Evaluation of Single-Injection Method of Inulin and Creatinine as a Renal Function Test in Normal Cats

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ABSTRACT. A single injection method for estimating glomerular filtration rate (GFR) by measuring plasma inulin and creatinine clearances was evaluated in 10 healthy cats. GFRs were estimated from the plasma clearance (PC) by dividing the injected dose of an indicator by the area under the plasma disappearance curve (AUC). AUC was determined by 2 common pharmacokinetic analyses, the two-compartment model and the trapezoidal rule. AUCs determined by these two methods were significantly correlated both in inulin (r=0.993) and creatinine (r=0.959). To minimize errors, GFR was estimated by PC only if AUC/10 was greater than the area under the curve from the final sampling time to infinitive (A2). GFRs determined by PC of inulin at final sampling time of 180 and 240 min were 3.61 ± 0.64 and 3.63 ± 0.67 ml/min/kg of body weight (mean ± SD), respectively. These values corresponded to the reference range reported for normal cats. In contrast, when creatinine was used as a maker, A2 was always greater than AUC/10 at any final sampling time and GFRs estimated using these AUCs of creatinine were significantly greater than those of inulin, suggesting creatinine may not be suitable indicator for the single injection method. — KEY WORDS: creatinine, feline GFR, inulin, single injection method.

Glomerular filtration rate (GFR) is one of the parameters of the renal function which most clinicians are interested to determine. GFR can be used to document suspected renal disease, to monitor the course of renal disease, and to modify drug dosages which are potentially toxic to the kidney. Urinary clearance of exogenous inulin is the accepted method to quantitate GFR [11, 19] and the urinary clearance of exogenously administered creatinine clearance has been demonstrated to also estimate GFR in dogs [12] and cats [22]. However, these urinary clearance procedures are cumbersome and impractical in most clinical situations because they require placement of an indwelling urinary catheter, an accurate collection of timed urine samples, and continuous infusion of the exogenous indicator [11]. In addition, GFR measurements which mandate urine collection are impossible or unreliable in patients with oliguria [14].

Single injection method of estimating GFR has been developed to obviate these constraints associated with the standard urinary clearance techniques in human [1, 7, 13, 18, 23], dogs [3, 4, 9, 14, 17], and cats [4, 5, 9, 21]. Generally, radioactive substances have been used as indicators of GFR, but the use of such substances usually are prohibited in a clinical situation. Nonradioactive indicators such as iohexol require a analytical techniques which are beyond the scope of veterinary clinician [4].

With the single injection methods, GFR is estimated as the plasma clearance of an indicator that mimics inulin. The plasma clearance (PC) is calculated by dividing the amount of indicator that was administered by the area under the plasma disappearance curve for the indicator. The area under the curve (AUC) can be calculated pharmacokinetically using a two-compartment model [14], the trapezoidal rule [6, 25], or least-squares regression analysis [4, 25].

A disadvantage of single injection methods is their tendency to overestimate the GFR [17, 19]. The rapid decline of the plasma concentration of the indicator during the early distribution phase is generally implicated in the overestimation [9]. However, a recent study in human patients demonstrated that the single injection method using inulin has the best reproducibility and good agreement with the standard urinary clearance of inulin [13].

The purpose of the study reported here was to assess the accuracy and utility of the single injection method of inulin and creatinine to estimate GFR in healthy cats. Inulin and creatinine were selected as indicators in this study because inulin is the reference standard for standard urinary clearance method and creatinine is a markers of GFR readily known to veterinary practitioners for the assessment of renal function [15]. Both indicators are not radioactive and commercially available, and the automated assays for both indicators can be readily performed in a clinical laboratory [10, 11, 15, 16, 24].

MATERIALS AND METHODS

Cats: Ten adult cats, five from cattery in my hospital and five from a local animal shelter, were used. All cats, eight males and two females, were maintained in individual cages and fed a commercial dry ration and canned fish meat and water ad libitum for at least 3 weeks before the present study.

All cats were healthy on the basis of physical examination, plasma biochemical profile, complete blood count (CBC), complete urinalysis, and serology for feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV).

Preparation of indicator infusate: For each experiment,
one gram of inulin and creatinine were reconstituted aseptically with 20 ml of sterile 0.9% NaCl, and administered via a cephalic vein at a dosage of 2 ml/kg of body weight (BW) as to provide plasma concentrations of inulin at 1 mg/ml and creatinine at 0.4 mg/ml as previously described [20].

**Experimental protocol:** Five days preceding experimental inoculation, cats were anesthetized by intravenous (IV) inoculation of droperidol at 0.25 mg/kg of BW and ketamine at 5 mg/kg of BW IV and intubated; anesthesia was maintained by inhalation of halothan in oxygen. Subsequently, a central venous catheter was placed percutaneously in the jugular vein for a blood collection. After recovery from the anesthesia, the cats were maintained for 4 days. Prior to study, each cat was fasted overnight prior to study, but freely accessible to water [11].

After an IV injection of the inulin-creatinine solution, plasma samples were collected through the jugular catheter at 0, 1, 5, 10, 15, 30, 45, 60, 75, 90, 120, 180, and 240 min to determine plasma concentrations of inulin and creatinine. All experiments were performed with cats in a conscious state.

**Inulin and creatinine assay:** Inulin was analyzed on 0.25 ml aliquote by an anthrone colorimetric assay as described previously [15]. Briefly, 1 ml of 10% trichloroacetic acid in solution was added to 250 µl of distilled water, inulin standard (0.1 mg/ml), and test plasma. After 5 min, they were centrifuged at 1,300 × g for 5 min. Fifty µl of 0.5% indol-3-acetic-acid in ethanol and 2 ml of 10 M HCl were then added to 250 µl aliquots of supernatant, and mixed, and incubated at 37°C for 75 min. After cooling to 25°C, absorbance of reagent blank, standard, and unknowns were read at 520 nm.

The concentration of plasma creatinine was measured on 0.25 ml of sample using Jaffe’s method [10].

**GFR estimation:** GFR was estimated by PC of inulin and creatinine according to the following formula [3]:

\[ \text{GFR (ml/min/kg of BW)} = \frac{D}{\text{AUC/BW (kg)}} \]

where D is the amount of injected inulin and creatinine (mg), and AUC (mg × min/ml) is the total area under the plasma disappearance curve. The two-compartment model [13] and the trapezoidal rule [6, 25] were used to determine AUC in this study. In both method, AUC is divided into two parts (Fig. 1). Major area (A1) corresponds to the area from time zero (t=0) to the last plasma sampling time (t=T). T was selected arbitrarily at 120 min, 180 min and 240 min in this study [13, 14]. Minor area (A2) corresponds in time from t=T to t= and can be calculated by the following formula [5]

\[ A2 = \frac{C(T)}{\beta} \]

where C(T) is the plasma concentration (mg/ml) of indicator at t=T and β is the slope of the plasma disappearance curve defined as a two exponential function of the form [17]:

\[ C(t) = A \exp(-\alpha t) + B \exp(-\beta t) \] (Fig. 1),

where C(t) is the plasma concentration of the indicator at time (t), A is the intercept at t=0 and α is the slope of the distribution phase, B is the intercept at t=0 and β is the slope of the elimination phase. AUC can then obtained by integration of C(t) from t=0 to t= and is given as

\[ \text{AUC} = \frac{A}{\alpha} + \frac{B}{\beta} \]

B and β were calculated by linear regression of the natural logarithm of last three corrected plasma samples. A and α were then obtained by subtracting the elimination component from the disappearance curve (Fig. 1).

A1 was also calculated as the sum of trapezoidal areas from the plasma disappearance curve using the formula:

\[ A1 = \sum_{i=0}^{T} 1/2 \times (t_{n} - t_{n-1}) \times (C_{n+1} + C_{n}) \]

where, Cn is the concentration of the plasma indicator at

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**Fig. 1. Analytical model of plasma disappearance curve.**
any time (Fig. 1) [14].

Statistical analysis: Values are expressed as mean ± SD. The mean plasma indicator concentrations versus time were compared with Tukey’s test. Variance was analyzed to assess differences in data obtained from the analysis of plasma disappearance curve of indicator at each final sampling time by the two-compartment model and the trapezoidal rule using Fisher’s test. AUCs determined by the two methods were compared using the linear regression. GFR was compared by paired-\( t \) test. A \( p \) value <0.05 was considered significant.

RESULTS

All cats were healthy and none of the cats showed adverse effects of the experimental procedure. BW of cats averaged 4.17 kg (range: 2.68 to 6.10 kg). Blood urea nitrogen (BUN) and plasma creatinine concentration were within reference range [11]; mean BUN and plasma creatinine were 12 mg/100 ml (8 to 18 mg/100 ml) and 1.20 mg/100 ml (0.72 to 1.43 mg/100 ml), respectively. FeLV and FIV were negative in all cats.

The influence of glucose on the inulin assay: Fructose fraction measured as inuline of glucose solutions averaged 0.53 ± 0.02 mg/100 mg of glucose (n=8). The degree of glucose interference increased up to 24% as inulin concentration decreased. Mean plasma baseline fructose fraction was 1.37 ± 0.33 mg/100 mg of blood glucose level (n=19), but no correlation was found between plasma fructose fraction and blood glucose concentration (r=0.022).

Analysis of plasma disappearance curve of inulin: Plasma inulin concentrations changed rapidly during the first 30 min (\( p<0.05 \)), and then decreased gradually (Table 1). There was a significant correlation between AUC determined by the two-compartment model and that computed with the trapezoidal rule (Fig. 2). There were no differences in the AUC determined when the final sampling time (T) was 120, 180, and 240 min. In contrast, A2 at T=120 min for both inulin and creatinine were 13% of AUC and this was significantly greater than the respective values at the other sampling times (Table 2). The mean GFR estimated by inulin plasma clearance was 3.61 ± 0.64 ml/min/kg of BW (T=180 min) and was not different from 3.63 ± 0.67 ml/min/kg of BW estimated at T=240 min. The distribution volume of inulin at t=0 was 92 ± 9 ml/kg of BW and 308 ± 76 ml/kg of BW during the elimination phase (Table 3), respectively. Both volumes were larger than the estimated plasma volume at 50 ml/kg of BW and the extracellular volume at 200 ml/kg of BW. Mean distribution constant from plasma \( t_{1/2} \) of the elimination phase was 57 ± 7 min. The correlation coefficient of \( t_{1/2} \) and GFR at T=180 and 240 min was r=0.328 and there was no significant difference between them.

Analysis of plasma disappearance curve of creatinine: The plasma disappearance for creatinine demonstrated a similar experimental decline as seen with inulin. There was

| Table 1. The plasma concentration of inulin and creatinine after bolus intravenous injection |
|-----------------|--------|--------|
| Time (min)      | Inulin (mg/dl) | Creatinine (mg/dl) |
| 1               | 134.2 ± 13.3a) | 47.85 ± 3.98 |
| 5               | 78.24 ± 13.00b) | 30.39 ± 3.44b) |
| 10              | 51.20 ± 8.40b) | 22.15 ± 2.91b) |
| 15              | 38.37 ± 7.56b) | 17.97 ± 2.37b) |
| 30              | 21.71 ± 4.42b) | 11.89 ± 2.03b) |
| 45              | 14.59 ± 3.4b) | 9.16 ± 1.73b) |
| 60              | 9.71 ± 3.21 | 7.42 ± 1.28 |
| 75              | 8.47 ± 2.12 | 6.54 ± 1.17 |
| 90              | 6.0 ± 1.65 | 5.84 ± 1.28 |
| 120             | 4.66 ± 1.14 | 4.98 ± 1.12 |
| 180             | 2.33 ± 0.58 | 3.72 ± 0.86 |
| 240             | 1.21 ± 0.34 | 2.91 ± 0.74 |

a) Data are expressed as mean ± SD in mg/dl.

b) Significantly different from preceding value at \( p<0.05 \).
a significant correlation between AUC determined by the two-compartment model and area computed with the trapezoidal rule (Fig. 2). There were no differences in area computed at final sample time equal to 120, 180, and 240 min. However, unlike the results for inulin, A2 was 27–40% of AUC at any time point and failed to satisfy the requirement for AUC/10 to be greater from A2.

**DISCUSSION**

BUN and plasma creatinine concentration are used commonly as filtration markers to evaluate renal function in clinical settings, but BUN is greatly affected by protein intake and protein metabolism [11, 19], and plasma creatinine is influenced by a lean body mass [19]. Both are not sensitive to early changes in GFR. More precise methods to evaluate GFR would facilitate the assessment and monitoring of renal dysfunction, however, the technical difficulties associated with the urinary clearance methods preclude such assessment. Other methods to measure GFR are suited only for a research setting or require use of biohazard substances [1, 2, 9, 25] and special analytical equipment [4, 18].

The single injection method evolved as an alternative to the standard urinary clearance methods in humans [1, 7, 13, 18, 23], dogs [1, 3, 17], and cats [2, 4, 9, 21]. Fettman et al. first demonstrated the advantage of this technique over standard GFR determination methods in cats [9], but later Rogers et al. criticized the validity of this single injection of inulin to measure GFR in cats [21]. More recently, Brown et al. demonstrated single injection methods of iohexol and inulin provided reliable estimates of feline GFR [4, 5]. Each of these previous studies was performed in anesthetized cats and each used a different indicator for GFR estimation.

### Table 2. AUC and GFR determined by analysis of plasma disappearance curves of inulin and creatinine

<table>
<thead>
<tr>
<th></th>
<th>AUC (mg, min/ml)</th>
<th>A2 (mg, min/ml)</th>
<th>GFR (ml/min/kg)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>T=120</td>
<td>T=180</td>
<td>T=240</td>
</tr>
<tr>
<td>Inulin</td>
<td>28.0 ± 5.1</td>
<td>28.4 ± 4.8</td>
<td>28.3 ± 5.0</td>
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<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Creatinine</td>
<td>22.5 ± 5.5</td>
<td>24.0 ± 5.1</td>
<td>24.6 ± 5.1</td>
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<tr>
<td>(n=10)</td>
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</tbody>
</table>

| a) Area under the curve. b) Area under the curve from t=0 to time of a final plasma sample. c) Significantly different from other two values of inulin (p<0.05). d) Significant difference between inulin and creatinine (p<0.05).

### Table 3. Experimental parameters determined from plasma disappearance analysis of inulin and creatinine using the two-compartment model

<table>
<thead>
<tr>
<th></th>
<th>Distribution rate constant (min⁻¹)</th>
<th>Distribution volume (ml)</th>
<th>t(1/2) (min)</th>
<th>β (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k₁₂(b)</td>
<td>k₂₁</td>
<td>k₁₀</td>
<td>t=0</td>
</tr>
<tr>
<td>Inulin</td>
<td>0.028(e)</td>
<td>0.024(e)</td>
<td>0.039</td>
<td>92 ± 9</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.037(e)</td>
<td>0.020</td>
<td>0.018</td>
<td>225 ± 30</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
<td>25 ± 0</td>
</tr>
</tbody>
</table>

| a) k₁₂, k₂₁, and k₁₀ are the distribution rate constants from the central compartment (X1) to peripheral compartment (X2), from X2 to X1, and X1 to urine, respectively. b) Distribution volume during the elimination phase. c) Plasma half-life. d) slope of the elimination phase. e) Significantly different from k₁₀ (p<0.05).
were highly reproducible in response to a constant dosage in these normal cats (Table 1). It is quite important for accurate measurement of A2 as C(T)/β that β remains a relatively small number with a narrow range of variability (Table 3). At the dosage used, AUC is less than 30 mg.min/ml (Table 2) and A2 is less than AUC/10. If C(T) of inulin and creatinine is greater than 3 mg/100 ml, AUC cannot be used to estimate GFR and additional plasma samples must be collected at the point under the curve is monoexponential. As GFR decreases, β will become smaller due to a prolongation of plasma t½ of indicator. Under these conditions, the dosage of inulin and/or creatinine must have decreased so that C(T) is low enough to satisfy the requirement of AUC/10>A2.

There were no differences for AUCs predicted by the two-compartment model and the areas determined by the trapezoidal rule for both inulin and creatinine (Table 2).

Although AUC of inulin and creatinine were similar at any T, the A2 of inulin at T=120 min was significantly greater than that of T=180 and 240 min as well as corresponding AUC/10 (Table 2). A2 of creatinine at any T was 27–39% of the AUC and always greater than AUC/10. GFR can be estimated using AUC of creatinine regardless of the fact AUC/10<2A2, but the corresponding GFRs were significantly greater than those of inulin (Table 2) and beyond the reference range for cats [12, 26]. Therefore, AUC of inulin at T=120 min and creatinine at any T in this study were not likely valid for GFR estimation.

The distribution volume at t=0 was approximately 9% of BW, suggesting inulin is distributed initially in plasma volume. The mean distribution volume during the elimination phase was 308 ml/kg of BW, which is approximately 50% greater than the estimated extracellular volume of 200 ml/kg of BW. This observation suggests that inulin is trapped into some tissues or organs in the central compartment [25]. Therefore, inulin fulfills the requirements of an indicator for the single injection method in cats.

In contrast to inulin, the distribution rate constant from central compartment to urine (kto) of creatinine was significantly less than that to prepheral compartment (kto) (Table 3), suggesting that creatinine did not equilibrate rapidly within the central compartment after a bolus IV injection and a lesser portion of the injected creatinine was eliminated by glomerular filtration. The finding that the distribution volume of creatinine during the elimination phase was 885 ± 160 ml/kg of BW suggested that a significant portion of injected creatinine was trapped in some compartments during this phase [25]. These findings predict that the pharmacokinetic behavior of creatinine may be different from that of inulin. Consequently, a two-compartment model may not be a suitable analytical method to describe creatinine excretion following IV injection and creatinine may not be an appropriate pharmacokinetic indicator for single injection method of GFR estimation.

In conclusion, inulin was shown to be a good indicator for a single injection GFR estimation in normal cats, and may be used as an alternative renal function test for cats with renal diseases. However, creatinine showed a quite different pharmacokinetic behavior and single injection techniques could not be validated for GFR estimation.

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