Imaging of Leukocytic Infiltration in Human Cerebral Infarcts

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SUMMARY The circulating white blood cells of patients with brain infarction were labelled in vitro with Indium-111 tropolonate; the cells were reinjected to study the inflammatory process by gamma camera imaging. Eight patients with acute cerebral ischemic infarct were studied during the first two weeks after the onset of neurological symptoms. In seven cases a well defined area of increased radioactivity was revealed in the infarcted hemisphere indicating active migration and tracking of labelled leukocytes in cerebral infarct. This method allows monitoring of the cellular inflammatory response in human cerebral infarcts and adds another imaging technique.

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IN VITRO LABELLING of peripheral leukocytes with Indium-111 and the study of cell circulation and homing after their reinjection by means of gamma camera imaging, is a technique widely used for detecting abscesses,1,2 areas of cellular infiltration in inflammatory diseases3,4 and, using a purified labelled lymphocyte preparation, clusters of lymphocytic infiltrates in patients with organ specific autoimmune disease.5,6 Recently, the inflammatory response to acute myocardial infarction in dogs and humans has been studied, using Indium-111 labelled leukocytes, thus providing new insight into the dynamics of this process.7,8 The leukocytic infiltration in areas of cerebral infarction is a well documented histopathological finding.9,10 In the early stages, granulocytes are present both in the small blood vessels and in the surrounding disintegrating parenchyma. Approximately 4 to 6 days after an infarct the granulocyte infiltration is replaced by mononuclear phagocytes which remain the predominant cell population for many weeks.

In the present study we investigated eight patients with cerebral ischemic infarcts, and we report here the dynamics of the in vivo leukocyte infiltration in the infarcted areas using Indium-111 tropolonate labelled white cells.

Material and Methods

Patients

Eight patients with acute cerebral infarcts had been examined during the first two weeks after the onset of neurological symptoms; one patient had this study repeated 30 days after stroke. The patients’ primary presenting neurological findings, timing of the investigations after symptoms, their results and clinical outcomes are reported in table 1.

Acute cerebral infarct was diagnosed on the basis of clinical history and focal neurological signs at physical examination. Before the Indium-111 labelled leukocytes study was carried out, cranial CT scan to confirm the clinical diagnosis, was completed. All patients were diagnosed as having non-hemorrhagic infarcts since the original and follow-up CT scans revealed only hypodense areas.

Conventional static brain scintigraphy with Technetium 99m was also done in order to evaluate the permeability of the blood-brain-barrier.

The recovery from cerebral infarct was classified in terms of good and poor outcome depending on the eventual improvement (good outcome) or stationary/worsened evolution (poor outcome) of the patients’ neurological signs and symptoms during the observation time (at least one month).

Two patients died and at autopsy the presence of an extensive area of ischemic brain damage with large surrounding edema was evident. Five normal volunteers (2 females and 3 males, mean age 55 ± 10) acted as a control group. Informed consent was obtained from all volunteers and from all patients or their next of kin.

Cell Separation and Leukocyte Labelling

Leukocytes were collected from 100 ml of peripheral blood anticoagulated with sodium citrate. Cell labelling was performed according to Peters et al11 with slight modifications.

Briefly, the white cell buffy coat was recovered after incubation of blood with dextran for 30 minutes at 37° C. Cells were washed twice in phosphate buffer saline (PBS) and the contaminating red cells were lysed by the use of Tris ammonium chloride by incubating the cell suspension for 5 minutes at 4° C. The plasma was also collected and from this the platelets were removed by centrifugation for 5 minutes at 2000 g. Purified white cells were then incubated with 100 μl of platelet free plasma and 100 μl of tropolonate at a concentration of 4.4 mM in hepes saline buffer (pH 7.3). Four hundred μCi of Indium-111 in less than 50 μl 0.04 M HCL were added to the cell suspension. Incubation was carried out for 5 minutes at room temperature. The labelling efficiency varied between 70% and 80% of the dose added in vitro. Therefore in all instances the injected dose was approximately 300 μCi. The white cells were finally washed twice and resuspended in 5–7 ml of autologous plasma for reinjection.
TABLE 1  Patient's Data

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Neurological signs</th>
<th>Indium-111 scans</th>
<th>Days after onset of symptoms</th>
<th>Results*</th>
<th>X-ray CT scan finding</th>
<th>Clinical outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(+) or (—)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>49</td>
<td>M</td>
<td>Left hemiparesis</td>
<td>8</td>
<td>+</td>
<td>Right subcortical grey and white matter infarct</td>
<td>Poor</td>
</tr>
<tr>
<td>Case 2</td>
<td>76</td>
<td>M</td>
<td></td>
<td>10</td>
<td>+</td>
<td>Right temporal infarct</td>
<td>Poor</td>
</tr>
<tr>
<td>Case 3</td>
<td>62</td>
<td>M</td>
<td>Left hemiplegia</td>
<td>I Study</td>
<td>+</td>
<td>ND</td>
<td>Right parietal-occipital infarct and left frontal infarct</td>
</tr>
<tr>
<td>Case 4</td>
<td>74</td>
<td>M</td>
<td>Right hemiparesis</td>
<td>2</td>
<td>+</td>
<td>ND</td>
<td>Right temporal-parietal infarct</td>
</tr>
<tr>
<td>Case 5</td>
<td>71</td>
<td>M</td>
<td>Left hemiplegia</td>
<td>II Study</td>
<td>30</td>
<td>28</td>
<td>Right temporal-parietal infarct</td>
</tr>
<tr>
<td>Case 6</td>
<td>69</td>
<td>F</td>
<td>Left hemiparesis</td>
<td>14</td>
<td>+</td>
<td>12</td>
<td>Right fronto-temporal infarct</td>
</tr>
<tr>
<td>Case 7</td>
<td>70</td>
<td>F</td>
<td>Right hemiplegia</td>
<td>4</td>
<td>+</td>
<td>8</td>
<td>Left temporal-parietal infarct</td>
</tr>
<tr>
<td>Case 8</td>
<td>47</td>
<td>F</td>
<td>Left hemiparesis</td>
<td>5</td>
<td>—</td>
<td>9</td>
<td>Right temporal infarct</td>
</tr>
</tbody>
</table>

* (+): abnormal uptake of the radiotracer; (—): absence of pathological uptake of the radiotracer.

Procedure and Imaging

Labelled leukocytes were injected in a cubital vein. Peripheral venous blood samples were collected immediately thereafter and after 2 and 20 hours to measure activity in whole blood, leukocyte and plasma fractions. At the same time intervals, antero-posterior and lateral brain scans were recorded. Brains scans were performed using a gamma camera (400 T General Electric) fitted with a high sensitivity collimator and interfaced on-line to a computer. Imaging was carried out using the two photopeaks of Indium-111 (173 and 247 Kev). The scanning time was 480 seconds collecting approximately 200,000 total counts for each study.

Regions of interest (ROI) 3.0 x 3.0 cms were placed in both hemispheres to calculate the rate of brain activity in each case studied. For each pair of ROI values so obtained, an absolute percent index of asymmetry (IA) was calculated as [200 (right-left)]/(right + left).

Results

In normal subjects brain images revealed symmetrical and constant hemispheric uptake of labelled leukocytes due to the blood pool activity.

In 7 out of 8 patients with ischemic cerebral infarcts a well defined brain asymmetry with greater uptake of the labelled leukocytes in the infarcted hemisphere was revealed (table 1). In these patients, at 2 hours after the reinjection of the labelled leukocytes, the activity measured in cerebral ROI was 3.78 ± 0.05 (counts log_{10} ± SEM) in non ischemic contralateral hemispheres and 3.86 ± 0.05 (counts log_{10} ± SEM) in the infarcted hemispheres (p < 0.001 paired T test) (fig. 1). The IA was, at 2 hours, 1.90 ± 0.40 (± SD) in the patients and it was 0.59 ± 0.13 (± SD) in normal subjects. This difference was statistically significant p at values of 0.001 (Student's T test).

In the scans completed 20 hours after the reinjection, persistent leukocytic infiltration was found in the infarcted hemisphere (IA was 2.03 ± 0.66), sometimes resulting in a more pronounced asymmetrical brain uptake (fig. 2).

In patient n° 8 images at either 2 or 20 hours failed to
show any leukocytic infiltration of labelled cells. The activity measured at 2 hours was 3.79 in the infarcted hemisphere and 3.78 in the contralateral hemisphere. This patient’s CT scan revealed an infarct smaller than that seen in the others and he made a good clinical recovery.

The patient n° 3 underwent a scan 30 days after the initial symptoms and still showed persistent blood-brain-barrier disruption but disappearance of leukocytic infiltration in the infarcted area (fig. 3).

The plasma activity was less than 5% of the total blood activity immediately after injection of labelled cells and did not change in the samples collected 2 and 20 hours later. However, peripheral leukocytic activity at 2 and 20 hours was 33% and 7% respectively of initial activity, indicating the homing of these cells in the target organs (fig. 4).

This is the consequence of the decrease with time of labelled cells in the circulation due to the migration of cells to the liver, spleen or, when present, to inflammatory sites.3

Discussion

The present study provides the first in vivo evidence of leukocytic infiltration in cerebral ischemic infarct in humans. As early as two hours after the injection of labelled leukocytes, the cells are visible in the infarcted hemisphere indicating an early localization of labelled leukocytes. Similar findings have been described with this tracer in other inflammatory lesions.1

The localization of labelled leukocytes in cerebral infarct appears to be an active phenomenon. The possibility that free Indium-111 tropolonate in the plasma may represent a source of artificial results has been ruled out. The plasma activity is very low and it remains stable with time.

Furthermore when the study of Indium-111 labelled leukocytes was repeated 30 days after the initial injection, disappearance of radioactivity in the presence of persistent blood-brain-barrier breakdown was observed. A similar finding (positive Technetium scintigraphy but negative leukocytic infiltration) was obtained in patient n° 8. This suggests that the infiltration of white cells in cerebral infarcted areas is not directly related to the disruption of the blood-brain-barrier.

Patients studied in early (48 hours) or late (but not later than 2 weeks) periods after stroke, showed a clear patch of leukocytic infiltration in the infarcted area. However, all patients examined (except patient n° 8) had extensive ischemic infarcts as documented by CT scans.

It is of interest to consider which types of white blood cells infiltrate areas of brain infarction. From histologic studies it is known that in (human and animal) acute cerebral infarcts (either ischemic or hemorrhagic) the polymorphonuclear infiltration, when present, occurs within 5 days, whereas in the later periods (6 days to three weeks) abundant phagocytic infiltration is the most prominent morphological finding.12-13 This is confirmed indirectly by an increase of lactoferrin, lysozyme and B2-microglobulin in cerebro-spinal fluid up to 18 days; these substances are mediated by granulocytes, macrophages and lymphocytes, respectively.14

No attempt has been made in our study to selectively label leukocyte subpopulations; therefore it is not surprising that positive scans were observed either two days or two weeks after the infarct. Such radioactivity was probably the result of indiscriminate labelling of different types of leukocytes (granulocytes, lymphocytes and monocytes).

There is paucity of information on the relationship that may exist between acute inflammation and cerebral infarct; the intensity of the inflammatory response to a cerebral infarct probably depends on the primary mechanism responsible for the ischemia (i.e. thrombosis or embolism). Embolism elicits more leukocyte response than thrombosis.15

The mechanism of cerebral infarct was not investigated in this study (angiography was not performed); however, the less extensive tissue damage suggested by CT scan in a patient without leukocytic infiltration,
suggests, in that patient, a thrombotic mechanism. Infiltrating leukocytes may augment tissue necrosis and induce further inflammatory response with the release of lysosomal enzymes capable of inducing proteolytic disruption of the viable tissue.\textsuperscript{16} Activated neutrophils transform molecular oxygen to highly reactive free radicals such as superoxide anion, hydroxyl radical, hydrogen peroxide and singlet oxygen. These toxic oxygen derivatives attack plasma membrane phospholipids and promote cellular injury or death.\textsuperscript{17-18}

Whether the extent of the tissue lesion and the clinical outcome correlate with the presence, and persistence of leukocytic infiltration remains to be studied.

In a recent study of experimental ischemic heart injury, the size of the infarct was proportional to the amount of the leukocytic infiltration; the authors suggested that the latter phenomenon may exacerbate the extent of tissue destruction.\textsuperscript{19} This mechanism may be considered also for ischemic brain disease even if experimental evidence has not yet been obtained. Indium-111 labelling technique of leukocytes may help to highlight the inflammatory reaction in a brain infarct; the technique could be useful to monitor the response to anti-inflammatory treatment when this therapy is considered.

Acknowledgment
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References
7. Thakur ML, Gottschalk A, Zarei BL: Imaging experimental myocardial infarction with Indium-111 labelled autologous leucocytes:
Fibromuscular Dysplasia of Cervico-Cephalic Arteries With Multiple Dissections and a Carotid-Cavernous Fistula.
A Pathological Study

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and J. Barbizet, M.D.‡

SUMMARY A rare case of widespread fibromuscular dysplasia (F.M.D.) is reported, involving the cervico-cephalic arteries associated with multiple dissections, saccular aneurysms and a carotid-cavernous fistula. A detailed post-mortem examination revealed FMD involvement of the intracranial vessels, not demonstrated by arteriography.

FIBROMUSCULAR DYSPLASIA (F.M.D.), an angiopathy of unknown etiology is diagnosed on angiographic grounds. FMD has been reported in most of the major arteries of the body. Since the first histologic study of FMD of the carotid artery, little pathologic information concerning FMD involving the cervicocephalic arterial tree has been published. Dissecting aneurysms are thought to represent a frequent complication of FMD but SATO and HATA found only 3 histological studies of such an association prior to their report. To our knowledge, the association of cervico-cephalic FMD with a carotid-cavernous fistula has been described once, without pathological examination. The present paper describes an autopsied case of bilateral cervico-cephalic FMD with multiple dissections and a carotid-cavernous fistula due to a saccular aneurysm rupture.

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