using lung and liver microsomes in the presence of paraquat, to generate superoxide and covalently bind a metabolite of DOPA.

Male Wistar rats (150–250 g) were killed by decapitation and their lungs were perfused with ice cold normal saline. The lungs and livers were removed, homogenised and the microsomal fraction was isolated by differential centrifugation. The covalent binding of DOPA to washed microsomal protein was measured according to the method of Dybing *et al.* (1976).

In the absence of paraquat the covalent binding of radioactivity (expressed as nmol of DOPA) to microsomal protein was 0.200 ± 0.012 nmol mg protein^{-1} min^{-1} (mean ± s.e. mean, $n = 11$) for lung and 0.401 ± 0.012 nmol mg protein^{-1} min^{-1} ($n = 7$) for liver. However, the addition of paraquat (5 mM) caused a 132% increase in binding with lung microsomes with only a 22.4% increase with liver microsomes. Superoxide dismutase (30 μg/ml) in the absence of paraquat completely inhibited the covalent binding while in the presence of paraquat (5 mM) significant superoxide dismutase insensitive covalent binding remained. Preliminary studies to ascertain the involvement of species other than superoxide in the covalent binding were therefore initiated. Benzoate, a hydroxyl radical scavenger and the singlet oxygen scavenger, 1,4-diazabicyclo-2,2,2-octane in the absence or presence of paraquat had no effect on the covalent binding. Catalase in the absence of paraquat significantly reduced the binding whereas in the presence of paraquat it had no effect.

The addition of glutathione, which can form a conjugate with the reactive intermediate of DOPA, completely inhibited covalent binding in the absence and presence of paraquat. If glutathione plays a similar role in the intact cell then it may be necessary to deplete intracellular glutathione before this technique can be used *in vivo* to identify the cell type or types in the lung involved in paraquat toxicity.

Reference

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Environmental factors affecting monooxygenase activity of microsomal fractions of human liver biopsies

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Considerable attention has been focused on the influence of environmental factors on hepatic monooxygenase activity in man. Most studies have been performed *in vivo* and thus little is known about functional changes in the liver itself. As part of an investigation into *in vitro* monooxygenase activity in microsomal fractions of human liver we have accumulated clinical and biochemical information on 49 patients undergoing routine liver biopsies (26 needle, 23 wedge) in whom histology showed only non-specific changes with no evidence of established liver disease. The influence of alcohol consumption, caffeine intake, drug ingestion and cigarette smoking on *in vitro* monooxygenase activity was analysed. Samples were assayed for cytochrome P-450 content, NADPH-cytochrome c reductase and aryl hydrocarbon hydroxylase (AHH) activities. We have previously shown that in biopsies with preserved hepatic architecture the range of monooxygenase activities were similar in needle and wedge samples (Boobis, Brodie, Davies, Fletcher & Saunders, 1978). Therefore the two groups of biopsies were treated as a single population for statistical analysis.

The higher the weekly alcohol consumption, which varied from 0 to 350 units per week (equivalent to 20 pints of beer or 1 bottle of whisky per day), the lower the microsomal cytochrome P-450 content when expressed per mg microsomal protein ($P < 0.01$) and per g liver ($P < 0.05$). Cytochrome P-450 content decreased by up to 70% with increasing alcohol ingestion. There was no accompanying change in NADPH-cytochrome c reductase activity with alcohol ingestion. This suggests that changes in cytochrome P-450 content were not simply due to non-specific loss of protein following fatty infiltration. AHH activity per nmol cytochrome P-450 was positively correlated with alcohol intake ($P < 0.05$). Thus alcohol may selectively induce this monooxygenase.

Coffee and tea ingestion (1 cup tea = 0.6 cup coffee) had no significant effect on any of the parameters measured. However, there was a trend for AHH activity to increase ($P = 0.057$) with increasing coffee

and tea intake. Drug ingestion also had no significant effect on monooxygenase function which was not unexpected as only one patient was receiving a known enzyme inducer. Cigarette smoking significantly increased AHH activity ($P < 0.05$) and cytochrome P-450 content when expressed per g/liver but not per mg microsomal protein but had no effect on NADPH cytochrome c reductase activity. In the smokers, there was no relationship between number of cigarettes smoked and the degree of AHH induction.

We have thus been able to show significant correlations between two environmental factors and *in vitro* monooxygenase activity in microsomal fractions of human liver.

ARB is an MRC Fellow

Reference

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Resolution and reconstitution of two forms of hepatic cytochrome P-450 from rabbits

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As part of a programme of investigation into the substrate specificity of cytochrome P-450 we have attempted to resolve individual forms of this haemoprotein from rabbit liver microsomes. Resolution of cytochrome P-450 and NADPH-cytochrome P-450 reductase was performed essentially as described by Johnson & Muller-Eberhard (1977). Solubilisation of the microsomal fraction with sodium cholate was followed by polyethylene glycol fractionation. Treatment of the precipitated material with Renex, a non-ionic detergent and chromatography on a hydroxylapatite-cellulose column enabled two fractions of cytochrome P-450 to be obtained. Detergent was removed by dialysis followed by adsorption on to calcium phosphate gel. NADPH-cytochrome P-450 reductase was purified by chromatography of the 25–60% ammonium sulphate precipitate of the supernatant of the appropriate polyethylene glycol fraction on DEAE-cellulose followed by adsorption on to calcium phosphate gel and concentration by ultra-filtration.

Two forms of cytochrome P-450 were partially purified with specific contents of up to 9.8 nmol/mg protein. SDS polyacrylamide gel electrophoresis revealed that one fraction (fraction 1) contained only a single band, molecular wt 54,000, whereas the second fraction (fraction 2) comprised several proteins, molecular weights between 45,000 and 60,000. Treatment of rabbits with 3-methylcholanthrene (80 mg/kg in corn oil injected intraperitoneally) increased fraction I whereas treatment of rabbits with phenobarbitone (1 mg/ml in drinking water for 4 days) increased fraction II. Fraction I had an absorption maximum of the carbon monoxide complex at 448 mm whereas the absorption maximum of fraction II was 450 nm.

Both fractions of cytochrome P-450 could be reconstituted to give aryl hydrocarbon hydroxylase and 7-ethoxycoumarin 0-deethylase activity. Activity was dependent on the presence of both cytochrome P-450 and the reductase fraction and was enhanced by the addition of lipid.

Fraction I from methylcholanthrene treated rabbits was more active than fraction I from phenobarbitone-treated or control animals in supporting aryl hydrocarbon hydroxylase activity (375 pmol $nmol^{-1}$ min^{-1}). Fraction II from phenobarbitone-treated animals was least active (86 pmol $nmol^{-1}$ min^{-1}). Fraction I from phenobarbitone-treated animals was most active in supporting 7-ethoxycoumarin 0-deethylase activity.

α-Naphthoflavone (500 μM) a selective inhibitor of monooxygenase activity, inhibited aryl hydrocarbon hydroxylase activity with fraction I from methylcholanthrene-treated animals by over 70% and from phenobarbitone-treated animals by 50%. With fraction II from both groups of animals 2 μM α-naphthoflavone inhibited aryl hydrocarbon hydroxylase activity by 50–80% but at higher concentrations enhanced activity supporting the suggestion by others that fraction II contains more than one form of cytochrome P-450.

We are currently investigating methods to further purify cytochromes P-450 from the microsomal fraction.

ARB is an MRC Fellow. KML is an MRC Student.

Reference

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