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Increase in renal cytochrome P-450 and NADPH cytochrome *c* reductase activity following drug inhibition of hepatic monooxygenase activity

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Cytochrome P-450 is the terminal oxidase of the microsomal monooxygenase system, which is responsible for drug metabolism. Cytochrome P-450 concentration in hepatic tissue may possibly influence the normally lower levels found in extra-hepatic sites, for example the reduction of hepatic cytochrome P-450 following partial hepatectomy in surgical models resulted in a compensatory increase at extrahepatic sites, especially the kidney [1]. Whether this extrahepatic compensatory effect follows treatment with drugs known to reduce hepatic cytochrome P-450 is not known.

Amiodarone (AD) is an anti-arrhythmic drug that accumulates in several tissues (mainly adipose tissue and liver) in a dose dependent manner [2–5]. The high incidence of dose dependent hepatic changes, including hepatic necrosis, following AD treatment suggests a direct hepatotoxic effect [6]. In animal studies AD has been found to reduce significantly hepatic levels of cytochrome P-450 and other drug metabolizing enzymes [7]. This reduction in hepatic cytochrome P-450 may result from its direct hepatotoxic effect.

In this study we investigate the possibility that drugs reducing hepatic cytochrome P-450 (using AD as a model) may also have an effect on renal cytochrome P-450 and NADPH cytochrome *c* reductase activity.

Materials and methods

Male Wistar rats, weighing 300–320 g, were obtained from Wellcome Research Animal Laboratories (Dublin). Animals were housed in rooms with alternating light and dark cycles (light 07.00–19.00). Rats were allowed free access to food and tap water. Animals, pretreated with AD (Cordarone X Intravenous) 200 mg/kg i.p. for 3 consecutive days, were killed by cervical dislocation 1 hr, 2 weeks and 4 weeks following cessation of treatment. A control group received normal saline during the same period (N = 6 in each group except for week 4 where N = 5).

Hepatic and renal microsomes were prepared by centrifugation as described previously [8, 9]. The following assays were conducted using fresh microsomes. The content of cytochrome P-450 in hepatic and renal microsomes was determined [9, 10] and levels are expressed as nmole/mg protein. NADPH-cytochrome *c* reductase activity was assayed by the method of Mazel [11] subsequently modified [12] and expressed as nmole/min/mg protein. Microsomal protein was measured by the Lowry method [13], against bovine serum albumin standards.

Statistical significance with respect to control was assessed using a Student's *t*-test for unpaired data.

Results and discussion

The effects of AD on the cytochrome P-450 and NADPH-cytochrome *c* reductase, both in kidney and liver, are presented in Fig. 1. No statistically significant difference in hepatic cytochrome P-450 was seen until 4 weeks fol-

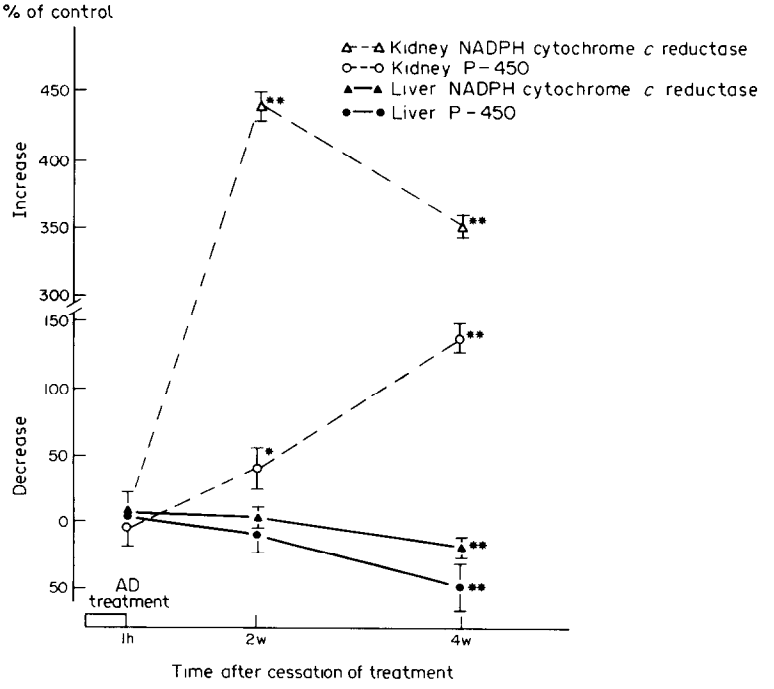

Fig. 1. Changes in hepatic and renal microsomal enzymes (NADPH cytochrome *c* reductase and cytochrome P-450) 1 hr, 2 weeks and 4 weeks after discontinuation of Amiodarone (200 mg/kg/day i.p.) treatment for three consecutive days. Changes are compared with control values. * $P < 0.05$, ** $P < 0.01$ (*t*-test for unpaired data).

lowing cessation of treatment, being decreased by 50% ($P < 0.01$) compared with the control group. Nevertheless there was an increase in the levels of renal cytochrome P-450 of 40% ($P < 0.05$, week 2) and of 135% ($P < 0.01$, week 4) after cessation of AD treatment. The activity of hepatic NADPH-cytochrome *c* reductase was decreased by 20% ($P < 0.01$) at week 4 and in kidney increased by 440% ($P < 0.01$) and 354% ($P < 0.01$) at weeks 2 and 4 respectively.

Using a pharmacological model (AD) of decreased hepatic cytochrome P-450 and NADPH cytochrome *c* reductase, our data further suggest a role for hepatic cytochrome P-450 levels in the regulation of renal cytochrome P-450. Of interest, the renal enzymatic activity is increased two weeks prior to a significant decrease in hepatic enzymes, perhaps due to the early detection of some endogenous inducer(s) by the kidney [1].

The inhibitory effect of AD on the hepatic drug metabolizing enzymes did not reach statistical significance until four weeks after discontinuing treatment. This may be explained by the complex pharmacokinetic behaviour of the drug, i.e. long elimination half-life, capacity of fat to act as reservoir, or even a delayed hepatotoxic effect.

The kidneys represent about 3–7% of the total cytochrome P-450 and NADPH cytochrome *c* reductase content in the rat [14]. The induction pattern of different enzyme systems in the same organ may vary and this may account for the disproportionate increase in NADPH cytochrome *c* reductase activity and cytochrome P-450 levels in the kidney following amiodarone treatment [15]. This disproportion may, however, be due to the existence of multiple forms of cytochrome P-450 where selective induction of some isoenzymes would result in a smaller increase in total cytochrome P-450 levels. Selective induction of some mono oxygenase activities in kidney microsomes following a reduction in hepatic cytochrome P-450 levels has been recently reported [1].

The pharmacological agent used in this study (AD) produces enzyme inhibition in association with direct hepatotoxicity; whether an increase in renal enzyme activity follows administration of a non hepatotoxic enzyme inhibitor remains to be seen.

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REFERENCES

1. G. Babany, V. Descatoire, M. Corbic, S. Gendre, C. Degott, D. Larrey, P. Letteron, J. C. Wandscheer, C. Funck-Brentano and D. Pessayre, *Biochem. Pharmac.* **34**, 311 (1985).
2. D. M. Holt, G. T. Tucker and P. R. Jackson, *Am. Heart J.* **106**, 840 (1983).
3. D. W. Holt, P. C. Adams, R. W. F. Cambell, A. R. Morley, J. Callaghan and G. C. A. Storey, *Br. J. clin. Pharmac.* **17**, 195P (1984).
4. D. M. Paton, D. R. Webner and M. Neutze, *Meth. Find. Exptl. Clin. Pharmac.* **6**, 41 (1984).
5. T. A. Plomp, W. M. Weirsinga and R. A. A. Maes, *Arzne-Forsch./Drug Res.* **35**, 122 (1985).
6. J. B. Simon, P. N. Manley, J. F. Brien, and P. W. Armstrong, *N. Engl. J. Med.* **311**, 167 (1984).
7. O. Grech-Belanger, *Res. Commun. Chem. Pathol. Pharmacol* **44**, 15 (1984).
8. J. R. Means, G. P. Carlson and R. C. Schnell, *Toxicol. Appl. Pharmacol* **80**, 293 (1979).
9. S. V. Jakobsson and D. L. Cinti, *J. Pharmac. exp. Ther.* **185**, 226 (1973).
10. T. Omura and R. Sato, *J. Biol. Chem.* **239**, 2370 (1964).
11. P. Mazel, in *Fundamentals of Drug Metabolism and Drug Disposition* (Eds. B. N. La Du, H. G. Mandel and F. L. Way), p. 546. Williams & Wilkins, Baltimore (1971).
12. 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).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
14. W. Hietanen, H. Bartsch, M. Ahotupa, S. S. Park and H. V. Gelboinon, *Drug Metabolism* (Ed. G. Siest), p. 141. Pergamon Press, Oxford (1985).
15. P. Jenner and B. Testa, in *Drug Metabolism: Chemical and Biochemical Aspects* (Ed. J. Swarbrick), p. 445. Marcel Dekker, New York (1976).

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Inhibition of epidermal growth factor binding to HeLa cells by auranofin*

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Auranofin (AF; 2,3,4,6-tetra-O-acetyl-1-thio-beta-D-glucopyranosato-*S*-[triethylphosphine] gold) is a novel, antirheumatic, lipophilic gold complex widely used in the treatment of rheumatoid arthritis [1].

The compound has inhibitory effects on several activation responses in human leukocytes which involve the Ca^{2+}/phospholipid-dependent protein kinase (protein kinase C; PK-C). For example AF inhibits platelet aggregation [1] and 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced superoxide anion release from human neutrophil polymorphs (PMN) [2] and monocytes [3]. The drug has little effect on superoxide release from PMN and monocytes [3] elicited by the Ca^{2+} ionophore A23187, which acts via Ca^{2+}/calmodulin-dependent pathways independently of PK-C [4].

In view of the apparent selective effect of AF on PK-C-dependent pathways, we are investigating the effect of AF on other cellular events modified by TPA and which involve activation of PK-C. One such event, well characterised in cultured cell lines, is the TPA inhibition of epidermal growth factor (EGF) binding [5]. TPA increases a PK-C mediated phosphorylation of the EGF receptor, a decrease in EGF binding and an inhibition of the intrinsic tyrosine kinase activity of the receptor [6]. In the present paper we show that AF does not interfere with TPA inhibition of EGF binding to HeLa cells, and is itself a powerful inhibitor of binding.

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