NEOVASCULARIZATION AND INCREASED UPTAKE OF $^{99m}$Tc IN EXPERIMENTALLY PRODUCED CEREBRAL HEMATOMA

Yoshinori Sugitani, Mototaka Nakama, Yoshihiro Yamauchi, Masatoshi Imaizumi, Tadaatsu Nukada, and Hiroshi Abe

Osaka University Medical School, Fukushima, Osaka, Japan

Experimental cerebral hematoma was prepared by injecting blood into the cerebral hemisphere in rats. During the course of experimental hematoma, the distribution of $^{99m}$Tc-pertechnetate and histological preparations were studied. The distribution of $^{99m}$Tc into the hematoma increased greater than that into the control hemisphere during 1–3 weeks after the preparation of hematoma. The major portion of $^{99m}$Tc was recovered in the supernatant fraction. During 1–3 weeks, newly formed capillaries were found in the hematoma lesions. Microglias appeared in the lesion during 3–4 weeks. Increased distribution of $^{99m}$Tc, into the hematoma hemisphere corresponded with the period when newly formed capillaries appeared.

These findings indicate that newly formed capillaries which appear in the course of experimental hematoma might be responsible for the development of a positive brain scan in patients with cerebrovascular accidents.

Brain scanning has become a routine clinical examination for detecting intracranial lesions (1,2). In patients with cerebrovascular accidents, it has been known that a latent period is often observed before the appearance of positive scan, and the peak incidence of positive scan occurs during the second to the third week after the onset (3–6). The mechanism of a positive brain scan appearing is not clear. Some authors (3,6,7) have speculated that an increased vascularization in the lesion may be responsible for the occurrence of the positive brain scans associated with a cerebrovascular accident.

To the best of our knowledge, histological observation and the subcellular localization of radioactive substance in the brain during the course of a cerebrovascular accident have not been reported. For the purpose of clarifying the mechanism of a positive scan associated with a cerebrovascular accident, the subcellular distribution of a radioactive substance ($^{99m}$Tc) was investigated in experimental cerebral hematoma using the zonal ultracentrifugation method. Histological examination of the brain lesion was also undertaken in this present report.

MATERIALS AND METHODS

Adult white male rats weighing 200–250 gm were used in this experiment.

Preparation of experimental cerebral hematoma. As shown in Fig. 1, the parietal scalp of the rat was incised longitudinally and a tiny hole was made on the right parietal bone at a point 4 mm laterally from the sagittal suture and 2 mm posteriorly from the coronal suture. Blood obtained from the tail vein was injected into the brain tissue in a volume of 0.3 ml through the hole at a depth of 4 mm. After these procedures, the parietal scalp was closed.

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For reprints contact: Y. Sugitani, First Dept. of Internal Medicine, Osaka University Medical School, 3 Dojimahamadori, Fukushima, Osaka 553, Japan.

FIG. 1. Preparation of experimental cerebral hematoma. Left figure indicates point of injection of blood in dorsal aspect and right picture shows position of hematoma to be prepared.
Subcellular distribution of $^{99m}$Tc-per technetate in the brain. The rats with cerebral hematoma were anesthetized with sodium pentobarbital and 200 $\mu$Ci $^{99m}$Tc was injected into the tail vein. Thirty minutes after injection, the rats were bled by decapitation. The brain was removed and washed with ice cold saline. Each hemisphere of the brain was weighed and homogenized with 10 ml of 0.25 M sucrose using a Teflon homogenizer. The ultracentrifugation procedures were followed as shown in Fig. 2, and tissue debris, mitochondrial, microsomal, and supernatant fractions were obtained. Radioactivity of each fraction was measured by ALOKA autowell scintillation counter.

Histological examination. The rats were sacrificed at 1 day, 1, 2, 3, and 4 weeks after the preparation of cerebral hematoma. The whole brain was removed, fixed in 8% formalin and stained with hematoxylin-eosin.

**Result**

As shown in Table 1, the rats with cerebral hematoma prepared by the injection of more than 0.4 ml blood died immediately after injection. In rats with cerebral hematoma with a volume of 0.3 ml, 32 out of 37 rats were alive for at least 4 weeks and 5 died. In later experiments, therefore, rats with 0.3-ml hematoma were used. In all rats studied here, the hematoma was found in the white matter of the right hemisphere, as shown in Fig. 1.

Total radioactivity in the brain varied considerably among the experimental rats. As represented by the ratio of hematoma to control radioactivity (H/C ratio), no significant difference in the radioactivity between the hematoma and control hemisphere was observed in the rats 1 day after the preparation. During the time of 1–3 weeks, the H/C ratios were higher than 1.0, indicating the increased distribution of $^{99m}$Tc into the hematoma hemisphere. At 4 weeks, the ratio returned to 1.0 (Fig. 3).

The subcellular distribution of $^{99m}$Tc is shown in Fig. 4. Seventy to 80% of the radioactivity of $^{99m}$Tc was found in the supernatant fraction. Percent distributions of $^{99m}$Tc into the particle fractions were less than 10%. The distribution patterns in the hematoma hemisphere were similar to those in the control hemisphere. No significant difference in the subcellular distribution pattern was observed during the course of experimental hematoma.

**Histological finding.** Histological studies of the

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<th>TABLE 1. MORTALITY RATE OF EXPERIMENTAL CEREBRAL HEMATOMA IN RAT*</th>
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<tr>
<td>Injected blood volume (ml)</td>
</tr>
<tr>
<td>0.2</td>
</tr>
<tr>
<td>0.3</td>
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<tr>
<td>0.4</td>
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* Blood obtained from tail vein is injected into cerebral hemisphere at point indicated in Fig. 1.

**FIG. 2.** Method of subcellular fractionation. Thirty minutes after injection of 200 $\mu$Ci of $^{99m}$Tc, rats are bled by decapitation. Each hemisphere of brain is homogenized with 10 ml of 0.25 M sucrose.

**FIG. 3.** Comparison of radioactivities of hematoma hemisphere to control hemisphere. Result is expressed as H/C ratio where H = total radioactivity of hematoma hemisphere and C = total radioactivity of control hemisphere. Each dot represents H/C ratio of individual rat.
hematomal hemisphere were examined at 2, 3, and 4 weeks after the preparation of hematoma and are shown in Figs. 5, 6, and 7, respectively. Histological lesion 2 weeks after the preparation revealed an increase in newly formed capillaries around the hematoma (Fig. 5). In addition to the finding of newly formed capillaries, microglia appeared in the histological section obtained after 3 weeks (Fig. 6). At 4 weeks, microglia increased in number, and connective tissues were observed while newly formed capillaries could not be found (Fig. 7). These histological findings were observed in all hematomal lesions studied here (four rats in each group).

Table 2 summarizes the present experiments. Increased distribution of 99mTc in the hematomal hemisphere observed at 1–3 weeks after the preparation corresponded with the period when newly formed capillaries appeared.
TABLE 2. CORRELATION BETWEEN HISTOLOGY AND $^{99m}$Tc UPTAKE RATIO AFTER THE PREPARATION OF EXPERIMENTAL CEREBRAL HEMATOMA

<table>
<thead>
<tr>
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<th>1 day</th>
<th>1 week</th>
<th>2 weeks</th>
<th>3 weeks</th>
<th>4 weeks*</th>
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<tbody>
<tr>
<td>Newly formed capillary</td>
<td>(−)</td>
<td>(+)</td>
<td>(+++)</td>
<td>(+++)</td>
<td>(−)</td>
</tr>
<tr>
<td>Newly formed microglia</td>
<td>(−)</td>
<td>(−)</td>
<td>(−)</td>
<td>(+)</td>
<td>(+++)</td>
</tr>
<tr>
<td>H/C ratio†</td>
<td>&gt;1.0</td>
<td>&gt;1.0</td>
<td>&gt;1.0</td>
<td>&gt;1.0</td>
<td>&gt;1.0</td>
</tr>
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</table>

* Time after preparation of experimental cerebral hematoma.
† Ratio of radioactivity in hematomal hemisphere (H) to that in control hemisphere (C).

DISCUSSION

The mechanisms of a positive brain scan associated with intracranial lesions are not fully understood. Bakay (8) has pointed out that differences in the vascular permeability, in the size of extracellular space, and in the cellular metabolism should be discussed. The pinocytotic activity of tumor tissue, blood content of tumor tissue, and peritumoral edema should also be considered. Cohn et al (9) demonstrated that the vascularity in the neoplasm was correlated with a positive brain scan and indicated that in the neoplastic lesions there might exist unusual permeability resulting from an undeveloped blood brain barrier. Quinn (10) stated that the loss of selective membrane impermeability was more responsible for the increased uptake of radioactive substance in the cerebral neoplastic tissues than the active cellular metabolism.

Contrary to the finding in neoplastic tissues, the brain scans performed in the early or late stage of patients with cerebrovascular accidents are negative. A positive brain scan is usually observed during the second to the third week after the onset in these patients. In cerebrovascular accidents, the disturbed blood brain barrier and the cerebral edema might exist in the early stage. In the present study, no significant differences in the distribution of radioactivity in the hematomal and control hemispheres were observed in the early stage and in the fourth week after the onset. These results indicated that cerebral edema might not be a major factor for the positive scan. Pinocytotic activity of microglia might not be expected to play a role for a positive brain scan since microglia appeared during the third to the fourth week when the equal distributions of radioactivity in the hematomal and control hemisphere were observed.

Quinn (10) has proposed two hypotheses for the mechanism of a positive brain scan in cerebral occlusive disease: the increased capillary permeability by revascularization of a previous ischemic area being caused by the further movement of an embolus along a vessel, and the retrograde flow through venous channels into capillaries previously occluded by edema. The newly formed immature vessels might have an undeveloped blood brain barrier. In the present histological examination, newly formed capillaries appeared significantly during the second to the third week after the preparation of hematoma. Corresponding with these periods, increased distribution of $^{99m}$Tc in the hematomal hemisphere was found. In the present experiments the comparison was made between the hematomal and control hemisphere. Further study of the distribution of radioactivity in subcellular fractions of the hematomal lesion would provide more precise information about the mechanism. The present experiment, however, indicated that $^{99m}$Tc activities in the intra- and extracellular soluble fraction, transduced from the newly formed capillary with an undeveloped blood brain barrier, might be responsible for the appearance of a positive brain scan in the patients with cerebrovascular accidents.

Recently, Baum (11) reported that $^{99m}$Tc can be used very successfully for autoradiography with Auger electrons. Whether the present results with zonal ultracentrifugation of the cerebral hematomal lesion are correlated with autoradiography remains to be elucidated.

REFERENCES


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The Scientific Program Committee welcomes the submission of abstracts of original contributions in nuclear medicine from members and nonmembers of the Society of Nuclear Medicine for the 21st Annual Meeting. Abstracts for both the regular scientific program and for works-in-progress papers will be published in the June issue of *The Journal of Nuclear Medicine*, necessitating earlier deadlines for abstracts than in previous years.

This year the Committee plans to divide the program into four major categories: Basic Science, Clinical Practice, Clinical Research, and Special Topics. Papers on the following subjects will be considered in these sessions:

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