DEVELOPMENT AND MASS SPECTROMETRIC EVALUATION OF A RADIOIMMUNOASSAY FOR 9α, 11α-DIHYDROXY-15-KETOPROST-5-ENOIC ACID

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1. Introduction

Several radioimmunoassays for prostaglandins have been developed recently [1–6]. Using these methods analyses of concentrations of primary prostaglandins in peripheral venous blood, in particular $PGF_{2\alpha}$**, have been carried out. However, the endogenous levels of $PGF_{2\alpha}$ obtained have not been verified by other methods. Studies on the production rate of $PGF_{2\alpha}$ under normal conditions and of plasma prostaglandins in humans during continuous infusion of $PGF_{2\alpha}$ have recently been reported [7–9]. The data from these mass spectrometric studies indicated that the reported endogenous plasma levels of $PGF_{2\alpha}$ obtained by radioimmunoassay might be by far too high. It was also recently demonstrated that PGE_2 and $PGF_{2\alpha}$ administered intravenously are rapidly converted into 11α-hydroxy-9, 15-diketoprost-5-enoic acid and 9α, 11α-dihydroxy-15-ketoprost-5-enoic acid, respectively [10, 11]. The mass spectrometric studies referred to above also showed that the latter keto-metabolite occurs in plasma in 20–30-fold higher concentration than the administered $PGF_{2\alpha}$. It therefore seems that monitoring of $PGF_{2\alpha}$ production might best be achieved by measuring the keto metabolite in plasma. An additional reason for this approach is that extensive conversion of prostaglandins to 15-keto-13, 14-dihydroderivatives occurs in many tissues.

The present report describes the development of a radioimmunoassay for 9α, 11α-dihydroxy-15-keto-prost-5-enoic acid. The method has been evaluated by quantitative mass spectrometry using the deuterium carrier technique [12].

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2. Experimental

[9β-^{3}H]9α, 11α-Dihydroxy-15-ketoprost-5-enoic acid, 10 mg, specific activity 0.2 mCi/mmole, was prepared by incubation of [9β-^{3}H]$PGF_{2\alpha}$ with the high speed supernatant from a swine kidney homogenate [13]. [17, 18-$^3H_2$]9α, 11α-Dihydroxy-15-ketoprost-5-enoic acid, 70 μCi, specific activity about 50 Ci/mmole, was prepared in the corresponding way from [17, 18-$^3H_2$]$PGF_{2\alpha}$, obtained as described earlier [14].

Ten mg of [9β-^{3}H]9α, 11α-dihydroxy-15-keto-prost-5-enoic acid was coupled with 40 mg of bovine serum albumin in the presence of 10 mg of 1-ethyl-3 (3-dimethylaminopropyl) carbodiimide [3]. By analysis of the tritium content of a small amount of the dialyzed and lyophilized material it was established that approx. 9 molecules of the prostaglandin metabolite was coupled to each BSA molecule.

The conjugate was dissolved in distilled water to a concentration of 5 mg/ml and was emulsified with an equal volume of Freund's complete adjuvant. Two rabbits were injected subcutaneously with the emul-

** *Abbreviations:*
Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$): 9α, 11α, 15-trihydroxyprosta-5, 13-dienoic acid; prostaglandin E_2 (PGE_2): 11α, 15-dihydroxy-9-ketoprosta-5, 13-dienoic acid; prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$): 9α, 11α, 15-trihydroxyprost-13-enoic acid; prostaglandin E_1 (PGE_1): 11α, 15-dihydroxy-9-ketoprost-13-enoic acid; prostaglandin A_2 (PGA_2): 15-hydroxy-9-ketoprosta-5, 10, 13-trienoic acid; prostaglandin B_2 (PGB_2): 15-hydroxy-9-ketoprosta-5, 8 (12), 13-trienoic acid; BSA: bovine serum albumin.

sion along the flank area once a week for four weeks. Two weeks after the last injection the animals were bled for the first time and then at weekly intervals. A detectable antibody titer was present in both cases at the first bleeding. The highest titers were obtained 10–12 weeks after beginning of the immunization, after which the titers gradually declined.

Anti-rabbit γ-globulin antibodies were simultaneously generated in a goat which was given weekly subcutaneous injections of rabbit γ-globulin for four weeks and then at monthly intervals.

The assay was carried out as follows: To 0.5 ml of a rabbit serum dilution (a dilution of 1:1000 gave a 65% binding of added ^{3}H-labelled compound) were added 0.1 ml of prostaglandin standard or sample and 0.1 ml of [17, 18-$^3H_2$]9α, 11α-dihydroxy-15-ketoprost-5-enoic acid (about 10,000 cpm). All solutions were made in 0.05 M Tris buffer, pH 7.8. After 3 hr at room temp. 0.1 ml of a 1:10 dilution of goat anti-rabbit γ-globulin serum was added and the tubes were kept for 12 hr at +4°. The formed precipitate was removed by centrifugation and 0.5 ml of the clear supernatant was assayed for radioactivity.

Serum samples, 0.5 ml, were extracted once with 0.5 ml of pentane, which was discarded, and subsequently acidified to pH 3 with 1 N HCl and extracted twice with 1 ml of ethyl acetate. The combined ethyl acetate phases were washed once with 1 ml of water and evaporated to dryness. The residue was taken up in 0.5 ml of 0.05 M Tris buffer, pH 7.8. Prior to extraction of the serum sample a small amount of [17, 18-$^3H_2$]9α, 11α-dihydroxy-15-ketoprost-5-enoic acid was added, and thus the recovery of the compound could be measured (generally 75–85%).

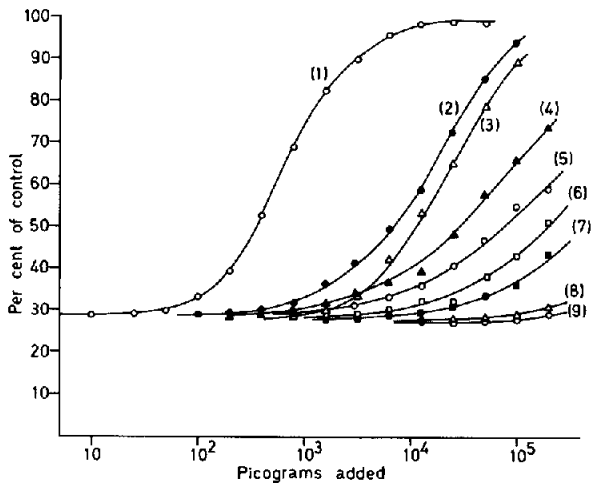

Fig. 1. Specificity of 9α, 11α-dihydroxy-15-ketoprost-5-enoic acid antiserum. Compounds tested were: (1) 9α, 11α-dihydroxy-15-ketoprost-5-enoic acid (15-dehydro-13, 14-dihydro-$PGF_{2\alpha}$; (2) 9α, 11α-dihydroxy-15-ketoprosta-5, 13-dienoic acid (15-dehydro-$PGF_{2\alpha}$); (3) 9α, 11α, 15-trihydroxyprost-5-enoic acid (13, 14-dihydro-$PGF_{2\alpha}$); (4) $PGF_{2\alpha}$; (5) $PGF_{1\alpha}$; (6) PGE_2; (7) PGE_1; (8) PGA_2; (9) PGB_2.

3. Results and discussion

Standard curves obtained with the method described above showed significant differences between 50 and 100 picograms of the compound (fig. 1). When other prostaglandins or prostaglandin metabolites were used to replace the unlabelled compound in the assay, the antiserum was found to cross-react to some extent only with 9α, 11α-dihydroxy-15-ketoprosta-5, 13-dienoic acid, 9α, 11α, 15-trihydroxyprost-5-enoic acid, and $PGF_{2\alpha}$ (fig. 1 and table 1).

Table 1
Specificity of antiserum directed against 9α, 11α-dihydroxy-15-ketoprost-5-enoic acid.

Prostaglandin	Picograms required to displace 50% of bound ^{3}H-9α, 11α-dihydroxy-15-keto-prost-5-enoic acid	Relative cross reaction (%)
9α, 11α-dihydroxy-15-ketoprost-5-enoic acid (15-dehydro-13, 14-dihydro-$PGF_{2\alpha}$)	600	100.0
15-Dehydro-$PGF_{2\alpha}$	16,000	3.8
13, 14-Dihydro-$PGF_{2\alpha}$	24,000	2.5
$PGF_{2\alpha}$	90,000	0.7
$PGF_{1\alpha}$	280,000	0.2
PGE_2	>500,000	< 0.1
PGE_1	>500,000	< 0.1
PGA_2	>500,000	< 0.1
PGB_2	>500,000	< 0.1

The assay was carried out as described in the Experimental section.

Structural alterations in the ring were easily recognized by the antiserum.

Serum samples that were analyzed for concentra-

tions of 9α, 11α-dihydroxy-15-ketoprost-5-enoic acid with this method were purified only by extraction and were not subjected to separation into different classes of prostaglandins by chromatographic procedures, due to the low degree of cross reactivity with compounds of the E-type and also with $PGF_{1\alpha}$ and $PGF_{2\alpha}$. 9α, 11α-Dihydroxy-15-ketoprost-5-enoic acid has been shown to be by far the most abundant metabolite of $PGF_{2\alpha}$ in serum, and other metabolites of $PGF_{2\alpha}$ are therefore not likely to interfere appreciably with the assay.

Table 2 shows the results obtained in an experiment where known amounts of 9α, 11α-dihydroxy-15-ketoprost-5-enoic acid were added to serum samples from a female subject prior to extraction. The obtained values have been corrected for recovery in each case. The concentration of the metabolite in serum prior to addition of the compound was 0–0.24 ng/ml according to this experiment. Additional results on normal blood levels will be reported later.

Table 2

Radioimmunoassay of known amounts of 9α, 11α-dihydroxy-15-ketoprost-5-enoic acid added to serum from a female subject.

Amount added (ng) per ml of serum	Serum level measured (ng/ml)	Mean ± SD
0	0	
0	0.24	
0.20	0.28	
0.20	0.34	
0.80	0.78	
0.80	0.88	
3.20	3.48	
3.20	3.02	
12.80	13.80	13.66 ± 1.04
12.80	13.60	
12.80	12.80	
12.80	13.20	
12.80	14.40	
12.80	14.20	
25.60	23.60	
25.60	28.40	

For a further evaluation results obtained with this method were in some cases compared with those obtained by a gas chromatographic–mass spectrometric method (based on addition of deuterium labelled carrier to the sample, cf. [8, 12, 15]. Six patients were given $PGF_{2\alpha}$ by intravenous infusion for several hours. Serum samples were collected prior to administration and after 3 hr of infusion, and these samples were extracted and analyzed by radioimmunoassay as described above.

The preinfusion values were in all cases less than 0.5 ng/ml serum using both methods. A comparison between the analyses of the three-hour samples is shown in fig. 2. As can be seen, the results obtained by the two methods compare favourably, however, the radioimmunoassay values were consistently somewhat lower.

The radioimmunoassay described thus provides for the first time a rapid and accurate method for analysis of the major metabolite of $PGF_{2\alpha}$, 9α, 11α-dihydroxy-15-ketoprost-5-enoic acid, in large numbers of plasma or serum samples. This will be of importance for studies of the physiological role of $PGF_{2\alpha}$ and also for pharmacokinetic studies.

Acknowledgements

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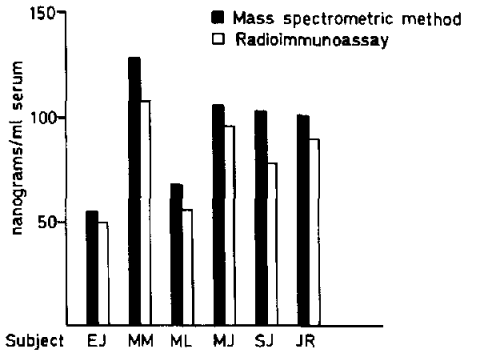

Fig. 2. Comparison between quantitative mass spectrometry and radioimmunoassay for 9α, 11α-dihydroxy-15-ketoprost-5-enoic acid. The serum samples were obtained from six patients receiving intravenous infusion of $PGF_{2\alpha}$, 75 μg/min.

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