Procalcitonin: Mysterious Protein in Sepsis

Misa Nakamura1*, Ryohei Kono2,3, Sachiko Nomura2,3, Hirotoshi Utsunomiya2,3

1 Department of Rehabilitation, Osaka Kawasaki Rehabilitation University, Osaka; 2Department of Strategic Surveillance for Functional Food and Comprehensive Traditional Medicine, Wakayama Medical University, Wakayama; and 3Second Department of Internal Medicine, Wakayama Medical University, Wakayama, Japan

Abstract

Septic shock is a leading cause of mortality in intensive care units, but can be difficult to distinguish from non-infectious conditions in critically ill patients admitted with clinical signs of acute inflammation. Procalcitonin (ProCT), the precursor of the hormone calcitonin, is composed of 116 amino acids and has a molecular weight of 13 kDa. In contrast to many pro-inflammatory cytokines (e.g., tumor necrosis factor alpha and interleukin-1 beta) that rise and decline in the early course of sepsis, serum ProCT is increased in patients with sepsis and those with systemic inflammation, correlates with the mortality and severity of illness, and remains elevated over a relatively long period of time. Hence, ProCT appears as an important marker of sepsis. In response to microbial infections, ProCT may play a causative role in the tissue damage because treatment with ProCT-neutralizing antiserum increases animal survival in the animal models of sepsis. Conversely, an anti-inflammatory effect of the ProCT has also been reported in humans stimulated with endotoxin. Awareness of the clinical effects of ProCT has led to increased interest in understanding its regulation and biological role. In this review, we first describe the biological characteristics of ProCT and then discuss its impact on the clinical medicine.

Keywords: Procalcitonin, sepsis, inflammation, cytokines, lipopolysaccharide

1. Procalcitonin (ProCT)

Calcitonin (CT) is produced by the thyroid C-cells and has an important role in the calcium homeostasis (1). The gene encoding the CT precursor ProCT, known as CALC-I, is localized on the short arm of human chromosome 11(2). The pre-mRNA from CALC-I gene contains 6 exons, while the mRNA of CT contains exons 1–4 and terminates after a polyadenylation site in exon 4. Another mRNA is also produced from this pre-mRNA by skipping exon 4, which includes exons 1–3, 5, and 6, and encodes a protein known as calcitonin gene-related peptide I (CGRP-I). After transcription, the CGRP-I sequence is excised from the mRNA by alternative splicing (3). The original product of the CALC-I gene is a 141 amino acid chain of preprocalcitonin, which is responsible for the production of ProCT in the C-cells of thyroid, and most likely also during inflammation. The signal sequence at the amino-terminus, together with its hydrophobic property, allows it binding to the endoplasmic reticulum where it is cleaved by an endopeptidase, giving rise to ProCT.

ProCT is composed of 116 amino acids and has a molecular weight of 13 kDa. It is further processed to a 57 amino acid peptide at the amino-terminus (termed aminoprocalcitonin (aminopro-CT)), a centrally placed 32 amino acid immature CT, and a 21 amino acid CT carboxyl-terminus peptide-I (CCP-I, designated catacalcin) (Fig. 1) (4). Practically all of the ProCT formed in the thyroid C-cells are converted into CT, so that no ProCT enters into the circulation and its level in healthy subjects is below the detection limit. ProCT has a long half-life of approximately 22-35 h in serum, contrasting with the short half-life of CT (4-5 min) (5).

Figure 1. Procalcitonin and its cleaved peptides.

2. ProCT and sepsis

Although small amounts of ProCT, aminopro-CT, CCP-I, and conjoined CT:CCP-I peptide are found in the peripheral circulation of healthy subjects, they are significantly increased 24–36 h following exposure to bacteria, lipopolysaccharide (LPS), or proinflammatory cytokines (6). Similar responses also occur in severe systemic conditions such as burns (7, 8), chemical pneumonitis (9), heat stroke (10) and sepsis (11, 12), while the response to viral infections is less significant and the response to fungal infections is variable.

Severe systemic inflammation seen under certain conditions is associated with high serum levels of ProCT peptides. The highest plasma levels of ProCT peptides are observed in acute bacterial infections and sepsis, but are not elevated in local bacterial infections (e.g., abscesses), viral or autoimmune inflammation, and cancer (13, 14). The plasma level of ProCT does not exceed 0.5 µg/l in healthy subjects, but can reach as high as 1000 µg/l in severe bacterial infections (15).
3. Molecular mechanisms of ProCT secretion

i) Regulation of ProCT expression

The regulation of ProCT expression is fundamentally different from the regulation of CT expression. In the C-cells of thyroid, elevated calcium levels as well as a number of other stimuli, such as the glucocorticoids, CGRP, glucagon, gastrin, or β-adrenergic stimulation, induce expression of the CT gene, while somatostatin and vitamin D suppress CT production (5). However, neither hypercalcemia nor any above stimuli lead to a release of ProCT during inflammation. The inflammatory release of ProCT can be induced in two main ways: one is the direct pathway induced by LPS or other toxins released by microbes, and another is the indirect pathway induced through cell-mediated host responses caused by inflammatory cytokines (e.g. interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α)) (16-18). The details of these mechanisms are still poorly understood. The level of ProCT increases selectively in the bacterial inflammatory processes, in contrast to the levels of cytokines which are not specific for particular types of inflammation. These findings suggest that several modulating factors might be involved in the expression of ProCT (5).

ii) Regulation of ProCT secretion

The normal expression of CT results almost exclusively from the transcription of CALC-I gene, which is restricted to the neuroendocrine cells, mainly the C-cells of thyroid. However, high level of CT-like immunoreactivity has been observed in the blood of patients with various extra-thyroid diseases (19). In hamsters, CT mRNA is ubiquitously and uniformly expressed in multiple tissues throughout the body during sepsis. Notably, CT gene transcription seems more widely up-regulated in sepsis than the transcriptions of classical cytokine genes (e.g. TNF-α and IL-6) (20, 21). These reports suggest a ubiquitous expression of CT mRNA in multiple tissues throughout the body in response to sepsis, while there is a relatively low and only transient expression of ProCT in the white blood cells (22, 23). Interestingly, however, the serum level of mature CT is not substantially elevated in microbial infection (24). The presence of microbial infection-specific response elements in the CALC-I gene promoter has been proposed, which, upon an inflammatory stimulus, could override the endocrine tissue-selective expression pattern (25). Fig. 2 shows a schematic diagram of CALC-I expression in non-neuroendocrine cells and in thyroid C-cells. In the classical neuroendocrine paradigm, the expression of CT mRNA is restricted to the neuroendocrine cells, mainly the C-cells of thyroid. Initially, the 116 amino acid prohormone ProCT is synthesized and subsequently processed to the considerably smaller mature CT. In the sepsis and inflammation, proinflammatory mediators induce CT mRNA expression in the non-neuroendocrine cells, which, in contrast to thyroid C-cells, lack secretory granules, thus the unprocessed ProCT is released in an unregulated constitutive manner. Similar to most cytokines, there is no intracellular storage for ProCT in sepsis (20).

Different from bacterial infections, viral infections usually induce only a modest increase of the circulating ProCT (1, 19, 27, 28). The release of interferon-γ (IFNγ) plays a pivotal role in the early antiviral defense mechanisms (29). For example, the marked release of IL-1β-mediated ProCT in human adipose tissue could be inhibited by 89% with co-administration of IFNγ (26). IFNγ secretion may inhibit CT mRNA induction, thereby reducing the increase of ProCT in response to an inflammatory stimulus during the host response to a viral infection (Fig. 2).

4. Pro-inflammatory effects of ProCT

The endotoxin of Gram-negative bacteria triggers humoral and cellular defense mechanisms; it activates coagulation and fibrinolysis, activates macrophages and neutrophils, and stimulates the release of various inflammatory mediators. The pro-inflammatory cytokines TNF-α and IL-1, IL-6 and IL-8 released by various cells have a key role in the pathogenesis of sepsis. At the initial stage of inflammation, the activity of pro-inflammatory cytokines is of benefit to the host, as they stimulate non-specific defensive mechanisms. LPS released by Gram-negative bacteria activates the translation of the mRNA for TNF-α and other cytokines in monocytes. In vivo, high concentrations of TNF-α and IL-1 are present 1-2 h after injection of LPS, together with activated monocytes and macrophages. IL-6 level also rises within hours of LPS injection. TNF-α, IL-1, IL-6, and IL-8 released by activated macrophages may accelerate septic conditions and bring about a fatal outcome caused by uncontrolled cytokine storm. TNF-α induces the oxidative burst in neutrophils and activates the production of reactive oxygen species in patients with systemic infection (30). ProCT increases the expression of certain surface markers on human neutrophils and lymphocytes (CD16 and CD14, respectively) similar to the pro-inflammatory action of LPS. ProCT also increases the concentration of intracellular calcium ions, similar to the action of the pro-inflammatory cytokine, IL-8 (31). In the study by Pincikova et al., ProCT has been found to decrease both phagocytic and candidacidal activities of the neutrophils in a dose-dependent manner (32).

The role of ProCT as a mediator of the sepsis cascade has received little attention. However, when ProCT is added to whole human blood, a marked dose-dependent increase of the pro-inflammatory cytokines above control levels is induced (33). A pro-inflammatory activity of the ProCT in the pathogenesis of sepsis has been suggested based on the immune-neutralization findings in two animal species (34). Severe peritonitis induced in hamsters by the intraperitoneal implantation of the pellets containing Escherichia coli is paralleled by the serum ProCT increases in a dose-dependent manner; at the highest dose of bacteria, serum ProCT level exceeds the control value by nearly 200-fold; and the ProCT level is correlated with the severity of both bacterial insult and mortality (35). In healthy hamsters,
intra venous administration of human ProCT has caused no evident adverse effects and no changes in the serum levels of IL-1β and TNF-α. In septic animals, ProCT injection has only modestly blunted IL-1β value and has not affected TNF-α, although the modality has increased markedly. When TNF-α is injected into healthy animals, there is a marked increase of the ProCT level. These results suggest that ProCT is a pro-inflammatory marker but does not secondarily enhance the level of TNF-α or IL-1β in peripheral blood (36).

5. Anti-inflammatory effects of ProCT

An anti-inflammatory effect of the ProCT has also been reported. Leukocytes are mediators of host defense against bacterial infections. During systemic inflammation or an episode of septic shock, these cells demonstrate alterations in chemotaxis, phagocytosis, and microbial killing (37-40). Release of the anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 is involved in the anti-inflammatory mechanisms associated with the control of immune response (41, 42). Increased IL-10 levels are correlated with the IL-6 levels in the sera of children with multi-organ failure in the course of sepsis (43). The release of anti-inflammatory cytokines seems to be an initial stage of the further control and limitation of the immune response in these patients (44).

Monocytes and neutrophils are stimulated by LPS and another pro-inflammatory product of the bacteria, formyl methionyl leucylphenylalanine peptide, which induces these cells to produce an important integrin, CD11b, a molecule that is involved in chemotaxis. Studies have shown that aminopro-PCT could decrease the expression of integrin CD11b in the monocytes and neutrophils after LPS challenge (45, 46). LPS is the principal component of the outer leaflet of the outer membrane of Gram-negative bacteria, and well-known as the most potent microbial mediator implicated in the pathogenesis of sepsis sequelae and septic shock. Therapeutic strategies for septic shock in humans are currently focused on the neutralization of the LPS molecule and thus its many deleterious effects (47). Recently, studies have shown that, when human recombinant ProCT is added to the endotoxin-stimulated human whole blood, there is a marked decrease of both the pro-inflammatory cytokine TNF-α and the anti-inflammatory factor IL-10 (46, 48). This indicates that ProCT may directly neutralize the LPS and hence the pro- and anti-inflammatory events triggered by LPS.

6. Usefulness of ProCT in clinical medicine

i) Usefulness as a biomarker of sepsis

Septic shock is a leading cause of morbidity in intensive care units, but can be difficult to distinguish from non-infectious conditions in critically ill patients who admitted with clinical signs of acute inflammation. Increased plasma ProCT level has been found in thyroidectomized patients with inflammation (19, 49). Serum ProCT concentration rises soon after sepsis sets in and remains elevated over a prolonged period of time. In contrast, many pro-inflammatory cytokines including TNF-α and IL-1β rise and decline early in the course of sepsis.

Exposure of human volunteers to endotoxin has illustrated the differences in the release and subsequent decrease of several humoral markers of critical illness. The serum level of the pro-inflammatory cytokine TNF-α increased at 1 h, reached a peak at 1.5 h, and was normalized by 24 h. Similar patterns of secretion occurred for the IL-1 receptor antagonist (IL-1ra), IL-6, granulocyte colony-stimulating factor, and several other cytokines with some of them being even more transient. The serum ProCT level was observed to increase by 3 h, peaked at 24 h, and subsequently declined very slowly and progressively. However, at day 7 after endotoxin exposure, all volunteers still exhibited a level of ProCT above the normal (50). In two of the volunteers who were studied for a longer period, the ProCT level did not normalize until the day 10–14 (51). The elevation of the short-lived acute phase cytokines contrasts with the extremely prolonged elevation of the serum ProCT after a systemic inflammatory episode in healthy humans. These findings suggest that measurement of the ProCT level would not only have advantages as an excellent marker of sepsis but also may offer a suitable target for therapeutic immunoneutralization, even several days after the severe inflammatory illness has commenced. Furthermore, studies have suggested that ProCT is a useful marker for the diagnosis of acute bacterial infection, especially sepsis (52, 53), but not for viral or fungal infection (54). These findings have led to the hypothesis that ProCT guidance could limit antibiotic overuse in most viral acute respiratory tract infections (55, 56).

ii) Antibiotic therapy

Interestingly, administration of ProCT to septic hamsters with peritonitis doubled the death rate, but treatment with ProCT-reactive antiserum increased the survival of the septic hamsters and pigs (57, 58). Martinez et al. have demonstrated that such immunoneutralization is effective even when the ProCT-reactive antiserum is administered after the pigs become moribund (59). In healthy animals, administration of similar doses of ProCT did not show any detrimental effects. These results suggest that immunoneutralization of ProCT may be a useful treatment in clinical situations where sepsis is already fully established.

In the septic hamster model, the reduction of the IL-1β but not the TNF-α by administration of ProCT (36) suggests that ProCT is a secondary mediator of the septic response and immunoneutralization of ProCT may be an inappropriate therapy for sepsis during the early phase. Given the clinical usefulness of ProCT, interest in the understanding of its regulation and biological role is steadily growing.

In conclusion, ProCT shows a high discriminative power for disease severity and therapeutic amelioration. This paper has reviewed the new insights of the biological and clinical events of sepsis and the potential modulation of the events by ProCT. A number of uncertainties still exist concerning the metabolism of inflammatory ProCT and its physiological role. Further studies will facilitate the applications of ProCT in clinical medicine.

References

Nakamura M et al. Procalcitonin in sepsis, JBCM 2013, 2(1):7-11


