4. E. de Lamirande and G. L. Plaa, *Proc. Soc. exp. Biol. Med.* **158**, 283 (1978).
5. C. L. Witzleben and W. H. Boyce, *Archs Path.* **99**, 492 (1975).
6. C. L. Witzleben and W. H. Boyce, *Archs Path.* **99**, 496 (1975).
7. E. de Lamirande, B. Tuchweber and G. L. Plaa, *Biochem. Pharmac.* **30**, 2305 (1981).
8. I. M. Yousef and R. K. Murray, *Can. J. Biochem.* **56**, 713 (1978).
9. I. M. Yousef, D. L. Bloxam, M. Oda, M. J. Phillips and M. M. Fisher, *Can. J. Biochem.* **53**, 989 (1975).
10. G. R. Bartlett, *J. biol. Chem.* **234**, 466 (1959).
11. G. H. Lathe and C. R. J. Ruthven, *J. clin. Path.* **11**, 155 (1958).
12. R. R. Sokal and F. J. Rohlf, *Biometry*, pp. 239–46. W. H. Freeman, San Francisco (1969).
13. G. Kakis and I. M. Yousef, *Gastroenterology* **75**, 595 (1978).
14. G. Kakis, M. J. Phillips and I. M. Yousef, *Lab. Invest.* **43**, 73 (1980).
15. A. M. Samuels and M. C. Carey, *Gastroenterology* **74**, 1183 (1978).
16. F. R. Simon, E. Supherland and R. Davis, in *Problems in Intrahepatic Cholestasis* (Eds P. Gentilini, H. Popper, S. Sherlock and U. Teodori), p. 30. Karger, Basel (1978).
17. M. J. Phillips, I. M. Yousef, G. Kakis, M. Oda and K. Funatsu, in *The Quantitative Aspects of Structure and Function* (Eds R. Preisig and J. Bircher), p. 185. Cantor, Berne (1979).
18. G. Szabo, *Nature, Lond.* **252**, 47 (1974).
19. I. M. Yousef, M. Lewittes, G. Kakis, C. C. Roy and B. Tuchweber, *Gastroenterology* **79**, 1069 (1980).
20. M. D. Apstein and A. R. Russo, *Hepatology* **2**, 143 (1982).
21. K. Miyai and W. W. Richardson, *Lab. Invest.* **32**, 527 (1975).
22. J. De Gier, J. G. Mandersloot and L. L. M. Van Deenen, *Biochim. biophys. Acta* **173**, 143 (1969).
23. T. N. Estep, D. B. Mountcastle, R. L. Biltonen and T. E. Thompson, *Biochemistry* **17**, 1984 (1978).

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Aldrin epoxidation kinetics in small samples of human liver

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In vitro studies of the activity of hepatic microsomal mono-oxygenases in man have been limited by the quantity of tissue that is conventially removed during diagnostic liver biopsy (rarely more than 60 mg wet wt is surplus to histological requirements) and by the sensitivity of methods for measuring the products of the enzyme reactions. Benz[*a*]pyrene hydroxylation, ethylmorphine demethylation and the kinetics of amylobarbitone hydroxylation have been measured in human liver biopsies [1–3]. Mono-oxygenases, including antipyrine hydroxylation [4], phenacetin-*O*-de-ethylase and 7-ethoxycoumarin de-ethylase [5], have also been investigated in tissue wedges obtained at laparotomy.

Aldrin epoxidation activity has been shown to be readily measured in needle biopsies of rat liver. The major metabolite, dieldrin, is detectable with a high degree of sensitivity by electron capture gas chromatography [6]. Wolff has demonstrated in the rat that aldrin epoxidation is readily inducible wtth phenobarbitone but not with 3-methylcholanthrene [6]. After purification of cytochrome P-450 forms, activity is limited to the phenobarbitone-inducible form of cytochrome P-450 [7]. These observations suggest that aldrin epoxidation might be a useful probe for investigating mono-oxygenase activity in small samples of human liver.

Materials and methods

Human liver, surplus to diagnostic requirements, was obtained at either diagnostic needle biopsy (six patients), or from a wedge biopsy removed during laparotomy (two patients). In all instances, liver biopsy was performed for suspected hepatic disease: in the six patients undergoing needle biopsy, no hepatic pathology was found and the liver was histologically normal. The two wedge biopsies were obtained from non-malignant tissue of patients undergoing partial hepatectomy for primary liver cell cancer.

Liver was either frozen immediately in liquid nitrogen and stored at −80°, or placed into ice-cold phosphate buffer (0.25 M potassium phosphate containing 0.15 M potassium chloride and 1.0 M EDTA, pH 7.25). The microsomal pellet was prepared as described by Boobis *et al.* [1] and resuspended in 0.25 M phosphate buffer containing 30% v/v glycerol. Enzyme activity was usually assayed immediately, although storage of microsomes at −80° for up to 2 months did not alter activity. Aldrin epoxidation was measured by a micro scale modification of the method described by Wolff *et al.* [6]. Incubations were for 20 min at 37° in a final vol. of 0.1 ml containing 0.005 M phosphate buffer (pH 7.5), 0.5 mM NADPH, microsomal protein (20–40 μg) and aldrin added in 1 μl methanol. Dieldrin was extracted into hexane and measured by electron capture gas chromatography. The lower limit of detection of the method was 0.5 pmoles dieldrin formed/mg microsomal protein/min. Protein was measured by a modification of the method of Lowry *et al.* [8]. Inhibitors (metyrapone and α-napthaflavone) were added to the incubation medium in 1 μl ethanol before addition of the substrate. Control incubations contained 1 μl ethanol.

Results and discussion

Aldrin epoxidation activities measured in microsomes prepared from 100-mg portions of wedge biopsy tissue were similar to those prepared from 10-mg portions of the same tissue [108.3 ± 7.3 (n = 4) and 104.9 ± 7.0 (n = 4) pmoles dieldrin/mg/min, respectively]. Aldrin epoxidation was linear with time to 30 min, and proportional to microsomal protein between 0.1 and 0.5 mg/ml at a substrate concn of 100 μM. Protein concns of less than 0.1 mg/ml resulted in variable activity measurements. Optimum activity was measured at pH 7.6, but between pH 7.4 and pH 7.8 activity was greater than 90% of the maximum. Activity was reduced by 97% when NADPH was omitted from the

Table 1. Kinetics of aldrin epoxidation in human liver biopsy tissue

Sex	Age	Biopsy wt (mg)	Protein content (mg/g)	Protein/ incubation (mg)	Aldrin epoxidation K_m (μM)	Aldrin epoxidation V_{max} (pmoles/mg/min)
Female	55	37.2	11.3	0.021	20.9	163.9
Male	49	17.2	15.3	0.017	21.4	116.2
Female	75	17.0	15.9	0.011	7.4	213.2
Male	40	17.3	20.5	0.014	3.3	66.0
Male	50	22.2	6.3	0.007	3.4	81.8
Female	57	17.4	21.8	0.025	2.8	119.9
Mean ± S.E.M.	54.3 ± 4.8	21.4 ± 3.3	15.2 ± 2.4	0.014 ± 0.003	9.9 ± 3.6	126.8 ± 22.1

Liver samples were histologically normal.

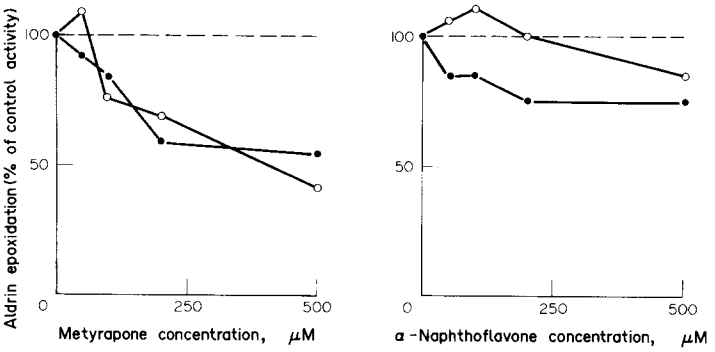

Fig. 1. Effect of metyrapone and α-naphthoflavone on human liver aldrin epoxidation activity. Results for two human liver samples are shown. Values are the means of three determinations at 100 μM aldrin.

incubation [103.3 ± 9.5 (n = 4) to 3.2 ± 1.8 (n = 4) pmoles dieldrin/mg/min], and was undetectable in the absence of oxygen, indicating that the enzyme is a cytochrome P-450-dependent mono-oxygenase.

The kinetics of aldrin epoxidation were investigated over a range of aldrin concns from 1 to 200 μM, in six liver biopsy samples with normal histology. Individual data are shown in Table 1. K_m and V_{max} values were determined by the double reciprocal plot. The apparent K_m was 9.9 ± 3.6 μM and V_{max} 126.8 ± 22.1 pmoles dieldrin formed/mg/min. There was considerable inter-individual variation in the apparent K_m values. Wolff *et al.* [6] have shown, in the rat, a five-fold reduction in apparent K_m from 18 to 3.5 μM with a similar reduction in protein concn (from 0.5 to 0.1 mg/ml). However, in our study we failed to detect any correlation between apparent K_m and the protein content of the incubation, nor was there any relationship between apparent K_m and the age of the patient. Fraser *et al.* [3] measured K_m values ranging from 1.1 to 6.4 mM for amylobarbitone hydroxylation in three samples of human liver and Boobis *et al.* [5] have reported wide inter-individual differences in the kinetics of phenacetin-*O*-de-ethylase and ethoxycoumarin de-ethylase. Aldrin epoxidation by human liver was inhibited by metyrapone to a greater extent than by α-naphthoflavone (Fig. 1). α-Naphthoflavone shows a greater affinity for cytochrome P-450 forms which are inducible by 3-methylcholanthrene [9], whereas metyrapone has affinity for other forms of cytochrome P-450. In rat liver microsomes aldrin epoxidase activity is mostly restricted to phenobarbitone-inducible cytochrome P-450 forms, which are inhibited by metyrapone but not by α-naphthoflavone [7].

Measurements of aldrin epoxidation in small samples of liver biopsy tissue offer a sensitive probe for the study of mono-oxygenase activity in man.

In summary, aldrin epoxidation can be measured in as little as 10 mg liver biopsy tissue available from a diagnostic needle biopsy in man. The human enzyme is a cytochrome P-450-dependent mono-oxygenase with K_m 9.9 ± 3.6 μM and V_{max} 126.8 ± 22.1 pmoles dieldrin formed/mg/min. Considerable inter-individual differences are observed.

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REFERENCES

1. A. R. Boobis, M. J. Brodie, G. C. Kahn, D. R. Fletcher, J. H. Saunders and D. S. Davies. *Br. J. clin. Pharmac.* **9**, 11 (1980).

2. G. C. Farrell, W. G. E. Cooksley and L. W. Powell, *Clin. Pharmac. Ther.* **26**, 483 (1979).
3. H. S. Fraser, F. M. Williams, D. L. Davies, G. H. Draffan and D. S. Davies, *Xenobiotica* **6**, 465 (1976).
4. A. R. Boobis, M. J. Brodie, G. C. Kahn, E-L. Toverud, I. A. Blair, S. Murray and D. S. Davies, *Br. J. clin. Pharmac.* **12**, 771 (1981).
5. A. R. Boobis, G. C. Kahn, C. Whyte, M. J. Brodie and D. S. Davies, *Biochem. Pharmac.* **30**, 2451 (1981).
6. T. Wolff, E. Deml and H. Wanders, *Drug Metab. Dispos.* **7**, 301 (1979).
7. T. Wolff, H. Greim, M. T. Huang, G. T. Miwa and A. Y. H. Farr, *Eur. J. Biochem.* **111**, 545 (1980).
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall *J. Biol. Chem.* **193**, 265 (1951).
9. S. A. Atlas and D. W. Nebert, *Archs biochem. biophys.* **175**, 495 (1976).

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Effects of niridazole and 5-nitroimidazoles on heart mitochondrial respiration

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Nitroimidazole derivatives are used extensively to treat infections caused by anaerobic protozoa and bacteria [1]. These drugs were found to be selectively adsorbed and have a cytotoxic action on anaerobes [2]. Recently a number of nitroimidazoles have been used as radiosensitizers of hypoxic cells [3], since these are believed to limit the effectiveness of radiation therapy in certain tumors [4]. However, in antineoplastic chemotherapy, large amounts of drugs are required [5], increasing the risk of toxic effects. In fact some nitroimidazoles have shown a weak carcinogenic effect [6, 7], whilst for most of them a mutagenic action has been demonstrated [8, 9]. Moreover ECG abnormalities were observed in patients under either niridazole or metronidazole therapy [10, 11]. Although the mechanism of antibacterial and antiprotozoal action is still under investigation, it is generally assumed that the nitrogroup of these compounds must be reduced and the highly reactive products possibly bind to DNA or other biopolymers [12, 13]. The reduction of the nitrogroup is a necessary step in the induction of mutagenicity in *Salmonella typhymurium* [14]. Adams *et al.* [15] pointed out a relationship between electroreduction potential and efficiency as radiosensitizers.

The purpose of the present paper was to study the effect of one nitrothiazole and five nitroimidazole derivatives on oxidative processes in rabbit heart mitochondria and the relationship with their electroreduction potentials.

Materials and methods. Female rabbits weighing 3–4 kg were used. While niridazole, ipronidazole, 1-methyl-2-formyl-5-nitroimidazole (MFNI), ornidazole and metronidazole were obtained from commercial sources, DA 3851 was a gift of De Angeli (Milano, Italy). Stock solutions were made in methanol or dimethylsulphoxide (niridazole). ADP, dinitrophenol (DNP) and respiration substrates were obtained from Sigma Chemical Co. (St. Louis, MO). All other reagents were of analytical grade. Mitochondria were prepared from rabbit heart, according to Tyler and Gonze [16], and rapidly utilized. Oxygen consumption was recorded at 30° using a Clark-type oxygen electrode obtained as an integral part of a thermostated vessel. The reaction mixture consisted of 0.25 M sucrose, 5 mM K_2HPO_4, 5 mM $MgCl_2$, 10 mM KCl, 0.5 mM EDTA and 10 mM Tris–HCl at pH 7.4, added to 1 mg mitochondria, in a total volume of 2.8 ml. Substrates were either 8 mM glutamate–malate or 20 mM succinate in the presence of 2 μM rotenone. Mitochondrial swelling was tested by measuring extinction changes at 546 nm [17] by means of a Zeiss PMQ 3 spectrophotometer. Mitochondrial protein was determined by the biuret method [18], using deoxycholate for solubilization.

The electroreduction potentials had been determined at pH 7.4 by means of cyclic voltametry using a mercury dropping electrode vs a saturated calomel electrode [8]. Only peaks between −200 and −900 mV had been considered.

Results and discussion. The structures, electroreduction potentials and per cent inhibitions of oxygen uptake are reported in Table 1. MFNI considerably affected mitochondrial respiration in the presence of glutamate–malate as substrate; effectiveness of inhibition of ADP-stimulated respiration was concentration-dependent (Fig. 1). Niridazole, ipronidazole, DA 3851 and ornidazole at concentrations up to 0.8 mM led to falls of only 10–20% in oxygen uptake; metronidazole did not seem to affect the process at all. The *in vitro* concentrations were fairly close to the serum levels which occur *in vivo* after administration of

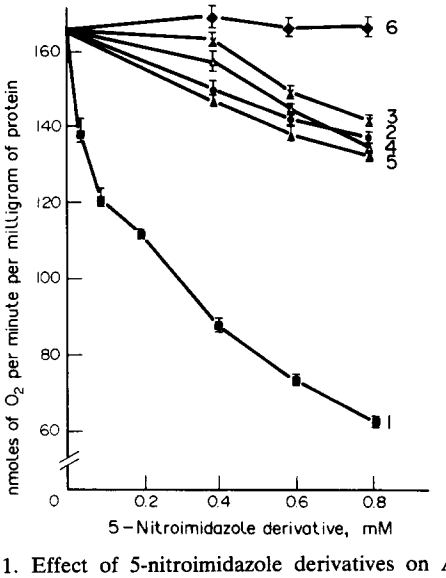

Fig. 1. Effect of 5-nitroimidazole derivatives on ADP-stimulated respiration. One milligram mitochondria was incubated at 30° in 2.8 ml of reaction medium (see Materials and Methods) containing 5-nitroimidazoles as indicated and 8 mM glutamate–malate as substrate. Respiration was stimulated by addition of 5 μl 0.1 M ADP. The points represent mean values ± S.E.M. for four separate experiments.