Measurement of Glomerular Filtration Rate by Free Vitamin B₁₂ Clearance

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We determined the vitamin B₁₂ clearance using an ultrafiltration technique and assessed whether the clearance of this vitamin B₁₂ could be used to estimate the glomerular filtration rate (GFR). Fourteen subjects (5 had renal disease, 7 had diabetes mellitus, one had liver cirrhosis and one had cholelithiasis) divided into two groups were studied (group 1, 5 patients without vitamin B₁₂ preloading; group 2, 9 patients with vitamin B₁₂ preloading). Vitamin B₁₂ clearance was significantly correlated with inulin clearance ($r = 0.81$, $p < 0.001$) in group 1; group 2 showed an even better correlation ($r = 0.94$, $p < 0.001$) with the presaturated vitamin B₁₂ binding protein. In group 2, the mean inulin and vitamin B₁₂ clearance values did not differ significantly (40.3 ± 13.6 vs 48.2 ± 17.2 ml/min), but there was a significant difference between mean inulin and creatinine clearance (40.3 ± 13.6 vs 64.9 ± 19.9 ml/min, $p < 0.05$). In conclusion, vitamin B₁₂ clearance appears to be a more reliable method of estimating GFR than creatinine clearance.

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Key words: inulin clearance, creatinine clearance

Introduction

The clearance of inulin and creatinine are the parameters most widely used for the measurement of the glomerular filtration rate (GFR). However, assessment of inulin clearance is a laborious method for clinical use, and creatinine clearance does not provide an adequate estimate of the GFR (1), especially in glomerulopathic patients (2).

Since Watkin et al (3) reported that the renal clearance of free vitamin B₁₂ is similar to that of inulin, several reports (4–8) have indicated the usefulness of vitamin B₁₂ clearance as a tool for the estimation of the GFR, in studies using radioactive vitamin B₁₂.

In the present study, we determined the vitamin B₁₂ clearance by an ultrafiltration technique and assessed whether it could be used to estimate the GFR.

Patients and Methods

Patients

Fourteen subjects described below were studied. They were divided into two groups: group 1 consisted of five patients without vitamin B₁₂ preloading, and group 2 consisted of nine patients with vitamin B₁₂ preloading.

Full informed consent of all subjects was obtained prior to the study and the study was approved by the regional ethics committee.

Group 1

The simultaneous renal clearance of creatinine, inulin, and vitamin B₁₂ was measured in five patients (4 males and 1 female). Four patients had diabetes mellitus and one had liver cirrhosis. Their mean age was 59 years, ranging from 43 to 75 years. After an overnight fast, the morning urine was discarded, and then a water load (10 ml/kg) was given before starting the procedure. They were given 0.1 ml of 1:1,000 epinephrine subcutaneously and then received 500 µg of vitamin B₁₂ subcutaneously at the same site, in order to maintain a constant blood level of vitamin B₁₂ by retarding its absorption. A priming dose of inulin (50 mg/kg) was given intravenously followed by continuous infusion at a rate calculated to maintain the plasma inulin concentration constant at 25 mg/dl. After a 60-min equilibration period, three clearance determinations were performed over 30 min each. Urine samples were collected by voluntary voiding and blood samples were taken at the mid point of each clearance period.

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Group 2

Group 2 consisted of four males and five females, with a mean age of 59 years (range: 41–72 years). Five patients had renal disease, three patients had diabetes mellitus, and one patient had cholelithiasis. After an overnight fast, the first urine was discarded, and then a water load (10 ml/kg) was given as with group 1. After being given 2,000 µg of vitamin B₁₂ intravenously, the subjects received 0.1 ml of 1:1,000 epinephrine and 1,000 µg of vitamin B₁₂ subcutaneously, and two clearance determinations of 60 min each were performed. Urine samples were collected by voluntary voiding and blood samples were taken at the midpoint of each clearance period, as in group 1.

Methods

1. Creatinine

Serum and urinary creatinine concentrations were measured colorimetrically by the modification of the manual Jaffe method using an autoanalyzer (Hitachi 736; Hitachi Ltd.) (9).

2. Inulin

Inulin clearance was measured by the anthrone method (10). Briefly, 500 ml of concentrated sulfuric acid was added to 200 ml of water, and 1.4 g of anthrone was dissolved. Protein-free plasma filtrates were prepared by adding 1 ml of 10% trichloroacetic acid (TCA) to 0.1 ml of heparinized plasma. Protein-free urines were prepared by adding 2 ml of 10% TCA to 0.02 ml of urine. Then, 4 ml of anthrone reagent was added to 0.1 ml of prepared sample, and the mixture was incubated in a water bath at 37°C for 50 min. The optical density of the incubated mixture at 630 nm was calculated by an inulin standard solution.

3. Vitamin B₁₂

Serum (1 ml) was subjected to ultrafiltration for 20 min at 3,000 × g, using an ultrafiltration membrane (Centricut, Kurabou, Osaka, Japan) that only allowed the passage of molecules with a mol wt <10,000. Free vitamin B₁₂ levels in the filtered samples were measured by a competitive protein binding assay (Ciba-Corning, USA). Free vitamin B₁₂ in the urine was also measured as it was in the serum after centrifugation for 5 min at 1,500 × g.

The preliminary experiment with free ⁵⁷Co-vitamin B₁₂ demonstrated that the percentage of recovery of vitamin B₁₂ in this assay was 81.7 ± 1.5% (mean ± SEM) and the coefficient of variation was 2.4%. Values were expressed as the mean ± SEM. Statistical analysis was performed by Student's *t*-test, and results were considered significant at *p* < 0.05.

Results

Serum vitamin B₁₂

The serum vitamin B₁₂ level did not change significantly between the first and third clearance periods in group 1 (1,165 ± 377 vs 1,293 ± 431 pg/ml) and between the first and second periods in group 2 (153,896 ± 20,478 vs 144,823 ± 23,231 pg/ml).

Relationship between inulin and vitamin B₁₂ clearance

In group 1, vitamin B₁₂ clearance was correlated significantly with inulin clearance (*r* = 0.81, *p* < 0.001) (Fig. 1). In group 2 with vitamin B₁₂ preloading, there was still a significant correlation between vitamin B₁₂ clearance and inulin clearance (*r* = 0.94, *p* < 0.001).

Fig. 1. Correlation between vitamin B₁₂ clearance and inulin clearance in subjects without vitamin B₁₂ preloading.

Fig. 2. Correlation between vitamin B₁₂ clearance and inulin clearance in subjects with vitamin B₁₂ preloading.

Table 1. Comparison of the Mean Vitamin B₁₂, Inulin, and Creatinine Clearances in Subjects with Vitamin B₁₂ Preloading*

	Vitamin B ₁₂ clearance	Inulin clearance	Creatinine clearance
1	2	9	19
2	5	13	12
3	3	5	9
4	72	41	71
5	64	27	55
6	81	89	117
7	33	33	70
8	7	13	25
9	167	133	205
Mean	48.2	40.3	64.9
SEM	17.2	13.6	19.9

* Each value is the average of two clearance periods. There is a significant difference between inulin and creatinine clearance ($p < 0.05$).

(Fig. 2).

Comparison of vitamin B₁₂, inulin, and creatinine clearance

In group 2, the inulin, vitamin B₁₂, and creatinine clearances were all compared. The mean inulin and vitamin B₁₂ clearance data of the nine patients did not differ significantly (40.3 ± 13.6 vs 48.2 ± 17.2 ml/min), but there was a significant difference between the mean inulin and creatinine clearance values (40.3 ± 13.6 vs 64.9 ± 19.9 ml/min, $p < 0.05$) (Table 1).

Discussion

Inulin and creatinine clearance are generally used for the estimation of the GFR. Because inulin is freely filtered at the glomerulus but not acted on by the tubules, inulin is believed to be the ideal marker for determining GFR. Although there are variations in inulin clearances measured in a given individual over time and diurnal variations (11), the inulin clearance has become the standard method for estimation of the GFR. However, the method is too laborious for routine use. The endogenous creatinine clearance is a simple method for estimation of the GFR, but it does not provide adequate information, especially in patients with renal insufficiency (2).

In this study, we assessed a simple method for the estimation of the GFR using the free vitamin B₁₂ clearance. Vitamin B₁₂ is firmly bound by plasma proteins in physiologic amounts and is not filtered by the glomeruli. Thus, only free vitamin B₁₂ can be excreted by the kidneys on the basis of glomerular filtration (4, 5), and the vitamin B₁₂ clearance has been shown in several reports (6–8) to correlate with the inulin clearance. At low levels of serum vitamin B₁₂, the error in estimating

vitamin B₁₂ is sufficiently large to warrant the inference that the urinary clearance of vitamin B₁₂ would be equivalent to the inulin clearance (3); we obtained a better correlation between the vitamin B₁₂ and inulin clearances after presaturation of vitamin B₁₂ binding protein in group 2.

The prior injection of a small dose of epinephrine retarded the absorption of vitamin B₁₂, and allowed a constant serum vitamin B₁₂ level to be maintained during the study by a single subcutaneous injection of the vitamin. The small dose of epinephrine did not produce any symptoms and was unlikely to affect renal function (12). This method required no constant infusion of vitamin B₁₂. Moreover, as an ultrafiltration technique was used to measure the free vitamin B₁₂ level (13), there was no need to handle radioactive material or to expose the subjects to radiation. Assessment of the vitamin B₁₂ clearance following a single subcutaneous injection of vitamin B₁₂ with epinephrine appears to be a simple and useful method for the estimation of the GFR.

References

- 1) Levey AS, Perrone RD, Madias NE. Serum creatinine and renal function. *Ann Rev Med* **39**: 465, 1988.
- 2) Shemesh O, Golbetz H, Kriss JP, Myers BD. Limitations of creatinine as a filtration marker in glomerulopathic patients. *Kid Int* **28**: 830, 1985.
- 3) Watkin DM, Barrows CH Jr, Chow BF, Shock NW. Renal clearance of intravenously administered vitamin B₁₂. *Proc Soc Exp Biol Med* **107**: 219, 1961.
- 4) Nelp WB, Wagner HN Jr. Use of radioactive vitamin B₁₂ to measure the glomerular filtration rate (GFR). *J Nuclear Med* **4**: 216, 1963.
- 5) Nelp WB, Wagner HN Jr, Reba RC. Renal excretion of vitamin B₁₂ and its use in measurement of glomerular-filtration rate in man. *J Lab Clin Med* **63**: 480, 1964.
- 6) Breckenridge A, Metcalfe-Gibson A. Methods of measuring glomerular-filtration rate: A comparison of inulin, vitamin-B₁₂, and creatinine clearances. *Lancet* **2**: 265, 1965.
- 7) Cutler RE, Glatte H. Simultaneous measurement of glomerular filtration rate and effective renal plasma flow with ⁵⁷Co-cyanocobalamin and ¹²⁵I-Hippuran. *J Lab Clin Med* **65**: 1041, 1965.
- 8) Patterson RM, Ray CT. Studies of the applicability of cobalt 57 labelled vitamin B12 (CoB12) for measuring glomerular filtration rate (GFR). *Clin Res* **13**: 80, 1965.
- 9) Chasson AL, Grady HJ, Stanley MA. Determination of creatinine by means of automatic chemical analysis. *Am J Clin Pathol* **35**: 83, 1961.
- 10) Davidson WD, Sackner MA. Simplification of the anthrone method for the determination of inulin in clearance studies. *J Lab Clin Med* **62**: 351, 1963.
- 11) Schuster VL, Seldin DW. Renal clearance. in: *The Kidney*, Seldin DW, Giebisch G, Eds. Raven Press, New York, 1992, p. 943.
- 12) Israelit AH, Long DL, White MG, Hull AR. Measurement of glomerular filtration rate utilizing a single subcutaneous injection of ¹²⁵I-iothalamate. *Kid Int* **4**: 346, 1973.
- 13) Davies AG, Leftley JW. Vitamin B₁₂ binding by microalgal ectocines: dissociation constant of the vitamin-binder complex determined using an ultrafiltration technique. *Marine Ecology Progress Series* **21**: 267, 1985.