Chronological Changes in Spontaneous Intracerebral Hematoma — An Experimental and Clinical Study

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SUMMARY A model of intracerebral hematoma that closely resembles the state in humans after spontaneous intracerebral hematoma was developed. Sequential changes in experimental intracerebral hematomas were compared with the in vivo findings in spontaneous intracerebral hemorrhage.

The clinical series consisted of 28 patients with spontaneous intracerebral hemorrhage observed by CT during their natural course from 1976 through 1978. The experimental series consisted of 26 adult mongrel dogs with intracerebral hematoma near the basal ganglia studied by CT and histological examinations.

In neither the clinical nor the experimental series was the time of decrease in density beginning in the periphery of the hematoma or the first appearance of ring enhancement and its concentric concentration toward the center of the hematoma affected by the size of the hematoma. In the experimental series, the tissue reaction near the periphery of the intracerebral hematoma showed constant processes: First, a necrotic layer appeared; this was then replaced by immature connective tissue with newly formed vessels and argentophil fibers, and finally the immature layer was gradually transformed into mature connective tissue with collagenous fibers. Ring enhancement was accompanied by the appearance of immature connective tissue and capillaries. This process of change was also unrelated to the size of the hematoma.

The following correlations were suggested from the chronological observation of CT images and the histological appearance: 1) acute stage — homogeneous high density extending to the periphery, appearance of the necrotic layer; 2) subacute stage — decreased density spreading from the periphery and formation of ring enhancement, appearance of immature connective tissue with argentophil fibers; 3) chronic stage — concentric concentration of ring enhancement and development of mature connective tissue with collagenous fibers.

COMPUTED TOMOGRAPHIC (CT— IMAGES of spontaneous intracerebral hemorrhage show dynamic structural changes with time after the formation of the hematoma. Therefore, CT images of the hematoma, together with histological studies, are helpful in determining the pathological mechanism of spontaneous intracerebral hemorrhage at a given chronological stage and also in determining the treatment, prognosis and differential diagnosis. However, the data available on the chronological stagings of spontaneous intracerebral hemorrhage have not often been put into clinical use, unlike those of cerebral infarction. The fact that human hematomas vary greatly in size due to considerable differences in their durations makes their classification difficult. When a hemorrhagic lesion is large, the hematoma takes much longer to turn into scars or cysts and to be absorbed. Thus, the clinical course should vary with the size of the hematoma. The chronological histologic classification varies from case to case depending on the time when the hematoma disappeared and turned into scars or cysts. CT images show the chronological changes of the hemorrhage in a living brain, which cannot be deduced from pathological analysis of a cadaver.

Therefore, in this study, we induced experimental intracerebral hematomas in dogs and made a chronological comparison of the CT images and histological findings. The data obtained in dogs were then compared with the CT images obtained in humans, and an attempt was made to classify the chronological stages of spontaneous intracerebral hematoma. The classification of sequential stages of spontaneous intracerebral hematoma based on sequential changes in CT images and the histological appearance of the periphery of the hematoma was proposed with three stages: the acute stage (within four days after hematoma formation), the subacute (days five to 14 after hematoma formation) and the chronic stage (15 days after hematoma formation).
Materials and Methods

The 28 patients with spontaneous intracerebral hemorrhage examined in this study were nonoperative subjects in whom hematomas were found in the basal ganglia. In these 28 cases, a total of 114 successive CT examinations were undertaken with EMI (1010) and ACTA (0100) scanners. Intracerebral hematomas were induced in the region near the basal ganglia in 26 adult mongrel dogs (weighing 10–15 kg). For this, a burr hole was made 4 cm behind the line of the supraorbital protuberances, 3 cm from the midline. Then a balloon was inserted 1.8 cm below the surface of the brain at an angle of 45° to the perpendicular. The balloon was gradually inflated with 3.5 ml of physiological saline and an intracerebral hematoma was induced by injecting coagulated venous blood into the brain while deflating the balloon. In the first five experimental dogs, hematomas were induced, but no high density intracerebral hematomas were observed on CT examinations. In the other 21 dogs (No. 6–No. 26), intracerebral hematomas of optimal size were induced in the target area. After perfusion-fixation, autopsies and histological examinations were carried out on 20 of the 21 dogs. A total of 31 CT examinations were successively performed on the dogs (No. 6–No. 26) with the EMI-1010 CT scanner (matrix 320x320, high-definition scan). Specimens for microscopic observation were stained with hematoxylin eosin (HE), silver stain (Bodian, Watanabe’s variation), Klüver-Barrera stain, Mallory’s azan stain and phosphotungstic acid hematoxylin (PTAH). Ultrathin sections were stained with 2% uranyl acetate and lead citrate and observed under an electron microscope (Hitachi HU-12).

Results

Observation of Ring Enhancement of Hematomas in CT Images

In human spontaneous hemorrhages of the basal ganglia, homogeneous high density areas decreased in density with time from the periphery as the hematomas contracted toward their center. Furthermore, a positive contrast enhancement image (known as a ring enhancement) was observed at the periphery of the hematomas in contrasted-CT (CE-CT) examinations performed at about the time when the density of the periphery started to decrease. The density of the periphery of the homogeneous high density area of the hematoma started to decrease 14 days or more after the hemorrhage. The earliest ring enhancement was observed on day 14. From days 14 to 20, ring enhancement was observed in 26 of 28 cases examined by the CE-CT method. No ring enhancement was seen in two cases in which the hematomas were less than 1 cm in diameter. When the hematomas were about 1 cm in diameter, no positive CE-CT image was seen after two months, whereas when they were more than 2 cm in diameter, they were. In one case, the diameter of the hematoma was 2.8 cm, and the CE-CT image was still detectable on day 200. Ring enhancement appeared on day 14 or later, and the ring gradually contacted, finally becoming a mere speck (fig. 1).

Experimentally induced high density areas of intracerebral hematomas in dogs, like those of human spontaneous intracerebral hemorrhage, decreased in density from the periphery. The experimentally induced intracerebral hematomas were 3.5 ml in size and less than 1 cm in diameter. After about four days, an abrupt decrease in the density of the periphery was observed. The earliest ring enhancement was noted on day 9. Twenty CE-CT examinations on day 9 or later were performed on nine dogs. In 19 of these examinations, made on eight of the dogs (all except dog No. 12), ring enhancement was observed. The ring also contracted with time and in case No. 16, a speck was detected in the CE-CT image on day 67 (fig. 2).

Gross Appearance of Hematomas

A 56-year-old male died of gastrointestinal bleeding 18 days after spontaneous intracerebral hemorrhage. In this case, ring enhancement was noticed 14 days after the stroke. The hematoma was surrounded by capsule-shaped tissue, which became thicker from the upper region of the hematoma through to its external region. These findings in spontaneous intracerebral hematomas are well known

Figure 3 shows a large section from the intracerebral hematoma of dog No. 13 stained with cresyl violet stain. In this case, ring enhancement was observed 12 days after induction of the hematoma, and autopsy was carried out for histological examination immediately after CT examinations. A ring of faintly stained capsule-shaped tissue had developed around the hematoma, which was located in the vicinity of the basal ganglia. These observations suggest that both the capsule-shaped tissue surrounding the hematoma of the human specimen and the ring of tissue stained with cresyl violet around the canine hematoma correspond to the region of ring enhancement seen in CE-CT observations.

Comparison of Sequential Changes in CT Image and Histological Findings of Hematomas

CT and Histological Images on Days 0 to 4

In CT images, the human spontaneous intracerebral hemorrhage on day 1 after the stroke appeared as a
FIGURE 2. The scheme of experiments in 26 dogs. Thirty-six double scans were carried out. Positive ring enhancement appeared nine days after hematoma formation in most cases.

FIGURE 3. Gross appearance of a dog's brain 12 days after formation of an intracerebral hematoma. A round, well-circumscribed hematoma stained with cresyl violet stain (Nissl's variation).

FIGURE 4. CT image in a dog 48 hours after experimental hematoma formation. The hematoma was homogeneous in density to its periphery. The lateral ventricle was deformed by compression by the high density area.

homogeneous high density area in the basal ganglia. In dogs, intracerebral hematomas in the early stage after their induction also appeared as homogeneous high density areas (fig. 4). The histological appearance of a perfusion-fixed intracerebral hematoma of a dog in which the CT image was taken 48 hours after hematoma induction is shown in fig. 5. Red blood cells had spread out to the boundary of the hematoma. Between the boundary tissue and the blood clot, there were also spaces, probably formed by fibrin, stained blue with PTAH stain, as well as plasma. In the periphery, a necrotic layer of destroyed brain tissue was seen surrounding the hematoma. This destruction was caused by continuous infiltration of red blood cells from the hematoma, secondary hemorrhage around capillary vessels, infiltration of blood plasma, etc. Outside this necrotic layer, there was what may be called a status spongiosus, a boundary layer of tissue with many intercellular spaces looking like vacuoles. This boundary layer did not have a clear border and gradually merged with the surrounding normal cerebral tissue.

CT and Histological Images on Days 5 to 14

In the CT images of human intracerebral hematoma on day 5 or later after the stroke, a homogeneous high-density region remained only in the central area of the hematoma. At the periphery, the density had decreased. When CE-CT examinations were performed on about day 14, the peripheral low density area had expanded toward the center and ring enhancement was
FIGURE 5. Histological appearance 48 hours after hematoma formation in a dog. The specimen is of the tissue surrounding the high density area of the hematoma (bottom) in a dog. A necrotic layer with infiltration of many red blood cells and secondary bleeding around the capillaries are observed in the area adjacent to the hematoma. The boundary with the normal cerebral tissue contained many small vacuoles. (HE stain × 150)

detected around the peripheral low density area. As in human intracerebral hematomas, the density of canine intracerebral hematomas started to decrease from the periphery within a few days. When the peripheral low density area had expanded and the central part was still of high density, ring enhancement was observed along the external region of the low density area (fig. 6). The histological image of the hematoma and its neighboring area nine days after the appearance of the ring enhancement is shown in figure 7-A,B. Where previously a necrotic layer had been seen, many fat cells and few connective fibers were present (fig. 7-A). These fibers stained with silver stain but not with Mallory's azan stain, and therefore seemed to be undeveloped connective fibers (fig. 7-B). Many new blood vessels surrounded by argentophili fibers were also observed.

CT and Histological Images on Day 15 and Later

Ring enhancements of human intracerebral hematoma, which appeared on about day 14, were then observed one after another. These rings gradually shrunk toward the center of the hematomas. Likewise, the ring enhancements of dog hematomas also decreased in diameter with time (fig. 8). The histological appearance on day 18, when the ring enhancement began to decrease, is shown in figure 9-A,B,C. The argentophil fiber layer of undeveloped connective fibers that was observed on day 9 had become considerably thicker (fig. 9-B). The center of this thick connective fiber layer stained deep blue with Mallory's azan stain, indicating that the connective fibers were mainly collagenous fibers (fig. 9-C). These connective fibers were observed outside many argentophil fibers, newly formed capillaries, fat granule cells and hemosiderin granule cells.

Electron Microscopic Images of Newly Formed Blood Vessels

Newly formed blood vessels in the region of ring enhancement were observed by electron microscopy. In the endothelium of newly formed capillaries, many marginal folds projected into the capillary lumen and tight junctions were shallower and more numerous than normal. The nuclei were larger than those in normal cerebral capillaries, and the ribosomes were evenly distributed in the cells. There were many pinocytotic vesicles (plasmalemma vesicles) in the cytoplasm of the endothelial cells, resembling those in the endothelium of muscle capillaries in structure and the microvacuoles in appearance (fig. 10).

Discussion

There have been few studies on the classification of chronological stages of spontaneous intracerebral hematoma. Spatz classified the histological changes in hematoma absorption into three terms: first term —
deformation and necrosis of surrounding tissues by compression of the hematoma, and peripheral edema; second term — absorption of the hematoma; third term — conversion of the hematoma to scars and cysts. He reported that the length of each term varied with the size of the hematoma and classified hematomas as large, intermediate and small. Uemura and Fukazawa classified the chronological stages of hematomas by applying corresponding CT images to Spatz's classification. Laster et al proposed a six-term classification based on density changes and the influence of steroids on the appearance of ring enhancement. In these classifications, the whole process from formation of the hematoma to its disappearance and to the stage of its turning into scars and cysts is distinguished into three or six terms on the basis of histological or CT observations. However, it is observed both clinically and histologically that the larger the hematoma in the early stage after stroke, the longer the period of hematoma absorption, and vice versa. Therefore, it seems that in Spatz's classification, the time of the disappearance of hematomas in the second term should vary according to the patient. This must also be true for the CT image; that is, the time when the high density changes to isodensity or low density and when ring enhancement appears should both vary with the size of the high density area after the stroke. For this reason, it is very likely that in Laster's classification, terms II through V vary from case to case.

Figure 7. Histological appearance of a specimen from a dog on day 9. This specimen was from the area of ring enhancement. New vascularization is seen, chiefly in the necrotic layer. Hemostatic granule cells are noted in the tissue surrounding the hematoma, but argentophil fibers are still immature and no collagenous fibers are seen. (A: HE stain x 200, B: silver stain x 200)

Figure 8. CT image in a dog on day 18, showing the intracerebral hematoma in the last stage. The area of high density has mostly changed to one of isodensity or low density. Ring enhancement shows concentric concentration.
In the experimentally induced hematomas in dogs in this study, the reactions of the tissue surrounding the hematomas were almost identical with small hematomas composed mainly of injured cerebral tissue and with hematomas of about 1 cm in diameter.

Initially, there was a necrotic layer bordering the blood clot. Then, undeveloped connective tissue composed of argentophil fibers appeared, followed by connective tissue composed of collagenous fibers. These argentophil fibers were also observed during the healing of wounds with the appearance of newly formed capillaries. The larger the damage, the longer it took for it to be repaired. Therefore, there is no doubt that the argentophil fibers and the newly formed capillaries remain for a long time. There may be a close relationship between the stage when the argentophil fibers appear and that when the ring enhancement appears.

In this study, the progressive changes in the tissues surrounding hematomas in humans were compared with those of CT images in experimental intracerebral hematomas in dogs. In the early stage of hemorrhage, the necrotic layer on the blood clot was relatively localized, and in the CT images, the periphery of the hematoma was of high density. But on day 5 and later, argentophil fibers, granule cells and newly formed capillaries gradually increased in the necrotic layer. On about day 9, the necrotic tissues were replaced by undeveloped connective tissues composed of granule cells, new capillaries and argentophil fibers. In the middle of the transformation, the high density area on the periphery began to decrease toward the center, and,
thus, the low-density area expanded. At about the time
when the newly formed capillaries increased, ring en-
\( \text{hancement was detected on CE-CT examination. Argentophil fiber networks with abundant newly formed} \)
\( \text{enhancement was recognized on days 6 to 14, and later, but according to many reports, it occurred on} \)
day 14 or later. Histologically, on about days 11 to 13, fat granule cells surrounded a clot of blood like epithe-
lum, and a layer of argentophil fibers was formed during the formation of new capillaries. The destruc-
tion of red blood cells began on day 11 or later.

In the experimentally induced hematomas in dogs, since the position of ring enhancement coincided exactly with the new connective tissue of the argentophil fiber layers and the endothelium of newly formed capillaries, the ring enhancement was presumably caused by increased permeability of newly formed capillaries. After the appearance of the ring enhancement on about day 14, the ring gradually contracted toward the center with time, and after a month, it became smaller than that at the time of hemorrhage. The contraction of the hemorrhagic lesion seems to be mainly attribut-
able to scavenging of red blood cells of the blood clot
and formation of scars, which result from change of the surrounding undeveloped connective tissues into collagenous fibers. The change of argentophil fibers into collagenous fibers is thought to begin on day 11.

As mentioned above, both in dogs with experimen-
tal intracerebral hematomas and in humans with sponta-
neous intracerebral hematomas, the chronological changes in the CT image at the periphery of the hema-
tomas corresponded well to those in the histological appearance. Therefore, it seems appropriate to classify the chronological stages of spontaneous intracerebral hematoma on the basis of changes in the CT image at the periphery of the hematoma and the process of sur-
rounding tissue reactions, neither of which are influ-
enced by the size of hematomas at the time of hemor-
rage (fig. 11).

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PATIENTS WITH FH are known to have increased risk of coronary heart disease as compared to normolipidemic subjects, but few clinical data are available on extracoronary atherosclerosis. Affected patients have premature coronary heart disease, which causes angina pectoris, myocardial infarction and sudden death. This is commonly seen in homozygotes between age 5 and 301-3 and in heterozygotes in their 40’s.4 Several studies confirm that the risk of coronary heart disease event in FH patients is 25 times higher than that in their unaffected relatives.5 Among unselected survivors of myocardial infarction, FH heterozygotes are between 3 and 6%.5-6 Using population genetic analysis of hyperlipidemia among survivors of myocardial infarction, Goldstein et al estimated a frequency of about 1 patient in 500 Caucasian individuals.7 This is a value not very far from that of Carter et al, who derived a frequency of about 1 homozygote in 200 persons.8 Few data are available on the frequency of premature cerebrovascular and/or lower limb vascular disease in FH patients.1 It is important not to ignore extracardiac localization of atherosclerosis in FH patients and to study the prevalence of vascular lesions in arteries other than coronaries. Carotids can be actually well investigated by noninvasive methods, such as echo-Doppler, able of detecting irregularities of extracranial neck arteries.

Patients and Methods

Thirty patients (15 males, 15 females) with FH have been investigated. The FH diagnosis was based upon a) elevated plasma and LDL-cholesterol concentrations, which were in all cases above 300 mg/dl and 200 mg/dl respectively; b) the presence of tendon and cutaneous xanthomas and/or a pedigree with evidence of vertical transmission of hypercholesterolemia. None of the patients under study had relatives with lipoprotein abnormalities of multiple types. Four have been diagnosed as homozygotes by skin fibroblast culture (three receptor-defective and one receptor-negative).

Venous blood was collected in all patients after 12–14 hours fasting in a drug-free period from at least three weeks, but during an hypocholesterolemic diet (cholesterol < 300 mg/day and P/S ratio > 1.0). Plasma lipoproteins were separated by preparative ultracentrifugation9 (very low density lipoprotein (VLDL) d < 1.006; low density lipoprotein (LDL): 1.006 < d < 1.063; high density lipoprotein (HDL) d > 1.063) and cholesterol and triglyceride determined by enzymatic authomated methods.10 Plasma apolipoprotein B and A concentrations have been measured by radial immunodiffusion.11 Both common and internal carotids have been studied by a Duplex Scanner ATL Mark V, which combines B mode echography and pulsed Doppler ultrasound for detection of vascular lesions even mild or arterial wall irregularities. The study was performed

Carotid Atherosclerosis In Familial Hypercholesterolemia

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SUMMARY Common and internal carotids have been studied by noninvasive method (echo-Doppler) in 30 normotensive patients with familial hypercholesterolemia (FH). Vascular lesions were detected in 14 patients (46%), who presented one or more lesions of different degree (between 1–15% and 16–49%). In one case, only one carotid had stenosis > 50%. Severity and number of stenosis were related to age and levels of hypercholesterolemia. FH patients with carotid lesions showed a significantly higher LDL-cholesterol (p < 0.01) and plasma apolipoprotein B (p < 0.001) concentrations and a significantly lower HDL-cholesterol (p < 0.05) and plasma apolipoprotein A (p < 0.001) levels as compared to those with normal echo-Doppler findings. These data indicate that investigation of arterial districts other than coronaries are useful in quantitative evaluation of atherosclerotic involvement.

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