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HIGH FIELD PROTON NMR INVESTIGATIONS OF THE METABOLIC PROFILES OF MULTIDRUG-SENSITIVE AND -RESISTANT LEUKAEMIC CELL LINES: EVIDENCE FOR DIMINISHED TAURINE LEVELS IN MULTIDRUG-RESISTANT CELLS

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High field proton (^{1}H) nuclear magnetic resonance (NMR) spectroscopy has for the first time been employed to investigate and compare the metabolic profiles of vinblastine-sensitive and -resistant T-lymphoid leukaemic cell lines (CCRF-CEM and CEM/VLB$_{100}$ respectively) and evidence is presented for a significantly lower taurine content in the CEM/VLB$_{100}$ resistant subline when expressed relative to that of its drug-sensitive parental counterpart. These data suggest differences in the nature and relative involvements of taurine biosynthetic pathways between the two cell lines, a phenomenon that may be related to their differing sensitivities towards chemotherapeutic agents such as adriamycin which promote the generation of cytotoxic reactive oxygen species (ROS) *in vivo*. However, the ^{1}H NMR data obtained provided no evidence for an increased metabolic consumption of hypotaurine (a metabolic precursor of taurine with powerful $^{\cdot}$OH radical scavenging properties) in CCRF-CEM cells since differences observed in the hypotaurine: taurine concentration ratio between the drug-sensitive and -resistant cell lines were not statistically significant. Furthermore, hypotaurine is unlikely to compete with alternative endogenous $^{\cdot}$OH radical scavengers present such as lactate since its level in either of the two cell lines investigated (ca. 6.0×10^{-8} mol./10^8 cells) is insufficient for it to act as an antioxidant in this context. The biochemical and therapeutic significance of these results are discussed.

INTRODUCTION

The antitumour activity of many chemotherapeutic agents has been generally ascribed to their ability to promote the generation of cytotoxic reactive oxygen species. Indeed, during chemotherapy, cell-activated drugs such as adriamycin or bleomycin release the superoxide anion ($O_2^{\cdot -}$)[1] which in turn gives rise to the production of hydrogen peroxide (H_2O_2) via its direct reduction by selected endogenous electron donors, or dismutation catalyzed by the enzyme superoxide dismutase (SOD). Once formed, H_2O_2 has the ability to generate the highly reactive hydroxyl radical ($^{\cdot}$OH) via the Fenton or Haber-Weiss reactions[2] which involve a "catalytic" source of redox-active iron ion complexes, or to directly

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consume critical α-keto acid anions such as pyruvate, 2-oxoglutarate or oxaloacetate[3], the latter reactions severely hampering the cellular synthesis of ATP from both glucose and glutamine[4]. In view of its potent oxidising actions, the hydroxyl radical has the ability to damage a wide variety of biomolecules, e.g. proteins, polyunsaturated fatty acids, polysaccharides and DNA[5].

The development of cellular resistance to antitumour drugs has been postulated to arise from a variety of diverse molecular mechanisms. The multidrug resistance (MDR) phenotype may appear as a result of amplification of the *MDR1* gene, which encodes for a cell transmembrane 170 kDa glycoprotein termed P-glycoprotein (PGP)[6]. PGP functions as an energy-dependent drug effluxing pump of broad specificity[7]. Decreased drug retention has been attributed mainly to an enhanced active efflux of the drug from the cells, which is thought to occur via PGP[6]. MDR has also been suggested to be of a multifactorial nature in a variety of tumour cells, and a protection mechanism against drug-mediated $O_2^{\cdot-}$ generation involving elevated SOD activity has been suggested in CEM/VLB_{100} resistant cells in addition to the PGP mechanism[8]. The MDR phenotype also appears to be associated with alterations in glutathione (GSH) levels, GSH distribution, and GSH metabolising enzymes[9,10]. Therefore, an enhanced antioxidant status in drug-resistant sublines relative to that of their drug-sensitive parental cell lines may play an important role in determining their susceptibility to damage induced by these chemotherapeutic agents.

The recent development of high field nuclear magnetic resonance (NMR) spectrometers with increased sensitivity, resolution and dynamic range has permitted the rapid multicomponent analysis of low-molecular-mass compounds of endogenous or, where appropriate, exogenous origin in biological samples. The technique generally requires no knowledge of sample composition prior to analysis, and much useful biochemical and clinical information can be obtained from high field NMR investigations of human body fluids[11-13]. High resolution NMR spectroscopy is also a well established and powerful tool for investigating cellular metabolism, providing detailed information regarding intracellular metabolite levels and fluxes[14-16]. The method can be performed either *in vivo*, or by the analysis of perchloric acid ($HClO_4$) extracts in which all components present give sharp signals that are readily identified and quantitated.

In this investigation we have conducted high field (500 MHz) proton (^1H) NMR analysis of $HClO_4$ extracts of the CCRF-CEM T-lymphoid leukaemia cell line and its vinblastine-resistant CEM/VLB_{100} subline to directly compare their metabolic profiles, and for the first time present evidence for differences in the intracellular concentrations of the β-amino acid taurine between them. The lower levels of taurine observed in the MDR CEM/VLB_{100} cells could be of biochemical significance in view of their increased resistance to drugs such as adriamycin, vinblastine, daunorubicin and etoposide.

MATERIALS AND METHODS

Cell Culture

The human T-cell lymphoblastic leukaemia cell line CCRF-CEM with no detectable PGP, and its CEM/VLB_{100} subline which was selected for and exhibits a 270-fold resistance to vinblastine, and expresses high levels of PGP[17] were employed in

these studies. The CEM/VLB_{100} cell line is a subclone of the parental line CCRF-CEM developed by Beck *et al.*[18] which exhibited identical growth rate and cell cycle phase to CCRF-CEM cells. Both drug-sensitive and -resistant cell lines were maintained in RPMI 1640 culture medium (Sigma) supplemented with 10% foetal calf serum (Sigma) in a humidified atmosphere of 5% CO_2 in air at 37°C. The chemical composition of the culture medium empolyed is given in Table 1. Individual, single cultures of the CCRF-CEM cell line and its CEM/VLB_{100} MDR subline were conducted together and equivalent cell numbers (2.00×10^8 cells for each line) collected at the same cell cycle stage on three separate occasions.

Preparation of Perchloric Acid ($HClO_4$) Extracts of the Drug-Sensitive and Resistant Cells

Subsequent to removal of the culture medium, both drug-sensitive CCRF-CEM and drug-resistant CEM/VLB_{100} cells with equivalent cell numbers (2.00×10^8 cells for each line) were washed twice with 0.9% (w/v) NaCl and frozen at −70°C. The two types of frozen cells were then scraped into an ice-cold 0.90 mol.dm^{-3} aqueous solution of $HClO_4$ (3.0 ml) separately. The mixture was retained at 0°C for a period of 15 min and then centrifuged (5,000 g) at 4°C for 5 min. The clear supernatant was removed and neutralised with 9.0 mol.dm^{-3} NaOH and further centrifuged at 8,000 g and 4°C for 15 min. The extracts were then lyophilized and the residue stored at −70°C prior to reconstitution in 0.80 ml of ^2H_2O for ^1H NMR analysis.

^1H NMR Analysis of $HClO_4$ Extracts Prepared from CCRF-CEM and CEM/VLB_{100} Cell Lines

0.80 ml aliquots of the ^2H_2O-reconstituted samples prepared as described above were placed in 5-mm diameter NMR tubes and treated with a known concentration of the internal standard sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)-propionate (TSP, 2.50×10^{-5} mol.dm^{-3}).

Proton NMR spectra were acquired using a JEOL JNM-GSX 500 spectrometer (University of London Intercollegiate Research Services (ULIRS), Biomedical NMR Centre, Birkbeck College, London, U.K.) operating at 500.16 MHz in quadrature detection mode and a probe temperature of 293 K. Each spectrum corresponds to 128 free induction decays (FIDs) using 32,768 data points, 3–7 μs pulses and a 5 s pulse repetition rate. The residual HOD/H_2O signal was suppressed by presaturation with gated decoupling during the delay between pulses.

Resonances were referenced to internal TSP ($\delta = 0.00$ ppm). The methyl group ^1H resonances of lactate ($\delta = 1.330$ ppm), valine ($\delta = 1.050$ ppm) or alanine ($\delta = 1.487$ ppm) served as secondary internal references in all cell line extract spectra. The identities of components responsible for the resonances present were routinely assigned by a consideration of characteristic chemical shift values, coupling patterns and coupling constants. Where appropriate, standard additions of authentic endogenous metabolites were made to confirm assignments. Metabolite concentrations were determined by electronic integration of their ^1H resonances and expressing the intensities relative to that of the internal TSP standard.

Average total intracellular concentrations of metabolites were calculated by estimating the mean volume of the cultured cells (v) using the expression $v = 4/3\,\pi r^3$, where r represents the mean cell radius. These calculations were

TABLE 1
Chemical composition of RPMI cell culture medium 1640 (pH 7.2, adjusted with 10% (v/v) aqueous sodium carbonate solution)

Constituent	Concentration ($mol.dm^{-3}$)
Amino acids	
L-Arginine	1.15×10^{-3}
L-Asparagine	3.78×10^{-4}
L-Aspartic acid	1.50×10^{-4}
L-Cystine	2.08×10^{-4}
L-Glutamic acid	1.36×10^{-4}
L-Glutamine	2.05×10^{-3}
Glutathione (reduced)	3.25×10^{-6}
Glycine	1.33×10^{-4}
L-Histidine	9.66×10^{-5}
L-Hydroxyproline	1.53×10^{-4}
L-Isoleucine	3.81×10^{-4}
L-Leucine	3.81×10^{-4}
L-Lysine hydrochloride	2.19×10^{-4}
L-Methionine	1.00×10^{-4}
L-Phenylalanine	9.08×10^{-5}
L-Proline	1.74×10^{-4}
L-Serine	2.85×10^{-4}
L-Threonine	1.68×10^{-4}
L-Tryptophan	2.45×10^{-5}
L-Tyrosine	1.10×10^{-4}
L-Valine	1.71×10^{-4}
Vitamins	
4-Aminobenzoic acid	7.29×10^{-6}
Biotin	8.19×10^{-7}
Choline chloride	2.15×10^{-5}
Cyanocobalamin	3.69×10^{-9}
Folic acid	2.27×10^{-6}
i-inositol	1.94×10^{-4}
Nicotinamide	8.19×10^{-6}
Pantothenate	1.05×10^{-6}
Pyridoxine hydrochloride	4.86×10^{-6}
Riboflavin	5.31×10^{-7}
Thiamine hydrochloride	2.96×10^{-6}
Inorganic cations and anions	
Na^+	1.164×10^{-1}
K^+	5.37×10^{-3}
Ca^{2+}	4.24×10^{-4}
Mg^{2+}	4.23×10^{-4}
NO_3^-	8.47×10^{-4}
HPO_4^{2-}	5.64×10^{-3}
SO_4^{2-}	4.06×10^{-4}
Cl^-	1.081×10^{-1}
HCO_3^-	2.381×10^{-2}
Miscellaneous	
Glucose	1.110×10^{-2}
Phenolsulphonphthalein	1.41×10^{-5}

conducted utilising the assumptions that the cells empolyed in these investigations were spheroid, and that their mean radius is similar to that of lymphoblasts ($r = 7 \times 10^{-6}$ m)[30]. In view of these assumptions, it should also be noted that the mean lymphoblast nucleus: cytoplasm cell diameter ratio is ca. 6:1[30].

Gamma-Radiolysis of Aqueous Hypotaurine and Taurine Solutions

Aqueous solutions of hypotaurine and taurine (1.00×10^{-2} mol.dm^{-3}) were subjected to gamma-radiolysis in the presence of atmospheric O_2 using a ^{60}CO source (Department of Immunology, London Hospital Medical College) at a total dose of 5.00 kGy (17.5 hr. at a dose-rate of 286 Gy hr.$^{-1}$). Under these experimental conditions the major primary radiolytic products generated are $^{\cdot}OH$ (G = 2.7), $e^-_{(aq.)}$ (G = 2.7) and $H^{\cdot}$ (G = 0.5) (equation 1). In the presence of atmospheric O_2, radiolytically-generated aquated electrons ($e^-_{(aq.)}$) are predominantly converted to $O_2^{\cdot -}$ (equation 2).

$$H_2O \rightarrow {}^{\cdot}OH,\ e^-_{(aq.)},\ H^{\cdot},\ H_2,\ H_2O_2,\ H_3O^+ \quad (1)$$

$$O_2 + e^-_{(aq.)} \rightarrow O_2^{\cdot -} \quad (2)$$

^{1}H NMR Analysis of Aqueous Hypotaurine and Taurine Solutions Prior and Subsequent to Gamma-Irradiation Treatment

^{1}H NMR measurements on control and gamma-irradiated aqueous solutions of taurine, and its metabolic precursor hypotaurine, were conducted on a Bruker AMX-600 (ULIRS, Queen Mary and Westfield College facility, University of London, U.K.) spectrometer operating in quadrature detection mode at an operating frequency of 600.13 MHz for ^{1}H. All spectra were recorded at a probe temperature of 298 K. A 0.60 ml aliquot of each sample was placed in a 5-mm diameter NMR tube and 0.07 ml of ^2H_2O was added to provide a field frequency lock. Typical pulsing conditions were: 64 FIDs using 32,768 data points, 30–40° pulses and a 3 s pulse repetition rate, the latter to allow full spin-lattice (T_1) relaxation of the protons in the samples investigated. The intense water signal was suppressed by presaturation with gated decoupling during the delay between pulses. Resonances in spectra were referenced to internal TSP.

Statistical Analysis of Metabolite Concentration Data

Individual CCRF-CEM and CEM/VLB$_{100}$ cell lines were matched on the basis of the date at which they were cultured, the particular batch of paired drug-sensitive and -resistant cultures acting as a "blocking" device for statistical analysis of the experimental ^{1}H NMR data generated.

For each of the date-matched cell lines, the taurine content of the CEM/VLB$_{100}$ cells was expressed as a percentage of that of the parental CCRF-CEM cells, the percentages transformed to $\sin^{-1}(\%)^{1/2}$ values to ensure normality of these data, and a *t*-test applied to ascertain the significance of the difference observed between the mean $\sin^{-1}(\%)^{1/2}$ value and that corresponding to 100.00% (90.00). 95% confidence intervals for the above arcsin-transformed data were also computed.

The hypotaurine content was expressed as a percentage of that of taurine for

each cultured cell line and these data were transformed to their corresponding $\sin^{-1}(\%)^{1/2}$ values. A paired sample Student's *t*-test was then performed to assess the significance of the difference in these values observed between the date-matched CCRF-CEM and CEM/VLB_{100} cell lines.

RESULTS

^1H NMR Evaluations of the Metabolic Status of CCRF-CEM and CEM/VLB_{100} Cell Lines

Typical 500 MHz ^1H NMR spectra of $HClO_4$ extracts prepared from drug-sensitive CCRF-CEM and drug-resistant CEM/VLB_{100} leukaemic cell lines are shown in Figures 1 and 2. These spectra contain many resonances assignable to a wide variety of low-molecular-mass metabolites, and illustrate the multicomponent analytical ability of the technique employed. Indeed, the high field (aliphatic) region of these spectra contains well-resolved, sharp signals assignable to isoleucine, leucine, valine, 3-D-hydroxybutyrate, threonine, lactate, alanine, lysine, acetate, glutamate, glutamine, N-acetylaspartate, succinate, citrate, taurine and hypotaurine, and the low field (aromatic) region includes those arising from tyrosine and formate. Further major ^1H resonances present in these spectra include those attributable to the polar $-\overset{+}{N}(CH_3)_3$ head groups of choline and phosphorylcholine located at 3.21 and 3.23 ppm respectively (the latter appearing as a small shoulder) and the methyl and methylene group protons of creatine (3.03 and 3.93 ppm) and phosphocreatine (3.05 and 3.95 ppm respectively). Other features of the spectra are resonances assignable to the H4, H6 and H2 protons of myoinositol (multiplet centred at 3.62 ppm (H4, H6) and a triplet at 4.08 ppm (H2)), and those corresponding to the α-CH protons of aliphatic amino acids such as glycine (3.60 ppm), isoleucine, leucine and valine (3.60–3.70 ppm), glutamate and glutamine (3.80 ppm) and aromatic amino acids such as tyrosine (ca. 4.0 ppm).

Differences between the two cultured cell lines were generally localised to the 2.6–4.2 ppm chemical shift range of spectra (Figure 2), the most notable being the high taurine content of the drug-sensitive CCRF-CEM cells which was found to be reproducibly lower in the drug-resistant CEM/VLB_{100} subline. A typical level of taurine for the CCRF-CEM cells was 2.9×10^{-7} mol./10^8 cells (estimated intracellular concentration 2.0×10^{-3} mol.dm^{-3}) and the mean value for the CEM/VLB_{100} subline when expressed as a percentage of that of the above date-matched drug-sensitive cell line was 60.8 ± 4.7% (mean ± standard error, $n = 3$, $p < 0.01$). 95% confidence intervals for the arcsin-transformed mean percentage value were 51.3 ± 12.1, the higher limit value being considerably less than that of the corresponding $\sin^{-1}(\%)^{1/2}$ value for 100.00% (90.00). Similar results were obtained when the taurine triplet resonances were normalised to those of the lactate or alanine-CH_3 groups, the intensities of which were similar for $HClO_4$ extracts of both cell lines when expressed relative to that of the internal TSP standard (e.g., Figure 1). The significantly lower taurine content of the CEM/VLB_{100} cells relative to that of the CCRF-CEM cell line suggests a difference in the level of its biosynthesis between the two cell types. Metabolic pathways involved in the biosynthesis of taurine in mammalian tissues include (1) oxidation of cysteine to 3-sulphinoalanine (cysteine sulphinic acid) and decarboxylation of the latter to hypotaurine which in turn is oxidised to taurine; (2) oxidation of cysteine to

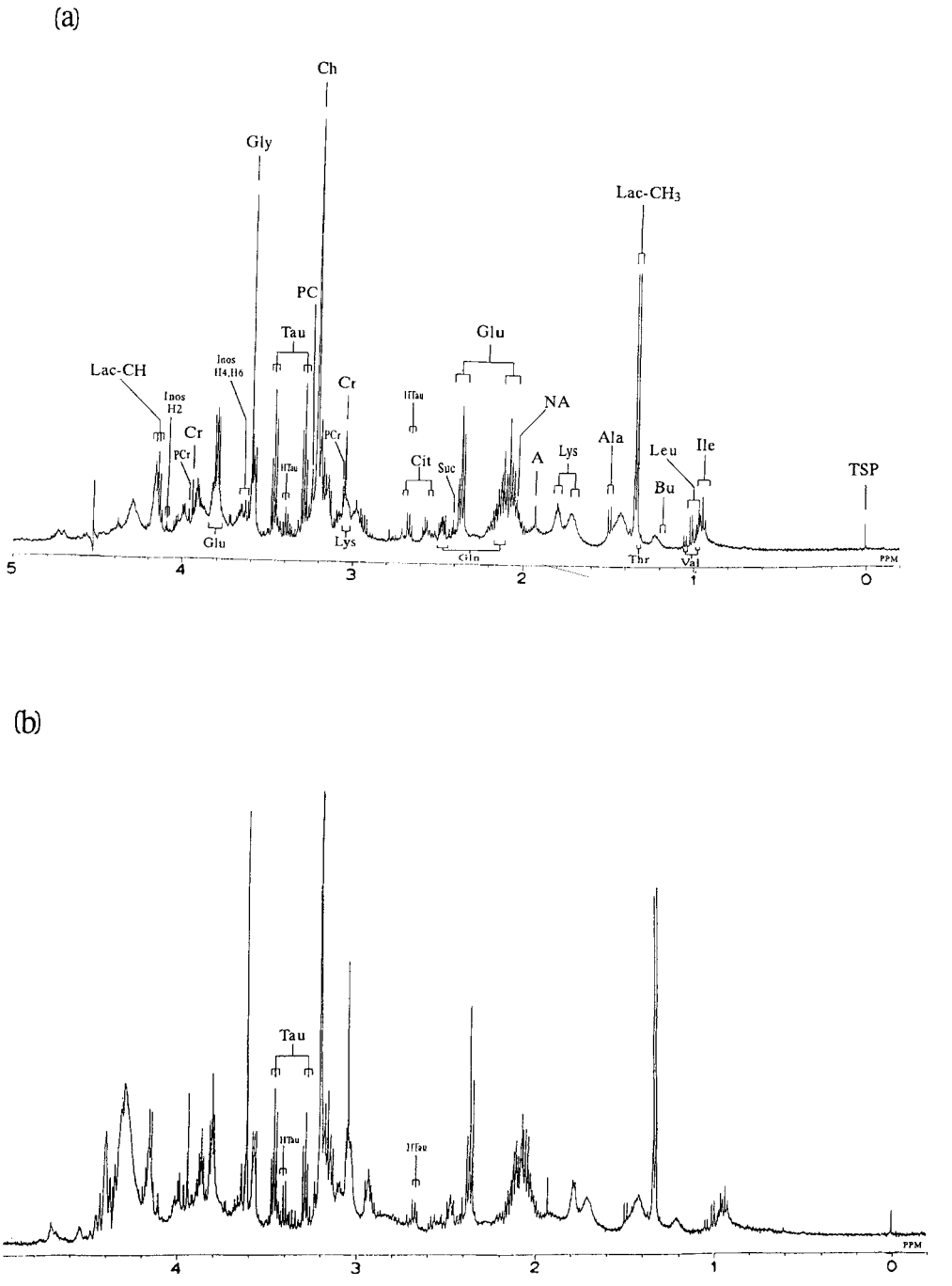

FIGURE 1a and 1b

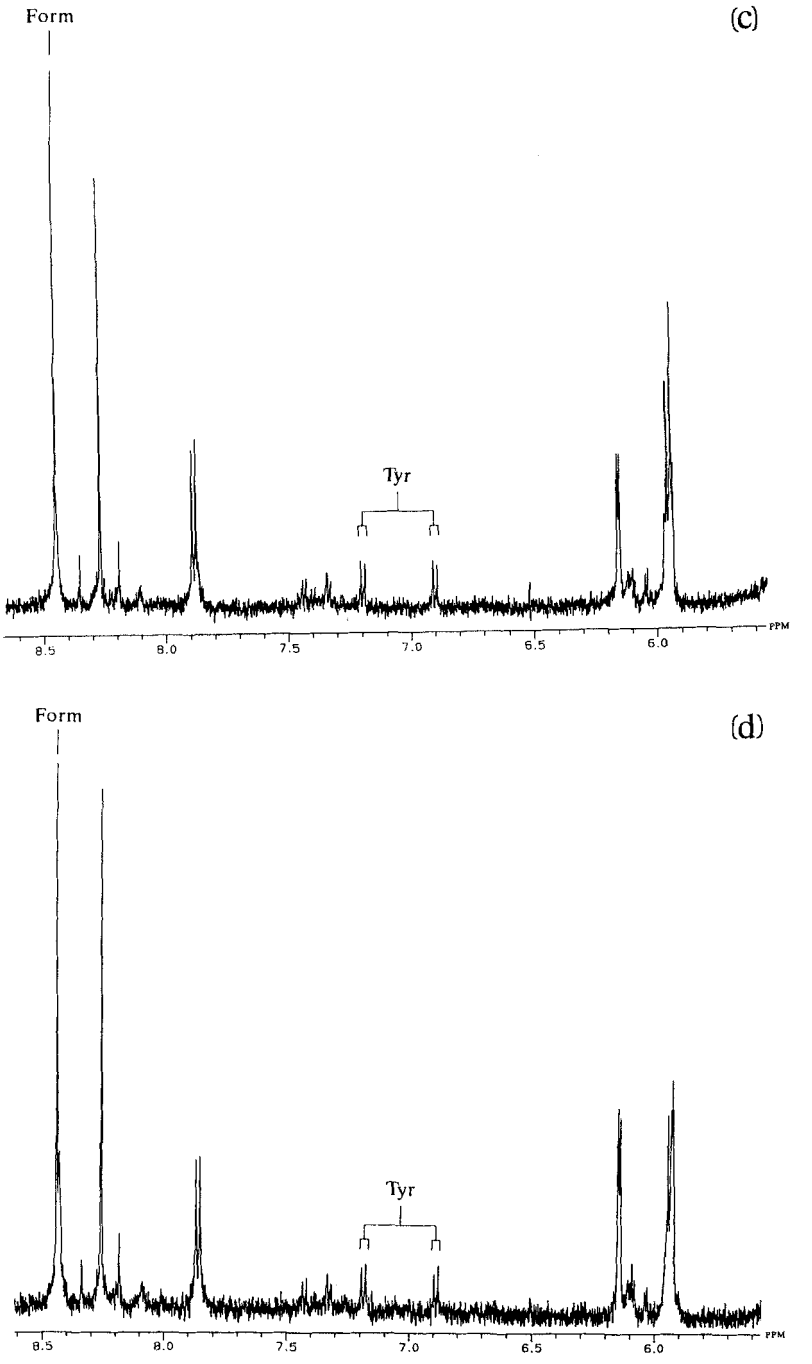

FIGURE 1 High field (0.00–5.00 ppm) regions of 500 MHz single-pulse ^{1}H NMR spectra of $HClO_4$ extracts prepared from (a) a chemotherapeutic drug-sensitive CCRF-CEM leukaemic cell line and (b) a date-matched multidrug-resistant CEM/VLB_{100} cell subline. The corresponding low field

3-sulphinoalanine and then to cysteate which is decarboxylated to form taurine; (3) reaction of cysteine with phosphopantothenate to generate phosphopantothenoyl cysteine, the latter undergoing cleavage to form cysteamine which is then oxidised to hypotaurine, and subsequently taurine. The enzymes required to perform each of the above transformations are available in mammalian tissue[19-21], and hypotaurine appears to be oxidised to taurine via an NADPH-dependent oxidase[22].

In order to obtain information regarding the relative contributions of the above taurine biosynthetic pathways operating in the CCRF-CEM and CEM/VLB_{100} cell lines, a comparative evaluation of their hypotaurine contents was conducted. Hypotaurine was readily detectable in ^{1}H NMR spectra of $HClO_4$ extracts of both types of cells (triplet resonances located at 2.66 and 3.40 ppm) and a typical value for the content of this metabolite in the CCRF-CEM cell line was 6.0×10^8 mol./10^8 cells (estimated intracellular concentration 4.2×10^{-4} mol.dm^{-3}). However, although the mean hypotaurine: taurine concentration ratio for the CEM/VLB_{100} subline (0.29 ± 0.09) was greater than that of the CCRF-CEM cells (0.16 ± 0.02), this difference was not statistically significant (paired sample Student's *t*-test performed on arcsin-transformed data).

Further metabolic precursors of taurine (3-sulphinoalanine, cysteate and cysteamine) were undetectable in 500 MHz ^{1}H NMR spectra of $HClO_4$ extracts of both types of cell line. Indeed, the detection of these species in biological samples by ^{1}H NMR spectroscopy is complicated by overlap of their resonances with those of alternative metabolites and, in the case of cysteate, a higher order (ABX) coupling system.

In view of the above metabolic pathways for taurine biosynthesis, it should also be noted that the bulk of the 3-sulphinoalanine is metabolised by transamination, generating pyruvate and sulphite (SO_3^{2-}) as products[19]. Moreover, hypotaurine itself may also be transaminated by a mammalian enzyme to form acetaldehyde and SO_3^{2-} [23,24]. Examination of the ^{1}H NMR spectra of $HClO_4$ extracts of the cultured cells, however, revealed that neither acetaldehyde, nor its corresponding hydrate, were detectable in either cell line.

High field ^{1}H NMR Analysis of Products Arising from the Reactions of Hypotaurine and Taurine with Radiolytically-Generated ·OH Radical

In view of the difference observed between the taurine contents of the CCRF-CEM and CEM/VLB_{100} cell lines, the chemical nature and relative levels of products derived from the reactions of radiolytically-generated ·OH radical with taurine, and its metabolic precursor hypotaurine, were investigated by high field ^{1}H NMR spectroscopy.

Proton NMR analysis demonstrated that exposure of aqueous hypotaurine solutions to radiolytically-generated ·OH radical generates taurine as a major

(5.10–8.70 ppm) regions of these spectra are shown in (c) and (d) respectively. Typical spectra are shown. Abbreviations: A, acetate-CH_3; Ala, alanine-CH_3; Bu, 3-D-hydroxybutyrate-CH_3; Ch, choline-$\overset{+}{N}(CH_3)_3$; Cit, citrate-CH_2; Cr, creatine-N-CH_3; Form, formate-H; Gln, glutamine β-and γ-CH_2; Glu, glutamate β-and γ-CH_2; Gly, glycine α-CH_2; HTau, hypotaurine ^-O_2S-CH_2 and $H_3\overset{+}{N}$-CH_2; Inos, inositol H4, H6 and H2 proton resonances; Ile, isoleucine terminal- and β-CH_3 groups; Lac-CH_3 and -CH, lactate-CH_3 and -CH groups; Leu, leucine-CH_3; Lys, lysine side-chain-CH_2 group resonances; NA, N-acetylaspartate-NHCOCH_3; PC, phosphorylcholine-$\overset{+}{N}(CH_3)_3$; PCr, phosphocreatine-$\overset{+}{N}(CH_3)_3$; Suc, succinate-CH_2; Tau, taurine ^-O_3S-CH_2 and $H_3\overset{+}{N}$-CH_2; Thr, threonine-CH_3; Tyr, tyrosine aromatic ring protons; Val, valine-CH_3.

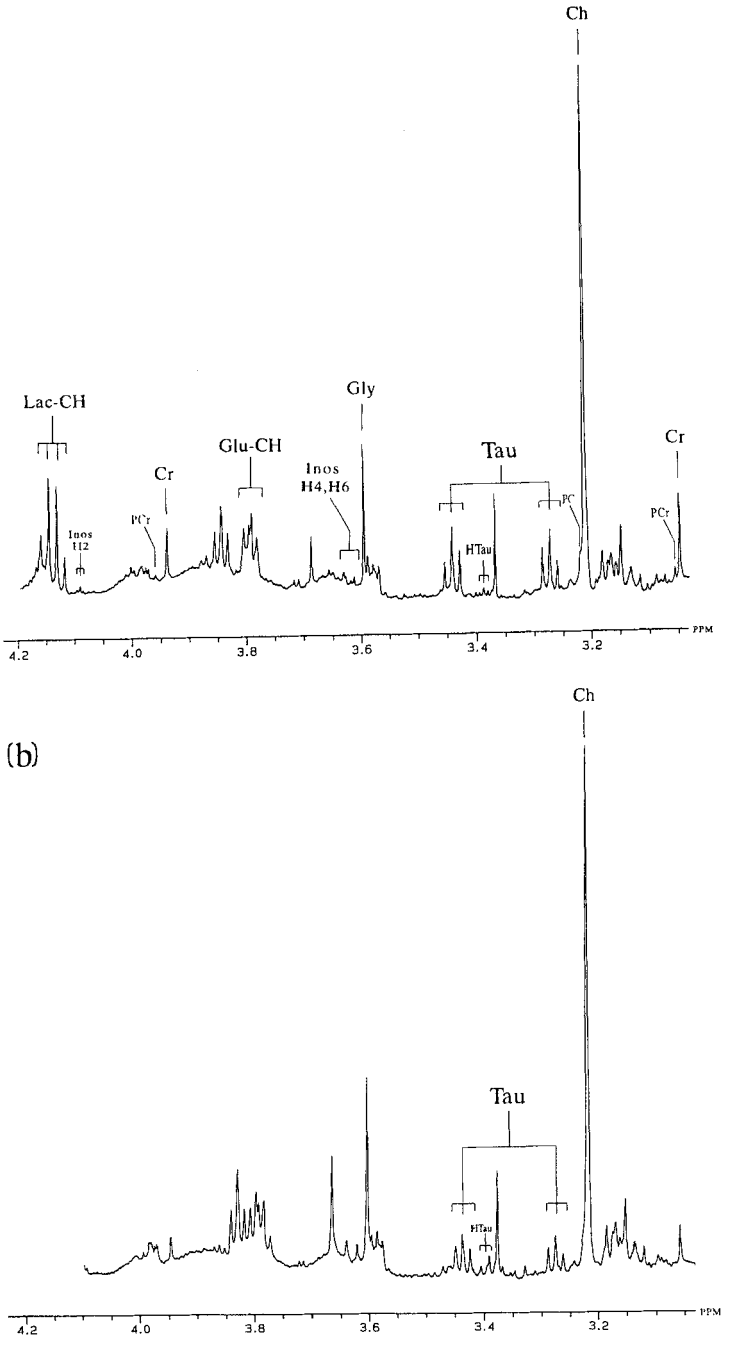

FIGURE 2 Expanded 2.60–4.20 ppm chemical shift regions of 500 MHz single-pulse ^{1}H NMR spectra of $HClO_4$ extracts prepared from (a) a chemotherapeutic drug-sensitive CCRF-CEM leukaemic cell line and (b) a date-matched multidrug-resistant CEM/VLB_{100} cell subline. Typical spectra are shown. Abbreviations: as Figure 1.

product (Figures 3(a) and (b)), confirming results obtained in previous investigations[22]. Indeed, a comparison of resonance intensities showed that >80% of the hypotaurine is oxidised to taurine under the experimental conditions employed here, consistent with the powerful ·OH radical scavenging capacity of the former[25]. The broad nature of the $-CH_2\dot{N}H_3$ proton signals of both the hypotaurine reactant and taurine reaction product present in the spectrum shown in Figure 3(b) is probably attributable to an exchange process, and we are currently conducting further experiments to investigate this phenomenon. Additional sharp triplet resonances at 3.84 and 4.31 ppm, and broader signals centred at 3.09 and 3.16 ppm in spectra of gamma-irradiated hypotaurine solutions demonstrated that further minor products were derived from the attack of radiolytically-generated ·OH radical on hypotaurine. Moreover, low levels of ethanol were also detectable in these spectra (methyl group triplet resonance located at 1.21 ppm), demonstrating that this species also arises from the radiolytic degradation of hypotaurine.

Fellman and Roth[22] have previously suggested a mechanism for the oxidation of hypotaurine to taurine by ·OH radical which involves the prior formation of a resonance-stabilised hypotaurine radical which dimerises, generating an intermediate sulphone species which in turn disproportionates to an equimolar mixture of taurine and hypotaurine.

With the exception of the very low levels of formate generated, gamma-radiolysis of aqueous solutions of taurine did not give rise to any NMR-detectable products (Figures 3(c) and (d)), an observation consistent with its poor ·OH radical scavenging ability[25]. However, gamma-radiolysis of this endogenous metabolite induced a curious increase in the line-widths of its ^1H resonances, an observation that is not simply explicable.

DISCUSSION

The multicomponent analysis of $HClO_4$ extracts of cultured cell lines by high field NMR spectroscopy offers substantial advantages over alternative analytical techniques in that it provides much useful information regarding their metabolic profiles, facilitating the detection of specific biochemical differences between two or more classes of cell line. Although Callaghan *et al.*[26] have previously employed high field ^1H NMR spectroscopy to compare the total lipid profiles of parental Chinese hamster ovary cell lines with those of corresponding cells selected and transfected for the multidrug resistance gene, to the best of our knowledge this is the first ^1H NMR investigation of the status and levels of polar, water-soluble metabolites present in cultured chemotherapeutic drug-sensitive and -resistant leukaemic cells. As detailed in this study, the technique is readily applicable to the facile detection and quantification of potential antioxidants present in cell lines, together with their metabolic precursors and products.

The ^1H NMR data obtained here clearly demonstrate that the taurine content of the drug-sensitive CCRF-CEM cell line is significantly greater than that of its drug-resistant CEM/VLB_{100} subline. In view of the powerful ·OH radical scavenging and other, antioxidant actions of hypotaurine,[25] an increased metabolic consumption of this metabolite and certain of its precursors may render the CCRF-CEM cells more susceptible to oxidative damage induced by chemotherapeutic agents such as adriamycin. However, with the available data we were unable to detect a significantly elevated hypotaurine:taurine concentration ratio in the

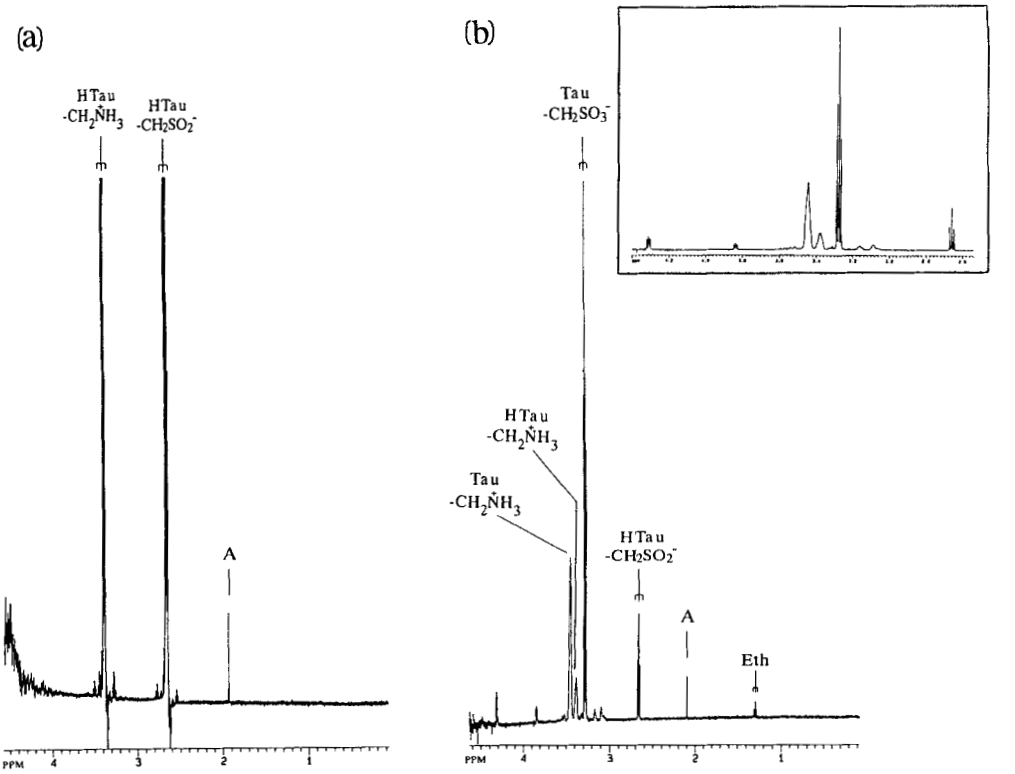

FIGURE 3a and 3b.

drug-resistant subline over that of the CCRF-CEM cells, and hence an alternative explanation for the difference in taurine contents observed is an increased involvement of a taurine biosynthetic pathway that does not involve hypotaurine as its metabolic precursor in the latter cell line, i.e., that involving the oxidation of 3-sulphinoalanine yielding taurine via cysteate. Such a phenomenon may be a direct reflection of the relative activities and affinities of the enzymes cysteine dioxygenase and 3-sulphinoalanine decarboxylase in the cell lines investigated, and experiments to assess this are currently in progress. The nature and relative levels of taurine biosynthetic pathways operating in the CCRF-CEM and CEM/VLB_{100} cell lines are likely to have major physiological and therapeutic consequences since hypotaurine is a potent competitive inhibitor of taurine uptake by cells, whereas cysteate exerts no influence on the transport of this metabolite[27].

With regard to the antioxidant capacity of hypotaurine, a further important consideration is the concentration of this species present in the two cell lines examined, i.e., although hypotaurine is a good scavenger of $^{\cdot}OH$ radical (second-order rate constant, $k_2 = 1.15 \times 10^{10}$ mol.$^{-1}$ dm^3 s^{-1}) and hypochlorous acid (HOCl)[25], its concentration is insufficient for it to function as an effective antioxidant *in vivo*. Indeed, lactate, which also reacts extremely rapidly with $^{\cdot}OH$ radical ($k_2 = 4.8 \times 10^9$ mol.$^{-1}$ dm^3 s^{-1})[28], is present at much higher levels in both cell lines (ca. 7.5×10^{-7} mol./10^8 cells); estimated intracellular concentration

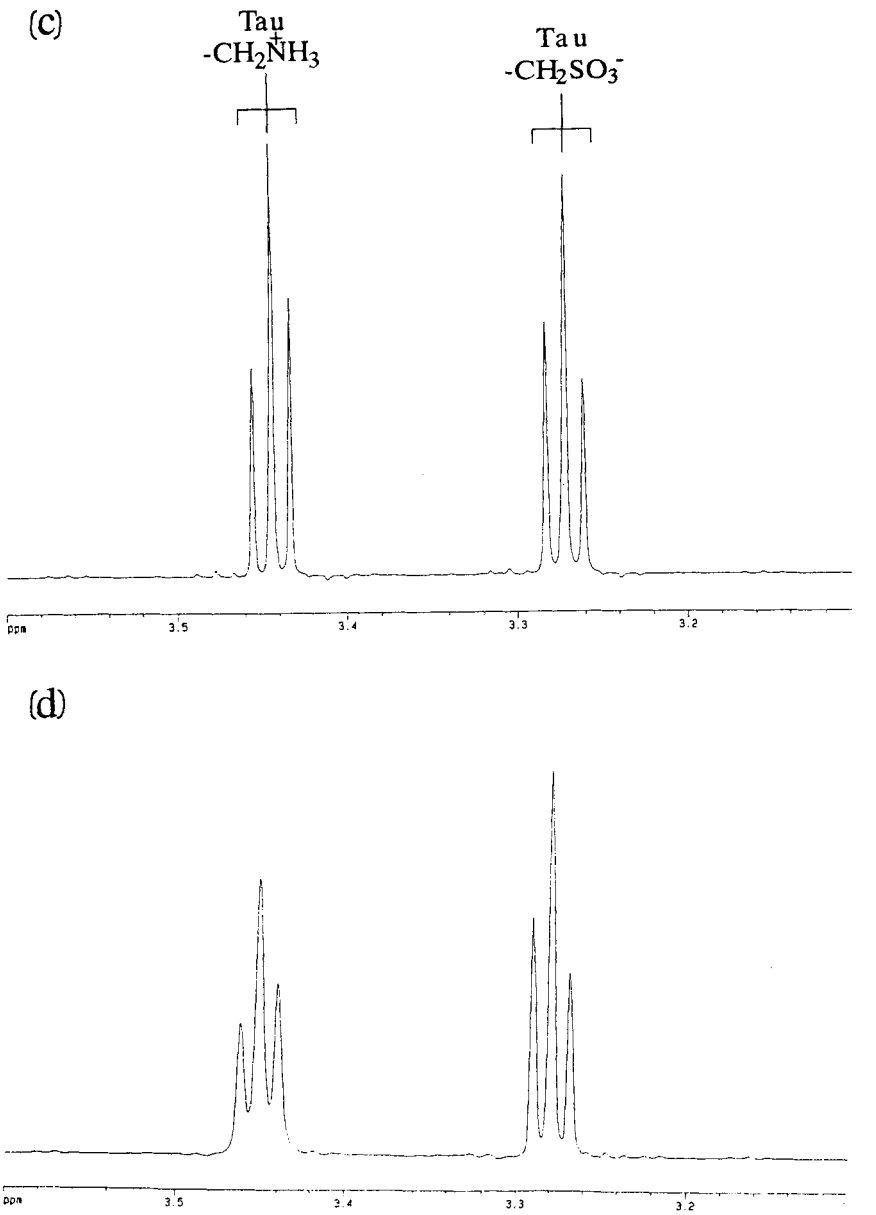

FIGURE 3 600 MHz ^{1}H NMR spectra of an aqueous solution containing 1.00×10^{-2} mol.dm^{-3} hypotaurine (a), before and (b), after gamma-radiolysis (5.00 kGy). Corresponding spectra of control and gamma-irradiated aqueous solutions of taurine (1.00×10^{-2} mol.dm^{-3}) are shown in (c) and (d) respectively. Abbreviations: as Figure 1, with Eth representing the -CH_3 group of ethanol. The inset in (b) shows the expanded 2.60–4.40 ppm region of the spectrum.

5.2×10^{-3} mol.dm^{-3}). Taurine itself is a poor antioxidant since it reacts slowly with $^{\cdot}OH$ radical ($k_2 = 2.42 \times 10^6$ mol.$^{-1}$ dm^{3}s^{-1}) and does not appear to scavenge H_2O_2 or $O_2^{\cdot -}$[25]. Moreover, the products of its reaction with HOCl (N-*mono*- and N-*di*chloroamines) are themselves sufficiently oxidising and can inactivate α-antiprotease[29], an important extracellular target of HOCl attack *in vivo*.

Proton NMR analysis of control and gamma-irradiated aqueous hypotaurine solutions confirmed that this metabolite readily reacts with $^{\cdot}OH$ radical forming taurine in high yield. In contrast, gamma-radiolysis of aqueous taurine solutions failed to generate significant quantities of any NMR-detectable products, an observation consistent with its very poor ability to scavenge $^{\cdot}OH$ radical.

In conclusion, the results obtained here indicate a perturbation of taurine biosynthesis in the cultured CEM/VLB$_{100}$ drug-resistant subline relative to that of its parental CCRF-CEM drug-sensitive cell line. Such a metabolic difference may play an important role in contributing towards the resistance of the former cell line towards chemotherapeutic agents which promote the generation of ROS *in vivo*.

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