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ORIGINAL ARTICLE

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The expression of P-selectin in inflammatory and non-inflammatory lung tissue

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Abstract An initial attachment of leucocytes to blood vessel walls is mediated by selectins. A feature of adhesion mediated by P-selectin is the "rolling" of leucocytes on the endothelium. The time dependent expression of p-selectin in lung tissue was investigated in five groups of cases with different causes of death: carbon-monoxide and cyanide intoxication (n = 11), drowning (n = 5), hanging (n = 9), pneumonia (n = 13) and polytrauma with blunt thorax trauma (n = 14). In paraffin-embedded archival specimens immunostaining was achieved using an adapted APAAP-immunoperoxidase technique together with a wet autoclave method. P-selectin detection was scored by a semiquantitative method evaluating the intensity and incidence of positively stained endothelial cells.

The distribution pattern of endothelial P-selectin of blood vessels in cases of pneumonia and septic shock were heterogenius and weak. In one case with lung contusion (survival time 3 h) moderate infiltrates of granulocytes were found near to septal and subpleural hemorrhages. In these inflammatory areas the positive endothelial immunostaining of small vessels was often weaker than in other lung segments or compared to the intensely stained platelets in corresponding vessels.

Key words Immunhistochemistry · P-selectin · Endothelium · PADGEM

Introduction

In recent years a remarkable expansion in knowledge of cell adhesion molecules in the vascular endothelium and in circulating leucocytes has occurred (Betz 1994, Betz et al. 1996, Kondo et al. 1996, Grellner et al. 1996). An important initial attachment of leucocytes to the blood vessel wall is mediated by selectins. Three forms of selectin can be distinguished. P-selectin is stored for rapid release in Weibel-Palade bodies of the endothelium and in the alpha granules of platelets (McEver et al. 1989). E-selectin is produced by the endothelium after cytocine activation and L-selectin is expressed on leucocytes (Gearing et al. 1993).

An initial feature of the adhesion mediated by P-selectin is the "rolling" of the leucocytes on the endothelium (Ley et al. 1991; von Andrian et al. 1991). The occurrence of p-Selectin on the cell membrane after endothelial activation with various chemoattractants such as histamine, thrombine, LPS or oxygen radicals suggests that this selectin is involved in different types of endothelial reaction. Therefore, the distribution of P-selectin could reflect certain stages of activation. This could be especially true for lung tissue since their vessels play a central role in various pathomechanisms (Kita 1987, Kita et al. 1989, Lindeman et al. 1995). The present study was performed to clarify the dynamics of endothelial P-selectin in inflammatory and non-inflammatory lung tissue.

Materials and methods

Materials

Human peripheral lung specimens from 52 cases were fixed for two days in 4% buffered formalin and embedded in paraffin. Autopsies had been performed up to 48 h postmortem in 1995 and 1996. Final diagnoses were made by further considering the reported circumstances, histological and toxicological findings. Thereafter the following major groups remained: carbon monoxide and cyanide intoxication (n = 11), drowning (n = 5), hanging (n = 9), pneumonia and sepsis (n = 13) and polytrauma with blunt thorax trauma (n = 14, i.e. traffic accidents). The corresponding survival times of trauma patients varied from seconds to three hours. Primary pulmonary diseases were excluded by histological investigations.

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Immunohistochemical methods

The primary antibody was designed for use on frozen sections only. For this reason the technique had to be adapted to formalin-fixed and paraffin-embedded material. In archival specimens adequate immunostaining was achieved using an APAAP-immunoperoxidase method.

Sections (4 μm thick) were deparaffinized in xylene and rehydrated with graded alcohols and finally in aqua dest. All specimens were investigated without (a) and with (b) a retrieval technique.

a. Slides were incubated in Tris buffer (0.05 M, pH 7.6).

b. Corresponding slides were placed in citrate buffer (pH 6.0) and pretreated for 10 min in a wet autoclave at 120° C. (Shin et al. 1991, Bankfalvi et al. 1994). After reaching room temperature the sections were washed in aqua dest and Tris buffer, followed by a 15 min proteinase K digestion (0.01%, pH 7.5, 37° C) to facilitate antigen retrieval and to increase membrane permeability to antibodies.

The primary anti-CD62p antibody (Serva, Lot No. E255-SA55) was applied to the sections at a dilution of 1 : 25 and incubated for 16 h at 4° C. After washing in Tris buffer, biotinylated immunoglobulins (20 min) and alkaline phosphatase-conjugated streptavidin (20 min) were added (Super Sensitive Kit BioGenex, CA). The P-selectin staining was visulalized by fast red (BioGenex, CA) for 5 min. The sections were counterstained with hematoxylin and coverslipped with aqueous mounting medium. Positive controls were thrombocytes as present in every slide. Sections without application of primary antibody were used as negative controls.

Analysis of immunostaining

P-selectin was scored by a semiquantitative method evaluating the intensity and incidence of positively stained endothelial cells. Since P-selectin is absent in the alveolar capillaries, this vascular region was not considered in the quantitative and qualitative assessments. The intensity of the pulmonary endothelium was graded as absent (0), mild (1), moderate (2), and intense (3); the incidence of vessels involved was graded as absent (0), less then 10% (1), 10%–50% (2), and more then 50% (3). A staining score was obtained (range 0–6) by adding both variables.

Results

Without using the retrieval technique the antigen detection in the endothelium was either poor or negative compared to positive thrombocytes. A close association between lung affection and the staining pattern of the pulmonal endothelium could not be detected.

Using the retrieval technique P-selectin positive endothelial cells were found in the pulmonary arteries, arterioles, precapillaries and postcapillary venules and veins. The endothelium of the alveolar capillaries was negative (Fig. 1). Unstained endothelium was not clearly distinguishable when these vessels contained positive thrombocyte aggregation (Fig. 4). Megakaryocytes and thrombocytes also showed intense positive staining. The mesothel of the pleura visceralis was negative.

In acute deaths (hanging, carbon monoxide and cyanide intoxication) lung vessels showed an overall occurrence of P-selectin with an intense homogeneous staining pattern. The five cases of drowning showed a slightly weaker intensity (Table 1).

Protracted deaths with pneumonia and septic shock showed an irregular distribution and weak intensity of the marker (Figs. 3–4).

The 14 cases with blunt thorax trauma could be divided into immediate deaths (n = 6), survival times of 5–30 min (n = 5) and survival times varying between 1–3 h (n = 3). All persons died due to polytrauma. In all these cases P-Selectin was strongly expressed in areas with and without erythrocyte extravasation (Fig. 2). In one case (survival time 3h) moderate infiltration of granulocytes was found subpleural and septal next to hemorrhages. In such areas the staining intensity of small vessels was weaker than in other lung segments. Despite this local phenomenon this was scored “high” because the whole slide was considered. A massive fat embolism of the lung was also found in this case.

Discussion

In accordance with the literature (McEver et al. 1989) megakaryocytes, platelets and non-activated and activated endothelium reacted P-selectin positive in our study. The intense P-selectin detection of thrombocytes without an immunhistochemical retrieval technique can be explained by the much higher concentration of selectin in this cell type (McEver et al. 1989). P-Selectin could not be detected in alveolar capillaries, which suggests that this specialised endothelium contains little or no P-selectin.

P-selectin is translocated from secretory granules of endothelial cells (Bonfanti et al. 1989, Geng et al. 1990) to the cell membrane within seconds after stimulation by thrombin and histamine and thus leads to an adhesion of leucocytes, which is terminated after approximately 1 h (Hattori et al. 1989). Unfortunately, light microscopical examination does not show, whether the P-selectin is located on the plasma membrane or in the cytoplasmatic granules.

The transient expression by the endothelium (Lorant et al. 1991; Hattori et al. 1989) is a mechanism for temporarily regulating leucocyte-endothelium adhesive interactions. The factors which control the reinternalisation of p-selectin in the endothelium are unclear. The question also arises why P-selectin was clearly decreased in the endothelium of inflammatory lung tissue. Two possibilities exist:

1. P-selectin is metabolised after reinternalisation.
2. There exists powerful enzymatic cleavage of P-selectin on the cell membrane.

The second theory is supported by previous investigations. Circulating sP-selectin which lacks a transmembrane domain is either another form (Gearing et al. 1993) or shearing products of the cell membrane associated P-selectin. In accordance with serum level increases in pneumonia (Sakamaki et al. 1995) we found P-selectin weakly positive in a respective group. A physiological enzymatic cleavage is verified for the other two cell-membrane-associated selectins (Kishimoto et al. 1989; Newman et al. 1993).

Fig. 1 Hanging, P-selectin positive veins and arteries, focal acute emphysema. Positive thrombocyte aggregation in an artery (APAAP, original magnification 40 ×)

Fig. 2 Polytrauma and lung contusion, survival time a few minutes. Massive erytrocyte extravasates. Intense P-selectin positive stained endothelium (APAAP, orig. magnification 100 ×)

Fig. 3 Fatal aspiration pneumonia, state one day after an intoxication, leucocytic bronchitis (above), P-selectin impoverished swollen endothelium in the associated vessels (APAAP orig. magnification 160 ×)

Fig. 4 Septic shock, beginning pneumonia. Massive damage of the peripheral circulation, capillaries closed with thromobyte aggregations, interalveolar haemorrhages. Weak P-selectin immunostaining of the swollen endothelium (APAAP, orig. magnification 200 ×)

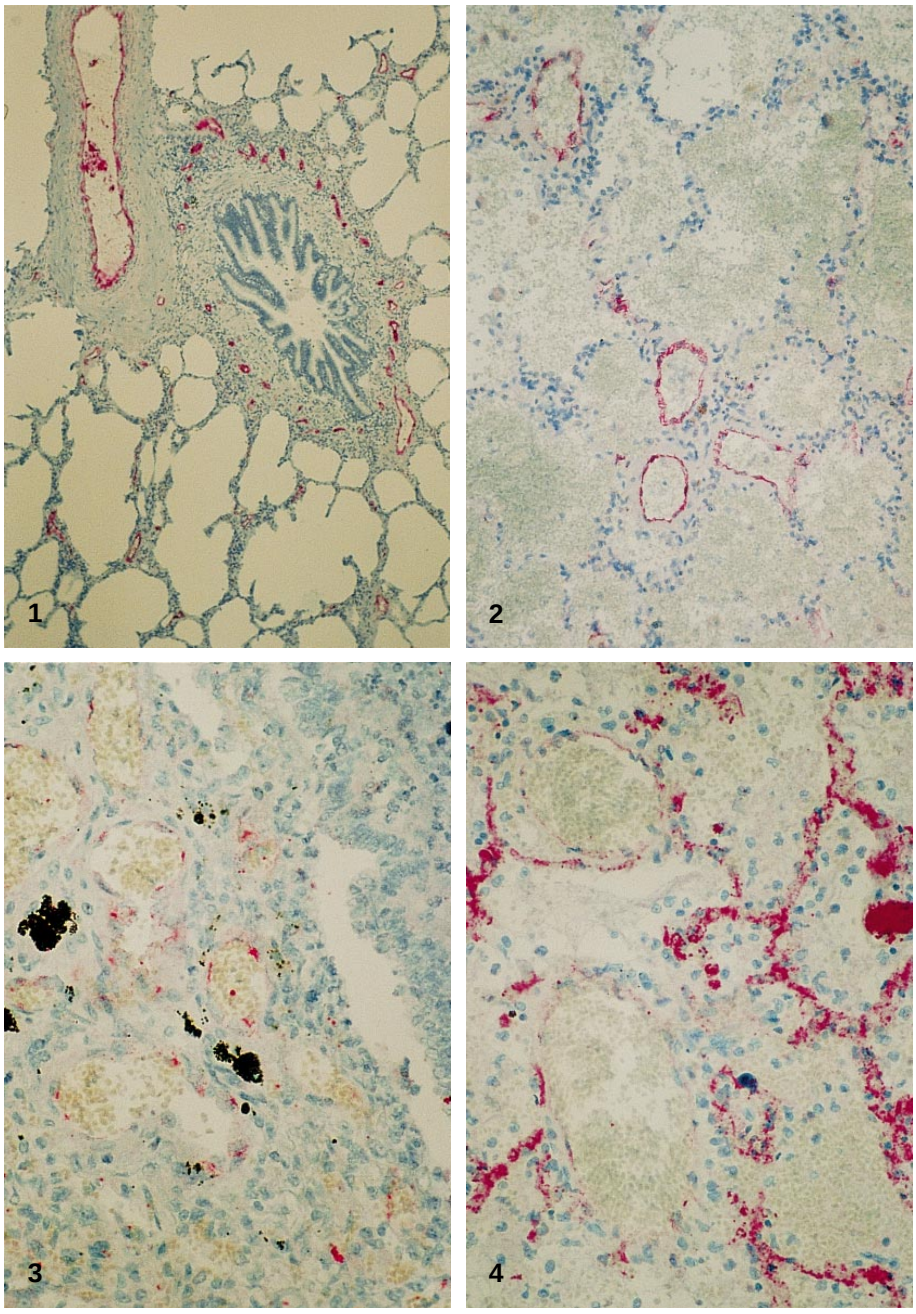

Table 1

Cause of death	Number (n = 52)	Mean value of *P*-selectin grading	Standard deviation
Carbon monoxide/ cyanide intoxication	n = 11	4.7	0.7
Polytrauma	n = 14	4.7	0.6
Hanging	n = 9	4.5	0.7
Drowning	n = 5	4.0	0.3
Pneumonia/septic shock	n = 13	2.4	1.1

Also, reinternalisation mechanisms of the endothelium may be impaired by inflammation agonists. Experimentally, it was shown that endothelium activation by oxidising radicals and peroxides results in a prolonged surface expression for several hours (Patel et al. 1991). Extravascular activated granulocytes which produce these radicals, would be able to recruit other circulating granulocytes by activation of the endothelium. This mechanism, which is initially a promotor of the healing process but later might impair the healing process, could only be terminmated by the splitting of P-selectin.

Thus, the irregular distribution of endothelial P-selectin found in pneumonia probably reflects different stages of the inflammatory process. In cases with lung contusion our results suggest that a visible loss of P-se-

lectin occurs a few hours after injury. Our study would therefore suggest that irregular distributions of P-selectin in association with hemorrhages, inflammatory infiltration and leucocyte sticking can be an important marker to establish vital origin and time dependence. Further studies and inclusion of other markers are needed to establish time dependence with forensic significance.

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