Correlation between radionuclides uptake and ultrastructural features of tight junctions in the capillary wall of meningiomas

P BAR-SELLA, D FRONT, E PEYSER, A TALMON, O ISRAEL, I NIR

From the Unit of Electron Microscopy, the Faculty of Medicine, Haifa, the Division of Nuclear Medicine and Department of Neurosurgery, Rambam Medical Center, Haifa, Israel

SUMMARY The uptake of Tc-99m pertechnetate and Co-57 bleomycin and the ultrastructure of small blood vessels were studied in 10 cases of meningioma. When thin sections of 120 contact areas between adjacent endothelial cells were examined, 92% of the intercellular contacts contained one to three punctate tight junctions. Freeze fracture preparations revealed fascia oculudens with one to four sealing strands. These findings are consistent with the high permeability the meningiomas displayed for both of the radiopharmaceuticals.

The normal brain capillaries, having a “blood barrier”, are not permeable to appreciable amounts of radiopharmaceuticals, contrast materials, and many other substances. The ultrastructural basis for this barrier is the presence of belts of tight junctions between the endothelial cells. Also, normal blood capillaries are non-fenestrated and have very few pinocytic vesicles. The vessels of tumour are different from those of the normal brain. Their increased permeability permits the visualisation of these tumours by radionuclide imaging and computed tomography and the penetration of chemotherapeutic agents from the blood. In a previous preliminary study we compared the ultrastructure of tumour vessels with the in vivo Tc-99m pertechnetate uptake. In the present study a group of highly permeable tumours, meningiomas, was selected in order to study in depth the ultrastructural nature of the intercellular contacts. These were correlated with tumour uptake of Tc-99m, a common scintigraphic agent and Co-57 bleomycin, a labelled chemotherapeutic drug.

Materials and methods

Patients with meningiomas, whose clinical condition made possible detailed scintigraphic assessments, were included in this study. Routine brain scintigrams were done with Tc-99m pertechnetate. Co-57 bleomycin with a specificity of 1mCi Co-57/mg bleomycin was prepared from Co-57 chloride (Amersham Arlington) and bleomycin (Bristol-Myers Co, Syracuse, NY), using a technique described previously. One mCi of Co-57 bleomycin was injected intravenously and the head of the patient was imaged at 30 s after the injection and at 2 h, in the anterior and both lateral views. Data was collected with an Anger type camera for 10 min in each view. Eight patients were investigated by using both pertechnetate and labelled bleomycin, and two patients only with pertechnetate.

The meningiomas obtained at surgery were prepared for electron microscopy as previously described. Freeze fracture replicas were made using the methods described by Moor et al. and Polak-Charcon et al. Thin sections of 120 contact areas between adjacent endothelial cells of brain tumours were examined, as well as 50 contact areas between endothelial cells of the normal human brain removed during surgery. The total length of the membrane apposition between adjacent endothelial cells, the number of fusion sites, and the length of the fusion sites were measured.

Results

Early and marked uptake of both Tc-99m pertechnetate and Co-57 bleomycin by tumour tissue and the lack of uptake by normal brain tissue occurred in all patients examined (figs 1 and 2). The small blood vessels—capillaries and post-capillary venules—of the normal brain appeared to be non-fenestrated with very few pinocytic vesicles and belts of tight junctions present between adjacent endothelial cells (fig 3). The average length of membrane apposition...
Correlation between radionuclides uptake and ultrastructural features of tight junctions

between the adjacent endothelial cells was 1.7 μm and the average length of membrane fusion sites was 1.3 μm. Measurements of 50 contact areas between adjacent endothelial cells are summarised in the table.

In the meningiomas the small blood vessels were found to be of the fenestrated type (fig 4) with numerous pinocytic vesicles (fig 5). Intercellular boundaries between adjacent endothelial cells showed short membrane appositions and punctate type tight junctions (figs 4 and 5). Fusion sites between adjacent endothelial cell membranes were few and short (table). However, in 92% of the observed intercellular boundaries, at least one fusion site was a punctate fusion site on the apposing endothelial cell membrane and one fenestrae can be clearly visualised. (× 9000)

Table Measurements of appositional areas of endothelial cells in small blood vessels in ten meningiomas

<table>
<thead>
<tr>
<th>No of appositional areas (% of total)</th>
<th>Length of appositional areas (μm)</th>
<th>No of appositional areas with fusion sites (% of total)</th>
<th>Number of fusion sites</th>
<th>Average length of fusion site (μm)</th>
<th>No of appositional areas without fusion sites (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56 (46.7%)</td>
<td>0-1-1-0</td>
<td>50 (41.6%)</td>
<td>1-3</td>
<td>0.045</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>46 (38.3%)</td>
<td>1-1-2-0</td>
<td>42 (35.3%)</td>
<td>1-3</td>
<td>0.045</td>
<td>4 (3.3%)</td>
</tr>
<tr>
<td>18 (15%)</td>
<td>2-1-3-0</td>
<td>18 (5%)</td>
<td>2-4</td>
<td>0.045</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>40 (80%)</td>
<td>1-0-2-0</td>
<td>40 (80%)</td>
<td>continuous line</td>
<td>1.3</td>
<td>none</td>
</tr>
<tr>
<td>10 (20%)</td>
<td>2-1-3-0</td>
<td>10 (20%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
site was observed. In cells with longer intercellular boundaries, the appositional areas were convoluted and included in two to four fusion sites. The length of each fusion site in the luminal-basal axis measured between 0.02 to 0.08 μm. The remaining appositional area was open (figs 4 and 5). In 8% of the thin sections there were no fusion sites at all between the adjacent cell membranes and completely open gaps

Fig 5 The endothelial wall of a small blood vessel of a menigioma. One punctate fusion site, and pinocytotic vesicles can be visualised on the endothelial cells. (× 53 000)

Fig 6 (a) Fractured membranes of endothelial cells showing a short strand of tight junction (fascia ocludens). In higher magnification (b) this tight junctional element appears as a discontinuous array of particles. (× 65 000)
were visualised.

In freeze fracture preparations variations were seen in the number, size and degree of the junctional elements. Tight junctions appeared mostly as "fascia ocludens" and were composed of one to four sealing strands (fig 6a). In only a few replicas five to six strands were seen and most areas contained only one junctional strand. There was some anastomosing of parallel strands (fig 7), but most fibrils were not anastomosed and contained discontinuous arrays of particles.

Discussion

All the meningiomas examined showed early and markedly increased uptake of Tc-99m pertechnetate (molecule with a volume of 40Å³) and Co-57 bleomycin (molecule with a diameter of 30 to 40Å) indicating increased permeability to these agents. Previously, the typical feature of the meningioma capillaries was considered to be the absence of tight junctions between adjacent endothelial cells. This correlated well with scintigraphic findings. Our observations of the thin sections and the use of freeze fracture techniques indicated the presence of tight junctions which is still consistent with very high permeability. In 90% of the appositional areas there was at least one short fusion site between the adjacent cells. In freeze fracture replicas the tight junctions appeared as "fascia ocludens" and were composed of one to four sealing strands.

Several studies correlated the freeze etch morphology of tight junctions between epithelial cells and their permeability as determined by electrical resistance measurements. Junctions with one to two sealing strands were considered as "very leaky" and those with one to six strands with depth of 0.1 to 0.8 μm in the apical basal direction were considered as "leaky" junctions. Using these criteria, the junctions between the endothelial cells of meningioma capillaries observed in our study can be defined as both leaky and very leaky. The appearance of the tight junctions as fascia ocludens may explain our previous observations of entirely open routes between the endothelial cells observed in thin sections. This may have been caused by a sectioning plane through a zone devoid of sealing strands. The fine structure of the junctional elements between the endothelial cells showed some special properties. Although they were discontinuous strands of random distribution similar to the structure of leaky venules in muscle, they had arrays of many protruding particles, like the structure of capillaries described in muscle with stronger cell to cell interaction.

It should be mentioned that although the vessels of the meningiomas studied also showed fenestration which may be associated with increased permeability, previous studies and our unpublished data show increased permeability to both pertechnetate and Co-57 bleomycin in non-fenestrated vessels. The role of pinocytic vesicles remains to be clarified.

It may be concluded that thin section findings of at least one fusion site between adjacent endothelial cell membrane in more than 90% of the intercellular boundaries with the laminal-basal axis of 0.1 to 0.8 μm and freeze fracture preparations with tight junctions appearing as fascia ocludens composed of one to four sealing strands are compatible with high permeability both to Tc-99m pertechnetate and Co-57 bleomycin. Such findings in brain tumours other than meningiomas may indicate that their vessels are permeable to chemotherapeutic drugs with diameter smaller than 40 Å. It has been suggested that in the chemotherapy of brain tumours, the blood brain barrier is not a factor. The present study defines further the ultrastructural characteristics of vessel permeability in brain tumours.

This study was supported by the Israel Cancer Association.

References

1 Brightman MW, Kiatzo I, Olsson I, Reese TS. The blood-brain barrier to proteins under normal and


