MAGNETIC RESONANCE IN MEDICINE 22, 394–403 (1991)

Alkalosis Monitored by ^{31}P NMR in a Human Glioma Cell Line Exposed to the Anti-tumor Drug 1,3-bis(2-Chloroethyl)-1-Nitrosourea

FERNANDO COMMODARI,*†‡ BRYAN C. SANCTUARY,* WILLIAM FEINDEL,† AND ERIC A. SHOUBRIDGE†

*Chemistry Department and †Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4

Received October 24, 1989; revised October 5, 1990

A transient alkalosis of similar magnitude to that observed *in vivo* has been observed using ^{31}P NMR and 2-deoxy-D-glucose-6-phosphate as a pH marker in a human glioma cell line, SKI-1, with demonstrated sensitivity to 1,3-bis(2-chloroethyl)-1-nitrosourea. At an effective dose of $5 \pm 1 \times 10$ μg/ml, an increase of 0.13 ± 0.05 pH units was observed within $4 \pm 1 \times 10$ min of introducing the drug into the perfusion chamber. Although the *in vitro* response is of a time course much faster than that *in vivo*, these results suggest that this immediate pH change could be an indicator of the cytotoxic action of the drug.

INTRODUCTION

The chloroethylnitrosoureas are clinically important anti-tumor agents used in the treatment of a variety of cancers (*1*). 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) is widely used in the treatment of primary brain tumors. About 40–60% of all cerebral gliomas are, however, resistant to BCNU (*2*). Even from an individual tumor, phenotypically distinct cell lines can be cultured with markedly different sensitivities to the drug (*3*). It would be useful to have an immediate metabolic indicator in cell lines which reflected the efficacy of chemotherapy. The mechanism of the antitumor activity of BCNU appears to be related to its lipid solubility (*4*) and its mode of decomposition (*5*). BCNU shows a different rate of decomposition in tumor than in normal brain (*4*). It is unstable at or above physiological pH, rapidly decomposing (*6, 7*) into 2-chloroethyldiazohydroxide, a compound which alkylates nucleic acids and proteins, and a protein carbamoylating agent, 2-chloroethylisocyanate. Alkylation leading to cross-linking of DNA appears to be a crucial feature of the anti-tumor action of BCNU (*8–10*). However, the degree of DNA-cross-linking does not always correlate well with toxicity (*11*). Carbamoylation has been suggested to play a role in cytotoxicity by interfering with DNA repair and RNA processing and by inhibition of a variety of enzymes (*12*).

Changes in intracellular pH (pH_i) have been demonstrated (*13, 14*) by ^{31}P NMR within hours of treatment in glioblastomas of patients being treated with BCNU. The exact nature of the pH changes depended upon the route of administration of the drug. Patients receiving the drug by a superselective intraarterial catheter (*13, 14*)

‡ Present address: Varian NMR Instruments, 25 Hanover Rd, Florham Park, NJ 07932.

0740-3194/91 $3.00

showed a transient intracellular alkalosis of 0.15 ± 0.06 pH units while in patients receiving intravenous BCNU a transient acidosis of 0.15 ± 0.03 units was observed (*14*). The interpretation of the *in vivo* data is complicated by the fact that glioblastomas are highly heterogeneous tumors with a variable number of cystic and necrotic *foci*, a mixed population of cell types, and a variable pattern of abnormal blood vessels. Furthermore, the nature of the volume-selective procedure used in human spectroscopy studies and the relatively large volumes required for adequate signal to noise leads to an ill-defined proportion of normal brain tissue in the selected volume. In order to overcome these problems and to define the molecular mechanism of the early pH response to BCNU, we are studying human glioma cell lines.

In this study, we have tested whether the *in vivo* pH changes can be reproduced *in vitro* in the BCNU-sensitive SKI-1 glioma cell line. We have also demonstrated that 2-deoxy-D-glucose-6-phosphate (2dG6P) can be used as a reliable pH marker (*15*) in such studies.

METHODS

Glioma SKI-1 Cells

Cultured human glioblastoma SKI-1 cells (*16*), originally isolated by J. Shapiro (Sloan Kettering Institute, Cornell University) were obtained from Dr. L. Panasci (Lady Davis Institute for Medical Research, Sir Mortimer B. Davis-Jewish General Hospital, Montreal, Quebec, Canada). The cells were grown at 37°C in a humidified 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) and 10% fetal calf serum. This cell line shows an ED_{30} (the concentration that reduces the number of colonies to 30% of control values) of 10.7 μg/ml for BCNU in the human tumor cloning assay (*16*). Cells ($9 \pm 1 \times 10^7$) were harvested mechanically at approximately 90% confluency and diluted in DMEM to a volume of 2.75 ml. They were embedded in low melting point agarose by mixing with 1 ml of a 2.25% (w/w) solution of agarose made with P_i (inorganic phosphate) free Krebs–Henseleit buffer and extruded as fibers (*17*). The cell–fiber system was perfused at 1 ml/min with P_i free buffer or, for introducing 2dG6P into the cells, with buffer containing 1 m*M* P_i and 3 m*M* 2-deoxy-D-glucose (2dG). The Krebs–Henseleit solution contained 5 m*M* glucose and was saturated with 95/5 O_2/CO_2 during perfusion. The perfusate return was not recycled. BCNU was prepared by dissolving 100 mg of carmustine (BiCNU, Bristol Laboratories, Candiac, Quebec, Canada) in 27 ml of sterile water and 3 ml of absolute ethanol. The resulting stock solution of 3300 μg/ml was stored as 2.5-ml aliquots at −87°C until needed. The cells were challenged with BCNU in P_i and 2dG free buffer after accumulating 2dG6P. The frozen BCNU aliquots were dissolved in 85 ml of P_i and 2dG free Krebs yielding an initial BCNU concentration of 94 ± 2 μg/ml and an ethanol vehicle concentration of 50 m*M* (0.3% v/v).

NMR Spectroscopy

A homebuilt probe in a wide-bore Varian VXR400S spectrometer system was used to acquire ^{31}P NMR spectra at ambient temperature (23°C). The probe consisted of an aluminium shell with a Helmholtz coil made of 12 gauge copper wire mounted on a Plexiglas base. The coil had a height of 2.9 cm. For added stability, the coil was

supported by Pyrex glass into which a homemade NMR tube would fit. The inner diameter of this tube was 1.33 cm leading to an NMR-sensitive volume of 2.8 ml. The NMR tube was positioned so that its base coincided with that of the coil. A pulsewidth of 22 μs (54°) was used with a recycle time of 1.3 s, 0.5 s for relaxation and 0.8 s for acquisition, over 13,500 Hz. The cells were restricted to the NMR-sensitive volume. Good signal to noise (S/N) was obtained with a line broadening of 45 Hz after 750 transients yielding a time resolution of 16.25 min/spectrum.

Titration Curves

Titration curves which relate pH with the relative ^{31}P chemical shifts of P_i and 2dG6P were obtained using 5 ml of an aqueous solution of cellular ionic strength comprised of 20 mM P_i (as KH_2PO_4), 150 mM KCl, 7 mM 2dG6P, and 10 mM $MgCl_2$. All chemical shifts were referenced to external methylene diphosphonate (MDP), at 0 ppm, in a 2-mm-o.d. (1-mm-i.d.) capillary containing 0.023 ml of a 100 mM solution in the sensitive volume leading to an effective NMR MDP concentration of about 0.82 mM. A pHM84 research pH meter (Radiometer, Copenhagen, Denmark) was used for all pH measurements. The titration curves were obtained using KOH and HCl to adjust the pH. The spectra and pH measurements were acquired at ambient temperature (23°C).

RESULTS

Titration Curves

The titration curve relating 2dG6P relative chemical shift to pH was obtained assuming Henderson–Hasselbach equilibrium: $\mathrm{pH} = \mathrm{p}K_a - \log((x - B)/(A - x))$, leading to the analytical expression

$$\mathrm{pH} = 6.05 - \log((x + 13.15)/(-16.79 - x)),$$

where $x = (\delta 2\mathrm{dG6P} - \delta\mathrm{MDP})$, -13.15 ± 0.05 (SD) ppm $= B$ and is the chemical shift (δ) of free "base" relative to methylene diphosphonate, MDP (δbase $-$ δMDP), and -16.79 ± 0.02 ppm $= A$ and is the relative shift of free "acid". The pK_a value for 2dG6P of 6.05 ± 0.02 was determined from a plot of pH as a function of x, by solving for x when $(x - B) = (A - x)$. The preceding expression accurately fitted the experimental points within ± 0.03 pH units from pH 5 to 7.5 (Fig. 1a).

An analytical expression for P_i for correlating pH to relative chemical shifts was also found. For P_i (Fig. 1b) the pK_a was 6.63 ± 0.02, B was -15.00 ± 0.05 and A was -17.468 ± 0.014. For all of the expressions the values of A and B were determined from the titration curves as averages of the end points for the free acid and base, respectively, that gave the best fits.

Effects of BCNU

Good quality spectra from about 10^8 cells were obtained in 16.25 min. A 2-h preperfusion with P_i and 2-deoxy-D-glucose resulted in the intracellular accumulation of about 1 mM of 2dG6P (e.g., Fig. 2a). Figure 2b shows the relative amount of intracellular P_i remaining after perfusing with P_i free Krebs. The intracellular P_i peak was not a reliable pH_i marker in well-perfused cells because of low S/N.

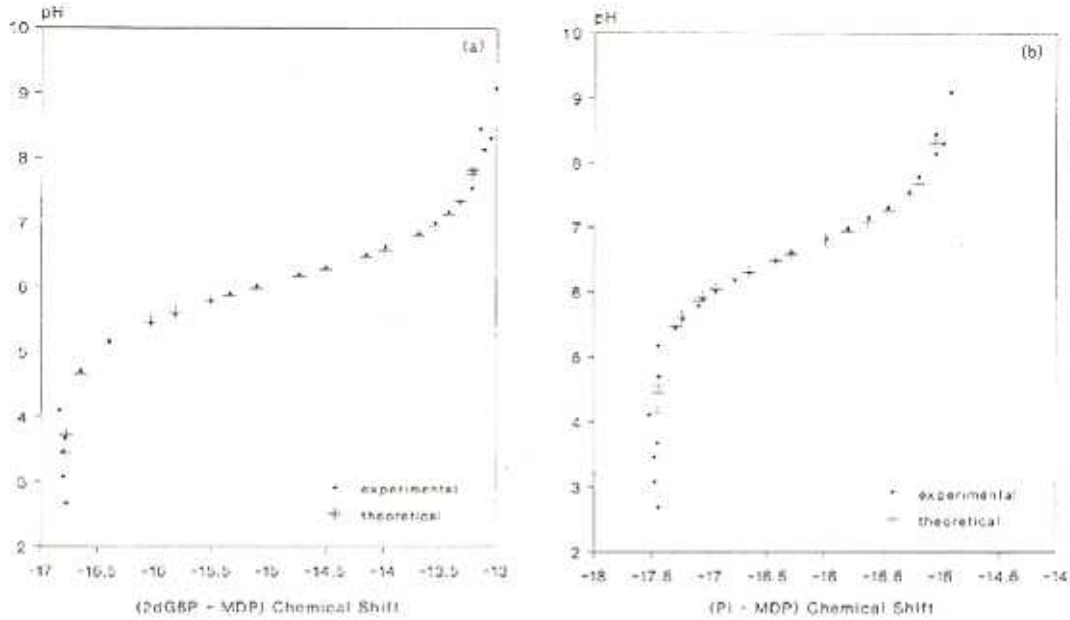

FIG. 1. Titration curves correlating intracellular pH with (a) 2-deoxy-D-glucose-6-phosphate (2dG6P) relative chemical shift (X) and (b) inorganic phosphate (P_i) relative chemical shift (Y). For 2dG6P, the predicted pH is given by: $pH = 6.05 - \log((X + 13.15)/(-16.79 - X))$. For P_i: $pH = 6.63 - \log((Y - 15.00)/(-17.468 - Y))$.

Figure 3 shows the time course of perfusion and the change in the $P_{i\,ex+in}$, $P_{i\,in}$, and 2dG6P levels prior to and following BCNU administration. There was a 34% loss in the level of 2dG6P over a period of about 5 h following the end of 2dG6P loading. The average ($n = 4$) hourly decrease in the 2dG6P level was $10 \pm 3\%$ in both the control and BCNU studies. This depletion of 2dG6P does not impair its use as a pH_i marker.

The results of four individual experiments showing the pH pre- and postinitial BCNU challenges are shown in Table 1. Prior to challenging the cells with BCNU, the average control pH_i was 7.33 ± 0.13. A transient alkalosis of 0.13 ± 0.05 pH units was observed within $4 \pm 1 \times 10$ min of adding the BCNU to the perfusate. This difference in pH between the cells prior to and after the initial BCNU challenges was found to be statistically significant ($P < 0.02$) by a paired t test. BCNU was added to the perfusate at an initial dose of 94 ± 2 μg/ml. Assuming a chemical half life of 40 min and first order decomposition (*18*); this resulted in an effective dose of $5 \pm 1 \times 10$ μg/ml. At the end of three such BCNU challenges, about 65 min apart, the average pH was 7.15 ± 0.15 ($n = 4$). Figure 4 shows the variation in pH following the administration of BCNU for one experiment.

Control experiments show that the drug vehicle, ethanol, is not responsible for the observed alkalosis (Table 2). In four different experiments the average preethanol pH_i was 7.34 ± 0.37. Following the addition of 50 mM ethanol, the average pH, pH_f, was 7.29 ± 0.35. This difference is not statistically significant at a 95% confidence level by a paired t test. Thus, the drug vehicle, ethanol, is not responsible for the alkalosis seen

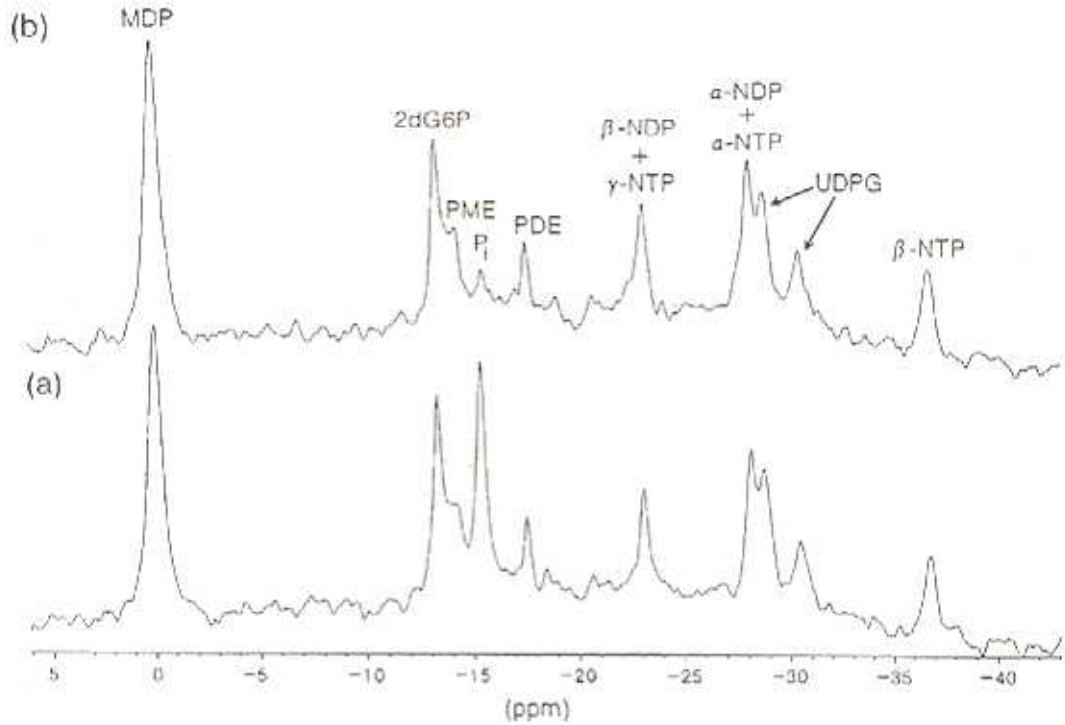

FIG. 2. (a) The level of intracellular 2-deoxy-D-glucose-6-phosphate (2dG6P, ~ 1 m*M*) following 2.2 h of preperfusion of SKI-1 glioma cells with 1 m*M* P_i and 3 m*M* 2-deoxy-D-glucose (2dG) containing Krebs–Henseleit saline. The spectrum corresponds to the time indicated by arrow number 2 in Fig. 3. (b) The level of intracellular P_i following the washout of the extracellular P_i with P_i free Krebs for 1.4 h. This spectrum corresponds to the time indicated by arrow number 3 in Fig. 3. MDP, methylene diphosphonate; PME, phosphomonoester; PDE, phosphodiester; NDP, nucleoside diphosphate; NTP, nucleoside triphosphate; and UDPG, uridine diphosphoglucose.

following an initial BCNU challenge. Since a straight horizontal line was the best fit to the points postethanol, an average pH value was used for pH_f.

Figure 5 shows that (a) the introduction of 2dG6P does not alter the intracellular pH (using endogenous P_i as a pH_i marker prior to the introduction of 2dG6P) and (b and c) the levels of high energy phosphates, phosphomonoester, phosphodiester, and uridinediphosphoglucose are not depleted with 2dG6P loading over the 2.51 ± 0.43-h period of 2dG6P loading used for the experiments represented in Table 1. Prolonged 2dG6P loading (greater than about 4 h) results in a decrease in α-NTP levels (Fig. 5b). The levels of these metabolites were also not decreased after challenging the cells with BCNU. Although the phosphodiester level was not altered during 2dG6P loading or the BCNU challenges, an initial decrease was observed during perfusion with P_i and 2dG free Krebs.

DISCUSSION

In this study, 2dG6P was used as a pH_i marker (*15*). This compound accumulates readily during perfusion with 2-deoxy-D-glucose. The loss of 2dG6P (in both BCNU

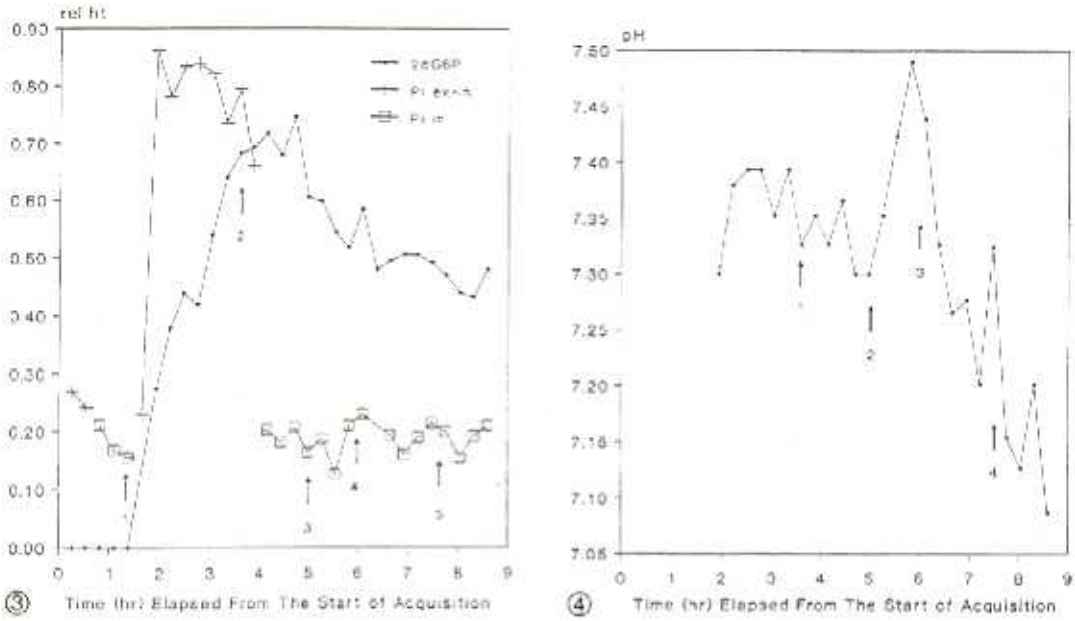

FIG. 3. Changes in the relative levels (rel ht = metabolite peak height/MDP peak height) of 2-deoxy-D-glucose-6-phosphate (2dG6P), overlapping resonances for medium P_i, $P_{i\,ex}$, and endogeneous intracellular P_i, $P_{i\,in}$ ($P_{i\,ex+in}$), and $P_{i\,in}$ in SKI-1 glioma cells. The cells were initially perfused with P_i and 2-deoxy-D-glucose (2dG)-free Krebs. Arrow 1 indicates the start of perfusion with 1 m*M* P_i and 3 m*M* 2dG containing Krebs. Arrow 2 indicates the start of the washout of the extracellular P_i with P_i and 2dG free Krebs. The arrows labeled 3, 4, and 5 indicate the introduction of (P_i and dG free) Krebs containing 95 μg/ml BCNU. Once $P_{i\,ex}$ is washed out, only $P_{i\,in}$ is seen. Upon perfusion with P_i containing Krebs, $P_{i\,in}$ is masked by the larger $P_{i\,ex}$.

FIG. 4. Intracellular pH (based on relative 2dG6P δ values) preceding and following the administration of BCNU measured during the experiment shown in Fig. 3. Arrow 1 indicates the start of perfusion with P_i and 2-deoxy-D-glucose (2dG) free Krebs, after perfusion with 1 m*M* P_i and 3 m*M* 2dG containing Krebs. The arrows labeled 2, 3, and 4 indicate the start of perfusion with (P_i and 2dG free) Krebs containing 95 μg/ml BCNU.

challenged cells and controls without BCNU) may be due to leakage, as is the case with methylphosphonate (*19, 20*), or the fluorinated pH markers (*21, 22*) or due to the action of a phosphatase (*23*). Since with continued perfusion with 2dG containing medium there is no decrease in the level of 2dG6P, even with the end of perfusion and cell death (Fig. 5d), the action of a phosphatase in living cells is the most likely cause of this 2dG6P depletion once 2dG6P loading is ended. Accumulation of 2dG6P does not change pH_i (Fig. 5a) or deplete the levels of high energy phosphates and other phosphate-containing compounds (Figs. 5b and 5c) in the SKI-1 cell line, over a 2.51 ± 0.43-h time course for 2dG6P loading. Also, it does not appear to inhibit glycolysis since when perfusion was terminated at the end of the experiments pH_i decreased to 6.64 ± 0.12 within 2 h (e.g., Fig. 5a). These findings are consistent with the results of other studies in which 2dG has been used to monitor metabolism. Loading rat brains with nontracer doses of 2dG6P (500 mg/kg 2dG, i.v.) does not result in alteration of pH_i, concentrations of high energy phosphates, or detectable toxicity

TABLE 1

A Comparison of the Average pH Values before and after Challenging Glioma SKI-1 Cells with BCNU

Cell sample	Cells $\times 10^{-7}$	t_f (min)	pH_i	pH_f	ΔpH	$[BCNU]_i$ μg/ml	$[BCNU]_f$ μg/ml
1	7.7	32.5	7.46	7.57	0.11	91	52
2	10.7	48.8	7.33	7.49	0.16	95	41
3	9.0	48.8	7.15	7.22	0.07	94	40
4	9.2	32.5	7.38	7.57	0.19	94	54
X	9.2	40.7	7.33*	7.46*	0.13	94	47
$\text{avg}(X_i - X)$	0.8	8.1	0.09	0.12	0.05	2	6
$\sigma_{(n-1)}$	1.2	9.4	0.13	0.17	0.06	2	7

Note. t_f, the time elapsed after the initial BCNU challenge; pH_i, the control pH prior to the initial BCNU challenge (average of four or five values); pH_f, the pH post-BCNU at time t_f; $[BCNU]_i$, the initial BCNU concentration in the perfusate; $[BCNU]_f$, the effective BCNU concentration after t_f min, assuming a 40-min chemical half life and first order decomposition of BCNU (*18*); X, the average of the values in the column; $\text{avg}(X_i - X)$, the mean deviation from the mean of the values in the column; $\sigma_{(n-1)}$, the unbiased standard deviation of the values in the column.

* The difference between these values is statistically significant ($P < 0.02$) by a paired t test.

(*24–27*). Navon *et al.* (*28*) have shown that uptake and phosphorylation of 2dG has little effect on high energy phosphates in ^{31}P spectra of MCF-7 tumor subcutaneously implanted in an athymic mouse (2 g/kg 2dG, i.p.). Although 2dG was shown (*29*) to be toxic to ADR breast cancer cells as evidenced by an irreversible depletion of high energy phosphates, it is not clear if these cells were also glucose starved.

TABLE 2

pH prior to and following 50 m*M* (0.3% v/v) Ethanol (Drug Vehicle) Challenge of the SKI-1 Glioma Cells

Cell sample	pH_i	pH_f	ΔpH
1	7.74	7.66	−0.08
2	7.56	7.51	−0.05
3	6.97	6.98	+0.01
4	7.09	7.02	−0.07
X	7.34	7.29	−0.05
$\text{avg}(X_i - X)$	0.31	0.29	0.03
$\sigma_{(n-1)}$	0.37	0.35	0.04

Note. pH_i, the pH (average of five values) before the introduction of vehicle, after loading the cells with 2dG6P; pH_f, the pH (average of five values) after the vehicle challenge; X, the average of the values in the column; $\text{avg}(X_i - X)$, the mean deviation from the mean of the values in the column; $\sigma_{(n-1)}$ = the unbiased standard deviation of the values in the column.

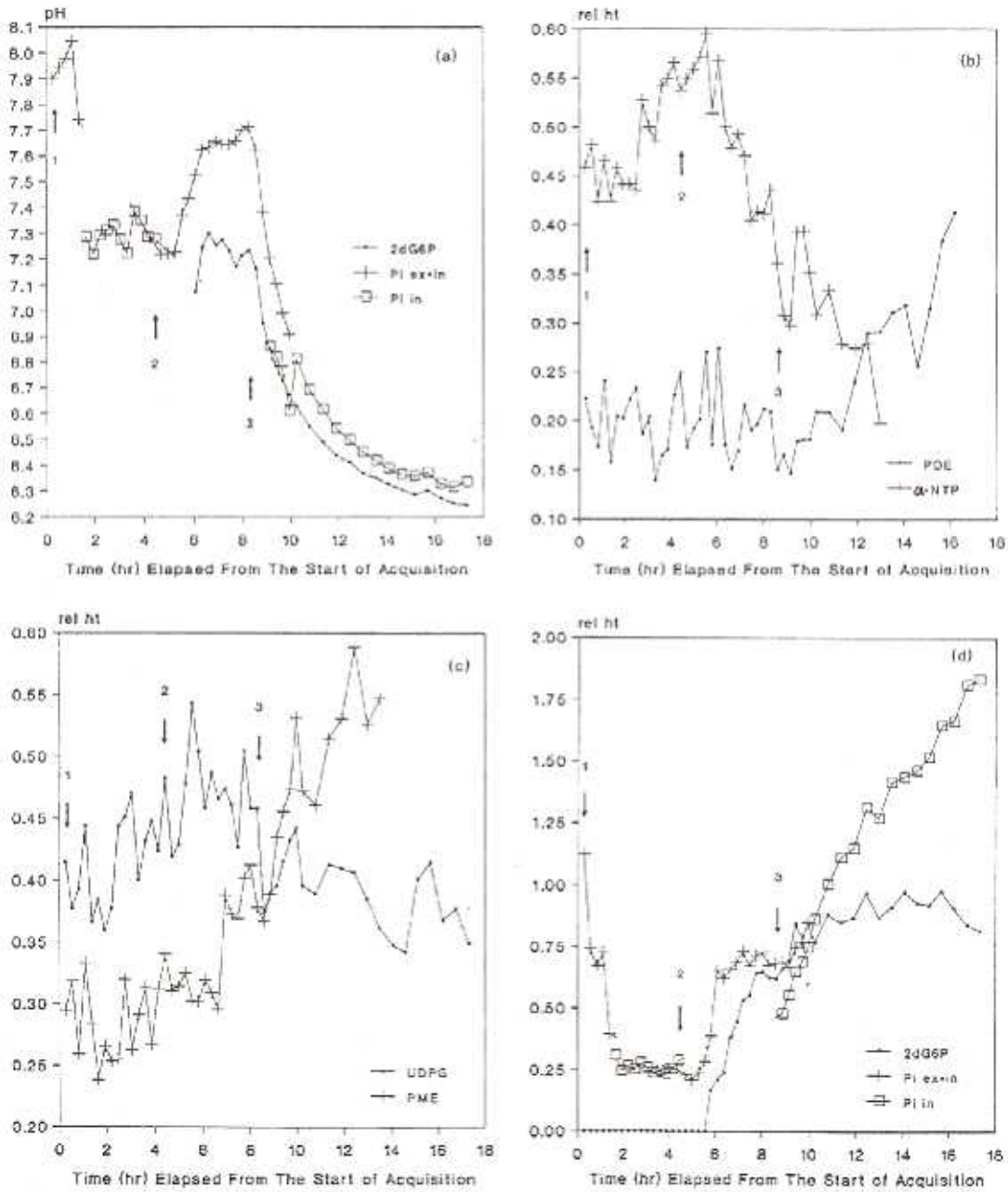

FIG. 5. Control data showing that (a) the introduction of 2dG6P does not alter the intracellular pH (using endogenous P_i as a pH marker prior to the introduction of 2dG6P), and that the levels of (b) phosphodiester (PDE) and high energy phosphates (α-NTP), and (c) uridine diphosphoglucose (UDPG) and phosphomonoester (PME) are not depleted with 2dG6P loading for 2.51 ± 0.43 h and (d) the variation in the relative levels (rel ht = metabolite peak height/MDP peak height) of 2-deoxy-D-glucose-6-phosphate (2dG6P), overlapping resonances for medium P_i, $P_{i\,ex}$, and endogeneous intracellular P_i, $P_{i\,in}$ ($P_{i\,ex+in}$), and $P_{i\,in}$ pre- and postcell death. The same time course is shown in all of the figures with arrows 1, 2, and 3 indicating, respectively, the washout of extracellular P_i, the start of 2dG6P loading, and the end of perfusion (cell death). During perfusion with P_i containing Krebs, the $P_{i\,ex}$ overlaps and obscures the $P_{i\,in}$, leading to the absence of points for $P_{i\,in}$ in (a) and (d). Likewise, with washout of $P_{i\,ex}$, only $P_{i\,in}$ remains. With cell death, the increase in $P_{i\,in}$ eventually obscures any $P_{i\,ex}$ in (a) and (d).

In this study, the P_i chemical-shift proved to be a nonreliable marker of intracellular pH. Although intracellular P_i, $P_{i\,in}$, was usually visible, the level of $P_{i\,in}$ relative to MDP was variable between experiments and even from one spectrum to another within the same cell sample. Often the level of $P_{i\,in}$ was too low (not much above the baseline noise) to provide an accurate chemical shift. Furthermore, when there was enough $P_{i\,in}$ to obtain accurate chemical shifts, the pH values obtained from these shifts did not always correlate well with those from 2dG6P shifts. This could be because the $P_{i\,in}$ shift observed may represent an average of various P_i components in the cell preparation (dead versus live cells). Phosphorylation of 2dG by hexokinase can only occur in the cytoplasm of living cells because incorporation into the cell requires the glucose carrier for facilitated diffusion (*26, 30, 31*). For this reason, 2dG6P may in fact be a better cytoplasmic pH marker than P_i.

A transient alkalosis of similar magnitude to that observed *in vivo* (*13, 14*) was observed in a glioma cell line with demonstrated sensitivity to BCNU (*16*). The effective concentration of BCNU that the cells were exposed to (about 40–54 μg/ml) is in the same range as that expected during intraarterial infusion (*32*). With repeated BCNU challenges an overall acidosis was observed which likely reflects the accumulation of lactic acid resulting from metabolic compromise. Although the time course of the *in vitro* response is much faster than that *in vivo*, these results suggest that the immediate pH change could be an indicator of the cytotoxicity of the drug.

The alkylating (DNA cross-linking) action of BCNU (*8–10*) is unlikely to produce the pH changes we have observed in this study or in the *in vivo* studies (*13, 14*); however, carbamoylation (*12*) of membrane proteins could lead to intracellular alkalinization resulting from a loss of membrane potential (*13*). Support for this idea has come from studies on BCNU-sensitive and -resistant cell lines in which dose-dependent morphological changes in membrane structure were demonstrated only in BCNU-sensitive lines (*32*). One may investigate the molecular mechanism of the intracellular alkalosis by performing interleaved ^{31}P, ^{23}Na, and ^{13}C NMR studies.

ACKNOWLEDGMENTS

This research was supported in part by a grant from the Neuro Foundation, Toronto, to E.A.S. who is a chercheur boursier with FRSQ, Quebec. We thank Ms. Louise Boulet for her expert assistance with cell culture.

REFERENCES

1. J. A. MONTGOMERY, in "Nitrosoureas: Current Status and New Developments" (A. W. Prestayko, S. T. Crooke, L. H. Baker, S. K. Carter, and P. S. Schein, Eds.), pp. 3–8, Academic Press, New York, 1981.
2. M. D. WALKER AND B. S. HURWITZ, *Cancer Chemother. Rep.* **54**, 263 (1970).
3. W. A. YUNG, J. R. SHAPIRO, AND W. R. SHAPIRO, *Cancer Res.* **42**, 992 (1982).
4. K. SAKO, M. DIKSIC, S. FARROKHZAD, L. YAMAMOTO, AND W. FEINDEL, *J. Neuro Oncol.* **3**, 229 (1985).
5. S. L. AUKERMAN, R. B. BRUNDRETT, J. HILTON, AND P. E. HARTMAN, *Cancer Res.* **43**, 175 (1983).
6. J. W. LOWN AND S. M. S. CHAUHAN, *J. O. C.* **46**, 5309 (1981).
7. J. W. LOWN AND S. M. S. CHAUHAN, *J. O. C.* **46**, 2479 (1981).
8. G. EISENBRAND, M. R. BERGER, J. FISCHER, M. R. SCHNEIDER, W. TANG, AND W. J. ZELLER, *Anti-Cancer Drug Design* **2**, 351 (1988).
9. J. W. LOWN, V. J. ALUMMOOTTIL, AND W. MCLAUGHLIN, *J. Med. Chem.* **23**, 798 (1980).

10. C. ZLOTOGORSKI AND L. C. ERICKSON, *Carcinogenesis (London)* **4**, 759 (1983).
11. W. J. BODELL, H. T. R. RUPNICK, J. RASMUSSEN, W. F. MORGAN, AND M. L. ROSENBLUM, *Cancer Res.* **44**, 3736 (1984).
12. C. DIVE, P. WORKMAN, AND J. V. WATSON, *Biochem. Pharmacol.* **36**, 3731 (1987).
13. D. L. ARNOLD, E. A. SHOUBRIDGE, W. FEINDEL, AND J. G. VILLEMURE, *Can. J. Neurol. Sci.* **14**, 570 (1987).
14. D. L. ARNOLD, E. A. SHOUBRIDGE, W. FEINDEL, AND J. G. VILLEMURE, *Invest. Radiol.* **24**, 958 (1989).
15. L. A. BAILEY, S. R. WILLIAMS, G. K. RADDA, AND D. G. GADIAN, *Biochem. J.* **196**, 171 (1981).
16. V. SKALSKI, J. RIVAS, L. PANASCI, A. MCQUILLAN, AND W. FEINDEL, *Cancer Chemother. Pharmacol.* **22**, 137 (1988).
17. D. L. FOXALL, J. S. COHEN, AND J. B. MITCHELL, *Exp. Cell Res.* **154**, 521 (1984).
18. J. A. MONTGOMERRY, R. JAMES, G. S. MCCALEB, AND T. P. JOHNSTON, *J. Med. Chem.* **10**, 668 (1967).
19. R. J. LABOTKA AND R. A. KLEPS, *Biochemistry* **22**, 6089 (1983).
20. M. DEFRONZO AND R. J. GILLIES, *J. Biol. Chem.* **262**, 11,032 (1987).
21. J. TAYLOR AND C. DEUTSCH, *Biophys. J.* **53**, 227 (1988).
22. C. DEUTSCH AND J. TAYLOR, *in* pp. 33–47, *Ann. N.Y. Acad. Sci.* **508**, 33 (1987).
23. G. E. DEMETROKOPOULOS, B. LINN, AND H. AMOS, *Biochim. Biophys. Acta* **6**, 65 (1982).
24. J. J. KOTYK, R. S. RUST, J. J. H. ACKERMAN, AND R. K. DEUEL, *J. Neurochem.* **53**, 1620 (1989).
25. J. J. H. ACKERMAN, "Cerebral Deoxyglucose Metabolism: ^{13}C ^{19}F ^{31}P Surface Coil NMR Spectroscopy of Rat Brain *in vivo*," SMRM Abstracts, pp. 77, Amsterdam, 1989.
26. K. KOGA AND I. MIURA, *Biochem. Biophys. Res. Commun.* **157**, 1258 (1988).
27. F. HALABI, J. P. SEGUIN, J. FONROGET, AND G. GOETHALS, *Spectrochimica Acta* **44A**, 699 (1988).
28. G. NAVON, R. C. LYON, O. KAPLAN, AND J. S. COHEN, *FEBS Lett.* **247**, 86 (1989).
29. O. KAPLAN, G. NAVON, R. C. LYON, P. J. FAUSTINO, E. J. STRAKA, AND J. S. COHEN, *Cancer Res.* **50**, 544 (1990).
30. D. M. KIPNIS AND C. F. CORI, *J. Biol. Chem.* **234**, 171 (1959).
31. J. A. HOERTER, C. LAUER, G. VASSORT, AND M. GUERON, *Am. J. Physiol. Cell Physiol.* **255**, C192 (1988).
32. B. H. SMITH, M. VAUGHAN, M. A. GREENWOOD, P. L. KORNBLITH, A. ROBINSON, N. SHITARA, AND P. E. MCKEEVER, *J. Neuro Oncol.* **1**, 237 (1983).