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ENHANCED ACETYLCHOLINESTERASE IN CHRONIC SUBDURAL HEMATOMAS

KAZUYA SHIRAISHI

Department of Neurosurgery, Soga Clinic

ABSTRACT

Hematomas and specimens were studied from 13 surgically-operated chronic subdural hematoma cases. Evacuated hematomas contained elevated potassium ions and slightly increased levels of Acetylcholinesterase (AChE), and the dural specimens including hematoma capsules stained positive for AChE using histochemical methods, especially in the inner membrane of the dura where the Masson Trichrome staining revealed damaged and irregular collagen fibers. This article suggests that the initial dural damage caused by trauma results in minor bleeding, which in turn causes a raised potassium ion concentration in the hemolytic fluid, which may also cause depolarization and elevated AChE in the dura.

Key Words: subdural hematoma, acetylcholinesterase, potassium ion, Masson staining, dural damage

INTRODUCTION

Etiologically, diabetic patients and/or alcoholic people are known to be apt to develop chronic subdural hematomas. Most textbooks have mentioned that they seem to have more chances of trauma than ordinary people. The dural reactions have been recognized as a sort of inflammation. However, it has already been realized among neurosurgeons that many patients have some minor head traumas, which are not so severe that they cause inflammation in the subdural space.[1)] The cause of the chronic subdural hematoma has been discussed from the stand point of the fibrinolytic system in the capsule.[1,2)] Actually, its hematoma contains a high concentration of fibrin degradation products (FDP). Within the capsule, continuous hemolytic episodes develop, and the capsule's hematoma expansion often causes brain herniation. This situation is why the irrigation operation is generally recommended to reduce the enhanced fibrinolytic cascade in the hematoma capsules. The cause of hematoma expansion should be recognized as a disorder of the fibrinolytic system. But, what is the initial disorder that causes a hematoma capsule? And, then, around the hematoma, what kind of reaction occurs during hematoma expansion after the initial capsule formation? The author has been interested in the dural reaction in this hematoma and has been investigating the dural specimens and hematoma fluid. Incidentally, the author found enhanced AChE in the hematoma and began to study the relationship between the dural reaction and hematomas with AChE in this clinical practice.

Correspondence: Kazuya Shiraishi, M.D.
Department of Neurosurgery, Soga Clinic
4-14-1 Ohyamacho, Monbetsu City, Hokkaido, 094 Japan

MATERIALS AND METHODS

Since November 1994, this clinic has treated 24 chronic subdural hematoma cases. Thirteen of these cases were used for this study, each of which had a clear traumatic history and had no associated metabolic disorders such as diabetes or liver dysfunction. All 13 cases were treated by single burr hole irrigation under local anesthesia consisting of 1% lidocaine. In all 13 cases, dural specimens and hematomas were successfully collected. The dura with capsule was excised without coagulation and fixed with 6% paraformaldehyde (pH 7.2). The hematoma was aspirated rapidly to the test tubes. Later, surgical specimens and hematomas were studied with histochemical and biochemical methods. Fixed dura and capsule were stained with H. & E., Masson-Trichrome, and AChE. AChE histochemistry was performed using the Tago[3] modification of the Karnovsky-Roots procedure.[3,4] Briefly, sections were rinsed in 0.1M maleate buffer, pH 8.0, and then incubated in a solution of 0.053 gm of acetylthiocholine iodide, 0.147 gm of sodium citrate, 0.075 gm of cupric sulfate, and 0.0164 gm of potassium ferricyanide in 1 liter of the 0.1M maleate buffer. To inhibit nonacetylcholinesterases, 0.0072 gm of ethopropazine was added to the incubation solution. After 50–60 min the incubation was stopped with a rinse in 0.1M Tris buffer, pH 7.6. The sections were then soaked in a solution of 0.008 gm of cobalt chloride in 100 ml of Tris for 10 min, rinsed again in the Tris buffer, and finally incubated for 10 min in a solution of 0.05 gm of diaminobenzidine (DAB) in 100 ml of Tris with 3.3 ml of 0.3% H_2O_2 solution added. The sections were taken through a final rinse, dehydrated and coverslipped with Permount. Using this method, normal dura did not have AChE staining.

All hematomas were analyzed by the biochemical analyzer (AU 560, Olympus corp.).

Table

No.	Age	Sex	Days until operation	Hematoma labs results			
				K^+ mEq	AChE IU/l	T-bil. mg/dl	LDH IU/l
1	78	M	40	5.8	152	3.1	450
2	64	M	29	7.1	168	5.1	262
3	72	F	45	4.4	92	4.7	305
4	81	F	48	4.7	90	4.2	250
5	58	M	20	7.5	174	4.7	272
6	77	M	29	7.2	164	3.0	305
7	74	F	34	6.9	165	2.9	208
8	78	M	30	6.5	155	3.8	211
9	68	F	44	5.5	142	5.5	307
10	62	M	20	7.8	162	5.1	406
11	81	M	13	7.9	165	5.0	372
12	74	M	12	7.4	151	3.0	354
13	73	M	42	5.9	102	4.2	330

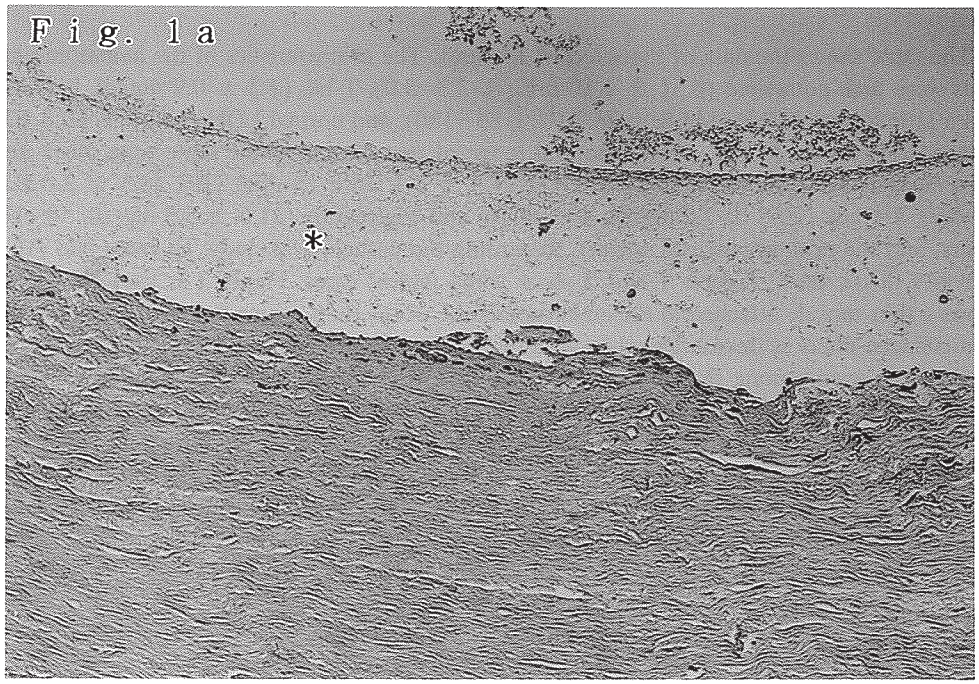
RESULTS

The table shows the data of the selected 13 cases. The characteristics of the hematoma analyses were high potassium (6.51 ± 1.10 mEq/l, 3.3–5.0), T-bil. (4.2 ± 0.9 mg/dl, 0.2–1.3), and a little higher AChE (144.8 ± 28.7 IU/l, 85–180), and normal LDH (310.1 ± 69.4 IU/l, 320–550) (average & S.D. ,N=13, normal range of this clinic). At the same time other items were measured and showed low titers; GOT (7.3 ± 3.1 IU/l, 15–40), GPT (8.5 ± 3.0 IU/l, 10–50), T-Chol. (20.8 ± 7.2 mg/dl, 130–220), and triglyceride (21.0 ± 5.5 mg/dl, 50–170) (titers, and the normal range of serum). Regarding the pathological study, all cases had AChE activities in the dura. Figures 1 and 2 show some typical cases. Figure 1 is case No.12, and shows a thin capsule attached to the inner membrane of the dura, which has folded and partially torn collagen fibers as demonstrated by Masson staining. Figure 2 is case No.4, and had the longest trauma term among the study group. The case had a thick capsule consisting of venous channels in the dura. Outside the venous channels, the inner membrane of the dura also showed corrugated collagen fibers. Both cases demonstrate AChE activity in the dura parenchyma, especially in the inner membrane. AChE activities were positive in the dura of all cases, irrespective of dural thickness or the duration of the hematoma. Control specimens (without maleate buffer incubation) showed no AChE staining.

DISCUSSION

AChE is known to develop not only in the cholinergic synapses but in the non-cholinergic systems.[5,6] It is one of non-specific enzymes in the central nervous system. In the peripheral nerves, AChE is a marker of axonal transport and with denervation its activities usually decrease.[7] The author at first thought that the AChE activities of the hematoma would decrease because of dilution by the cerebrospinal fluid and the partial denervation to the dura caused by trauma, but the data were quite different. Data shows that the fluid has a low content of hemoglobin (1.5 ± 1.1 g/dl), white blood cells (250 ± 120/mm^3), albumin (0.55 ± 0.20 g/dl), and blood sugar (65 ± 34 mg/dl) (N=20). The products related to fibrinolytic cascades, such as FDP, d,d-dimers, and bilirubins, are higher than normal serum. But incidentally, two years ago, the author found that AChE activities in the hematoma are a little higher than normal serum. Other biochemical items, such as GOT, GPT, total cholesterol and triglyceride, decreased in hematomas. In this study, the author has mentioned enhanced AChE in the hematoma and the dura, though the cause of it has not been well known. The secretion of AChE was reported to be induced by depolarizing agents[8] and, in the chronic subdural hematoma, the potassium ion is the most likely agent because the initial hematoma does develop and the hemolysis cascade is thought to continue during the hematoma expansion.[1,2] Another possibility could be cellular damage of the brain parenchyma. But the data did not show elevated LDH (one of intracellular proteins), so continuous cellular damage cannot be a plausible reason for enhanced AChE. Actually, in clinical practice, the brain parenchyma of the chronic subdural hematoma is known to have no damage except compression.[9] As Masson stainings showed, dural inner membrane had damage in collagen fibers and there seemed to be leaking blood in the dural vessels.

Not only at the beginning of the traumatic changes, but in the chronic state, localized vascular damage of the dura must have existed, after which damaged dura might have induced the production of potassium ions. The author also found cases which showed a granulation formation within the dura (data not shown). This fact indicates that the initiation of hematoma capsule can be in the dura as the result of dural damage, which could induce small granulation in the dura



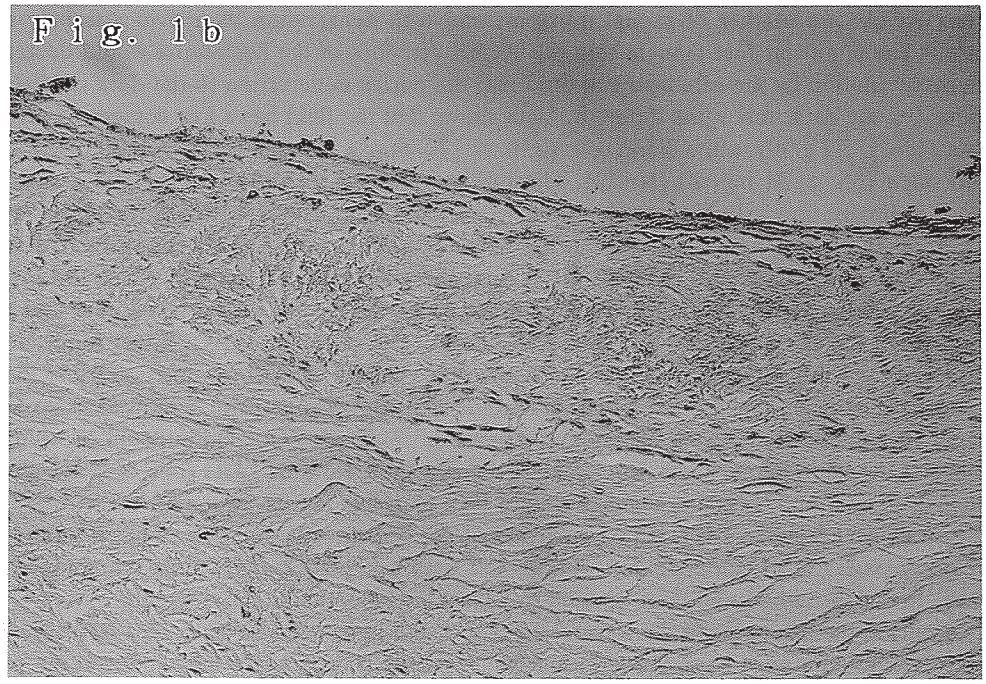
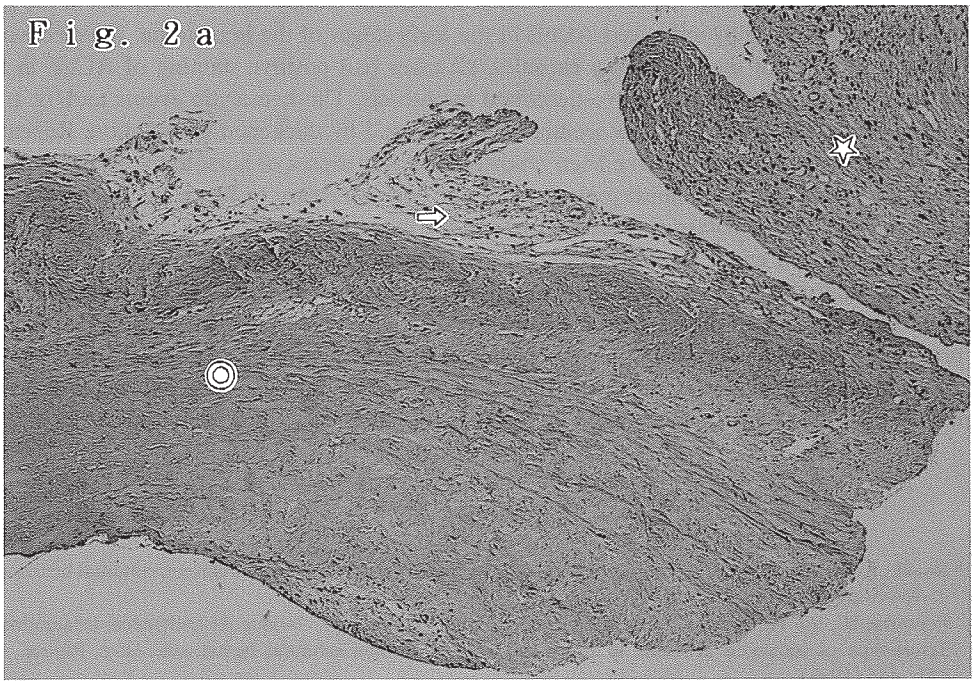


Fig. 1. Case No.12. Masson staining, X40(a), and AChE staining X40(b)
a: A thin capsule (*) attached to the inner membrane of the dura. The dura was damaged and blood leaked. b: The dura had positive staining for AChE.



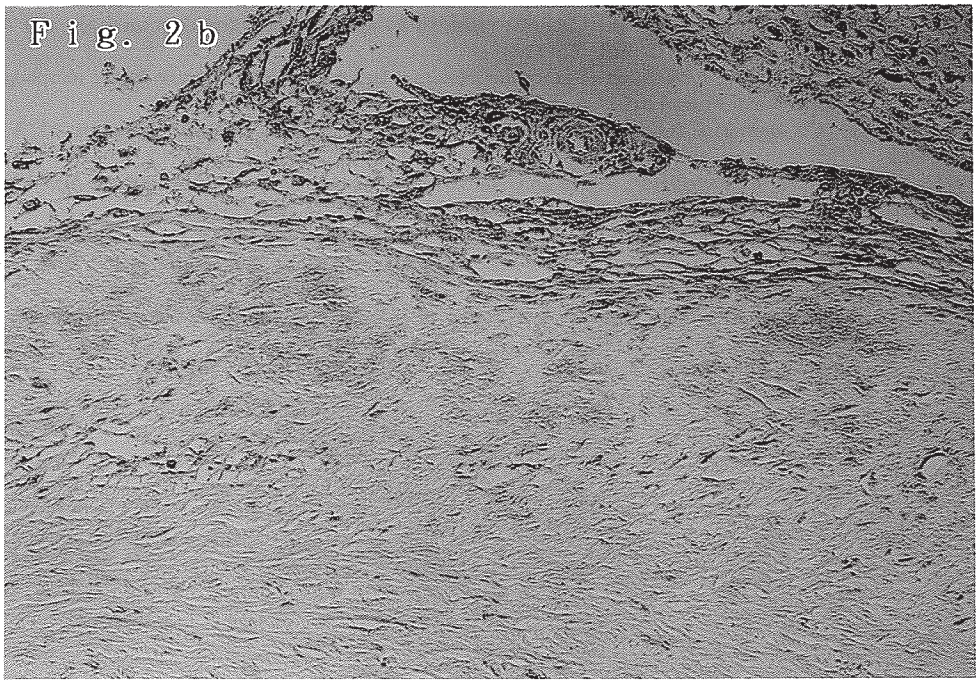


Fig. 2 Case No.4. Masson staining, X10(a), and AChE staining X40(b)
a: A thick capsule attached to the dura. Note the three layers; dura (◎), hematoma capsule (☆), and the venous cchannels (⇒). b: The dura had positive staining for AChE.

where an elevated potassium ion concentration in turn might induce hemolyitc events in the granulation, accompanying by enhanced AChE in the early stages of the hematoma. Irrespective of the size or duration of the hematoma capsules, all cases had positive staining of AChE in the dura, which would support the above-described hypothesis. The hemolytic events and/or continuous activation of potassium ions seemed to be followed by thickened granulation. Repeated hemolytic events and bleedings have been reported as major causes of hematoma expansion.[1,2)]

From the stand point of clinical practice, it is not only necessary to block the hemolytic cascade but also to inhibit potassium ion production, for example, by using the anti-diabetic agent (Sulfonylurea) which might prevent the expansion of the hematoma in order to block the initial granulation. Actually, diabetic patients suffer from more cases of this hematoma than normal people. The treatment of diabetes may be effective not only by decreasing blood sugar but also by preventing the initial granulation of chronic subdural hematomas. Controlled diabetic patients are known to have lower mortality than who are not treated.[9)] A newly developed potassium channel blocker for the dura might be the cure for chronic subdural hematomas.

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