Immunoneutralization of procalcitonin or its component peptides: a promising treatment of sepsis

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ABSTRACT

Sepsis and the severe systemic response syndrome are very common illnesses that are responsible for a great amount of morbidity and death. These closely related conditions are characterized by a remarkable increase in the prohormone ProCT (procalcitonin). ProCT is both a marker of sepsis and a harmful mediator of the disease. In the present issue of Clinical Science, in a study in rats with endotoxin shock, Tavares and Miñano used an antibody to a segment of N-ProCT (aminoprocalcitonin) that is part of the ProCT molecule, and confirmed that immunoneutralization of ProCT saves the animals from this severe illness. Furthermore, they extensively studied the epiphenomena associated with this immunoneutralization.

Sepsis and the closely related condition SIRS (systemic inflammatory syndrome) yearly affects 18 million people throughout the world [1]. In the U.K., the estimated number of deaths from sepsis ranges from 35 000 to 64 000 [2]. The death rate for this deadly condition exceeds that of lung cancer and breast cancer. Of patients with sepsis in the U.K., nearly 50 % die during their initial hospitalization. The medical cost of this disease is estimated to be £2 billion yearly.

Sepsis is a systemic illness due to an attack of host pro-inflammatory cytokines and other humoral substances commonly induced by a bacterial infection [3]. The symptomatology includes fever or hypothermia, tachypnea, tachycardia and/or leucocytosis or leucopenia. These symptoms and signs are called SIRS. When the cause is presumed to be infection, the diagnosis is considered to be sepsis [3]. Sepsis and severe SIRS often lead to dangerous and fatal complications: hypotension, heart failure, intravascular coagulation and/or coma; this is termed MODS (multiple organ dysfunction syndrome).

Distinguishing clinically between SIRS and sepsis is often difficult or impossible; they are probably identical conditions on a hierarchical continuum. In both of these related conditions, high serum levels of pro-inflammatory cytokines [e.g. TNF-α (tumour necrosis factor-α), IL (interleukin)-1, IL-6 and IL-8] are commonly detected. They arise from leucocytes and other body cells.

In 1993, very high levels of the prohormone ProCT (procalcitonin) were found to be increased by ten, to hundreds, to tens of thousands fold in nearly all patients with sepsis and also in many with severe SIRS (e.g. burns, pancreatitis, pneumonia, extensive surgery, multitrauma and some non-bacterial infections such as malaria) [4]. ProCT is a 116-amino-acid polypeptide arising from the CALC-I gene. It is composed of an N-terminal peptide [N-ProCT (aminoprocalcitonin)], a centrally placed peptide, CT (calcitonin), and a CT C-terminal peptide (CCP-1). Intact ProCT circulates at low levels in the blood of healthy individuals, along with its liberated components N-ProCT, CT and CCP-1 [5].

Key words: adrenocorticotropic hormone (ACTH), aminoprocalcitonin peptide (N-PCT), cytokine, mortality, procalcitonin, septic shock.

Abbreviations: ACTH, adrenocorticotropic hormone; CGRP, calcitonin gene-related peptide; CT, calcitonin; CCP-1, CT C-terminal peptide 1; IL, interleukin; LPS, lipopolysaccharide; N-ProCT, aminoprocalcitonin; ProCT, procalcitonin; SIRS, systemic inflammatory syndrome; TNF-α, tumour necrosis factor-α.

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ProCT is stimulated by the pro-inflammatory cytokines that have been evoked by bacterial products. They include endotoxin [LPS (lipopolysaccharide)] from the cell wall of Gram-negative bacteria, lipoteichoic acid from Gram-positive bacteria, other constituents of micro-organisms and necrotic body cells. These substances can originate from external infection or from endogenous translocation of bacterial toxins across the gut wall.

PCR studies in humans and animals with sepsis reveal, in nearly every tissue, an up-regulation of mRNA for the CALC-1 gene, thus giving rise to massive serum levels of ProCT. In essence, the entire body becomes an endocrine organ. Sensitive immunoassays have been developed to measure serum ProCT in sepsis, the levels of which are nearly always increased, tend to correlate with severity, decrease with improvement, and persist as long as the illness lasts [5].

Although ProCT is not harmful to healthy animals, if it is given to animals that already have sepsis, the mortality increases to nearly 100%. Multiple in vitro studies have demonstrated relevant actions of ProCT that may play a role in sepsis: induction of pro-inflammatory-like effects in leucocytes, a decrease in phagocytic activity of neutrophils, inhibition of neutrophil migration, local increase in pro-inflammatory cytokines, and an increase in NO. Furthermore, ProCT blocks the action of the hormone CGRP (calcitonin gene-related peptide). This peptide increases in the serum of patients with sepsis and exerts effects that would be potentially beneficial in this illness (e.g. phagocytosis, decreased TNF-α, dilation of coronary arteries etc.). By blocking CGRP, the greatly increased ProCT nullifies CGRP activity [5].

The induction of severe peritonitis by a toxigenic strain of Escherichia coli has been found to be fatal in hamsters in a dose-related manner. Then, antibody raised to a central segment of CT peptide residing within the mid-portion of ProCT was administered and nearly all hamsters with sepsis survived [6].

Subsequently, adult pigs with sepsis were infected by instillation of measured faecal material plus additional E. coli. All animals died within 7–8 h. When animals with sepsis were treated with an antibody to N-ProCT, there was a marked amelioration of the critical physiological and laboratory parameters. At the time of killing at 15 h, the only surviving animals were those that had antibody administered (85% short-term survival compared with 0% of controls) [7]. In a similar experiment in which the antiserum was withheld until 4 h, a time at which controls with sepsis were moribund, an identical amelioration of the critical parameters occurred and survival was increased (80% compared with 0% of controls) [8].

The paper by Tavares and Miñano [9] in the present issue of Clinical Science confirms and expands upon these previous hamster and pig studies, utilizing the rat as the experimental animal, and exploring ancillary metabolic phenomena consequent to the immunotherapy. When sepsis due to bacterial infection occurs in rats, the response is known to be fairly heterogenous [10]. Instead, the authors [9] chose to induce a more reproducible LPS-induced shock syndrome that is similar to early sepsis.

In their paper, the authors reported that the N-ProCT peptide exerted toxic effects [9]. These conclusions are largely based upon the finding that the immunoneutralization of a portion of N-ProCT (amino acids 44–57) by an anti-N-ProCT antibody diminished the LPS-induced early release of TNF-α and IL-1β, and also increased the protective anti-inflammatory factors ACTH (adrenocorticotropic hormone) and IL-10. However, N-ProCT is an integral component of ProCT. All N-ProCT is either within intact ProCT or is cleaved out of this polypeptide prohormone to form a free peptide. Hence, as the authors showed in Figure 5 and Table 1 in their paper [9], one cannot neutralize the N-ProCT segment without appreciably neutralizing ProCT.

In addition, the authors demonstrated that, in both blood and in the hypothalamus, LPS increased the expression of the Calc-1 gene (the gene that produces ProCT) [9]. As a result, ProCT was markedly increased. They then observed that anti-N-ProCT-(44–57) diminished gene expression and completely nullified ProCT secretion in the long-term survivors (Table 1 in [9]).

Other than the direct evidence that the intracerebral infusion of N-ProCT directly induces an anorectic effect, attributing the modulation of ACTH and IL-10 to the effect of this peptide is indirect, because it is based on neutralization of the putative offender. Direct proof of the toxicity of N-ProCT would entail administering this peptide to LPS-treated rats and observing an augmented mortality.

Although this important paper [9] does not prove the toxicity of the N-ProCT peptide in sepsis, it confirms the reports of beneficial effects of immunoneutralization of ProCT by an antibody to a segment of the N-ProCT component of ProCT, reveals that it also occurs in the rat, confirms that delayed therapy is effective, demonstrates that this phenomenon is also applicable in the LPS shock syndrome, raises the issue of possible intracerebral changes in sepsis, and explores further important aspects of the humoral pathophysiology of this disease.

It is evident that the therapeutic neutralization of ProCT offers considerable promise in sepsis. It is easily and rapidly measurable [11], and allows for confirmation of the diagnosis of sepsis and for the evaluation of its severity. Furthermore, the long-lasting persistence of serum ProCT in sepsis and in severe SIRS allows for a broad window of therapeutic opportunity. These
characteristics have not been demonstrated for any other mediator of sepsis.

REFERENCES


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