Research

Recombinant activated protein C treatment improves tissue perfusion and oxygenation in septic patients measured by near-infrared spectroscopy

Abele Donati[1], Michela Romanelli[1], Laura Botticelli[1], Agnese Valentini[1], Vincenzo Gabbanelli[1], Simonetta Nataloni[1], Tiziana Principi[1], Paolo Pelaia[1], Rick Bezemer[2] and Can Ince[2]

[1]Department of Neuroscience, Intensive Care Unit, Marche Polytechnical University, Via Tronto 10/A, 60020 Torrette di Ancona, Italy
[2]Department of Intensive Care, Erasmus MC University Hospital Rotterdam, 3000 Rotterdam, The Netherlands

Corresponding author: Abele Donati, a.donati@univpm.it

Abstract

Introduction: The purpose was to test the hypothesis that muscle perfusion, oxygenation, and microvascular reactivity would improve in patients with severe sepsis or septic shock during treatment with recombinant activated protein C (rh-aPC) ($n = 11$) and to explore whether these parameters are related to macrohemodynamic indices, metabolic status or Sequential Organ Failure Assessment (SOFA) score. Patients with contraindications to rh-aPC were used as a control group ($n = 5$).

Materials and methods: Patients were sedated, intubated, mechanically ventilated, and hemodynamically monitored with the PiCCO system. Tissue oxygen saturation (StO_2) was measured using near-infrared spectroscopy (NIRS) during the vascular occlusion test (VOT). Baseline StO_2 ($StO_{2\ baseline}$), rate of decrease in StO_2 during VOT ($StO_{2\ downslope}$), and rate of increase in StO_2 during the reperfusion phase ($StO_{2\ upslope}$) were determined. Data were collected before (T0), during (24 hours (T1a), 48 hours (T1b), 72 hours (T1c) and 96 hours (T1d)) and 6 hours after stopping rh-aPC treatment (T2) and at the same times in the controls. At every assessment, hemodynamic and metabolic parameters were registered and the SOFA score calculated.

Results: The mean ± standard deviation Acute Physiology and Chronic Health Evaluation II score was 26.3 ± 6.6 and 28.6 ± 5.3 in rh-aPC and control groups, respectively. There were no significant differences in macrohemodynamic parameters between the groups at all the time points. In the rh-aPC group, base excess was corrected ($P < 0.01$) from T1a until T2, and blood lactate was significantly decreased at T1d and T2 (2.8 ± 1.3 vs. 1.9 ± 0.7 mmol/l; $P < 0.05$). In the control group, base excess was significantly corrected at T1a, T1b, T1c, and T2 ($P < 0.05$). The SOFA score was significantly lower in the rh-aPC group compared with the controls at T2 (7.9 ± 2.2 vs. 12.2 ± 3.2; $P < 0.05$). There were no differences between groups in $StO_{2\ baseline}$. $StO_{2\ downslope}$ in the rh-aPC group decreased significantly at all the time points,

and at T1b and T2 (−16.5 ± 11.8 vs. −8.1 ± 2.4%/minute) was significantly steeper than in the control group. $StO_{2\ upslope}$ increased and was higher than in the control group at T1c, T1d and T2 (101.1 ± 62.1 vs. 54.5 ± 23.8%/minute) ($P < 0.05$).

Conclusions: Treatment with rh-aPC may improve muscle oxygenation ($StO_{2\ baseline}$) and reperfusion ($StO_{2\ upslope}$) and, furthermore, rh-aPC treatment may increase tissue metabolism ($StO_{2\ downslope}$). NIRS is a simple, real-time, non-invasive technique that could be used to monitor the effects of rh-aPC therapy at microcirculatory level in septic patients.

Introduction

During sepsis, the microcirculatory and mitochondrial dysfunction plays a key role in the development of severe sepsis, septic shock, and multiple organ failure [1]. This condition is characterized by microcirculatory perfusion heterogeneity, arteriovenous shunting, and impaired autoregulation mainly due to disturbed coagulation, inflammation, and leukocyte–endothelium interaction [1-3]. It is now well established in septic patients that restoring basic macrohemodynamic parameters, such as blood pressure, in itself does not lead to improved patient outcome, and that normalization of microcirculatory and mitochondrial function may be necessary as an endpoint [2,4].

To this end, recombinant activated protein C (rh-aPC) has been used to restore the coagulative cascade, the inflammatory response, leukocyte adhesion and migration, and endothelial function. In previous studies, rh-aPC has been shown to decrease end-organ dysfunction and mortality if administered in the early stages of sepsis [5-7].

NIRS = near-infrared spectroscopy; rh-aPC = recombinant activated protein C; SOFA = Sequential Organ Failure Assessment; StO_2 = tissue oxygen saturation; $StO_{2\ downslope}$ = rate of decrease in tissue oxygen saturation; $StO_{2\ upslope}$ = rate of increase in tissue oxygen saturation; VOT = vascular occlusion test.

Near-infrared spectroscopy (NIRS) is a rapid, continuous, and non-invasive monitoring system of hemoglobin oxygen saturation in muscle and the brain, and has been used to assess the presence and extent of both circulatory and metabolic disorders in intensive care patients and trauma patients [8,9]. The monitoring system uses near-infrared light (680 to 800 nm) to illuminate tissue, which is mainly absorbed by hemoglobin and myoglobin [10]. Due to the selected wavelength range and the high corresponding spectral absorbance by (de)oxyhemoglobin, the NIRS measurements are confined to vessels with a diameter <500 µm.

Using NIRS, oxyhemoglobin can be distinguished from de-oxyhemoglobin because of their differing optical absorption spectra. The ratio of oxyhemoglobin concentration to deoxy-hemoglobin concentration is used to calculate a parameter called tissue oxygen saturation (StO_2), describing the oxygenation of the microvasculature in a certain volume of (muscular) tissue. In addition to steady-state StO_2 values, NIRS can be used in combination with a vascular occlusion test (VOT), which consists of a baseline phase, an ischemia phase, a reperfusion phase, and a reactive hyperemia phase. Using this methodology in many studies of sepsis, it has been demonstrated in a variety of ways that, following a brief period of ischemia, there is an anomalous tissue reperfusion profile due to disturbed microcirculatory functioning [11,12].

The purpose of the present study was to test the hypothesis that rh-aPC treatment corrects tissue perfusion and micro-circulatory reperfusion in septic patients, evaluated with NIRS in combination with a VOT, and to explore whether the NIRS parameters are related to macrohemodynamic indices, metabolic status, and Sequential Organ Failure Assessment (SOFA) score.

Materials and methods
Patients
The study was designed as a prospective observational investigation. For the experimental group (rh-aPC group), we enrolled all patients admitted to the 12-bed polyvalent intensive care unit of the University Hospital of Ospedali Riuniti, Ancona, Italy with a diagnosis of severe sepsis or septic shock – based on the criteria of the International Sepsis Definitions Conference ACCP/SCCM [13] – that could receive rh-aPC treatment (continued infusion of 24 µg/kg/hour for 96 hours). We included patients with two or more sepsis-related organ failures (that is, cardiovascular, pulmonary or renal dysfunction, thrombocytopenia, metabolic acidosis with high lactates) or sepsis-correlated acute respiratory distress syndrome. Patients with absolute or relative contraindications to rh-aPC therapy were enrolled into the control group. At the onset of severe sepsis or septic shock, the Acute Physiology and Chronic Health Evaluation II score was calculated.

All patients were sedated, intubated, and mechanically ventilated. They were hemodynamically monitored by arterial femoral catheter with the PiCCO system (Pulsion, Munich, Germany). All patients received fluid challenge, and, if necessary, continuous infusion of inotropic (dobutamine) and vasopressor (norepinephrine) agents to maintain a normal cardiac index and intrathoracic blood volume index and to maintain the mean arterial pressure between 70 and 100 mmHg.

Near-infrared spectroscopy
StO_2 was measured by a tissue spectrometer (InSpectra™ Model 325; Hutchinson Technology Inc., Hutchinson, MN, USA). The spectrometer consists of light detection circuitry and an optical cable that transmits light to tissues and receives scattered light from tissues. The maximum depth of the tissue volume sampled is estimated to be equal to the distance between the sending and receiving fibers of the probe (probe spacing). A probe spacing of 15 mm was used, with the probe placed on an adhesive surface on the skin of the volar surface of the forearm at the level of the brachio-radial muscle. The VOT was applied using a sphygmo-manometer cuff around the same arm that was inflated to 60 mmHg above the systolic arterial pressure to obtain an arterial occlusion (stagnant ischemia) until StO_2 decreased to 40%. StO_2 was monitored continuously before (baseline) and during (ischemia) pneumatic compression and after cuff release (reperfusion).

Data were analyzed using InSpectra™ software to plot and measure the StO_2 curve characteristics; that is, baseline StO_2 ($StO_{2\,baseline}$), rate of decrease in StO_2 during the VOT ($StO_{2\,downslope}$), and rate of increase in StO_2 during the reperfusion phase ($StO_{2\,upslope}$). Data were collected before (T0), during (24 hours (T1a), 48 hours (T1b), 72 hours (T1c), and 96 hours (T1d)), and 6 hours (T2) after rh-aPC treatment (that is, 102 hours from T0), and at the same times in the controls. At all time points (except at 96 hours) the following measurements were obtained: mean arterial pressure, dose of norepinephrine, arterial blood lactate and base excess, cardiac index and intrathoracic blood volume index, and SOFA score.

Statistical analysis
Results are expressed as the mean ± standard deviation, and as the median (first to third interquartile range) for catechol-amines. Parametric statistics were applied for all parameters – except for catecholamines, for which nonparametric statistics were utilized. A two-way analysis of variance test was used to assess differences between groups; a paired *t* test was applied to test differences between times within each group, while an unpaired *t* test with Welch correction when indicated was applied to test differences at each time between groups. The Friedman test was used to test significant differences during time within each group, the Wilcoxon test was used to test differences between each

Table 1

Patient characteristics

Patient	Diagnosis at admission	Contraindication
rh-aPC group		
1	Postoperative triple aortocoronary bypass	
2	Mediastinitis after odontogenic abscess	
3	Postoperative liver trauma	
4	Severe acute respiratory distress syndrome	
5	Severe trauma	
6	Severe trauma	
7	Postoperative urgent aortocoronary bypass	
8	Postoperative esophagectomy	
9	Postoperative intestinal perforation	
10	Necrotic-hemorrhagic pancreatitis	
11	Pulmonary aspergillosis	
Control group		
1	Intracerebral hematoma	Craniotomy
2	Postoperative cerebral aneurysm	Craniotomy
3	Postoperative abdominal aortic aneurysm	Risk of bleeding
4	Septic shock for cholecystitis	Risk of intracranial bleeding
5	Pancreatitis	Risk of bleeding

rh-aPC, recombinant activated protein C.

time point and T0, and the Mann–Whitney U test was used to test for differences between groups. $P < 0.05$ was considered statistically significant.

Results

We studied 11 patients (four female and seven male) with severe sepsis or septic shock who received rh-aPC therapy (continued infusion of 24 μg/kg/hour for 96 hours) and a control group of five patients (two female and three male) who could not receive rh-aPC because of contraindications. The patient characteristics are presented in Table 1. On admission to the intensive care unit, the mean Acute Physiology and Chronic Health Evaluation II score was 26.3 ± 6.6 for the rh-aPC group and 28.6 ± 5.3 for the control group, with a risk of death of $41.0 \pm 22.9\%$ and $57.6 \pm 25.7\%$, respectively. The mortality rate was 36.4% in the rh-aPC group and 60% in the control group.

Four patients in the rh-aPC group (Table 1, Patients 2, 4, 9, and 11) and one patient in the control group (Table 1, Patient 4) presented severe sepsis/septic shock on intensive care unit admission, while the other patients developed sepsis after admission to the intensive care unit.

From the two-way analysis of variance test, significant differences between groups were found for $StO_{2\,downslope}$ ($P < 0.01$), $StO_{2\,upslope}$, the SOFA score ($P < 0.05$) and the mean arterial pressure ($P < 0.001$).

The Friedman test showed that the norepinephrine and dobutamine rates significantly decreased only in the rh-aPC group ($P < 0.01$), and not in the control group (Table 2).

The SOFA score, compared with T0, was significantly lower at T1c and T1d (10.1 ± 2.3 vs. 8.8 ± 2.0 and 8.0 ± 2.3; $P < 0.05$) and at T2 (7.9 ± 2.2; $P < 0.01$) (Figure 1). At T2 the SOFA score was significantly reduced compared with the control group (7.9 ± 2.2 vs. 12.2 ± 3.2; $P < 0.05$). In the control group, no differences were found with respect to baseline values.

There were no significant differences in the macrohemo-dynamic parameters (cardiac index and intrathoracic blood volume index) at T0, during therapy, and at T2 (Table 2). The mean arterial pressure was no different at T0 between groups, while it was significantly increased during treatment only in the rh-aPC group (T2 93.8 ± 12.8 vs. T0 81 ± 10.9 mmHg) (Figure 2).

With regard to metabolic acidosis in the rh-aPC group, base excess was significantly corrected ($P < 0.01$) after 24 hours from T0 and remained corrected until T2 (Figure 3a). In the control group, base excess was significantly corrected at T1a, T1b, T1c, and T2 ($P < 0.05$) (Figure 3a). Blood lactate was significantly decreased in the rh-aPC group at T1d and T2 (2.8 ± 1.3 vs. 1.9 ± 0.7 mmol/l; $P < 0.05$) (Figure 3b).

Table 2

Cardiac index, ITBVI, norepinephrine dose, and dobutamine dose before, during, and after rh-aPC treatment

|	T0	T1a	T1b	T1c	T1d	T2
rh-aPC group						
Cardiac index	4.6 ± 1.3	4.6 ± 1.3	3.8 ± 0.7	4.1 ± 1.7	4.0 ± 1.0	4.1 ± 0.7
ITBVI	883 ± 207	874 ± 189	884 ± 203	922 ± 223	876 ± 213	872 ± 176
Norepinephrine*	0.22 (0.16 to 0.35)	0.22 (0.15 to 0.38)	0.22 (0.14 to 0.34)	0.22 (0.11 to 0.24)	0.17 (0.02 to 0.23)	0.14 (0.01 to 0.21)
Dobutamine*	3.4 (1 to 4.8)	2.8 (1 to 3.25)	2.1 (0 to 3.4)	2.1 (0 to 3.1)	2 (0 to 3.1)	2 (0 to 3.1)
Control group						
Cardiac index	4.2 ± 0.6	4.1 ± 1.1	4.3 ± 0.7	4.2 ± 0.5	4.1 ± 0.8	4.1 ± 0.7
ITBVI	892 ± 249	894 ± 211	926 ± 208	897 ± 265	913 ± 280	891 ± 260
Norepinephrine	0.17 (0.06 to 0.37)	0.37 (0.11 to 0.5)	0.27 (0.11 to 0.37)	0.27 (0.11 to 0.37)	0.37 (0.11 to 0 to 4)	0.37 (0.11 to 0.4)
Dobutamine	4.2 (0 to 5.3)	0 (0 to 3.7)	0 (0 to 3.7)	2.3 (0 to 3.7)	2.3 (0 to 3.7)	2.3 (0 to 3.7)

Data are presented as the mean ± standard deviation or as the median (first to third interquartile range). Data were collected before (T0), during (24 hours (T1a), 48 hours (T1b), 72 hours (T1c), and 96 hours (T1d)), and 6 hours (T2) after recombinant activated protein C (rh-aPC) treatment (that is, 102 hours from T0). ITBVI, intrathoracic blood volume index. *$P < 0.01$, Friedman test in the rh-aPC group.

Figure 1

Two-ways ANOVA between groups p<0.05
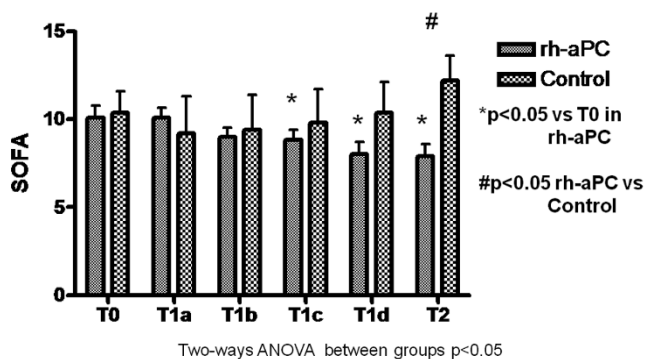
Sequential Organ Failure Assessment score before, during, and after recombinant activated protein C treatment. The Sequential Organ Failure Assessment (SOFA) score in the recombinant activated protein C (rh-aPC) group before, during, and after rh-aPC treatment, and in the control group at the same times. Data were collected before (T0), during (24 hours (T1a), 48 hours (T1b), 72 hours (T1c), and 96 hours (T1d)), and 6 hours (T2) after rh-aPC treatment (that is, 102 hours from T0). ANOVA, analysis of variance.

Figure 2

Two-ways ANOVA between groups p<0.001
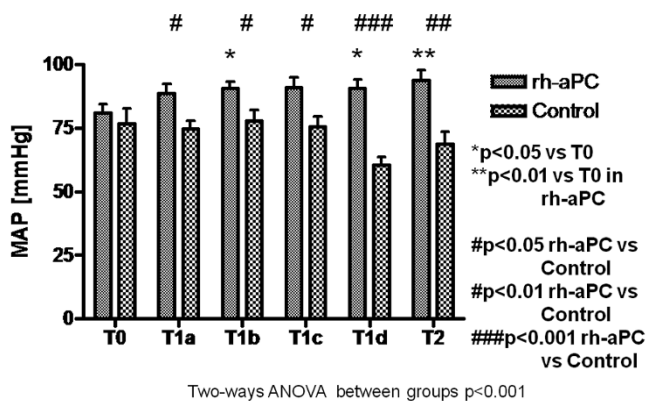
Mean arterial pressure before, during, and after recombinant activated protein C treatment. Mean arterial pressure (MAP) in the recombinant activated protein C (rh-aPC) group before, during, and after rh-aPC treatment, and in the control group at the same times. Data were collected before (T0), during (24 hours (T1a), 48 hours (T1b), 72 hours (T1c), and 96 hours (T1d)), and 6 hours (T2) after rh-aPC treatment (that is, 102 hours from T0). ANOVA, analysis of variance.

StO_2 baseline was significantly higher at T1a, T1c and T2 (Figure 4), while in the control group it was significantly higher at T2. StO_2 downslope decreased significantly at all the time points (Figure 5a) only in the rh-aPC group, and it was significantly steeper in the rh-aPC-treated patients than in the control patients at T1b and T2 (−16.5 ± 11.8 vs. −8.1 ± 2.4%/minute). StO_2 upslope increased significantly in the rh-aPC group at T1b, T1c, T1d and T2 (Figure 5b), and

was significantly higher than in the controls at T1c, T1d and T2 (101.1 ± 62.1 vs. 54.5 ± 23.8%/minute).

Discussion

The present prospective observational study investigated the effects of rh-aPC treatment on the SOFA score, macrohemodynamic parameters, and metabolic acidosis in severe sepsis and septic shock. Additionally, and more importantly in the

Figure 3
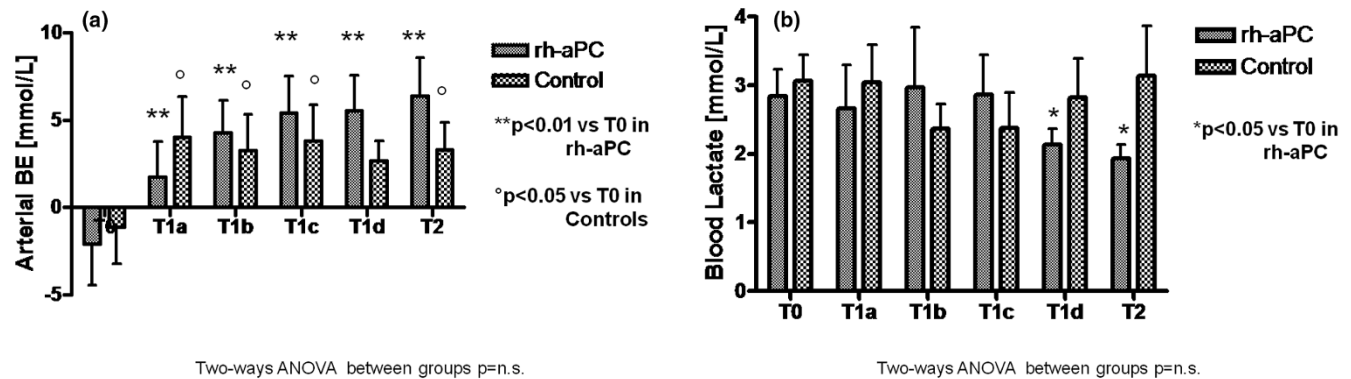
Base excess and blood lactate before, during, and after recombinant activated protein C treatment. **(a)** Arterial base excess (BE) and **(b)** blood lactate in the recombinant activated protein C (rh-aPC) group before, during, and after rh-aPC treatment, and in the control group at the same times. Data were collected before (T0), during (24 hours (T1a), 48 hours (T1b), 72 hours (T1c), and 96 hours (T1d)), and 6 hours (T2) after rh-aPC treatment (that is, 102 hours from T0). ANOVA, analysis of variance; n.s., not significant.
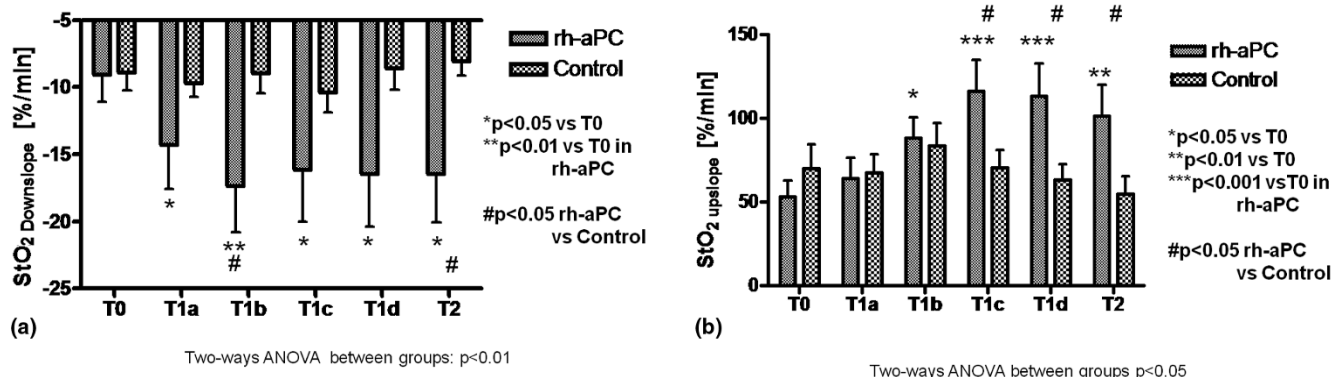
Figure 4
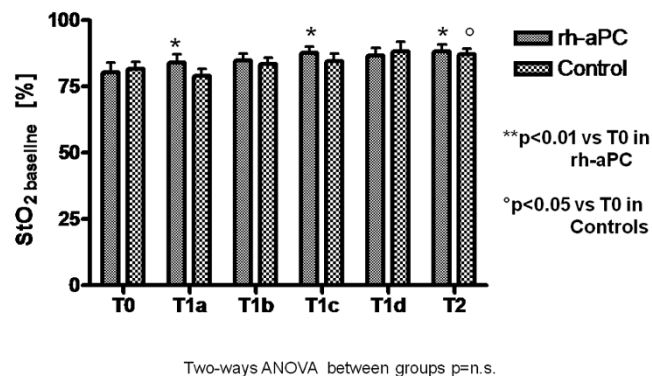
Baseline tissue oxygen saturation before, during, and after recombinant activated protein C treatment. Baseline tissue oxygen saturation (StO_2 baseline) in the recombinant activated protein C (rh-aPC) group before, during, and after rh-aPC treatment, and in the control group at the same times. Data were collected before (T0), during (24 hours (T1a), 48 hours (T1b), 72 hours (T1c), and 96 hours (T1d)), and 6 hours (T2) after rh-aPC treatment (that is, 102 hours from T0). ANOVA, analysis of variance; n.s., not significant.

context of sepsis, the tissue oxygenation, metabolism, and microvascular reperfusion dynamics were assessed using NIRS in combination with a VOT to study any beneficial effects of rh-aPC therapy at the microcirculatory level. It was shown that rh-aPC treatment significantly lowered the SOFA score, increased the mean arterial pressure, and reduced the blood lactate concentration. Furthermore, rh-aPC had positive effects on the VOT-derived StO_2 parameters; both StO_2 downslope and StO_2 upslope increased significantly, indicating raised oxygen consumption/metabolism and indicating improved microvascular reperfusion following ischemia.

Early goal-directed therapy focused on restoring macrohemodynamics has been shown to be insufficient in preventing cellular hypoxia and organ failure due to the heterogeneous nature of sepsis-related microcirculatory dysfunction. It has been shown that improvement in hemodynamic parameters with vasoconstrictors, such as norepinephrine, could make tissue perfusion worse [14], and several studies have demonstrated the positive effects of vasodilators on microcirculatory recruitment even in hemodynamically resuscitated septic patients [15]. rh-aPC treatment has been shown to improve end-organ function and to decrease mortality if started in the early stages of sepsis [5-7] by restoring the coagulative cascade, the inflammatory response, leukocyte adhesion and migration, and endothelial function. In addition to the anticoagulant, profibrinolytic [16], and anti-inflammatory effects and the antioxidant properties [17,18], rh-aPC acts at the microcirculation level to enhance the proportion of perfused capillaries and improve local autoregulation [1,19-21]. These studies were mostly carried out in animals. De Backer and co-workers [22] were the first to show the beneficial effects of rh-aPC on microcirculatory perfusion by direct observation of the sublingual microcirculation of septic patients using OPS imaging. To our knowledge, however, the direct effects of rh-aPC treatment on tissue oxygenation have not been studied before.

Many studies have shown the relevance of StO_2 in the assessment of the metabolic and microcirculatory state in septic patients. Doerschug and colleagues [23], De Blasi and colleagues [10], Skarda and colleagues [24], and Pareznik and colleagues [25] all showed that tissue oxygen consumption was lower in septic patients than in nonseptic patients or healthy volunteers and that septic patients have slower tissue reoxygenation following ischemia. In addition, Creteur and colleagues [12] demonstrated that persistent

Figure 5

Tissue oxygen saturation increase and decrease before, during, and after recombinant activated protein C treatment. **(a)** Rate of decrease in tissue oxygen saturation ($StO_{2\ downslope}$) and **(b)** rate of increase in tissue oxygen saturation ($StO_{2\ upslope}$) in the recombinant activated protein C (rh-aPC) group before, during, and after rh-aPC treatment, and in the control group at the same times. Data were collected before (T0), during (24 hours (T1a), 48 hours (T1b), 72 hours (T1c), and 96 hours (T1d)), and 6 hours (T2) after rh-aPC treatment (that is, 102 hours from T0). ANOVA, analysis of variance.

alteration of StO_2 resaturation correlated with worse outcome and multiorgan failure.

The steady-state tissue oxygenation ($StO_{2\ baseline}$) did not change as a result of rh-aPC treatment, which indicates that the balance between tissue oxygen delivery and consumption is unaltered by rh-aPC infusion. $StO_{2\ downslope}$, in contrast, increased significantly after starting the rh-aPC therapy, indicating increased cellular oxygen consumption. Additionally, $StO_{2\ upslope}$ increased significantly due to rh-aPC treatment, which indicates the improved ability of the microcirculation to be reperfused after a brief period of ischemia. Microvascular function is therefore improved by rh-aPC treatment. This finding is also supported by the reduced SOFA score and lactate levels.

The present study has some limitations: firstly, the small number of patients – in particular in the control group, where patients affected by head trauma and intracranial hypertension could have an altered systemic hemodynamic; and secondly, because the NIRS technique itself has some limitations. $StO_{2\ downslope}$ has been asserted to indicate the muscle oxygen consumption, but oxygen consumption cannot be directly measured as the amount of hemoglobin in the respective muscle blood volume is not known. The parameter being measured is the oxygen consumption rate extrapolated from the decrease in saturation of hemoglobin (StO_2 decrease rate, %/minute), which is an index of the basic metabolism of the thenar muscle. Moreover, whether the concentration of hemoglobin affects the oxygen consumption rate is not known. One argument regarding the reperfusion rate is that NIRS does not measure blood flow and it must be assumed that an increase in StO_2 reflects endothelium-dependent vasodilation. The extent to which comorbidites

such as atherosclerosis, age, gender or mental stress may influence this parameter is not known. Regardless of these limitations, the ability to provide a non-invasive, reproducible estimate of the oxygen consumption rate of skeletal muscle at the bedside renders this technique potentially useful in clinical practice.

Conclusion

Treatment of septic patients with continuous infusion of rh-aPC may improve tissue oxygenation, cellular metabolism, and microvascular reactivity, and may significantly reduce the SOFA score and lactate levels. NIRS in combination with a VOT was able to detect microcirculatory and metabolic changes associated with sepsis and rh-aPC treatment.

Competing interests

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Acknowledgement

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