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The effect of blood flow modification on intra- and extracellular pH measured by ^{31}P magnetic resonance spectroscopy in murine tumours

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Summary Intra- and extracellular pH (pH_i and pH_e) were measured simultaneously by ^{31}P magnetic resonance spectroscopy (MRS) in CaNT tumours before and after blood flow modification. Before modification, pH_i was 7.1 ± 0.09 ($n = 11$) and pH_e [measured with an MRS-visible extracellular marker, 3-aminopropyl phosphonate (3-APP)] was 6.7 ± 0.05 ($n = 8$). Chemical shift imaging and localised MRS experiments showed that the 3-APP signal was only from the tumour, not surrounding tissue. After modification by vascular occlusion, independent of whether tumours were maintained at room temperature (22-24°C) or kept warm (33-35°C), there was a decrease in pH_i and pH_e with pH_i decreasing to a greater extent. Qualitatively similar results were found using flavone acetic acid (FAA) as a blood flow modifier; only four out of nine tumours responded to FAA. Concomitant with the reduction of the pH gradient after modification was a decrease in the phosphorylation state of the adenine nucleotides measured either as ATP/P_i by MRS or $[ATP]/[ADP][P_i]$ in tumour extracts. These results indicate that the intracellular uptake of chemotherapeutic drugs which are dependent on the transmembrane pH gradient will not be enhanced in cells made ischaemic as a result of vascular shutdown.

Keywords: intracellular pH; extracellular pH; magnetic resonance spectroscopy; murine tumour; blood flow modifier

In the quest for new anti-cancer strategies for solid tumours many approaches have been tried using pH (for review see Wike-Hooley *et al.*, 1984). These include various strategies for altering pH: lowering the pH to make the tumour cell more sensitive to a particular treatment modality, e.g. hyperthermia (Hiraoka and Hahn, 1989), or accentuating pH gradients (ΔpH) between intra- and extracellular compartments (Gerweck *et al.*, 1991). To achieve the latter various methods have been tried: inducing hyperglycaemia to increase lactic acid production and thus lower pH (Evelhoch *et al.*, 1984; Hwang *et al.*, 1991; Jahde *et al.*, 1992); inhibiting the Na^+/H^+ exchanger with amiloride and its analogues (Newell *et al.*, 1992; Maidorn *et al.*, 1993) and using compounds [e.g. hydralazine, flavone acetic acid FAA] that specifically reduce blood flow to tumours (Vorhees and Babbs, 1982; Evelhoch *et al.*, 1988; Parkins *et al.*, 1994*a*) to make the tumours more anoxic and therefore more acid. By using these various strategies, it is hoped that the pH differences can be exploited either by activating cytotoxic agents selectively within tumours (Tannock and Rotin, 1989; Newell *et al.*, 1992) or by altering the distribution of drugs that are weak acids or bases (Gerweck *et al.*, 1991) in such a way that they will be taken up more effectively by the tumour than by the normal surrounding tissue. Several drugs (e.g. mitomycin C, chlorambucil) have been shown to have increased cytotoxicity *in vitro* in isolated cell experiments at low extracellular pH (pH_e) (Maidorn *et al.*, 1993; Parkins *et al.*, 1993, 1994*b*). These drugs have also caused growth delay *in vivo* in some tumour types (Newell *et al.*, 1992; Parkins *et al.*, 1994*b*).

Following the classical experiments of Warburg in the 1920s, for many years tumours were thought to be acidic, but it is now generally accepted (Vaupel *et al.*, 1989; Griffiths, 1991) that tumour intracellular pH (pH_i) is close to neutrality. ^{31}P magnetic resonance spectroscopy (MRS) has confirmed non-invasively that pH_i in both human and animal tumours is on the alkaline side of neutrality: pH 7.1-7.2 (Vaupel *et al.*, 1989; Griffiths, 1991; Evelhoch, 1992; Negendank, 1992) which is *similar* to that in most normal tissues. Confirmation that the measurement of tumour pH by MRS is largely representative of pH_i has been made in animal tumours (Stubbs *et al.*, 1992). It is now possible, with the aid of an MRS-visible extracellular marker 3-aminopropyl phosphonate (3-APP) (Gillies *et al.*, 1994) to measure pH_e *in vivo*. 3-APP is not toxic to C6 glioma or Ehrlich ascites tumour cells at concentrations up to 20 mM (Gillies *et al.*, 1994). Thus it may be used for monitoring the course of the ΔpH of solid tumours *in vivo* after therapy. Since *in vitro* and *in vivo* experiments with CaNT tumour cells have shown dependence of drug cytotoxicity on pH_e, enhancement of cell kill at low pH_e and dependence of ischaemia-induced cell death on temperature (Parkins *et al.*, 1993, 1994*a*,*b*), the purpose of the work reported here was to monitor pH_i and pH_e simultaneously by ^{31}P MRS in CaNT murine tumours before and after blood flow modification. The ΔpH was monitored in three cohorts of mice, before and up to 2 h after total vascular occlusion. The core temperature was maintained at preocclusion values (33-35°C) or allowed to cool naturally to room temperature (22-24°C) after cessation of blood flow. The results showed that the ΔpH decreased after vascular occlusion and that there was a decrease in both pH_i and pH_e after treatment with FAA in the four out of nine tumours that responded to FAA.

Materials and methods

Tumours

Moderately differentiated murine adenocarcinoma NT (CaNT) tumours were grown subcutaneously on the lower dorsum of syngeneic CBA/Gy fTO mice and examined when they were about 10 mm in diameter. The mice were divided into two groups for MRS measurements and freeze clamping. The mice were anaesthetised intraperitoneally (i.p.) with ketamine (50 mg kg^{-1}) (Parke-Davis, UK) and diazepam (25 mg kg^{-1}) (Phoenix Pharmaceuticals, UK) to avoid motion artefacts during MRS measurements.

Injection of 3-APP and FAA

The mice were injected with 0.3 ml of 128 mg ml^{-1} of 3-APP (Sigma, UK) i.p. (12-15 μmol g^{-1} body weight), 30 min before the spectra were collected. In the studies with FAA

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(provided by Lipha Pharmaceutical, Lyon, France), this was also administered i.p. (200 mg kg^{-1}) but 10–15 min after the 3-APP.

Total vascular occlusion

Because remotely controlled non-magnetic occlusion devices are difficult to make, vascular occlusion was achieved by an intravenous (i.v.) injection of a lethal dose of euthatal via an in-dwelling tail vein cannula with a line for remote injection inserted before the mouse was placed in the magnet.

MRS measurements

MRS measurements were made on a Sisco 200–330 at 4.7 T using image-guided localised spectroscopy by ISIS (image-selected *in vivo* spectroscopy) (Ordidge *et al.*, 1986), with adiabatic pulses, a recycle time of 3 s and a gradient strength of 7.5×10^{-4} T cm^{-1}. On average a volume of 0.8 cm^3 was selected using a two-turn 1 or 2 cm solenoid coil (depending on tumour size and shape). Pre- and post-occlusion spectra were obtained with an interleaved ISIS localisation acquired in 320 scans (total) with the transmitter frequency set on α-NTP in one spectrum (Figure 3a) and on 3-APP in the other (see Figure 3b). Because the chemical shift difference between 3-APP and α-NTP was >30 p.p.m. there was a significant chemical shift artefact and the double interleaved ISIS acquisition was used to minimise this problem (see Maxwell *et al.*, 1994, and Discussion). To obtain adequate signal/noise for the 3-APP signal it was necessary to double the dose (to 0.3 ml of 128 mg ml^{-1}) 12–15 μmol g^{-1} body weight given by Gilles *et al.* (1994) which produced a peak between 32 and 34 p.p.m. (relative to α-NTP). The mice tolerated this increased dose very well and there were no deaths that could be attributed to it.

For the chemical shift imaging (1D-CSI) experiment, 64 transients were acquired for each of 32 phase-encoding steps over a 4 cm field of view. The acquisition time was 128 ms with a repetition time of 2 s and 8 kHz spectral width.

Data processing

The MR spectra were analysed using VARPRO, a time domain fitting routine (van der Veen, *et al.*, 1988). Because the chemical shift artefact causes more distortion to the β-NTP peak than the γ-NTP, the latter has been used to calculate the NTP/P_i ratios. The contribution from free NDP is considered negligible and likely to be in the micromolar range (Stubbs *et al.*, 1989).

pH measurements

pH_i was measured from the difference in chemical shift between the P_i resonance and that of α-NTP at −7.57 p.p.m. according to Pritchard *et al.* (1983). The value for pH_e was measured from the chemical shift difference between 3-APP and α-NTP. In the occlusion experiments, the reference signal (α-NTP), disappeared after 30–40 min and in these cases the chemical shift value obtained for the α-NTP peak before the α-NTP signal disappeared was used for calculation of pH_i and pH_e. For this analysis a standard curve was constructed *in vitro* by setting up solutions at 19 pH intervals between pH 4.93 and 9.01 containing 0.154 M sodium chloride, 5 mM potassium chloride, 30 mM Pi, 30 mM ATP, 30 mM PCr, 30 mM magnesium chloride and 30 mM 3-APP. ^{31}P MR spectra were collected under our standard conditions from the solutions in a glass sphere, similar in shape to the tumours we examined. The chemical shift was referenced to α-ATP.

In vivo *experiments*

Temperature was monitored throughout the experiments via a rectal probe and maintained by a bath of circulating warm water. During vascular occlusion mouse core temperatures were maintained at preocclusion values (33–35°C) or allowed to cool naturally to room temperature (22–24°C). During the FAA experiments the core temperature was maintained at about 31–33°C.

Statistical analyses

The results from the *in vivo* experiments were analysed using the Student's *t*-test and are reported as mean ± s.e.m.

Freeze clamping

Two hours after vascular occlusion some of the tumours were freeze clamped. Extracts of the tumours were assayed for adenine nucleotides by high-performance liquid chromatography (HPLC), lactate according to Bergmeyer (1974) and P_i according to Lowry and Lopez (1946) as modified by Chandra Rajan and Klein (1976).

Results

Occlusion Studies

The results in Figure 1 show the chemical shift dependence on pH for 3-APP. The data were fitted to the Henderson–Hasselbalch equation to obtain an estimated pK_a of 6.91 for 3-APP with a limiting acid chemical shift of 34.30 p.p.m. and base chemical shift of 31.11 p.p.m. Using similar experimental conditions, Gillies *et al.* (1994) reported a slightly higher value (7.1) for the pK_a of 3-APP. They also noted that standard curves constructed at different temperatures or ionic strengths did not significantly affect the values reported for $pK_{a3\text{-}APP}$.

Preliminary experiments were performed to ascertain that 3-APP was present in the tumours. The CSI experiment (Figure 2), which gives an overall view of where the MR signal is coming from, demonstrates that the majority of the 3-APP signal is from the tumour along with signal from P_i and the α-, β- and γ-phosphates of NTP. The PCr signal comes only from the muscle of the body wall and there appears to be no 3-APP signal coming from this region. The sensitivity of this experiment is insufficient to make any statements about the homogeneity of the 3-APP in the tumour.

The values for pH_i of CaNT tumours in the absence and presence of 3-APP were 7.1 ± 0.09 (n = 11) and 7.08 ± 0.06 (n = 8) respectively, indicating that the presence of 3-APP did

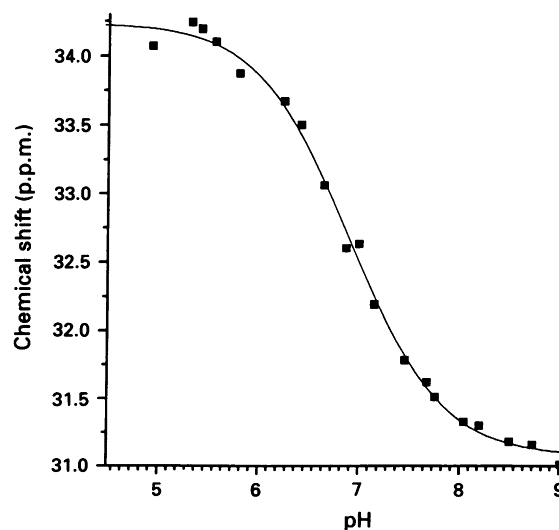

Figure 1 pH dependence of the chemical shift of 3-APP. $pK_{a3\text{-}APP}$ was calculated as 6.91 (see Materials and methods for details). It should be noted that phosphonate resonances shift to lower frequencies at higher pH, the opposite to phosphates, which shift to higher frequencies at higher pH values (Prichard *et al.*, 1983).

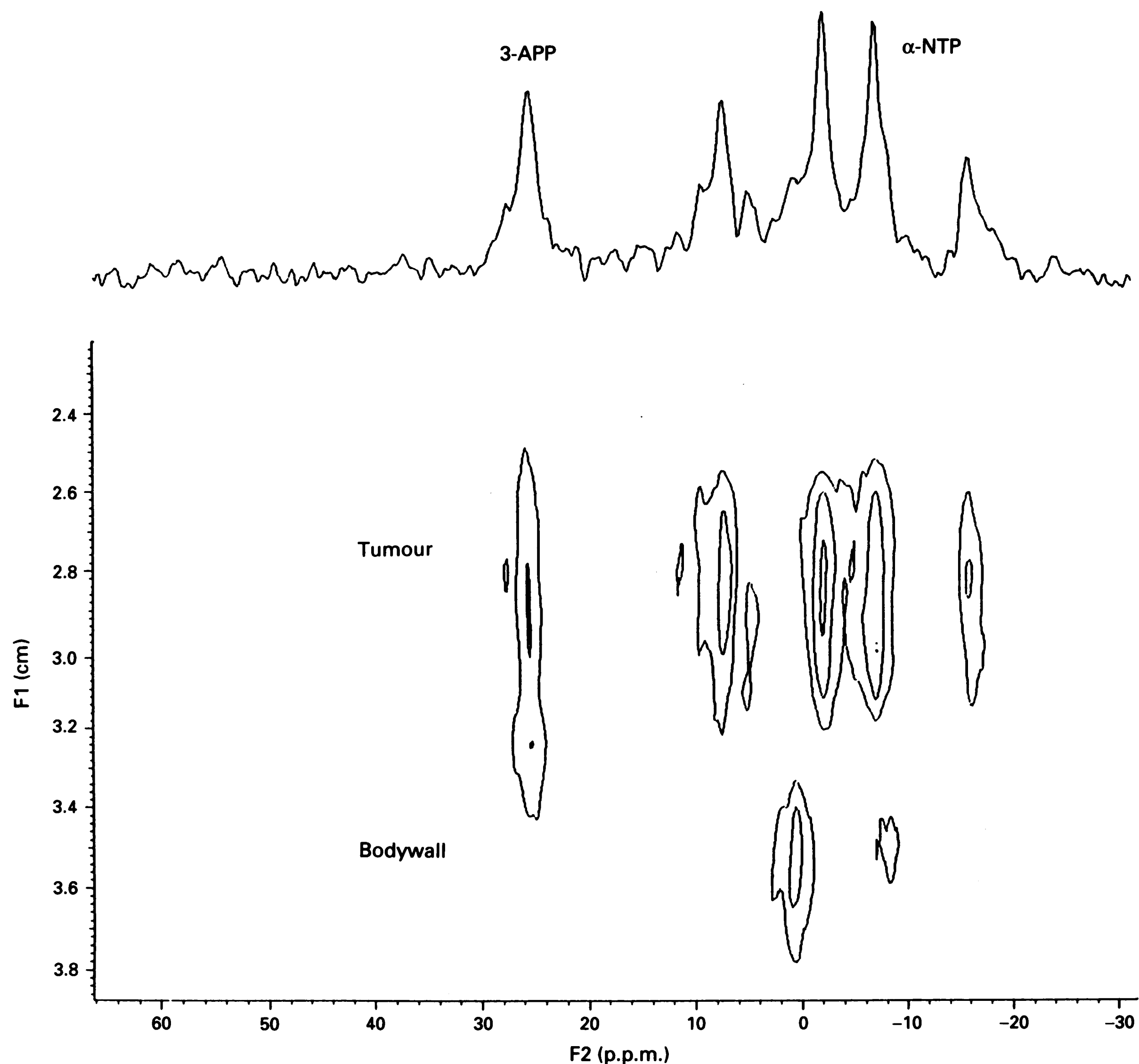

Figure 2 A one-dimensional CSI contour plot indicating the location of the metabolites measured over the different anatomical regions of the mouse. The spectrum at the top is of the metabolites summed over the whole region.

not affect pH_i. When pH_i and pH_e were measured simultaneously in the CaNT tumours, the value for pH_e was 6.7 ± 0.05 ($n = 8$) which is about 0.4 pH units more acid than pH_i and is significantly different from pH_i ($P<0.005$). Spectra obtained with an interleaved ISIS acquisition (see Materials and methods for details) from one of the tumours examined are shown in Figure 3a and b. The findings are consistent with the results of Gillies *et al.* (1994) for RIF-1 tumours.

The plots in Figure 4, before and up to 128 min after occlusion, demonstrated that *both* pH_i and pH_e decreased after occlusion. The values for pH_i after occlusion decreased on average by 0.51 and 0.68 pH units for the room temperature and 33–35°C experiments respectively. On the other hand, pH_e decreased by less, 0.35 for the room temperature and 0.41 for the 33–35°C experiments. Values for pH_{pot} measurements reported previously (Parkins *et al.*, 1994*a*) also showed decreases in pH_{pot} after occlusion, but in those experiments a relatively larger decrease (0.5 pH units) was observed at 33–35°C when compared with room temperature (0.2 pH units) after 120 min occlusion.

Although the ΔpH (i.e. the difference between pH_i and pH_e) appeared smaller in both the tumours maintained at room temperature and tumours maintained at 33–35°C after occlusion, decreasing from 0.31 ± 0.13 to 0.17 ± 0.015 and from 0.53 ± 0.04 to 0.28 ± 0.13 respectively, these differences were not statistically significant.

Concomitant with the decrease in pH_i and pH_e after occlusion, there was a decrease in NTP/P_i ratio with time (Figure 5). Spectra from one of the tumours before and after occlusion, with the temperature maintained at 33–35°C, are shown in Figure 6. There are significant decreases in γ-NTP/P_i with time up to 48 min post-occlusion (Figure 5) at both temperatures, after which no further changes were observed. The rate at which the NTP/P_i ratio decreased at the higher temperature was not significantly different from the NTP/P_i ratio of the tumours maintained at room temperature.

Metabolites measured in extracts of CaNT tumours at the end of the experiment (Table I) showed that there had been significant breakdown of adenine nucleotides after occlusion ($P<0.01$) although no significant differences were observed between tumours maintained at room temperature and tumours maintained at 35°C. This is in accordance with the observations made by MRS (Figure 5) that no further decreases in NTP/P_i were observed after 48 min of occlusion when a plateau appears to have been reached.

FAA studies

pH_i and pH_e were also followed for 2 h after treatment of the animals with FAA, an agent that reduces blood flow by coagulopathy (Murray *et al.*, 1989). The NTP/P_i ratios (Figure 7) and pH_i and pH_e measurements obtained indicated that there were two different responses to FAA. Nine mice

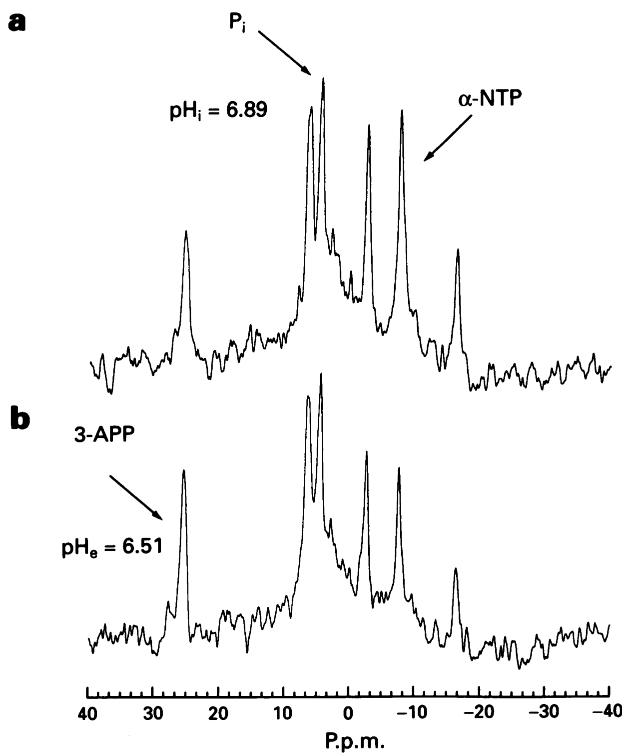

Figure 3 pH_i and pH_e of CaNT murine tumour measured by ^{31}P MRS. ^{31}P MR spectra were obtained with an interleaved ISIS localisation acquired with the transmitter frequency set on α-NTP in the upper spectrum (**a**) and on 3-APP in the lower spectrum (**b**) 30 min after the mice were injected with 3-APP. pH_i was measured from the chemical shift difference between α-NTP and P_i in the upper spectrum. pH_e was measured from the chemical shift difference between 3-APP in the lower spectrum and α-NTP in the upper spectrum to correct for the chemical shift artefact (for further details see text).

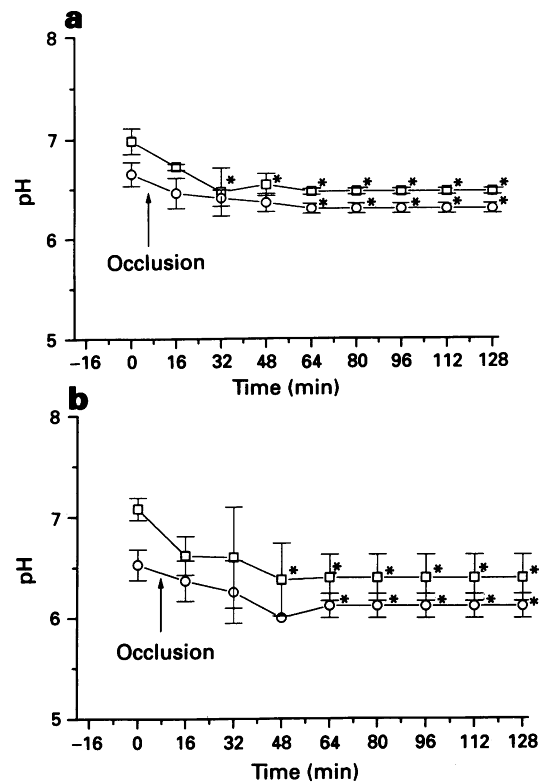

Figure 4 (**a**) Time course of pH_i (□) and pH_e (○) measured before and after vascular occlusion with mouse core temperature maintained at room temperature (22–24°C). Symbols represent mean ± s.e.m. ($n = 3$). (**b**) Time course of pH_i (□) and pH_e (○) measured before and after vascular occlusion with mouse core temperature maintained at 33–35°C. Symbols represent mean ± s.e.m. ($n = 3$). *Indicates significant difference ($P<0.05$) from values observed at 0 min.

were treated with FAA, five showed no significant decrease in the NTP/P_i ratio 2 h after treatment whereas in the other four tumours the NTP/P_i ratio started to decrease after 68 min and was significantly different from the control values after 100 min of treatment. In the group of four tumours, the pH_i correspondingly decreased (but not significantly) from 7.08 ± 0.02 to 6.85 ± 0.05 and the pH_e from 6.66 ± 0.08 to 6.23 ± 0.16 by the end of the experiment. In contrast, in the non-responders, pH_i (7.24 ± 0.1 at the beginning of the experiment and 7.16 ± 0.05 at the end) was virtually unchanged as was pH_e (6.75 ± 0.05 at the beginning and 6.59 ± 0.05 at the end).

The decreases in the responders were not significant but in the same direction and of a similar magnitude to those found for total vascular occlusion (see Figure 4). This is probably because FAA takes a long time to exert its effect (relative tumour perfusion ~21% of control after 200 mg kg^{-1}; see Hill *et al.*, 1989). However it is not possible to look at later times in these experiments because the 3-APP has to be given before the FAA, when the vasculature is still patent. The 3-APP gradually disappears from the tumour with time leaving no marker of extracellular space at the later times and the signal/noise deteriorates.

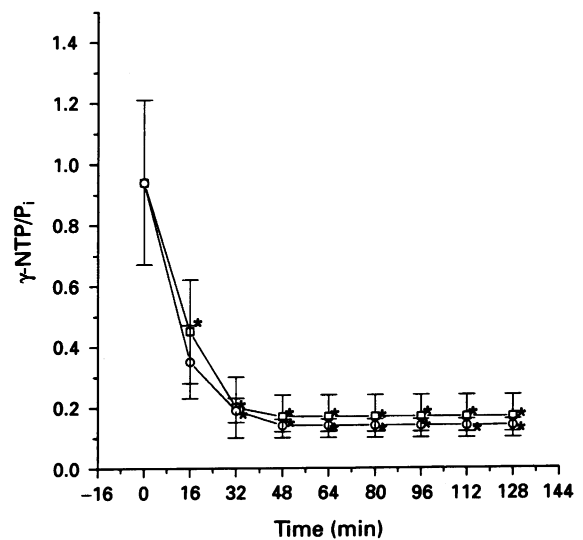

Figure 5 Time course of γ-NTP/P_i ratios measured before and after vascular occlusion with mouse core temperature maintained at either 22–24°C (□) or 33–35°C (○). ($n = 3$) for each point at each temperature. Symbols represent mean ± s.e.m. *Indicates significant difference ($P<0.05$) from values observed at 0 min.

Discussion

Direct non-invasive measurements of pH_i in tumours have changed the view that existed for many years after Warburg's experiments of the 1920s, namely that tumours were 'acidic' (for review see Griffiths, 1991). Now it is possible to make direct, non-invasive (except for injecting the marker, 3-APP) measurements of pH_e. However these measurements do represent volume-average pH values and this may not be ideal in tumours that are known to be histologically heterogeneous. However, some strength lies in using volume averages, especially since they are directly comparable with subsequent metabolic assays done on freeze-clamped tumours which also represent volume averages. One drawback of the volume-average method is that pH_e values obtained by ^{31}P MRS are likely to be different from pH_e values obtained by

Table I Effect of vascular occlusion for 2 h on adenine nucleotides [ATP]/[ADP][Pi] and lactate in extracts of CaNT tumours

Treatment	*Total adenine nucleotides* ($\mu mol\ g^{-1}$)	*[ATP]/[ADP][P_i]* M^{-1}	*Lactate* ($\mu mol\ g^{-1}$)
Control ($n = 3$)	2.16 ± 0.14	1107 ± 268	7.8 ± 0.6
Occlusion ($n = 5$) at 22–24°C	1.2 ± 0.08*	51 ± 8.2*	21 ± 2.3*
Occlusion ($n = 7$) at 33–35°C	0.99 ± 0.17*	80 ± 31*	19 ± 2.2*

*Significantly different from control value ($P<0.05$).

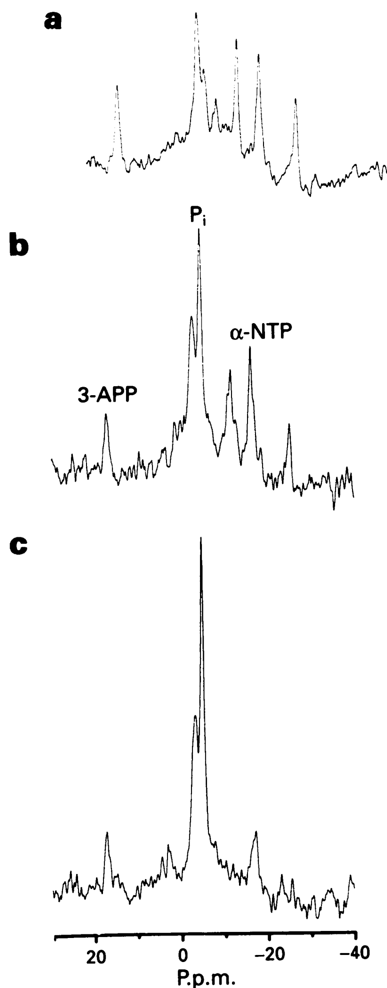

Figure 6 Representative ^{31}P spectra showing preocclusion (**a**) and the decrease in γ-NTP/P_i with time 16–32 min (**b**) and 32–48 min (**c**) post occlusion. Mouse core temperature was maintained at (33–35°C).

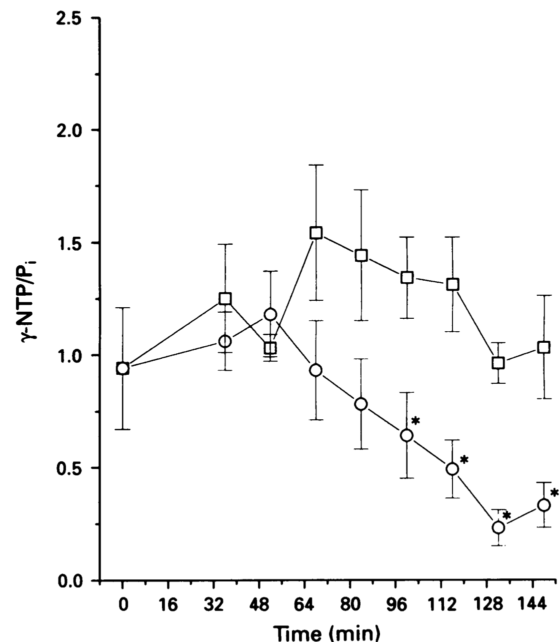

Figure 7 Time course of γ-NTP/P_i ratios measured after treatment with 200 mg kg^{-1} of FAA. (○) are the responders ($n = 4$) and (□) are non-responders ($n = 5$). The 0 min value was obtained from control animals with no treatment ($n = 3$). Symbols represent mean ± s.e.m. *Indicates significant difference ($P<0.05$) from values observed at 36 min, the first post-FAA treatment value obtained.

microelectrodes since microelectrode measurements are invasive and can only sample a few microenvironments within a tumour. The values found in untreated tumours for pH_e by ^{31}P MRS were somewhat lower than those found by microelectrode studies in the same tumour type (although performed on different batches at different times (see Parkins *et al.*, 1994*a*), and this may reflect differences between the techniques. The wide range of pH (5.8–7.52) found in tumours by microelectrode measurements (see Wike-Hooley *et al.*, 1984) suggests that they are sampling a mixture of compartments (see also Vaupel *et al.*, 1989) whereas the range of pH_{APP} is very narrow (6.5–6.8) in untreated tumours (see Gillies *et al.*, 1994 and this paper).

In spite of these discrepancies both microelectrodes and 3-APP methods confirm that the extracellular compartment is more acid than the intracellular compartment (see also Vaupel *et al.*, 1989; Griffiths, 1991; Stubbs *et al.*, 1994). Further studies, in which pH_e will be measured on the same tumour by both ^{31}P MRS-3-APP and microelectrodes are planned to begin shortly and we hope these studies will resolve the differences between the two methods.

In this study, ISIS localisation of the tumours was chosen to ensure that the intense PCr signal from the body wall did not contribute to the tumour spectra (Howe *et al.*, 1992). However, because the chemical shift difference between 3-APP and α-NTP is >30 p.p.m. there would be a significant chemical shift artefact (Howe *et al.*, 1992). The consequence of this would be that the volumes from which the 3-APP and the α-NTP signals were obtained would be displaced by 2 mm (in three dimensions). In a 0.8 cm^3 volume of interest (chosen for most of our experiments), the volumes from which the signals arose would have overlapped by only 43%. To ensure that the chemical shift artefact was minimised and that α-NTP and 3-APP signals came from an identical volume, the double interleaved ISIS acquisition (Maxwell *et al.*, 1994) was used for all ISIS experiments. It should be noted that the chemical shift artefact is negligible when calculating pH_i because the chemical shift difference between P_i and α-NTP is <10 p.p.m.

In theory, absolute concentrations can be obtained from ^{31}P MRS since the area under the signals in the MR spectrum are directly proportional to the concentration of the

metabolites. However, since ISIS localisation was used, the results presented here have been expressed as peak ratios (i.e. NTP/P_i) since absolute sensitivity depends on coil loading and the relative position of the selected voxel to the coil. It should be noted that this ratio (NTP/P_i) only approximates the energy status of the tumour in real time. In order to obtain information about the metabolic status of the tumour that might approximate thermodynamic information, [ADP] would have to be ascertained. While [ADP] cannot be determined directly from *in vivo* ^{31}P MR spectra, total [ADP] (free + bound) can be ascertained by freeze clamping and making extracts of the tumour with subsequent assays of ATP, ADP and P_i. Although this does not give true thermodynamic information, it does give the direction, and some indication of the magnitude of the changes observed.

An additional caveat is that the MR spectrum includes all the nucleotide phosphates under the peaks which accounts for the conventional label of NTP, although the major proportion of this peak is ATP (Stubbs *et al.*, 1989). However, the extracts provide data on ATP since HPLC separates ATP from other trinucleotides. The maintenance of adenine nucleotides in tumours compared with control tissue (e.g. liver or kidney) over relatively long periods of ischaemia is supported by anaerobic glycolysis (Williamson *et al.*, 1970; Weber *et al.*, 1971) and the expected large increase in lactate and fall in $[ATP]/[ADP][P_i]$ was observed in these tumours.

Lowering pH_e in isolated cell experiments enhances cytotoxicity of certain anti-cancer drugs e.g. doxorubicin, melphalan (for review see Wike-Hooley *et al.*, 1984). Similarly agents that cause vascular occlusion (e.g. FAA), subsequently increase the cytotoxic effect of drugs (such as mitomycin C and chlorambucil *in vivo* (Parkins *et al.*, 1993, 1994*b*). Now that it is possible to monitor pH_i and pH_e simultaneously, we have investigated the effect of total vascular occlusion on pH_i and pH_e at room temperature (22–24°C) and at 33–35°C. The values for pH_e of CaNT tumours were up to 0.4 pH units more acid than pH_i. This in itself would enable a drug which was a weak acid (eg. chlorambucil), to partition more favourably into the more alkaline intracellular compartment. However the energy of ATP hydrolysis $[ATP]/[ADP][P_i]$ required to maintain the proton gradient is decreased in tumours compared with normal tissues (Stubbs *et al.*, 1994) and after vascular occlusion, this ratio ($[ADT]/[ADP][P_i]$) decreases even further. This combined with the inability of the tumour to remove products of ischaemia such as lactate, contributes to the collapse of ΔpH, caused largely by a decrease in pH_i. In the tumours which respond to FAA there is also a decrease, to about the same extent, in both pH_i and pH_e.

These results clearly indicate that tumour regions subjected to ischaemia do not display an enhanced differential between pH_i and pH_e. Thus, for chemotherapeutic drugs with weak acid functions such as chlorambucil, whose increased activity at reduced pH_e *in vitro* is primarily due to increased uptake as a result of the larger transmembrane ΔpH, no enhanced toxicity in such regions should be expected. However, as mentioned earlier, the tumour cytotoxicity of chlorambucil is enhanced if combined with agents which induce tumour ischaemia such as FAA (Parkins *et al.*, 1994*b*). Two possible explanations for this result are: (i) if chlorambucil is given before an agent which induces tumour ischaemia (i.e. when the vasculature is still patent), then the pharmacokinetics of the agent could be altered by 'trapping' of the drug in the tumour; (ii) it is known that with FAA a small percentage of vessels remain functional and these supply the tumour cells which elicit tumour regrowth following FAA treatment alone. Thus if the majority of the tumour is made ischaemic, acidic metabolites could diffuse and reduce the pH_e in such areas, although, because they still have an adequate blood supply, the cells could still maintain their pH_i. In this scenario chlorambucil would have increased toxicity against this remaining subpopulation.

The data obtained show that there are two different responses to FAA – a significant decrease in NTP/P_i ratio and a non-significant decrease in pH_i or pH_e for responders compared to a non-significant decrease in NTP/P_i ratio and no change in pH_i or pH_e for non-responders after FAA treatment. However, this interpretation should be taken with caution because it is possible that the results do not indicate two completely separate responses, but rather the two extremes of the response within a continuum.

Simultaneous and non-invasive monitoring of volume average pH_i and pH_e of solid tumours *in vivo*, demonstrates that pH_i is more alkaline than pH_e and that both decrease after vascular occlusion, or in response to FAA, concomitant with a decrease in the $[ATP]/[ADP][P_i]$.

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