Quantitative assessment of blood-brain barrier permeability in multiple sclerosis using 68-Ga-EDTA and positron emission tomography.

C Pozzilli, S Bernardi, L Mansi, et al.

*J Neurol Neurosurg Psychiatry* 1988 51: 1058-1062
doi: 10.1136/jnnp.51.8.1058
Quantitative assessment of blood-brain barrier permeability in multiple sclerosis using 68-Ga-EDTA and positron emission tomography

C POZZILLI,* S BERNARDI,* L MANSI,‡ P PICOZZI,† F IANNOTTI,‡ B ALFANO,† L BOZZAO,* GL LENZI,* M SALVATORE,‡ P CONFORTI,‡ C FIESCHI*

From the Department of Neurological Sciences,* University of Rome ‘La Sapienza’, Institute of Neurosurgery,‡ University of Naples, National Cancer Institute ‘Fondazione Sen. G. Pascale’,† Naples, Italy

SUMMARY Fifteen patients with definite multiple sclerosis were examined with high volume delayed (HVD) CT scan and positron emission tomography (PET) using 68-Ga-EDTA as a tracer. The passage of 68-Ga-EDTA across the blood-brain barrier (BBB) was measured by using multiple graphical analysis. This method permits the simultaneous calculation of a blood to brain influx constant Ki (ml/g min⁻¹) and of the plasma volume Vp (ml/g⁻¹). Focal areas of abnormal CT enhancement and pathological accumulation of 68-Ga-EDTA were visualised in four patients who were all examined during a clinical exacerbation of the disease. The mean Ki value measured in these areas was 12.5, SD 3.4 indicating a moderate but significant increase of BBB permeability compared with the value found in normal tissue (3.2, SD 0.9). No parallel increase in Vp values was found in these pathological areas. Quantitative data obtained with PET seems to provide further insight into the study of BBB function in multiple sclerosis.

In postmortem perfusional studies utilising trypan blue, Broman first demonstrated a disturbance of blood-brain barrier (BBB) permeability in some multiple sclerosis plaques.¹

Subsequently, using CT scan after the intravenous injection of a contrast medium, regions of abnormal enhancement, reflecting extravasation of iodine through a damaged BBB, have frequently been reported in patients with multiple sclerosis.²⁻⁴ The detection of these enhancing areas was found to be markedly increased when the patients were studied during recent clinical exacerbation⁵ and when a high volume delayed CT scanning technique (HVD-CT) was employed.⁶⁻⁸ However, enhanced CT shows only a BBB alteration without being able to quantify the alteration itself.

The measurement of BBB permeability in humans has recently been obtained using positron emission tomography (PET).⁹⁻¹³ In a previous paper¹⁴ we described a simple and non invasive method for quantifying BBB permeability using 68-Ga-EDTA and PET. The technique was found to be sensitive and accurate for the measurement of BBB disruption associated with brain tumours.

The purpose of the present investigation was to elucidate the value of 68-Ga-EDTA and PET in the study of BBB abnormalities associated with multiple sclerosis.

Material and methods

Patients A total of 15 patients with multiple sclerosis, three men and 12 women, aged 17 to 51 years (mean 30 yr) and four age-matched control subjects were selected for the study. The diagnosis of definite multiple sclerosis was determined by the clinical criteria of Schumacher et al.¹⁵ CSF studies and evoked potentials were available in all patients. Magnetic resonance imaging was performed on five patients.

Eight patients experienced a clinical exacerbation at the time of the study; two were in remission and five had a chronic progressive form of multiple sclerosis. All patients had clinical or CT scan suggestion of at least one supratentorial area of the demyelinating process, although in the five patients with the chronic progressive form of the disease, the clinical decline was evidenced mainly by neurological
Quantitative assessment of blood-brain barrier permeability in multiple sclerosis using 68-Ga-EDTA and PET

Table 1  HVD-CT and PET scan findings in 15 patients with definite multiple sclerosis

<table>
<thead>
<tr>
<th>Disease's activity</th>
<th>HVD-CT scan</th>
<th>PET scan focal abnormal 68-Ga-EDTA-uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low density areas</td>
<td>Large CSF</td>
</tr>
<tr>
<td>Exacerbation (n = 8)</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Remission (n = 2)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Chronic progression (n = 5)</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Total (n = 15)</td>
<td>12</td>
<td>10</td>
</tr>
</tbody>
</table>

signs and symptoms related to a spinal cord involvement. Patients had no steroid or other pharmaceutical treatment at the time of the study; in those with acute relapses, the beginning of therapy was postponed until the completion of the HVD-CT and PET study. Consent to participate in the study was signed by all patients and volunteers prior to initiation. HVD CT scan After a non contrast CT scan, routine enhancement (40 gm of iodine) was administered and a CT scan was performed. When the scan terminated, a second dose of contrast medium (40 gm of iodine) was administered and a delayed (45-60 minutes) scan was also obtained. PET scan The complete procedure used for studying BBB permeability with 68-Ga-EDTA and PET has been described elsewhere. After a transmission scan to allow for attenuation correction, 6-8 mCi of 68-Ga-EDTA were injected intravenously. Then 16 scans, 300 seconds each, were performed on a single ring Neuro-ECAT scanner (CTI) with a spatial resolution of 8.7, 1.0 mm (mean, SD) full width at half maximum (FWHM) in a slice of 16 MM. The tomographic level of the slice examined was selected on the basis of CT enhancement, when present. Otherwise, a standard slice through the body of the lateral ventricles, approximately at OM + 6 cm was chosen.

The measurement of 68-Ga-EDTA passage across BBB was achieved by multiple time graphical analysis. This method allows for the simultaneous calculation of a blood to brain influx constant Ki (ml g⁻¹ min⁻¹) and of the plasma volume Vp (ml g⁻¹). The tracer time activity curve in the blood, corresponding to the input function, was calculated by a non-invasive method that avoids arterial sampling. This method utilises a region of interest (ROI) drawn on the sagittal sinus and one venous blood sample. The time activity curve derived from the sinus image was converted in absolute terms by measuring the actual activity of the syringe blood withdrawn at a fixed time. The values measured with this procedure are comparable to those derived using standard arterial sampling.

The spatial activity distribution in the scanned plane was reconstructed using a filtered back projection with the ramp filter and an attenuation correction measured by transmission scan. After reconstruction, parametric images of Ki and Vp were obtained utilising the entire 16 scans dynamic sequence.

Small ROIs containing a minimum of 30 pixels were placed on the areas showing an abnormal 68-Ga-EDTA uptake in the parametric images. The values obtained from those ROIs were plotted in a graphic form according to the multiple time graphical analysis method, and used to calculate Ki and Vp. The pattern of the plotted data was linear in all the measurements thus indicating the progressing accumulation of the tracer in the brain during the entire scanning period. The Ki values of these regions were considered of pathological significance when exceeding the 2SD of the mean of the normal values.

Additionally in all patients and in normal subjects, four standard ROIs were placed on the frontal and parietal lobes and averaged in order to obtain a whole-brain Ki and Vp value for each subject.

Fig 1  PET and HVD-CT scans of a patient with multiple sclerosis examined during an exacerbation of the disease. Note the focal increase of 68-Ga-EDTA uptake corresponding to the left periventricular CT enhanced area.
Results

Table 1 shows CT and PET scans abnormalities in multiple sclerosis patients divided into three subgroups according to the activity of the disease.

Focal areas of pathological accumulation of 68-Ga-EDTA were visualised in four patients, all examined during a clinical exacerbation of the disease. The location of the pathological areas was clearly related to the clinical relapse in three of these cases. In one patient, presenting with left hemiparesis, PET scan showed evidence of bilateral lesions. Extent and location of abnormal PET areas roughly corresponded with the enhanced areas seen on HVD-CT images, as shown in fig 1.

A dynamic PET sequence showing multiple areas of increased 68-Ga-EDTA uptake is reported in fig 2. In this patient, an early 68-Ga-EDTA pathological uptake is clearly detected on the right hemisphere, whereas multiple pathological areas on the left hemisphere appear only in the delayed scans. Ki value measured in the area of right hemisphere was three times higher than the corresponding value in the left hemisphere, suggesting a different degree of regional BBB impairment. Table 2 shows mean Ki and Vp values in brain areas of multiple sclerosis patients, showing a significant increase of BBB permeability. The increased Ki value in these areas was not accompanied by a parallel increase of the vascular space (Vp), which was within the normal range.

Table 2 Mean Ki and Vp values in “abnormal” ROIs of multiple sclerosis patients (a) and in “normal” ROIs of control subjects (b)

<table>
<thead>
<tr>
<th></th>
<th>Ki x 10^-4 (ml.g^-1.min^-1)</th>
<th>Vp (ml.g^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) ROIs with increased B-B-B permeability (n = 7)</td>
<td>12.5, SD 3.4*</td>
<td>3.3, SD 0.3</td>
</tr>
<tr>
<td>(b) ROIs with normal B-B-B permeability (n = 4)</td>
<td>3.2, SD 0.9</td>
<td>3.7, SD 0.7</td>
</tr>
</tbody>
</table>

*p < 0.001 (t test)

Whole brain Ki and Vp values of multiple sclerosis patients and four normal subjects are shown in table 3. No significant difference was found for either the parameters between normal subjects or each of the multiple sclerosis subgroups.

Discussion

PET has been employed to assess BBB integrity in patients with cerebral infarction, brain tumours and dementia. Recently, Brooks et al found normal BBB permeability in multiple sclerosis. However, their patients were in remission at the time of the study.

As frequently reported we also have observed a correlation between BBB alteration and clinical activity of multiple sclerosis. Both HVD-CT and PET studies clearly showed areas of BBB abnormalities in four of the eight patients examined during “acute exacerbation” before the initiation of antinflammatory treatment. The negative findings in the remaining four patients could be explained by the fact that clinical exacerbations were associated with new symptoms reflecting an involvement of brain stem in two cases, and optic nerve and spinal cord in one case each. The absence of a pathological BBB increased permeability is justified in two patients examined during clinical remission and in five patients with chronic progressive multiple sclerosis whose clinical decline was shown mainly by signs and symptoms related to spinal cord involvement.

In the present series enhancing areas demonstrated by the HVD-CT scan were also detected by PET. However, a comparison of these two techniques was not the aim of this study and it is further limited at the present time since a single ring positron camera was
Quantitative assessment of blood-brain barrier permeability in multiple sclerosis using 68-Ga-EDTA and PET

Although pathological studies have demonstrated that only "fresh plaques" show a BBB disruption, it is still unknown whether a relationship may exist between the entity of BBB damage and the "age" of the plaque. Theoretically one may argue that the temporal evolution of BBB damage in multiple sclerosis may reflect the underlying inflammatory reaction, thus resulting in a greater BBB alteration during the "peak" of the cellular infiltration. Future sequential quantitative PET studies will be necessary in order to clarify further this issue.

In conclusion, this study demonstrates that PET using 68-Ga-EDTA is a sensitive method for the detection and measurement of BBB dysfunction associated with multiple sclerosis and may be usefully employed to evaluate the effect of therapies on BBB abnormalities during the exacerbation of the disease.

The authors are indebted to Aldo Bartiromo for his technical contribution. Mrs Patrizia Franco edited the manuscript. This work was supported by a grant from "Ministero Pubblica Istruzione, Progetti di Interesse Nazionale 40%", and by a grant from the National Council of Research (CNR) "Progetti Strategici".

References

Pozzilli, Bernardi, Mansi, Picozzi, Iannotti, Alfano, Bozzao, Lenzi, Salvatore, Conforti, Fieschi


