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Measurements of Blood–Brain Barrier Permeability in Patients Undergoing Radiotherapy and Chemotherapy for Primary Cerebral Lymphoma

R.J. Ott, M. Brada, M.A. Flower, J.W. Babich, S.R. Cherry and B.J. Deehan

Positron emission tomography (PET) has been used to measure changes in regional blood–brain barrier (BBB) permeability in patients with primary cerebral lymphoma undergoing radiotherapy and chemotherapy. The method employed is to measure the rate of wash-out of a radioactive tracer (^{68}Ga-EDTA) from blood into brain tissue using time-sequence PET imaging. Preliminary studies carried out on patients with more common primary cerebral tumours show that time-activity data are reproducible to ~10%. Measurements made in 2 patients with primary cerebral lymphoma treated with initial chemotherapy showed significant changes in permeability in the region of the tumour. Within 5 weeks of the start of treatment, permeability values reached the levels of normal brain. No changes in BBB permeability in normal brain were seen immediately after radiotherapy.
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INTRODUCTION

THE BLOOD–BRAIN barrier (BBB) acts as a selective functional barrier which allows a limited number of naturally occurring substances access to brain tissue. Many substances, including a wide variety of drugs and toxins, are also excluded from entering the brain by the BBB. The integrity of the BBB is therefore fundamental to the regulation of material exchange between the blood and the brain tissue and plays an important role in preserving normal brain function. Substances such as glucose and essential aminoacids which are required in brain tissue are transported across the BBB by specific membrane transport systems. The permeability of the BBB in normal brain to other agents depends both on the lipid solubility of the compound and on its molecular weight.

In pathological conditions, such as primary or secondary intracranial tumours, the BBB is disturbed, leading to a large local increase in capillary wall permeability. As a result, substances which are usually excluded from the brain can enter the tissue. This alteration in permeability forms the basis for imaging lesions by exploiting the accumulation, in regions of BBB disruption, of compounds such as contrast agents [in computed tomography (CT) and magnetic resonance imaging (MRI)] or radiopharmaceuticals (in nuclear medicine) which are normally excluded by intact BBB. Much interest to date has centred around the imaging of cerebral tumours which disrupt the BBB, leading to local increases in permeability. The use of dynamic positron emission tomography (PET) imaging combined with a suitable model [1, 2] means that BBB permeability can now be measured quantitatively *in vivo* for the first time.

Drug resistance probably accounts for the disappointing results of chemotherapy in most primary intracranial tumours, although poor drug access may also contribute. Primary cerebral lymphoma (PCL), which is histologically identical to large-cell non-Hodgkin lymphoma (NHL) presenting peripherally, may, in terms of chemoresponsiveness, be one of the exceptions among primary brain tumours. Yet with conventional radiotherapy and chemotherapy, the long-term control of this tumour has been elusive [3], while at peripheral sites it is often curable, particularly if localised [4].

Some authors have demonstrated chemoresponsiveness of recurrent PCL, occasionally with conventional drugs effective against NHL [5] and in other instances with high-dose methotrexate [6]. To exploit the potential effectiveness of chemotherapy in PCL, we performed a pilot study of initial chemotherapy in patients presenting with PCL [3]. The drugs used in the selected treatment regimen (methotrexate/doxorubicin/cyclophosphamide/vincristine/prednisone/bleomycin, MACOP-B) [7] are known for their effectiveness in diffuse large-cell systemic NHL, but most of the agents do not cross the intact BBB.

We have applied the technique of measurement of BBB permeability with PET scanning to assess the sequential quantitative changes within the tumour and surrounding normal brain during therapy of PCL, a primary cerebral tumour known to respond initially to chemotherapy. Because of the rarity of PCL, we have studied the reproducibility of the method in patients with other primary cerebral tumours. 2 patients with active high grade astrocytomas and 1 with supracellular germinoma in remission were selected. The findings may serve as a guide to designing future treatment strategies in PCL which has so far defied most therapeutic attempts.

Theory

The theory of the technique has been outlined recently by Webb *et al.* [8]. Using ^{68}Ga-EDTA, it is possible to measure the transport of the tracer from plasma to tumour and normal brain tissue and, in addition, to measure the cerebral plasma volume associated with the region under study. The basis of the method is given by equation 1.

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$$C_{PET}(T) = K_i(1-V_p/(1-CHt))\int_0^T C_p(t)\exp^{-k_2(T-t)}dt + C_p(T)V_p \quad (1)$$

where C_{PET} is the concentration (Bq/ml) of the tracer at time T in the region of interest (ROI) as measured by the PET camera; C_p is the isotope concentration (Bq/ml) in the plasma; K_i is the influx constant or rate of uptake (ml/min/g) of ^{68}Ga-EDTA into brain from the blood; V_p is the plasma vascular volume (ml/g) including those compartments in parallel with the BBB complex which rapidly exchange with the plasma; CHt is the cerebral haematocrit; and k_2 is the outflux constant (per min) from cerebral tissue into the blood. K_i is related to the permeability × surface area product (PS) of the tissue. If the value of PS is much less than the tissue blood flow, $K_i = PS$. This relationship holds in normal brain and for all tumour sites studied to date [9].

This relationship was first proposed by Patlak [2]. If the value of k_2 is zero, i.e. the transport of the tracer is unidirectional, then the ratio of $C_{PET}(T)/C_p(T)$ against the time integral in equation 1 for different values of T will show a linear relationship and, using standard fitting methods, K_i and V_p may be estimated. This is known as a multiple time graphical analysis (MTGA) [2]. If the MTGA plot does not show a clear linear phase, this indicates a significant backflux from brain to blood (non-zero k_2). The magnitude of this backflux will depend very much on the nature of the BBB breakdown and on the time scale of the measurements.

It has been shown [10] that measurements in regions of tumour made with ^{68}Ga-EDTA over a short timescale (~10–15 min) are dominated by the values of K_i and V_p and that little information can be obtained about k_2. Measurements extending beyond 40 min can allow all three parameters to be estimated using equation 1. Accurate extraction of the values of K_i, V_p and k_2 requires a more sophisticated iterative procedure [10] in the presence of significant backflux.

Iannotti *et al.* [9] proposed that in order to overcome the requirement for arterial plasma measurements to determine the value of C_p, it was possible to measure a single venous blood sample to calibrate the activity levels in an ROI over the sagittal sinus obtained during brain imaging. We propose a further modification to this which involves frequent venous sampling from early times. This leads to a more accurate determination of the venous activity levels than can be obtained from ROI analysis of the sagittal sinus on sequential PET images. Strictly, both methods are inaccurate with respect to equation 1 due to the difference between arterial and venous concentrations in the time period up to 5 minutes after injection. This inaccuracy means that the values of K_i, V_p and k_2 measured here are proportional rather than equal to those defined in equation 1.

PATIENTS AND METHODS

Imaging and quantification

60–150 MBq ^{68}Ga-EDTA was injected intravenously as a bolus. Patients were scanned in an individually moulded cabulite (cellulose acetate) mask as used for immobilisation during radiotherapy. Head fixation was reproducible to within 3 mm. Time sequence scanning was performed using the MUP-PET large area positron camera [11] and a series of volume images (64^3 matrices, 6 mm voxels) produced during a total period of 35–40 min. Each scan took 5–10 min. Venous blood sampling was taken to determine the time course of C_p. The blood samples were centrifuged and a 1 ml aliquot of plasma measured in a sodium iodide well-counter to determine the plasma activity concentration in Bq/ml. Cerebral haematocrit was determined, in each case, as 0.69 × peripheral haematocrit [9].

Images were reconstructed using a back projection and deconvolution method [12] and corrected for attentuation using an approximate weighting function during image reconstruction. Image cross-sections were chosen to display the tumour and the sagittal sinus and ROI analysis carried out on tumour, normal brain, background outside the brain and on the sagittal sinus. All ROI data were corrected for system dead-time and for radioisotope decay. A 20 cm diameter cylindrical phantom containing a known concentration of radioactivity was scanned under the same conditions as patients and images reconstructed using identical parameters. From this it was possible to obtain a calibration factor to allow the time-activity curves for each ROI to be expressed in Bq/ml.

Clinical studies

Prior to PET imaging, informed consent was obtained from each patient.

Reproducibility. To establish the reproducibility of the technique 3 patients with primary intracranial tumours were scanned on two occasions each. Patient 1 was a 29-year-old woman treated between December 1986 and May 1987 with combined chemotherapy and radiotherapy for a suprasellar germinoma. At the time of PET scanning she was free of disease with a residual unenhancing defect in the suprasellar region. Single tomographic scans were taken 1 week apart with no treatment during this period.

Patient 2 was a 20-year-old man with high-grade astrocytoma deep in the left parietal lobe which was partially excised. Time sequence tomographic scans were performed prior to radiotherapy and immediately on completion. He received involved field radiotherapy to a tumour dose of 55 Gy in 36 fractions over 4 weeks on a twice daily accelerated radiotherapy schedule. During this time there was no change in the patient's clinical status. The post-treatment brain CT demonstrated some increase in the low density region surrounding the tumour but no marked alteration in the enhancing tumour.

Patient 3 was a 57-year-old man with recurrent high grade astrocytoma in the left temporal lobe. He was treated 2 years previously with radical radiotherapy (55 Gy in 33 fractions over 6.5 weeks) and following a first recurrence 7 months after completion of radiotherapy with lomustine/cisplatin/vincristine/procarbazine chemotherapy. Time sequence tomographic scans were taken on two occasions at a 1 week interval during which time he did not receive any active treatment. The dose of corticosteroids (dexamethasone) was being reduced and his clinical condition did not significantly alter.

Monitoring of changes through therapy. Changes in BBB permeability were assessed in 2 patients with PCL treated on a protocol of combined modality therapy (CMT), the results of which have been reported elsewhere [3]. Both patients were married heterosexual males and neither had AIDS or its predisposing factors. Patient 4 was 42 years old and had enhancing lesion lying anterior to the frontal horn of the left lateral ventricle with apparent subependymal tumour spread along the lateral and third ventricular surfaces. Diagnosis was made by a stereotactic needle biopsy and histology was diffuse malignant lymphoma composed of small cleaved cells (group E on Working

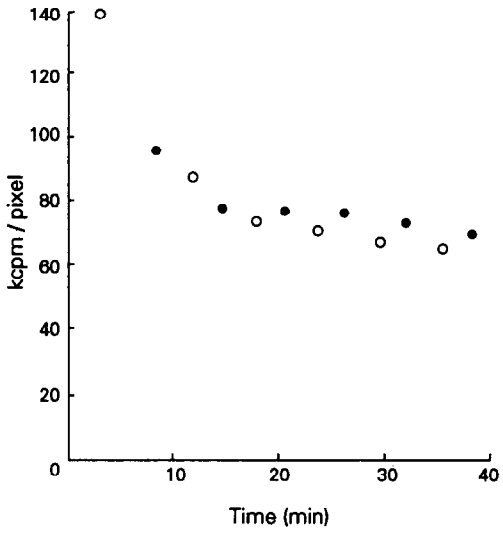

Fig. 1. Time-activity data from tumour ROI for two studies, 1 week apart ROI (patient 3). ○ = study 1, ● = study 2.

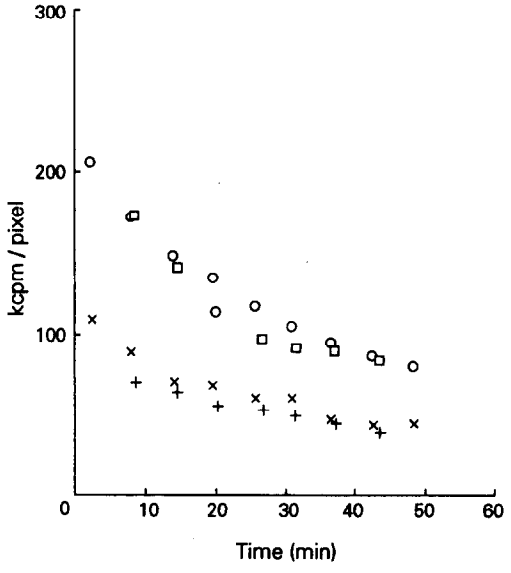

Fig. 2. Time-activity data from tumour pretherapy (○) and post-radiotherapy (×) and sagittal sinus (SS) ROI before (□) and after radiotherapy (+) (patient 2).

Formulation). Patient 5 was 63 years old, and had multiple enhancing masses. Burr-hole biopsy confirmed diagnosis of PCL (diffuse large and small cell lymphoma, group F). Both patients were treated with MACOP-B weekly chemotherapy regimen [7] alternating cyclophosphamide and doxorubicin (weeks 1, 3 and 5) with methotrexate and vincristine (weeks 2 and 6) and bleomycin and vincristine (week 4) together with oral prednisolone. Both patients received a 6-week course of chemotherapy. After a further 4-week break both patients underwent a course of radiotherapy: patient 4 received craniospinal irradiation (30 Gy to whole brain, 30 Gy to the spine and 55 Gy to tumour site); patient 5 received 40 Gy to the whole brain. Both patients were scanned at weekly intervals prior to the start and on the day just prior to weeks 2, 3 and 4 of chemotherapy. The PET scan was repeated prior to the start of radiotherapy and at 6 weeks after completion of treatment.

Patient 4 recurred in the left parietal lobe 10 months after diagnosis and died at 12 months of uncontrolled intracranial disease. Patient 5 remains alive at 17 months from diagnosis.

RESULTS

Reproducibility

The data associated with the preliminary evaluation study indicate the level of reproducibility. Figure 1 shows time-activity curves from the tumour ROI analysis of patient 3, illustrating the level of count density as a function of time for two scans taken 1 week apart. The data are normalised to the injected activity in this figure. Values for K_i and V_p could not be obtained from the first scan of this patient due to the technical difficulties during blood sampling. The second scan provided values of 600 (S.D. 50) $\times 10^{-4}$ ml/min/g (K_i) and 490 (40) $\times 10^{-3}$ ml/g (V_p). Figure 2 shows measurements made for patient 2 undergoing radiotherapy. The data are normalised to injected activity and indicate little change in time-activity measurements between the two scans. These results are supported by the minimal changes in tumour vascularity as seen on the X-ray CT. Analysis of the time-activity data for this patient gave values of 67 (27) $\times 10^{-4}$ ml/min/g (K_i) and 470 (20) $\times 10^{-3}$ ml/g (V_p) from the first study and 50 (5) $\times 10^{-4}$ ml/min/g (K_i) and 510 (10) $\times 10^{-3}$ ml/g (V_p) from the second. The tomographic images obtained from patient 1 also show that measurements of activity in various ROI in the brain are reproducible to 10%. As no time course study was performed in this patient, no values of K_i and V_p are available.

Monitoring of changes through therapy

Figure 3 shows two MTGAs carried out on patient 4. Within errors the data can be fitted adequately using a linear response and the values of k_2 are small in both studies. Figure 4 shows an image of patient 4 showing BBB damage at the site of tumour seen on brain CT (Fig. 5). Both images were taken immediately prior to the start of therapy. Table 1 shows the values of K_i and V_p for the 2 lymphoma patients. Plots of the values of K_i against time after start of treatment (Figs 6, 7) show a significant fall in the permeability of the tissue during chemotherapy. These data show that the value of K_i reaches that of normal brain after 5

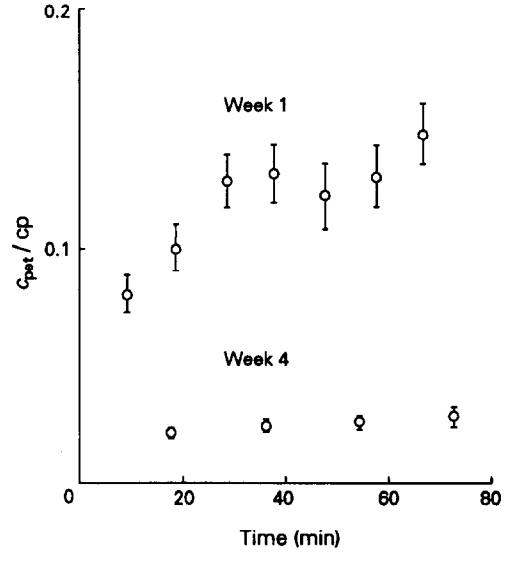

Fig. 3. Multiple time graphical analysis curves for patient 4 for data obtained at weeks 1 and 4.

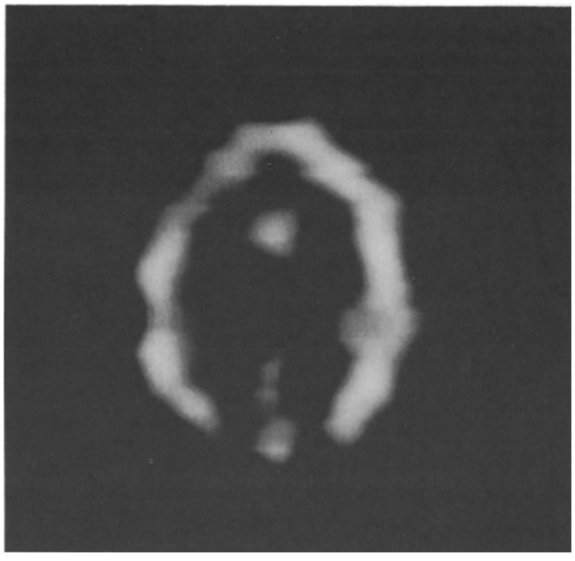

Fig. 4. 6 mm thick transaxial PET section through the brain of patient 4 taken prior to therapy.

weeks from the beginning of treatment. A repeat X-ray CT at 6 weeks after start of chemotherapy showed a considerable reduction in the size of the enhancing mass with little BBB damage visible. The high K_i value in patient 4, week 21 was due to tumour recurrence (Fig. 6).

DISCUSSION

The basic methodology described here has been used elsewhere [1, 9] to measure single values of K_i, V_p and k_2. These previous results indicate that, for brain tumours, K_i can vary between 2×10^{-4} and 120×10^{-4} ml/min/g. This is to be compared with normal brain values of $2–5 \times 10^{-4}$ ml/min/g. Similarly, tumour values for V_p appear to be 0.02 and 0.2 ml/g as compared with normal brain varying between 0.02 and 0.05 ml/g. Finally the values for k_2 appear to lie in the range 0.01–0.04 min. The accuracy of these measurements is clearly determined by the sensitivity of the parameters to the variables in the study. For instance, the accuracy of k_2 is very much determined by the length of the study, whereas values of V_p and K_i are determined by the early time frames.

It has been shown [10] that it is possible, with MUP-PET, to estimate these parameters for ^{68}Ga-EDTA washout to approximately 20% for a 30 ml tumour, using a scanning protocol of 5 minute frames. Shorter time-frames at the early part of the studies would improve the precision of the values obtained for K_i and V_p but the present sensitivity of MUP-PET precluded such measurements. Uncertainties in CHt lead to small errors in the parameters which can be neglected. More significant are inaccurate corrections for scatter and accidentals which may lead to systematic errors in all three parameters. At present,

Table 1. Values of K_i and V_p obtained from scans of 2 patients with PCL

		Week							
		1	2	3	4	5	9	21	22
Patient									
4	K_i*	20	15	5	5	–	5	110	–
	V_p†	185	205	130	30	–	140	150	–
5	K_i*	80	40	65	25	5	–	–	15
	V_p†	130	190	85	135	185	–	–	120

K_i in 10^{-4} ml/min/g, V_p in 10^{-3} ml/g.
Scans performed 1 day prior to week shown.
Chemotherapy carried out from weeks 1–6.
Radiotherapy carried out between 5th and 6th measurement.
S.E. estimated to be * 5×10^{-4} ml/min/g and † 30–40 $\times 10^{-3}$ ml/g.

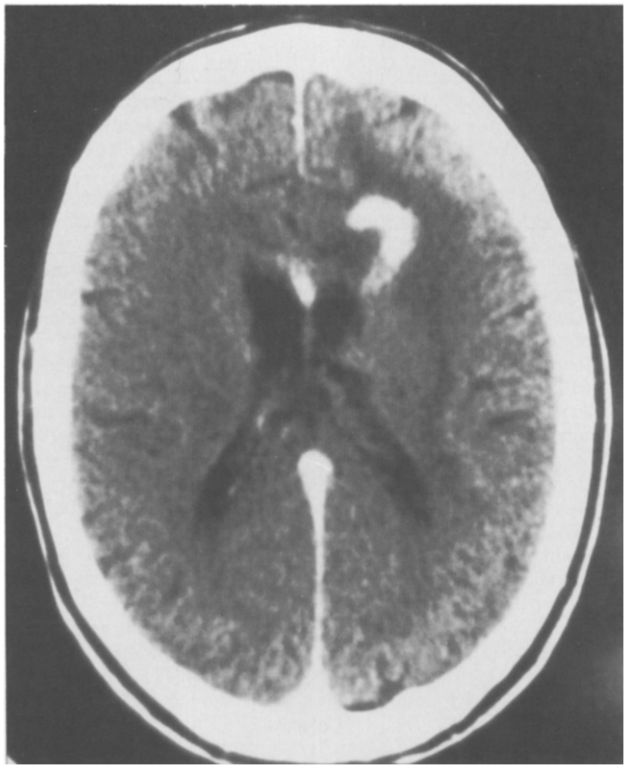

Fig. 5. CT of patient 4 taken at time of first PET scan in Fig. 4.

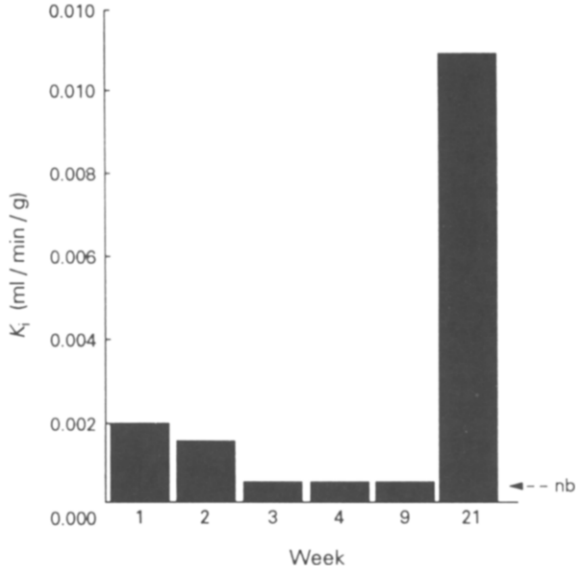

Fig. 6. Values of K_i through therapy of patient 4. nb shows permeability level of normal brain.

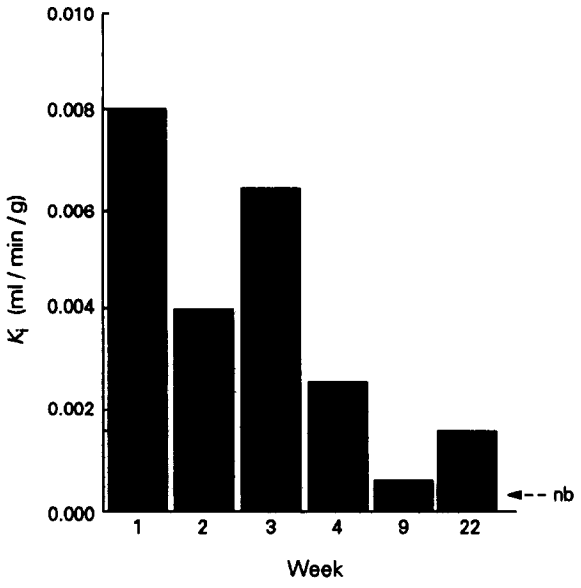

Fig. 7. Values of K_i through therapy of patient 5. nb shows permeability level of normal brain.

these corrections are made assuming a uniform distribution of background events across the 3-D image and that ROI placed outside the regions of the brain accurately sample scatter and accidental event rates [10]. Errors in these measurements can lead to large uncertainties in the values of K_i and V_p for normal brain tissue, where the concentration of radioactivity gives count rates close to that from scatter and random events. The use of venous rather than arterial plasma samples is most likely to affect the estimates of V_p. The magnitude of these errors will depend on the exact form of the time-activity curves but could be of the order of 15–60% for V_p and 5–15% for K_i.

Given these levels of uncertainty, however, it can be seen from Figs 6 and 7 that in 2 patients with PCL the value of K_i dropped significantly throughout the period of chemotherapy. The reduction in the value of K_i corresponded with the apparent disappearance of lesion enhancement on CT. It is difficult to equate such changes with the disappearance of tumour rather than the simple normalisation of BBB at the tumour site. The absence of enhancement does not exclude the presence of underlying tumour, whereas the presence of enhancement usually correlates with the presence of active tumour. We will correlate the disappearance of BBB lesions with tumour activity using aminoacid metabolism studies in the near future.

Experience in the use of chemotherapy in PCL is limited [3, 5, 6]. Our results indicate a fast repair of BBB in the region of tumour with permeability reaching the normal brain value within 4 weeks of therapy. The molecular weight of Ga-EDTA is 360, so this tracer is excluded by intact BBB. Agents used in MACOP-B chemotherapy, particularly doxorubicin and cyclophosphamide, are hydrophilic, of molecular weight 543 and 361, respectively, and are unlikely to penetrate the normal BBB. Return of BBB to normal brain values suggests that continuing use of these agents beyond 4 weeks is unlikely to achieve penetration of a significant amount of drug to the primary tumour site and consequently to the residual lymphoma cells. The absolute value of K_i may also be an important factor determining drug access to tumour but it will be necessary to study a large number of patients to answer this question. PET studies of surrounding normal brain indicate that these agents would also penetrate these regions very poorly. If lymphoma cells are present outside the enhancing CT margin and the area of high BBB permeability seen on a PET scan, conventional chemotherapy in this region is unlikely to be effective. In both lymphoma patients there was no change in BBB permeability in normal brain during chemotherapy, or before and immediately after radiotherapy. The values of the parameters obtained by this methodology are within the physiological range expected [9].

These results must be interpreted with caution as they are based only on 2 patients with PCL. Nevertheless, the results indicate that BBB permeability may be an important determinant of outcome in PCL treated with chemotherapy. If future studies confirm our observations then, as PCL is a chemosensitive tumour [3, 5, 6], new treatment strategies should consider modifying BBB permeability and the use of more lipophilic agents. Further studies of alteration of BBB permeability in chemosensitive secondary tumours within the brain (e.g. teratoma, small cell lung carcinoma) may also help in establishing the role of chemotherapy in these situations.

CONCLUSION

Measurements of BBB permeability can be carried out quantitatively using dynamic PET imaging. Despite limited sensitivity, the MUP-PET positron camera was used to evaluate changes in BBB permeability of patients undergoing treatment for primary cerebral tumours. The results indicated dramatic changes in permeability in the region of the tumour in 2 patients with PCL responding to chemotherapy. The indications are that, within 5 weeks of the start of treatment, tumour-associated BBB permeability reaches a level found in normal brain, at which time little therapeutic benefit is expected from further conventional chemotherapy. No change in global BBB permeability was seen immediately after radiotherapy. The errors on the measurements indicate that changes in BBB permeability of the order of 20% would be detected.

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Ablation of Human Choriocarcinoma Xenografts in Nude Mice by Antibody-directed Enzyme Prodrug Therapy (ADEPT) with Three Novel Compounds

Caroline J. Springer, Kenneth D. Bagshawe, Surinder K. Sharma, Frances Searle, Joan A. Boden, Pari Antoniw, Philip J. Burke, Gordon T. Rogers, Roger F. Sherwood and Roger G. Melton

Three novel prodrugs have been designed for use as anticancer agents. Each is a bifunctional alkylating agent which has been protected to form a relatively inactive prodrug. They are designed to be activated to their corresponding alkylating agents at a tumour site by prior administration of an antitumour antibody conjugated to the bacterial enzyme carboxypeptidase G2 (CPG2) in a two-phase system called antibody-directed enzyme prodrug therapy (ADEPT). The K_m and V_{max} values for three different antibody–CPG2 conjugates were determined in relation to each prodrug. The K_m values ranged from 4.5–12 μmol/l and the V_{max} from 0.5–1.6 μmol/U/min. Athymic *Nu/Nu* mice with palpable transplanted human choriocarcinoma xenografts, which are resistant to conventional chemotherapy, were treated with anti-human chorionic gonadotropin antibodies conjugated to CPG2. This was followed by each of the three novel prodrugs. Significant increase in survival was obtained in three of the regimens tested using only one course of treatment. This demonstrates the potential of a tumour-localised bacterial enzyme to activate protected alkylating agents in order to eradicate an established human xenograft.
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INTRODUCTION

CANCER CHEMOTHERAPY is hampered by the low therapeutic index of most anticancer drugs. Selective generation of a cytotoxic active drug from an inactive prodrug at the tumour site has become, therefore, an important goal. Ideally, the drug, once generated, should interact quickly at the site of its formation, for if it exhibits slow uptake at the tumour and/or has high diffusibility away from the tumour the advantage will be lost [1].

Antibody-directed enzyme prodrug therapy (ADEPT) [2, 3] separates the cytotoxic function from the targeting function in a two-phase system which has several benefits over a one-phase chemo- or radioimmunoconjugate. Radioisotopes linked to antibodies cause normal tissue cytotoxicity during their clearance phase and before their preferential retention in tumours can dominate the distribution pattern. With chemoimmunoconjugates only a limited number of cytotoxic molecules can be linked to an antibody molecule, internalisation of the drug–antibody conjugate may present problems and it is necessary to achieve targeting to most cells in the tumour mass. The amplification inherent in the enzyme component of an antibody–enzyme conjugate may compensate for the low proportion of administered antibody retained in tumours *in vivo*. Alkylating agents are good candidates for ADEPT in that their cytotoxicity is dose-related and they can be given repeatedly with less induced resistance than other classes of anticancer agents [4].

A series of three novel prodrugs 4-[*bis*(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid, 4-[2-chlorethyl)(2-mesyloxyethyl)amino]benzoyl-L-glutamic acid and 4-[*bis*(2-chloroethyl)amino]benzoyl-L-glutamic acid has been synthesised [5] for use in ADEPT. Each drug is a bifunctional alkylating agent in which the activating effect of the ionised

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