Anti-Cancer Drugs 1998, 9, pp. 449–456

Preclinical paper

Characterization of multidrug resistance and monitoring of tumor response by combined ^{31}P and ^{1}H nuclear magnetic resonance spectroscopic analysis

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A combined ^{31}P and ^{1}H nuclear magnetic resonance (NMR) spectroscopic study was carried out in drug-sensitive and adriamycin- and mitoxantrone-resistant P388 murine leukemic cells. Typical spectral changes characteristic of multidrug resistance were observed in resistant cells compared to drug-sensitive cells. Quantitative comparison of phosphate metabolites ATP, phosphocreatine, phosphomonoesters and phosphodiesters revealed a significant alteration in the metabolism of resistant cells. The elevated levels of energy metabolites supported the energy-dependent process of drug efflux in resistant cells. An increased rate of glycolysis in resistant cells as indicated by the elevated lactate level further supported this. The near total loss of energy metabolites and marked decrease in phospholipid metabolites in sensitive cells upon treatment with drug compared to unaltered metabolite levels in resistant cells suggested that the spectral changes can reveal the subtle differences in tumor response between drug-sensitive and -resistant cells. The results substantiate the potential of this non-invasive method to characterize the multidrug resistance phenotype and monitor tumor response. [© 1998 Lippincott-Raven Publishers.]

Key words: Metabolites, multidrug resistance, NMR, tumor response.

Introduction

Development of multidrug resistance (MDR) is one of the major reasons in the failure of cancer chemotherapy. Although several mechanisms of resistance have been described for MDR such as overexpression of P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP), altered topoisomerase II activity, elevated glutathione, increased DNA repair, etc., the *mdr1* gene product P-gp is by far the most common factor in MDR.[1] Many differences in the biochemical pathways between drug-sensitive and -resistant cells have been identified to be involved in multidrug resistance.[2,3] Studies of these biochemical alterations, which occur when tumor cells become drug resistant, might be useful as prognostic markers in the development of drug resistance and could allow one to predict response to therapy or modify cellular biochemistry to improve therapeutic efficacy.

A number of tumor characteristics contribute to the variation in therapeutic response. Unfortunately, many of the techniques used to monitor these characteristics in tumor systems are invasive and either cannot be applied to humans or are applied with significant difficulty and discomfort. Consequently it is difficult to implement such techniques to provide indices of tumor responsiveness to chemotherapeutic agents. Nuclear magnetic resonance (NMR) spectroscopy provides the ability to investigate the metabolic processes in cancer cells and tissues in a non-invasive manner.[5]

We have earlier shown the feasibility of monitoring therapeutic response of tumors by high-resolution ^{1}H NMR spectroscopy.[5] We have also reported the characterization of *mdr* using conventional screening methods, such as measurement of drug transport, cytotoxicity, DNA damage and repair in drug-resistant cells.[6,7] In the present study, ^{31}P and ^{1}H NMR spectroscopy was employed to monitor levels of high-energy phosphate and phospholipid metabolites both in intact cells, and in perchloric acid extracts of drug-sensitive and MDR (P388/ADR and P388/MTN) murine lymphocytic leukemic cells. Since P-gp-mediated drug resistance is an energy-dependent process, changes in energy requirements and metabolism may be expected. Here, we report the presence of metabolites typical for the *MDR* phenotype and the

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response-specific markers of drug sensitivity and resistance.

Materials and methods

Chemicals

Adriamycin (ADR) was purchased from Farmitalia Carlo Erba (Milan, Italy). Mitoxantrone (MTN) was a kind gift from Dr F Durr (American Cyanamide, New York, NY). Diphenyl phosphate, D_2O and ATP were obtained from Aldrich (Milwaukee, WI) and minimum essential medium from Himedia Laboratories (Bombay, India). All other reagents used were of analytical grade.

Tumor system

The parental drug-sensitive P388 murine leukemic cell line (P388/S) originally obtained from the Tumor Repository, Frederick Cancer Research Facility, NCI, MD, was maintained *in vivo* by serial transplantation of 1×10^5 cells i.p. in syngenic DBA/2 mice. The animals were given standard pellet diet and water *ad libitum*. ADR (P388/ADR) and MTN-resistant (P388/MTN) sublines were obtained from the Cancer Research Institute (Bombay, India). The resistance was checked periodically, and these sublines were completely refractory to *in vivo* ADR and MTN treatment.

Ascites cells were aspirated on day 7 post-transplantation from mice bearing P388/S, P388/ADR and P388/MTN leukemic cells by peritoneal lavage with cold isotonic saline. Then the cells were washed twice with saline and finally suspended in MEM medium. Viability of the cells were checked using 0.4% Trypan blue dye.

Perchloric acid extraction

For perchloric acid (PCA) extractions, all the solutions were prepared in deionized water that contained no detectable paramagnetic impurity. Cell extracts were made from approximately 5×10^8 cells at 4°C as reported by De Jong *et al.*[8] Ice-cold PCA (10%) was added to the cell pellet, and the cell mixture was vortexed at the beginning and end of the 20 min period. The extracts were neutralized with 6 N KOH, centrifuged at 8000 *g* at 4°C to remove $KClO_4$ precipitate, freeze dried and stored at −70°C, until the spectra were recorded. The samples were dissolved in D_2O, just prior to loading the sample. For the preparation of cell extract after drug treatment, the animals were treated with 5 mg/kg of ADR i.p., 24 h prior to cell harvest and cell extracts were prepared as mentioned above.

^{31}P and ^{1}H NMR spectroscopy

The extracts were dissolved in 1 ml of D_2O and loaded in a 5 mm NMR tube. ^{31}P NMR spectra were recorded on a Bruker MSL-300 Spectrometer at 121.5 MHz at 15°C with deuterium lock. The operating conditions were a 55° flip angle, 40 s repetition time, 1200 transients and 8 K data points; 1 mM diphenyl phosphate was used as a reference standard.

^{1}H NMR spectra were recorded at 300 MHz, with 0.075% w/w trimethylsilylpropane-sulfonic acid (TSP) as a standard at 0 p.p.m. The operating conditions were a 90° flip angle, 3 s repitition time, 1000 transients and 16 K data points.

^{31}P spectra of intact cells was recorded as follows. After washing with isotonic saline and centrifugation, cells were suspended in phosphate-free buffer (pH 7.2) and loaded in a 25 mm NMR imaging tube. The spectra were recorded at 55° flip angle, 1 s repetition time and 1500 transients.

Results

^{31}P NMR spectra of cell extracts

The resonances were identified based on literature values and on the resonances of pure compounds added to the PCA extract of the cells. Peak heights were reproducible and, thus, were used for spectral analysis.

^{31}P NMR spectra of PCA extract of drug-sensitive, ADR- and MTN-resistant cells are shown in Figure 1(A, B and C, respectively). Diphenyl phosphate added to the extract helped simplify peak identification on the basis of chemical shift values and to quantitate the phosphate metabolite contents in the extract. The main resonances originated from phosphocholine (PC), phosphoethanolamine (PE), inorganic phosphate (Pi), glycerophosphocholine (GPC), glycerophosphoethanolamine (GPE) and ATP (α, β, and γ).

^{31}P NMR spectra of P388/ADR- and P388/MTN-resistant cells exhibited striking differences in the levels of phosphate metabolites from the drug-sensitive P388/S cells. The spectra revealed elevated levels of phosphocreatine (PCr) and ATP, and decreased levels of phosphomonoesters (PC and PE) and phosphodiesters (GPE and GPC) in both the P388/ADR- and P388/MTN-resistant cells when compared

with that of sensitive cells. The phosphate metabolite levels quantitated from ^{31}P MRS and the concentrations were normalized to 10^8 cells (Table 1). There was a 4.5- and 3-fold increase in the PCr level in P388/ADR- and P388/MTN-resistant sublines, respectively, when compared with P388/S cells. The level of ATP was about 1.5-fold higher in both the resistant cells. In contrast, there was about 2-fold decrease in PC and PE levels in both the drug-resistant cells compared to sensitive cells. The level of GPC was about 3-fold less in ADR- and MTN-resistant cells compared to sensitive cells. The concentration of GPE was 0.74 μmol in drug-sensitive cells, whereas in both the drug-resistant cells GPE the signal was absent. A 50% decrease in the DPDE level in both the resistant sublines was observed. The α-ADP peak was not observed in both the drug-resistant cells. Quantitation of PCr in some experiments was difficult due to its low intensity and it therefore was susceptible to baseline noise. Similarly, the signal intensities of GPE and GPC were very low in the resistant cells in some experiments.

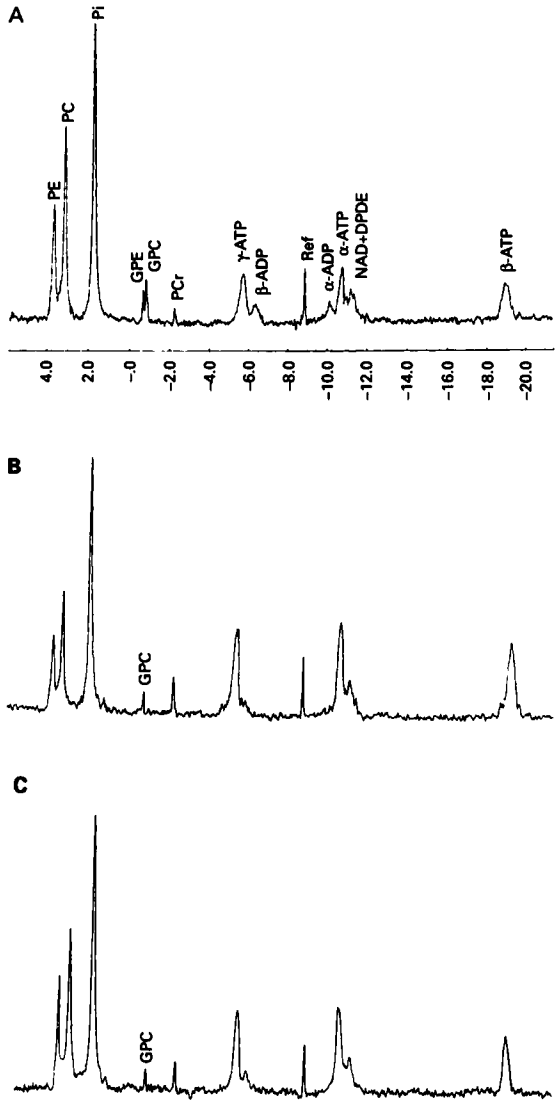

Figure 1. ^{31}P NMR spectra (121.5 MHz) of the PCA extract of (A) P388/S, (B) P388/ADR and (C) P388/MTN leukemic cells.

^{31}P NMR spectra of intact cells

^{31}P NMR spectra of intact cells were recorded, in order to ensure that the NMR spectra of PCA extracts reflect the actual data of the intact cells and no artifacts from the extraction procedure were found. The ^{31}P NMR spectra of intact drug-sensitive and ADR-resistant cells are shown in Figure 2 (A and B). Here again, the signal intensities arose mainly from ATP, Pi, phosphomonoesters and phosphodiesters. The PCr resonance was not observed in either of the spectra. The peaks obtained, in general, were very broad and the resolution was not better when compared to PCA cell extracts. Besides, the level of PCr was lower and it is possible that due to peak broadening no typical peak for PCr could be detected in intact cells. The distinct peaks were integrated from each spectrum and the mean concentration of these energy and phospholipid metabolite levels calculated based on the peak area (Table 2).

The phosphomonoesters PC and PE appeared together and, therefore, the values were calculated as total area. Phosphomonoesters were about 2-fold higher in drug-sensitive cells compared with that of the resistant subline. A decreased level of phosphodiesters were observed in P388/ADR cells. GPC and GPE were 4- and 2-fold higher, respectively, in drug-sensitive cells when compared with the ADR-resistant counterpart. In contrast, the energy metabolite ATP was higher by 1.2- to 1.3-fold in P388/ADR cells than P388/S cells. The values obtained with the PCA extracts of cells were generally in good agreement with intact cells.

^{31}P NMR spectra of cell extracts after drug treatment

^{31}P NMR spectra of the PCA extract of drug-sensitive, ADR- and MTN-resistant cells 24 h after ADR treatment (5 mg/kg, i.p) are shown in Figure 3 (A, B and C, respectively). A noticeable change in the spectra of drug-sensitive cells after drug treatment was observed when compared with the spectra obtained prior to drug treatment, whereas in both the drug-resistant

Table 1. Concentration of phosphate metabolites in PCA extracts (μmol/10^8 cells)[a] of drug-sensitive, ADR- and MTN-resistant P388 leukemic cells

Metabolite	P388/S	P388/ADR	P388/MTN	P388/S P388/ADR	P388/S P388/MTN
PE	2.58±0.51	1.23±0.36	1.42±0.22	2.10[c]	1.82[c]
PC	3.30±0.35	1.80±0.23	2.31±0.17	1.83[c]	1.43
GPE	0.74±0.26	ND	ND	–	–
GPC	1.13±0.32	0.31±0.08	ND	3.65[b]	–
PCr	0.23±0.10	1.05±0.22	0.76±0.24	0.22[b]	0.31[b]
γ-ATP	1.75±0.37	2.70±0.18	2.23±0.44	0.65	0.78
α-ATP	1.89±0.39	2.93±0.45	2.45±0.18	0.64	0.77
β-ATP	1.58±0.23	2.36±0.36	2.16±0.42	0.67	0.73
β-ADP	0.32±0.12	0.23±0.07	0.21±0.04	1.40	1.52
NDA+DPDE	0.65±0.16	0.38±0.10	0.32±0.05	1.71	2.03

[a]Mean±SD of six experiments.
ND, not detectable.
[b]$p<0.01$ (Student's *t*-test); [c]$p<0.001$.

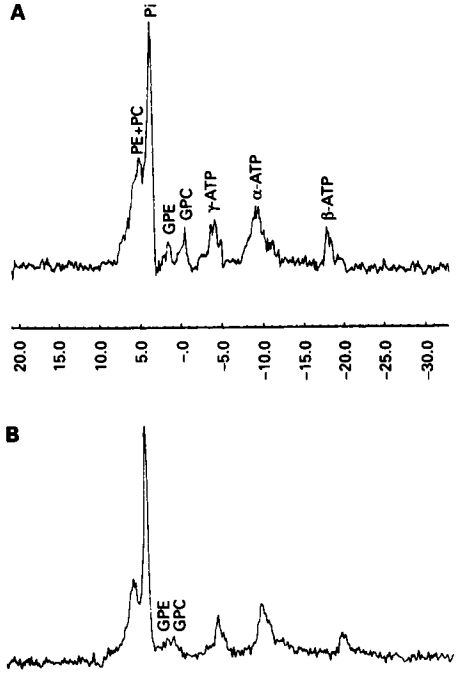

Figure 2. ^{31}P NMR spectra (121.5 MHz) of intact (A) P388/S and (B) P388/ADR leukemic cells. Peak assignments are as in the legend to Figure 1.

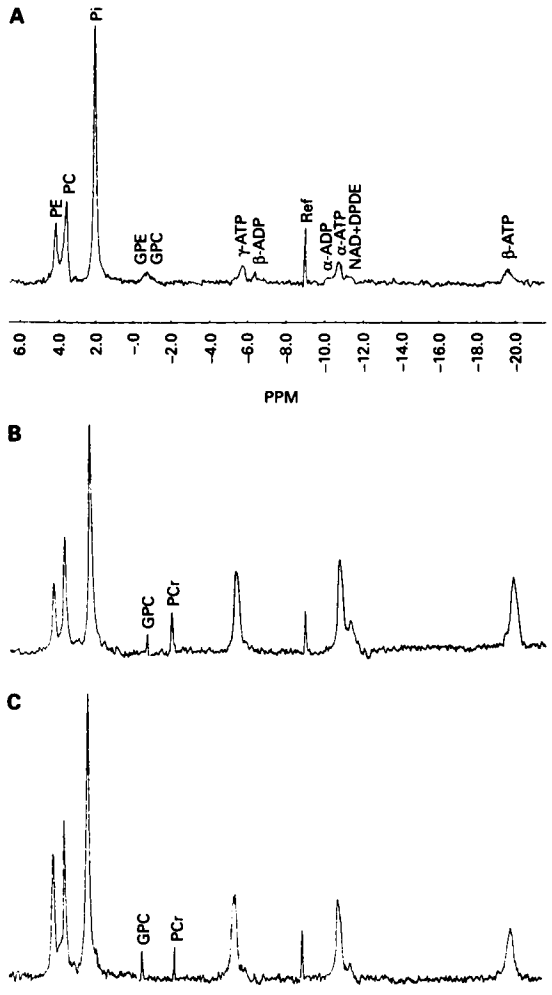

Figure 3. ^{31}P NMR spectra (121.5 MHz) of the PCA extract of (A) P388/S, (B) P388/ADR and (C) P388/MTN leukemic cells 24 h after treatment with ADR. Peak assignments are as in the legend to Figure 1.

sublines very little change in the spectra was observed between before and after drug treatment (Table 3).

PC and PE levels decreased from 3.3 and 2.6 to 1.4 and 0.8 μmol, respectively, in drug-sensitive cells. This was a decrease of more than 60% of control. Similarly, for the energy metabolites ATP and PCr, levels decreased by more than 70%. The PCr signal was not detectable in sensitive cells after ADR treatment, and the GPC and GPE levels also decreased by more than 75%.

^{1}H NMR spectra of perchloric acid extract

Typical high resolution ^{1}H NMR spectra of the PCA extract of drug-sensitive, ADR- and MTN-resistant cells are shown in Figure 4 (A, B and C, respectively). In the high field aliphatic region (0–5 p.p.m.) resonances of various amino acids, creatine, PCr, choline, lactate and acetate were clearly distinguishable in all the samples studied. Since the resolution between methyl groups of choline, PC and GPC signals was not optimal, rendering individual integral measurements difficult, these three peaks were measured together. Choline content was calculated by subtracting the PC and GPC contents, which were determined by ^{31}P NMR, from the value obtained in ^{1}H NMR for all three compounds. Similarly, methyl groups of Cr and PCr had identical chemical shifts and Cr content was determined as mentioned above.

The concentrations of various metabolites and the precursors measured in the PCA extract of P388/S, P388/ADR and P388/MTN cells are given in Table 4. The level of lactate in the drug-resistant cells was found to be elevated by 2-fold implying the role of enhanced rate of glycolysis. The PCA extract of drug-resistant cells showed higher levels of glutamate, a potential substrate for detoxification, and amino acids glycine and leucine compared to drug-sensitive cells. There was no significant change in the level of choline, a precursor for choline synthesis. However, Cr was 1.5-fold higher in resistant cells compared to sensitive cells. The level of PCr+Cr was found to be 2-fold higher in resistant cells compared to sensitive cells. The levels of PC+GPC were found to be about 65% only in resistant cells compared to sensitive cells. Thus, the results of ^{1}H NMR spectra were in agreement with the ^{31}P NMR.

^{31}P NMR spectra of drug-resistant cells did not show any alteration in the metabolite level on treatment with drug. This showed that there was not any further change in the metabolism as a result of drug treatment. This may be due to the metabolic adaptation

Table 2. Phosphate metabolite levels in the intact cells of drug-sensitive and ADR-resistant murine leukemic cells (relative to the peak area of β-ATP)[a]

Metabolite	P388/S	P388/ADR	P388/S P388/ADR
PME (Pc+PE)	3.98±0.62	2.3±0.42	1.7
GPE	0.32±0.12	0.14±0.05	2.3[b]
GPC	0.80±0.26	0.19±0.06	4.2[a]
γ-ATP	1.35±0.18	1.67±0.21	0.8
α-ATP	1.70±0.23	2.18±0.33	0.8
β-ATP	1.0	1.0	1.0

[a]Mean±SD of four experiments.
[b]$p<0.001$ (Student's *t*-test); [c]$p<0.01$.

Table 4. Concentration of metabolites in PCA extracts (μmol/10^8 cells)[a] of drug-sensitive, ADR- and MTN-resistant P388 leukemic cells

Metabolite	P388/S	P388/ADR	P388/MTN
Lactate	0.17±0.02	0.37±0.04[b]	0.31±0.03[b]
PC+GPC+C	7.87±0.93	5.32±0.43	5.96±0.77
PCr+Cr	0.94±0.17	2.28±0.39[b]	1.87±0.32
Gly	1.43±0.24	2.36±0.30	2.41±0.35
Gln+Glu	0.36±0.02	0.53±0.07	0.51±0.08
Calculated			
C	3.44±0.52	3.20±0.13	3.27±0.27
Cr	0.71±0.11	1.13±0.11	1.11±0.16

C, choline; Cr, creatine; Gly, glycine; Gln, glutamine; Glu, glutamic acid.
[a]Mean±SD of at least four experiments.
[b]$p<0.001$ compared to P388/S cells.

Table 3. Concentration of phosphate metabolites in PCA cell extracts (μmol/10^8 cells)[a] of drug-sensitive, ADR- and MTN-resistant murine leukemic cells after ADR treatment

Metabolite	P388/S	P388/ADR	P388/MTN	P388/S P388/ADR	P388/S P388/MTN
PE	0.8±0.13	1.41±0.18	1.35±0.34	0.57	0.59
PC	1.4±0.34	1.95±0.43	2.17±0.46	0.72	0.64
GPE	0.15±0.05	ND	ND	–	–
GPC	0.27±0.03	0.29±0.05	ND	0.93	–
PCr	ND	1.12±0.24	0.76±0.17	–	–
γ-ATP	0.4±0.1	2.63±0.19	2.34±0.58	0.15[b]	0.17[b]
α-ATP	0.6±0.14	2.76±0.52	2.38±0.24	0.22	0.25
β-ATP	0.43±0.07	2.12±0.39	2.29±0.34	0.20[b]	0.19[b]
NAD+DPDE	0.15±0.03	0.48±0.11	0.46±0.06	0.31	0.33

[a]Mean±SD of at least three experiments. ND, not detectable.
[b]$p<0.001$ (Student's *t*-test).

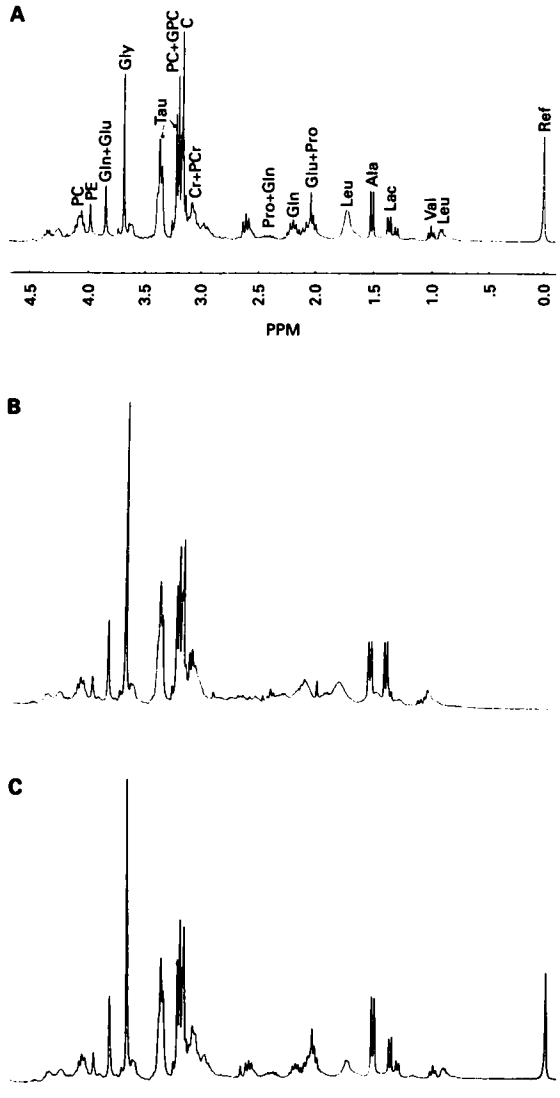

Figure 4. NMR spectra (300 MHz) of the perchloric acid extract of (A) P388/S, (B) P388/ADR and (C) P388/MTN leukemic cells. Val, valine; Leu, leucine; Ala, alanine; Lac, lactate; Pro, proline; Glu, glutamic acid; Gln, glutamine; Tau, taurine; C, choline; Cr, creatine; Gly, glycine.

associated with development of drug resistance. Since we did not observe any change in the ^{31}P NMR study after drug treatment, we presumed that there would not be any change in ^{1}H NMR analysis as well after drug treatment.

Discussion

Due to its non-invasive nature, considerable attention has been given to employ NMR as a diagnostic tool and to monitor tumor response by measuring metabolite levels. Towards this approach, we have made an attempt to identify the NMR detectable metabolite profile typical for the MDR cells and also observe the effect of drugs on these metabolite levels. There were consistent and reproducible differences in the levels of PCr, ATP, PME, PDE and lactate between drug-sensitive and -resistant cells which reflected major alterations in the control of metabolism of the cell. Although both intact cells and cell extract gave a similar picture, the observed differences in the PCA extract were more pronounced due to the clear resolution of their signals in unambiguous chemical shift position.

In the ^{31}P spectra the signal intensity of PC was dominant over PE in the PME region. GPE level was, in fact, absent to very little in ADR- and MTN-resistant sublines. Differences in both precursors (PC and PE) and degradation products (GPC and GPE) pointed to alterations in the control of phospholipid turnover. We have reported earlier that both the resistant cells displayed increased efflux capacity compared to sensitive cells.[6,7] Since enhanced efflux capacity of the cells is suggested to be the main mechanism responsible for drug resistance, it is understandable that this property of resistant cells is associated with alteration in membrane metabolism and, thus, could explain the decreased concentrations of the PMEs and PDEs.[9]

Unlike normal cells, tumor cells depend more on glycolysis as a source for ATP production. The increase in the ATP level in P388/ADR and P388/MTN cells may also be attributed to the increased demand for ATP, since the drug efflux mechanism is an energy-dependent process. A 2-fold increase in the lactate level as shown by ^{1}H NMR spectra supported an enhanced rate of glycolysis in both the resistant cells and its greater dependency on glucose metabolism. The increased PCr was probably used to maintain the ATP pool at a stable level via creatine kinase which is in agreement with the finding of a higher PCr level in drug-resistant cell lines.[8,10] Another proposed mechanism for MDR involving increased drug detoxification is also an energy-dependent process. A moderate increase in the glutamate level in resistant cells supported increased drug detoxification. Thus, the increase in energy metabolites level is consistent with the increased drug efflux and detoxification in resistant cells. These findings are in agreement with an earlier report.[11]

The results obtained after drug treatment indicated that the most sensitive marker for tumor response is the PME/PDE levels. The marked decrease in the PC levels suggests the marked decrease in the synthesis of

PC. The decrease in the level of PDE is mainly due to a decrease in the PME level. Increased levels of PMEs have been hypothesized to be associated with intensified cell membrane synthesis and cell proliferation.[12] The decline in the PME contents after therapy in the drug-sensitive cells further confirms this hypothesis.

Significantly reduced levels of ATP and PCr also indicated a response of the drug-sensitive cells, which may be explained as follows. Because of the drug toxicity, cell proliferation is affected and, therefore, tumor cells do not require any energy. This means tumor cells kill in the drug-sensitive cell line, which was in agreement with the increased life span of drug-sensitive tumor bearing animals on treatment with ADR. However, the same ADR concentration had little effect on ATP and PCr levels in the drug-resistant cells compared to untreated resistant cells. The results obtained in sensitive and resistant cells after treatment with drug were in conformity with other reports of *in vivo* NMR experiments.[13,14]

It was interesting to note that although DNA strand breaks were observed in both the resistant cells, albeit at reduced levels when compared with sensitive cells,[6,7] there was very little change in the ^{31}P NMR spectra of resistant cells on treatment with ADR. It may be construed that the DNA strand breaks induced by ADR at the given dose may not cause lethal damage to resistant cells. Earlier, we have reported enhanced DNA repair efficiency of drug-resistant cells[6] and it is possible that in 24 h, there could be complete repair of damaged DNA. Alternatively, in the light of recent developments on the molecular mechanism of ADR action, it can be hypothesized that the mutated p53 may interfere with resistant cells by blockade at the G_0 or G_1 phase of the cell cycle. It is also possible that *bcl2* overexpression may protect the drug-resistant cells from sustained damage.[15,16] These may result in the loss of susceptibility of the resistant cells to undergo drug-induced apoptosis or programmed cell death. However, further studies are required to confirm these observations. The exact reason for the spectral changes is a subject of ongoing investigation; nonetheless it can be concluded from the results that the spectral changes were only due to the effect of drug, as these changes were not to be observed in both P388/ADR- and P388/MTN-resistant tumor cells.

The spectra were recorded from different cell generations over a period of 1 year and reproducibility of the spectra show that ^{31}P NMR spectroscopic phenotype was well conserved in these cells. The results demonstrated that P388/S cells invariably give ^{31}P NMR spectra significantly different from those of P388/ADR and P388/MTN cells. This was in agreement with the result of others using different cell lines.[17,18] The most convincing evidence was reported by Kaplan *et al.* who showed that transfection of cells with the *mdr1* gene was enough to induce spectral changes, observable by ^{31}P NMR in drug-resistant cells.[19]

Combined analysis of both ^{1}H and ^{31}P NMR spectra of extracts of drug-sensitive and MDR resistant cells enabled quantitative comparisons between the concentrations of major metabolites, as well as their precursors. The NMR results also showed that MTN-resistant cells behave similar to ADR-resistant cells. The metabolite levels partially reflect the degree of resistance. However, further studies are required to confirm the assessment of degree of resistance by NMR.

The present study demonstrates the usefulness of NMR spectroscopy to characterize MDR and monitor tumor response in drug-sensitive and -resistant cells by studying the biochemical changes associated with MDR. Characterization of MDR by NMR in P388 and its resistant sublines is, perhaps, the first report. The results support the usefulness of ^{31}P and ^{1}H NMR spectroscopy in understanding the differences in the metabolism between the drug-sensitive and -resistant tumor cells, and these metabolic changes predict sensitivity of tumors. It is envisaged that these non-invasive magnetic resonance studies may have direct relevance to *in vivo* and clinical MRS studies of human tumors.

Conclusion

A consistent increase in the high-energy metabolites and decrease of phospholipid metabolite levels were characteristic features of the MDR phenotype that can be used as a marker. A marked decrease in the levels of the above metabolites in drug-sensitive cells compared to a marginal change in resistant cells on drug treatment suggested that measurement of these metabolites can also be used to monitor tumor response. Combined ^{1}H and ^{31}P NMR analysis facilitated quantitation of not only phosphophate metabolites but also their precursors. The spectral resolution was superior in PCA extracts and the results were generally in good agreement with intact cells. This study suggests that due to its non-invasive nature, NMR can be used routinely for *in vivo* characterization of drug resistance and to monitor tumor response.

Acknowledgments

The authors thank Dr N ChandraKumar and Dr S Sendhil Velan, Central Leather Research Institute, Madras for their technical assistance in NMR experiments. Financial assistance from the Council of Scientific and Industrial Research, Government of India, is gratefully acknowledged.

References

1. Roepe PD. The role of the MDR protein in altered drug translocation across tumor cell membranes. *Biochim Biophys Acta* 1995; **1241**: 385-405.
2. Steen GR. Response of solid tumors to chemotherapy monitored by *in vivo* ^{31}P nuclear magnetic resonance spectroscopy: a review. *Cancer Res* 1989; **49**: 4075-85.
3. Daly PF, Cohen JS. Magnetic resonance spectroscopy of tumors and potential *in vivo* clinical application: a review. *Cancer Res* 1989; **49**: 770-9.
4. Negendank WG, Padavic-Shaller KA, Li C, *et al.* Metabolic characterization of human non-Hodgkin's lymphomas *in vivo* using proton-decoupled phosphorus magnetic resonance spectroscopy. *Cancer Res* 1995; **55**: 3286-94.
5. Senthilvelan S, Saravanan K, Jagannathan NR, Nagarajan B. Monitoring therapeutic response of tumors by nuclear magnetic resonance spectroscopy. *Med Sci Res* 1991; **19**: 609-11.
6. Venkatesan PV, Nagarajan B. Flow injection analysis quantifies multidrug resistance phenotype. *Cancer Lett* 1995; **97**: 93-8.
7. Venkatesan PV, Nagarajan B. Assessment of cross-resistance in adriamycin- and mitoxantrone-resistant cells by flow injection analysis. *J Clin Biochem Nutr* 1995; **19**: 123-30.
8. De Jong S, Mulder NH, de Vries EGE, Robillard GT. NMR spectroscopy analysis of phosphorus metabolites and the effect of adriamycin on these metabolite levels in an adriamycin-sensitive and -resistant human small cell lung carcinoma cell line. *Br J Cancer* 1991; **63**: 205-12.
9. Tausch-Treml R, Köpf-Maier P, Baumgart F, *et al.* ^{31}P nuclear magnetic resonance spectroscopy, histology and cytokinetics of a xenografted hypopharynx carcinoma following treatment with cisplatin: comparison in three sublines with increasing resistance. *Br J Cancer* 1991; **64**: 485-93.
10. Cohen JS. Phospholipid and energy metabolism of cancer cells monitored by ^{31}P MRS: possible clinical significance. *Mayo Clin Proc* 1988; **63**: 1199-206.
11. Lyon RC, Cohen JS, Faustino PJ, Megnin F, Myers CE. Glucose metabolism in drug-sensitive and drug-resistant human breast cancer cells monitored by magnetic resonance spectroscopy. *Cancer Res* 1988; **48**: 870-7.
12. Daly PF, Lyon RC, Faustino PJ, Cohen JS. Phospholipid metabolism in cancer cells monitored by ^{31}P NMR spectroscopy. *J Biol Chem* 1987; **262**: 14875-8.
13. Evelhoch JL, Keller NA, Corbett TH. Response specific adriamycin sensitivity markers provided by *in vivo* ^{31}P nuclear magnetic resonance spectroscopy in murine mammary adenocarcinomas. *Cancer Res* 1987; **47**: 3396-401.
14. Wehrle JP, Li SJ, Rajan SS, Steen RG, Glickson JD. ^{31}P and ^{1}H NMR spectroscopy of tumors *in vivo*: untreated growth and response to chemotherapy. *Ann NY Acad Sci* 1987; **508**: 200-15.
15. Reeve RG, Xiong J, Morgan J, Bleehen NM. Expression of apoptosis regulatory genes in lung tumor cells lines: relationship to p53 expression and relevance to acquired resistance. *Br J Cancer* 1996; **73**: 1193-200.
16. Linn SC, Honkoop AH, Hoekman K, Van der Valk P, Pinedo HM, Giaccone G. p53 and P-glycoprotein are often coexpressed and are associated with poor prognosis in breast cancer. *Br J Cancer* 1996; **74**: 63-8.
17. Cohen JS, Lyon RC, Chen C, *et al.* Differences in phosphate metabolite levels in drug-sensitive and -resistant human breast cancer cell lines determined by ^{31}P magnetic resonance spectroscopy. *Cancer Res* 1986; **46**: 4087-90.
18. Kaplan O, van Jijl PCM, Cohen JS. Information from combined ^{1}H and ^{31}P NMR studies of cell extracts: differences in metabolism between drug-sensitive and drug-resistant MCF-7 human breast cancer cells. *Biochem Biophys Res Commun* 1990; **169**: 383-90.
19. Kaplan O, Jaroszewski JW, Clarke R, *et al.* The multidrug resistance phenotype: ^{31}P nuclear magnetic resonance characterization and 2-deoxyglucose toxicity. *Cancer Res* 1991; **51**: 1638-44.

(Received 25 November 1997; revised form accepted 26 February 1998)