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BRIEF COMMUNICATION

Changes in insulin-like growth factor-1 and IGF-binding protein-3 in camel plasma during dehydration in the presence and absence of losartan

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Abstract In the present study, the effect of 20 days of dehydration in the presence or absence of losartan (angiotensin II AT1 receptor antagonist) on insulin-like growth factor-1(IGF-1) and insulin-like growth factor-binding protein-3(IGFBP-3) in plasma of the one-humped camel was studied. Eighteen male camels, 3–4 years of age, were divided into three equal groups: control, dehydrated, and dehydrated–losartan-treated groups. The control camels were given food and water ad libitum. The two dehydrated groups underwent 20 days of water deprivation but were given food ad libitum. The dehydrated–losartan-treated camels were given losartan injection (Merck, USA), intravenously at a dose of 5 mg/kg body weight daily for 20 days. Our results demonstrated a progressive decrease in the circulating levels of IGF-1 and IGFBP-3 in the dehydrated and dehydrated–losartan-treated animals across dehydration compared to their basal levels and time-matched control. On day 5 of dehydration, the IGF-1 level in the losartan-treated group showed a decrease of 60 % and the dehydrated group showed 45 % decrease from their baseline levels and time-matched control. On day 10 the decrease in the losartan-treated animals reached 74 % and for the dehydrated was 62 %. On day 20 the decrease in the losartan-treated was 89 % and for the dehydrated reached 80 % from their baseline levels and time-matched control. Dehydration in the presence or absence of losartan caused a decrease in the circulating level of IGFBP-3. The decrement reached 26 % on day 10 and 20 for the treated camels, while the decrease for the dehydrated was 22 % on day 10 of dehydration and reached 29 % on day 20 compared to their baseline levels and time-matched control. In conclusion, dehydration alone, or in presence of Angiotensin II AT1 receptor blocker caused significant decrease in the circulating levels of IGF-1 and IGFBP-3 compared to their basal values and to time-matched controls. Losartan enhanced the effect of dehydration mainly in the early phase of dehydration for both parameters; albeit, no significant differences between the two dehydrated groups was observed. Finally, these findings suggest an essential role of IGF-1and IGFBP-3 in the dehydration state of these dromedarian camels.

Keywords Camel · Dehydration · IGF-1 · IGFBP-3 · Losartan

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Introduction

The one-humped camel (*Camelus dromedarius*) is traditionally and economically an important animal in the Gulf region. The camel is known for its ability to survive water deprivation for long periods. Indeed, camels can tolerate a loss of water corresponding to 30 % of body weight, whereas other mammals may die from circulatory failure when water loss reaches 12 % of their body weights (Schmidt-Nielsen et al. 1956). All vertebrates maintain plasma osmolality and extra cellular volume primarily by regulating the ingestion and excretion of water and electrolytes; an elevation in plasma osmolality, and consequent

cellular dehydration, is the most potent stimulus of thirst (Antunes et al. 2004).

Insulin-like growth factor-I (IGF-I) is produced primarily by the liver as an endocrine hormone and in target tissues as a paracrine or autocrine. IGF-1production is stimulated by growth hormone (GH) and can be retarded by under nutrition. IGF-I mediates chondrogenic effects of growth hormone in promoting long bone growth, and regulate the proliferation and differentiation of various cell types, including bone marrow cells. Regarding its amino acid sequence, IGF-I shows partial homology with the pro-insulin peptide, and also exhibits insulin-like bioactivities (Froesch et al. 1996; Daughaday and Rotwein 1989). IGF-1 is expressed at low concentrations in the stromal elements of normal pancreas tissue (Kroc 1998; Bergmann et al. 1995). Insulin-like growth factor-binding proteins (IGFBPs) is a family of proteins that possess unique biologic properties, and are well-described modulators of IGF activity at the cellular level (Han at el. 1992).

Losartan has a tendency to reduce IGFBP-3 levels and to increase free IGF-I levels (Zandbergen et al. 2006). The maintenance of fluid, electrolyte, and circulatory homeostasis during dehydration is partly dependent upon changes in the circulating level of hormones with known effects on sodium and water balance. Another study on IGF-1 and IGFBP-3 indicated that IGF-I regulates islet β-cell growth, survival, and metabolism and hence protects against type 1 diabetes. It also been found that the combined effect of IGF-I and IGFBP-3 gave more efficient protection from destruction of β-cell and this complex could be future treatment for the prevention of type-1 diabetes (Chen et al. 2004).

The aim of the present study was to determine the effect of dehydration in the presence or absence of angiotensin II receptor AT1 antagoniost (losartan) on plasma IGF-1 and IGFBP-3 in the dromedary camel and to compare them with a group of hydrated controls. Although a number of studies have been carried out on IGF-1 and its receptors in the one-humped camel as well as hormonal levels with and without dehydration, we are not aware of studies assessing directly IGF-1 and IGFBP-3 responses in presence of angiotensin II receptor AT1 antagonist to sustained dehydration in the camel.

Materials and methods

Eighteen male camels, 3–4 years old and body weights range (290–348 kg) were included in this study. The camels were kept in a corral during the hot summer of the Arab Gulf region, when the temperature varies between 40 and 50 °C. Camels were divided into three equal groups: dehydrated, dehydrated–losartan treated, and controls camels. The control camels were allowed free access to food and water. While, the dehydrated groups, with and without losartan were denied water access for 20 days, but food was given ad libitum. All camels were maintained on dry hay for the first week of the experiment and green-hay for the remainder of the study. The dehydrated- losartan treated camels were given losartan injection (Merck, USA), intravenously at a dose of 5 mg/kg body weight daily for 20 days. Blood samples were collected from all camels by venipuncture into cold K3-EDTA vacutainers at 2 days before the start (baseline value) and on days 5, 10, and 20 of dehydration. Blood was centrifuged at (−4 °C) and plasma was extracted and stored as aliquots at −80 °C until analysis. Assays for plasma IGF-1 and IGFBP-3 were performed using commercial radioimmunoassay kits from (Peninsula Laboratories, CA, USA). Prior to assay plasma, samples were extracted on Sep-Pak C18 columns, pre-equilibrated with 1 % trifluoroacetic acid, after elution with 60 % acetonitrile in 1 % trifluoroacetic acid and evaporated to dryness. The dried samples were reconstituted in assay buffer and the immunoreactivity of IGF-1 and IGFBP-3 were assessed using kits specific for these proteins (Peninsula Laboratories). The hormone and its binding protein levels were expressed as nanograms per milliliter plasma. The study protocol was approved by the Animal Ethics Committee of the UAE University.

Statistical analysis

The recorded values for all groups were expressed as mean± SEM. Differences between the three groups of camels for IGF-1 and IGFBP-3 levels were determined by one-way anova using SPSS version 19 with time as a repeated factor. Statistical significance was assumed at $P<0.05$.

Results

Our results demonstrated that differences in the measured levels of plasma IGF-1 and IGFBP-3 at baseline between the three groups were not statistically significant. The control group did not show any significant differences in both IGF-1 and IGFBP-3 levels from the baseline levels at all-time points of the experiment. Compared with baseline values and time-matched controls, the dehydrated–losartan-treated camels showed a decrease of 60 % and the dehydrated camels showed a decrease of 45 % in the circulating levels of IGF-1 on day 5 of dehydration. On the 10th day, the decrease in the dehydrated–treated animals reached 74 % and the dehydrated 62 % from their respective baseline values and from the time-matched control. At the end of the dehydration period (day 20), the level of IGF-1 was severely dropped in the treated group reaching a reduction

Table 1 Plasma IGF-1 (nanograms per milliliter) in control, dehydrated–treated, and dehydrated camels at baseline, day 5, 10, and 20 of dehydration

Camels group	Baseline level	Day 5 level	Day 10 level	Day 20 level
Control	331.0±92	319.8±79	326.3±54	334. 7±33
Dehydrated–treated	319.7±25	126.5±19*	82.8±15**,****	32.40±11***,****
Dehydrated	315.7±25	173.0±73	118.3±28**,****	62.0±22***,****

Data are shown as mean±SEM

*P<0.05 level of significance for basal versus losartan and dehydrated; **P<0.01 level of significance for basal versus losartan and dehydrated; ***P<0.001 level of significance for basal versus losartan and dehydrated; ****P<0.001 level of significance for control versus losartan and dehydrated

of nearly 90 % of the baseline value and time-matched controls. The decrease for the dehydrated group reached nearly 80 %. No significant differences in IGF-1 levels were observed between the dehydrated–treated and the dehydrated group at all-time points (Table 1).

The effect of 20 days of dehydration in presence or absence of losartan on the circulating IGFBP-3 was 26 % and 29 % respectively compared to their basal values and to the time-matched controls. The effect of both dehydration and losartan on the treated camels was significant on day 10 of dehydration and remained steady (26 %) to the last day of the experiment. The dehydrated group showed 22 % decrease on day 10 and 29 % on day 20 of dehydration compared to their baseline value and to time-matched controls. The decrease in IGFBP-3 level for the dehydrated group was greater than for the dehydrated-treated group; albeit, no significant differences were seen between them (Table 2).

Discussion

The one-humped camel is a typical desert animal that has developed physiological and anatomical adaptation to cope with the harsh climatic changes of the desert. IGF-1 is a low molecular mass polypeptide, found in highest concentration in serum (Ryan and Costigan 1993). It is synthesized and secreted primarily by the liver via a growth hormone-dependent process and circulates in the blood complexed to a specific carrier protein (Nissley and Rechlor 1984). In our earlier publication (Al Haj Ali et al. 2003), we demonstrated that the one-humped camel unlike other mammals has a high concentration of IGF-1 receptors in the duodenal mucosa compared to other parts of the digestive tract. It also possesses a higher concentration of IGF-1 receptors in its mucosa compared to the muscle layer. This could be a significant feature necessary for the regenerative ability of the duodenal mucosa in the one-humped camel; as the duodenum is the first part of the gut that comes in contact with the roughages.

In the present study, we observed no significant changes in the level of plasma IGF-1 concentration in the control animals compared with their baseline level after 20 days of the experiment. In contrast, the effect of dehydration with or without losartan on the circulating levels of IGF-1 in the dehydrated- treated and dehydrated groups were profound from the early days of dehydration. On day 5 of dehydration, both dehydrated group of camels showed a decrease of 45 % for the dehydrated and 60 % for the treated compared to their baseline and control. The level of IGF-1 was declining steadily as the days of dehydration continued. On day 20 of dehydration, the IGF-1 concentration for the dehydrated group reached 80 % of the baseline level as well as from the time-matched controls; while, the dehydrated–losartan-treated group showed higher reduction (89 %) in IGF-1 level. Reasons for these major drop in IGF-1 is not well-known,

Table 2 Plasma IGFBP-3 (nanograms per milliliter) in control, dehydrated–treated, and dehydrated camels at baseline, day 5, 10, and 20 of dehydration

Camels group	Baseline level	Day 5 level	Day 10 level	Day 20 level
Control	410.0±19	396.8±32	406.2±21	405.0±13
Dehydrated–treated	406.7±71	387.8±15	300.4±15*,***	302.6±21**,****
Dehydrated	396.0±31	397.0±42	308.6±13*,***	281.6±19**,****

Data are shown as mean±SEM

*P<0.05 level of significance for basal versus losartan and dehydrated; **P<0.001 level of significance for basal versus losartan and dehydrated; ***P<0.01 level of significance for control versus losartan and dehydrated; ****P<0.001 level of significance for control versus losartan and dehydrated

but possible explanation for this could be due to the reduced basal metabolic rate as a result of reduced food and water intake, and this could affect the liver function and thus reduction in IGF-1 production. Another possible explanation could be that these dehydrated camels reduce all their body synthetic processes as an adaptation mechanism to preserve water and reduce energy required. In a previous publication, we showed that dehydration for 20 days resulted in 39.1 % decrease in body weight for the dehydrated–losartan-treated camels and 34.5 % for the dehydrated camels as the result of reduced feed intake and complete water deprivation (Al Haj Ali et al. 2011).

The effect of dehydration alone on IGFBP-3 after 20 days of dehydration was 29 % reduction from the baseline level. The effect of dehydration with losartan caused a decrement of 26 % in the dehydrated–treated group at the end of experiment. It is known that the endocrine system mediates many of the physiological responses to the homeostatic and climatic demands of salt and water transport (McCormick and Bradshaw 2006). Previous studies have suggested a role of IGF-1 in the regulation of intestinal water uptake reduction and osmoregulation in fishes (Link et al. 2010). It was also indicated that hyperosmotic stress can increase growth hormone sensitivity in the gills and liver of flounders, and consequently improve IGF-I production (Meier et al. 2009). Even though the GH/IGF-1 axis has been suggested to be essential in the homeostasis of the water balance, very little is known about the role of IGF-1 in dehydration. From the present study, it is evident that dehydration resulted in a decrease in both IGF-1 and IGF1BP-3, which may reflect a disturbance in growth hormone level in this dehydrated camels. The effects caused by losartan on the IGF-1 and IGFBP-3 levels suggest a possible role of the renin–angiotensin system in the present context. It is previously shown that endurance horses with dehydration and electrolyte disturbances exhibited activation of the renin–angiotensin–aldosterone–vasopressin axis (Munoz et al. 2010). Moreover, studies in several species have demonstrated that intracerebroventricular administration of AT1 receptor antagonist drugs inhibit homeostatic responses to thermal dehydration (McKinley et al. 2001). The effects of the AT1 blocker on IGF-1 is not clear but earlier studies have shown that IGF-1 may up-regulate the AT1-receptor (Müller et al. 2000) and this may account for an important interaction that may have implications for the observed effect of losartan.

In conclusion, dehydration alone or in presence of angiotensin II AT1 receptor blocker caused significant and progressive decrease in plasma IGF-1 and IGFBP-3, indicating a linear correlation between the state of dehydration and the decrease in IGF-1 and IGFBP-3 levels. Losartan enhanced the effect of dehydration, albeit, no significant differences between the treated and dehydrated groups was seen. Finally, our results suggest an essential role of IGF-1and its binding protein-3 in water and electrolyte homeostasis in the dromedarian camel.

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Declaration of interest None.

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