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Department of Biochemistry and Molecular Biology, Medical University of Lublin

BOLESŁAW FLORIAŃCZYK

Structure and diagnostic value of procalcitonin

Procalcitonin (PCT), a protein of 116 amino acids with molecular weight of 13 kDa, was discovered 27 years ago as a prohormone of calcitonin produced by C-cells of the thyroid gland and intracellularly cleaved by proteolytic enzymes into the active hormone. Circulating levels of PCT in healthy subjects are below detection limit. Since 1993 when its elevated level was found in patients with bacterial infection, PCT became an important protein in the detection and differential diagnostics of inflammatory states. The production of PCT during inflammation is linked with a bacterial endotoxin and with inflammatory cytokines (TNF, IL-6). PCT detectable in the plasma during inflammation is not produced in C-cells of the thyroid. The probable site of PCT production during inflammation are the neuroendocrine cells in the lungs or intestine.

There is no evidence of plasma PCT binding to cellular receptors of calcitonin, and the role of PCT in calcium and phosphate metabolism during sepsis is still not clear (4,8).

STRUCTURE OF PROCALCITONIN

Procalcitonin (PCT) is a 116 amino acid protein with a molecular weight of approximately 13 kDa (Fig. 1 and Tab.1). It comprises the sequence of calcitonin at position 60 to 91 (32 amino acids).

- Pro – Phe – Arg – Ser – Ala – Leu – Glu - Ser – Ser – Pro – Ala
- Asp – Pro – Ala – Thr – Leu – Ser – Glu – Asp – Glu – Ala – Arg –
- Leu – Leu – leu - Ala – Ala – Leu – Val – Gln – Asp – Tyr – Val –
- Gln – Met – Lys – Ala – Ser – Glu – Leu – Glu – Gln – Glu – Gln –
- glu - Arg – Glu – Gly – Ser – Ser – Leu – Asp – Ser – Pro – Arg –

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- Ser – Lys – Arg - Cys – Gly – Asn – Leu – Ser – Thr - Cys – Met –
- Leu – Gly – Thr – Tyr - Thr – Gln – Asp – Phe – Asn – Lys – Phe –
- His – Thr – Phe – Pro – Gln - Thr – Ala – Ile – Gly – Val – Gly – Ala –
- Pro - Gly – Lys – Lys – Arg – Asp – Met – Ser – Ser – Asp – Leu – Glu –

Arg – Asp – His – Arg – Pro – His – Val – Ser – Met – Pro – Gln –

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Asn – Ala - Asn

1 –57 aa – N-ProCT
60 – 91 - Calcitonin
96 –116 – Katacalcin
↓ - cleavage of endopeptidases

Fig. 1. The amino acid sequence of procalcitonin

Table 1. Amino acid present in procalcitonin

Symbol	Name	Number of residues
Ala	Alanine	11
Asn	Asparagine	4
Asp	Aspartic acid	8
Arg	Arginine	8
Cys	Cysteine	2
Gln	Glutamine	4
Glu	Glutamic acid	12
Gly	Glycine	6
His	Histidine	3
Ile	Isoleucine	1
Leu	Leucine	11
Lys	Lysine	5
Met	Methionine	4
Pro	Proline	8
Phe	Phenyloalanine	4
Ser	Serine	13
Thr	Threonine	6
Tyr	Tyrosine	2
Val	Valine	4

Table 2. Plasma and serum concentrations of PCT

Procalcitonin concentration	
0.1 – 0.5 ng/ml	Normal plasma and serum concentration
0.5 - 2.0 ng/ml	Moderately increased
> 3 ng/ml	high value
> 30	Very high elevated

Human procalcitonin is encoded by the Calc-l gene. After transcription of the CALC-1 gene, the primary transcript is processed into mRNA, endocing a 141-aminoacid protein with a molecular weight of aproximate 16 kDa. The protein is assigned preprocalcitonin and comprises a signal sequence (aminoacid 1-25), the N-terminal region of procalcitonin called "N-ProCT", the sequence of calcitonin, and the C-terminal region of procalcitonin, called "katacalcin" – Fig. 2 (1).

The signal or leader sequence is a hydrophobic sequence and it mediates binding of the protein to the endoplasmic reticular. The endoplasmic reticulum is the most important structure of the cell processing exocrine peptides. The signal sequence is degraded in the endopeptidase and the remaining proteon is procalcitonin (116 amino acids, Fig. 3). The midportion of this molecule comprises the amino acid sequence of calcitonin at position 60 to 91. This sequence is flanked by polybasic amino acids (Lys-Arg and Gly-Lys-Lys-Arg). These sequences are signal sequences for specific proteolysis by the enzyme prohormone convertase, resulting in the main

cleavage products of PCT: N-ProCT (67 aa), calcitonin (32 aa), katacalcin (21 aa) and their combinations (5).

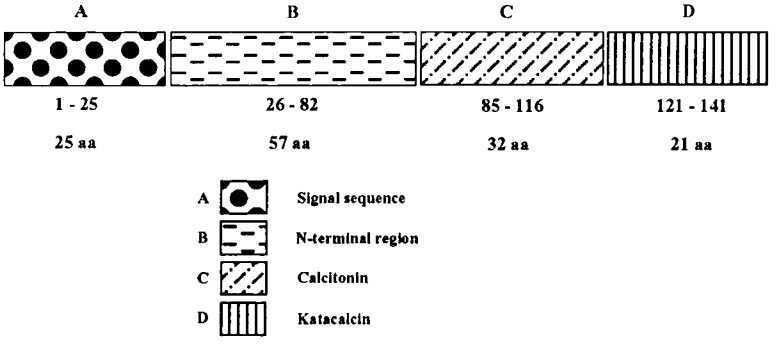

Fig. 2. Preprocalcitonin

The final structure of calcitonin originates upon formation of a cyclic structure by cystein residues (cys 1 – cys 7) and the cleavage of the C-terminal glycin by a carboxypeptidase and amidation of this molecule (peptidyl glycine amidating monooxygenase, PAM) – Fig. 3.

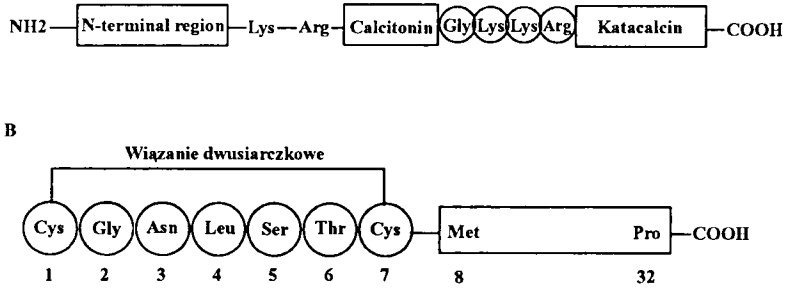

Fig. 3. Preprocalcitonin (A) and calcitonin (B)

At normal metabolic conditions the hormone calcitonin is secreted into circulation, regulated by calcium-dependent stimuli.

With systemic bacterial infections, in sepsis and multiorgan disfunction, high concentrations of calcitonin-precursor peptides, but no increassed calcitonin-levels were found in plasma (5). Among these peptides procalcitonin is the main peptide with a plasma half-life time of about 25 to 30 hours *in vivo*.

PROCALCITONIN IN PLASMA

Plasma concentrations of the prohormone procalcitonin in healthy individuals are very low in the picogram range - < 0.1 ng/ml - and below detection limit of the presently used PCT

assay. With severe infections and in sepsis plasma concentrations of procalcitonin ranging from 1 ng/ml to above 1000 ng/ml are found (Table 2). Very high increased PCT values can be seen only in severe acute bacterial infections and sometime in the hyperinflamatory phase of multiorgan dysfunction syndrome and sepsis.

Procalcitonin induction was stimulated by i.v. injection of small amounts of bacterial endotoxin (E. Coli 0113H10k, 4 ng/kg body weight). PCT can be detected in the plasma two hours after the injection of endotoxins. Within 6 to 8 hours PCT concentrations rapidly rise and a plateau is reached after approximately 12 hours. Within the following two to three days PCT values decrease to their normal values (3,7).

PROCALCITONIN IN DIAGNOSIS

Blood concentrations of procalcitonin are increased in systemic inflammation, especially when this is caused by bacterial infection. Studies of its behaviour in patients with bacterial sepsis have led to the proposal that it may be a useful marker of systemic bacterial infection, with greater specificity and sensitivity than acute phase proteins such as C-reactive protein (8).

Circulating levels of PCT in healthy subjects are below detection limit. Since 1993 when its elevated level was found in patients with bacterial infection, PCT has become an important protein in the detection and differential diagnostics of inflammatory states. The production of PCT during inflammation is linked with a bacterial endotoxin and with inflammatory cytokines (TNF, IL-6). PCT detectable in the plasma during inflammation is not produced in C-cells of the thyroid. The probable site of PCT production during inflammation are the neuroendocrine cells in the lungs or intestine. There is no evidence of plasma PCT binding to cellular receptors of calcitonin, and the role of PCT in calcium and phosphate metabolism during sepsis is still not clear (2,4,6).

BACTERIAL INFECTIONS

With severe bacterial infections high plasma concentrations of PCT are found without significant change in plasma calcitonin. Procalcitonin is a very stable protein (*in vivo* and *in vitro*) and it is not degraded to hormonal active calcitonin in plasma. Its half-life time *in vivo* is about 25 to 30 hours. PCT, stimulated by bacterial inflamation, is most likely not produced by C-cells of the thyroid. With bacterial inflamation and in sepsis, other cleavage products of the calcitonin precursor peptides are found in the plasma. However, PCT represents the main product of these peptides.

The search for sensitive and specific markers of systemic infection has shown that procalcitonin levels are increased in sepsis, and, consequently, this plasma protein has come into the focus of clinical research (2, 3).

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SUMMARY

Procalcitonin (PCT), a protein of 116 amino-acids was discovered as a prohormone of calcitonin produced by C-cells of the thyroid gland and intracellularly cleaved by proteolytic enzymes into the active hormone. Circulating levels of PCT in healthy subjects are below detection limit. Blood concentrations of procalcitonin are increased in systemic inflammation, especially when this is caused by bacterial infection. Studies of its behaviour in patients with bacterial sepsis have led to the proposal that it may be a useful marker of systemic bacterial infection, with greater specificity and sensitivity than acute phase proteins such as C-reactive protein.

Struktura oraz wartość diagnostyczna prokalcytoniny

Prokalcytonina (PCT) to białko zbudowane ze 116 aminokwasów. Białko zostało odkryte jako prekursor kalcytoniny. Produkowane jest przez komórki C tarczycy. Wytworzenie hormonu kalcytoniny następuje wewnątrzkomórkowo na drodze częściowej proteolizy. U zdrowych osób poziom krążącej prokalcytoniny jest bardzo niewielki (poniżej wykrywalnego stężenia). Wzrost stężenia PCT we krwi następuje w zapaleniach, szczególnie spowodowanych zakażeniami bakteryjnymi. Na podstawie badań i obserwacji stwierdza się, że w przypadku zakażeń bakteryjnych prokalcytonina może być lepszym markerem niż białko C-reaktywne.