Simultaneous measurement of renal clearance and plasma clearance of $^{99m}$Tc-labelled diethylenetriaminepenta-acetate, $^{51}$Cr-labelled ethylenediaminetetra-acetate and inulin in man

M. REHLING, M. L. MØLLER, B. THAMDRUP, J. O. LUND AND J. TRAP-JENSEN

Department of Clinical Physiology and Department of Nephrology, Frederiksberg Hospital, Copenhagen, Denmark

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Summary

1. The present investigation was undertaken to study the kinetics of $^{99m}$Tc-labelled diethylenetriaminepenta-acetate (DTPA) as compared with inulin and $^{51}$Cr-labelled ethylenediaminetetra-acetate (EDTA).

2. Twenty patients with various degrees of decreased renal function were studied. The renal clearance, plasma clearance and volume of distribution of all three tracers were measured after a simultaneous single injection.

3. The average renal clearance ratio $^{99m}$Tc-DTPA to inulin was 0.97; the average renal clearance ratio $^{99m}$Tc-DTPA to $^{51}$Cr-EDTA was 1.02.

4. In all patients the plasma clearance of inulin exceeded that of $^{99m}$Tc-DTPA. No difference was seen between the plasma clearance of $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA. The plasma clearance of all three tracers overestimated the simultaneously measured renal clearance; on average this was, for $^{99m}$Tc-DTPA 5.7 ml/min, for $^{51}$Cr-EDTA 6.0 ml/min and for inulin 8.1 ml/min. The plasma clearance of $^{99m}$Tc-DTPA correlated well with the renal clearance of inulin, but overestimated this by 3.5 ml/min on average.

5. The volume of distribution of inulin was less than that of $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA. No difference was seen between the volume of distribution of $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA.

6. It is concluded that the difference in the kinetics of $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA in patients with decreased renal function was small and without clinical relevance. The two radioactively labelled tracers can replace each other in assessment of glomerular filtration rate (GFR). The close correlation between the plasma clearance of $^{99m}$Tc-DTPA after a bolus injection and the renal clearance of inulin justifies the use of the single-injection technique in assessment of GFR.

Key words: $^{51}$Cr-labelled ethylenediaminetetra-acetate, glomerular filtration rate, inulin, plasma clearance, renal clearance, $^{99m}$Tc-labelled diethylenetriamine-acetate, volume of distribution.

Introduction

The renal clearance of inulin obtained during constant intravenous infusion has long been accepted as the reference measure of the glomerular filtration rate (GFR). This technique, however, involves a somewhat complicated chemical analysis and a quantitative urine collection during the clearance periods. In order to circumvent these difficulties, a plasma clearance technique has been invented to evaluate GFR. This method consists of an intravenous bolus injection of a radioactively labelled tracer, e.g. $^{51}$Cr-labelled ethylenediaminetetra-acetate (EDTA), and collection of venous blood samples during the next 5 h [1]. Urine collection is not needed. The plasma clearance of $^{51}$Cr-EDTA agrees well with the renal clearance of
Inulin except in patients with severely decreased renal function [1, 2].

In recent years there has been a growing interest in assessment of single kidney function by renography with the \( \gamma \)-camera directly followed by measurement of GFR using the plasma clearance technique. Due to its radiophysical properties \( ^{51}\text{Cr}-\text{EDTA} \) is not suitable for studies using a \( \gamma \)-camera. \( ^{99m}\text{Tc}-\text{labelled} \) diethylenetriaminepenta-acetate (DTPA) is well suited for this purpose as well as for estimation of GFR using the plasma clearance technique [3].

In a previous study we compared the plasma clearances of four different \( ^{99m}\text{Tc}-\text{DTPA} \) preparations with that of \( ^{51}\text{Cr}-\text{EDTA} \) using a single-injection technique [3]. Only one preparation yielded results identical to those obtained with \( ^{51}\text{Cr}-\text{EDTA} \). In order to further elucidate the use of this \( ^{99m}\text{Tc}-\text{DTPA} \) preparation for estimation of GFR the present study compares the plasma clearance, renal clearance and volume of distribution of \( ^{99m}\text{Tc}-\text{DTPA} \) with those of \( ^{51}\text{Cr}-\text{EDTA} \) and inulin.

Methods

Subjects

Twenty patients, ten females and ten males, aged 49 to 78 years (median 67 years), were studied. All had been unilaterally nephrectomized as treatment for nephrolithiasis (eight patients), renal carcinoma (six patients) or renovascular hypertension (six patients). None had diabetes mellitus, oedema, ascites or lower urinary tract obstruction. The patients were in steady state concerning body weight and renal function as judged by repeated determination of serum creatinine.

Protocol

After an overnight fast, the patients were given 300 ml of water/h throughout the study. The patients rested supine, but were allowed to stand up during voiding. A polyethylene catheter was inserted into a cubital vein in both arms. Depending on GFR and the body weight 35-55 ml of a 10% inulin solution (Laevosan Gesellschaft, Austria) was injected from \( t = -1 \) to \( t = 1 \) min. At \( t = 2 \) min 54 \( \mu \text{Ci} (2 \text{ MBq}) \) of \( ^{99m}\text{Tc}-\text{DTPA} \) (CIS, France) per kg body weight and 100 \( \mu \text{Ci} (3.7 \text{ MBq}) \) of \( ^{51}\text{Cr}-\text{EDTA} \) (Amersham, U.K.) were injected simultaneously. The volume of the radioactive tracers varied from 1.5 to 2.5 ml. The catheter was flushed through with 20 ml of a 0.9% NaCl solution. The exact volume of tracer injected was calculated by the weight loss of the syringe divided by the specific gravity of the solution. For inulin a specific gravity of 1.044 g/ml was used. Blood samples were drawn from the contralateral arm at 5, 10, 20, 40, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min. The urine was collected and time of voiding noted. Urine and plasma blanks were obtained from samples taken before the injection of inulin. Blood glucose was measured at 0, 120 and 240 min. Inulin in plasma and urine was determined spectrophotometrically after deproteinization [4]. The radioisotope activity was counted in plasma samples of 2 ml with a well-crystal scintillation detector to a statistical accuracy of 1%. The time–plasma concentration curves were described as a sum of three exponential functions, with the peeling-off technique [1]. The smallest rate constant (final slope) was calculated over the period from 150 to 300 min.

In each urine collection period the renal clearance \( (C_{i})_r \) was calculated according to the equation:

\[
C_{i} = \frac{U_i \times V_i}{\int_{t_i}^{t_{i+1}} P(t) \, dt}
\]

where \( U \) denotes urine concentration, \( V \) volume of urine, \( P(t) \) plasma concentration and \( i \) the period number. Delay in the urinary tract dead space was taken to be 4.5 min [5]. The mean transit time from the brachial artery to the cubital vein was taken to be 1.6 min [6]. Consequently, the \( t_i \) values were subtracted 2.9 min [eqn. (1)]. The renal clearance \( (C_i)_r \) was finally calculated as the mean of the renal clearances obtained in the interval from 60 to 300 min after the injection.

The total plasma clearance \( (C_p) \) was calculated as the ratio between the injected amount of tracer \( (Q_0) \) and the total area under the plasma concentration curve \( (P(t)) \):

\[
C_p = \frac{Q_0}{\int_{0}^{\infty} P(t) \, dt}
\]

The volume of distribution \( (V_d) \) of a tracer in an open biological system is given by the product of the flow \( (F) \) out of the system and the mean transit time \( (\bar{t}) \) of the tracer in the system. Taking the plasma concentration as representative of the
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concentration in the outflow, $V_d$ can be calculated as

$$V_d = F \times \bar{t} = \frac{Q_0}{\int_0^\infty tP(t) \, dt} \times \frac{1}{\int_0^\infty P(t) \, dt}$$

(3)

The plasma concentration in the outlet is ideally the arterial concentration. Although the arterial and venous concentrations at a given time are not identical owing to extraction of tracer in the kidney, the

$$\int_0^\infty P(t) \, dt$$

values will be identical. Thus the plasma concentration in a vein can be used.

The concentration of inulin is measured spectrophotometrically in plasma water. However, for protein precipitation the plasma samples are diluted 10-fold reducing the relative volume of the proteins proportionally. Thus the concentration of inulin measured will overestimate the plasma concentration by only about 0.5%. This has not been corrected for.

Statistics

All comparisons were made with the Wilcoxon test for paired differences which were considered significant if a $2P$ value less than 0.05 was obtained.

Results

Seventy-one renal clearance periods were obtained in 20 patients. For each patient the renal clearance was calculated as the mean of two to five clearance periods (median four). The diuresis during the clearance periods varied from 1.9 to 9.3 ml/min (median 4.7).

The renal clearance, plasma clearance, final slope, volume of distribution (as % body weight) and total amount of tracer excreted during 5 h (as % of injected amount) for all three tracers are shown in Table 1.

The renal clearances of all three tracers are shown in Fig. 1. The average clearance ratio $^{99m}$Tc-DTPA to inulin was 0.97; the average clearance ratio $^{99m}$Tc-DTPA to $^{51}$Cr-EDTA was 1.02.

The plasma clearances of all three tracers are shown in Fig. 2. The plasma clearance of all three tracers was significantly greater than the simultaneously measured renal clearance: on average for $^{99m}$Tc-DTPA 5.7 ml/min, for $^{51}$Cr-EDTA 6.0 ml/min and for inulin 8.1 ml/min (Fig. 3). The difference between $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA was not significant.

The final slope for inulin was significantly greater than that for $^{99m}$Tc-DTPA which was greater than that for $^{51}$Cr-EDTA.

| TABLE 1. Renal clearance, plasma clearance, final slope, volume of distribution (% of body weight) and the amount of tracer excreted during 5 h (% of injected amount) of inulin, $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA (median and range) |
|---|---|---|
| Renal clearance (ml/min) ($n = 20$) | 43.6 ± 11.0-76.1 | 42.0 ± 13.2-68.5 | 40.8 ± 11.8-73.8 |
| Plasma clearance (ml/min) ($n = 20$) | 53.8 ± 25.1-83.5 | 47.9 ± 19.8-81.1 | 46.2 ± 20.5-81.1 |
| Final slope ($10^3$/min) ($n = 20$) | 4.06 ± 1.92-6.52 | 3.24 ± 1.38-5.97 | 3.22 ± 1.43-6.45 |
| Volume of distribution (% body weight) ($n = 20$) | 17.1 ± 4.06 | 19.4 ± 1.92 | 20.7 ± 1.38-5.97 |
| Total amount of tracer excreted during 5 h (% of injected amount) ($n = 12$) | 54.9 ± 14.1-20.1 | 50.2 ± 15.8-23.9 | 45.7 ± 15.8-24.8 |
| * Denotes significant difference between $^{99m}$Tc-DTPA and inulin. § Denotes significant difference between $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA. |
The volume of distribution of inulin was smaller than that of $^{99m}$Tc-DTPA. The volume of distribution of $^{99m}$Tc-DTPA did not differ from that of $^{51}$Cr-EDTA.

The total amount of inulin excreted by the kidney during the 5 h in % of the dose administered exceeded that of $^{99m}$Tc-DTPA which again exceeded that of $^{51}$Cr-EDTA. This variable for inulin could only be measured in 12 patients as in eight patients the inulin concentration in the first urine voided after the injection was too high to be measured spectrophotometrically.

Fig. 4 shows the plasma clearance of $^{99m}$Tc-DTPA compared with the simultaneously
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Renal clearance (ml/min)

**FIG. 3.** Plasma clearance compared with the simultaneously measured renal clearance for (a) $^{99m}$Tc-DTPA, (b) $^{51}$Cr-EDTA and (c) inulin.

**FIG. 4.** Plasma clearance of $^{99m}$Tc-DTPA compared with the renal clearance of inulin. $y = 0.93x + 6.8$, $r = 0.97$.

measured renal clearance of inulin. The correlation coefficient was 0.97 and the regression equation was $y = 0.93x + 6.8$. The plasma clearance of $^{99m}$Tc-DTPA overestimated the renal clearance of inulin by 3.5 ml/min on average.

The blood glucose was within normal range in all patients and varied on average by 0.5 mmol/l during the study.

**Discussion**

The renal clearance of the tracers was measured during a period of decreasing plasma concentration. In this situation the concentration of tracer in a peripheral vein will exceed that in an artery [7]. In four normal subjects the venous concentration of inulin was 7.4% higher than the arterial after a single injection [8]. Thus a renal clearance calculated from venous plasma concentration will underestimate the true clearance. However, all our patients had decreased renal function with GFR values from 11 to 76 ml/min, so the difference between the venous and the arterial concentration of tracer was probably lower than 7.4%. Furthermore we made a correction for the transport delay from the artery to the vein. Therefore we consider our renal clearance values to be underestimated maximally by 2-3%.

We found the ratio between the renal clearance of $^{99m}$Tc-DTPA and inulin to be 0.97. The difference between the two radioactively labelled tracers was not significant. This agrees well with
the literature where the clearance ratio $^{51}$Cr-EDTA to inulin is found to vary from 0.85 to 1.01 during studies at constant infusion [9-19].

The reason why the renal clearance of $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA is less than that of inulin is not obvious. Several factors may decrease the renal clearance of the radioactively labelled tracers. However, binding to plasma proteins is less than 0.5% and binding to erythrocytes seems to be independent of the concentration in plasma and the diuresis [22, 23]. Less than 1% of $^{51}$Cr-EDTA has been found dissociated in vivo [9]. The $^{99m}$Tc-DTPA preparation has a labelling yield of over 99%. A greater amount of free $^{99m}$Tc-pertechnetate is unlikely since the renal clearance of $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA did not differ.

The plasma clearance has been measured according to eqn. (2). If a final slope has not been reached within the time of examination the total area under the plasma curve will be underestimated and the plasma clearance overestimated. For all tracers we found that the plasma clearance overestimated the renal clearance, but to a greater degree for inulin. This supports the view that a final slope has not been reached, particularly not for inulin, which has a much smaller diffusion coefficient in water than the radioactively labelled tracers [24]. In agreement with our earlier investigations we found the plasma clearance of $^{99m}$Tc-DTPA to equal that of $^{51}$Cr-EDTA [3]. The final slope for $^{99m}$Tc-DTPA was slightly higher than that of $^{51}$Cr-EDTA. This is still in accordance with our earlier studies in patients with GFR values comparable with those studied here [3]. Hillson et al. [25] have compared the plasma clearance of $^{99m}$Tc-DTPA with that of $^{51}$Cr-EDTA in 45 patients using a single-injection, single-exponential method. The clearance of $^{99m}$Tc-DTPA was about 11% lower than that of $^{51}$Cr-EDTA. This agrees with our earlier investigation using the same technique and the same DTPA preparation (Diagnostic Isotopes, Bloomfield) [3].

We found a close correlation between plasma clearance of $^{99m}$Tc-DTPA and renal clearance of inulin. The plasma clearance of $^{99m}$Tc-DTPA overestimated the renal clearance of inulin by 3.5 ml/min on average.

The volume of distribution of inulin was less than that of $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA. This might be explained by the fact that inulin is a bigger molecule with an elongated configuration [26]. The volume of distribution of inulin is usually measured with a constant-infusion technique. However, the volume of distribution we found agrees well with the literature [27].

During the study, the relative amount of inulin excreted in the urine exceeded that of $^{99m}$Tc-DTPA, which again exceeded that of $^{51}$Cr-EDTA. This can simply be explained by the differences in renal clearance and volume of distribution of the tracers.

In conclusion, the present study has shown small differences without clinical relevance in the kinetics of $^{99m}$Tc-DTPA and $^{51}$Cr-EDTA in patients with decreased renal function. Therefore the two radioactively labelled tracers can replace each other in assessment of GFR.

The renal clearance of $^{99m}$Tc-DTPA was on average 3% smaller than that of inulin. However, the plasma clearance of $^{99m}$Tc-DTPA after a bolus injection correlated well with the renal clearance of inulin. This justifies the use of the single-injection technique in assessment of GFR.

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