Effect of Dehydration in the Presence and Absence of the Angiotensin Receptor Blocker Losartan on Blood Constituents in the Camel

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Abstract
Aim: Dromedary camels are extremely well adapted to periods of water deprivation. The physiological mechanisms underlying this adaptation, however, are imperfectly understood. It is likely that the renin-angiotensin system plays an important role although few studies have addressed this possibility in the camel. Accordingly, the effects of long term dehydration alone and with angiotensin II type 1 receptor blocker, losartan, on whole blood and serum constituents were studied in camels. Methods: Twenty eight male camels 3-4 years old were studied while under shade during summer in the Gulf-region, where the ambient temperature was above 40 degree Celsius. The camels were divided into three groups: a control group (n=6) was allowed free access to feed and water, a dehydration group (n=16) was given food ad-lib during 20 days of total water deprivation, and a dehydration plus losartan (losartan) group (n=6) which received losartan 5 mg/Kg daily by intravenous injection during 20 days of dehydration. Results: The body weight of the losartan group decreased by nearly 39.1% across dehydration whereas the reduction in body weight for the dehydration group was nearly 34.5% compared to controls. There was a significant increase in the packed cell volume (p<0.05) and leucocytes count (p<0.01) in the losartan group compared to controls. However, the mean corpuscular volume was significantly higher (p<0.05) in the dehydration group compared to controls. We observe major, statistically significant increases in serum urea (p<0.01) and creatinine (p<0.05) levels in the dehydration and losartan groups compared to controls. By the end of the period of water restriction, serum levels of gamma glutamyl transferase were significantly (p<0.01) lower in the losartan group compared to controls. Conclusion: The results of our experiment show that dehydration alone or in combination with Angiotensin II receptor blocker has major effects on the biochemical and hematological parameters of the camel blood.

Keywords: Blood, camel, dehydration, losartan, and serum.

Introduction
The one-humped camel (Camelus dromedarius) is widely distributed in the Gulf countries. It is well known that camels are able to survive water deprivation for long periods of time without ill effects. Indeed, it has been shown that the camel can tolerate a loss of
water corresponding to 30% of its body weight whereas other mammals often die from circulatory failure when water loss involves 12% of body weight. The camel's physiological adaptation to its desert environment is due in part to its extremely low rate of water turnover which is accomplished by minimal use of evaporative cooling, low urinary output, and its ability to extract water from undigested feed residues. Furthermore, the camel can change its body temperature in response to alterations in the ambient temperature in order to save water.

It is clear that thirst and secretion of antidiuretic hormone (ADH) are pivotal to the maintenance of body fluid homeostasis under many circumstances. Regulation of both thirst and ADH secretion is largely, if not exclusively, under the regulatory control of plasma and tissue osmolality and circulatory volume. Whereas it is known that the renin-angiotensin system, which is activated during dehydration, is capable of modulating thirst and ADH secretion under some circumstances, its role in response to sustained dehydration in the camel is not clear. The unique ability of the camel to survive extreme water deprivation suggests that extrapolation of data from other species might not be applicable to the camel.

Our hypothesis was that the rennin-angiotensin system (RAS) is an important regulator of electrolyte balance across water deprivation in camels. With the advent of drugs such as losartan which specifically block the angiotensin II AT-1 receptor, it is possible to dissect the physiological role of the RAS under various circumstances including dehydration. Accordingly, we measured selected biochemical and hematological indices across 20 days of dehydration in camels with or without concomitant administration of losartan compared with control, non-dehydrated, camels.

Materials and Methods

Twenty eight healthy male camels, aged 3-4 years, were studied during the summer months (June and July) in the United Arab Emirates. They were kept in a shaded corral and divided into three groups. A control group (n=6) was allowed free access to feed [Hay for the first week and thereafter Lucerne (green Alfalfa) until the end of the experiment] and water throughout the study. The losartan group (n=6) underwent 20 days of total water deprivation but free access to feed during which time they received a daily intravenous injection of losartan (5mg/kg) in normal saline. The dehydration group (n=16) was also allowed free access to feed during 20 days of water deprivation. Body weights were calculated at baseline and thereafter every third day using the formula, live weight (Kg) = Shoulder height x chest girth x hump girth x 50^2. Blood samples were obtained from the external jugular vein without apparent discomfort to the camels between 0800 and 1000 hours two days before the start of the study (day-zero) and again on the 20th day of dehydration or equivalent in control camels. Blood was collected into two vacutainers, one containing K$_3$-EDTA for the measurement of hematological indices (including packed cell volume (PCV), hemoglobin concentration (Hb), total erythrocytes (RBC) and leukocytes (WBC) count, using fully automatic hematologic analyzer (CELL-DYN 3700 system)-ABBOTT-USA)) and the second vacutainer without anticoagulant for the determination of biochemical indices including blood urea, creatinine, total protein (TP), gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT) and total bilirubin (TBili) using an ACE chemistry analyzer (Alfa Wassermann-USA).

The study was approved by the animal ethics committee of the Faculty of Medicine and Health Sciences, United Arab Emirates University.

Statistical Analysis

Mean and standard errors were calculated for all parameters. One way analysis of variance with Tukey’s test for pairwise comparisons adjusted for multiple comparisons was used for comparisons among groups at day 0 (baseline) and day 20. Statistical significance was accepted at p<0.05.

Results

Differences in the measured indices at baseline between the three groups were small and
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EFFECTS OF DEHYDRATION IN THE CAMEL

Table 1. Changes in Hematological Parameters of Dehydrated and Dehydrated Losartan Treated Compared to Control Camels

<table>
<thead>
<tr>
<th></th>
<th>Control Basal Day</th>
<th>Control Day 20</th>
<th>Losartan Basal Day</th>
<th>Losartan Day 20</th>
<th>Dehydrated Basal Day</th>
<th>Dehydrated Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>25.95±0.86</td>
<td>25.67±0.51</td>
<td>26.05±1.06</td>
<td>30.50±1.96*</td>
<td>26.66±0.67</td>
<td>28.99±1.97</td>
</tr>
<tr>
<td>Hb (g/liter)</td>
<td>12.05±0.38</td>
<td>12.03±0.28</td>
<td>12.33±0.60</td>
<td>14.32±1.20</td>
<td>13.21±0.37</td>
<td>13.52±0.56</td>
</tr>
<tr>
<td>MCH Pg</td>
<td>13.53±0.26</td>
<td>13.62±0.16</td>
<td>13.53±0.26</td>
<td>13.48±0.16</td>
<td>13.96±0.16</td>
<td>14.19±0.21</td>
</tr>
<tr>
<td>MCHC g/dl</td>
<td>46.42±0.63</td>
<td>46.93±0.33</td>
<td>47.20±0.63</td>
<td>46.63±0.90</td>
<td>46.86±0.38</td>
<td>46.57±0.25</td>
</tr>
<tr>
<td>MCV Fl</td>
<td>29.18±0.70</td>
<td>28.97±0.25</td>
<td>28.72±0.70</td>
<td>29.00±0.47</td>
<td>29.82±0.43</td>
<td>30.45±0.45*</td>
</tr>
<tr>
<td>RBC M/ul</td>
<td>8.89±0.41</td>
<td>8.86±0.13</td>
<td>9.10±0.41</td>
<td>10.57±0.80</td>
<td>9.47±0.25</td>
<td>9.69±0.34</td>
</tr>
<tr>
<td>WBC K/ul</td>
<td>10.70±1.21</td>
<td>11.57±0.71</td>
<td>10.53±1.64</td>
<td>15.23±0.70**</td>
<td>10.22±1.23</td>
<td>13.42±0.87**</td>
</tr>
</tbody>
</table>

Values are mean ± SE (standard error of the mean) for all the three groups. Control (n=6), Losartan (n=6) and Dehydrated (n=16). Significant difference from control (Day 20) is denoted by *p<0.05, **p<0.01. Not statistically significant. Mean body weight of the control animals increased progressively during the 20 day study from 302±16 to 318±14 Kg. By contrast, mean body weight decreased progressively during the 20 days of dehydration from 290±8 to 190±6 Kg for the dehydration group and from 348±21 to 212±5 Kg in the losartan group. Our results demonstrated that the packed cell volume was significantly higher (p<0.05) at day 20 in the losartan group compared to controls. By day 20 the leucocyte count was significantly higher in the losartan and the dehydrated group (p<0.01) whereas the mean corpuscular volume was significantly higher (p<0.05) in the dehydration group compared to controls (Table 1). Serum urea and creatinine levels were statistically significantly higher on day 20 (p<0.01 and p<0.05) in both dehydration and losartan groups compared to control, but there were no differences between the two dehydration groups (Table 2). Our results also demonstrated that gamma-glutamyltransferase (GGT) levels were significantly lower (p<0.01) and (p<0.05) on day 20 in the losartan group compared to the control and dehydration group respectively. However, circulating levels iron, copper, calcium, alanine aminotransferase, total biliru-

Table 2. Biochemical Changes in Serum of Control, Dehydrated Losartan Treated and Dehydrated Camels

<table>
<thead>
<tr>
<th></th>
<th>Control Basal Day</th>
<th>Control Day 20</th>
<th>Losartan Basal Day</th>
<th>Losartan Day 20</th>
<th>Dehydrated Basal Day</th>
<th>Dehydrated Day 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT u/l</td>
<td>20.83±2.17</td>
<td>33.60±2.4</td>
<td>18.17±1.60</td>
<td>15.17±5.39**#</td>
<td>19.29±2.56</td>
<td>33.31±3.48*</td>
</tr>
<tr>
<td>ALT u/l</td>
<td>19.33±1.70</td>
<td>16.0±2.4</td>
<td>21.50±3.44</td>
<td>21.0±4.3</td>
<td>20.08±0.91</td>
<td>21.1±1.9</td>
</tr>
<tr>
<td>T. bili Mg/dl</td>
<td>0.17±0.03</td>
<td>0.2±0.02</td>
<td>0.17±0.01</td>
<td>0.2±0.09</td>
<td>0.16±0.01</td>
<td>0.2±0.04</td>
</tr>
<tr>
<td>T.P g/dl</td>
<td>6.30±0.16</td>
<td>5.6±0.1</td>
<td>6.19±0.22</td>
<td>7.14±0.4</td>
<td>6.61±0.15</td>
<td>6.76±0.1</td>
</tr>
<tr>
<td>Urea Mg/dl</td>
<td>15.67±0.42</td>
<td>11.17±1.3</td>
<td>13.33±1.33</td>
<td>39.2±6.8**</td>
<td>16.50±0.86</td>
<td>34.9±3.11**</td>
</tr>
<tr>
<td>Creatinine Mg/</td>
<td>1.43±0.10</td>
<td>1.52±0.15</td>
<td>1.28±0.13</td>
<td>3.68±1.24*</td>
<td>1.32±0.06</td>
<td>2.39±0.25*</td>
</tr>
<tr>
<td>Albumin g/dl</td>
<td>3.14±0.07</td>
<td>2.8±0.01</td>
<td>3.09±0.12</td>
<td>3.4±0.10</td>
<td>3.10±0.12</td>
<td>3.3±0.10</td>
</tr>
<tr>
<td>Fe (µg/dl)</td>
<td>59.33±4.29</td>
<td>62.83±5.3</td>
<td>52.90±6.41</td>
<td>60.33±5.30</td>
<td>60.78±3.27</td>
<td>59.10±3.40</td>
</tr>
<tr>
<td>Cu (µg/dl)</td>
<td>68.67±1.74</td>
<td>79.17±2.5</td>
<td>61.80±1.24</td>
<td>75.83±6.50</td>
<td>65.38±1.37</td>
<td>75.2±2.20</td>
</tr>
<tr>
<td>Ca Mg/dl</td>
<td>8.98±0.69</td>
<td>9.22±0.05</td>
<td>10.05±0.22</td>
<td>10.90±0.20</td>
<td>10.14±0.14</td>
<td>10.89±0.09</td>
</tr>
</tbody>
</table>

Values are mean ± SE for all the three groups. Control (n=6), Losartan (n=6) and Dehydrated (n=16). Significant difference from control (Day 20) is denoted by *p<0.05, **p<0.01 and from Dehydrated (Day 20) is denoted by *p<0.05.
bin and total protein were not significantly different between the three groups by the end of the study period (Table 2).

Discussion

Camels are adapted to endure water deprivation for sustained periods of time without ill effects. Whereas a number of studies have been carried out on the blood chemistry and hematology of the one-humped camel with and without dehydration\textsuperscript{10,11} the results are often conflicting presumably because of disparate methods of analysis, differences in seasons chosen for the studies as well as gender and age of the camels. In addition, we are not aware of studies assessing directly the potential role of the renin-angiotensin system in responses to sustained dehydration in the camel. Accordingly, we have examined hematological and biochemical variables before and during a standardized period of dehydration and determined whether these responses are affected by blockade of angiotensin II type 1 receptors using losartan.

In an earlier study comparing the effects of dehydration on cattle and camels\textsuperscript{12} it was noted that when cattle were dehydrated for 9 days they lost 20% of their plasma volume causing the packed cell volume and total protein to rise by 20% and the albumin concentration to increase by a lesser extent (8%). The camel’s plasma volume does not fall to the same extent and the packed cell volume changes little due partly to the ability of the red blood cells to shrink\textsuperscript{13}. Apart from the plasma volume, hematocrit reflects RBC number and size\textsuperscript{14}. In the present study the hematocrit did not change significantly during 20 days of dehydration in line with the observations of Yagil and ZineFilali\textsuperscript{13,14}. However, a significant change in hematocrit was observed in the losartan-treated camels across dehydration. The most obvious explanation is that losartan-treated camels lost more water and hence developed a lower plasma volume than the dehydration-alone animals. This possibility is supported by the greater loss of weight across dehydration in the losartan-treated animals (mean 136 Kg, nearly 39.1% of body weight) than the dehydrated-alone group (mean 100 Kg, nearly 34.5% of body weight) and the greater increase in serum creatinine. Furthermore, plasma protein concentration, a useful indicator of plasma water volume\textsuperscript{14}, increased slightly though not significantly more in the losartan versus dehydration group. Alternatively, it is possible that the size and/or shape of red blood cells might be affected during dehydration in the presence of the angiotensin II receptor blocker indicating that the renin-angiotensin system may play a role in the morphology of red blood cells during dehydration in the camel. The change in plasma protein concentration with dehydration in the present study was greater (by approximately 5%) than in other reported studies\textsuperscript{13,15,16} most likely because the period of dehydration was longer.

The relatively high MCHC in camel erythrocytes may contribute to their resistance to hemolysis which would be an important adaptation to life in the desert environment\textsuperscript{17}. It has been reported that the camel erythrocyte is highly resistant to hemolysis in hypotonic saline solutions and that this characteristic is important during rapid rehydration following sustained dehydration\textsuperscript{18}. Several years later the unique nature of the camel erythrocyte was confirmed when they were shown to be more resistant to hypotonic solutions under conditions of dehydration than the erythrocytes of normal, hydrated camels\textsuperscript{19}.

Several lines of evidence indicate that a local bone marrow RAS contributes to regulation of both normal and malignant haematological processes\textsuperscript{20}. The potential for RAS-mediated haematopoiesis was established in in vivo studies where infusion of Angiotensin II reconstituted hematopoietic precursors in bone marrow of mice with irradiation-induced myelosuppression\textsuperscript{21}. Dehydration is known to stimulate the RAS in camels\textsuperscript{8}. Thus the significant increase of WBC observed in the dehydrated camels could possibly be due to the stimulation of the RAS. However, the significant in-
crease of WBC observed in the losartan group could possibly be due to the effect of RAS mediated through a different receptor than that blocked by losartan. Recently, it was reported that angiotensin receptors (AT₁ and AT₂) antagonists alone or in combination failed to block completely the effects of angiotensin II suggesting that another angiotensin II receptor may also be functional in leukocytes.

In conclusion, 20 days of dehydration in camels resulted in substantial weight loss which was greater, albeit not significant, in the losartan-treated animals in which the hematocrit also increased significantly. Losartan enhanced the effects of dehydration to increase the PCV, serum urea and creatinine although levels of statistical significance were not achieved. These data suggest that blockade of angiotensin II type 1 receptors compromises water retention during dehydration in the camel. Our data show that dehydration has major effects on biochemical and hematological indices and that the renin–angiotensin system plays a role in some responses to dehydration in the camel.

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