Leukocyte Infiltration in Acute Hemispheric Ischemic Stroke

Pao-Yu Wang, MD; Chia-Hung Kao, MD; Ming-Yan Mui, MD; and Shyh-Jen Wang, MD

Background and Purpose: The dynamics of leukocyte infiltration in human cerebral ischemia were studied using technetium-99m hexamethylpropyleneamine oxime (99mTc HMPAO)–labeled leukocyte brain single-photon emission computed tomography (SPECT).

Methods: Twenty-two patients diagnosed as having hemispheric ischemic stroke were examined with 99mTc HMPAO brain SPECT for cerebral blood flow study and 99mTc HMPAO–labeled leukocyte brain SPECT for the study of leukocyte infiltration. Three patients with chronic hemispheric ischemic stroke received one examination. Nineteen patients with acute hemispheric ischemic stroke received their initial examination within the first week after onset. Follow-up examinations were performed at intervals of 1–3 weeks whenever possible.

Results: In patients with chronic hemispheric ischemic stroke, leukocyte infiltration was not seen in areas of perfusion defect. In patients with acute hemispheric ischemic stroke, leukocyte infiltration was seen in areas of perfusion defect during the acute stage, which persisted for no less than 5 weeks after onset and then declined.

Conclusions: A new method to study and monitor the process of leukocyte infiltration in acute cerebral ischemia using 99mTc HMPAO–labeled leukocyte brain SPECT is described. This method shows that leukocyte infiltration in acute hemispheric ischemic stroke is a dynamic process that persists for no less than 5 weeks and then declines. (Stroke 1993;24:236–240)

Key Words: cerebral ischemia • leukocytes • tomography, emission computed

It has been implied that leukocytes contribute to postischemic damage in a number of tissues. Leukocyte infiltration in cerebral ischemia has been demonstrated in experimental animal studies.1,2 Leukopenic animals showed better recovery of electrophysiological functioning and no areas of very low blood flow after cerebral ischemia,3–7 suggesting active participation of leukocytes in postischemic neuronal damage. In humans, impaired filterability of white cells,8–11 increased granulocyte adhesion,12 and activation of leukocytes after stroke13 have been reported. High leukocyte count in peripheral blood was claimed to be predictive of the risk of further strokes.14–16 Leukocyte infiltration in human cerebral infarcts have been reported using conventional static brain scintigraphy and leukocyte labeling with indium-111 troponolactate.17

We studied the dynamics of in vivo leukocyte infiltration using technetium-99m hexamethylpropyleneamine oxime (99mTc HMPAO)–labeled leukocyte brain single-photon emission computed tomography (SPECT).

Subjects and Methods

Preparation of Leukocytes

Autologous leukocytes were isolated and labeled with 99mTc HMPAO with minor modifications of the technique of Uno et al.18 Briefly, 36 ml fresh venous blood was drawn and anticoagulated with 4 ml acid-citrate-dextrose. A concentrated pellet of leukocytes was obtained after differential speed centrifugation. 99mTc HMPAO was formed by reconstituting a commercial vial of HMPAO (Ceretec, Amersham International Plc., United Kingdom) with 5 ml of 30 mCi fresh 99mTc pertechnetate. After incubating the pellet of leukocytes in 10–15 mCi 99mTc HMPAO for 15 minutes at room temperature, the labeled leukocytes were separated by high-speed centrifugation. The labeled leukocytes were resuspended in 5 ml of 0.9% saline solution for re inoculation into the patient. The in vitro viability study of labeled leukocytes by the trypan blue rejection was greater than 90%, the recovery ratio of the labeled leukocytes in total blood after 40 minutes of intravenous injection was 42.0±4.7% (mean±1 SD), and the labeling efficiency was 72±7% (mean±1 SD).19

Brain SPECT Imaging

Two separate brain SPECT scans were performed. The scan for the cerebral blood flow study was performed 5–10 minutes after intravenous injection of 10–15 mCi 99mTc HMPAO initially; then the scan for the study of leukocyte infiltration was performed 24 hours after the injection of 99mTc HMPAO–labeled leukocytes the following day. An APEX 609R system (Elscint Ltd.,
Haifa, Israel) was used for data acquisition and reconstruction. An Elscint APC-4R high-resolution collimator and Elscint APC-3R medium resolution collimator were used for $^{99m}$Tc HMPAO brain SPECT and $^{99m}$Tc HMPAO–labeled leukocyte brain SPECT, respectively. Data were acquired in a 64×64 matrix through a 360° rotation at an angular interval of 6°. Reconstruction was performed by a standard back projection using a Hanning filter for a 64×64 matrix image. Images of transverse axial, coronal, and sagittal planes were obtained in each study. The transverse axial plane was parallel to the orbitomeatal line. In each plane, 16 images were obtained.

**Region of Interest Analysis**

We selected transverse axial slices at the same level from $^{99m}$Tc HMPAO brain SPECT and $^{99m}$Tc HMPAO–labeled leukocyte brain SPECT for region of interest (ROI) analysis. The slice that showed maximal perfusion defect in $^{99m}$Tc HMPAO brain SPECT was chosen; then ROIs were drawn with a manual cursor to include the area of perfusion defect and the homologous area of the contralateral hemisphere. The same ROIs were drawn in the $^{99m}$Tc HMPAO–labeled leukocyte brain SPECT. For semiquantitative measurement, the differences of radioactivities in ROIs were calculated as the asymmetry index (AI) with the following equation: AI = (right−left)/(right+left).

**Patients**

Twenty-two patients diagnosed as having hemispheric ischemic stroke of the middle cerebral artery territory were examined with both $^{99m}$Tc HMPAO brain SPECT and $^{99m}$Tc HMPAO–labeled leukocyte brain SPECT. The diagnosis was confirmed after clinical history, neurological examination, cranial computed tomography, and $^{99m}$Tc HMPAO brain SPECT. There were 20 men and two women aged between 59 and 78 years (mean age, 67.3 years). Three patients with chronic hemispheric ischemic stroke, of durations longer than 7 months (ranging from 7 months to 3 years), received one examination. Nineteen patients with acute hemispheric ischemic stroke received their first examination within the first week after onset,

**FIGURE 1.** Computed tomographic (CT), technetium-$^{99m}$ hexamethylpropyleneamine oxime ($^{99m}$Tc HMPAO) brain single-photon emission computed tomographic (SPECT), and $^{99m}$Tc HMPAO–labeled leukocyte brain SPECT scans in a case of chronic hemispheric ischemic stroke. Top panel: Plain CT showed a chronic infarct in the territory of the right middle cerebral artery. Middle panel: $^{99m}$Tc HMPAO brain SPECT showed a perfusion defect in the territory of the right middle cerebral artery, corresponding to the infarcted area shown by CT. The area of perfusion defect and the homologous area of the contralateral hemisphere were outlined by a manual cursor, and then the asymmetry index of cerebral blood flow was calculated as described in the text. Bottom panel: $^{99m}$Tc HMPAO–labeled leukocyte brain SPECT showed no definite leukocyte infiltration in the area of perfusion defect shown by $^{99m}$Tc HMPAO brain SPECT. The same regions of interest were outlined, and the asymmetry index of leukocyte infiltration was calculated as described above.
except one patient. Follow-up examinations were performed at intervals of 1–3 weeks whenever possible. In acute stroke patients, seven patients were examined once, seven patients twice, one patient three times, and four patients more than three times.

Statistical Analysis

The Mann-Whitney U test was performed to compare the AI of the chronic stroke with the AI determined the first week after the onset of acute stroke. The Wilcoxon matched-paired signed rank test was performed to compare the AI of the first week with that of other weeks after the onset of acute stroke.

Results

In those patients with chronic hemispheric ischemic stroke, leukocyte infiltration was not seen in areas of perfusion defect (Figure 1). However, in all cases of acute hemispheric ischemic stroke, leukocyte infiltration was present in areas of perfusion defect during the acute stage (Figure 2). The AI of leukocyte infiltration is summarized in Table 1. There was a statistically significant difference in AI between chronic hemispheric ischemic stroke and the first week after the onset of acute hemispheric ischemic stroke. No statistically significant differences in AI were present when comparing the first week after onset with other weeks in acute hemispheric ischemic stroke. But the leukocyte infiltration persisted for no less than 5 weeks after onset and then declined as shown in Figure 3.

Discussion

Pozzilli et al.17 had demonstrated leukocyte infiltration in human cerebral infarcts. In their study, however, the relation between the perfusion defect and leukocyte infiltration was not studied. Of the eight patients they studied, only one patient was examined twice, so the serial changes of leukocyte infiltration were not explored. Our study clearly shows the dynamics of leukocyte infiltration in acute hemispheric ischemic stroke,
High leukocyte infiltration, as measured with $^{99m}$Tc HMPAO-labeled leukocyte brain SPECT, predicted a poor prognosis in acute human cerebral infarcts. Leukocyte infiltration in acute ischemia causes further ischemic neuronal damage through several possible mechanisms, including impaired microcirculation due to capillary occlusion by leukocytes, release of chemical mediators such as granular enzymes, reactive oxygen metabolites, and products of membrane phospholipases. Cytokines and expression of intercellular adhesion molecule-1 on the endothelial cells are presumably implicated in the processes of leukocyte infiltration, but the precise mechanisms that mediate and regulate leukocyte infiltration are still unknown. Potential therapeutic interventions of leukocyte infiltration and its harmful effects are in development now, such as the use of leukocyte adhesion antibody and platelet-activating factor antagonists.

In conclusion, a new method to study and monitor the process of leukocyte infiltration using $^{99m}$Tc HMPAO-labeled leukocyte brain SPECT has been described. This method shows that leukocyte infiltration in acute hemispheric ischemic stroke is a dynamic process that persists for no less than 5 weeks and then declines.

### References

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### TABLE 1. Asymmetry Indexes of Leukocyte Infiltration in Hemispheric Ischemic Stroke

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean±SD</th>
<th>No. of examinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic stroke</td>
<td>0.07±0.03*</td>
<td>3</td>
</tr>
<tr>
<td>Acute stroke†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.21±0.10</td>
<td>18</td>
</tr>
<tr>
<td>2</td>
<td>0.23±0.09</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>0.19±0.08</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>0.20±0.04</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>0.17±0.11</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>0.08±0.01</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>0.07</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>0.11</td>
<td>1</td>
</tr>
<tr>
<td>11</td>
<td>0.06</td>
<td>1</td>
</tr>
</tbody>
</table>

*p=0.0208 by the Mann-Whitney U test comparing the asymmetry index of the chronic stroke with that determined the first week after the onset of acute stroke. Values of p ranged from 0.1088 to 1.000 by the Wilcoxon matched-pair signed rank test comparing the asymmetry index of the first week with that of other weeks after the onset of acute hemispheric ischemic stroke.

†Time of examination after stroke onset in weeks.

which persisted for no less than 5 weeks and then declined. Also, SPECT gives better spatial resolution of leukocyte infiltration than the conventional static brain scan.

Leukocyte infiltration in areas of human cerebral infarction is already documented histopathologically. In early stages, polymorphonuclear leukocytes are the main leukocyte subpopulation participating in the acute inflammation of stroke. Approximately 4–6 days after an infarct, polymorphonuclear leukocytes are replaced by the mononuclear leukocytes, which remain as the main leukocyte subpopulation for many weeks. In our study, mixed leukocytes were used for labeling, without efforts to selectively label different leukocyte subpopulations; thus, the dynamic process of leukocyte infiltration that we explored in the present study represents infiltration of mixed leukocyte subpopulations.

![Figure 3. Line graph showing trend of leukocyte infiltration in acute hemispheric ischemic stroke. The asymmetry index (AI) of leukocyte infiltration is plotted against the time of examination. Leukocyte infiltration persisted for no less than 5 weeks and then declined. C represents the AI of chronic hemispheric ischemic stroke. Bars represent ±1 SD.](http://stroke.ahajournals.org/)

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