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Subarachnoid Haemorrhage Induced Proliferation of Leptomeningeal Cells and Deposition of Extracellular Matrices in the Arachnoid Granulations and Subarachnoid Space

Immunhistochemical Study

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Summary

Subarachnoid haemorrhage (SAH) often leads to subarachnoid fibrosis and resultant normal pressure hydrocephalus; however, how subarachnoid fibrosis occurs is unknown. We examined the changes within arachnoid granulations (AGs) and the subarachnoid space (SAS) chronologically at the parasagittal region obtained from patients with SAH at autopsy and made comparison with controls by immunostaining for cytokeratin, specific marker for leptomeningeal cells and by the elastica Masson-Goldner methods. Within a week some AGs were torn, and many inflammatory cells filled the AGs and SAS. Cytokeratin positive cells were scarce. During the next two weeks cytokeratin positive cells increased. After three weeks, AGs and SAS were filled by dense deposits of extracellular matrices surrounded by multiple layers of leptomeningeal cells.

Keywords: Subarachnoid haemorrhage; leptomeningeal cells; arachnoid granulations.

Introduction

It is well known that hydrocephalus occurs a few weeks to months after subarachnoid haemorrhage in some cases. It has been reported that normal pressure hydrocephalus is induced after subarachnoid fibrosis, which was revealed by histopathological studies [2, 9]. But no one has elucidated how subarachnoid fibrosis occurs. There are few studies on which cell types or which kinds of fibres participate in subarachnoid fibrosis. Watanabe et al. reported that the leptomeningeal (LM) cells proliferated in the subarachnoid space (SAS) after subarachnoid haemorrhage [10]. Histological examination showed the accumulation of collagen during fibrosis and the LM cell seems to be one of the cells which produces collagen [7]. LM cells have many functions, that is, proliferation and production of extracellular matrix (ECM) such as type I, III, and IV collagen in response to infection, trauma, or neoplastic infiltration [7], phagocytic activity [2], influence on the growth of medulloblastoma [6], secretion of trophic factors for embryonic neurons [4] and neuroblasts [1], structural support of the central

Figs. 1 a, 2 a, 3 a, and 4 a are immunostainings for cytokeratin. Figs. 1 b, 2 b, 3 b, and 4 b are elastica Masson-Goldner method. *D* means dura mater. Scale bar is 100 μm. Fig. 1. (a) In control group arachnoid granulations (AGs) were recognized at the parasagittal region (arrow head). Cells in and lining the granulations were immunoreactive for cytokeratin. (b) Loose collagen fibres were recognized in AGs (arrow). Erythrocytes and inflammatory cells were not present in AGs

Fig. 2. (a) In group I AGs stained less deeply than those in controls (arrow head). Cytokeratin positive cells were not so frequently recognized. (b) Many erythrocytes and inflammatory cells filled AGs. Some AGs were torn and inflammatory cells leaked out (arrow). Fibrous tissue did not increase

Fig. 3. (a) In group II AGs were distended and looked round compared with control specimens (arrow head). Torn AGs were not recognized. Many cyotkeratin positive cells were present in AGs. (b) Collagen fibres slightly increased (arrow)

Fig. 4. (a) In group III AGs were also distended and looked round compared with control specimens (arrow head). Cytokeratin positive cells in AGs were present but not so obviously as compared with those in group II. (b) Dense collagen fibres were recognized in the subarachnoid space and AGs (arrows)

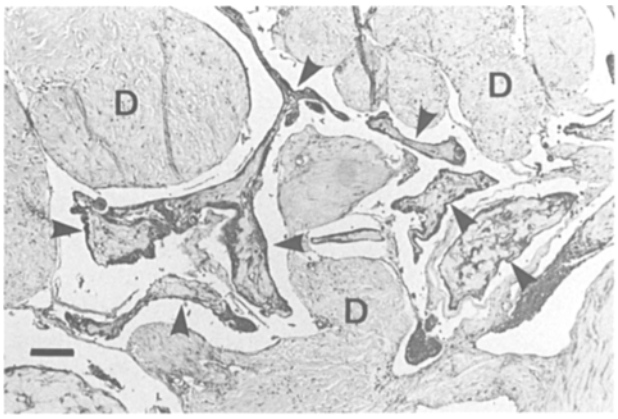

Fig. 1 a

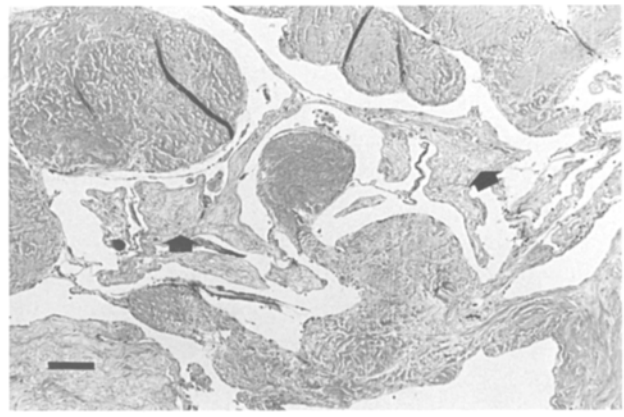

Fig. 1 b

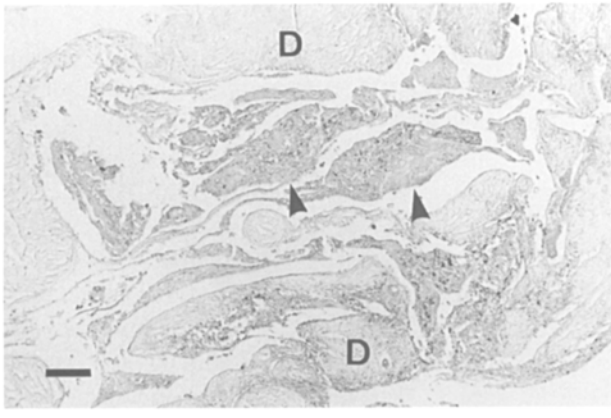

Fig. 2 a

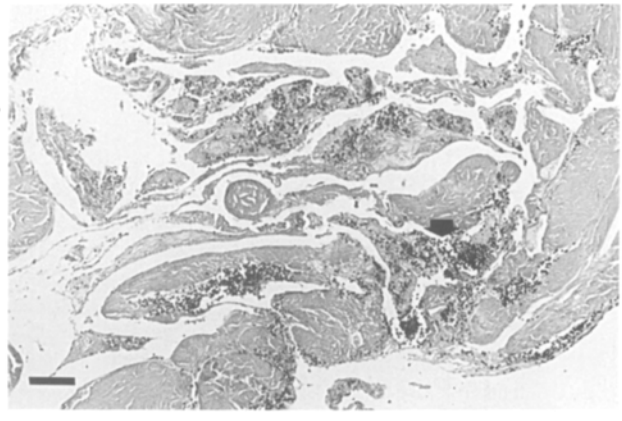

Fig. 2 b

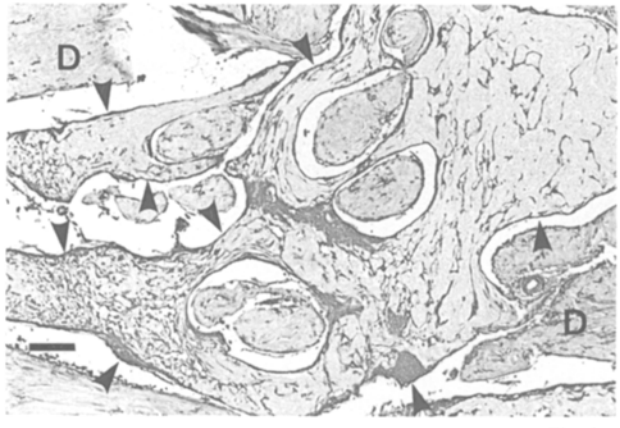

Fig. 3 a

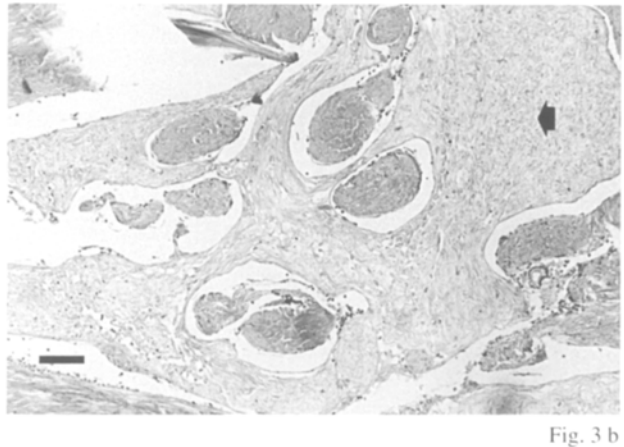

Fig. 3 b

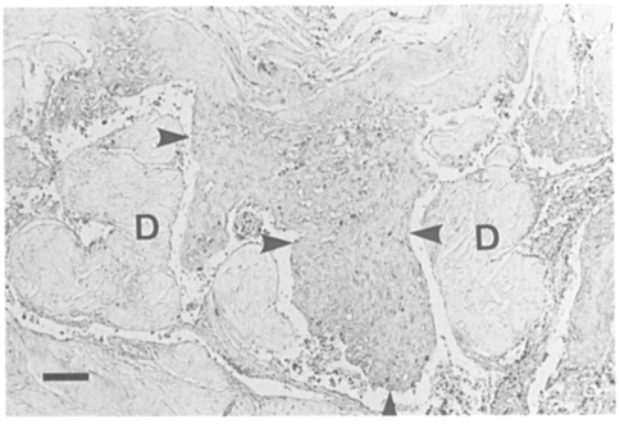

Fig. 4 a

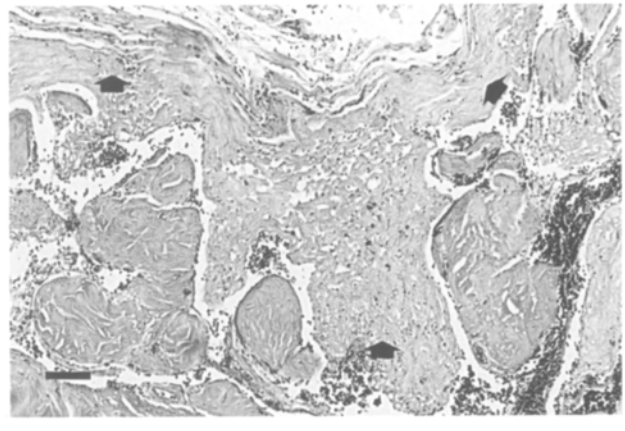

Fig. 4 b

nervous system [7], and the circulation of cerebrospinal fluid [5]. These data led us to the hypothesis that leptomeningeal cells, which are broadly recognized in the subarachnoid space, proliferate and produce extracellular matrices so that finally hydrocephalus occurs following subarachnoid fibrosis. To verify this hypothesis we examined the leptomeninges of the parasagittal region, especially the arachnoid granulation and SAS, obtained at autopsy from patients with subarachnoid haemorrhage and other diseases but no intracranial haemorrhagic lesions by immunohistostaining for cytokeratin, specific marker for leptomeningeal cells, and by the elastica Masson-Goldner method.

Materials and Methods

Leptomeninges of the parasagittal region were obtained at autopsy from patients with subarachnoid haemorrhage (12 cases) and other diseases, as shown in Table 1 (3 cases). We divided the patients with subarachnoid haemorrhage into three groups according to the time interval from the onset of SAH to death, as follows: group I 0–7 days (7 cases), group II 8–21 days (2 cases), and group III 22-days (3 cases). X-ray computer tomography (X-CT) showed hydrocephalus in 2 cases. The tissue specimens were fixed in 10% formaldehyde and embedded in paraffin. Serial sections were made and stained by the elastica Masson-Goldner method.

Immunohistostaining for cytokeratin was also performed by the avidin-biotin peroxidase complex method. The sections were deparaffinized and hydrated. To inhibit the internal peroxidase activity the sections were put into methanol containig 0.3% hydrogen peroxidase for half an hour. Predigestion was not performed. Mouse monoclonal anit-human cytokeratin antibody (DAKO A/S Glostrup, Denmark) was used at a 1 : 50 dilution and incubated overnight in the refrigerator. The second antibody, biotinized anti-mouce antibody (Vector Laboratories, Inc., Burlingame, USA), was used at a 1 : 100 dilution and incubated for one and a half hours at room temperature.

Results

Representative aspects of each group are shown. In the control group arachnoid granulations (AGs) were recognized in the parasagittal region (Fig. 1 a). Cells in and lining the arachnoid granulations were immunoreactive for cytokeratin (Fig. 1 a). Loose collagen fibres were recognized in AGs in elastica Masson-Goldner preparations (Fig. 1 b). Erythrocytes and inflammatory cells were not present in AGs (Fig. 1 b). In group I cytokeratin positive cells and fibrous tissue were rarely encountered (Fig. 2 a, b). Many erythrocytes and inflammatory cells filled AGs (Fig. 2 b). Some AGs were torn (Fig. 2 b). In group II AGs were distended and looked round compared to control specimens (Fig. 3 a). Torn AGs were not recognized. Many cytokeratin positive cells were present in AGs (Fig. 3 a). Collagen fibres increased (Fig. 3 b). In group III AGs were also distended and looked round compared to control specimens (Fig. 4 a). Cytokeratin positive cells in AGs were present but not so frequently when compared with those in group II (Fig. 4 a). Dense collagen fibres were recognized in the subarachnoid space and AGs (Fig. 4 b).

Discussion

Our evidence is thought compatible with our hypothesis, namely that LM cells proliferate after SAH and finally dense collagen deposition occurs in AGs and SAS. From the viewpoint of the healing process we consider the events leading to subarachnoid fibrosis to happen as follows. First of all, rupture and so destruction of AGs occurs, possibly as the result of physical pressure from the SAH and from chemical effects of digestive enzymes released from inflammatory cells. The inflammatory process begins and various chemical factors are released into the central nervous system. In fact, cerebrospinal fluid of patients with SAH contains some chemical factors [3, 8]. LM cells, which might be stimulated by these chemical factors, proliferate [7] to repair the AGs and get rid of some other agents [2], such as degenerative products of SAH. Then, proliferated LM cells produce extracellular matrices [7] and finally cause obstruction to the circulation of cerebrospinal fluid and suppression of its production.

References

1. Gensburger C, Labourdette G, Sensenbrenner M (1986) Influence of meningeal cells on the proliferation and maturation of rat neuroblasts in culture. Exp Brain Res 63: 321–330
2. Hammes EMJ (1944) Reaction of the meninges to blood. Arch Neurol Psychiat 52: 505–514
3. Kitazawa K, Tada T (1994) Elevation of transforming growth factor-b1 level in cerebrospinal fluid of patients with communicating hydrocephalus after subarachnoid hemorrhage. Stroke 25: 1400–1404
4. Matthiessen HP, Schmalenbach C, Muller HW (1991) Identification of meningeal cell released neurite promoting activities for embryonic hippocampal neurons. J Neurochem 56: 759–768
5. Murphy M, Chen J-N, George DL (1991) Establishment and characterization of a human leptomeningeal cell line. J Neurosci Res 30: 475–483
6. Rutka JT, Dougherty DV, Giblin JR, Edwards MB, McCulloch JR, Rosenblum ML (1987) Growth of a medulloblastoma on normal leptomeningeal cells in culture: interaction of tumor cells and normal cells. Neurosurgery 21: 872–878

7. Rutka JT, Giblin JR, Dougherty DV, McCulloch JR, DeArmond SJ, Rosenblum ML (1986) An ultrastructural and immunocytochemical analysis of leptomeningeal and meningioma cultures. J Neuropathol Exp Neurol 45: 285–303
8. Suzuki M, Ogawa A, Sakurai Y, Nishino A, Uenohara K, Mizoi K, Yoshimoto T (1992) Thrombin activity in cerebrospinal fluid after subarachnoid hemorrhage. Stroke, Lett 23: 1181–1182
9. Vessal K, Sperber EE, James AEJ (1974) Chronic communication hydrocephalus with normal CSF pressures: a cisternographic-pathologic correlation. Ann Radiol 17: 785–793
10. Watanabe K, Kowada M (1975) Long-term observation of arachnoid villus after subarachnoid hemorrhage–an electron microscopic study in dog. No To Shinkei 27: 491–498

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Comment

This is a well written, well illustrated very concise article.

The methods used are straight forward and the results are convincing.

The results are very much in line with the expectations of the authors (and the expectations of most other neurosurgeons) and lack therefore the sense of surprise, that is felt when something really new and unexpected is encountered.

This is, however, no reason to discard the results as unimportant, since the confirmation of a plausible hypothesis by way of carefully performed post mortem histological examinations, as the authors did, is very valuable.

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