Inhibition of tetraphenylphosphonium-induced NMR-visible lipid accumulation by chlorpromazine.

N. Sathasivam, S. Brammah[ψ], L.C. Wright* and E.J. Delikatny
Department of Cancer Medicine and *Center for Infectious Diseases and Microbiology, University of Sydney.
[ψ]CSAHS Electron Microscopy Unit, Concord Hospital, NSW, Australia.

INTRODUCTION

Cationic lipophilic compounds such as tetraphenylphosphonium chloride (TPP), selectively accumulate in the mitochondria of tumour cells (1,2) disrupting the membrane potential, leading to cell death. Previous studies have shown that treatment of human breast cell lines with TPP induces the formation of NMR-visible lipid (3,4). In this study, the effect of TPP treatment ± an inhibitor of triglyceride synthesis, chlorpromazine (CPZ) on HBL-100 human breast cells was investigated using ^{5}H NMR, lipid analysis and electron microscopy.

METHODS

Cell Culture: HBL-100 cells were grown in RPMI 1640 buffered with 0.22% $NaHCO_3$ and supplemented with (v/v) 10% FBS, 1% glutamine, 0.1% gentamycin, 0.25% insulin.

NMR Spectroscopy: Cells were seeded ($2.5x10^5$ cells/ml), TPP ± CPZ in ethanol added 5 h later. The cells were harvested after 48 hours. $1x10^7$ cells were taken for lipid analysis and the remaining cells rinsed 3x and resuspended in a volume of 600 µl PBS/D_2O in a 5-mm NMR tube. Spectra were obtained on a Bruker DRX 360 spectrometer (256 acquisitions, sw 3600 Hz and a 90° pulse with gated pre-saturation of the residual water signal). Peak height ratios were plotted against drug treatment.

Lipid Analysis: Lipid was extracted from the cells using chloroform/methanol (2:1) and analyzed by thin layer chromatography (TLC) using the solvent system hexane:diethyl ether:acetic acid (90:15:1 v/v/v). Plates were stained with 0.03% (w/v) Coomassie Brilliant Blue R-250.

Electron Microscopy (EM): $2.5x10^5$ cells/ml were seeded and drugged as above. 48 h later, cells were fixed with 2.5% glutaraldehyde and stained with uranyl acetate and lead citrate.

RESULTS

Previous studies had demonstrated that a subcytotoxic concentration of 6.25 µM TPP produced the largest increase in NMR-visible lipid in HBL-100 cells (4). Similarly, the 1D ^{1}H NMR spectra of HBL-100 cells treated with 6.25 µM TPP showed substantial increases in mobile lipid resonances, especially at 1.3 ppm (methylene) and 5.3 ppm (olefinic). Cells cultured in TPP and CPZ (12.5 or 25 µM) showed a reduction in the lipid resonances, producing spectra similar to the control (Figure 1). Internal peak height ratios of the methylene relative to the methyl resonance showed significant increases ($p<0.01$, Student's t-test) with TPP treatment. Dose-dependent decreases ($p<0.05$) in the ratios were observed in the presence of CPZ. Treatment with CPZ alone did not cause any significant changes in these ratios (Figure 2).

TLC of lipid extracts showed that TPP treatment caused significant increases in whole cell triglycerides, which was attenuated by the presence of CPZ. EM of TPP-treated cells showed distorted mitochondria and many cytoplasmic lipid droplets. CPZ-treated cells appeared more bullet-shaped with few lipid droplets, multilamellar lipid inclusions (myelinoid bodies, which are probably representative of lysosomal activity) and membranous material in the cytoplasm and in large vacuoles. TPP and CPZ-treated cells showed no normal mitochondria, a large presence of myelinoid bodies and membranous vacuoles but fewer lipid droplets than the TPP treatment indicating that triglyceride production had been reduced with the addition of CPZ.

DISCUSSION AND CONCLUSION

CPZ has been reported to be an inhibitor of lysosomal phospholipases and of phosphatidic phosphohydrolase, the enzyme involved in the conversion of phosphatidic acid to diglyceride (5). Since the final step in triglyceride synthesis involves the condensation of a diglyceride with an activated fatty acid, inhibition of either of these enzymes would reduce the amount of substrates available for triglyceride synthesis. ^{1}H NMR showed that mobile lipid decreased in the presence of CPZ with TPP and than this was accompanied by a decrease in whole cell triglycerides. This observation indicates that either of these enzymes may be involved in processing of lipids released during the cytotoxicity process.

EM of TPP-treated cells showed many lipid droplets with few myelinoid bodies. This appears to be dose-dependent, as is the appearance of NMR-visible lipid (3,4). Previous studies (6) and unpublished data have shown that higher (cytotoxic) concentrations of TPP and other cationic lipophilic compounds produced increased myelinoid bodies but fewer lipid droplets. The involvement of lipids in drug-induced cytotoxicity is complex, however NMR is proving to be a useful tool to study the processes involved.

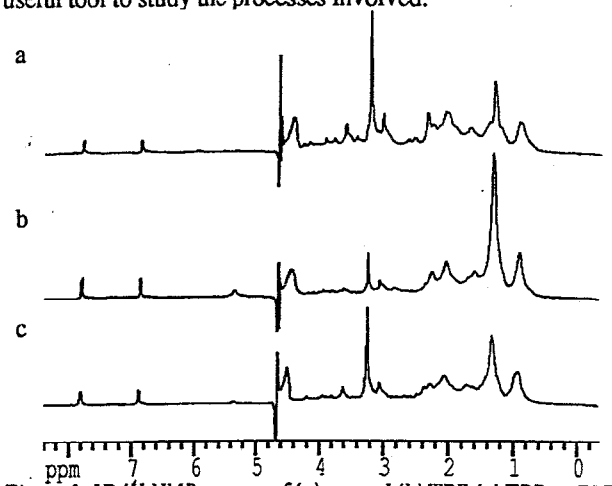

Figure 1. 1D ^{1}H NMR spectra of (a) control (b) TPP (c) TPP + CPZ-treated HBL-100 cells.

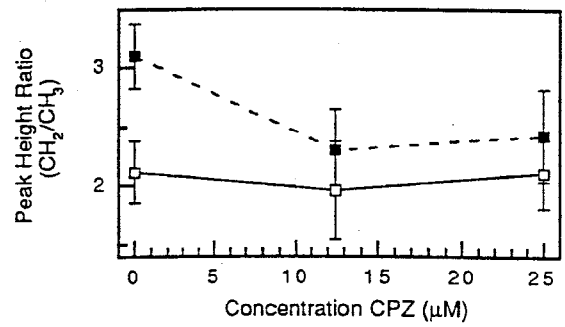

Figure 2. Ratio of methylene/methyl resonances versus CPZ concentration. ■ : TPP and CPZ; □ : CPZ alone.

REFERENCES

1. Modica-Napolitano, J.S., Aprille, J.R., *Cancer Res.*, 47, 4361, 1987. 2. Rideout, D.C., Calogeropoulou, T., *et al.*, *Anti-Cancer Drug Design*, 4, 265, 1989. 3. Delikatny, E.J., Roman, S.K., *et al.*, *Int. J. Cancer*, 67, 72, 1996. 4. Roman, S.K., Jeitner, T.M., *et al.*, *Int. J. Cancer*, 73, 570, 1997. 5. Gimes, G., Toth, M., *Acta Physiol. Hung.*, 83, 151, 1995. 6. Cooper, W.A., Brammah, S., *et al.*, *Proc. Soc. Magn. Reson.* 1, 267, 1996.