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Pulmonary myelomonocyte subtypes in drowning and other causes of death

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Abstract Three immunohistochemically different myelomonocytic subtypes, i. e. MRP8, MRP14 and 27E10 were quantitatively evaluated in the intraalveolar, alveolar-interstitial and alveolar-intracapillary lung compartments. Lung sections from 5 major groups with defined causes of death, i. e. *d*rowning and death during *i*mmersion (DI), *c*erebral/intracranial *h*aemorrhages (CH), *s*udden *c*ardiac *d*eaths (SCD), *h*anging and *t*hrottling (HT) and *i*mmediate *t*rauma *d*eaths (ITD) were stained and the positive cells counted. The results show clear differences of the cell numbers on average. Among the different compartments the intracapillary cell count exhibits the highest numbers. If the cell counts are compared to the different causes of death, DI shows the highest values and ITD the lowest. The individual values, however, show considerable variations in all compartments and especially in the low cell count range. Within the DI group two subgroups can be differentiated, one having low and the other one having high cell numbers. This can be due to the type of agony, i.e. drowning versus immersion/hydrocution, or to resuscitation attempts or to a combination of both factors.

Key words Macrophage subtypes · Lung compartments · Drowning · Immunohistochemistry

Introduction

The diagnosis of drowning mainly focuses on the lungs where external appearance is classical. Depending on the salt content, two entities have been distinguished, i.e. the "emphysema aquosum" in fresh water drowning and the "oedema aquosum" in salt water drowning. Paltauf's diffuse haemorrhages is also a well known criterion for the diagnosis of freshwater drowning. Swelling, formation of intracytoplasmatic vesicles and desquamation of the pneumocytes and endothelia, hemolysis and perivascular and peribronchial oedema are typical for freshwater drowning but can only assist where autolysis is not apparent (Reh 1970, Mueller 1975; Brinkmann et al. 1983). Discontinuity of parenchymatous damage and capillary anaemia or phagocytotic activity of intraalveolar macrophages can increase the specificity of these changes (Brinkmann and Butenuth 1982; Püschel et al. 1982; Fechner et al. 1983). The length of the agony period in rapid or slow drowning seems to be associated with quantitative differences only (Janssen 1984). Death in the water can be further subdivided into "classical" drowning and so-called hydrocution where the latter is often associated with cardiac reflex mechanisms.

In a recent study, increased numbers of 27E10 and 25F9 positive macrophages were described in the intravascular and interstitial compartment in cases of protracted asphyxia (Du Chesne et al. 1996). Macrophage transformation into polynuclear giant cells as described by Janssen (1984) and Reh (1969) is a contentious issue (Betz et al. 1993; Grellner et al. 1996). Betz et al. (1994) demonstrated that alveolar macrophages including polynuclear giant cells showed very similar frequencies in fatal asphyxia (strangulation, throttling) and in control cases (severe head injury).

Immunohistochemical characterisation of myelomonocytes in different compartments of the lungs (alveolar spaces, interstitium, alveolar capillaries) has not yet been reported. Drowning can take a longer course than other asphyxial deaths and is also combined with distinct osmotic and hydropic changes (Brinkmann and Butenuth 1982; Püschel et al. 1982). Therefore, drowning may have effects on the myelomonocytes, which could be used for differential diagnosis. We used a panel of antibodies to differentiate myelomonocytes and to evaluate quantitative differences between different causes of death.

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Table 1 Drowning cases in the order of decreasing MRP8, MRP14 and 27E10 positive cell numbers in the alveolar and interstitial compartment. BAC – Blood alcohol concentration

Case	Sex	Age years	Location/circumstances	Time in water	Resuscitation	MRP8+MRP14 +27E10 Σ alv. + int.
1	M	12	swimming pool /diving activities with hyperventilation	< 20 min	+	40
2	M	34	swimming pool/abundant food in the stomach	< 30 min	+	15
3	M	2	pond	< 1 h	+	13
4	M	36	canal/psychosis, handcuffs	< 2 h	–	12
5	F	62	pond/oligophrenia	< 1.5 h	–	8
6	M	6	swimming pool/unilateral otitis media	< 20 min	+	7
7	M	46	bathtub/BAC 2.29%	< 1 d	–	7
8	M	74	bathtub/organic psychosis, moderate angiocardiopathy	< 2 h	–	6
9	F	63	swimming pool/moderate angiocardiopathy	< 30 min	+	6
10	M	57	bathtub/suicidal intoxication (valium)	< 1.5 h	–	5
11	M	53	ditch/BAC 3.03%	< 1.5 h	–	5
12	M	36	river/BAC 4.11%	< 2 d	–	4
13	M	57	ditch/chronic alcoholism, delirious state	< 2 h	–	4
14	M	54	pond/BAC 2.96%	< 6 h	–	3
15	M	52	ditch/BAC 3.71%	< 6 h	–	3
16	F	43	river/epilepsy	< 1 d	–	2

Materials and methods

Three peripheral lung specimens from three different lobes were fixed in 4% buffered formaline, embedded in paraffin and cut into 4 µm sections. Final diagnoses from 43 deaths were made by evaluating the scene circumstances, post-mortem, histological and toxicological findings. The post-mortem intervals were less than three days and cases with primary lung disease or advanced autolysis were excluded. An exact anamnesis as to smoking habits could not be obtained. The following subgroups were formed:

1. Drowning and immersion deaths (DI) in fresh water, $n = 16$. Individual ages 2–74 yrs, estimated agony periods up to 10 min, emphysema aquosum in all cases, individual data see Table 1.
2. Death by hanging and throttling (HT), $n = 9$. Individual ages 19–74 yrs, estimated agony periods 5 to 8 min.
3. Sudden cardiac death (SCD), $n = 6$. Individual ages 46–66 yrs, either no history of prodromata or chronic cardiac insufficiency, high degrees of stenosis in the main coronary arteries, probably physical activity at the time of death, no acute infarction (histology, gross anatomy, toxicology), diagnosis by exclusion, agony times unknown.
4. Cerebral haemorrhages (CH), $n = 5$. Individual ages 1 month–4 years, estimated survival times 30 min–2 h, subarachnoidal, subdural or cerebral haemorrhages.
5. Immediate trauma death (ITD) such as decapitation with immediate death, $n = 7$. Individual ages 25–49 yrs, survival times below 1 min.

No resuscitation attempts were made in the individual cases included in groups 2–5.

Immunohistochemical methods

The antibody reactions were performed using the avidin-biotin-complex (ABC) method (Hsu et al. 1981). Primary antibody dilutions and pretreatment: MRP8 and MRP14 1:100, 27E10 and 25F9 1: 25 and 30 min proteinase K 0.01%.

Characterisation of the antibodies

The *m*igration inhibiting factor *r*elated *p*roteins MRP8 and MRP14 are expressed intracellularly in granulocytes and monocytes. In stimulated cultures of monocytes, the expression of MRP14 increases after several hours (Odnik et al. 1987; Hessian et al. 1993, Roth and Sorg 1992) and reaches a maximum after 3 days (Zwadlo et al. 1985).

Table 2 Mean number of cells per field. () = standard deviation

MRP8	Alveolar capillaries	Alveolar	Intertstitial
DI	14.1 (7.0)	1.8 (1.7)	1.9 (1.7)
CH	14.0 (5.8)	2.3 (0.8)	1.3 (0.4)
SCD	12.3 (8.2)	1.0 (0.8)	0.2 (0.4)
HT	5.3 (2.5)	0.7 (0.8)	0.2 (0.4)
ITD	4.8 (2.6)	0.4 (0.5)	0.0 (0.0)
MRP14	**Alveolar capillaries**	**Alveolar**	**Intertstitial**
DI	14.2 (6.1)	2.2 (3.2)	2.0 (1.5)
CH	10.2 (3.5)	1.5 (0.5)	0.5 (0.5)
SCD	9.0 (2.7)	0.8 (0.4)	0.0 (0.0)
HT	9.6 (3.7)	1.1 (0.9)	0.6 (0.8)
ITD	3.1 (2.0)	0.2 (0.4)	0.0 (0.0)
27E10	**Alveolar capillaries**	**Alveolar**	**Intertstitial**
DI	3.9 (6.0)	0.5 (1.0)	0.4 (1.0)
CH	4.5 (3.9)	0.5 (0.5)	0.0 (0.0)
SCD	4.8 (4.5)	0.2 (0.4)	0.0 (0.0)
HT	3.0 (3.3)	0.1 (0.3)	0.0 (0.0)
ITD	2.6 (4.3)	0.3 (0.7)	0.0 (0.0)

– The 27E10 antigen is produced by the heterodimer formation of MRP8 and MRP14 and the expression in stimulated monocyte cell lines exhibits a maximum after 2–3 days (Bhardwei et al. 1992; Zwadlo et al. 1986). 27E10 is absent in resident mononuclear phagocytes in healthy tissues.

– The 25F9 antigen is not present on freshly isolated blood monocytes but reacts with mature macrophages in various tissues; the expression in stimulated monocyte cell lines exhibits a maximum after 7–8 days (Zwadlo et al. 1985).

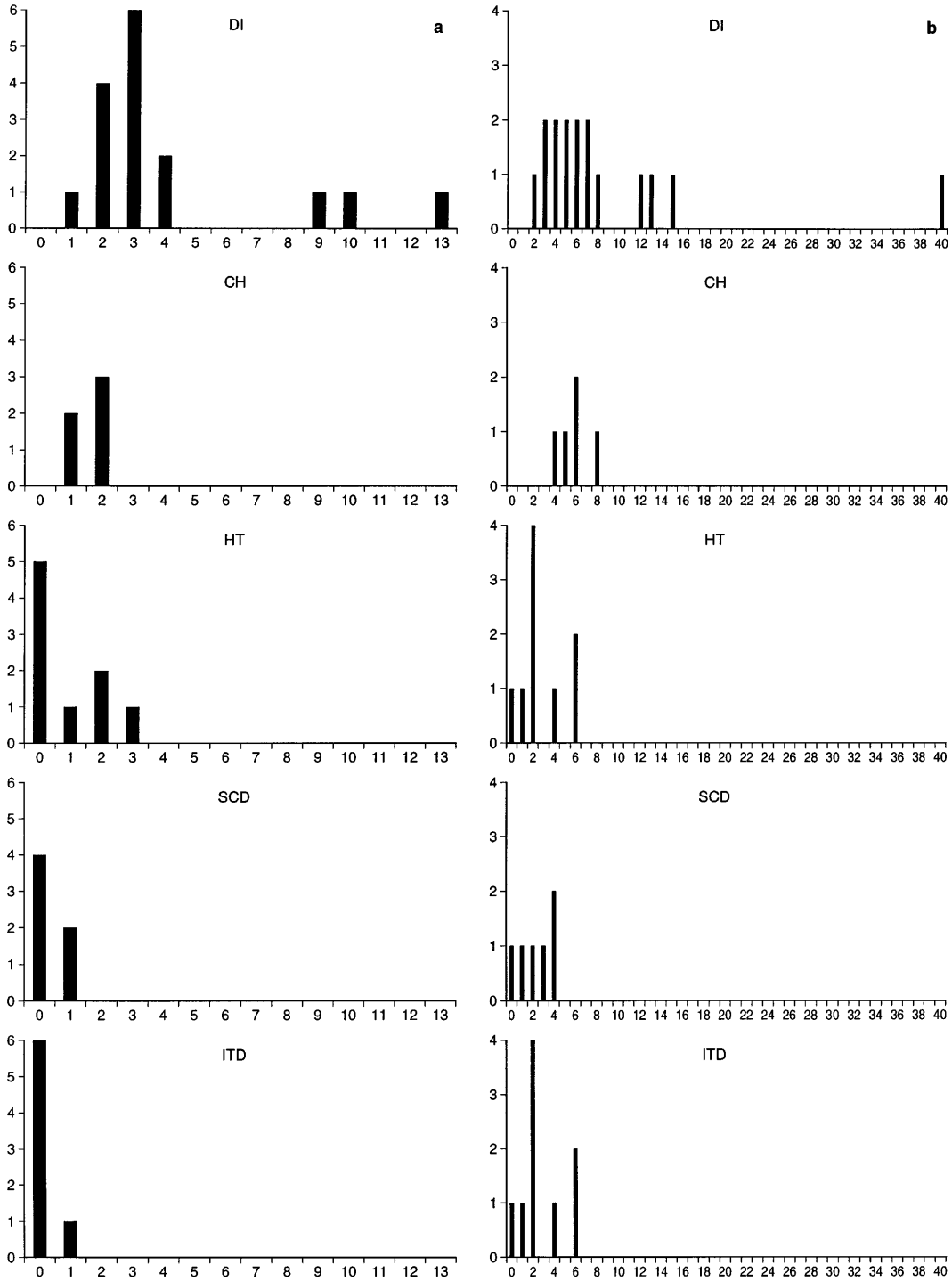

Fig. 1 **a** Sum values of MRP8, MRP14 and 27E10 positive cells in the interstitium. Y axis = number of cases; X axis = number of cells. **b** Sum values of MRP8, MRP14 and 27E10 positive in the alveolar and interstitial compartment. Y axis = number of cases; X axis = number of cells

Analysis of immunostaining

Twelve visual fields per section in 400 × magnification were randomly selected and all positively stained cells were counted. Only fields without bronchi and their arteries were considered, i.e. mainly the parenchyma. Three compartments (intraalveolar, alveolar capillaries, interstitial compartment) were evaluated separately. The results are given for all positive cells since the stained myelomonocytes could not always reliably be differentiated. A detailed evaluation of 25F9 was not performed because with this antibody, only alveolar macrophages and very few interstitial macrophages were stained. These cells may represent resident phagocytes.

Results and discussion

ITD showed the lowest cell counts while DI showed the highest in all 3 compartments and with all cell subtypes used (Table 2). The remaining groups have an intermediate position with CH close to DI (Table 2). For the *intracapil-*

lary compartment, the highest cell count triples (MRP8) or quadruples (MRP14) the lowest while 27E10 shows a 80% increase only (Table 2). The *alveolar* cell counts are associated with similar differences while the *alveolar interstitum* exhibits calculable numbers in DI and CH only (Table 2).

The results of the cell counts in the *intracapillary* compartment surpasses the values in the *interstitial* and *alveolar* compartments by approximately one order of magnitude if the same markers are compared (Table 2). Consequently, the establishment of sum values including all three compartments would level differences between the causes of death if they exist on the alveolar or interstitial level. Therefore, the *interstitial* cell counts of all three markers were evaluated in isolation (Fig. 1a) and in combination with *alveolar* cell numbers (Fig. 1b). Five deaths due to drowning show interstitial cell counts above 4 (Fig. 1a), which exceeds the cell counts in all other causes of death. If interstitial and alveolar cells are counted in combination, 4 deaths exceed the number of 11 cells while all cases except for one due to other causes of death are below 7 (Fig. 1b). The DI subgroup appears to be heterogeneous. A few cases show high cell counts but in the majority of cases intermediate numbers of cells are counted. This heterogeneity is also revealed by a comparison of the standard deviations, which are by far the highest in DI (Table 2). Therefore, the DI group was re-evaluated with regard to associated factors (Table 1). Low cell numbers were associated with high blood alcohol levels and with manifest cerebral diseases. High cell numbers showed an association with resuscitation attempts but it remains uncertain whether the form and the site of DI has an effect on whether resuscitation was attempted or not.

If the markers used are correlated with the different causes of death, MRP14 seems to most rapidly appear since only ITD show low cell numbers (Table 2). MRP8 already shows 2 or 3 and 27E10 shows 3 or 4 unaffected subgroups (Table 2). This is corroborated by the characterisation of these markers as either early stage or delayed stage markers (Zwadlo et al. 1985, 1986; Odnik et al. 1987; Roth and Sorg 1992).

While the ranking of the causes of death relative to the cell counts follows a time-dependent order from ITD (agony period < 1 min.) to hanging (approx. 5 min.), SCD (minutes to half an hour) and CH (a few hours), the top-ranked DI (5–10 min) does not follow this chronological order. This would lead to the assumption that local trigger mechanisms due to chemotactic agents play a more important role in DI than in CH. In both groups hypoxia or acidosis, catecholamine reactions and interstitial oedema could be factors which modulate the stimulated cell accumulation (Brinkmann et al. 1981). Together with a longer agony time, these factors could also explain the increase of cells in all compartments of the subgroup DI plus resuscitation. Other factors such as an increase of the pulmonary arterial and capillary wedge pressure (Madert et al. 1982), osmotic cytotoxic effects and disintegration of membranes with pathological connections to other lung compartments (Brinkmann et al. 1982; Fechner et al. 1983) could also increase the cell count in DI. Although all cases of DI showed emphysema aquosum, the differentiation of a short and a longer agony period is possible. This would greatly influence the release of local trigger mechanisms as well as water-induced post-osmotic destruction.

If this distinction between the two subgroups in DI is maintained in a larger number of cases, the association either to the mode of DI (drowning/immersion) or to resuscitation attempts will probably be more obvious. Also, if the high cell numbers found in 5 and 4 DI cases respectively are substantiated by a larger series, this could become a new marker for vital origin of water aspiration.

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