammation [2, 3]. This compound is unstable and has been shown to be rapidly decomposed non-enzymatically into 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) in biological fluids [4]. Therefore, it is important and necessary for the study of physiological and pathological roles of PGI_2 in tissues to establish a precise method for quantitative analysis of 6-keto $PGF_{1\alpha}$ in biological fluids.

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Many assay methods for the measurement of 6-keto $PGF_{1\alpha}$ and PGI_2 have been developed to date; for example, radioimmunoassay, bioassay using human platelet aggregation, and gas chromatography—electron impact mass spectrometry [5]. Of these methods, gas chromatography—electron impact mass spectrometry was the most accurate and reliable method for the determination of 6-keto $PGF_{1\alpha}$. However, the mass fragmentograms were easily interfered with by fragment ions from substances in biological samples.

Chemical ionization (CI) mass spectrometry has shown a great potential advantage in the determination of molecular weight and molecular structure of labile compounds [6, 7]. Because the CI mass spectra are much simpler, interfering substances in the samples could be reduced in the mass fragmentograms of prostaglandins. Recently we reported the qualitative analysis of 6-keto $PGF_{1\alpha}$ by CI mass spectrometry and showed the superiority of ammonia gas as a reagent gas [8].

In this paper, we will demonstrate the successful measurement of 6-keto $PGF_{1\alpha}$ in biological fluids by capillary gas chromatography—CI mass spectrometry.

MATERIALS AND METHODS

The following were obtained from commercial sources: N-methyl-N'-nitro-N-nitrosoguanidine (Aldrich, Milwaukee, WI, U.S.A.); O-methylhydroxylamine hydrochloride (Wako, Tokyo, Japan); trimethylsilyl (TMS) imidazole (Pierce, Rockford, IL, U.S.A.); bis-TMS-trifluoroacetamide (Applied Science Labs., State College, PA, U.S.A.); [^{3}H]6-keto $PGF_{1\alpha}$, 150 Ci/mmol (The Radiochemical Centre, Amersham, Great Britain).

6-Keto $PGF_{1\alpha}$ and 6-keto [5,8,9,11,12,14,15-$^2H_7$]$PGF_{1\alpha}$ (6-keto $PGF_{1\alpha}$-d_7) were kindly supplied by Ono Pharmaceutical Co., Osaka, Japan, and Toray Industries, Kamakura, Japan, respectively.

Preparation of sample from carrageenin-induced granuloma containing 6-keto $PGF_{1\alpha}$

Granuloma was induced in male Sprague—Dawley strain rats weighing 150—180 g by the method described in a previous paper [9]. Exudate of 4-day-old granuloma (4—8 ml) was collected in a glass tube containing indomethacin to give a final concentration of 2×10^{-5} *M* and 200 ng of 6-keto $PGF_{1\alpha}$-d_7. The sample was acidified to pH 3.0 with 1 *N* HCl and was extracted with 8 volumes of ethyl acetate twice. The resulting organic phase was evaporated to dryness under reduced pressure. Residues were dissolved in a small amount of ethanol and applied quantitatively to thin-layer chromatography (TLC) plates (HPTLC plates, silica gel 60; E. Merck, Darmstadt, G.F.R.). The plates were developed first with a solvent system of methylene chloride—diethyl ether—petroleum ether (b.p. 30—60°C) (1:1:1, v/v), to remove neutral fatty acid, and then developed successively with solvent C which was the organic phase of ethyl acetate—2,2,4-trimethylpentane—acetic acid—water (11:5:2:10, v/v). The silica gel corresponding to migrated 6-keto $PGF_{1\alpha}$ was scraped off and the prostaglandin was extracted with methanol—chloroform (1:1, v/v). The extract was applied to a TLC plate and the plate was developed

again with solvent C. The silica gel corresponding to 6-keto $PGF_{1\alpha}$ was scraped off and the prostaglandin was extracted with methanol. Recovery of 6-keto $PGF_{1\alpha}$ throughout the entire procedure was estimated by using parallel exudate samples to which 0.1 μCi of [^{3}H]6-keto $PGF_{1\alpha}$ had been added at the beginning of the procedure. The overall recovery of 6-keto $PGF_{1\alpha}$ was 82%.

Preparation of the derivatives for gas chromatography—mass spectrometry

The samples were methylated with diazomethane which was freshly prepared from N-methyl-N'-nitro-N-nitrosoguanidine by the method of Fales et al. [10], converted to methoxime derivatives with O-methylhydroxylamine hydrochloride and finally trimethylsilylated with a mixture of TMS imidazole and bis-TMS-trifluoroacetamide as described previously [8].

Gas chromatography—mass spectrometry

A Shimadzu-LKB Model 9000A equipped with a CI source was used. The data processing system included a GC-MS-PAC 300 DG consisting of an Okitac 4300 mini-computer with 16 K core and a magnetic disk.

A glass capillary column (30 m × 0.3 mm I.D.) coated with SE-30 was used. The column with a solventless injection device [11] was connected to the gas chromatograph—mass spectrometer. The temperatures of column, injection port and ionization chamber were kept at 270°C, 300°C and 270°C, respectively. The flow-rate of helium gas was 30 ml/min. The CI mass spectra were obtained at an electron energy of 500 eV, an emission current of 400 μA and an accelerating voltage of 3.5 kV. Ammonia was used as a reagent gas at 0.8 Torr.

RESULTS AND DISCUSSION

The CI mass spectrum of 6-keto $PGF_{1\alpha}$, using ammonia as a reagent gas, is shown in Fig. 1A. The quasi-molecular ion (QM^+) was recorded at *m/e* 630 with weak intensity. The fragment ion at *m/e* 598 was presumably due to [QM^+—CH_3OH]. The ions at *m/e* 540, 450 and 360 were formed by successive eliminations of trimethylsilanol from QM^+ (*m/e* 630). The base peak was the ion at *m/e* 540. As demonstrated in Fig. 1B, the ion at *m/e* 547 was the base peak in the CI mass spectrum of 6-keto $PGF_{1\alpha}$-d_7. Therefore, quantification of 6-keto $PGF_{1\alpha}$ by mass fragmentography was done to trace the peaks of *m/e* 540 and 547. The quantitative analysis was made by measuring the peak height ratio of the ions at *m/e* 540 and 547. The calibration curve was linear from 40 to 400 pg of 6-keto $PGF_{1\alpha}$.

Fig. 2 shows mass fragmentograms of 6-keto $PGF_{1\alpha}$ in the exudate of rat carrageenin-induced granuloma and human plasma. In both samples there was practically no interference from endogenous substances in the biological fluids. The content of 6-keto $PGF_{1\alpha}$ in exudate of rat carrageenin-induced granuloma was 17.3 ± 6.5 ng/ml (n = 5). For human plasma, the study was made in triplicate, 6-keto $PGF_{1\alpha}$ being extracted from 50 ml of plasma each time (taken from a healthy male volunteer aged 25 years) using the same procedures as for the granuloma exudate. The content of 6-keto $PGF_{1\alpha}$ in human plasma was 400 ± 85 pg/ml (n = 3).

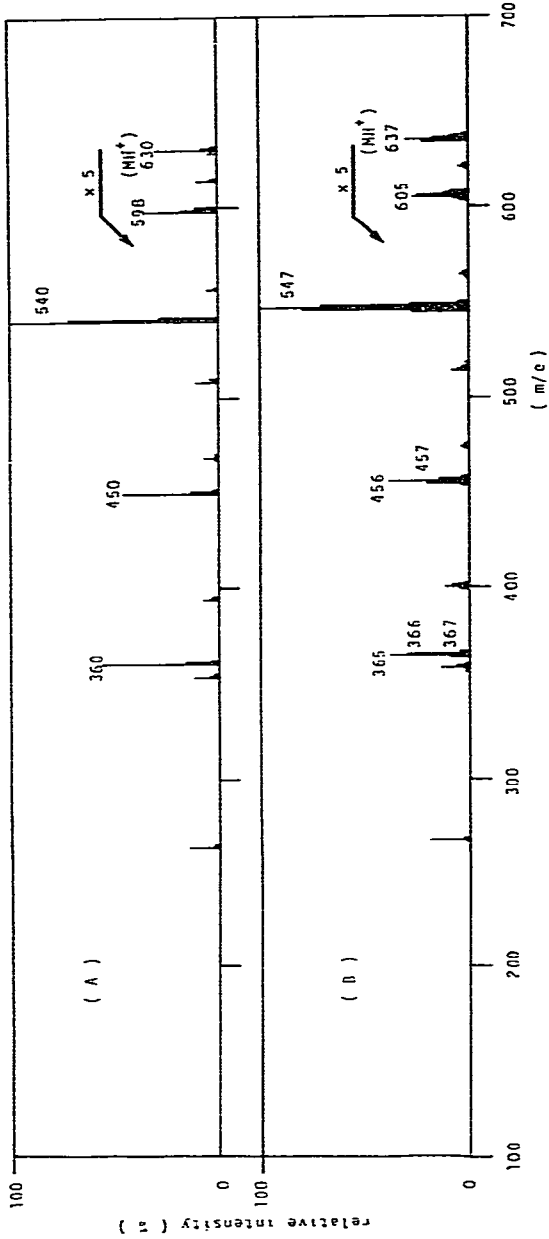

Fig. 1. Ammonia CI mass spectra of the methyl ester—methoxime—TMS derivatives of 6-keto $PGF_{1\alpha}$ (A) and 6-keto $PGF_{1\alpha}$-d_7 (B).

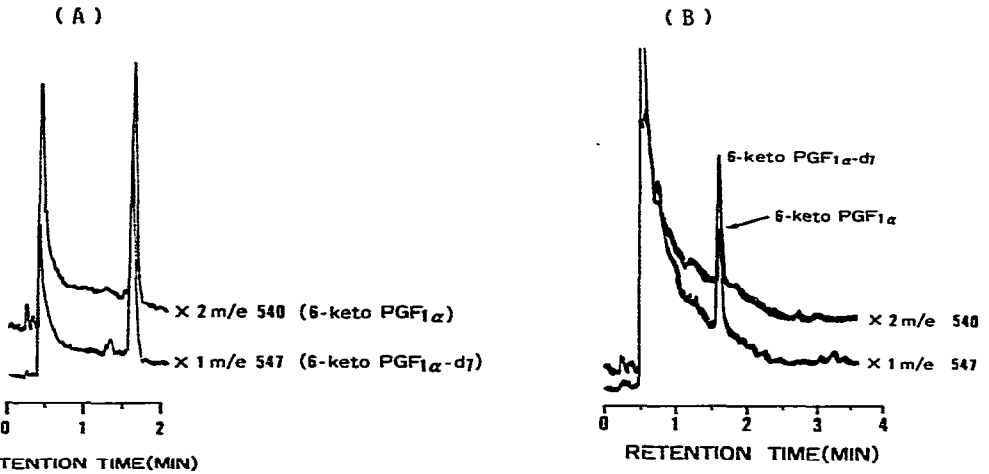

Fig. 2. Mass fragmentograms of the methyl ester—methoxime—TMS derivatives of 6-keto $PGF_{1\alpha}$ from the exudate of rat carrageenin-induced granuloma (A) and human plasma (B).

Further efforts have been made in our laboratory to reduce interfering substances and to increase the recovery of 6-keto $PGF_{1\alpha}$ by using high-performance liquid chromatography. The procedure will contribute to minimizing the starting volume of plasma. The level of 6-keto $PGF_{1\alpha}$ in carrageenin-induced granuloma was similar to the data of Ohuchi et al. [12], measured by radioimmunoassay.

CI mass spectrometry has recently been applied to the structural elucidation and quantitative determination of various biological compounds in terms of increased specificity and sensitivity [6, 7]. Especially ammonia CI mass fragmentography should be a most specific method for the determination of prostaglandins because ammonia CI mass spectra are most simple [6] and interference from other biological compounds could be minimized. Recently, we reported the CI mass spectrum of the methoxime—TMS derivative of 6-keto $PGF_{1\alpha}$ and obtained the molecular ion as the base peak [8]. However, the detection limit with this derivative was found to be approximately 100 times higher than with the methyl ester—methoxime—TMS derivative.

Fitzpatrick [13] reported that glass capillary columns could be used for prostaglandin separation and that the detection limit using such columns was about ten times lower than that obtained with packed columns. For this reason we successfully devised an assay method for 6-keto $PGF_{1\alpha}$ by capillary gas chromatography—ammonia CI mass fragmentography as its methyl ester—methoxime—trimethylsilyl derivative. As demonstrated by the results, the detection limit was decreased to 40 pg.

The work to minimize plasma sample volume is currently under way in our laboratory with the aim of studying plasma 6-keto $PGF_{1\alpha}$ content in cerebrovascular and cardiovascular diseases.

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