Circulating angiotensin-converting enzyme, von Willebrand factor antigen and thrombomodulin in exertional heat stroke

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1. Military recruits frequently succumb to exertional heat stroke during intensive training. Since widespread endothelial injury is often associated with exertional heat stroke, the relationship between changes in three circulating endothelial cell markers (angiotensin-converting enzyme, von Willebrand factor antigen and thrombomodulin) and exertional heat stroke was studied.

2. Twelve recruits who had succumbed to exertional heat stroke during basic physical training (5000 m running) were included in the study. Another 10 age-matched healthy subjects who had gone through the same physical training regimen were selected as controls.

3. Blood was withdrawn on admission and on discharge for analyses of angiotensin-converting enzyme, von Willebrand factor antigen and thrombomodulin. Other physiological parameters and biochemical analyses reflecting renal and liver functions were also recorded.

4. Our results indicated that these subjects with exertional heat stroke exhibited impaired liver function as revealed by the significant elevation of both serum glutamic oxaloacetic transaminase (P<0.05) and serum glutamic pyruvic transaminase (P<0.05) as compared with normal healthy control subjects. Unfortunately, these values remained mostly somewhat elevated on discharge, although serum glutamic oxaloacetic transaminase was reduced dramatically. Indices of kidney functions, including creatinine clearance and uric acid and phosphorus secretion, were not significantly different from those observed in healthy controls.

5. Circulating angiotensin-converting enzyme activities in exertional heat stroke patients on admission were significantly lower than in normal subjects (10.68±2.15 versus 21.21±3.18 nmol hippuric acid min⁻¹ ml⁻¹, P<0.05). In contrast, von Willebrand factor antigen, thrombomodulin and plasma renin activity were significantly elevated compared with corresponding values in healthy subjects (P<0.001, 0.05 and 0.01 respectively). The values of these endothelial markers returned to normal on discharge.

6. These data suggest that the decrease in angiotensin-converting enzyme and increase in von Willebrand factor antigen and thrombomodulin associated with exertional heat stroke may reflect endothelial injuries. Since endothelial injuries may trigger haemostatic failure and/or disseminated intravascular coagulation, the monitoring of these values might be useful for the evaluation of endothelial status in patients suffering from exertional heat stroke.

INTRODUCTION

Vigorous exercise or rigorous physical exertion such as that encountered in intensive military training in hot and humid environments can lead to exertional heat stroke (ExHS) [1, 2]. Victims of ExHS exhibit widespread endothelial cell injury in the heart, brain, lung and kidney as demonstrated histologically [3, 4]. It has been suggested that the endothelial cell damage due to an elevated body temperature may be the triggering mechanism of disseminated intravascular coagulation (DIC) in victims of heat stroke [4]. It is also known that clotting dysfunction peaks at 16–18 h after the acute phase of heat stroke [5]. The present study was therefore undertaken to examine whether alterations of endothelial specific markers such as angiotensin-converting enzyme (ACE), thrombomodulin (TM) and von Willebrand factor antigen (vWF:Ag) [6–8] are associated with ExHS.

Circulating ACE is produced mainly by vascular endothelium [9]. Depressed ACE activity is observed in patients with temporal arteritis [10], a
demonstrated in patients with diseases involving endothelial cell injury. Furthermore, an increase in circulating vWF:Ag and a decrease in ACE have been demonstrated in patients with diseases involving injury to the vascular endothelium, such as systemic sclerosis and scleroderma [11, 12]. vWF:Ag is a glycoprotein synthesized by endothelial cells [13]. Although platelets and megakaryocytes also contain vWF:Ag, the endothelium probably produces most of the vWF:Ag in the circulation [14]. A high plasma level of vWF:Ag is widely accepted as an indicator of endothelial cell injury [15–17]. TM, which neutralizes thrombin clotting activity and acts as a cofactor for thrombin-catalysed activation of anticoagulant protein [18], is a surface glycoprotein of endothelial origin. Since TM is not normally secreted from cells but only as a result of cell injury [19], it has been suggested that circulating TM might be a more reliable marker of endothelial cell injury than vWF:Ag [20]. The purpose of this study was to examine these circulating endothelial specific markers in patients with ExHS.

SUBJECTS AND METHODS

Subjects

All procedures and protocols involving human subjects were approved by the Human Ethics Committee of The National Defense Medical Centre. Taking blood samples from the patients for biochemical analyses was part of the routine treatment procedure. Consent for participation was obtained from healthy control subjects as well as from the ExHS patients upon recovery of consciousness. Twelve recruits who had succumbed to ExHS during basic physical training (5000 m running) were included in the study. Their ages ranged between 19 and 22 years (mean 20.1 ± 0.2). Another 10 age-matched (mean age 19.9 ± 0.3 years) healthy subjects who had gone through the same physical training regimen were selected as controls. Water had been readily available during the training sessions. Criteria for the diagnosis of ExHS included hyperthermia (body temperature >40°C), disorientation or other significant alterations of mental status and positive urinary orthotolidine test without microscopic haematuria. After emergency field treatments, which included cooling and fluid replenishment by military physicians, the patients were delivered to the nearby Army 802 General Hospital within 20 min.

Methods

Blood samples were drawn on admission before therapy or as cooling commenced. Venous blood for the determination of vWF:Ag and TM was collected into siliconized tubes containing 1:10 volume of 0.129 mol/l trisodium citrate, and centrifuged at 2000g for 20 min at 4°C to obtain platelet-poor plasma. Two sets of plasma were prepared: one was for immediate biochemical analyses and the other set was aliquoted and frozen at −70°C for later measurements of ACE, vWF:Ag, TM and renin activity. Patients were subsequently cooled rapidly with a hypothermic blanket and/or ice sponging, fanning and were massaged. Serum uric acid, creatinine (Cr), transaminase and alkaline phosphatase were determined using a Sequential Multiple Autoanalyzer (Technicon Instrument, Tarrytown, NY, U.S.A.).

Serum ACE activity was determined using a spectrophotometric assay according to procedures described by Cushman and Cheung [21]. In brief, serum samples were incubated with hippuryl-L-histidyl-L-leucine (an ACE substrate) for 1 h. Plasma thrombomodulin levels were measured by an enzyme immunoassay (Asserachrom Thrombomodulin Kit, Diagnostica Stage, Asnieres, France).

Plasma vWF:Ag levels were measured with ELISA kits obtained from Diagnostica Stage (Asnieres, France). Plasma renin activity (PRA) was analysed using a radioimmunoassay kit (NEA105, DuPont, MA, U.S.A.).

After a therapy period of 14 ± 2 days, ExHS patients exhibiting stable physical condition were discharged. Another set of blood samples was drawn for the measurement of ACE, TM, vWF:Ag and PRA on discharge. All data are expressed as means ± SEM.

The significance of differences between groups was tested by Student's t-test. Probability (P) values of less than 0.05 were considered statistically significant.

RESULTS

The patients' mean core temperature was 41.2 ± 0.3°C on admission. Four of the 12 ExHS patients were acclimatized and three were anhidrotic. Water had been readily available during the training sessions. Time for cooling (core temperature to less than 38.5°C) was less than 30 min in all except two patients, who required between 30 and 45 min. Time for recovery of consciousness was between 1 and 3.5 h. Table 1 presents the general clinical data
of the ExHS patients on admission. None of the subjects had any history of cardiac, hepatic or renal diseases. Table 2 shows the biochemical data in 12 ExHS patients and 10 age-matched healthy control subjects. There was no significant difference in packed cell volume (PCV) between ExHS and controls, although 2 of 12 ExHS patients had PCV higher than 45%. In the 12 patients with ExHS, mean serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) values on admission were significantly higher than those in healthy control subjects. Mean serum albumin levels were significantly lower in ExHS patients on admission than in control subjects ($P < 0.01$). These parameters returned to values not significantly different from those of control subjects on discharge. Creatinine, alkaline phosphatase, phosphorus and uric acid exhibited no changes in these patients on admission and on discharge.

Table 3 shows the changes in circulating ACE, vWf:Ag, TM and PRA levels on admission and on discharge in 12 patients. Compared with control subjects, circulating ACE levels were significantly lower in ExHS patients on admission (10.68 ± 2.15 versus 22.14 ± 3.23 nmol hippuric acid min$^{-1}$ml$^{-1}$, $P < 0.05$), but returned to a level not significantly different from that in healthy control subjects (22.14 ± 3.23 nmol hippuric acid min$^{-1}$ml$^{-1}$) on discharge. Circulating vWf:Ag concentrations on admission were significantly higher (1.23 ± 0.05 versus 0.63 ± 0.05%, $P < 0.001$), but decreased significantly on discharge (0.63 ± 0.05%, $P < 0.001$). Similarly, TM levels in ExHS patients were higher than in healthy control subjects, but decreased to values not significantly different from those in healthy control subjects on discharge. Compared with control subjects, circulating PRA was markedly higher (3.72 ± 1.62 versus 1.50 ± 1.02 pmol angiotensin I h$^{-1}$ml$^{-1}$, $P < 0.01$) in ExHS patients on admission, but returned to normal (1.50 ± 1.02 pmol angiotensin I h$^{-1}$ml$^{-1}$) on discharge.

**DISCUSSION**

Although widespread endothelial cell injury has been demonstrated in ExHS for two decades [3, 4], scant attention has been paid to the changes in endothelial markers in these victims. Our results show that circulating vWf:Ag and TM levels were elevated while ACE activity was decreased in acute ExHS.

Although haemoconcentration is often observed in heat stroke, the overall mean PCV values in our patients were not significantly different from those in the healthy control subjects despite the fact that 2
of the 12 patients had elevated PCV values. This is probably because the recruits had a ready water supply during the training and had received emergency treatment which included cooling and fluid replenishment before hospitalization. The relatively small sample size in our study may also have affected the statistical analyses. However, the absence of PCV elevation in association with heat stroke has also been repeatedly observed, sometimes in much larger samples [3, 22-24]. Based on the PCV values, haemoconcentration was not apparent at the time of blood sampling. Thus, the elevation of circulating vWfAg and TM in our ExHS patients appeared to be a real increase and not entirely related to haemoconcentration.

SGPT and SGOT levels were elevated, indicating hepatic dysfunction, in our patients on admission. Physical exercise by man in high ambient temperature could result in body temperature rising to above 40°C, leading to a 20% reduction in liver function, which could be accounted for by diversion of blood flow from the splanchnic area to the skin [25]. Heating the liver to about 43°C by extracorporeal circulation in dogs results in a reduction in hepatic clearance, as indicated by a decrease in indocyanine Green clearance [26]. Impaired hepatic clearance could therefore have contributed to the elevation of TM or vWfAg observed in our study. However because there was no correlation between SGPT or SGOT and the circulating levels of vWfAg or TM (data not shown), the elevation in vWfAg or TM was unlikely to be accounted for by a reduction in hepatic clearance. Likewise, the lack of correlation between serum Cr and vWfAg or TM in ExHS suggested that impaired renal clearance was unlikely to be the cause of these elevations.

In the present study, the circulating TM and vWfAg levels were elevated significantly in ExHS patients on admission, suggesting a generalized endothelial cell injury in patients with ExHS. It is known that hyperthermia can induce lipid peroxidation and oxygen radical formation [27]. In vitro study has demonstrated that oxidative injury can induce the release of TM from the endothelial cell surface [19].

In contrast to increased circulating levels of TM and vWfAg, decreased ACE activity was observed in patients with ExHS. Decreased ACE activities can also be found in patients with acute illness, such as acute renal failure from rhabdomyolysis [28]. Heat stroke is associated with the release of tumour necrosis factor (TNF) [29]. In an animal model, injection of TNF can lower the serum zinc level [30]. Since ACE is a zinc-dependent peptidylpeptide hydrolase [31], a low serum zinc level can suppress serum ACE activity [32]. Zinc deficiency might also potentiate the hyperthermia-induced inactivation of ACE activity [33]. We therefore speculate that increased TNF [34] in ExHS might trigger the zinc deficiency and therefore suppress the serum ACE activity.

Circulating ACE is produced mainly by vascular endothelium [8]. Because the pulmonary capillaries represent the largest vascular bed, most circulating ACE could be of pulmonary endothelial origin [8]. ACE is situated on the luminal surface of endothelial cells and has direct access to circulating plasma solutes [35]. In hyperthermia-induced pulmonary oedema, there is an increase in transendothelial albumin leakage associated with damage to the endothelial lining [36]. The leaked albumin may form a complex with ACE on the surface of the endothelium, thus decreasing the serum ACE activity [37]. The decreased serum albumin level observed in our ExHS patients on admission supported this likelihood. Furthermore, serum ACE activity is decreased in the adult respiratory distress syndrome (ARDS) [38, 39]. A reduction in serum ACE correlated with histological evidence of endothelial loss has also been demonstrated [39]. It was suggested that low serum ACE may be a consequence of increased pulmonary vascular resistance leading to fewer molecules of ACE released in ARDS [40]. Increased pulmonary vascular resistance or ARDS has been demonstrated in heat stroke victims [41]. Thus, a pulmonary contribution, as part of generalized endothelial injury, to the lowering of circulating ACE cannot be completely discounted.

Excessive renin activity had been found in ExHS patients [42], and one might argue that this may give rise to depletion of ACE. In our ExHS patients, although PRA was elevated on admission, it was not inversely correlated with ACE activity. Thus, the decrease in circulating ACE in ExHS could have been accounted for by factors other than accelerated consumption.

In other chronic diseases with injury of vascular endothelium, such as systemic sclerosis [11] and scleroderma [12], a decrease in circulating ACE levels associated with an increase in vWfAg [12] has also been demonstrated. The presence of circulating factors inhibiting ACE synthesis has been hypothesized [11, 12].

The observations in this study of increased circulating TM and vWfAg levels with decreased ACE values on admission suggested endothelial cell injuries in patients with ExHS. Since endothelial injuries may trigger haemostatic failure and/or DIC, we propose that the measurement of circulating TM, vWfAg and ACE can be used to assess the state of endothelial cell injury in ExHS.

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