Ultrastructure of Collagen Fibers in the Outer Membrane of Recurrent Chronic Subdural Hematoma

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Abstract

The three-dimensional structure of the collagen fibers in the outer membrane of recurrent chronic subdural hematoma was studied by scanning electron microscopy (SEM). Specimens obtained at surgery were treated with NaOH at room temperature to digest away all cellular components and expose the collagen fibers. SEM observation of the dural side of the outer membrane showed the collagen fibers were woven into a compact feltwork with a dense arrangement. The fiber bundles had a honeycomb structure framed by the collagen fibers. Observation of the hematoma side found the collagen bundles had a sparse wavy appearance. The arrangement of the collagen fibers on the dural side is different from that on the hematoma side. The thick outer membrane may be formed by granulation resulting from inflammatory reaction. Collagen fibrillar networks are not fragile, and may reinforce the outer membrane of the recurrent hematoma.

Key words: chronic subdural hematoma, collagen fiber, outer membrane, scanning electron microscopy

Introduction

Chronic subdural hematoma is one of the most common diseases in neurosurgical practice, but the pathogenesis is not well understood. Morphological studies of the hematoma membrane using transmission electron microscopy (TEM) have been reported.7,11,12,14,17) Normally, the outer membrane of the hematoma consists of loose collagen fibers and proliferation of dural border cells.2,13) Macrocipillaries in the outer membrane are important in the enlargement of hematoma.5,7,11) The endothelial cells of the macrocapillaries are abnormally permeable and fragile, and the junction between the adjacent endothelial cells is loose,14) with gap junctions and absence of the basement membrane.16,17) These endothelial gap junctions promote the enlargement of chronic subdural hematoma, because blood components such as erythrocytes and plasma leak repeatedly from the macrocapillaries through the endothelial gap junctions.12) However, TEM cannot easily demonstrate the three-dimensional structure of the outer membrane.

This study investigated the three-dimensional structure of the collagen fibers in the outer membrane of recurrent hematoma using scanning electron microscopy (SEM).

Clinical Materials and Methods

Specimens of outer membrane and dura mater were obtained from three patients, aged 20, 61, and 69 years, who underwent surgery for recurrent chronic subdural hematoma. The outer membrane was resected through the craniotomy. The thick, brownish membrane was commonly encountered after opening the dura mater. The outer membranes were all carefully resected from the central part of the hematoma, avoiding any mechanical injury to the specimens. The specimens were fixed in 2% paraformaldehyde, 2.5% glutaraldehyde, and 0.1 M cacodylate buffer (pH 7.3) for 1 day. Samples were observed by SEM using conventional methods.

Some samples were treated with aqueous sodium hydroxide at low temperature to digest all cellular elements, leaving the connective tissue fiber arrangements.10,15) Specimens were immersed in a 10% aque-
ous solution of NaOH for 5–7 days at room temperature (about 20°C), and then rinsed in distilled water for a day. The specimens were post-fixed with a 2% OsO₄ in 0.1 M cacodylate buffer (pH 7.4) for 2 hours, dehydrated by a series of graded concentrations of acetone, transferred to isoamyl acetate, and critical-point dried using liquid carbon dioxide. The dried specimens were coated with gold by ion sputtering.

The outer membranes were observed from three different directions: the dural side, transverse section, and hematoma side using a HITACHI S-800 SEM (HITACHI Co., Hitachi, Ibaraki) at an accelerating voltage of 20 kV. The direct magnification range was 1000–7000 times.

Results

I. SEM observations of whole specimens
The surface of the dural side was smooth and the collagen fibers were partially exposed (Fig. 1). The surface of the hematoma side was irregular and the covering cells possessed short microvilli on the surface. Many caveolae had invaginated the cell membrane (Fig. 2A). Some red blood cells were located in the lacunae and spilled over the hematoma cavity (Fig. 2B).

II. SEM observations of connective fibers
The bundles of collagen fibers were clearly visible by SEM. The dural side had a dense arrangement of collagen fibers and the fibers were woven into a compact network (Fig. 3). The transverse section showed the fiber bundles had a honeycomb structure, and cylindrical structures framed by the collagen fibers (Fig. 4). The collagen fibrillar networks were strong and reinforced the outer membrane. The hematoma side had collagen bundles running in all directions. The fibers were loosely interwoven compared with those of the dural side. The larger, sinuous collagen bundles consisted of small parallel fibrils under high magnification (Fig. 5).
Discussion

Our SEM study of the outer membrane of recurrent chronic subdural hematoma showed that the dural side was characterized by a dense network of collagen fibers, the inside by a honeycomb structure of fibers with cylindrical structures, and the hematoma side by a loose network of fibers.

Smooth muscle cells in the dural side of the membrane produce much collagen, which is characteristic of healing processes. The granulation process involved in the healing of subdural hematoma would account for the thick collagen tissue observed. The resultant dense network of collagen fibers provide the outer membrane with similar strength to the dura mater.

Friede and Schachenmayr described that the outer membrane resulted from the proliferation of the dural border cells. The outer membrane of the hematoma was formed within the split dural border cell layer. The dura mater has dense connective tissue and is formed mainly by the great preponderance of the fibers over the cellular and matrix components. Therefore, the dural side of the outer membrane was formed of dense collagen fibers. These structural characteristics indicated the dural side of the outer membrane of the hematoma originating from the dura mater. In contrast, the hematoma side of the outer membrane seemed to be reinforced by thick collagen tissue as a result of granulation during the healing process of the chronic subdural hematoma. SEM observations of the transverse section showed many cylindrical structures. This characteristic arrangement appeared to be collagen fibers surrounding the macrocapillaries following thickening of the outer membrane by granulation.
proliferation of the collagen fibers reinforced the macrocapillaries in the recurrent membrane.

The activation of coagulofibrolysis in chronic subdural hematoma has been studied. It is commonly accepted that local hyperfibrinolysis may be important in the development of chronic subdural hematoma. The infiltration of eosinophils into the outer membrane is also an important factor in the repair and healing process. On the hematoma side, many caveolae were seen. The caveola is the chain of communicating vesicles which transport various cellular factors. Therefore, the elements of fibrolysis apparently pass from the outer membrane to the hematoma cavity.

References


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Commentary

Takahashi and colleagues are to be congratulated on a beautiful demonstration of anatomic details of collagen formation in chronic subdural hematoma (CSDH) membranes by scanning electron microscope. This diagnosis has posed difficult questions about its pathophysiology since its recognition; this paper may help address some of these, although like most studies it raises others.

The very dense outer surface of the CSDH appears to bear a close relationship to the adjacent dura, perhaps arising from dural border cells. The penetration of this dense collagen layer by apparently tubular structures fits with the known highly vascular nature of these membranes. This study does not answer the question of how deeply these vessels penetrate to the CSDH cavity. Indeed, the dense collagen would seem almost protective of these vessels, although it is generally felt that these vessels are the source of hemorrhage and CSDH enlargement.

The method of formation and role of the inner membrane caveolae is not clarified by the manuscript. If they are the pathway for transudation and exudation of vascular components from dural vessels, it is not clear to me why they do not traverse the entire CSDH membrane to and through the outer dense layer. Given the excellent detail which the authors provide, perhaps future studies can expand on their observations.
This paper deals with the ultrastructure of the outer membrane of recurrent chronic subdural hematoma from a new aspect: the structure of collagen fibrillar networks. The excellent SEM pictures show that the frameworks of collagen fibers of the outer membrane of the hematoma side are looser than the dural side. It is reasonable that the loose structure of collagen fibers may reduce the capacity to resist the rupture of the macrocapillaries. It is hoped that the relationship between the local hyperfibrinosis and this loose structure of collagen fibers of the outer membrane will be proved at the next stage.

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