Abstract

Objectives. Since atrial natriuretic factor (ANF) is detected in pleural fluid of human congestive heart failure patients, we studied in rabbit whether the parietal pleura is a site of production of ANF.

Results. The results show immunopositive mesothelial cells and PCR method shows the ANF transcript in parietal pleura.

Conclusions. Thus, we conclude that ANF in the pleural fluid is synthesized by mesothelial cells and it would be involved in the turnover of pleural fluid (Dent. Med. Probl. 2004, 41, 2, 209–212).

Key words: parietal pleura, atrial natriuretic factor, immunohistochemistry, PCR, rabbit.

The parietal pleura is an organ constituted by a thin mesothelial layer and a thick connective submesothelial layer with numerous blood and lymphatic vessels involved in the pleural fluid patency. This function is regulated by different local biochemical factors [1] so that the fluid and electrolytes permeate freely between the normal mesothelial cells and the endothelium as the mean barrier. Among examined biochemical factors, an increased level of ANF and its C- and N-terminal fragments were detected by radioimmunoassay in the pleural fluid of six patients with congestive heart failure [2, 3].

ANF, secreted by heart, lung [4], prostate [5], parotid gland, CNS, is formed in a proteolytic release by final 28 aminoacids from C-terminal of a 126 aminoacids precursor protein (pre-hormone). Moreover, the pre-hormone of ANF may be the
source of several other peptides which also exhibit ANF-like activity. ANF releases vascular smooth muscle, increases the diuresis and natriuresis, has the central role in suppression of fluid and salt intake and regulates volume distribution by shifting fluid between the extravascular and intravascular compartments.

In the pleural fluid of patients with congestive heart failure, Vesely [3] found high concentration of ANF nearly equal to the concentration in plasma of these patients but higher than that of persons without congestive heart failure. Vesely [3] hypothesized that ANF was produced either by the lung and released in pleural cavity, or by the capillary leakage from venules or capillaries. Today, the synthesis site of ANF in pleural fluid is not known. As in the parietal pleura, the mesothelial cells have much organelles involved in the synthesis of proteins [6], we think that the parietal pleura might, also, be the site of ANF synthesis, which released directly into the pleural fluid might be involved in the patency of pleural fluid. We carried out the study with immunohistochemistry and PCR analysis for the ANF localization in the rabbit parietal pleura.

Material and Methods

We have studied the parietal pleura of six adult rabbits weighing about 1500 g each with immunohistochemical methods and PCR analysis.

Immunohistochemistry

Three rabbits had been anesthetized with fentanyl, ventilated and perfused with Bouin’s solution. The samples were fixed in Bouin’s solution, dehydrated in a graded series of ethanol and embedded in paraffin. Seven micro thick sections were used for ANF immunostaining and were carried out with avidin-biotin peroxidase or streptavidin methods. Endogenous peroxidase was blocked by incubation with PBS containing 0.3 hydrogen peroxide for 5 min in the room temperature. Following blockade of the non specific binding by normal goat serum, the sections were treated with ANF (ser-Tyr) polyclonal antibody (rabbit antiserum, Poenix Pharmaceutical), at various dilutions (1/500, 1/600, 1/800) in 0.05 M Tris Buffer pH 7.2 for 12 hours at 4°C, subsequently the sections were washed in PBS (three times, 15 min). The reaction was revealed with avidin-biotin or streptavidin system; binding was demonstrated by amino-ethyl carbazole substrate. The negative controls were performed using treatment with non immunized antiserum and omission of the primary antiserum.

Reverse Transcription – PCR

Reverse transcription (RT) PCR analysis was performed on the samples of costal pleura (weight 1 g) removed from other rabbits and immediately immersed into liquid nitrogen. The tissues were homogenised. mRNA was obtained by RNAzol (Boeringer) and the integrity of this preparation was assessed with agarose gel electrophoresis, mRNA was quantified with standard absorbance at 260 nm. 1 microgram of each mRNA preparation was used for cDNA synthesis. RT was performed under standard conditions using random primers and murine leukemia virus reverse transcriptase. cDNA was amplified with PCR in 35 cycles. The cycle parameters were 1 min at 94°C and 1 min at 72°C. 1/10 of each PCR product was electrophoresed in 2% agarose gel and stained with ethidium bromide. The molecular weight of amplified products was estimated by comparison with the pattern of markers (with known molecular weight) migration. Glucose-6-phosphate-dehydrogenase (G6PD) mRNA was amplified and used as positive control. In fact, this gene is constitutively expressed. This excludes the possibility that the difference in ANF PCR products was due to the technical problems arising by RNA amplification.

Results

The costal pleura covers the ribs and intercostal spaces. It is constituted by a single layer of mesothelial cells and a submesothelial layer of loose connective tissue (Fig. 1, 2). In the submesothelial layer over the ribs, there are numerous blood and lymphatic vessels (Fig. 2); in the mesothelial layer over of the intercostal spaces, there are lymphatic lacune (Fig. 1) as well as specialized vessels like a flat cisterne, which open into pleural cavity by stomata. The immunohistochemical stainings for ANF show mesothelial cells, which cover both ribs and intercostal spaces with immunopositive granules (Fig. 1, 2). By high magnification, these granules are located in the perinuclear area (Fig. 3, 4).

In addition, we studied the expression pattern of ANF transcript in the costal pleura and RT-PCR experiments show high levels of ANF transcript in accordance with immunohistochemical data (Fig. 5).

Discussion

It is demonstrated by AA that the lung and the inferior airways are the sites of ANF synthesis. Recently, Vesely [3] observed high ANF levels,
nearly equal to plasma concentrations, in the pleural fluid of patients affected by congestive heart failure. To explain the presence of ANF in the pleural fluid, Vesely [3] hypothesized that ANF could be released in pleural space either by pulmonary capillaries or by pneumocytes which directly secrete ANF in pulmonary fluid and reversed in pleural fluid. Vesely [2, 3] found it more probable that the lung was the site of ANF synthesis, which was carried out into pleural space and would be involved in the mechanism of the removal of pleural fluid from the pleural cavity. Till today specific researches have not revealed the evidence for the site of ANF synthesis. This research, firstly, shows that the parietal pleura is the site of ANF synthe-
sis. Indeed, the immunohistochemical stainings show immunopositive−ANF granules in mesothelial cells and this immunopositivity is confirmed by PCR methods results.

We retain, therefore, that ANF produced by mesothelial cells may be secreted into pleural fluid where it may share to ionic composition and also we retain that ANF may play a role in submesothelial vessels to regulate the transport of the pleural fluid. Indeed, the parietal pleura of rabbit, in opposition to the human pleura [7] over the ribs, presents a thick submesothelial layer with numerous blood and lymphatic vessels. Over the intercostal spaces there are lymphatic lacunae that communicate with the pleural cavity by stomata [6, 8]. Therefore, via paracrine mechanism, ANF secreted by mesothelial cells, after binding to receptors of smooth muscle of blood and lymphatic vessels would induce vasodilation and decrement of their contractility. Also, ANF, by natriuretic activity, determines electrolyte transport varying the composition and the volume of the pleural fluid. In conclusion, ANF secretion by mesothelial cells would participate in normal turnover of pleural fluid. Moreover during pathologic events, when the pulmonary interstitium is filled with fluid, if would play a defensive role removing the excess fluid from the pleural cavity [9].

References


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