Metabolic Changes in Cerebral Gliomas Within Hours of Treatment With Intra-Arterial BCNU Demonstrated by Phosphorus Magnetic Resonance Spectroscopy

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ABSTRACT: A 40-year-old female with a recurrent mixed astrocytoma/oligodendroglioma was treated with intra-arterial BCNU at six week intervals. Phosphorus magnetic resonance spectroscopy was performed before, and on two occasions after her third treatment.

Before treatment, phosphodiesters were 25% less than normal and intracellular pH was 7.14 (normal 6.97 ± 0.02). Eight hours following treatment phosphocreatine and phosphodiesters were reduced by ~40% and pH_i increased to 7.24. Thirty-two hours after treatment, phosphocreatine and phosphodiesters had reversed their decline, but pH_i had increased further to 7.35. MRI and x-ray CT scans did not show any change during this period.

This study demonstrates that chemical changes can be observed in a glioma by magnetic resonance spectroscopy shortly after chemotherapy in a clinical setting and before changes are observable by imaging modalities. This approach evidently offers a possible means of monitoring the acute metabolic response of tumours to chemotherapy or other forms of treatment by a non-invasive repeatable quantitative method.

RÉSUMÉ: Altérations dans les gliomes cérébraux induites dans des heures après le BCNU: mise en évidence de la spéctroscopie à la résonance aimantée au phosphore. Nous avons traité une femme âgée de 40 ans, atteinte d'un astrocytome/oligodendrogliome mixte, par le BCNU intra-artériel aux intervalles de six semaines. La spéctroscopie à la résonance aimantée fut réalisée avant, et deux fois après le troisième traitement.

Avant la traitement, les phosphodiésters étaient 25 pour 100 moins que le niveau normal et le pH intracellulaire était 7,14 (n = 6,97 ± 0,02). Huit heures après le traitement, le phosphocréatine et les phosphodiésters étaient réduits de 40 pour 100 environ, et la pH_i s'est augmenté à 7,24. Trente-deux heures après le traitement, le phosphocréatine et les phosphodiésters avaient inversé son déclin, mais le pH_i avait changé jusqu'à 7,35. L'image à résonance aimantée et la tomodensitométrie ne montraient pas d'altérations pendant cette période.

Cette étude démontre que les altérations chimiques peuvent être mis en évidence dans un gliome par la spéctroscopie à résonance aimantée bientôt après la chimiothérapie avant que les altérations aux techniques radiographiques soient constatées. Cette méthode fournie possiblement un moyen de surveiller la réponse métabolique aigüe de tumeurs à la chimiothérapie ou à des autres types de traitement, en employant une méthode non invasive, quantitative, et reproducible.

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The response of brain tumours to radio or chemotherapy can now be evaluated earlier and more objectively by brain imaging using computerized x-ray tomography or magnetic resonance. But since, in living systems, chemical changes must precede structural changes, metabolic changes in tumours under treatment would reasonably be expected to provide an earlier index of therapeutic response or toxic effects of treatment. We have used phosphorus magnetic resonance spectroscopy (MRS) to monitor the metabolic changes of brain tumours *in vivo* after chemotherapy. We find that important changes may occur within hours of drug administration, long before structural changes appear on imaging modalities.

CASE REPORT

A 42-year-old woman had excision of a right temporo-parietal tumour which proved to be an astrocytoma, grade 2 to 3. She then received standard radiotherapy. Three years later a recurrence was a mixed oligodendroglioma/astrocytoma by needle biopsy. Astrocytes predominated by 4:1, some foci of necrosis were seen, and the tumour graded 2 on the Kernohan scale. Clinically the tumour was behaving more aggressively than grade 2. The patient started on an experimental treatment protocol (see below) in which 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) was delivered directly into the middle cerebral artery supplying the tumour. This treatment was repeated at six to eight week intervals. Phosphorus MRS was performed before and on two occasions after her third treatment.

Following her fourth treatment, she developed massive swelling necessitating surgical intervention and further resection of tumour. The pathological appearance was similar to that shown earlier by biopsy. Post-operatively she developed hydrocephalus and required a shunt. She was doing well when last seen four months after resection.

METHODS

Intra-arterial BCNU

The patient was treated as part of an ongoing experimental protocol for chemotherapy of high grade gliomas that had recurred following surgery and radiotherapy.[1] Under angiographic control, a catheter was placed into the middle cerebral artery just proximal to the trifurcation. BCNU (100 mg dissolved in 30 cc of D5W) was then infused continuously for about three hours. As the patient was responding to treatment, the infusion was repeated at 6-8 week intervals. Phosphorus MRS examination was performed before, and at 8 hours and 32 hours after her third infusion.

In our protocol for intra-arterial administration of BCNU, we had reduced the risk of toxic reaction recently reported in another clinical trial[2] by selectively infusing the drug above the ophthalmic artery and by using a relatively low dose of BCNU diluted in D5W rather than ethanol.

Magnetic resonance

Combined magnetic resonance imaging and spectroscopy was performed on a 1.5 tesla Philips Gyroscan. Phosphorus spectra were obtained with a small phosphorus tuned headcoil placed inside the usual proton imaging headcoil. A phosphorus MRS was acquired immediately after proton MRI to determine the location of the tumour, without moving the patient or repositioning the coils. The spectrum originated from a block-shaped volume of interest selected using a modification of the ISIS technique,[3] as described by Luyten et al.[4]

Data analysis

The area of each of the resonances (peaks) in the phosphorus spectrum reflects the amount of any metabolite resonating at that frequency. However, determining the area under each of the peaks in a brain spectrum is difficult because of the presence of several overlapping peaks of uncertain line-width on the downfield side of the spectrum. In the absence of a satisfactory solution to this problem, a simple empirical method of quantitation was used in which spectra were processed in a standard way and peak intensities were then measured from an appropriately drawn baseline. The standard process consisted of: a) removing broad components of the spectrum via a low frequency background subtraction equivalent to subtracting an 80 Hz broadened version of the spectrum from itself (i.e., a convolution difference) b) filtering out noise by multiplying the free induction decay (FID) by a function resulting in 15 Hz Gaussian line-broadening in the spectrum c) fourier transformation of the FID and d) phase correction of the final spectrum. This method yields reproducible values for control data in which the standard deviation of peak intensities relative to gamma-ATP varies by less than 10% of the mean for each peak.

Intracellular pH (pH_i) was determined from the position of the inorganic phosphate peak relative[5] to the phosphocreatine peak using the titration curve of Garlick et al,[6] where $pH = 6.75 + \log ((p_i \text{ shift} - 3.29)/(5.70 - p_i \text{ shift}))$.

RESULTS

Figure 1 shows a normal proton MR image and a normal phosphorus MR spectrum derived from a large centrally located block-shaped volume that is illustrated in the plane of the slice by the rectangle. The MRI provides mainly anatomical information based on the distribution and properties of water. The MRS provides metabolic information. The spectrum consists of seven peaks representing mainly (from right to left): beta, alpha and gamma phosphates of ATP, phosphocreatine (PCr), phosphodiester compounds (PDE), inorganic phosphate (P_i) and phosphomonoester compounds (PME) (See Glonek et al[7] for a detailed analysis of peak composition). Since PCr is in equilibrium with ATP via the reaction catalyzed by creatine kinase ($PCr + ADP + H^+ \rightleftharpoons ATP + creatine$), the ratio of PCr to ATP reflects the cellular energy state of the tissue. Because P_i is a weak acid that titrates in the physiological pH range, it exists in the cell in both acidic and basic forms. These two forms resonate at different frequencies, but are in sufficiently fast exchange that a single peak appears in the MR spectrum. The exact position of this peak is a function of the relative amounts of the acidic and basic forms present, and can be used as an indicator of the pH environment of the P_i. As almost all of the P_i is intracellular, the position of the P_i peak provides a measure of intracellular pH.[6]

Normal ratios of phosphate containing metabolites and normal pH_i are shown in Table 1. Figure 2 shows representative slices of MRI scans done before and after treatment with the volumes selected for spectroscopy illustrated. There is a cystic area in the right temporal lobe, probably related to the previous surgery, surrounded by an extensive area of high signal intensity involving the insula, frontal lobe, and basal ganglia. The lateral ventricle is slightly compressed. There was no significant change before and after treatment in these or other slices.

Figure 3 shows the phosphorus spectra from volumes of interest centered on the tumour. Before drug administration, phosphodiesters were 25% less than in normal brain and the intracellular pH was 7.14. The normal intracellular pH of human brain is 6.97 ± 0.02 (mean $\pm$ SD, $n = 7$). The concentrations of other phosphate-containing metabolites were within normal limits. Eight hours following treatment, phosphocreatine and phosphodiesters were reduced by 40% while pH_i increased to 7.24. Thirty-two hours after treatment, phosphocreatine and phosphodiesters were back to within 20% of control, but pH_i continued to increase to 7.35. These metabolite and pH changes are illustrated graphically in Figure 4.

DISCUSSION

This case demonstrates that the phosphorus MR spectrum may change as a direct result of chemotherapy in a clinical setting. These chemical changes may precede any structural alterations observable by imaging modalities. We have previously reported changes in the metabolic state of human brain tumours in association with treatment/growth, however, the relationship to treatment itself was not entirely clear due to the long intervals between MRS examinations.[8] It has been shown

in animal models[9,10] and we have observed in patients (unpublished observations) that the energy state may become severely impaired in tumours before any treatment, presumably as a result of the tumour outgrowing its nutritional supply. Changes demonstrated on MR spectra weeks or months after a therapeutic intervention may therefore reflect tumour growth and not necessarily a response to treatment.

There is precedent for MRS changes in response to chemotherapy of tumours in animals.[11,12] The nature of these changes has varied depending on the model and drug dose; the latter is often so large in the animal studies that it is difficult to extrapolate to a clinical setting. We are aware of two reports on MRS of human tumours in vivo before and after chemotherapy.[13-14] These studies, done without the benefit of image-guided localization, were possible because the superficial nature of the tumours (a rhabdomyosarcoma on a hand and an abdominal neuroblastoma) made them accessible to surface coils. Changes in these cases correlated with an obvious reduction in tumour size. The present report is unique in demonstrating in a clinical setting the potentially important contribution of MRS in revealing a metabolic change before structural changes become evident, either by clinical examination or by imaging modalities.

The most significant metabolic change in this tumour following BCNU treatment was a large increase in intracellular pH.
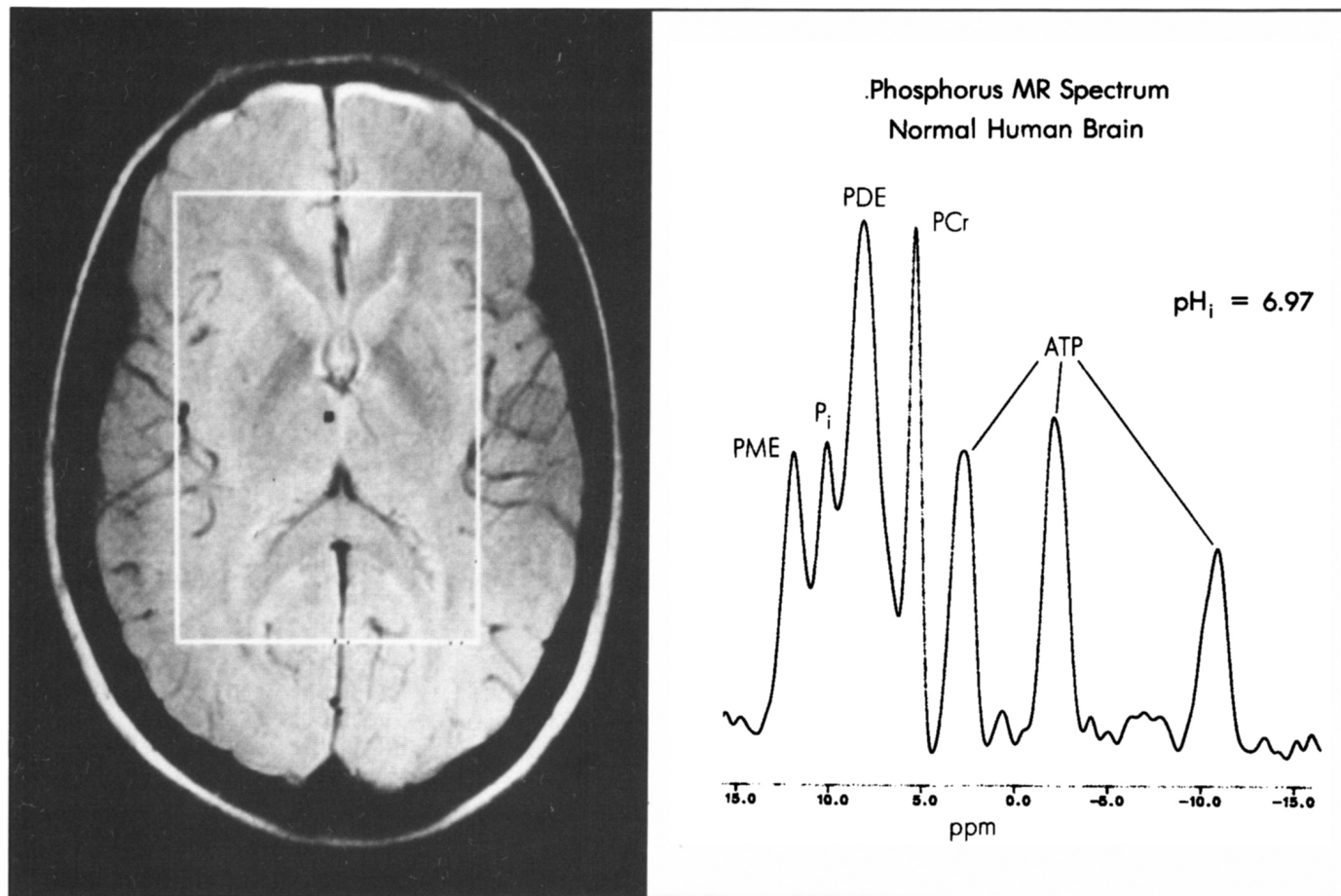

Figure 1 — Proton MRI and phosphorus MRS of a normal brain at 1.5 Tesla. MRI obtained using a spin echo sequence with TR = 1200 msec and TE = 50 msec. MRS obtained using a modified ISIS method for localization[4] with TR = 3 sec. Processing of the FID included convolution difference and gaussian multiplication as described in methods.
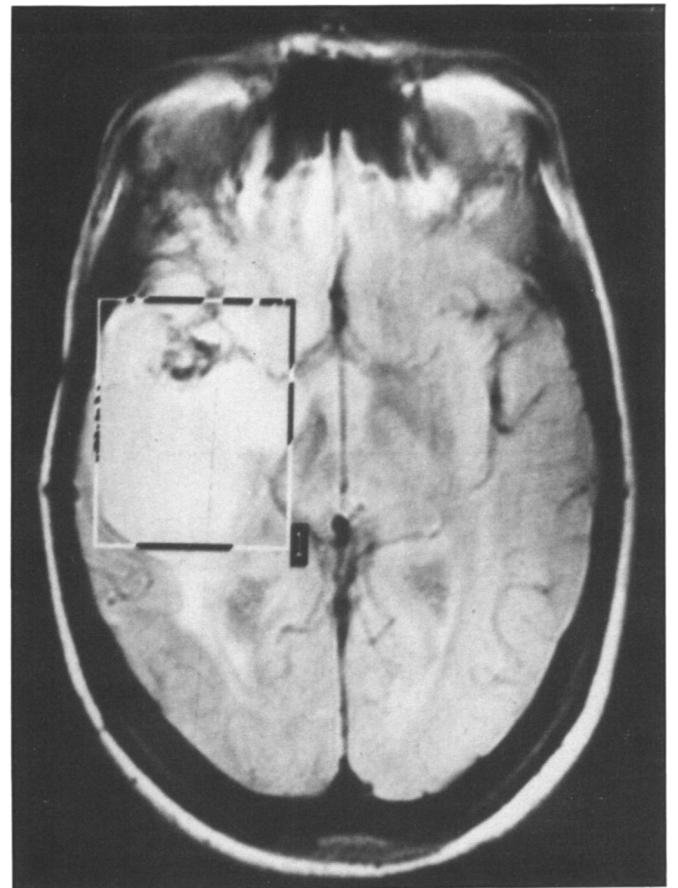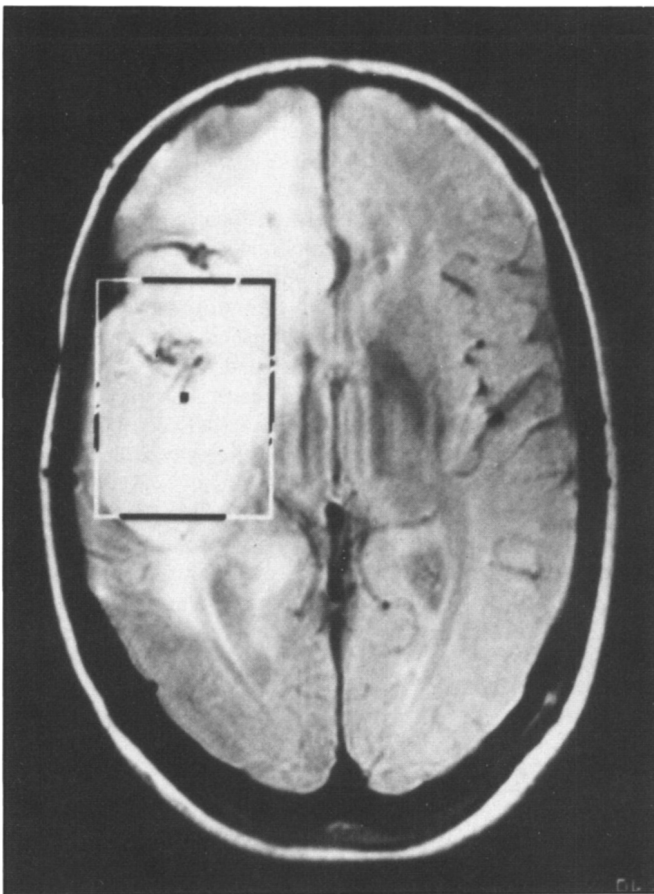
Figure 2 — MRI scans before (left) and after (right) intra-arterial BCNU showing no change in the tumour appearance. The rectangles indicate the volume of interest (in that slice) from which the phosphorus spectra were obtained. (TE = 50 milliseconds, TR = 1200 milliseconds).

Table 1: pH_i + Peak Intensities Relative to g-ATP

	pH_i	PME	P_i	PDE	PCr	a-ATP	b-ATP	PCr/P_i
MEAN (n = 7)	6.97	0.96	1.13	1.91	1.95	1.12	0.69	1.73
l SD	0.02	0.06	0.08	0.09	0.12	0.05	0.04	0.09

pH_i = Intracellular pH
PME = Phosphomonoesters
p_i - Inorganic phosphate
PDE = Phosphodiesters
PCr = Phosphocreatine

Intracellular pH is normally maintained above the equilibrium pH by active net proton extrusion.[15] Further increase of the intracellular pH could result from four general mechanisms: (1) more active cellular proton extrusion (2) an increase in cellular buffering capacity (3) a net change in cellular strong organic acid concentration (e.g. lactate) or (4) an increase in the equilibrium potential for hydrogen ion.

Malignant gliomas before any intervention tend to be more alkaline than normal brain.[16,17] The reason for this is not known but may be due to active proton extrusion associated with cellular synthetic processes[18] or malignant transformation.[19] The alkalosis following BCNU treatment could result from a further activation of proton extrusion. However, the fact that alkalosis may follow cellular injury resulting from stroke[20] and different forms of chemotherapy[21] suggests a non-specific passive process.

Which other mechanism could be responsible for the alkalosis following treatment? Cellular buffering capacity results mainly from the presence in the cell of weak acids (e.g. proteins) and bicarbonate in equilibrium with CO_2. This is unlikely to undergo acute change large enough to explain the observed alkalosis in this patient. Assuming a buffering capacity of 30 mM/pH unit in brain, a net decrease of 6-9 mM organic acids would be required to produce a pH_i increase of 0.2 - 0.3 units. Although a decrease of this magnitude in the concentration of lactic or other strong acids could result in a rebound alkalosis such concentrations are unlikely to be present in the tumour. The persistance of alkalinization of tumours for many weeks after treatment (unpublished observations) also argues against such a mechanism. Although cells are normally maintained more alkaline than their equilibrium pH, a loss of membrane potential secondary to redistribution of potassium and other ions would, as the membrane potential becomes more positive than the equilibrium potential for hydrogen ion (approximately - 24 mV), result in progressive passive alkalinization of the cell. We suspect that the pH changes observed in this patient reflect severe membrane damage and equilibration of the cell interior with the extracellular space.

BCNU decomposes above pH 6 to produce at least two reactive moieties, a diazo-hydroxide that will alkylate DNA and an isocyanate that will rapidly carbamoylate proteins.[22] Alkylation of DNA is unlikely to produce metabolic changes
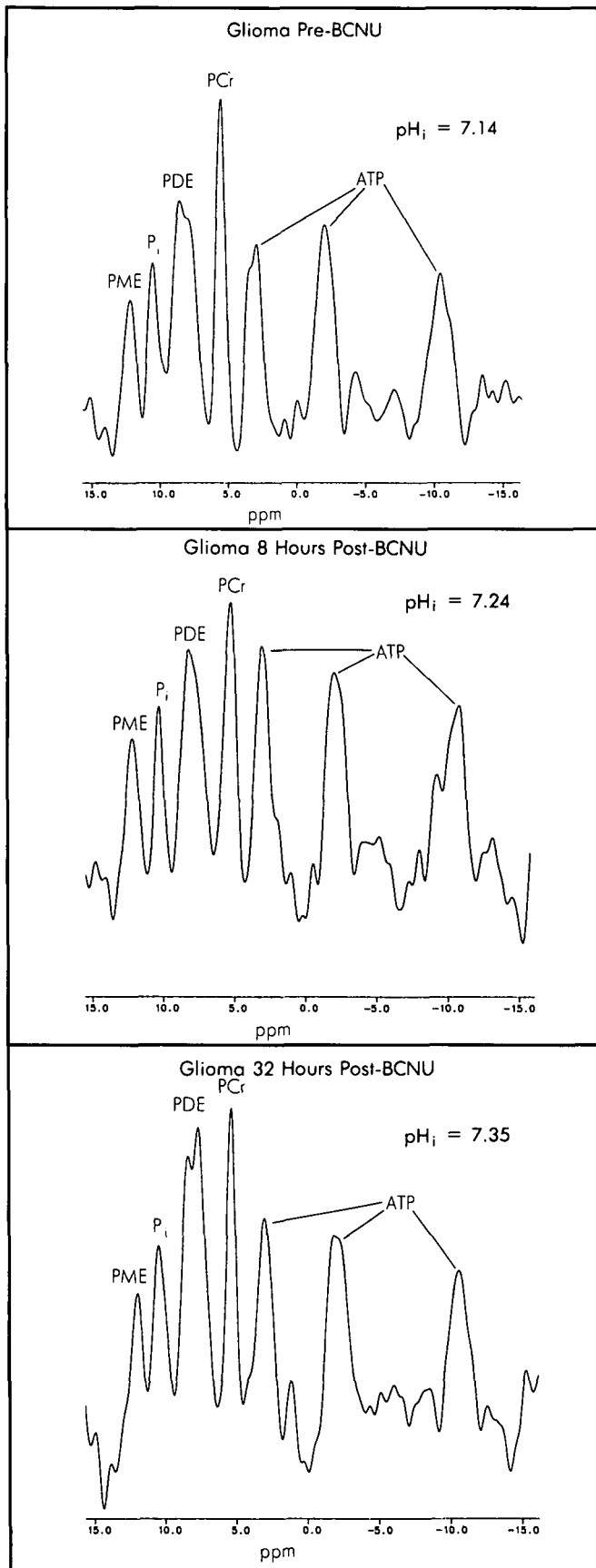
Figure 3 — Phosphorus MRS before, 8 and 32 hours after intra-arterial BCNU showing progressive alkalosis and impaired energy state. (TR = 3 seconds. Total acquisition time approximately 25 minutes for each spectrum.)

within hours of drug administration. Carbamoylation of membrane and cytoplasmic proteins could damage membranes and result in redistribution of ions enough to result in loss of the membrane potential and alkalinization. There is support for this mechanism from electron microscopic studies of glioma-derived cell lines exposed to BCNU *in vitro* where severe membrane and cytoplasmic changes occur within minutes in a dose dependent fashion.[23] The high local concentrations of BCNU obtained with intra-arterial administration may be responsible for the occurrence of this phenomenon in our patient.[24]

The lack of total hemispheric dysfunction after the infusion, as well as some preliminary unpublished observations, suggests that the BCNU effects described above are relatively specific for the tumour over normal brain. This specificity may result, in part, from the pH differences between gliomas and normal brain at the time of treatment, since intracellular alkalosis would accelerate the breakdown of BCNU into active compounds that could be trapped or bound in the tumour cells. If this is correct, the pre-treatment pH may prove a useful predictor of chemotherapeutic response or toxicity to the tumour. The fact that acute metabolic changes occur after treatment suggests that MRS may provide an early monitor of therapeutic efficacy.

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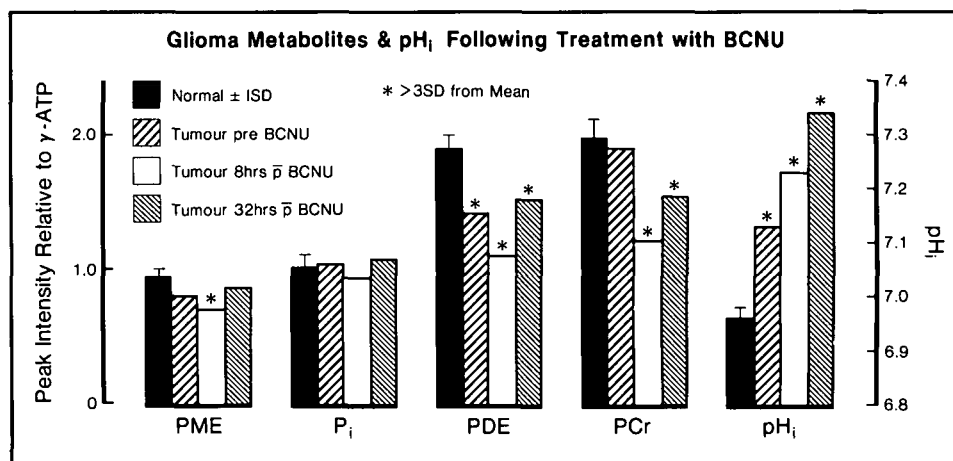
574

Figure 4 — Changes in individual metabolites on serial examinations over 32 hours (hatched bars). Normal value for each metabolite (black bars). Metabolite levels are expressed in arbitrary units relating the intensity of each peak to that of gamma-ATP. pH$_i$ is expressed in pH units.

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