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Original article

Measurement of Glomerular Filtration Rate by Rapid Intravenous Injection of a Newly Developed Inulin Fraction

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Abstract

Objective: Since the conventional drip-infusion method for measuring inulin clearance (Cin) has problems related to its accuracy and performance, we explored a more accurate and concise method by rapid intravenous injection of a newly developed inulin fraction (Inulead®), in which spot urine sampling was omitted and the administration period of inulin was shortened from 120 to 5 minutes.
Patients and Methods: Twenty seven patients (M/F: 15/12, 67.8 ± 12.9 years old) admitted to the Nephrology ward were enrolled in this study. Inulead®, 1500 mg dissolved in 150 mL of saline, was intravenously administered in 5 minutes. Then, sequential blood samplings and urine collection were performed for 24 hours. Cins were calculated by the following three formulae: (1) a pharmacokinetic analysis using a two compartments model based on the plasma inulin concentration to determine Cin, which was the administered dose divided by the area under the curve (AUC) from 0 to ∞, (2) urinary inulin excretion divided by the AUC for 24 hours and (3) the Bayesian method using a three-point set of plasma inulin concentrations to predict the change of inulin concentration to determine Cin as in 1. These Cins were compared with levels of estimated GFR (eGFR), creatinine clearance (Ccr), serum β2 microglobulin (β2MG) and serum cystatin C (Cys C).
Results: Cins obtained by the above three methods were well correlated with each other (r. = 0.9088–0.9998) and with eGFR (r. = 0.8286 − 0.8650), Ccr (r. = 0.821 − 0.864), 1/β2MG (r. = 0.631 − 0.752) and 1/CysC (r. = 0.830 − 0.857). The averaged differences of each Cin from eGFR were distributed between − 4.4 and − 4.5 mL/min.
Conclusion: Since the Cins by rapid inulin injection showed satisfactory correlation and differences with other GFR parameters, this method will be a good alternative to the drip infusion method, and may reduce the burden of patients and medical staff.

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Introduction

Estimated GFR (eGFR) has become popular for rough evaluation of renal function not only in Japan[1)], but also in other countries[2)]. It is certainly useful in stratifying patients or any specific populations, but it has limitations in predicting true GFR in an individual patient[3–5)] or in healthy subjects[6)] . Among methods for measuring GFR in an individual case, inulin clearance (Cin) is still a golden standard[4,7,8)]. A newly developed inulin fraction product, Inulead®(Fuji Yakuhin Co., Ltd., Saitama, Japan) has been available in Japan since December 2007, and a protocol for measuring GFR with this product has been proposed[1,9)]. However, this recommended protocol requires a 2-hour infusion of inulin and a 15-minute interval between alternating collections of urine and blood samples, which may disturb the widespread use of Cin in general medical facilities. Horio *et al*. proposed to decrease the points of urine sampling, but this arrangement does not seem to be a great help in reducing the burden of patients and medical staff[10)] because the method still requires blood and urine sampling under continuous hydration of inulin. Moreover, in the drip infusion method, it is practically difficult to maintain the plateau levels of plasma inulin that are an essential part of the conception of clearance.

For more convenient and accurate use of Cin in measuring GFR, we explored a more concise measurement of Cin by rapid injection of inulin, in which 1500 mg of inulin was intravenously administered over the course of 5 minutes. This protocol requires more frequent blood sampling

than the drip infusion protocol, but spot urine sampling under continuous hydration of inulin can be omitted. Instead, urine was simply collected for 24 hours to measure the total amount of urinary inulin excretion. We expect that this arrangement can make measurement of Cin more convenient, more accurate and more widespread in Japan and other countries.

Subjects and Methods

Of the patients admitted to the Nephrology Division of Toride Kyodo General Hospital from March 2009 to January 2010, 27 patients who gave the informed consent for this study were enrolled. The patients who did not give the informed consent and whom the attendant physicians recognized as inappropriate for this study, such as those in an unstable general condition or with a limited admission period, were excluded.

Because this examination required sampling for 24 hours, the patients' standard dietary and water restrictions, in accordance with their diseases and conditions, and prescribed drugs were continued. Two to three hours after breakfast (between 9–10 AM), the patients voided urine just before receiving inulin. From the next voiding, urine of each patient was collected for 24 hours to measure urinary inulin excretion. An infusion pump (TOP-2200, Terumo Corporation, Tokyo, Japan) was used to intravenously administer 1500 mg of an inulin product, Inulead®, dissolved in 150 mL of saline through the cephalic or basilica vein over the course of 5 minutes. Blood samples comprising 1.5 ml of whole blood (0.7 ml of plasma), were obtained via an indwelling needle (22G, SR-0T2225C, Terumo Corporation, Tokyo, Japan) in the contralateral cephalic or basilica vein, attached to an extension tube (SLX2-50, TOP Corporation, Tokyo, Japan) before and 0, 5, 10, 15, 30, 60, 120, 480 and 1440 min after administration of Inulead®. Plasma and urine inulin were measured by an established enzyme method[11].

Cin was calculated by the following three methods. The first method was pharmacokinetic inulin clearance (PKCin), in which the plasma concentration of inulin was simulated by the non-linear multiple points least squares (MPLS) method[12] based on the two compartments model using GraphPad Prism 4 (GraphPad Software, San Diego, CA, U.S.A). The obtained parameters were weighted by 1/Y in accordance with preliminary observations from the preceding 25 cases. Then, PKCin was calculated using the following equation.

PKCin = Administered dose of inulin / AUC (0–∞) ··· (1)

The second method for obtaining Cin was to calculate it using the following equation[13].

Cin = Urinary excretion of inulin for 24 hours / AUC in 24 hours ····· (2)

In this equation, the AUC for 24 hours was calculated by the trapezoidal rule. The AUC (0–24 hrs) by MPLS and that by the trapezoidal rule were well correlated (r = 0.997 in the preceding 25 cases). Hereafter, the Cin obtained from equation 2 was abbreviated as Uin/AUC. The third method of obtaining Cin (Cin-BM) was to calculate it by the Bayesian method using Multi-Bayes (www.kobegakuin.ac.jp/~pharm/asc/excel/bayes_m.xls) [14], in which a three-point set of plasma inulin levels was selected to calculate GFR, according to the level of eGFR, as follows.

eGFR > 15 ml/min/1.73 m^2: 120, 240, and 480 min

15 mL/min/1.73 m^2 ≥ eGFR ≥ 10 mL/min/1.73 m^2: 120, 240 and 720 min

eGFR < 10 mL/min/1.73 m^2: 240, 720, and 1440 min

The obtained plasma concentration of inulin was applied to the equation for predicting the concentration of inulin (C) at time t in the two compartment model[15],

$C = Ae^{-\alpha \cdot t} + Be^{-\beta \cdot t}$.

As an initial formula for analysis, A = 24 (50) mg/dL, α = 0.08 (0.01) /min, B = 13 (100) mg/dL and β = 0.017 (0.01) /min were set based on the preliminary observations, with the values in parentheses representing the variance in individual samples. Finally, Cin-BM was obtained using equation 1 based on analysis of a predicted concentration curve.

The Cins obtained by these three methods were compared with each other and were also compared with creatinine clearance (Ccr), 1/serum β2 MG, 1/serum cystatin C by the Pearson's linear regression analysis and the Bland-Altman (difference) plot[16] using Excel 2007® (Microsoft, Inc., Redmond. WA, the U.S.A.). Serum and urinary creatinine (Cr) were measured by the established enzyme method. Serum β2 microglobulin was measured by the densitometry method. Serum cystatin C was measured by the nephrometry method. These samplings were performed within a few weeks before or after measuring Cin. The values are shown as means ± SD, unless otherwise specified.

This study was approved by the ethics committee of Toride Kyodo General Hospital, and registered in the University hospital Medical Information Network-Clinical Trials Registry (UMIN-CTR) as UMIN 000001702 on Feb 15, 2009.

Results

As summarized in Table 1, the enrolled patients included 15 males and 12 females with a mean age of 67.8 ± 12.9 (27–84). Their main diseases were chronic glomerulonephritis in 8 cases, nephrosclerosis in 5 cases, diabetic nephropathy in 8 cases and other diseases in 6 cases. Three patients were

Table 1 Patient characteristics

Sex	Male 15, Female 12
Age (years old)	67.8 ± 12.9 (27 – 84)
BUN (mg/dL)	38.3 ± 20.2 (10 – 88)
Serum Cr (mg/dL)	3.84 ± 3.38 (0.84 – 11.84)
eGFR (mL/min/1.73 m^2)	23.3 ± 18.3 (2.8 – 82.6)
Height (cm)	157.4 ± 10.2 (139.0 – 183.0)
Body weight (kg)	60.6 ± 13.5 (37.0 – 94.8)
Body surface area (m^2)	1.60 ± 0.21 (1.26 – 2.17)
Ccr (mL/min/1.73 m^2)	28.8 ± 22.4 (3.1 – 103.5)
Serum β2MG (mg/L)	9.4 ± 6.9 (0.9 – 24.5)
Serum CysC (mg/L)	2.69 ± 1.31 (0.89 – 5.47)

The range is indicated in parentheses. Body surface area (BSA) in each patient was calculated by the equation proposed by Du Bois.

removed from the analysis of Uin/AUC because they could not complete urine collection for 24 hours due to cognitive disorder. No adverse reaction was experienced during the entire examination period in any of the patients.

In the MPLS method, the simulated models were well fitted to the measured values in each case (averaged r^2. = 0.9758). The averaged distribution volume (Vd) of inulin was 11.8 L (19.0% of BW), slightly larger than the estimated extracellular volume (ECV), which was consistent with a previous report[13]. The coefficient of variation (CV) of Vd was less than 30% and was not affected by the level of renal function in each patient.

As Figure 1 shows, the obtained PKCin and Cin-BM were extremely well correlated (r. = 0.9998) or rather identical. PKCin and Uin/AUC were also closely correlated (r = 0.9088), but less identical than PKCin and Cin-BM.

All of the measured Cins were well correlated with the other parameters for GFR (Figure 1 and Table 2). As Figure 2 and Table 3 show, the differences of PKCin, Cin-BM, and Uin/AUC from eGFR were −4.5 ± 10.6 mL/min/1.73 m^2 (−14.8 ± 36.7%), −4.6 ± 10.6 mL/min/1.73 m^2 (−15.2 ± 37.5%) and −4.5 ± 9.6 mL/min/1.73 m^2, respectively. Moreover, the calculated Cins and other GFR parameters were comparable in most cases, but big differences were observed between the Cins and eGFR in one patient who was in the first remission phase of minimal change nephrotic syndromeand was receiving steroid therapy (open circles in Figure 2).

Discussion

Inulin is a general term defined as a polysaccharides with a molecular weight ranging from 3000 to 10000. The newly introduced inulin product Inulead® is more purified than other inulin products and consequently has a stricter range of molecular weight, between 3000 and 8000, than those of previously available products. In humans, the distribution of administered inulin is almost equal to the extracellular fluid (ECF) in the body, and its elimination is absolutely dependent on the renal function.

Since the word “clearance” is scientifically defined as the elimination velocity of a substrate period divided by the concentration of its substrate, renal clearance of a substance can be measured in either the plateau or non-plateau phase of its serum (plasma) level. In the plateau phase, clearance can be calculated as follows.

Clearance = urinary excretion/serum concentration in a certain period

Although its calculation is simple, one of major concerns regarding clearance is the difficulty in maintaining plateau levels of the plasma concentration of a substance. Cin has the similar problem, even if the administration doses are arranged based on the stature and renal function of the individual patient or the administration period is extended[13]. In the non-plateau phase, clearance could be calculated by such methods as reported in this study. PKCin reflects systemic clearance, rather than renal clearance. Meanwhile, Uin/AUC more directly reflects renal clearance. These differences may explain the more close relation of Uin/AUC with other parameters of renal function than that found for PKCin, although such differences were negligible in previous reports[13,17]. Theoretically, PKCin and Uin/AUC should be equal if inulin is eliminated solely by the kidney. Therefore, the observed differences between PKCin and Uin/AUC might reflect delayed distribution of inulin in the extracellular space in the case of PKCin < Uin/AUC or inappropriate urine collection by spontaneous voiding only, not by installing a urinary catheter, in the case of both PKCin < Uin/AUC and PKCin > Uin/AUC, as the previous reports suggested[17,18].

Measurement of Cin can be made much easier by applying statistical model analysis, such as the Bayesian method, to predicting the elimination curve of the plasma inulin level for each patient. In this method, a few points (three points in this study) of blood sampling are enough, and no urine sampling is needed to determine GFR. Cin-BM might be less reliable than PKCin and Uin/AUC, but it does provide a more convenient approach for determining real GFR in an individual patient compared with the other measurements of Cin. In fact, the obtained Cin-BM showed extremely good correlations with PKCin (Figure 1). Blood sampling for determining the plasma inulin concentration could be reduced more by using prediction formulae, such as by Jacobsson, but such efforts will yield less accuracy in measuring GFR[19]. The most appropriate method for measuring Cin

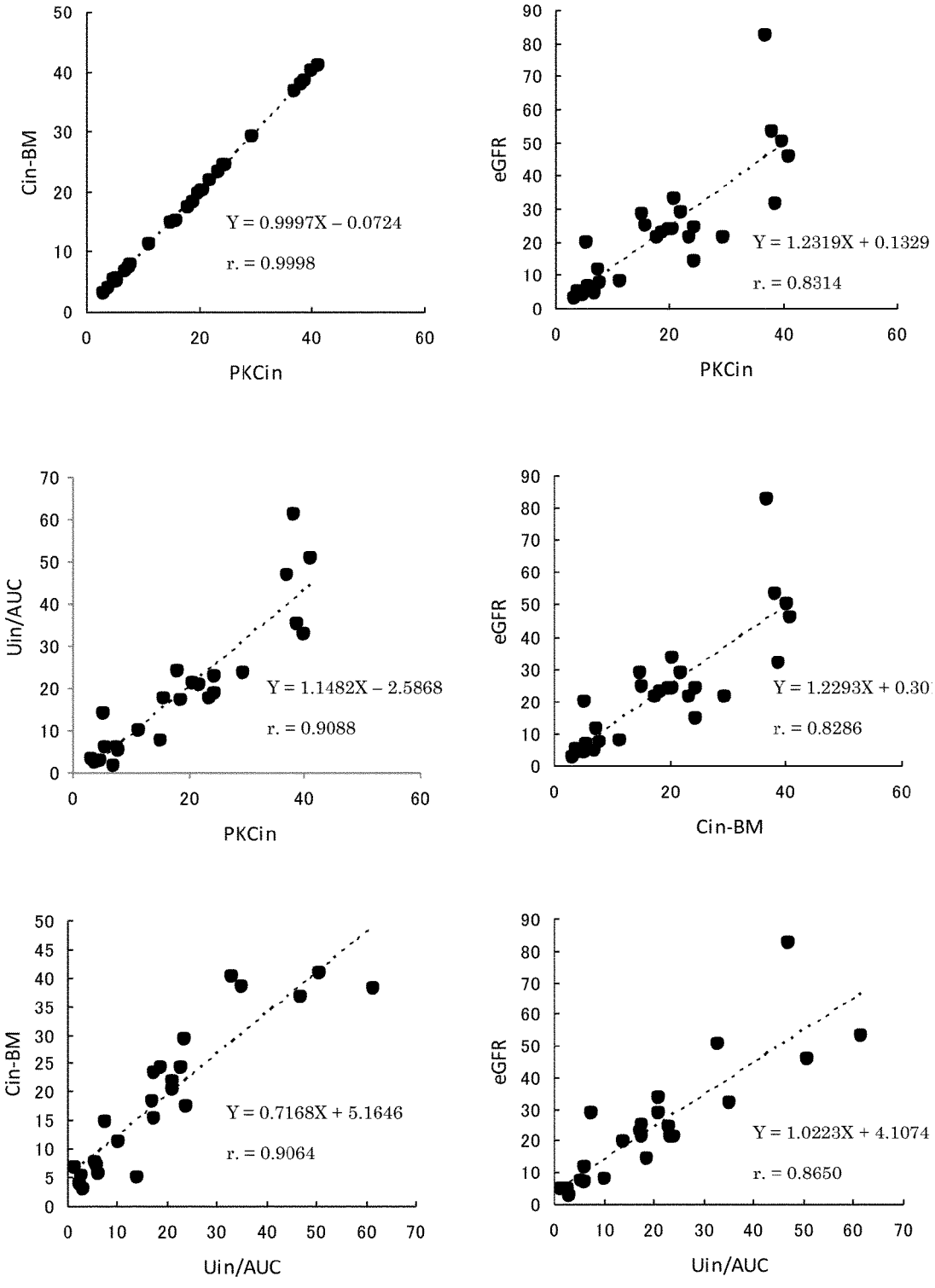

Figure 1 Correlation of PKCin, Uin/AUC, Cin-BM, and eGFR.

Table 2 Correlation coefficients of parameters for GFR

	PKCin	Cin-BM	Uin/AUC	eGFR	Ccr	β2MG
PKCin						
Cin-BM	0.9998					
Uin/AUC	0.909	0.906				
eGFR	0.831	0.829	0.865			
Ccr	0.825	0.821	0.864	0.970		
β2MG	0.634	0.631	0.752	0.909	0.887	
CysC	0.857	0.856	0.830	0.956	0.932	0.828

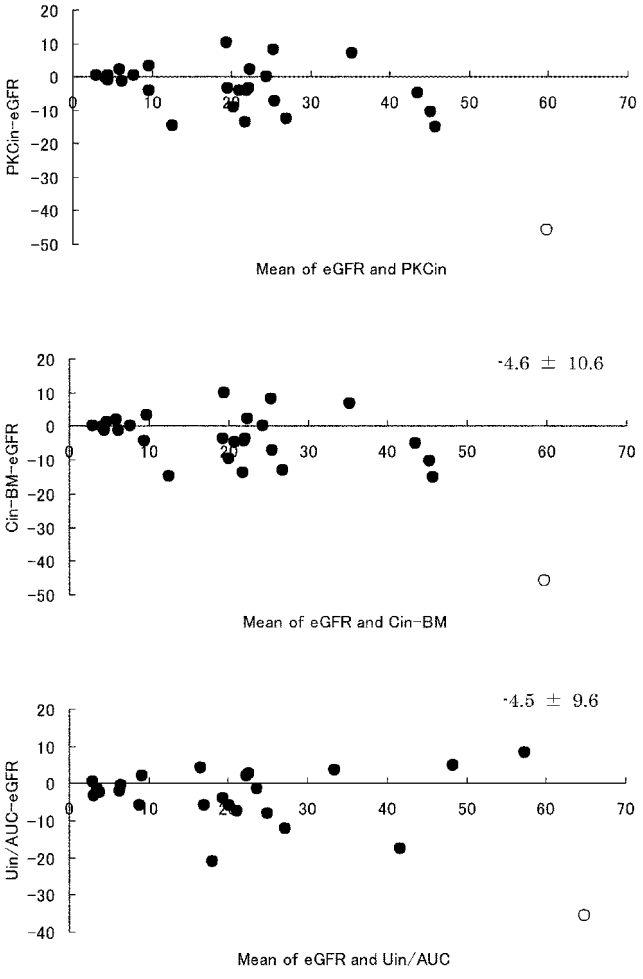

Figure 2 Bland-Altman plots of PKCin, Cin-BM, Uin/AUC, and eGFR.
The mean ± SD of the difference between each Cin and eGFR is presented in the upper right corner. Open cirle: The case in first remission of minimal change nephritic syndrome showed a big difference between Cins and eGFR.

in each clinical setting should be selected with due consideration given to the limitations of each Cin measurement.

The Cins in this study were well correlated with other parameters reflecting glomerular filtration rate. The observed correlations were comparable with those in previous reports in which Cin measured by the constant-infusion method was compared with other parameters in Japanese patients[9] and living kidney donors[6]. The correlations of Cins with eGFR were also similar to those in the report in which the equation for eGFR was originally developed; the equation was based on Cin obtained by the constant infusion method. The correlation coefficients in those studies were between 0.50 and 0.85.

Direct comparison of Cins reported here with Cin obtained by the drip infusion method was practically difficult because urinary excretion of inulin is disturbed by impaired renal function, which means that a longer interval period (> one week or longer) between measurements is required. Otherwise, residual inulin in the plasma from the first measurement may interfere with the second measurement. The good correlation of Cins and eGFR in this report indirectly suggests the similarities of the Cins obtained by both the rapid injection and drip infusion methods, since eGFR was originally derived from Cin by the drip-infusion method[1]. Moreover, the drip infusion method has a substantial problem as a standard technique for measuring Cin in maintaining plateau levels of plasma inulin, as described above. Therefore, we thought the Cins obtained by the protocol reported here was provided with sufficient advantages and accuracy for measuring GFR.

Although GFR is certainly affected by eating and drinking, it was not practically possible to avoid eating and drinking for 24 hours. In addition, measurement of inulin possibly interferes with several metabolites, such as fructose, but it was also difficult to prohibit foods containing such metabolites. For convenience in routine clinical settings, the protocol allowed patients to eat and drink during the measurement period. The levels of inulin in blood and

Table 3 Differences of parameters for GFR

	PKCin	Cin-BM	Uin/AUC	eGFR
PKCin				
Cin-BM	0.1 ± 0.2 (0.4 ± 2.9)			
Uin/AUC	–0.3 ± 7.0 (10.7 ± 42.0)	0.4 ± 7.1 (–10.3 ± 42.9)		
eGFR	–4.5 ± 10.6 (–14.8 ± 36.7)	–4.5 ± 10.6 (–15.2 ± 37.5)	–4.4 ± 9.6 (–26.0 ± 38.6)	
Ccr	–10.0 ± 14.1 (–32.6 ± 42.1)	–9.0 ± 14.9 (–30.5 ± 44.6)	–10.6 ± 12.3 (–46.5 ± 29.2)	–5.5 ± 6.4 (–18.8 ± 24.8)

The values represent the differences between the parameters indicated by the columns and rows and are presented as mL/min/1.73m^2. Percentages are indicated in parentheses.

urine samples stayed in reasonable range, and no case was removed from the analysis. However, these concerns should be further clarified to identify inappropriate cases for measuring Cin, in which the levels of inulin in samples might not fit in predictable ranges.

As Figure 2 shows, big differences were observed between the Cins and eGFR in one patient who was in the first remission phase of minimal change nephrotic syndrome and was receiving steroid therapy. The Ccr of this patient was 103.5 mL/min. Glomerular hypofiltraton[20] and accelerated tubular secretion of creatinine[21] have both been reported in nephrotic syndrome. These conditions might be sustained even after the remission phase of nephrotic syndrome. More cases like this need to be accumulated to determine whether this observation is based on the actual condition of glomeruli in the recovery phase of nephrotic syndrome or derived from trouble related to a technical failure.

In conclusion, we found close correlations and similarity in their levels between Cins measured by rapid injection of inulin and other parameters for GFR. Since the method reported here would be a more convenient alternative to Cin obtained by drip infusion of inulin, the most suitable approach should be selected for predicting glomerular filtration rate in varied clinical settings.

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