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Characterization of local hyperfibrinolysis in chronic subdural hematomas by SDS-PAGE and immunoblot

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Fibrinogen, fibrin monomer, and D dimer were analyzed in 41 cases of chronic subdural hematoma (SDH) to characterize local rebleeding, coagulation, and fibrinolysis using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting. Chronic SDH's were divided into five groups according to their appearance on computerized tomography: high-density, isodensity, low-density, mixed-density, and layering types. The concentration of fibrinogen, which indicates rebleeding, was higher in the mixed-density (15.7 ± 3.4 mg/dl (mean ± standard error of the mean)) and layering (15.7 ± 2.6 mg/dl) types of hematoma, and lower in the low-density hematomas (1.4 ± 0.6 mg/dl) compared with the isodense hematomas (6.9 ± 1.1 mg/dl). Fibrin monomer, which indicates coagulative activity, had a distribution similar to that of fibrinogen: 87 ± 22, 18 ± 8, 175 ± 40, and 177 ± 23 μg/ml in isodense, low and mixed-density, and layering types of hematomas, respectively. The D dimer, which indicates fibrinolytic activity, was higher in the layering hematoma type (2032 ± 384 μg/ml), and lower in low-density hematomas (301 ± 164 μg/ml) compared to isodense (1310 ± 256 μg/ml) and mixed-density (1039 ± 207 μg/ml) types of hematomas.

These observations suggest the following characterization of each type of chronic SDH. The layering type is active, with a high tendency to rebleed and for hyperfibrinolytic activity. The mixed-density type has a high tendency to rebleed with lower hyperfibrinolytic activity than the layering type. The low-density hematoma is stable with a low tendency to rebleed and to fibrinolytic activity.

KEY WORDS • chronic subdural hematoma • rebleeding • coagulation • fibrinolysis • fibrin/fibrinogen degradation products • D dimer

THE etiology of chronic subdural hematomas (SDH's) is still not fully understood. Ito, *et al*.,[6] stated that local hyperfibrinolysis prevents complete hemostasis and causes rebleeding into the hematoma cavity. Local hyperfibrinolysis of chronic SDH has been characterized by high concentrations of tissue-type plasminogen activator[3,5] and plasmin-α_2-plasmin inhibitor complex.[14]

Many factors are involved in blood coagulation and fibrinolysis, causing fibrinogen to change to fibrin monomer, fibrin, and fibrin/fibrinogen degradation products (FDP's) (Fig. 1). Fibrinogen and fibrin monomer concentrations have not been reported in chronic SDH. Although FDP and D dimer have been documented in chronic SDH, no comparison has been made between fibrinogen degradation products (FgDP) and D dimer.

In this study, fibrinogen and its derivatives contained in the hematoma were quantified by means of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and immunoblotting. Rebleeding, coagulation, and fibrinolysis in chronic SDH were analyzed and related to the appearance of the hematoma on computerized tomography (CT).

Materials and Methods

Chronic Subdural Hematomas

Forty-one chronic SDH's were studied in 34 patients who ranged in age from 39 to 80 years (average 70 years). There were 27 men and seven women. The interval from the time of head injury to operation ranged from 21 to 335 days (average 77 days) in 20 cases. Histories of head injury were not well documented in the other 14 cases. None of the patients included in this study had a hemostatic disorder.

Preoperative CT examination was performed 2 to 12 hours before the hematoma sample was obtained. The chronic SDH's were classified into five types according to their appearance on CT (Fig. 2): high density (two cases); isodensity (12 cases); low density (eight cases); mixed density (nine cases); and layering (10 cases). The upper layer of the layering type exhibited low density and the lower layer high density.

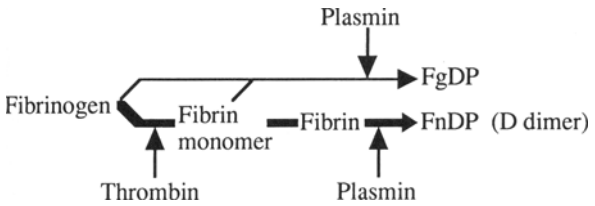

FIG. 1. Schematic diagram illustrating the conversion of fibrinogen into fibrin monomer, fibrin, fibrinogen degradation products (FgDP), and fibrin degradation products (FnDP) (D dimer). *Arrows* indicate the enzymes that mediate each reaction.

Study Methods

Details of the electrophoresis, immunoblotting techniques, and quantification of the patterns have been described previously.[13] In brief, hematoma samples were collected in a tube containing sodium citrate. After centrifugation, the supernatant was prepared for electrophoresis with 2% SDS. Polyacrylamide gel (5%) was used for electrophoresis, according to Laemmli's method.[10] After electrophoresis was performed, proteins were transferred from the gel to a nitrocellulose membrane by Western blotting.[9] Immunostaining of fibrinogen, fibrin monomer, and FDP was conducted using goat antiserum to human FDP-D and FDP-E, and peroxidase-conjugated rabbit antiserum to goat immunoglobulin G. Each band was compared with the previous description[4,11] for identification. The concentration of fibrinogen, fibrin monomer, and FDP fragments (Cx) was calculated as: $Cx = (Dx \div D_{total\ FDP}) \times C\ FDP$, where Dx was the density of the objective band on the nitrocellulose membrane measured by densitometry, Dtotal FDP the sum of the densities of the FDP fragments, and C_{FDP} the concentration quantified using the latex fixation method after fibrinogen had been removed from the sample.

The concentrations of fibrinogen, fibrin monomer, and D dimer were used as indicators of rebleeding, coagulation, and fibrinolysis, respectively (see Discussion). Data are presented as the mean ± standard error of the mean. Each value was assessed by one-way analysis of variance (ANOVA). Differences were regarded as statistically significant at $p < 0.05$. High-density hematoma types were excluded from the statistical analysis because of their rarity.

Results

Fibrinogen in Chronic SDH's

Fibrinogen, fibrin monomer, and FDP fragments were identified by Western blot analysis of the chronic SDH's using anti-FDP antibodies (Fig. 3 *left*); densities of the bands were measured and calculated as shown in Fig. 3 *right*.

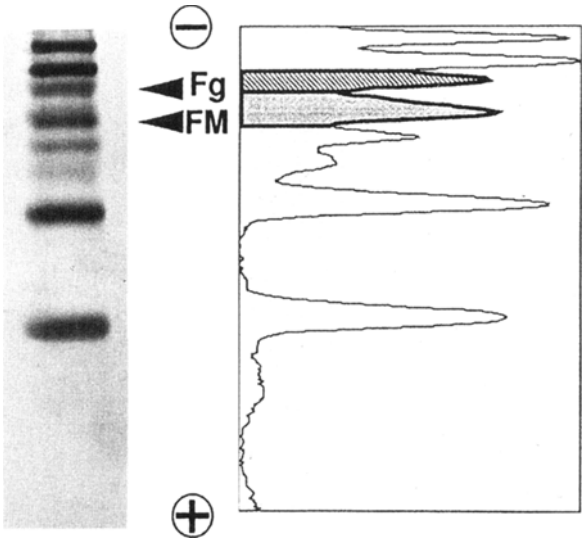

FIG. 3. Results of electrophoresis procedures to identify fibrinogen (Fg), fibrin monomer (FM), and fibrin/fibrinogen degradation products (FDP) in chronic subdural hematomas. *Left:* Western blot analysis using anti-FDP-D antibody. Bands not identified indicate FDP. *Right:* Densitometry results of the immunostaining pattern; *shading* represents the relative concentrations of fibrinogen, fibrin monomer, and fibrin/fibrinogen degradation products.

The average concentration of fibrinogen in the supernatant fluid of chronic SDH's was 9.8 ± 1.4 mg/dl (mean ± standard error of the mean), which was lower than normal values in peripheral blood (Table 1). The value in the mixed-density hematomas was significantly higher (15.7 ± 3.4 mg/dl) than that in the low-density hematomas (1.4 ± 0.6 mg/dl), and the value in the layering hematomas (15.7 ± 2.6 mg/dl) was significantly higher than that in both the isodense hematomas (6.9 ± 1.1 mg/dl) and the low-density hematomas ($p < 0.05$) (Fig. 4). These findings indicate that the recent rebleeding was extensive in mixed-density and layering hematomas and small in low-density hematomas.

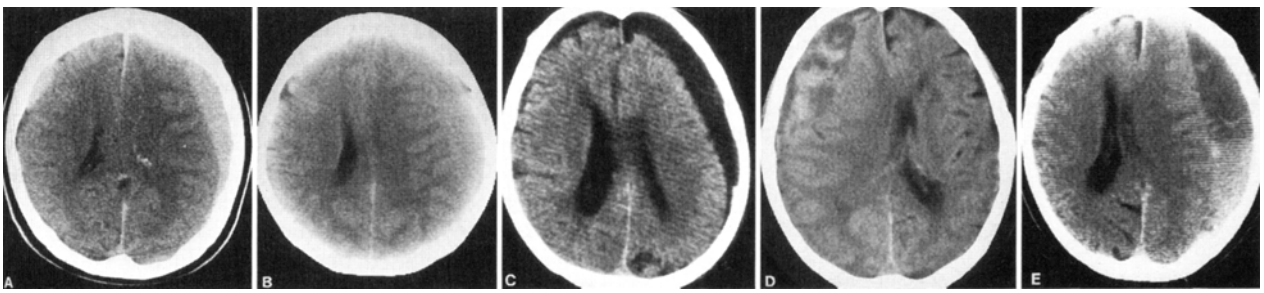

FIG. 2. Computerized tomography scans demonstrating examples of five types of chronic subdural hematoma: high-density (A), isodensity (B), low-density (C), mixed-density (D), layering (E).

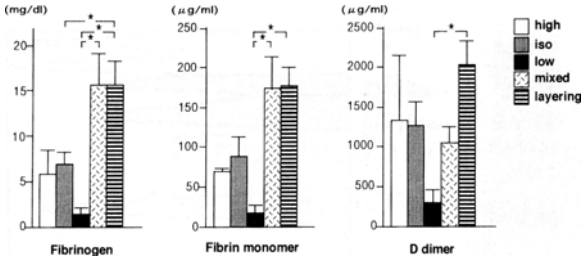

FIG. 4. Bar graph showing concentrations of fibrinogen, fibrin monomer, and D dimer in each type of chronic subdural hematoma: high-density, isodense (iso), low- and mixed-density, and layering. Each column represents the mean ± standard error of the mean. *Asterisks* indicate statistically significant differences ($p < 0.05$).

TABLE 1

*Concentrations of fibrinogen, fibrin monomer, and FDP in 41 chronic subdural hematomas**

Factor	Values in Chronic SDH†	Normal Values in Peripheral Blood
fibrinogen (mg/ml)	9.8 ± 1.4	200–300
fibrin monomer (μg/ml)	114.0 ± 16	<20
FDP (μg/ml)	1355.0 ± 173	<10
FgDP (μg/ml)	136.0 ± 17	—
D dimer (μg/ml)	1220.0 ± 159	<1

* FDP = fibrin/fibrinogen degradation product; SDH = subdural hematoma; — = not available; FgDP = fibrinogen degradation product.

† Values are mean ± standard error of the mean.

Fibrin Monomer in Chronic SDH's

The average concentration of fibrin monomer was 114 ± 16 μg/ml (Table 1), which was far beyond the upper limit of normal values in peripheral blood. As indicated in Fig. 4, the values in the mixed-density (175 ± 40 μg/ml) and layering types of hematoma (177 ± 23 μg/ml) were significantly higher than in low-density hematomas (18 ± 8 μg/ml, $p < 0.05$). The fibrin monomer value in isodense hematomas was 8 ± 22 μg/ml. These findings suggest that coagulative activity was high in mixed-density and layering types of hematomas and low in low-density hematomas.

D Dimer and FDP in Chronic SDH's

The average concentration of FDP in the 41 chronic SDH's was 1355 ± 175 μg/ml (Table 1), which was far beyond the upper limit of normal values in peripheral blood, and varied widely from 16 to 4760 μg/ml. According to the electrophoretic pattern, fragments of D dimer could be distinguished from fragments of FgDP. The average concentration of D dimer was 1220 ± 159 μg/ml, which was higher than that in peripheral blood. The D dimer value in the layering type of hematoma (2032 ± 384 μg/ml) was significantly higher than in the low-density type of hematoma (301 ± 164 μg/ml). The values in isodense (1310 ± 256 μg/ml) and mixed-density (1039 ± 207 μg/ml) hematomas tended to be lower than in the layering type of hematoma (Fig. 4). The average percentage of D dimer to total FDP was 88.1% ± 0.8%. No correlation was observed between the ratio of D dimer to FgDP and the CT appearance. These findings indicate that fibrinolytic activity was high in the layering hematoma, moderate in the isodense and mixed-density hematomas, and low in the low-density hematoma.

Discussion

Parameters of Rebleeding, Coagulation, and Fibrinolysis

The cycle of rebleeding, coagulation, and fibrinolysis has contributed to the development and maintenance of chronic SDH.[5] The use of SDS-PAGE with immunoblotting has made analysis of the details of these events possible by identifying fibrinogen and its derivatives. Three parameters were selected: fibrinogen as an indicator for recent rebleeding; fibrin monomer as an indicator for coagulative activity; and D dimer as an indicator for fibrinolytic activity. A high concentration of fibrinogen in chronic SDH's is evidence of continuous rebleeding, because fibrinogen is introduced into the cavity of the hematoma only by repeated bleeding and should disappear immediately through the action of thrombin changing it into fibrin. Fibrin monomer and D dimer, as indicators of coagulative activity[15] and fibrinolytic activity,[1] respectively, have already been accepted and used in the diagnosis of disseminated intravascular coagulation.

Appearance on CT and Electrophoretic Pattern

The relationship between the appearance of the hematoma on CT and its electrophoretic pattern was examined. Each group of CT scans was characterized in relation to rebleeding and coagulative and fibrinolytic activities.

In the layering type of chronic SDH, a large amount of recent rebleeding had occurred and coagulative and fibrinolytic activities were high. Kao[8] reported that the layering type of hematoma may be remarkable for a significant amount of rebleeding and may herald acute clinical deterioration. Saito, *et al.*,[14] stated that, in layering hematomas, the plasmin-α_2-plasmin inhibitor complex was higher than in any other type of chronic SDH. These results agree with ours. The hyperfibrinolytic activity seems to cause frequent, copious rebleeding, leading to severe signs and symptoms.

Mixed-density hematomas exhibit a large amount of recent rebleeding and high coagulative and low fibrinolytic activity. Ito, *et al.*,[7] suggested that mixed- and high-density hematomas indicate recent rebleeding, because clots or sediments obtained from the hematomas included many ^{51}Cr-labeled erythrocytes administered intravenously. The amount of recent rebleeding could be estimated from the concentration of fibrinogen. Mixed-density hematomas show much

recent rebleeding despite a lower fibrinolytic activity than in the layering type. The tendency to rebleed is determined not only by the balance between coagulation and fibrinolysis, but also by other factors, such as vulnerability of the capillaries in the outer membrane, intracranial pressure, and blood pressure. Blood clots were frequently found during surgery in the higher-density part of the mixed hematomas. Coagulation activity may be high enough relative to fibrinolysis to form fibrin in these hematomas.

In low-density hematomas, the tendency to rebleed and for coagulative and fibrinolytic activities were all moderately low. Nakamura, *et al.*,[12] reported that every chronic SDH that resolved without surgery showed low density on follow-up CT. These authors also indicated that the fibrinolytic activity of low-density hematomas decreased with regression of the thickness of the cavity. Their findings support our results.

Isodense hematomas were seen most frequently in this series of 41 chronic SDH's. The parameters for rebleeding and coagulative and fibrinolytic activities showed higher values than those of the low-density type but lower than those of the layering type. Fujioka, *et al.*,[2] reported that isodensity appeared in the later phase, following high- or low-density, and did not resolve naturally. Isodense hematomas seem to neither improve nor worsen, because rebleeding is moderate and the balance between coagulative and fibrinolytic activities is maintained.

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