BRIEF REPORT

OPEN

Alterations in the Renin-Angiotensin System in Experimental Septic Shock

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OBJECTIVES: To analyze dynamic changes in the renin-angiotensin system (RAS) during septic shock, focusing on angiotensin-converting enzyme (ACE) activity and the balance between angiotensin peptides, using a mass spectrometry method.

DESIGN: Experimental septic shock model induced by peritonitis in swine.

SETTING: Experimental Laboratory, Department of Intensive Care, Erasme Hospital, Université Libre de Bruxelles.

SUBJECTS: Forty time points from eight mechanically ventilated pigs.

INTERVENTIONS: Septic shock was induced using intraperitoneal instillation of autologous feces, followed by standardized fluid resuscitation, norepinephrine infusion, antibiotic administration, and peritoneal lavage.

MEASUREMENTS AND MAIN RESULTS: The induction of sepsis resulted in a significant increase in plasma renin activity and levels of angiotensin I and II, with a significant decrease in ACE activity observed from 4 hours post-resuscitation and a notable rise in the angiotensin I/angiotensin II ratio at 12 hours. Additionally, a shift toward the angiotensin-(1–7) axis was observed, evidenced by an increased angiotensin-(1–7)/angiotensin II ratio.

CONCLUSIONS: The study highlighted dynamic shifts in the RAS during septic shock, characterized by reduced circulating ACE activity, elevated angiotensin I/II ratio, and a shift toward the angiotensin-(1–7) axis. These findings suggest an adaptive response within the RAS, potentially offering new insights into sepsis management and therapeutic targets.

KEYWORDS: angiotensin II; angiotensin-(1–7); dipeptidyl peptidase 3; sepsis; septic shock

Recent advances have been made in understanding the pathophysiology of septic shock, particularly in relation to the renin-angiotensin system (RAS), which is described in the Supplement (**Fig. S1**, http://links.lww.com/CCX/B420) (1–3). An increased angiotensin I/angiotensin II ratio has been associated with worse outcomes (4). The primary hypothesis proposed to explain this finding is a potential defect in angiotensin-converting enzyme (ACE), the enzyme responsible for the conversion of Angiotensin I to Angiotensin II (5).

In a recent multicenter study involving 72 pediatric patients with septic shock, it was found that 69% of them had undetectable ACE activity on day 1 after diagnosis. Furthermore, diminished ACE activity was significantly associated with a composite outcome of persistent acute kidney injury, need for renal replacement therapy, or mortality by day 7 (6). However, this study did not report the concentrations of other RAS peptides in relation to ACE activity. Especially, the ratio of angiotensin-(1–7)/angiotensin II has been described as a surrogate of the balance between the classical RAS, mediated by angiotensin II, and the alternative RAS, mediated by angiotensin-(1–7) (7).

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KEY POINTS

Question: What are the dynamic changes in the renin-angiotensin system (RAS) peptides and angiotensin-converting enzyme (ACE) activity during experimental septic shock?

Findings: The study found significant increases in plasma renin activity and angiotensin I/angiotensin II ratio, along with a significant decrease in ACE activity. Additionally, there was a shift toward the angiotensin-(1–7) axis, indicated by an increased angiotensin-(1–7)/angiotensin II ratio.

Meaning: These results suggest an adaptive response within the RAS during septic shock, highlighting potential new therapeutic targets in terms of modulating the balance between different angiotensin peptides.

Although several RAS alterations have been linked to outcomes, like renin levels, the disturbances in the RAS induced by sepsis remain poorly understood (8). Large animal models are valuable tools for both understanding the pathogenesis of sepsis and evaluating candidate therapeutics. Therefore, the primary objective of our investigation was to analyze the changes in RAS peptides and the activities of circulating enzymes that occur during experimental septic shock.

METHODS

The study protocol was approved by the local animal ethics committee (CEBEA, protocol number 723N, laboratory LA1230406, 2019) at the Université Libre de Bruxelles and followed recommendations for translational research in sepsis.

Experimental Procedure

Sepsis was induced by intraperitoneal instillation of 3 g/kg of autologous feces in eight young adult pigs (Sus scrofa domesticus; RA-SE Genetics, Lokeren, Belgium) weighting 52 ± 2 kg, as previously described (9). Both sexes (seven males/one female) were included. Pigs were used due to the similarity to cardiovascular system in humans. No animal was excluded from the analysis. "Shock" time point was defined by severe hypotension, when mean arterial pressure (MAP) reached less than or equal to 50 mm Hg. Severe hypotension between MAP 45 and 50 mm Hg was left untreated for 1 hour. Then fluid resuscitation was started with the objective to restore the pulse pressure variation (PPV) to less than or equal to 13% over a 20-minute period and norepinephrine infusion was then started aiming at keeping the MAP between 65 and 75 mm Hg. At this point, broad-spectrum antibiotic and peritoneal lavage were initiated. Fluid resuscitation was reestablished whenever PPV exceeded 13% in case of hypotension. Resuscitation was continued for 12 hours, with blood samples taken at 4, 8, and 12 hours after the start of norepinephrine (**Fig. S2**, http://links.lww.com/CCX/B420). The total duration of the experiment was 27 ± 2 hours. Animals were euthanized under deep anesthesia at the end of the experiment.

RAS Analysis

We conducted RAS equilibrium analysis and circulating ACE activity quantification in 40 samples in a specialized laboratory (Attoquant Diagnostics, Vienna, Austria) using liquid chromatography mass spectrometry from heparinized plasma (10). Circulating dipeptidyl peptidase 3 (cDPP3) concentration was measured as previously described (4TEEN4 Pharmaceuticals GmbH, Hennigsdorf, Germany) (11).

Statistical Analysis

Analysis of variance for data normally distributed or Kruskal-Wallis test were used to compare the means of different time points with the baseline. Data were analyzed using Prism (GraphPad Software, Boston, MA).

RESULTS

Sepsis Induction

The mean time to reach shock (MAP ≤ 50 mm Hg) was 5.4 ± 1.7 hours. The hemodynamic and biological variables related to septic shock induction are reported in **Table 1**. Despite the initiation of full resuscitation, circulatory failure gradually worsened from 4 to 12 hours with incremental requirements of norepinephrine and rising arterial lactate concentrations (Table 1).

RAS Analysis

There was a significant rise in plasma renin activity (PRA) from 401 ± 250 pmol/L/hr angiotensin I formation at

TABLE 1.
Hemodynamics, Biological Variables, and Peptides of the Renin-Angiotensin System

Variables	Baseline	Shock	4 hr	8 hr	12 hr
Heart rate (beats/min)	102 ± 13	163 ± 17[d]	146 ± 16[d]	145 ± 11[c]	152 ± 16[d]
Temperature (°C)	36.7 ± 1.2	39.3 ± 1[a]	37.4 ± 1.3	37.5 ± 1.3	37.7 ± 1.2
Mean arterial pressure (mm Hg)	68 ± 4	49 ± 1[d]	72 ± 3[a]	69 ± 3	67 ± 3
Norepinephrine (μg/kg/min)			0.41 ± 0.16	0.53 ± 0.24	0.83 ± 0.52
Cardiac output index (mL/min/kg)	114 ± 15	61 ± 11	206 ± 60[c]	215 ± 51[c]	216 ± 51[c]
Right atrial pressure (mm Hg)	8 ± 3	4 ± 2[a]	9 ± 3	9 ± 2	9 ± 3
Pulmonary artery occlusion pressure (mm Hg)	8 ± 3	4 ± 2[a]	10 ± 3	10 ± 3	11 ± 3
Urine output (mL/kg/hr)	2.7 ± 1.1	0.5 ± 0.2[b]	2.4 ± 0.9	3.4 ± 2.4	1.8 ± 0.8
Central venous saturation (%)	63 ± 5	50 ± 8[c]	72 ± 3[a]	67 ± 6	69 ± 5
Lactate (mmol/L)	1 ± 0.2	1.4 ± 0.6	1.5 ± 0.4	1.8 ± 0.7	2.3 ± 1.4[b]
Albumin (g/L)	26 ± 4	24 ± 4	8 ± 3[d]	7 ± 2[d]	7 ± 2[d]
Hematocrit (%)	27 ± 3	43 ± 2[d]	24 ± 5	23 ± 5	21 ± 4[a]
Creatinine (mg/dL)	0.9 ± 0.1	1.7 ± 0.2[d]	1.3 ± 0.3[b]	1.1 ± 0.1	1.2 ± 0.2
Angiotensin I (pmol/L)	54 ± 32	569 ± 217[d]	162 ± 98	261 ± 62	619 ± 346[d]
Angiotensin II (pmol/L)	258 ± 194	2874 ± 935[d]	807 ± 377	1295 ± 370[b]	1431 ± 574[c]
Aldosterone (pmol/L)	94 ± 56	644 ± 107[d]	154 ± 35	162 ± 37	268 ± 98[c]
Angiotensin III (pmol/L)	11 ± 8	95 ± 22[d]	19 ± 9	39 ± 22[b]	40 ± 13[b]
Angiotensin IV (pmol/L)	11 ± 8	107 ± 32[d]	35 ± 13	61 ± 37[b]	58 ± 24[b]
Angiotensin-(1–7)(pmol/L)	9 ± 7	111 ± 40[d]	24 ± 15	50 ± 20	119 ± 59[d]
Angiotensin-(1–5) (pmol/L)	49 ± 3	388 ± 121[d]	55 ± 27	84 ± 38	102 ± 30
Circulating dipeptidyl peptidase 3 (ng/mL)	17 ± 6	53 ± 22[a]	48 ± 18[a]	51 ± 17[a]	54 ± 18[a]

[a]$p < 0.05$ compared with baseline.
[b]$p < 0.01$ compared with baseline.
[c]$p < 0.001$ compared with baseline.
[d]$p < 0.0001$ compared with baseline.
Data are expressed as mean ± SD.

baseline to 6643 ± 2606 pmol/L/hr angiotensin I formation at shock time point, as well as elevated levels of angiotensin I (from 54 ± 32 pmol/L/hr at baseline to 569 ± 217 pmol/L/hr at the shock time point) and angiotensin II (from 258 ± 194 pmol/L/hr angiotensin I formation at baseline to 2874 ± 935 pmol/L angiotensin I formation at the shock time point). These changes were observed in conjunction with an increase in aldosterone levels (**Fig. 1*A*** and Table 1). Other RAS peptides, including angiotensin III, angiotensin IV, angiotensin-(1–5), and angiotensin-(1–7), also increased at the shock time point. Subsequently, PRA and RAS peptides decreased close to baseline values by 4 hours and only moderately increased thereafter (Fig. 1*A* and Table 1). Throughout the experiment, the angiotensin I/angiotensin II ratio remained close to its baseline value, although it significantly increased at 12 hours (**Fig. 1*B***). Similarly, the angiotensin-(1–7)/angiotensin II ratio increased at 12 hours (Fig. 1*B*). Circulating ACE activity was stable at shock (67 ± 11 vs. 69 ± 11 nmol/L × hr of angiotensin II formation at baseline) but decreased progressively until reaching 16 ± 9 nmol/L × hr of angiotensin II formation at 12 hours ($p < 0.0001$). cDPP3 concentration increased from the shock time point and remained higher during the resuscitation phase.

DISCUSSION

In an experimental swine septic model, we observed dynamic activation of the RAS, characterized by an

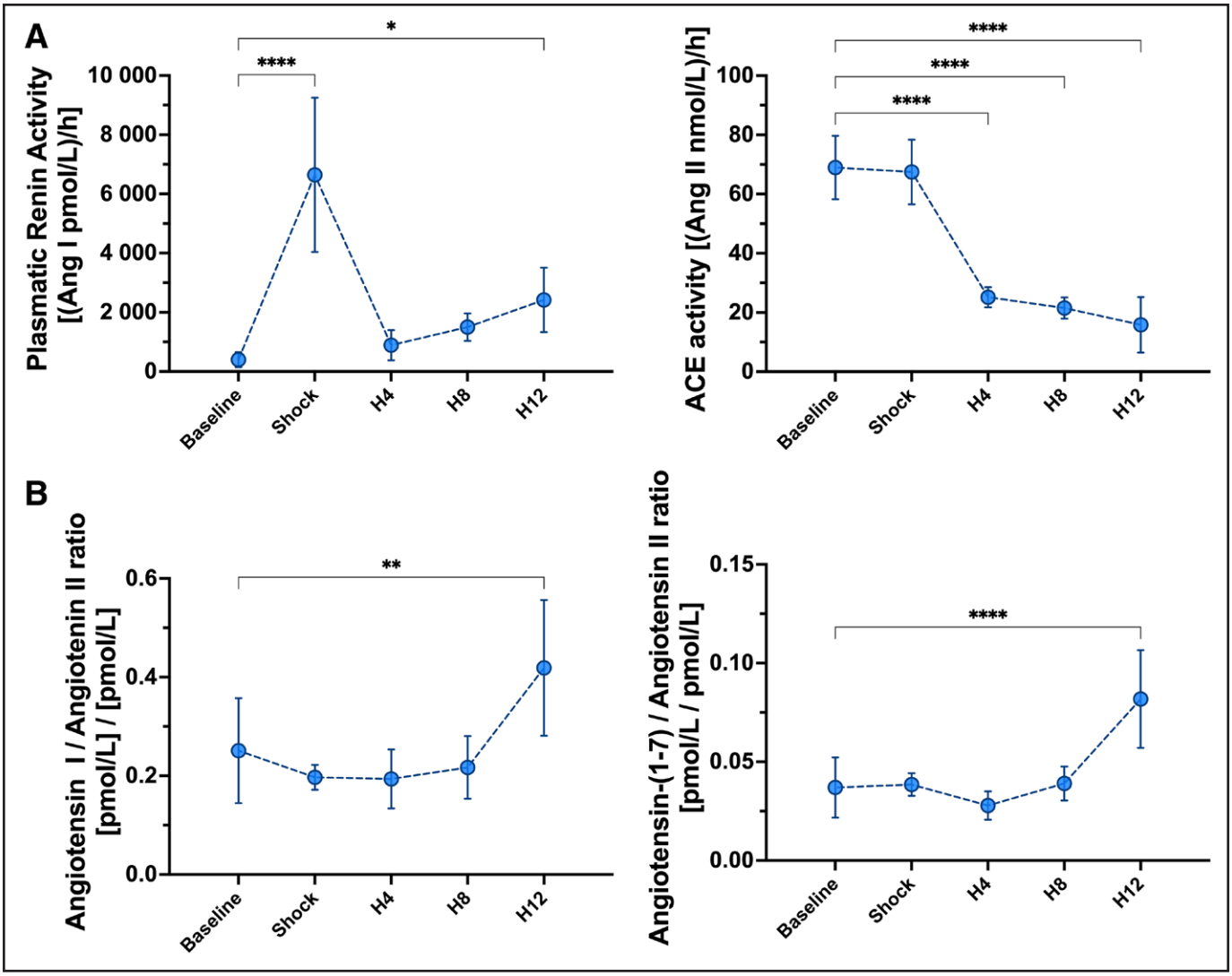

Figure 1. Renin-angiotensin system equilibrium analysis, plasma renin activity and angiotensin-converting enzyme (ACE) activity **A**, Plasma renin activity and circulating ACE activity. **B**, Angiotensin I (Ang I)/angiotensin II (Ang II) ratio, and Ang-(1–7)/Ang II ratio. Data are expressed as mean ± SD. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$ compared with baseline. H4 = 4 hr, H8 = 8 hr, H12 = 12 hr

increase in most of its peptides. We noted a decrease in circulating ACE activity and an increase in the angiotensin I/angiotensin II ratio. Additionally, there was a notable shift toward the angiotensin-(1–7) axis, evidenced by an increase in the angiotensin-(1–7)/angiotensin II ratio.

The alteration of the RAS observed dynamic changes. First, there was a proportional increase in most of the peptides of the RAS following sepsis induction at the shock time point, which might be related to renin secretion following hypoperfusion. Then, a decrease in most of the peptides could be explained by hemodilution induced by fluid resuscitation and/or by the depletion of preformed renin stores released at the shock onset. Later in the course of the experiment, changes in the ratios between angiotensin I/angiotensin II and between angiotensin-(1–7)/angiotensin II were observed.

We observed an early decrease in circulating ACE activity (from 4 hr), similar to observations in humans (6). However, the angiotensin I/II ratio was only affected at the latest time point (12 hr), suggesting that circulating ACE is not a good surrogate for total ACE activity (since ACE is predominantly a membrane-bound endothelial enzyme) and/or that other factors could influence the angiotensin I/II ratio. cDPP3 cleaves angiotensin II but not angiotensin I, thereby affecting the angiotensin I/II ratio (12). Increased cDPP3 concentration during human sepsis has been associated with a worse prognosis

(13). Similar increases have been also observed in this animal study and might contribute, in part, to the alteration observed in the angiotensin I/angiotensin II ratio (14).

ACE2 provides homeostatic downregulation of circulating angiotensin II levels by cleaving the C-terminal phenylalanine to form angiotensin-(1–7), thus offering a potential explanation for both the increased angiotensin I/II and the increased angiotensin-(1–7)/angiotensin II ratios. Angiotensin-(1–7) plays a vital role as the primary mediator responsible for counterbalancing the effects of angiotensin II and has been shown to prevent the development of experimental septic shock (15). However, the implications of a shift toward higher angiotensin-(1–7) levels during sepsis are not fully understood (7). In the context of acute respiratory distress syndrome related to COVID-19, an increase in ACE2 activity and angiotensin-(1–7) concentration has been reported and correlated with interleukin-6 levels and severity. In this study, the increase in the angiotensin-(1–7)/angiotensin II ratio was higher in severe COVID-19 patients compared with nonsevere patients. This activation was hypothesized to be a response to injury (16). It is plausible that a similar activation of the ACE2/angiotensin-(1–7) axis could be observed in sepsis, which might act as an anti-inflammatory adaptation mechanism (7). Despite being associated with theoretical vasodilatory effects, angiotensin-(1–7) administration has not been associated with systemic vasodilatory effects in a randomized controlled trial performed in COVID-19 patients (17) or in an experimental septic shock model (15), an effect that could be mediated by nitric oxide blockade (18).

The impact of this shift toward the alternative RAS on hemodynamics requires further investigation.

We performed a high-quality method of peptide quantification using a clinically relevant septic shock model. Circulatory failure and organ dysfunction were observed, and full resuscitation was performed using targets used in clinical practice. However, there are some limitations in our study, including the absence of ACE2 activity quantification, and limitations related to the use of healthy animals without comorbidities, which may not fully reflect the pathophysiological conditions observed in clinical settings.

Overall, these data illustrate a biological profile of RAS disturbance observed during experimental sepsis. Further investigation could be directed toward exploring whether RAS alterations can be targeted by specific therapeutics. For instance, a potential ACE/angiotensin II deficiency might be addressed through exogenous angiotensin II infusion (5). Alternatively, determining whether the shift to the angiotensin-(1–7) axis is adaptive or detrimental could be explored through additional research (7).

CONCLUSIONS

Experimental sepsis is associated with increased angiotensin I, angiotensin II, and angiotensin-(1–7) concentrations and a decrease in circulating ACE activity that may contribute to the increase of angiotensin I/angiotensin II ratio. A concurrent shift toward angiotensin-(1–7) may be a signal of ACE2/angiotensin-(1–7) axis activation. These findings suggest that the restoration of angiotensin II might be a therapeutic target in patients with septic shock.

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