

Plasma Clearance of Nonradioactive Iohexol as a Measure of Glomerular Filtration Rate¹

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(J. Am. Soc. Nephrol. 1995; 6:257–263)

ABSTRACT

Renal clearance of inulin is the best available indicator of GFR but cannot be used routinely for clinical purposes and is also difficult to perform for clinical investigation when repeated measurements are required. The aim of this study was to find a reliable alternative to inulin clearance that would allow one to avoid the use of radioactivity and problems related to the continuous infusion of the marker. The plasma clearance of unlabeled iohexol, a nonionic contrast agent, was used. Forty-one patients (creatinine clearance 6 to 160 mL/min per 1.73 m²) underwent simultaneous measurements of renal clearance of inulin and plasma clearance of iohexol. Iohexol was given as a single iv dose, and blood samples were drawn up to 600 min after the administration. Iohexol concentrations (by HPLC) were analyzed by a two-compartment, open-model system. A highly significant correlation between the plasma clearance of iohexol and the renal clearance of inulin over a wide range of GFR values was found. By analyzing the data with a simplified method that uses a one-compartment model corrected with the Bröchner-Mortensen formula, an excellent correlation with the inulin clearance was also observed. When only patients with moderate to severe renal failure were considered, a significant correlation between the two methods was found. A further comparison between GFR determined with iohexol and iopromide, a new low-osmolality, low-viscosity contrast medium, was also performed in a subgroup of patients. A highly significant correlation between the plasma clearance of iohexol

and iopromide over a wide range of GFR values was found. These findings indicate that the proposed method of measuring GFR by the plasma clearance of unlabeled iohexol or iopromide is a good alternative to the inulin clearance technique.

Key Words: GFR, contrast media, inulin, single-injection techniques, plasma clearance

The rapid development of therapeutic principles in nephrology has led to increasing demands for precise measurements of GFR. The rigorous assessment of GFR requires the measurement of the renal clearance of an exogenous marker that fulfills the criteria for an ideal filtration substance (1,2). Inulin, a fructose polymer, is freely filtered at the glomerulus but is not acted on by the tubules, is not synthesized or degraded by the body, and is physiologically inert (2). For these reasons, inulin clearance has become the standard for measuring GFR. However, the inulin clearance method cannot be considered practical for routine clinical purposes. It requires continuous iv infusion and sometimes bladder catheterization of the patients, who must be at bedrest. Furthermore, although automated methods for inulin determination have been developed, they are not routinely available in hospital chemistry laboratories (3,4). Given all of these drawbacks, it is not surprising that alternatives to the standard inulin clearance were sought. To overcome the need of constant infusion and urine collection, a single-injection method for GFR measurement based only on the total area under the curve of plasma marker concentrations versus time has been proposed (2). To this purpose, radioactive markers, such as [⁵¹Cr]EDTA, have been demonstrated to be reliable for the accurate determination of GFR, with values that are comparable with standard inulin clearance (5–8). Although simple and rapid, this method requires radiolabeled tracers, which complicate the procedure (special licensing, complicated handling, storage and disposal of waste) and exclude certain patients, such as pregnant women, from the investigation (9). Contrast agents that are eliminated from plasma mainly by glomerular filtration have previously been used as markers for the determination of GFR (10–19). However, the lack of suitable assay techniques required the use of radioactive tracers or impractically large doses of the tracer contrast. Recently, this limitation has been overcome by the development of sensitive methods that allow the determination of plasma clearance after a single injection of a small dose of nonradioactive compounds

¹ Received November 11, 1994. Accepted January 3, 1995.

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1046-6673/0602-0257\$03.00/0

Journal of the American Society of Nephrology
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(20,21). Thus, measurements of GFR, in patients with normal to moderately impaired renal function, by the plasma clearance of the two x-ray contrast agents iohalamate (22,23) and iohexol (21) have an excellent correlation with the plasma clearance of [^{51}Cr]EDTA, the most commonly used in clinical practice. Iohexol shares with iohalamate a similar kinetics profile but, from a practical point of view, is more attractive than iohalamate because of its reported lower allergenic potential. Even though the reason for this less pronounced effect remains unclear, the possibility exists that this may be because of the lower ability of iohexol to release histamine and serotonin and/or the activation of the complement cascade compared with ionic contrast agents (24). This study has been designed (1) to evaluate whether the plasma clearance of unlabeled iohexol is a reliable alternative in humans to renal inulin clearance—the standard method for the accurate measurement of GFR—avoiding problems related to the continuous infusion of the tracer and urine collection; and (2) to establish whether equations derived from already published models that calculate plasma clearance from the late phase of the plasma decline (23) were predictive of GFR, when applied to data generated by this study.

METHODS

Patients

Forty-one outpatients (30 men and 11 women) aged 20 to 62 yr and regularly monitored at the Division of Nephrology, Ospedali Riuniti Bergamo, were studied. The study protocol was described in detail to all patients before admission, and informed consent to perform the study was obtained in each instance. Patients had a wide range of renal function (creatinine clearance, 6 to 160 mL/min per 1.73 m²), and their clinical diagnosis included diabetic nephropathy, immunoglobulin A nephropathy, membranoproliferative glomerulonephritis, focal glomerulosclerosis, polycystic kidney disease, and essential hypertension. Four patients had moderate edema due to nephrotic syndrome. None had lower urinary tract obstruction or urinary tract infections. Patients with a history of allergy to iodine were excluded from entry. All studies were performed at the Clinical Research Center for Rare Diseases, Villa Camozzi, Ranica, Italy.

Study Protocol

In all patients, simultaneous measurements of renal clearance of inulin and plasma clearance of iohexol were performed. Sequential renal clearances of inulin were established under a steady state of diuresis induced by oral water administration adjusted in order to maintain urine flow relatively constant throughout the study (25). After an overnight fast, patients were given a water loading; then, a polyethylene catheter was inserted into a cubital vein in both arms for marker injection and blood sampling collection, respectively, and they rested supine but were allowed to stand up during voiding. A zero time blood sample for an inulin blank was obtained before the injection of the priming dose. The loading dose of inulin, depending on GFR and body surface area, was followed by a constant infusion of 10% inulin solution. The sustained dose of inulin was started to maintain constant plasma concentration of ~0.15 mg/mL.

After a 60-min equilibration period, patients were asked to void, and blood samples for inulin concentrations were drawn. Then, five carefully timed urine collections (2 h each) were begun and blood samples were collected at the beginning and at the end of each clearance period. At the end of the inulin stabilization period, 5 mL of iohexol solution (Omnipaque 300; 647 mg of iohexol per milliliter corresponding to 300 mg of iodine per milliliter; Schering, Milan, Italy) was also injected over 2 min, followed by 10 mL of saline solution. Blood samples were then taken from the contralateral arm at 5, 10, 20, 30, 45, 60, 90, 120, 180, 240, 300, 450, and 600 min, and after centrifugation at room temperature (2,000 × *g* for 20 min) plasma was separated and stored at -20°C for the HPLC determination of iohexol concentration. Systolic and diastolic blood pressure and heart rate were measured every hour during the study period.

In a subgroup of 29 outpatients (25 men and 4 women) aged 20 to 62 yr, simultaneous clearance studies were performed with iohexol and iopromide, a triiodobenzene derivative analog of iohexol. These patients received an additional 5-mL (3.117 g) iv dose of iopromide (Ultravist 300; Schering) together with iohexol injection.

Laboratory Procedures

Plasma and urine concentrations of inulin were measured by the thiourea-resorcinol method as reported elsewhere (26). Renal inulin clearance was calculated by the standard formula. The five clearance periods for each patient were averaged to provide a single value. The intra-assay variability of the inulin clearance measurement was usually about 2%.

Plasma concentrations of iohexol and iopromide were determined by HPLC as previously reported (23) with minor modification. Plasma samples were deproteinized by adding 4 vol of 5% perchloric acid and centrifuging. Twenty microliters of the supernatant was chromatographed with a System Gold HPLC with a model 160 UV detector set at 254 nm (Beckman, Fullerton, CA) and a 250 × 4 mm column packed with Lichrosorb C-18 (Merck, Darmstadt, Germany). Iohexol was eluted by a mixture of deionized water/acetonitrile (96:4 by volume, adjusted to pH 2.5 with phosphoric acid), pumped at a rate of 1.5 mL/min. Internal calibration curves of iohexol and iopromide were prepared for each set of samples.

The plasma clearance of iohexol (Cl_p) was calculated by the formula: $\text{Cl}_p = \text{Dose}/\text{AUC}$, where AUC is the area under the plasma concentration-time curve. The plasma profiles were analyzed by a two-compartment, open-model system. All data were fitted by a nonlinear regression iterative program (Carl Peck, Uniformed Services University, Bethesda, MD) and flooded according to Sacchi-Landriani *et al.* (27) on an HP-86 computer (Hewlett-Packard, Corvallis, OR). The clearance of iohexol was also estimated with the measurements from the timed period 120 to 600 min after the injection, according to a one-compartment model (Cl_1) by the formula: $\text{Cl}_1 = \text{Dose}/\text{AUC}$. According to Bröchner-Mortensen (28), plasma clearances were then estimated by the formula: $\text{Cl}_p = (0.990778 \times \text{Cl}_1) - (0.001218 \times \text{Cl}_1^2)$. The agreement between the plasma clearance of iohexol and the renal clearance of inulin was also estimated by the use of linear regression analysis. A further estimate of agreement between the methods was performed by plotting the difference between the two methods against the average of the two methods for each patient (29). Limits of agreement were calculated as mean difference $\pm 1.96 \times \text{SD}$ of the differences.

Statistical Analysis

Results were analyzed by *t* test and linear regression analysis, as appropriate. Regression estimates were also performed after log transformation of the clearance values. *P* values less than 0.05 were considered significant. The 95% confidence intervals of the regression estimate were calculated to test the hypothesis of the slope = 1 and the intercept = 0.

RESULTS

Standard curves for plasma iohexol and iopromide profiles were constructed with human plasma with known amounts of iohexol and iopromide. Linearity was found over the investigated range for iohexol and iopromide (from 1.5 to 700 $\mu\text{g/mL}$), and calibration curves had correlation coefficients better than 0.99. The detection limit was 0.5 $\mu\text{g/mL}$.

The accuracy of the method was calculated as the percent error from the true value, by processing daily three samples containing a known amount of compound, and was usually less than 3%. The precision of the assay was good, and the coefficient of variation never exceeded 2.2%.

As shown in Figure 1, both iohexol and iopromide eluted from the chromatographic column as two peaks, reflecting the isomers present in the pharmacologic preparation. For practical purpose, the major peak, eluting at about 5 min for iohexol and at about 11 min for iopromide, was used for the clearance

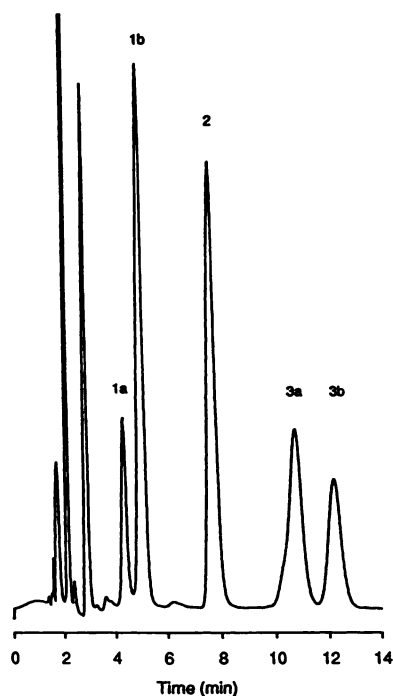


Figure 1. Chromatogram of a plasma sample containing iohexol (19.41 $\mu\text{g/mL}$) (Peaks 1a and 1b, two isomers), the internal standard (Peak 2), and iopromide (18.70 $\mu\text{g/mL}$) (Peaks 3a and 3b, two isomers).

calculation of each compound. However, repeated determinations of clearance using the minor peaks of both compounds consistently yielded identical results, indicating that both isomers are handled in the same manner by the body.

Figure 2 shows the plasma curves of iohexol after a single iv injection in three representative patients with different degrees of renal function. These plasma profiles were best described by a two-compartment pharmacokinetic open model, and they were linear (elimination phase), at least from 120 min after the injection of iohexol in all patients studied.

As shown in Figure 3A, the plasma clearance of iohexol, calculated by the two-compartment, open-model system, showed a good correlation with the renal clearance of inulin. The above regression estimate was also performed after log transformation of the clearance values. The standard error of the slope estimate established that 95% confidence intervals included 1.0 (that is, the line of identity). Thus, the models cannot be rejected by the data at a significance level of 0.05. When GFR was estimated by using the one-compartment model corrected according to the method of Bröchner-Mortensen (28), results comparable to those from the two-compartment model were obtained. Thus, the regression line of the plasma iohexol clearance (modified one-compartment) versus the plasma iohexol clearance (two-compartment) was $y = 1.02x - 1.04$ ($r = 0.996$). After log transformation, the equation was $y = 1.01x - 0.03$ ($r = 0.997$), and the slope was not statistically different from 1. A highly significant correlation was also observed between plasma iohexol clearance estimated by the modified one-compartment model and renal inulin clearance (Figure 3B).

We have also assessed whether the plasma iohexol technique is reliable in patients with renal insufficiency. In one series of 20 measurements from patients with creatinine clearance lower than 40 mL/min per 1.73 m^2 , a significant correlation between the

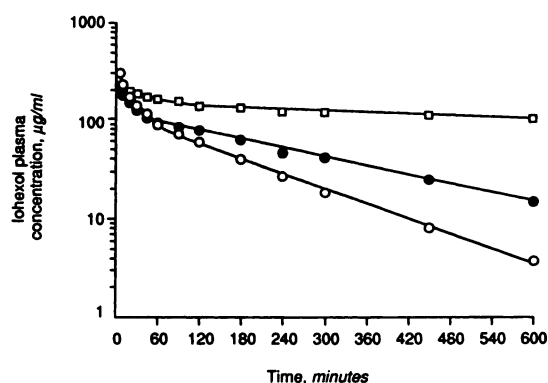


Figure 2. Iohexol plasma profile after the iv injection of the marker in three representative patients with different renal function. Patient 1 (open squares), 8.90 mL/min per 1.73 m^2 ; Patient 2 (closed circles), 77.85 mL/min per 1.73 m^2 ; Patient 3 (open circles), 115.67 mL/min per 1.73 m^2 .

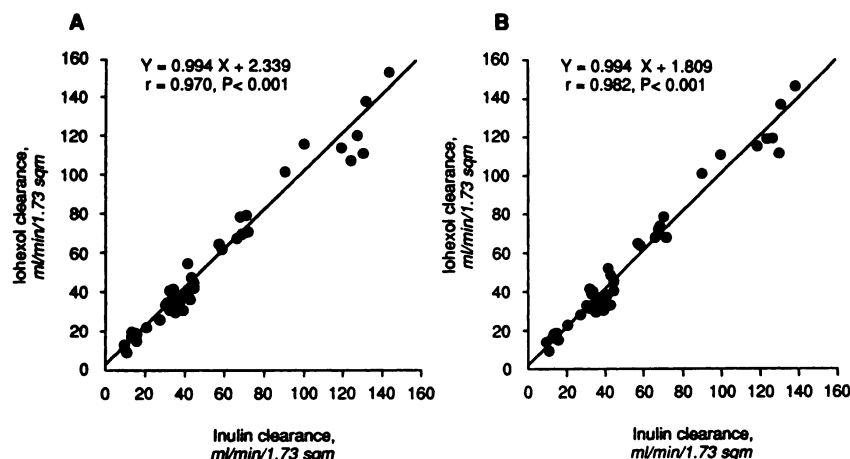


Figure 3. Correlation between plasma clearance of Iohexol and renal clearance of Inulin. (A) Iohexol clearances estimated by the two-compartment, open-model system. (B) Iohexol clearances calculated by the one-compartment, open-model system and then corrected by the Bröchner-Mortensen equation.

plasma clearance of Iohexol and inulin clearance was found, with a scattered distribution of the data around the line of identity (Figure 4).

To further reduce the number of blood samplings, we evaluated whether considering three points (60, 90, and 120 min) from the plasma Iohexol disappearance curve to estimate GFR and including a correlation factor, estimated from the intravascular space and distribution volume in the formula, as previously reported for the plasma clearance of Iothalamate (23), allowed an accurate determination of renal function. The clearance correlation coefficient (r) with two-compartment plasma Iohexol was 0.952, and the slope was significantly lower than 1 ($y = 0.79x + 4.91$). The error in predicting GFR ranged from -47 to 244% . Because from our data, the elimination phase began from 120 min, we applied this model to the first three points of the linear phase of our plasma Iohexol profile (120, 180, and 240 min). An improvement in the correlation coefficient to 0.991 was found, but the

slope of the regression versus two-compartment Iohexol clearance was statistically different from 1 ($y = 0.83x + 0.32$). The error in predicting GFR still ranged from -34 to 62% . When GFR was estimated by using the three-point model corrected according to the Bröchner-Mortensen equation, the correlation coefficient was 0.986 and the 95% confidence interval included 1 ($y = 0.96x + 5.48$), but the intercept was statistically different from 0. The associated error in GFR prediction ranged from -15 to 125% .

We then examined the agreement between the one-compartment Iohexol clearance and the renal clearance of inulin by plotting the difference between the two methods against the mean and calculated the limits of agreement from the mean difference and the standard deviation of the differences (29) (Figure 5). The mean difference between the two methods was -1.02 mL/min per 1.73 m², and the 95% limits of

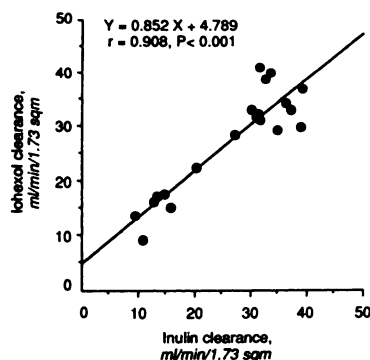


Figure 4. Correlation between Iohexol one-compartment model clearance modified with Bröchner-Mortensen formula with the renal clearance of Inulin for patients with renal function <40 mL/min per 1.73 m².

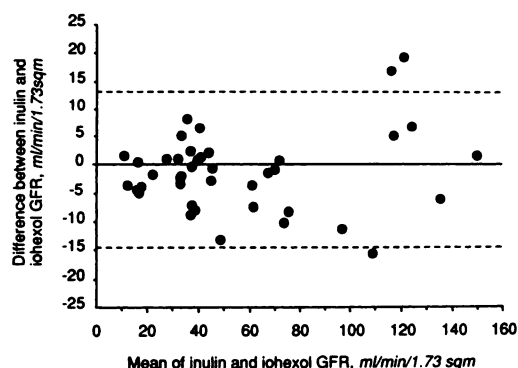


Figure 5. Difference between the plasma clearance of Iohexol (one-compartment, open-model system) and the renal clearance of Inulin versus the mean of the two methods in all patients studied. Mean difference (straight line) and 95% limits of agreement (dashed lines) are shown.

agreement varied from -15 to 12 mL/min per 1.73 m².

Figure 6 shows the plasma curves of iothexol and iopromide after a single iv injection in a representative patient. These plasma profiles were quite similar and were best described by a two-compartment pharmacokinetic open model; they were linear (elimination phase), at least from 120 min after the injection of iothexol in all patients studied.

As shown in Figure 7A, the plasma clearance of iothexol, calculated by the two-compartment open model system, showed a high correlation with the plasma clearance of iopromide. The standard error of the slope estimate established that 95% confidence intervals included 1.0 (that is, the line of identity). Thus, the models cannot be rejected by the data at a significance level of 0.05. When GFR was estimated by using the one-compartment model corrected according to the method of Bröchner-Mortensen (28), results comparable to those from the two-compartment model for both compounds were obtained. Thus, the regression lines of plasma clearance (modified one compartment) versus plasma clearance (two compartments) were $y = 1.021x - 0.376$ ($r = 0.996$) for iothexol and $y = 0.999x + 0.086$ ($r = 0.995$) for iopromide. After log transformation, the equations were $y = 1.000x + 0.001$ ($r = 0.998$) for iothexol and $y = 1.022x - 0.092$ ($r = 0.998$) for iopromide, and the slope was not statistically different from 1. A highly significant correlation was also observed between iothexol and iopromide plasma clearances, estimated by the modified one-compartment model (Figure 7B).

DISCUSSION

These data indicate a highly significant correlation between the plasma clearance of iothexol, estimated by the two-compartment model, and the renal clearance of inulin over a wide range of GFR values. This indicates that the total plasma clearance of iothexol, calculated from serial measurements of plasma concen-

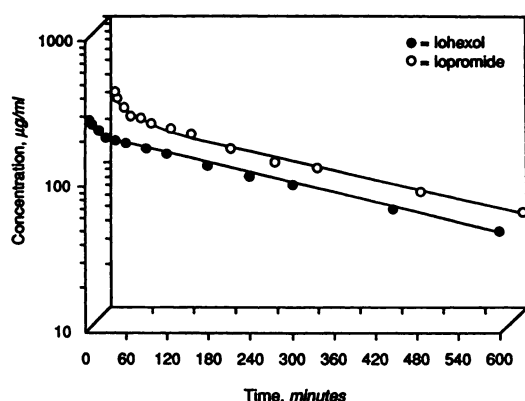


Figure 6. Iothexol and iopromide plasma profiles after the iv injection of the markers in a representative patient with reduced renal function. GFR: iothexol, 35.02 mL/min per 1.73 m²; iopromide, 37.28 mL/min per 1.73 m².

trations after a single rapid iv injection, is equal to GFR, suggesting that the compound is excreted solely by glomerular filtration without tubular secretion or reabsorption.

The plasma clearance technique has the advantage of avoiding the need for urine collection and the continuous infusion of the marker of glomerular filtration. The plasma clearance of a given marker, however, requires multiple blood samples to estimate GFR from the slope of the disappearance concentration profile. This plasma curve generally consists of two compartments, an early phase that reflects the distribution and a late one related to the excretion of the marker, and thus with its clearance rate (30). Even though a complete kinetic profile could provide more precise information, numerous plasma collections make this method expensive and cumbersome. Abbreviated kinetic profiles have been proposed to predict GFR from the plasma disappearance curve that would overcome the disadvantage of multiple blood samplings. Therefore, we analyzed the applicability to iothexol of a simplified method for the measurement of GFR already described for [⁵¹Cr]EDTA and iothalamate that uses a one-compartment model (22,28). The clearance was calculated from the slope of the terminal plasma disappearance curve, which requires only a few (six) blood samples, according to a one-compartment open model, and then the clearance values were corrected with the Bröchner-Mortensen formula (28). The finding of an excellent correlation between the one-compartment model corrected with the Bröchner-Mortensen formula and the two-compartment model confirms the reliability of this simplified technique.

The estimation of GFR by plasma clearance techniques relies on the assumptions that the extrarenal clearance of the marker is negligible. Thus, the reliability of the technique might be reduced in patients with renal insufficiency. We addressed this issue by considering our patients according to different degrees of GFR and comparing the plasma clearance of iothexol with the renal clearance of inulin for each subpopulation. The finding of a significant correlation between the plasma clearance of iothexol and inulin clearance in patients with moderate to severe renal failure (creatinine clearance, <40 mL/min per 1.73 m²) and of a scattered distribution of the results around the line of identity suggests that extrarenal routes of excretion of the marker are negligible and that blood sampling over a long period allows the possible limit of a single-injection clearance method, when clearance is low relative to body size, to be overwhelmed. These findings are consistent with previously published data (31) and with the recent observation by Nilsson-Ehle and Grubb that the extrarenal elimination of iothexol is negligible, even under extreme conditions, i.e., when GFR is as low as 2 to 3 mL/min (32).

Recently, Isaka and coworkers (23) have shown in patients and normal subjects an excellent correlation

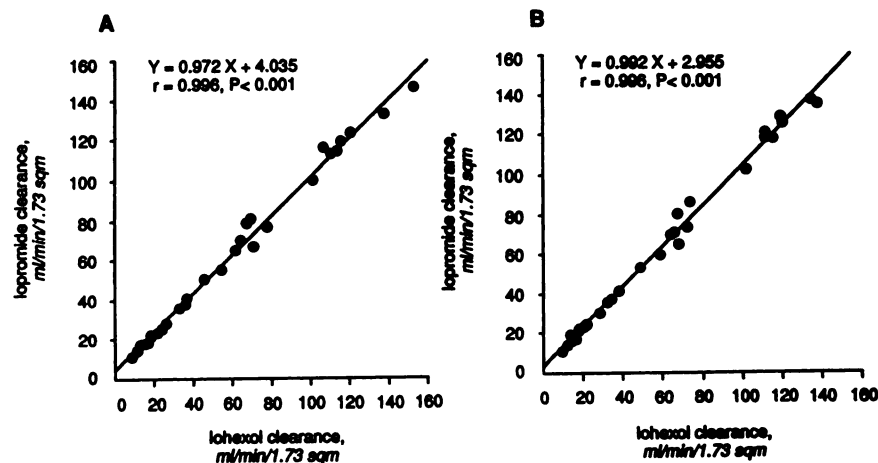


Figure 7. Correlation between plasma clearance of Iohexol and Iopromide. (A) Clearances estimated by the two-compartment, open-model system. (B) Clearances calculated by the one-compartment, open-model system and then corrected by the Bröchner-Mortensen equation.

of the plasma clearance of Iothalamate with the renal clearance of inulin, when GFR was estimated by a modified method that considers only the late phase of the plasma disappearance curve at 60, 90, and 120 min after the injection of the marker. These results were obtained when a correlation factor, which is estimated from the intravascular space and distribution volume, was also considered in the calculation, which is required because the elimination rate of the marker is determined not only by the filtration rate but also by the ratio between the central and the second compartment. We have applied the same modified plasma clearance estimation (60, 90, and 120 min) to assess whether this is a general and suitable alternative that could also be extended to Iohexol clearance. However, the regression line of the predicted over the measured GFR failed to show a good agreement between the two methods, with an unacceptable error in GFR prediction. This may be because we have been unable to find a single patient in whom the disappearance profile of plasma Iohexol was linear before 120 min after injection. Actually, this behavior was consistent with our previous findings with Iothalamate that documented a linearity of the profile, at least from 120 min after the marker injection in all 19 patients studied (22). Taking into account this difference in the linearity of the plasma disappearance curve, we extended the modified plasma clearance estimation to the first three times of our log/linear profile (120, 180, and 240 min). Even in this part of the elimination phase, the predicted GFR was unacceptable as compared with the measured GFR. When the three-time model (120, 180, and 240 min) was corrected according to the Bröchner-Mortensen equation, the slope of the regression line improved and reached the line of identity, but the error in GFR prediction remained unacceptable.

As an alternative to repeated blood sampling for

GFR determination, it has been recently shown (33) that a late (at 24 h after administration), single-point sampling strategy gives useful clearance estimation for clinical purposes. Even though this approach seems attractive because of its simplicity and few technical demands, it may suffer from the disadvantage of requiring nonhospitalized patients to be back at the medical center the day after the marker injection.

When one compares two methods for measuring GFR, the agreement between the two is usually evaluated by calculating the correlation coefficient (r). However, r does not measure the agreement but only the strength of a relationship between two variables. Actually, the correlation depends on the range of values considered for the analysis, and data with quite high correlation may be in poor agreement (29). However, the acceptable limit of agreement is not established. When we compared the one-compartment plasma Iohexol clearance with the renal clearance of inulin, the limit of agreement was small. Therefore, we judged the agreement between the two methods to be acceptable for clinical purposes.

These data also indicate that Iopromide could be a valid alternative to Iohexol as filtration marker. Indeed, we have shown a highly significant correlation between the plasma clearance of Iohexol and that of Iopromide as measured by a two-compartment model over a wide range of GFR values. The findings that plasma profiles of Iohexol and Iopromide were identical suggest that the two compounds are handled in humans in almost the same manner.

In summary, the proposed method of measuring GFR by the plasma clearance of unlabeled Iohexol is a good alternative to the inulin clearance technique and may be particularly suitable in clinical practice and for follow-up studies requiring repeated measurements of GFR. It has the advantage of using as a

filtration marker a nonionic, low-osmolality contrast agent that, as reported by clinical studies, offers significant benefits in patient comfort and patient safety (24). The overall performance of the analytical method we used to assay iohexol is very good in terms of both accuracy and precision, and furthermore, the ease and speed of sample preparation and the rapidity of the chromatographic step (less than 9 min when iohexol is analyzed) allow GFR determination for a single subject (*i.e.*, analysis of five to six samples) in about 1.5 h. Thus, the technique provides a useful alternative for accurate GFR measurements, avoiding the inconvenience and restrictions associated with the handling of radiolabeled tracers. The abbreviated kinetic disappearance curve (six sampling points [120 to 600 min]), one-compartment model corrected with Bröchner-Mortensen formula) further simplifies the procedure of iohexol, eliminating the disadvantage of multiple blood samplings. A further limited sampling strategy (three points corrected with Bröchner-Mortensen) does not adequately reflect the GFR both in patients with moderate to severe renal insufficiency and in those with normal renal function, leading to major errors in the estimation of the real GFR values.

ACKNOWLEDGMENTS

We thank Schering SpA, Milano, Italy, for kindly providing iohexol and iopromide. Dr. Carmen S. Amuchastegui is a recipient of a fellowship in memory of 'Avv. Tobia Naddeo.'

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