Angiotensin Converting Enzyme Activity and Nitric Oxide Level in Serum Patients with Dehydration

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Abstract

Angiotensin converting enzyme (ACE) and nitric oxide (NO) have been suggested to be involved in the regulation of fluid homeostasis. In the present investigation, ACE activity and NO levels were determined in serum of 20 patients (10 men and 10 women) with dehydration caused by gastroenterocolitis and 20 healthy individuals (10 men and 10 women). Serum and tissue ACE activity was determined by spectrophotometric method using hippuryl-l-histidyl-l-leucine (Hip-His-Leu) as a substrate. NO synthesis was determined by measuring the products of NO, nitrite and nitrate. The concentration of nitrites was determined by classic colorimetric method using Griess reagent. Nitrate concentration was determined indirectly by their reduction with elementary zinc into nitrite. Results have shown that serum ACE activity in patients with dehydration (36,46±2,74 U/L) is statistically higher then in healthy individuals (28,71±1,77 U/L, $p<0,05$). The average level of nitrites/nitrates in serum of patients with dehydration (30,57±1,05 μM; mean ± SEM) is also statistically higher then in healthy individuals (12,44±0,60 μM, $p<0,0001$). There was no correlation between ACE activity and NO production. The results indicate that ACE and NO may participate in the regulation of the alteration in blood flow and in the regulation of the water balance in patients with dehydration.

KEY WORDS: Angiotensin converting enzyme, nitric oxide, serum, dehydration

Introduction

A major interest in the study of homeostasis has focused on the physiological regulation of body fluid balance (1). Dehydration is a classic homeostatic challenge that leads to a series of well characterized endocrine responses. Thus, many studies have shown that dehydration enhances plasma vasopressin levels (2, 3) and aldosteron levels (4). The renin-angiotensin system is a master regulator of human physiology. This system plays an important role in the interrelated hormonal mechanisms that regulate blood pressure and electrolyte/blood volume homeostasis. Angiotensin converting enzyme (ACE; kininase II, EC 3.4.15.1) is one of key elements of the renin-angiotensin system and an important element of the kallikrein-kinin system. This enzyme removes the carboxy terminal dipeptide from the decapeptide angiotensin I to generate angiotensin II, a potent vasoconstrictor, and degrades bradykinin, a vasodilator (5). Earlier data on serum ACE activity in experimental dehydration were rather equivocal. Ibarra-Rubu and coworkers (6) did not find alterations of the serum ACE activity in rats under experimental dehydration. However, our preliminary study has shown that serum ACE in rats submitted to water deprivation for five days is significantly higher than in control group (7). Nitric oxide (NO) is a highly reactive inorganic free radical, produced by many cells in the organism. NO is synthesized from L arginine by NO sinthase which is made up of at least three isoforms (8). Previous studies have demonstrated that nitric oxide plays important roles in the regulation of the cardiovascular, nervous, immune, and other systems (9). Nitric oxide has also been implicated in the control of the secretion of hormones and evidence is accumulating that it contributes to the regulation of the secretion of renin and vasopressin, hormones that play key roles in the control of sodium and water balance (10). In the macula densa cells in the juxtaglomerular apparatus, there is a large production of NO from neuronal NOS (11). Recent studies have suggested that nitric oxide not only contributes to the regulation of basal renin secretion, but also participates in the renin secretory responses to activation of the renal baroreceptor, macula densa, and beta adrenoceptor mechanisms that regulate renin secretion (10). It was found that osmotic stimulation increased nitric oxide synthase activity in the supraoptic and paraventricular nuclei of the hypothalamus and in the posterior pituitary gland (12). Mornagui and co-workers (13) have found the increase NO level in serum of male Wistar rats submitted to water deprivation for three days. Similar results were obtained in other studies (14). Correlation between ACE activity and NO level in serum was not studied. Thus, the aim of the present study was to investigate the effect of dehydration on ACE activity and NO level in serum.

Materials and Methods

A control group consisted of 20 subjects of both sexes (10 men and 10 women) aged 35-45 years, who were healthy according to subjective and objective findings. A study group included 20 patients with dehydration caused by gastroenterocolitis of both sexes (10 men and 10 women), aged 35-45 years. The diagnosis of dehydration was made on the basis of clinical examination and biochemical findings at the Clinic for Infectology, University of Sarajevo Clinics Centre. Blood samples for the determination of serum ACE activity and NO level were taken from the cubital vein. After coagulation and centrifugation at 2000 g for 5 min, the serum was frozen at 20°C until the determination of ACE activity. Blood samples for the determination of NO concentration were diluted 1:1 (vol/vol) with 0,9% saline, protein-precipitated (30% $ZnSO_4$, 0,05 ml per ml of blood), centrifuged at 2,000 g for 10 minutes and frozen at -20°C until the determination of NO level. The serum activity of ACE was determined by the spectrophotometric method of Filipović (15) using hippuryl-l-histidyl-l-leucine as a substrate. The results were expressed in units corresponding to 1 nmol of hippuric acid that was released by the hydrolysis of hippuryl-l-histidyl-l-leucine per minute and ml of serum. The NO level in the blood and tissue was determined by measuring nitrite concentrations, a stable metabolic product of NO with oxygen. Conversion of NO_3- into NO_2- was done with elementary zinc. NO_2- concentration in serum and tissue was determined by classic colorimetric Griess reaction. Briefly, equal volumes of samples and Griess reagent were mixed at room temperature. After 5 min, the absorbance was measured at 570 nm using Perkin Elmer 550 S spectrophotometer. The concentration of nitrite was determined by a standard curve prepared with sodium nitrite (1-200 μM). The results of measurement was expressed as a mean ± SEM. Differences between the means were statistically compared by Student's t-test, and differences at $p<0,05$ were considered significant. Correlation coefficients were determined by employing Spearman's test.

Results

Figure 1 shows that serum ACE activity was significantly increased in patients with dehydration, and the mean value was by 27% higher than in control group healthy subjects (p<0,05). As shown in Figure 2. NO concentration in serum was significantly increased in patients with dehydration, and the mean value was by 146% higher than in control group healthy subjects (p<0,001).

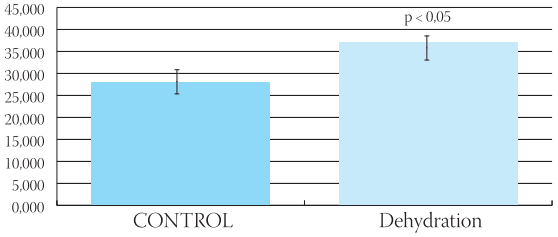

FIGURE 1. Serum angiotensin converting enzyme activity in patients with dehydration.
Data are presented as a mean ± SEM.

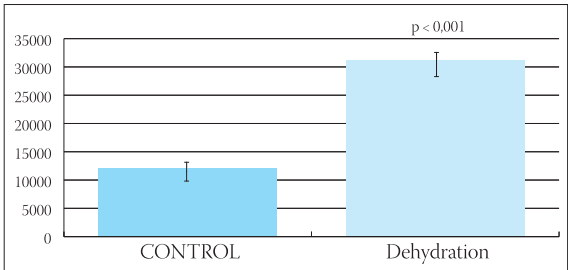

FIGURE 2. NO level in serum in patients with dehydration
Data are presented as a mean ± SEM.
Control – healthy subjects (N=20)

Discussion

Our study clearly showed that the serum ACE activity was significantly increased in patients with dehydration in comparison with values determined in control group healthy subjects. Also, we previously shown that activity of this enzyme was significantly increased in rats submitted to water deprivation for five days (7). However, our results are not in agreement with the results of Ibarra-Rubu and co-workers (6) who did not find alterations of the serum ACE activity in rats under experimental dehydration. The mechanism of the increased ACE activity in patients with dehydration is not clear. It is well known that vascular ACE is an ectoenzyme mainly expressed in the endothelial cells. In addition, a soluble ACE is found in serum, which is presumably derived from the membrane-bound form (16). Disequilibri¬um of electrolytes during dehydration probably alters the electrostatic forces that hold the enzyme to the membrane, resulting in a higher enzyme release from vascular endothelium. Some other mechanisms may be responsible for the increased enzyme release in serum. It is possible that secondary vascular changes could lead to an increase in the activity of serum ACE. Haemoconcentration, which occurs during dehydration, could also be a possible factor for observed increase in ACE. In our study, we found that NO concentration in serum of patients with dehydration was significantly higher than in control group. These results are in the agreement with the results of previous experimental studies (13, 14). Mornagui and co-workers (13) have shown that beside the increase NO level in serum there is a significant negative correlation between serum nitrite and nitrate concentration and serum volume in male Wistar rats submitted to water deprivation for three days. Theses authors suggest that dehydration increase serum NO probably by activation of nitric oxide synthases. We suggest that the continuous release of NO derived from the vascular endothelium may participate in the regulation of the alteration in blood flow, fluid and nutrient metabolism caused by water deprivation. Also, we found no correlation between ACE activity and NO level in serum of patients with dehydration. Opposed actions for NO and angiotensin II in vascular contraction are well documented. In addition, previously studies have shown that NO negatively modulates the renin angiotensin system by inhibiting ACE activity. On the other hand, angiotensin II positively stimulates NO synthesis and release (17). Although the reasons for higher values in both ACE activity and NO level in serum of patient with dehydration then in healthy subjects is not clear, this finding appears to deserve further investigation.

Conclusion

The results indicate that ACE and NO may participate in the regulation of the alteration in blood flow and in the regulation of the water balance in patients with dehydration.

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