Production of platelet-activating factor in patients with sepsis-associated acute renal failure

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Abstract

Background. Studies in experimental animals have suggested that platelet-activating factor (PAF) is a mediator of sepsis-associated acute renal failure (ARF). In the present study we have evaluated whether an increased concentration of PAF within circulation or urine of septic patients correlated with the worsening of renal function.

Methods. The concentration of PAF and selected cytokines (TNF, IL-1, IL-6, IL-8) was evaluated in blood and urine of 12 patients with septic shock and ARF for 4 consecutive days.

Results. The data obtained indicate that blood and urinary concentrations of PAF and of IL-1, IL-6 and IL-8 were significantly higher in septic patients than in controls subjects and in patients with chronic renal failure. The concentration of TNF was significantly increased only in urine. A significantly positive correlation was found among blood concentration of PAF and heart rate (r=0.4193, P<0.017), serum creatinine (r=0.3671, P<0.038), serum IL-6 (r=0.5475, P<0.005) and urine excretion of IL-8 (r=0.3984, P<0.044), whereas a negative correlation was present with the number of circulating platelets (r=−0.4285, P<0.018). Moreover, a positive correlation among the concentration of PAF in urine and the serum concentration of IL-6 (r=0.5654, P<0.006) and urine excretion of IL-6 (r=0.6589, P<0.0008) and IL-8 (r=0.6371, P<0.0004) were found.

Conclusions. These results demonstrate in humans during ARF associated with septic shock the production of PAF, a mediator that has been previously implicated in the pathogenesis of experimental endotoxin-induced shock and renal injury. The observation that blood and urinary concentrations of PAF correlated with some of the clinical and laboratory parameters related to the severity of ARF and sepsis suggests that PAF may contribute to the development of renal injury in septic patients.

Key words: acute renal failure; cytokines; humans; platelet activating factor; septic shock

Introduction

Platelet activating factor (PAF), a phospholipid mediator of inflammation with a wide range of biological activities, has been implicated in the pathogenesis of septic shock [1]. Blockade of PAF receptors in experimental animals inhibits and reverses endotoxin-induced hypotension and renal insufficiency, and significantly improves survival [1,2]. The synthesis of PAF during Gram-negative sepsis may be initiated either by the direct stimulatory effect of endotoxin or by the release of inflammatory cytokines [3–5]. Tumour necrosis factor (TNF) and interleukin-1 (IL-1) induce PAF synthesis by monocyte/macrophages, polymorphonuclear neutrophils (PMN), endothelial cells and glomerular mesangial cells [6,7]. Moreover, bacterial lipopolysaccharides (LPS) directly stimulate the synthesis of PAF by a CD14-LPS binding protein (LBP) pathway in monocytes, PMN, endothelial and mesangial cells [4]. PAF receptors are desensitized in platelets of patients with increased PAF plasma concentration and severe sepsis [8–10]. The presence of PAF in the circulation has been correlated with the severity of disease [9] and with the occurrence of an intravascular coagulation [10]. The administration of a PAF receptor antagonist in septic patients with severe thrombocytopenia was shown to increase significantly the number of circulating platelets [10]. Moreover, it has been reported that the rate of metabolism of PAF to its inactive metabolites is reduced concomitantly with a worsening of clinical outcome in septic patients [11]. Finally, PAF may decrease ventricular contractility.
[12], systemic and renal haemodynamics [13], increase vascular permeability, and promote neutrophil adhesion in endotoxaemia and septic shock [5]. No studies are available in humans on the involvement of PAF in acute renal failure (ARF) associated with sepsis.

The aim of the present study was to evaluate whether an increased concentration of PAF within circulation correlated with the impairment of renal function in septic patients. For this purpose 12 patients with septic shock and ARF were studied by monitoring PAF and cytokine concentration in blood and urine for 4 consecutive days.

Subjects and methods

Patients and controls

Twelve severely ill patients (aged 49–87 years, 70.8 ± 4.3 years, mean ± SEM) with septic shock and with or without multiple organ dysfunction syndrome (MODS) defined according to criteria of ACCP/SCCM Consensus Conference [14] were studied (Table 1). All patients presented clinical and laboratory evidence of serious infection (Table 1). Patients presenting one of the following conditions were excluded from the study: positivity for HBsAg, or HCV, or HIV; presence of systemic diseases—e.g. diabetes mellitus or autoimmune disease; metastatic or advanced cancer patients; pregnancy; burns, or allograft. In all septic patients the first day of study protocol was the day of diagnosis of ARF, defined as a serum creatinine higher than 2.0 mg/dl and/or urine output lower than 400 ml/day in the presence of anamnestic data of normal renal function (serum creatinine below 1.0 mg/dl). The causes of sepsis, the score APACHE III [15], the presence of MODS, defined as involvement of two or more organs [16], and the outcome are reported in Table 1. All patients received conventional therapy including crystalloid solutions, broad-spectrum antibiotics and other drugs including diuretics and vasopressor agents.

As controls, levels of PAF and cytokines in blood and urine were studied in two groups of subjects: (i) eight healthy sex-and age-matched volunteers (Group Healthy); (ii) eight patients affected by stabilized chronic renal failure (Group CRF) with a similar impairment of renal function of Group ARF (serum creatinine: Group CRF 3.1 ± 0.5 mg/dl vs Group ARF 2.51 ± 0.21, \(P > 0.05\)) and without signs of active nephritis.

Study protocol

The study protocol was approved by the local institutional Ethic Committee. The 12 patients of Group ARF were followed for 4 days by monitoring clinical and biochemical parameters as well as by measuring PAF and cytokines in blood and urine. The collection of clinical parameters and of blood and urine samples of patients was performed in early morning. Clinical parameters included body temperature, mean arterial pressure, heart rate, respiratory rate, and urine output. As biochemical data, serum creatinine, BUN, fibrinogen, fibrinogen degradation products (FDP) and haemochromocytometer examination were evaluated. Creatinine, BUN, fibrinogen, and haemochromocytometer examination were performed by standard laboratory methods. FDP were evaluated by a semiquantitative assay (Murex, Dartford, Kent, UK). The same protocol was applied to the controls (Group Healthy and Group CRF).

Purification and characterization of PAF

Freshly drawn blood (20 ml) and urine (10 ml) samples obtained at each time were immediately processed for PAF extraction. Blood samples were collected in citric acid–dextrose solutions (ratio 1:6) and immediately centrifuged at 200 g (20 min at 22 °C). Platelet-rich rich plasma were removed and centrifuged at 1000 g for 20 min, while platelet pellets were resuspended and washed twice in a Ca²⁺–Mg²⁺–freeTris–Tyrode solutions. In blood PAF was extracted from platelet-free plasma (PAF-Plsm) or from below 1.0 mg/dl and/or urine output lower than 400 ml/day in the presence of anamnestic data of normal renal function (serum creatinine below 1.0 mg/dl). The causes of sepsis, the score APACHE III [15], the presence of MODS, defined as involvement of two or more organs [16], and the outcome are reported in Table 1. All patients received conventional therapy including crystalloid solutions, broad-spectrum antibiotics and other drugs including diuretics and vasopressor agents.

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Table 1. Clinical and laboratory data of septic patients with ARF

<table>
<thead>
<tr>
<th>Patient (n)</th>
<th>age/sex (years)</th>
<th>Primary disease</th>
<th>APACHE III (Score)</th>
<th>MODS</th>
<th>Infective agent</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61/M</td>
<td>Sepsis in myocardopathy</td>
<td>40</td>
<td>No</td>
<td>Actinobacter hoffi</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>92/M</td>
<td>Pneumonitis</td>
<td>39</td>
<td>No</td>
<td>Staphylococcus aureus</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>84/M</td>
<td>Pneumonitis in myocardopathy</td>
<td>58</td>
<td>Yes</td>
<td>Staphylococcus aureus</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>83/M</td>
<td>Sepsis in facial trauma</td>
<td>61</td>
<td>No</td>
<td>Pseudomonas aeruginosa</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>62/M</td>
<td>Sepsis in meningioma</td>
<td>88</td>
<td>No</td>
<td>Pseudomonas aeruginosa</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>63/F</td>
<td>Pancreatitis in cholangitis</td>
<td>49</td>
<td>Yes</td>
<td>Proteus mirabilis</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>87/F</td>
<td>Sepsis in acute pyelonephritis</td>
<td>98</td>
<td>No</td>
<td>Proteus mirabilis</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>70/F</td>
<td>Sepsis in UTI</td>
<td>61</td>
<td>Yes</td>
<td>Pseudomonas aeruginosa</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>49/M</td>
<td>Pneumonitis in post-surgery</td>
<td>112</td>
<td>Yes</td>
<td>Staphylococcus aureus</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>82/F</td>
<td>Sepsis in post-cardiac surgery</td>
<td>103</td>
<td>Yes</td>
<td>Negative</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>71/M</td>
<td>Mediastinitis</td>
<td>106</td>
<td>Yes</td>
<td>Pseudomonas aeruginosa</td>
<td>Yes</td>
</tr>
<tr>
<td>12</td>
<td>46/M</td>
<td>UTI in polycystic kidney</td>
<td>–</td>
<td>Yes</td>
<td>Negative</td>
<td>No</td>
</tr>
</tbody>
</table>

Mean ± SEM | 70.8 ± 4.3 | 74.1 ± 8.4 |
biologically active material extracted from platelets and plasmas in different samples was characterized by comparison with synthetic PAF by the following criteria: (i) induction of platelet aggregation by a pathway independent both from ADP and from arachidonic acid/thromboxane A2-mediated pathways; (ii) specificity of platelet aggregation as inferred from the inhibitory effect of 5 mM WEB 2170 or CV 3988, two different PAF receptor antagonists [6]; (iii) TLC and high-pressure liquid chromatography behaviour and physico-chemical characteristics such as inactivation by strong bases and by phospholipase A1, acids, weak bases and 5 min heating in boiling water [6,7]; (iv) the chemical identity with synthetic PAF evaluated by HPLC-tandem mass spectrometry [4].

**Immunossay of TNF, IL-1, IL-6 and IL-8**

The plasma and urinary concentrations of TNF, IL-1, IL-6 and IL-8 were measured using an enzyme-linked immunosorbent assay (ELISA, Quantikine, R&D Systems, Inc., Minneapolis MN, USA) according to the manufacturer’s suggestion. All samples were tested in duplicate. The lowest concentration of detectable cytokine in sample from the zero value were: TNF 4.4 pg/ml; IL-1 0.3 pg/ml; IL-6 0.7 pg/ml; IL-8 18 pg/ml (plasma) and 3 pg/ml (urine). A single calibration curve for each type of sample (plasma and urine) was constructed. Samples below the detection limit were assumed to have a mean value between zero and the lowest detectable concentration.

**Statistical analysis**

The data were evaluated when appropriate by ANOVA followed by Student—Newman—Keuls multiple comparison test, by Student’s t test and by linear regression analysis. Values were expressed as mean ± SEM. P values <0.05 were considered statistically significant.

**Results**

Clinical and laboratory data of patients with ARF and septic shock are given in Table 1. Of these patients, seven developed MODS and the mortality rate was 50% (6 of 12 patients).

**Concentrations of PAF and TNF, IL-1, IL-6 and IL-8**

Table 2 shows the blood and urinary concentrations of PAF in septic patients of Group ARF and in controls (Group Healthy and Group CRF). In septic patients of Group ARF the levels of blood and urinary PAF were measured for 4 consecutive days. The blood and urine levels of PAF were significantly higher in septic patients (Group ARF) than in controls (Group Healthy and Group CRF) (Table 2). No significantly different concentrations of PAF between Group Healthy and Group CRF were detected in blood and urinary samples. The amounts of PAF-PRP (6.2 ± 3.7 pg/ml) and PAF-urine pg/creatinine mg ratio (18.5 ± 4.7 pg/mg) were not significantly different from that of control groups (Table 2) when the survived patients were studied about 1 year after recovery.

The levels of IL-6 and of IL-8 in plasma and urine were constantly and significantly higher in Group ARF than in controls (Table 3), whereas IL-1 was increased on day 4. The concentration of TNF was inconstantly increased in patients of Group ARF but only the urinary excretion of TNF reached a statistical significance (Table 3). In Group ARF, the mean amount of PAF-PRP and levels of creatinine during days 1–4 were significantly higher in patients with MODS than in patients without MODS (Figure 1).

**Correlation between PAF concentration and clinical, laboratory and immunological parameters**

We evaluated in septic patients (Group ARF) the correlation among blood and urinary concentrations of PAF and of several clinical, biochemical and immunological parameters. A significantly positive correlation was found among PAF-PRP and heart rate, serum creatinine, serum IL-6 (Figure 2) and urine IL-8 pg/creatinine mg (Figure 3), whereas a negative correlation was present with platelet number (Figure 4). No significant correlation was found with PAF-urine pg/creatinine mg (r = 0.2922, P = 0.11391), with the clinical parameters such as body temperature (r = 0.2240, P = 0.2519), mean arterial pressure (r = 0.0205, P = 0.9174), respiratory rate (r = -0.6846), urine output (r = -0.2429, P = 0.2130) and APACHE III score (r = -0.0259, P = 0.9514), with the biochemical data such as BUN (r = 0.0593, P = 0.7643), fibrinogen (r = -0.3044, P = 0.1152), FDP (r = 0.0531, P = 0.8080), and leukocyte count (r = -0.1463, P = 0.4574), and with serum and urinary immunological parameters such as TNF (serum, r = -0.0356, P = 0.8599; urine, r = 0.2264, P = 0.2560), and IL-1 (serum, r = -0.1142, P = 0.6039; urine, r = 0.1104, P = 0.6430), serum IL-8 (r = -0.2568, P = 0.1960), and urinary IL-6 (r = 0.2049, P = 0.3728).

A significantly positive correlation was found among PAF-urine pg/creatinine mg and serum IL-6 and with urine IL-8 and IL-6 pg/creatinine mg (Figure 3). No significant correlation was found among PAF-urine pg/creatinine mg and clinical parameters such as body temperature (r = -0.3223, P = 0.1011), mean arterial pressure (r = -0.0526, P = 0.7903), heart rate (r = 0.3201, P = 0.0926), respiratory rate (r = 0.0010, P = 0.9967), urine output (r = -0.4086, P = 0.0593) and APACHE III score (r = 0.3718, P = 0.4114), with the biochemical data such as BUN (r = 0.3579, P = 0.06683), serum creatinine (r = 0.2757, P = 0.1638), fibrinogen (r = 0.0953, P = 0.6363), FDP (r = 0.1203, P = 0.5667), leukocyte count (r = 0.1210, P = 0.5476) and platelet number (r = -0.0815, P = 0.8680) and with serum and urinary immunological parameters such as TNF (serum, r = 0.2936, P = 0.2017; urine, r = 0.2812, P = 0.1553), IL-1 (serum, r = 0.1326, P = 0.5463; urine, r = -0.0359, P = 0.8806), and serum IL-8 (r = -0.0714, P = 0.7234).

**Discussion**

The results of the present study suggest a correlation between the concentration of PAF in plasma and urine
Table 2. Blood and urine levels of PAF in septic patients (Group ARF, days 1–4) and in control patients (Group Healthy and Group CRF)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Group ARF (n=12)</th>
<th>Group CRF (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group Healthy (n=8)</td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>PAF-Plts (pg/ml)</td>
<td>0.0</td>
<td>133.7 ± 23.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>152.1 ± 37.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PAF-Plsm (pg/ml)</td>
<td>0.0</td>
<td>244.4 ± 105.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>208.6 ± 98.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PAF-PRP (pg/ml)</td>
<td>7.7 ± 4.6</td>
<td>375.1 ± 126.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>361.0 ± 120.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PAF-Urine (pg/ml)</td>
<td>7.7 ± 4.6</td>
<td>404.3 ± 101.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>259.3 ± 76.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>PAF-Urine/creatinine (pg/mg)</td>
<td>20.5 ± 3.7</td>
<td>20.5 ± 3.7</td>
<td>20.5 ± 3.7</td>
</tr>
</tbody>
</table>

*P<0.05 Group ARF vs Group Healthy (ANOVA followed by Student–Newman–Keuls multiple comparison test);<sup>a</sup>
*P<0.05 Group ARF vs Group CRF (ANOVA followed by Student–Newman–Keuls multiple comparison test);
Group Healthy vs Group CRF (ANOVA followed by Student–Newman–Keuls multiple comparison test); P>0.05.

PAF-Plts, PAF associated with platelets; PAF-Plsm, PAF extracted from platelet-free plasma; PAF-PRP, PAF extracted from platelet-rich plasma; urine PAF/creatinine (pg/ml), PAF/creatinine in urine expressed as pg of PAF/mg of creatinine by litre of urine.

Table 3. Blood and urine levels of cytokines in septic patients (Group ARF, Days 1–4) and in control patients (Group Healthy and Group CRF)

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Group ARF (n=12)</th>
<th>Group CRF (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group Healthy (n=8)</td>
<td>Day 1</td>
<td>Day 2</td>
</tr>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF&lt;sub&gt;a&lt;/sub&gt; (pg/ml)</td>
<td>19.1 ± 4.0</td>
<td>63.1 ± 37.7</td>
<td>73.8 ± 49.5</td>
</tr>
<tr>
<td>IL-1β (pg/ml)</td>
<td>3.5 ± 0.7</td>
<td>93.3 ± 46.9</td>
<td>58.3 ± 28.1</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.2 ± 0.8</td>
<td>127.8 ± 32.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.2 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>10.4 ± 1.3</td>
<td>145.3 ± 61.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.0 ± 47.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF&lt;sub&gt;a&lt;/sub&gt; (pg/mg*Cr)</td>
<td>2.5 ± 0.3</td>
<td>49.4 ± 29.1</td>
<td>104.4 ± 40.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-1β (pg/mg*Cr)</td>
<td>2.9 ± 0.5</td>
<td>50.0 ± 30.6</td>
<td>168.1 ± 82.0</td>
</tr>
<tr>
<td>IL-6 (pg/mg*Cr)</td>
<td>2.7 ± 0.5</td>
<td>143.7 ± 50.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.9 ± 15.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IL-8 (pg/mg*Cr)</td>
<td>5.9 ± 0.5</td>
<td>788.6 ± 321.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>381.1 ± 173.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*P<0.05 Group ARF vs Group Healthy (ANOVA followed by Student–Newman–Keuls multiple comparison test);<sup>a</sup>
*P<0.05 Group ARF vs Group CRF (ANOVA followed by Student–Newman–Keuls multiple comparison test);
Group Healthy vs Group CRF (ANOVA followed by Student–Newman–Keuls multiple comparison test); P<0.05.

Fig. 1. Concentrations of PAF-PRP (pg/ml) and of serum creatinine (mg/dl) in septic patients of Group ARF, days 1–4, according to absence or presence of MODS. Values are means ± SEM. *P<0.05.
Fig. 2. Relationship in patients of Group ARF between blood concentrations of PAF and heart rate, serum creatinine, platelet count, and serum IL-6. A significant positive correlation was found between PAF-PRP (pg/ml) and heart rate (beats/min), serum creatinine (mg/dl), and serum IL-6 (pg/ml), and a negative correlation between PAF-PRP and platelet count.

and the severity of acute renal failure in patients with septic shock. In humans, acute renal insufficiency is one of the most important complications observed in sepsis. The mechanism of renal injury in sepsis are complex and involves both the bacterial products and the host response. Haemodynamic changes, namely persistent hypotension leading to renal ischaemia play a primary role but not exclusive role in causing the fall in glomerular filtration rate as it has been shown that renal involvement can develop independently of endotoxiaemia, although endotoxaemia may contribute to the severity of acute renal failure [5]. A direct renal action of bacterial endotoxin has been suggested [17]. Indeed, glomeruli and/or glomerular mesangial cells, that express mCD14, may be stimulated by the LBP—LPS complex to synthesize cytokines (IL-1, TNF, IL-6) [18], chemokines such as IL-8, monocyte chemoattractant protein-1 (MCP-1), RANTES, growth-related oncogene-alpha (GROα) and growth-related oncogene-beta (GROβ) [19], and PAF [3,4]. Also tubular epithelial cells that do not express mCD14, can be directly stimulated by endotoxin to produce oxygen radicals and proinflammatory cytokines via the interaction with sCD14 [20]. These cells may produce also several chemokines including macrophage inflammatory protein-1 (MCP-1), RANTES, cytokine-induced neutrophil chemoattractant (CINC), macrophage inflammatory protein-2 (MIP-2) and IL-8 [19]. Furthermore, renal function is affected by increased concentrations of angiotensin II and noradrenaline, and of mediators such as eicosanoids, cytokines (TNF, IL-1, IL-6, IL-8), endothelin, NO, and PAF produced both by resident glomerular cells or by circulating leukocytes [5]. Experiments performed ex vivo on isolated perfused kidney suggest that endotoxin causes a slight increase of renal vascular resistances and does not significantly affect glomerular filtration rate (GFR) and Na reabsorption [17] despite the enhanced expression of mRNA for MCP-1, IL-1 and TNF [18]. In contrast, in vivo endotoxin causes a profound alteration in renal haemodynamics, suggesting the role of extrarenal factors including the extrarenal generation of mediators, the contribution of circulating inflammatory cells or the requirement of plasma as source of sCD14 and LBP for the full expression of the actions of endotoxin [1,4,13]. The reduction of GFR induced by LPS in isolated perfused kidney requires the presence of PMN and depends on the generation of oxygen radicals [21]. Another mediator produced by endotoxin-stimulated leukocytes, potentially involved in the
pathogenesis of acute renal failure, is PAF [1,2]. PAF is synthesized from mesangial cells, endothelial cells, and leukocytes stimulated by endotoxin, porins and endotoxin-induced cytokines such as TNF and IL-1 [3,4,6,7]. PAF can act directly on isolated glomeruli inducing reduction of planar surface due to contraction of mesangial cells [22]. TNF and IL-1 are also capable of contracting mesangial cells by a mechanism involving the production of PAF [22]. In turn, PAF promotes the synthesis of thromboxane A₂ and generation of oxygen radical [5]. PAF infusion in vivo causes the fall of GFR and RBP, the reduction of urine output, and of Na⁺ excretion [13].

The involvement of PAF in renal injury induced by endotoxin has been established in rats by Wang and Dunn [2], who demonstrated that PAF-receptor blockade completely prevented the reduction in glomerular filtration rate, in renal plasma flow and in filtration fraction induced by infusion of doses of endotoxin not able to affect systemic haemodynamics. The ability of PAF to induce systemic and renal haemodynamic alterations [2,13], to increase vascular permeability and to enhance leukocyte adhesion to endothelial cells [5] suggests that in septic patients this mediator contribute in promoting microvascular injury occurring in sepsis. Moreover, it has been recently shown that PAF mediates or potentiates certain effects of proinflammatory cytokines involved in the pathogenesis of septic shock, including TNF and IL-1 [6,7].

In the present study we investigated intravascular concentrations of PAF in patients with septic shock and acute renal failure. Previous studies demonstrated high concentrations of PAF in blood and bronchoalveolar lavage during human sepsis [8–10] that correlated with the occupancy of platelet receptors [8]. Moreover, it has been shown that the rate of catabolism of PAF is reduced during septic shock [11]. We found that the concentration of PAF in septic patients during a 4-day follow-up was persistently increased not only in plasma, but also in association with platelets and in urine. Moreover, the platelet-rich plasma as well as the urinary concentration of PAF correlated with the severity of renal failure and with several clinical and immunological parameters known to be altered in sepsis. The most relevant finding of the present study was the strong correlation among the PAF-urine/creatinine ratios and urine IL-6/creatinine ratio, serum IL-6 concentration and urine IL-8/creatinine ratio (Figure 3). Previous studies have shown that both IL-6 and IL-8 can be considered good indexes of the renal inflammatory injury [23]. An other interesting observation was the lack of link between the concentration of
PAF in blood and urinary concentration of PAF. This observation suggest an intrarenal production of PAF excreted by urine during sepsis. Indeed, PAF may be synthesized either by different populations of resident cells after stimulation with bacterial products or by proinflammatory cytokines [3–5] or by inflammatory cells recruited in the kidney [6,7]. Moreover, the intravascular PAF concentrations was significantly and persistently increased in patients with MODS in respect to patients without MODS (Figure 1, Table 2) and directly correlated with serum creatinine (Figure 2). Moreover, intravascular PAF also correlated with serum concentration of IL-6. IL-6, as well as the ability of peripheral blood mononuclear cells to produce cytokines after stimulation with LPS, are considered indexes of acute inflammatory response and of survival of critic septic patients [24,25]. In contrast, the serum levels of TNF, IL-1 and IL-8, were inconstantly increased during the 4-day follow-up. These data are in accord with a described poor relationship of these cytokines with disease gravity score and outcome [24,25].

Previous studies in septic disseminated intravascular coagulation have shown in septic patients a negative correlation between concentration of PAF in blood and numbers of circulating platelets. We also observed in our patients a negative correlation with the number of circulating platelets, suggesting a PAF-dependent activation of platelets. In contrast, no relationship with FDP and fibrinogen was found. It has been recently shown that the administration of PAF antagonist significantly reversed thrombocytopenia in an experimental model of disseminated intravascular coagulation and in septic patients [10].

In the studied patients we observed a positive correlation between heart rate and PAF-PRP concentration (Figure 2), but not with mean arterial pressure, although PAF is known to be hypotensive. This relationship between heart rate and PAF-PRP concentration may depend on a direct effect of PAF on cardiac muscle [12] that expresses specific PAF receptors [26].

In conclusion, the present study demonstrate that blood and urinary concentrations of PAF correlated with the degree of renal impairment and of renal inflammatory injury. This observation, in the light of information derived from experimental animals, suggests that PAF may contribute to the development of renal injury in septic patients.

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References

24. Casey LC, Balk RA, Bone RC. Plasma cytokine and endotoxin...
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