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THE EFFECT OF VARIOUS MODIFIERS ON RAT MICROSOMAL PEROXIDASE KINETICS

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Unsaturated fatty acid hydroperoxides (lipid peroxides) are thought to result from single-electron oxidation of intracellular membrane phospholipid components by such species as singlet oxygen, hydroxyl radicals or hydrogen peroxide (1-5) . Such lipid peroxides are also believed to exert various deleterious effects upon cell structure and function including mitochondrial enzyme inactivation (6), destruction of cytochromes including P-450 (6,7) and more recently, the cytotoxicity and cardiotoxicity associated with adriamycin (8). Studies using linoleic acid hydroperoxide (LAHPO) as a model substrate, have demonstrated a role for cytochrome P-450 in the rapid lipid peroxidase-activity of hepatic microsomal fractions (7). Together with cytosolic glutathione peroxidase (9, 10) and non-enzymic decomposition (9), cytochrome P-450 thus has the potential of attenuating the toxic actions of endogenous peroxides.

Many therapeutic agents are however, substrates for the cytochromes P-450 and as such, may act as inhibitors of P-450-mediated lipid peroxidase activity. It has been claimed that ligands which produce type II spectral changes, such as aniline and imidazole, produce greater inhibition of the microsomal lipid peroxidase than type I ligands, such as hexobarbitone and aminopyrine (7). It is the purpose of this communication to describe the effects phenobarbitone, hexobarbitone, aniline and SKF 525A additions upon the kinetics of rat hepatic microsomal lipid peroxidase activity.

METHODS

Microsomes from fasted mature male Wistar rats were prepared according to Hrycay and O'Brien (7) and stored at 0°C under nitrogen as suspensions in 0.1M Tris-HCl buffer (pH 7.4) containing 2mM EDTA and 25% (v/v) glycerol. Preparation and quantitation of LAHPO was performed according to O'Brien (9). Peroxidase activity was measured at 23°C using N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD) as hydrogen donor and LAHPO as substrate (7). The final reaction volume (3ml) contained EDTA (1mM), TMPD (0.2mM), microsomal protein (1.25mg) and LAHPO (0.1-2.7mM) with and without various modifiers in 0.05M Tris-HCl buffer (pH 7.4). The rate of TMPD oxidation to Wurster's blue free radical (extinction coefficient $11.6mM^{-1}$ cm^{-1}) was followed spectrophotometrically at 610nm (11) during the first minute of reaction (7) and corrected for the rate of reaction in the absence of LAHPO. Apart from control measurements

the effect of selected modifiers on microsomal peroxidase activity was also studied. The modifiers employed were phenobarbitone (0.5, 5 and 25mM) hexobarbitone (0.5mM), aniline (5mM) and SKF 525A (0.5mM). The kinetic constants describing the relationship between rate of reaction and LAHPO concentration (K_m and V_{max}) were determined by least-squares regression analysis of double-reciprocal (Lineweaver-Burk) plots. The effects of modifiers of peroxidase activity were assessed statistically by comparisons (student's t-test) of both graphical intercepts and the kinetic parameters derived from these values.

RESULTS

In all experiments, double-reciprocal plots of LAHPO decomposition by rat liver microsomes were linear (Figs. 1A and 1B). Fig. 1A shows the effect of increasing phenobarbitone concentration on LAHPO decomposition. V_{max} (nmol min^{-1} mg^{-1} protein) and K_m (mM) values (mean ± S.D.) in the absence of modifiers were 225 ± 31 and 1.27 ± 0.17 respectively. Fig. 1B shows the effect of aniline (5mM), hexobarbitone (0.5mM) and SKF 525A (0.5mM) on LAHPO decomposition.

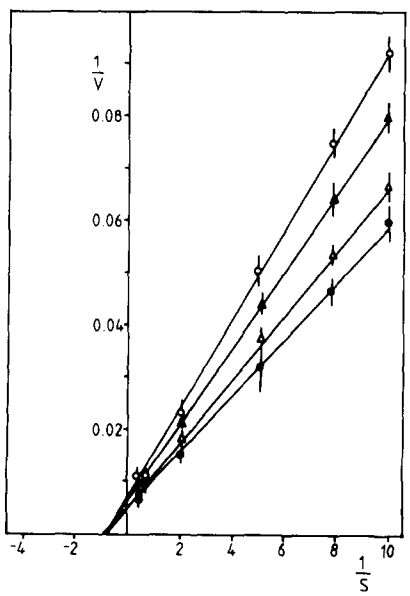

FIGURE 1A: Lineweaver-Burk plots of rates of lipid peroxide decomposition (V: nmol min^{-1} mg^{-1} microsomal protein; S: LAHPO concentration, 0.1 - 2.7mM) in the absence (●) and presence of phenobarbitone (0.5mM Δ; 5mM ▲; 25mM o). Bars indicate S.E.M. (n = 4).

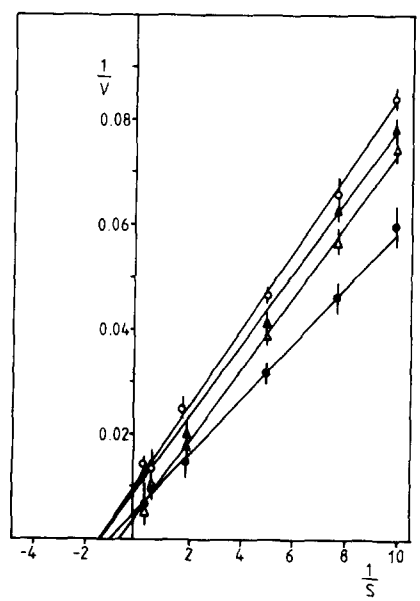

FIGURE 1B: Lineweaver-Burk plots of rates of lipid peroxide decomposition (V: nmol min^{-1} mg^{-1} microsomal protein S: LAHPO concentration, 0.1 - 2.7mM) in the absence (●) and presence of SKF 525A, 0.5mM (Δ); aniline, 5mM (▲); hexobarbitone, 0.5mM (o). Bars indicate S.E.M. (n = 4).

Phenobarbitone showed competitive inhibition at 0.5mM (V_{max} 280 ± 43, n.s.; K_m 2.21 ± 0.33, $2P < 0.025$) but non-competitive inhibition at 25mM (V_{max} 134 ± 14, $2P < 0.025$; K_m 1.23 ± 0.13, n.s.). Both aniline (5mM) and hexobarbitone (0.5mM) produced a decrease in V_{max} (aniline 107 ± 12; hexobarbitone 85 ± 4; $2P < 0.005$) and concomitant decrease in K_m (aniline 0.66 ± 0.31; hexobarbitone 0.65 ± 0.21; $2P < 0.025$). In contrast, SKF 525A (0.5mM) produced purely competitive inhibition of LAHPO decomposition (V_{max} 351 ± 132, n.s.: K_m 3.52 ± 0.38, $2P < 0.005$). For all individual experiments the standard errors of both slope and intercept were less than 5%, and thus statistical analysis was not a significant source of variance in the treatment of these data.

DISCUSSION

Several intracellular mechanisms exist which catalyse the decomposition of peroxides of the unsaturated fatty acid component of membrane phospholipids, of which cytochrome P-450 seems to be predominant (7). The preliminary data presented herein shows that substrates for cytochrome P-450, irrespective of the spectral changes they elicit (type I or II), can inhibit microsomal lipid peroxidase activity. Phenobarbitone, for example, produced competitive inhibition at low concentrations (0.5mM) and non-competitive inhibition at higher concentrations (25mM). The most dramatic effect observed was with hexobarbitone (0.5mM) which produced a 70% reduction in V_{max} for LAHPO decomposition.

These data are hard to reconcile with a single enzymic form of microsomal lipid peroxidase in the rat. One explanation for this might be that two or more forms of cytochrome P-450 are able to catalyse LAHPO decomposition and thus the apparent K_m and V_{max} values quoted here result from a multienzymic system, which is only partially inhibited by the modifiers employed.

Of particular interest are the toxicological implications of drug inhibition of lipid peroxide decomposition. Rational drug therapy often dictates the administration of two or more drugs concomitantly. An important source of toxicity resulting from drug interaction may be the combination therapy of a quinone-containing drug such as the antineoplastic agent adriamycin (which promotes oxygen reduction (12) and stimulates lipid peroxidation (8)) with a peroxidase inhibitor such as phenobarbitone. The modifiers employed in this study are in many respects arbitrary, phenobarbitone and SKF 525A, whose interactions with cytochrome P-450 are well-documented and a representative type I (hexobarbitone) and type II (aniline) ligand. There is some reason to suspect therefore, that many other drugs might inhibit lipid peroxidase activity and thus potentiate the toxic effects of quinone-containing drugs such as adriamycin.

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