LUNG THROMBOPOÏESIS IN PREGNANT RABBITS DURING SEPTIC SHOCK – AN ULTRASTRUCTURAL STUDY

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The aim of the present study was the comparative analysis of morphological changes found in the pulmonary microcirculation of pregnant rabbits in the course of experimental septic shock induced by endotoxin administration. Sixteen female rabbits, white Dutch, c.3 kg of mean body weight were used in the experiments. The endotoxin of Escherichia coli, serotype S.0127: 138 (Sigma) was applied intraperitoneally in a single dose of 100 μg/kg b.w. Morphological examinations were based on the ultrastructural analysis with the transmission electron microscope. Severe damage of endothelial cells (necrosis inclusive) was observed in endotoxin-treated rabbits. The vascular lumen in these animals showed cellular aggregation of neutrophils and platelets in particular, as well as microthrombi. Some of the blood vessels showed fragments of megakaryocytes. An increased tendency towards the development of these changes was noted in pregnant rabbits. The study confirmed that the lungs may be an important site of extramedullary thrombopoiesis in the course of septic shock especially during pregnancy.

Key words: rabbits, pregnancy, endotoxin, lungs, microcirculation, thrombopoiesis.

Adult respiratory distress syndrome (ARDS) is characterized by pulmonary insufficiency associated with lung microvascular injury, leukosequestration, interstitial formation of oedema, and fibrin-platelet aggregates in the microvasculature. This syndrome complicates multiple disease states such as sepsis, trauma and burns.

Endotoxin is known to participate in various events associated with the inflammatory response at the alveolar level. In these inflammatory processes, endotoxin is implicated as a major factor that initiates the complex cytokine networks resulting in various pathological processes, including ARDS (13, 15).

Much evidence suggests that the neutrophil may be a mediator of endotoxin-induced lung injury (7). Neutrophils, increased in the lungs, are activated in the early phase after endotoxin administration and may release toxic products able to injure cells (3, 8, 12). In vivo, neutrophil depletion experiments reveal that the neutrophil may play an important role in endotoxin lung injury (4). These findings, however, are difficult to interpret since neutropenia does not insure tissue depletion of neutrophils and agents that reduce granulopoiesis may also be toxic to other tissues, including vascular endothelium (2, 9).
Tumor necrosis factor-α (TNF-α) has recently been implicated in the pathogenesis of shock and tissue injury (7). Endotoxin is a potent stimulator of TNF-α production, which has been demonstrated in the sera of humans and experimental animals after endotoxin administration (10). Pulmonary microvessels are important target sites for the permeability-increasing effects of bacterial endotoxin and TNF-α. The pulmonary vascular response to TNF-α appears to be independent of polymorphonuclear leukocyte (PMN) (6, 11, 14). The gross and histopathologic damage seen in the lungs, intestine, kidneys, pancreas, and adrenals in TNF-treated animals resembles that induced by endotoxemia. Our previous study confirmed the fact that the pulmonary thrombopoiesis was present after the injection of TNF-α (16).

The aim of the present study was the comparative analysis of morphological changes found in the lung capillary bed of pregnant rabbits in the course of experimental septic shock induced by administration of endotoxin.

Material and Methods

The experiments were carried out on 16 female rabbits, white Dutch, with mean body weight of c.3 kg. The animals were maintained in a well sunlit room, at 18-20°C on standard granulated diet. Group I included five non-pregnant rabbits, injected intraperitoneally (i.p.) with the endotoxin of Escherichia coli, serotype S.0127: 138 (Sigma) in a single dose of 100 μg/kg b.w. in 15 ml PBS. Group II - five pregnant rabbits - received the same dose of the endotoxin as group I. Group III consisted of three non-pregnant rabbits, given i.p. a single dose of 15 ml PBS. Group IV - three pregnant rabbits received once i.p. 15 ml PBS. All experimental animals were anaesthetized by i.p. administration of 100 mg sodium pentobarbital after 12 hours following endotoxin treatment.

Morphological examinations were based on the ultrastructural analysis with the transmission electron microscope (TEM). For TEM examinations specimens were collected from the perihilar parts of both lungs. Small blocks of 1mm³ vol. were refixed in cold 2.5% glutaraldehyde solution and osmium tetroxide. After dehydration in alcohol-acetone series and embedding in epones, they were sectioned and contrasted with lead citrate and uranyl acetate, and examined with an electron transmission microscope Opton 900 PC.

This study was approved by the Institutional Review Board at the Medical Academy of Białystok, Poland, for welfare of animal subjects. All procedures were in strict accordance with guidance on the welfare and use of laboratory animals and were approved by the local Animal Welfare Organisation.

Results

Ultrastructural part of the lungs in animals from groups III and IV did not show any abnormalities. In the endotoxin-treated non-pregnant rabbits (group I) increased activity of endothelial cells prevailed. The nuclei of these cells were
frequently enlarged and indented towards the lumen of blood vessels. Their cytoplasm had numerous pinocytic vesicles. The cytoplasmic membrane of some of the endothelial cells formed digitate processes or/and tiny vesicular indentations (Fig. 1). Endothelial cells were severely damaged. The vascular lumen showed cellular aggregation of neutrophils and platelets (Figs 1 and 2). Some of the blood vessels showed fragments of megakaryocyte (MK) cytoplasm (Fig. 3). The endotoxin-treated pregnant rabbits (group II) had all the changes mentioned above, although more intensified. Severe damage to endothelial cells was observed, including focal necrosis. The vascular lumen showed domination of neutrophils, frequently combined with damaged endothelium (Fig. 4). The neutrophils revealed features of destruction. Cell degranulation was also observed. In places, granulocytes which adhered to the damaged endothelium produced microthrombic-like changes, together with fibrin and blood platelets (Fig. 5). The features of endothelial damage were relatively rare in blood vessels which showed accumulations of platelets or MK fragments. Sporadically MK fragments blocked the vascular lumen.

Fig. 1. Fragment of the interalveolar septum with a blood vessel containing a granulocyte (PMN). Severely damaged endothelial cells (EN) are also seen. Group I, TEM, x4400.
Fig. 2. Blood platelets (pl) found in the vascular lumen do not tend to combine with one another or endothelium. Group I, TEM, x7000.

Fig. 3. Fragment of a megakaryocyte (MK) and blood platelet (pl). Group I, TEM, x7000.
Fig. 4. A neutrophil (PMN) present in the vessel of an interalveolar septum is tightly attached to endothelium. The alveolar lumen shows a slight amount of oedematous fluid (oe) and fragment of macrophage (AM). Group II, TEM, x4400.

Fig. 5. The vascular lumen shows blood platelets fused together or joined to endothelium - status after vascular perfusion. Group II, TEM, x4400.
Discussion

Damage to vascular endothelium and platelet accumulation in the lung capillary bed provide favourable conditions for the formation of platelet thrombi observed in a number of lung tissue injuries, particularly in ARDS. However, little attention has been paid to the problem of local (pulmonary) thrombopoiesis and its significance in platelet thrombus formation. Most MKs released from the bone marrow pass with the blood to the lungs. In the terminal segments of the pulmonary microcirculation, the vascular diameter is much smaller than the MK one. Trowbridge (19), taking this into account, indicated three possible outcomes, namely MK disintegration, MK deformation, or vessel occlusion by MKs. The study clearly demonstrated the MK disintegration and the formation of numerous blood platelets in the lung capillary bed. If such disintegration occurs in vessels with endothelial damage, conditions are provided for thrombus formation. Our observations confirm the hypothesis that MKs may shed platelets and larger fragments of cytoplasm within the pulmonary capillary bed and provide evidence that MKs with copious cytoplasm can occlude lung vessels. The effect of the endotoxin on the pulmonary circulation in animals is well known. However, there are no detailed morphological reports on their effect on the pulmonary circulation in pregnant animals. It is known that even physiological pregnancy disturbs considerably numerous systemic mechanisms that contribute to e.g. septic shock (1). Maintenance of the blood in the fluid state in such an extremely dangerous condition as septic shock allows survival. Pregnancy seems to alter the activity of haemostasis, thus increasing the risk of disseminated intravascular clotting (5, 17, 18). We assume that microthrombi found in our study in the pulmonary capillaries, being particularly common in pregnant rabbits, may indicate pregnancy-related haemostasis disorders.

References