DOXORUBICIN FREE RADICAL FORMATION AND LIPID PEROXIDATION IN RAT HEPATOCYTES

T.J. Lavelle, J. Basra J.R. Brown* and L.H. Patterson, School of Pharmacy, Leicester Polytechnic, Leicester LE1 9BH and *Department of Pharmaceutical Chemistry, Sunderland Polytechnic, Sunderland SR2 7EE.

The biological activities of doxorubicin, a clinically useful anthraquinone antitumour agent, are often related to metabolic doxorubicin semiquinone generation resulting in reactive oxygen formation and subsequent peroxidative attack on cell membrane lipids. Xanthine oxidase (EC 1.2.3.2) and cytochrome P450 reductase (EC 1.6.2.3) are able to one-electron reduce doxorubicin to generate free radicals (Pan et al,1979). However, it has been suggested that 2-electron reduction by DT diaphorase (EC 1.6.99.2) may detoxify quinone based compounds by limiting autooxidation and concomitant reactive oxygen formation by converting the available quinone into the more stable hydroquinone (Lind et al, 1982). Since hepatocytes are rich in both one and two electron reductases we have used them as a model system to investigate doxorubicin free radical formation.

Sprague Dawley male rat hepatocytes were isolated as described by Fry (1981) and their DT diaphorase, xanthine oxidase and cyt.P450 reductase activities determined by standard methods. Electron spin resonance (esr) spectrometry was used to directly investigate doxorubicin free radical formation in isolated hepatocytes using esr operating parameters essentially as previously described (Oldcorne, Brown and Patterson, 1984). The effect of doxorubicin on hepatocyte lipid peroxidation was measured as described previously (Patterson et al,1983).

In freshly isolated rat hepatocytes the activity of cyt.P450 reductase was 41.8nmol.cyt.c reduced min^{-1} $mg\ protein^{-1}$, xanthine oxidase 5.7nmol uric acid formed min^{-1} $mg\ protein^{-1}$ and diaphorase 64.3nmol dichlorophenylindolphenol reduced min^{-1} $mg\ protein^{-1}$. After 4h incubation in protein supplemented medium about 70% of these enzyme activities were retained. Doxorubicin (300μM) when incubated with hepatocytes ($3x10^6$) that immediately beforehand had been de-aerated with N_2 generated an unresolved doxorubicin free radical (g=2.0045) esr spectrum, which was collapsed on reaeration of the esr flat cell. No esr spectrum was observed in hepatocytes in the absence of doxorubicin. Lipid peroxidation in freshly isolated hepatocytes ($1.3x10^6\ ml^{-1}$) incubated for up to 3h with doxorubicin (3μM) was only slightly greater (15%) than in control hepatocytes (0.59 nmol malondialdehyde $mg\ protein^{-1}$, $p<0.05$); whereas 100μM doxorubicin had no effect. Hepatocyte viability was greater than 70% after 3-4h incubation in Williams E medium in the presence or absence of doxorubicin.

The results demonstrate that rat hepatocytes shown to possess enzymes associated with quinone one and two electron reduction can metabolically generate doxorubicin free radicals. However, this event does not greatly affect lipid peroxidation; a result that is consistent with previous observations (Meredith and Reed,1983). It would appear that the presence of doxorubicin in isolated hepatocytes does not necessarily result in doxorubicin free radical mediated lipid peroxidation.

Pan,S-S., Pedersen,L. and Bachur,N.R. (1981) Molec. Pharmacol. 19:184-186
Lind,C., Hochstein,P. and Ernster,L. (1982) Arch. Biochem. Biophys. 216:178-185
Fry,J.R. (1981) In: Methods in Enzymology, vol 77, ed.Jakoby, Acad.Press pp130-137
Oldcorne,M.A., Brown,J.R. and Patterson,L.H. (1984) Biochem.Soc.Trans. 12:680-681
Patterson,L.H., et al, (1983) Biochem. Biophys. Res. Commun. 110:399-406
Meredith,M.J. and Reed,D.J. (1983) Biochem. Pharmacol. 32:1383-1388