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DIRECT DETERMINATION OF URINARY 6-KETO-PROSTAGLANDIN-$F_{1\alpha}$ BY A NEW RADIOIMMUNOASSAY METHOD

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Clinical testing of a new RIA kit for the determination of 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) has been performed. The statistical characteristics of the calibration curves, the intra- and interassay variation coefficients and the recovery studies point to the reliability and applicability of this test. Using this RIA-kit, the urinary 6-keto-$PGF_{1\alpha}$ content could be detected without applying a prior extraction procedure and chromatography, and the results obtained (urinary excretion of 6-keto-$PFG_{1\alpha}$ of control subjects and of patients with chronic glomerular diseases) corresponded well with various other methods.

Introduction

Prostanoids and their metabolites can be assayed in blood (serum or plasma) and in urine samples.[1] Several chemical methods and radioimmunoassays have been developed for their determination. Owing to the instability and rapid degradation of the prostanoids in vivo, the stable metabolites are now regarded as indicators of the biosynthesis of prostaglandins. Their routine determination is hindered by the fact that in the majority of assays the prostanoids have to be extracted and concentrated, usually they must be separated by chromatography[2] and direct assay can be performed only in the urine.

Prostacyclin (PGI_2) is a powerful vasodilator and an inhibitor of platelet aggregation in humans.[3] This prostanoid is not metabolised during its passage through the lungs, but it is rapidly hydrolyzed to 6-keto-$PGF_{1\alpha}$ under physiological conditions.[4,5] In addition to this, the biological activity of prostacyclin might be ex-

*The kit is available from the Institute of Isotopes of the Hungarian Academy of Sciences, H-1525, Budapest, P.O.B. 77, Hungary.

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tinguished by metabolism within the kidney and liver, resulting in metabolites other than 6-keto-$PGF_{1\alpha}$.[6] As 6-keto-$PGF_{1\alpha}$ is a stable hydrolysis product, its determination offers a convenient method for studying prostacyclin metabolism in man. This study was undertaken to test the clinical applicability of a new RIA method for the determination of 6-keto-$PGF_{1\alpha}$ which has been developed in the Isotope Institute of the Hungarian Academy of Sciences. Furthermore, our aim has been to assay the 6-keto-$PGF_{1\alpha}$ content of urine in controls and in patients with chronic glomerular diseases and to compare the data with the results of others, using different methods.

Materials and methods

For the determination of 6-keto-$PGF_{1\alpha}$ a new RIA kit was used (code-number: RK–16), which has been developed in the Isotope Institute of the Hungarian Academy of Sciences.* The assay was performed as recommended by the producer.

The kit contains the following reagents:

lyophilised antiserum (rabbit),

125-J-6-keto-$PGF_{1\alpha}$ (tracer),

phosphate buffer concentrate,

dextran-coated charcoal (DCC) suspension.

The steps of the assay are shown in Table 1. After measuring the radioactivity of the samples, the B/B_0% was calculated. By plotting B/B_0% against various standard concentrations, calibration curves were constructed on semilogarithmic graph paper. The amount of 6-keto-$PGF_{1\alpha}$ of each unknown sample was computed by interpolation from the standard curve.

Table 1
Protocol of the radioimmunoassay

Reagent	Standard	Tube sample	B_0	NSB	Total
Buffer	200	200	300	300	400
Standard	100	–	–	–	–
Sample	–	100	–	–	–
Tracer	100	100	100	100	100
Antiserum	100	100	100	–	–
	Vortex, incubate at +4 °C overnight				
Charcoal suspension	300	300	300	300	–
	Vortex, centrifuge at +4 °C 15–20 minutes at a minimum of 1000 g				

Volumes are expressed in microliters.

The cross-reaction and the optimal antibody concentration were checked in the Isotope Institute. The following objectives were set forth: (1) to compute the statistical characteristics of standard curves and to determine the non-specific binding (NSB); (2) to study the reliability of this method, to test the intra- and interassay variation coefficients; (3) to study the recovery of added 6-keto-$PGF_{1\alpha}$; (4) to determine the urinary content of excreted 6-keto-$PGF_{1\alpha}$ in control subjects and in patients with chronic glomerular diseases.

Results

Testing 20 kits, the computed data for the calibration curves are shown in Table 2. Fig. 1 demonstrates a typical calibration curve. Table 2 shows that the average binding at zero concentration was 31.7% (S.D. = 3.75). The range of

Table 2
Computed data for calibration curves using a RIA method for the determination of 6-keto-$PGF_{1\alpha}$

Concentration of 6-keto-$PGF_{1\alpha}$ pg/0.1 ml	Mean percentage of bound 6-keto-$PGF_{1\alpha}$ (n = 20)	S.D.	V.C.
500	5.04	0.6	11.0
250	9.39	1.0	10.7
125	17.55	20.6	11.7
62.5	28.74	2.3	8.2
31.25	44.97	2.6	5.8
16	62.09	4.3	6.9
8	78.62	4.0	5.0
4	89.43	2.5	2.8
2	94.61	2.8	2.9
0	31.72	3.8	11.8

Table 3
Data of logit-log lines

	Range	Mean (n = 20)
V. C.	1.6–7.0	3.3
Correlation coefficient	0.9307–0.9990	0.989
Tangent	−2.252 and −2.836	−2.4761
Slope ($180° - \alpha$)	66.06–70.58	67.93
Sensitivity (pg/0.1 ml)	0.6–5.5	1.9

binding varied from 5.04 to 94.61% between the extreme concentrations. Following logit-log plotting of the curves, statistical calculations were performed, too. These results are shown in Table 3, while Fig. 2 shows a logit-log line. The proper variation coefficients and the steep slope (68^0) of the logit-log line are to be emphasized. Non-specific binding (calculated against total activity) varied between

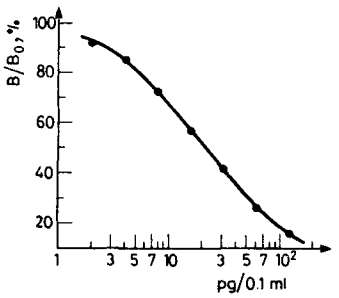

Fig. 1. 6-keto-$PGF_{1\alpha}$ standard curve

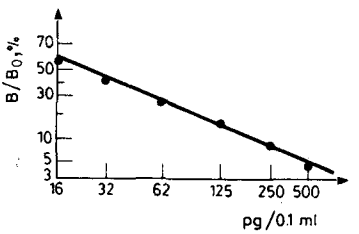

Fig. 2. logit-log line

0.02 and 0.58% (average mean: 0.22%), exceeding 0.50% only in a single case. If the calculation was performed versus zero concentration, the range of NSB ranged between 0.07 and 1.67% (average mean: 0.68%).

The reliability of the method was established by the intra- and interassay variation coefficients (Tables 4. and 5). Performing 50 tests the average mean of the intraassay variation coefficient was 8.5%. Data in detail are given in Table 4. Interassay variation coefficients were determined in seven instances. The mean value of interassay VC proved to be 12.7% (range: 1.37–26.17%).

To evaluate further the validity and accuracy of the RIA method our studies were completed with recovery assays. For this purpose a urine pool containing less than the minimum detectable concentration of 6-keto-$PGF_{1\alpha}$ (less than 2 pg/0.1 ml) was used. From this pool, after the addition of different quantities of

Table 4
Intraassay variation coefficients

Concentration range of 6-keto-$PGF_{1\alpha}$	Mean V. C.	Mean S. D.
3–100 pg/0.1 ml (n = 50)	8.5	2.1
3–20 pg/0.1 ml (n = 24)	9.4	1.2
20–50 pg/0.1 ml (n = 17)	9.6	3.2
50–100 pg/0.1 ml (n = 9)	3.9	2.5

6-keto-$PGF_{1\alpha}$, solutions of various concentrations (100, 50 and 10 pg/0.1 ml) were obtained. After this we determined the 6-keto-$PGF_{1\alpha}$ content of the different solutions. The results of the recovery experiments are shown in Table 6. Analysis of the specimens showed variable recoveries depending on the 6-keto-$PGF_{1\alpha}$ concentration of the solution.

Table 5
Interassay variation coefficients

Number of series	n	Mean concentration of 6-keto-$PGF_{1\alpha}$, pg/0.1 ml	S. D.	V. C.
1	3	5.65	0.8	14.9
2	4	7.72	0.6	7.4
3	3	14.55	0.2	1.4
4	5	17.55	2.2	13.0
5	4	23.82	4.8	20.3
6	5	25.09	5.5	26.1
7	5	51.63	2.9	5.7
	Mean:		2.6	12.7

Table 6
Recovery of added 6-keto-$PGF_{1\alpha}$ at various concentrations

Concentration of added 6-keto-$PGF_{1\alpha}$	n	Mean, pg/0.1 ml	S. D.
100 pg/0.1 ml	6	76.20	8.2
50 pg/0.1 ml	5	43.60	6.2
10 pg/0.1 ml	6	13.80	2.1

In subsequent studies we determined the urinary 6-keto-$PGF_{1\alpha}$ content of control hospitalized subjects. All persons remained supine during a 4-hour period of urine collection from 8.00–12.00 a.m. Fluids were withheld for the preceding 8 hours except 200 ml water given at 8.00 a.m. At 8.00 a.m. the subjects voided and during the urine collecting period no medication was given. After urine collections had been completed, aliquots were immediately frozen at –20 °C for subsequent 6-keto-$PGF_{1\alpha}$ assay. Knowing the total volume of the voided urine, we calculated the quantity of the excreted 6-keto-$PGF_{1\alpha}$. Table 7 shows the results. The mean of

the urinary 6-keto-$PGF_{1\alpha}$ content/4 hours was 50 ng is in the range: 36.5–70 ng in healthy subjects.

The urinary content of 6-keto-$PGF_{1\alpha}$ of hospitalized women with chronic glomerular diseases of different types was also determined. The serum creatinine of these patients exceeded 200 nmol/l, in the urine the excreted amount of Na varied between 100–200 mequ/day. In Table 7 it is shown that the urinary 6-keto-$PGF_{1\alpha}$ excretion of these patients was significantly less ($p < 0.001$) than that found in control subjects.

Table 7
Urinary 6-keto-$PGF_{1\alpha}$ content of control subjects and of patients with glomerular diseases

6-keto-$PGF_{1\alpha}$	Control subjects (n = 9)	Patients with chronic glomerular diseases (n = 17)
Mean ng/4 hours	50.22	6.12
Range	36.58–70.21	0–39.52
S. D.	9.5	11.6
95% confidence limits	42.89–57.55	0.17–8.07

Discussion

6-keto-$PGF_{1\alpha}$ is a stable hydrolysis product of PGI_2. About 16% of radiolabeled PGI_2 infused into the renal artery can be recovered as 6-keto-$PGF_{1\alpha}$ in urine.[1,8]

PGI_2 seems to be the major cycloxygenase product in human glomeruli[7,9] and the bulk of evidence suggests that urinary 6-keto-$PGF_{1\alpha}$ levels reflects primarily renal PGI_2 production, however, a certain proportion may be derived from the systemic circulation. Though in the urine beside 6-keto-$PGF_{1\alpha}$ other metabolites of PGI_2 can be measured (as 2,3-dinor-6-keto-$PGF_{1\alpha}$),[10] usually the 6-keto-$PGF_{1\alpha}$ excretion is assessed and it is assumed that the determination of this metabolite reflects endogenous prostacyclin production.

A direct determination of prostanoids and their metabolites seems to be feasible only in urine. According to MITCHELL et al.[11] 6-keto-$PGF_{1\alpha}$ suffers intensive degradation during extraction and they suggested that caution must be exercised in the interpretation of results obtained after an extraction procedure. Thus a direct assay for urinary 6-keto-$PGF_{1\alpha}$ appears not only to be possible, but the execution

of such a procedure is advisable. On this basis, the determination of 6-keto-$PGF_{1\alpha}$ by radioimmunoassay without previous extraction and chromatography seemed worth undertaking.

The RIA method applied by us seems to be suitable for the determination of 6-keto-$PGF_{1\alpha}$. The low non-specific binding and the data of the calibration curves support this assumption; furthermore, the intra- and interassay variation coefficients point to the reliability of this method. These data are in accordance with observations of NADLER et al.[12]

As we have not performed extraction and chromatography, special attention has been paid to the recovery of 6-keto-$PGF_{1\alpha}$, which was found to be 76–78% in the majority of experiments. These experimental results are in agreement with the data of MACDONALD et al.[13] and CIABBATONI et al.[14] These authors observed 60–70% recovery, although they performed extraction and chromatography prior to radioimmunoassay.

Subsequently we determined urinary 6-keto-$PGF_{1\alpha}$ of control subjects and the mean value of this metabolite excreted during a 4-hour period was 50 ng. In previous experiments the range of the mean value of the excreted metabolite was 16–45 ng/4 hours.[12,14] Our experimental results are somewhat higher than those found by others although they performed extraction and chromatography for the separation of 6-keto-$PGF_{1\alpha}$. The small difference between our data and the above values might be explained by the direct assay adopted by us, in which a certain quantity of 2,3-dinor-6-keto-$PGF_{1\alpha}$ could be detected, too. In previous experiments it was established that applying a RIA method for the determination of 6-keto-$PGF_{1\alpha}$ an about 30% cross reactivity existed with 2,3-dinor-6-keto-$PGF_{1\alpha}$.

Previous data point to a decreased 6-keto-$PGF_{1\alpha}$ excretion in patients with chronic glomerular diseases.[14] Our experimental results corroborate these findings. The lowered excretion of this metabolite may reflect a reduced glomerular mass available for PGI_2 synthesis, though other hypotheses have been advanced for the reduction in glomerular production of prostacyclin too.[14]

Summarizing our results, it may be concluded that even in the absence of the prior extraction procedure, the RIA method adopted by us is suitable for the determination of urinary 6-keto-$PGF_{1\alpha}$ content.

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