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REGULAR ARTICLE

Assessment of glomerular filtration rate in normally hydrated and dehydrated dromedary camel by plasma exogenous creatinine clearance test

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Abstract

The main objective of this study was to assess glomerular filtration rate (GFR) in the camels (*Camelus dromedarius*) under free water access and dehydration conditions (after a 34 days-period of water deprivation) using plasma exogenous creatinine clearance without urine collection. Trials were carried out on six non-pregnant, non-lactating and healthy female camels. Creatinine was administered as an IV bolus at a dose of 16 mg/kg body weight. Blood samples were collected at predetermined times over 24 h post-injection. Plasma creatinine concentration was analysed using Jaffé method. Creatinine clearance was calculated by pharmacokinetic analysis using a non-compartmental approach. Water deprivation induced a significant 15%-decrease in body weight but did not affect haematocrit and total plasma proteins. Mean corpuscular volume increased and red blood cells number decreased in dehydrated conditions. Dehydration produced a significant 30%-increase in plasma creatinine and mean residence time and a significant 20%-decrease in GFR. In conclusion, water deprivation decreased glomerular filtration and plasma exogenous creatinine clearance test could be used as a practical method for GFR assessment in dromedary camel in field conditions.

Key words: *Camelus dromedarius*, Creatinine, Dehydration, Glomerular filtration rate, Kidney

Introduction

Dromedary camel is well adapted to extreme ambient temperature and associated plasma osmotic fluctuations. Indeed, this animal species can endure long periods without water in desert conditions. When water is available, it is often brackish due to high evaporation rate. Moreover, food available in such arid environments is frequently very salty and considered as unpalatable by other domestic species (Yagil, 1986).

Adaptation of the dromedary camel to dehydration results from unique anatomical and physiological features. When long term water deprived, this animal is able to ensure homeostasis and maintain water balance by specific mechanisms reducing water losses (reduced urine production with increased urine concentration, limited sweating, decreased basal metabolism, changes in body temperature, etc). The urinary excretion of metabolites that need large quantities of water (e.g., glucose, urea, phosphorus) for their elimination is also decreased (Bengoumi and Faye, 2002). Kidney function by regulating water balance and urine excretion therefore plays a major role in the camel's adaptation to extreme environmental conditions (Yagil, 1993; Bengoumi et al., 1993).

The best overall indicator of kidney function is the glomerular filtration rate (GFR) which can be assessed using an appropriate marker like creatinine. Plasma creatinine is indeed used for indirect GFR assessment. GFR can be also directly estimated by urinary clearance of creatinine. However, this approach is tedious and time consuming and requires collection of urine. For these reasons, alternative plasma clearance methods based only on repeated blood sampling after

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administration of the marker has been proposed. The plasma exogenous creatinine clearance test has been used for GFR assessment in dogs (Watson et al., 2002) and cats (Le Garreres et al., 2007), but has never been performed in dromedary camel.

The present study aims i) to assess GFR in dromedary camel under normal and dehydrated conditions using plasma exogenous creatinine clearance as an estimate and ii) to evaluate the practicability of this method in dromedary camel for GFR testing.

Materials and Methods

The study was performed over 2 periods. During the first period, GFR was assessed under normal watering conditions. In the second period, dehydration was induced by total water deprivation and GFR was measured after 34 days of dehydration.

Animals

This trial was carried out in Institut Agronomique et Vétérinaire Hassan II in Rabat-Morocco on six non-lactating female dromedary camels older than 7 years. Animals were fed before and during this experiment with 2 kg/animal/day concentrated feed and 3 kg/animal/day wheat straw which contains few quantity of water (10%). Water was given *ad libitum* during normal hydration period. Body weight was assessed using barymetric measurements on the day of GFR testing (Schwartz and Dioli, 1992).

Induction of dehydration by water deprivation

Water was removed the day after completion of blood sampling for the GFR testing performed in normal conditions. Animals were water deprived for 34 days. Throughout the study, the animals were housed in a barn where the ambient temperature was maintained between 20°C and 23°C. For animal welfare reasons, animals were examined every day to collect body temperature and to observe their behaviour, to detect any adverse effect and discomfort (eg, apathy and pain).

GFR testing

The injectable creatinine solution was prepared as follow: 40 g of anhydrous creatinine (creatinine free base anhydrous-crystalli, C2455, Sigma-Aldrich, France) was progressively dissolved in 500 ml of distilled water, and sterilized by filtration using 0.22 µm paper filter. The prepared solution (at a final concentration of 8 g/100 ml) was intravenously injected at a nominal dose of 16 mg/kg of body weight corresponding to 20 ml of the solution/100 kg of body weight. This dose was chosen according to results from a pilot study. The volume to be injected to each animal was calculated according to the body weight measured on the same day. The solution was injected *via* a catheter inserted in the left jugular vein. Immediately after the end of administration, the catheter was rinsed with distilled water (around 100 ml) and removed. An aliquot of the solution was kept for measurement of the creatinine concentration in the solution.

For injection and first blood samples, animals were kept in sternal recumbency position. Animals were fed until creatinine administration but no food was offered during the day of the GFR testing.

Blood (8-10 ml) was collected from the right jugular vein in vacuum tubes with anticoagulant (heparin) at 0 (just before injection for determination of basal plasma creatinine concentration), and then at 2, 6, 10, 20, and 40 min and 1, 1.5, 2, 4, 6, 8, 12, 18 and 24 h after administration.

Blood was centrifuged (3000g/min during 15 min) within 30-45 min after sampling. Plasma was stored at -20°C until laboratory analyses. After the last blood sampling, animals were untied and food was given; water was distributed *ad libitum* for each animal.

Other tested variables

Blood was collected before administration of creatinine and every week for measurement of total plasma proteins, haematocrit, red cells number (RCN) and mean corpuscular volume (MCV) which were used as indicators of dehydration.

Assays

Plasma creatinine concentration was analyzed using Jaffe method. Haematocrit was determined using haematocrit tubes after centrifugation at 3000g during 10 min. Total plasma proteins concentration was determined using refractometer. RCN was counted on total blood using Neubauer cells counter and MCV was calculated as the ratio of haematocrit and RCN.

Pharmacokinetic analysis

Pharmacokinetic analyses were performed using WinNonlin Software (Version 5.2, Build 200701231637 Core version 18 Sept 2006) by a non-compartmental approach. The basal concentration of creatinine determined just before administration (time 0) was subtracted from the plasma creatinine concentration observed after administration of creatinine. The area under the curve (AUC) of plasma creatinine concentration versus time was determined using trapezoidal rule with extrapolation to infinity (Watson et al., 2002).

The AUC was calculated by adding area of each trapeze defined by successive time points (T_n and T_{n+1}) and corresponding to plasma creatinine concentrations (C_n and C_{n+1}) (Creton, 2008).

$$\text{Aire}_{\text{trapèze}} = \frac{[C_n] + [C_{n+1}]}{2} \times (T_{n+1} - T_n)$$

$$\text{Aire}_{\text{totale}} = \sum_{i=1}^{n} \text{Trapèze}_i$$

Linear extrapolation from last points to infinity was done. Extrapolated AUC was calculated as follow:

AUC extrapolated = Clast/ λz and so:

$$\text{AUC} = \sum_{t=0}^{t_{last}} \frac{[C_n] + [C_{n+1}]}{2} \times (T_{n+1} - T_n) + \frac{[C_{last}]}{\lambda_z}$$

Clast is the last observed concentration (at time Tlast), and λz is the slope of the elimination phase determined from last points of the plasma creatinine concentration *vs* time curve.

Plasma creatinine clearance was calculated by dividing administered dose by AUC (Watson et al., 2002). Steady state volume (Vss) of distribution and mean residence time (MRT) were obtained by standard equations (Watson et al., 2002). These two parameters are linked together and to GFR by the following formula (Creton, 2008): Vss = DFG x MRT

Statistical analysis

Statistical analysis was performed using Excell software. Comparisons of tested variables between normal and dehydrated conditions were performed using Student's t test. $P<0.05$ was considered for the difference to be significant and results are expressed as mean ± standard error.

Results

Effect of water deprivation on body weight, haematocrit, total proteins, RCN and MCV

The 34-day water deprivation induced a significant decrease in the mean body weight by 15% ($p<0.001$), RCN by 33% ($p<0.01$) and MCV by 34% ($p<0.05$) (Table 1). Haematocrit and total proteins were not significantly affected by dehydration.

Table 1. Body weight, hematological and plasma variables 6 adult camels dromedaries before and after 34 days of water deprivation.

(data are expressed as mean ± SD)

	Before	After
Weight (kg)	391±51	333±52***
Haematocrit (%)	26.5±1.517	27.5±2.429
Total Proteins (g/100ml)	42.667±2.658	45.167±1.169
RCN (10^6/mm3)	8.617±1.402	5.783±0.739**

In bold are indicated the statistically significant differences induced by dehydration.

** : p<0.01 *** : p<0.001

Effect of water deprivation on plasma basal concentration and kinetics of creatinine

Water deprivation induced a significant ($p<0.05$) increase in mean plasma creatinine concentrations by 30%. Two camels (B and F) however did not show any increase for this parameter (Table 2).

Table 2. Plasma creatinine in 6 adult camels dromedaries before and after 34 days of water deprivation.

Animals	Before		After	
	mg/dl	µmol/l	mg/dl	µmol/l
A	0.69	61	1.59	140
B	1.45	128	1.33	118
C	1.17	104	1.72	152
D	1.15	102	1.56	138
E	1.15	102	1.59	140
F	1.46	129	1.41	124
Mean	1.18	104	1.53*	135*
SD	0.28	25	0.14	12

In bold are indicated the statistically significant differences induced by dehydration.

*: p<0.05

Table 3. Glomerular Filtration Rate in 6 adult camels dromedaries before and after 34 days of water deprivation.

Animals	GFR mL/min/kg Before	After	% change
A	1.23	0.90	-26.8
B	1.64	1.20	-26.8
C	1.18	1.25	5.9
D	1.58	1.30	-17.7
E	1.08	0.81	-25.0
F	1.27	0.91	-28.3
Mean	1.33	1.06*	-19.8
SD	0.23	0.21	5.4

In bold are indicated the statistically significant differences induced by dehydration.

* : $p<0.05$

The extrapolated part of the AUC to infinity represented 9.3 ± 6.3% and 11.2 ± 4.9% of the total AUC in normal and dehydrated conditions, respectively. Plasma exogenous creatinine clearance (i.e. GFR estimate) was significantly ($p<0.05$) lower by 20% following dehydration. Changes in GFR showed large inter-individual variability from -28% to 6% (table 3). MRT increased significantly by 53% following water deprivation from 317 ± 73 min to 484 ± 131 min whereas the Vss remained unchanged (409 ± 65 ml/kg before vs 496 ± 79 ml/kg after water deprivation).

Discussion

The present study was performed under controlled ambient conditions (temperature, feeding, water intake) using the same animals before and after induction of dehydration.

Dehydration is generally reported to induce changes in body weight, haematocrit, and total proteins according to its severity. The 34-day water deprivation period induced a 15%-decrease of body weight. These results were different from those previously published by Bengoumi et al. (1993) who reported that a 14-day water restriction reduced body weight by 35%. However, in this latter study, the ambient temperature was higher (45°C) and thus a more severe dehydration was expected. In other animal species, body weight decreased also in response to dehydration. Only a 3-day water deprivation period causes a decrease in body weight by 21% in indigenous male goats in Saudi Arabia, 18% in Sudanese male goats and 20% in Bedouin non lactation female goats (Alamer, 2006).The loss in body weight in dromedary camel was supposed to be due only to water loss and not to tissue substance losses, in contrast to what was reported in most animal species which stop eating once water is no more available (Schmidt Nielsen et al., 1956). According to Djegham and Belhadj (1986), dromedary camel resistance to water deprivation was due to its ability to mobilise its water storage and to transfer it from one to another compartment. Thus, the dromedary camel is able to loss until 25% of total body water without any dehydration-associated clinical sign.

In the present study, haematocrit values remained within the usual values [20-33%] as described by Yagil et al. (1974a). They were lower than those reported by Bengoumi (1993) (30% in hydration state and 38% in dehydration state), but again the dehydration conditions were different. In the present study, the 34-day water deprivation period did not affect significantly the haematocrit. This variable appears therefore to be a relatively insensitive indicator of moderate dehydration in the dromedary camel. It cannot be excluded however that haematocrit could increase in other conditions (higher ambient temperature, prolonged water deprivation period) leading to severe dehydration, as previously observed for goats (Alamer, 2006). Bengoumi et al. (1993) showed in the dromedary camel that haematocrit did not change during the first dehydration week, then increased significantly from 30% to 38% after 14 days water deprivation with ambient temperature of 40.1°C. Rehydration induced a progressive return to pre-dehydration values from 32% to 30%, respectively, after 12 hours and 4 days. In contrast, a decrease has been reported also in camels between 7 and 12 days of water deprivation. Haematocrit then returned to normal value (Mahmud et al., 1984). Similar results were also observed by Yagil et al. (1974b) who suggested that haematocrit decrease was explained by the decrease in red cells size resulting from increased blood tonicity.

RCN remained within previously described usual values, i.e. between $3.8\times10^6/mm^3$ and 12.6×10^6 $/mm^3$ (Yagil et al., 1974a), but dehydration caused a significant decrease in RCN. Similar findings were reported by Yagil et al. (1974a). The increase in MCV after dehydration results from the decrease in RCN as the haematocrit remained unchanged.

The plasma creatinine values observed in normally watered animals were similar to those previously published by Ben Romdhane et al. (2003). Water deprivation induced a significant 30%-increase in plasma creatinine concentration. The values observed in the present study were close to those reported by Bengoumi (1993) (116 µmol/l) in normally hydrated but lower than those in dehydrated animals (437 µmol/l). Again, the dehydration conditions were more drastic in this latter study. Different results about the effect of dehydration on plasma creatinine in the dromedary camel have been previously published. Yagil and Berlyne (1977) stated that moderate dehydration did not affect plasma creatinine concentrations, while severe dehydration induced a significant increase by 61%. In fact as in other mammals, Sulivan (1974) noticed increase of plasma creatinine resulting from decreased renal elimination in dehydrated camels (Yagil et Berlyne, 1977).

The moderate increase of creatinine observed in this study after 34 days of water deprivation was in contrast with what has been reported in other species living in arid environment after short period of water deprivation. A 72 h-dehydration in indigenous goats in Saudi Arabia induced an 81%-increase in plasma creatinine concentration. Also, an 88%-increase was reported in Awassi sheep after only 5 days of water deprivation. In Barki sheep, a 13%-increase was observed but only after 3 days of water deprivation (Alamer, 2006). These comparisons could lead to the conclusions that the kidney function in the dromedary camel is less sensitive to dehydration than the other species. Nevertheless, the basal plasma concentration of creatinine is a hybrid parameter depending not only on the renal excretion of creatinine but also on its production and volume of distribution (Watson et al, 2002). Therefore, interpretation of changes in basal plasma creatinine concentration in terms of renal function alteration should be done cautiously.

One of the distinct advantages of this study is that direct assessment of GFR has been performed in both conditions. The plasma exogenous creatinine clearance test was here selected for measuring GFR for the following reasons: i) it requires only repeated blood sampling without any urine collection, ii) plasma creatinine can be easily measured, iii) creatinine is stable in blood and plasma allowing delays for centrifugation and storage (Braun et al., 2003), iv) it is a cost-effective method as the injectable solution and assay for creatinine are not expensive compared to those for other markers (e.g., inulin, iohexol), v) creatinine is an endogenous compound which was shown in dogs to have an excellent tolerance when injected as an IV bolus at 160 mg/kg (Watson et al., 2002), and vi) the creatinine clearance can be, if needed, manually calculated without any specific pharmacokinetic software (Watson et al., 2002). Although no commercially available formulation of creatinine exists, the major advantage of the plasma exogenous creatinine clearance test therefore is that it is an easily practicable method in field conditions, as illustrated here for the dromedary camel. No adverse effect was observed following creatinine administration in any animals in normal and dehydrated conditions, and tolerance of repeated blood sampling in this animal species appears to be good. The extrapolated part of the AUC observed in normally hydrated and dehydrated camel was less than 15% of the total AUC, as previously recommended (Watson et al., 2002), indicating that performing the last blood sample at 24h post-injection is appropriate. From λz, the mean elimination half-life (data not shown) was estimated to 4.4 h in normally hydrated and 6.3 h after induction of dehydration. Approximately 4 times the elimination half-life is required to clear 94% of the exogenous creatinine injected. Therefore, in order to minimize the proportion of the AUC extrapolated, the last blood sampling should be performed between 16 and 24 h post-injection according to the hydration status.

In our study, 34 days of water deprivation in camels induced a significant but moderate decrease (20%) in GFR (1.33±0.22 ml/min/Kg in hydration status and 1.06±0.21 ml/min/Kg in dehydration status). Bengoumi (1993) observed a 60%-decrease in endogenous creatinine urinary clearance after 13 days of water deprivation. Yagil and Berlyne (1977) reported as well that severe dehydration (T°= 36.9°C, 10 days) induced a decrease in urinary creatinine clearance by 72%. These results were in agreement with those of Alamer in sheep and goats (2006). GFR decrease is less important in our study compared to these previous data. This discrepancy could be explained by the dehydration conditions (less severe in the present study) and the method used for GFR assessment. Urine collection in dromedary camel is indeed tedious with risk of inaccurate estimation of the urine volume collected traduced by a lower value of GFR and therefore an underestimation of renal clearance.

Conclusion

In conclusion, GFR decreased moderately after a 34-day water deprivation period confirming the

adaptation of dromedary camel to water scarcity. This study demonstrated practicability and good tolerance of plasma exogenous creatinine clearance testing in the dromedary camel which could be used for improving knowledge about GFR in various physiological and clinical settings in this animal species.

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