Differences in hepatic drug accumulation and enzyme induction after chronic amiodarone feeding of two rat strains: role of the hydroxylator phenotype?

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- 1 It has previously been shown that the extent of hepatic phospholipidosis induced by chronic amiodarone treatment correlates with the degree of drug accumulation in liver tissue.
- 2 To investigate a possible influence of pharmacogenetic factors, biochemical and morphological investigations were carried out in two rat strains differing in debrisoquine hydroxylation.
- 3 Plasma and liver tissue concentrations of amiodarone and its main metabolite, desethyl-amiodarone, were significantly higher in rats with deficient hydroxylation. Microsomal enzyme induction, drug cytochrome P-450 complex formation and typical ultrastructural features of phospholipidosis were only seen in rats with deficient hydroxylation and in a more sensitive species, the guinea-pig.
- 4 It remains to be seen whether deficient debrisoquine hydroxylation in man is associated with an increased susceptibility to amiodarone side effects.

Introduction

Chronic treatment with the antiarrhythmic drug amiodarone has been linked in some cases to severe liver disease in man (Lim *et al.*, 1984; Poucell *et al.*, 1984; Simon *et al.*, 1984; Rigas *et al.*, 1986; Babany *et al.*, 1986). The type of liver disease associated with chronic amiodarone treatment resembles that induced by perhexiline maleate (Pessayre *et al.*, 1979); it is characterized histologically by changes similar to those of alcoholic hepatitis. Ultrastructural findings are suggestive of phospholipid storage. The guinea-pig has been shown to serve as a valuable animal model for the study of amiodarone-induced phospholipidosis (Pirovino *et al.*, 1988). Chronic oral treatment with amiodarone leads to accumulation of amiodarone, desethyl-amiodarone (DEA) and phospholipids (PL) in the liver which seems to be due to both lysosomal drug-PL complex formation and to inhibition of phospholipases A1 and A2 (Luellmann *et al.*, 1978; Hostetler & Matsuzawa, 1981; Heath *et al.*, 1985; Hostetler *et al.*, 1986). We have recently shown that the extent of the hepatic phospholipidosis, as assessed morphometrically, correlates strictly with the accumulation of amiodarone in the liver (Pirovino *et al.*, 1988).

The rarity of severe liver disease in man as a result of treatment with amiodarone has led us to consider the possible influence of pharmacogenetic factors on plasma and tissue concentrations of the drug and its main metabolite DEA. It has been shown that perhexiline-induced liver disease and neuropathy are more frequent in poor hydroxylators when tested with debrisoquine or sparteine (Shah *et al.*, 1982; Morgan *et al.*, 1984). This observation is paralleled by an increased phospholipid accumulation in nervous tissue of rats with a similar genetic hydroxylation defect (Meier *et al.*, 1986). We wondered whether the same holds true for the phospholipidosis induced by amiodarone. Since the metabolism of amiodarone following its desethylation to DEA is poorly known, it could well be that deranged hydroxylation taking place at a further step could influence the expression of the adverse effects of the drug. Furthermore, the genetic hydroxylation deficiency may be linked to deranged desalkylation

with a consecutive increase in concentration of the parent drug, as has been shown for the N-demethylation of amiflamine in some rat strains by Alvàn *et al.* (1984).

To study this question, we chose the comparison between Sprague-Dawley (SD) and Dark Agouti (DA) rats. Female animals of the latter strain are known to represent an animal model of a genetic hydroxylation deficiency of the debrisoquine type (Al-Dabbagh *et al.*, 1981; Küpfer & Preisig, 1983). We investigated the effects of chronic amiodarone treatment on the liver both morphologically and biochemically. At the same time, we studied the effect of chronic amiodarone feeding on microsomal enzymes. It has previously been shown that short-term treatment with high intraperitoneal doses of amiodarone leads to the formation of an inactive cytochrome P-450Fe(II)-amiodarone metabolite complex in rats (Larrey *et al.*, 1986). This observation may be responsible for some of the drug interactions reported in man (Mason, 1987).

Methods

Animals

Female Dark Agouti (DA) rats and female Sprague-Dawley (SD) rats, 3 months old and weighing 150–250 g, were from the Versuchstierzucht, Hannover, and from the Deutsche Versuchstierfarm, Tuttlingen, FRG, respectively. Four groups of animals consisting of 6 rats each were investigated. They received standard rat chow or the same food regimen with amiodarone added in a concentration of 0.86 g kg⁻¹ of food (NAFAG, Gossau, Switzerland). This dose, resulting in an approximate daily intake of 20–30 mg amiodarone, was chosen in accordance with previous studies in our laboratory showing that this dose was well tolerated by the rats even on a longterm basis. Animals were kept on a 12 h light-dark cycle in temperature- and humidity-controlled quarters. They were maintained on this regimen for 5 months before being killed by bleeding after ether anaesthesia. Experimental and control animals were killed at the same time of day. The animal experiments were performed in accordance with the guidelines laid down by the Swiss Academy of Medical Sciences.

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Morphological methods

For light microscopy, pieces of liver tissue were fixed in 10% formalin, dehydrated in ethanol and embedded in paraplast. Sections were stained with hematoxylin-eosin and with periodic acid Schiff (PAS). For electron microscopy, small tissue blocks (about 1 mm³) were fixed in 3.1% phosphate buffered glutaraldehyde (pH 7.4), rinsed with buffer, postfixed in osmium tetroxide, rinsed, dehydrated in graded ethanol and embedded in epon using propyleneoxide as an intermedium. Sections of about 1 µm were stained with toluidine blue for light microscopic control, and ultrathin sections (ca. 50 nm) were stained with uranylacetate and lead citrate for electron microscopic examination.

Extraction of amiodarone and desethyl-amiodarone from plasma

Blood was taken by cardiac puncture and centrifuged for 10 min at 1500 *g*. Plasma was stored at -20°C. Amiodarone and its major metabolite DEA were quantitated according to a method described previously (Stäubli *et al.*, 1983): briefly, after addition of the internal standard L 8040/1, 2-ethyl-3- (3, 5-dibromo-4- γ -di-n-propylaminopropoxybenzoyl) benzothiophene (Sanofi SA, Montpellier, France), 500 µl of plasma were extracted with diethylether at pH 3. The ether phase was evaporated at 40°C under a gentle stream of nitrogen. The residue was dissolved in 100 µl of mobile phase of which 10 µl was injected into the high-performance liquid chromatography (h.p.l.c.) column.

Recovery of amiodarone and DEA from spiked plasma was 80 to 85%, recovery of internal standard 76 to 82%. The day to day coefficient of variation was 4.4% and 6.7% for amiodarone and DEA respectively at concentrations ranging from 0.47 to 4.5 µmol l⁻¹.

Extraction from tissue

Approximately 200 mg of liver tissue was dried between Kleenex tissues and weighed. The tissue was finely minced with scissors and homogenized with 5 ml of distilled water in a 10 ml Dounce glass homogenizer (20 strokes); 500 µl of this homogenate was extracted and chromatographed as described for plasma.

H.p.l.c. conditions for separation of amiodarone and desethyl-amiodarone

The h.p.l.c. system consisted of a Waters M-45 pump, a WISP model 712 autosampler and a Kratos Spectroflow 757 u.v. detector operated at 254 nm. A 25 cm h.p.l.c. column with 4 mm inner diameter filled with Nucleosil 100-5C18 was used (Macherey Nagel, Oensingen, Switzerland). The mobile phase consisted of 7 ml ammonia 25% (w/v) dissolved in 993 ml of h.p.l.c. grade methanol. Flow rate was 1.0 ml min⁻¹. Retention times for amiodarone and DEA were 6.5 and 5.5 min, respectively, and 8.5 min for the internal standard (L 8040/1).

Determination of total phospholipid content

Total PL content of the liver homogenate was assessed according to a fluorimetric assay method using 1,6-diphenyl-hexatriene as marker (Jouanel *et al.*, 1980). Since we have shown previously that high concentrations of amiodarone interfere with this assay (Pirovino *et al.*, 1988), we used an additional method by measuring PL-derived phosphate with malachite green. Briefly, PL-phosphorus of the tissue homogenates was precipitated and ashed according to the procedure outlined by Boehringer Mannheim FRG for their phosphorus test combination. The resulting inorganic orthophosphate was then determined in an assay mixture containing ammonium heptamolybdate and malachite green dissolved in polyvinyl alcohol. This most sensitive colorimetric measurement of inor-

ganic orthophosphate is based on the complex formation of phosphomolybdate with the basic malachite green dye. Absorbance of the samples was determined at 610 nm and compared with a test curve using phosphatidylcholine as a standard. The method, allowing phosphate determinations in the nanomolar range, was performed according to van Veldhoven & Mannaerts (1987).

In vivo phenotyping procedure

DA- and SD-rats were phenotyped after oral administration of 1 mg debrisoquine (as hemisulphate) in 500 µl of an aqueous solution by gavage. Urine was collected during 24 h while rats were kept in metabolic cages. During the phenotyping procedure, the rat chow was withheld, but the rats had access to water *ad libitum*.

G.l.c. conditions for separation of debrisoquine and 4-OH-debrisoquine

A gas chromatograph Carlo-Erba model 4100 with ECD detection and a Hewlett-Packard-Integrator model 3392A were used. The column was a 1.8 m/2 mm i.d. glass-column filled with 3% SE-30 on Chromosorb 80/100 W HP.

The carrier gas was a mixture of 95% argon and 5% methane at a flow rate of 35 ml min⁻¹. The injector temperature was 275°C, the detector temperature was 275°C and the column temperature was isothermal at 200°C.

Analyses of debrisoquine and 4-OH-debrisoquine were carried out after derivatization using hexafluoroacetylacetone according to the g.l.c. method described by Erdtmansky *et al.* (1975).

Microsomal enzymes

After preparation of microsomes, the amount of uncomplexed, complexed and total cytochrome P-450 and the activity of NADPH-cytochrome c reductase were measured according to methods described previously (Larrey *et al.*, 1986).

Chemicals

Amiodarone hydrochloride was generously provided by Sanofi, Montpellier, France. Debrisoquine was kindly supplied by Hoffman-La Roche, Basel, Switzerland. All other chemicals were of p.a. grade and were purchased from Fluka or Merck Chemicals.

Statistics

Results are expressed as mean $\pm$ 1 standard deviation ($x \pm 1$ s.d.). Differences were quantified by the Student's *t* test with *P* < 0.05 accepted as the level of significance.

Results

Debrisoquine hydroxylation and its modification by chronic amiodarone feeding (Figure 1)

All animals were phenotyped at the beginning and at the end of the 5 months feeding period. Hydroxylation, as expressed by the ratio of debrisoquine to 4-OH-debrisoquine excreted in the urine, was slower in the DA rats (metabolic ratio [MR]: 0.88 ± 0.07 in DA rats vs. 0.14 ± 0.07 in SD rats). The defect in hydroxylation in DA rats became more pronounced after 5 months of control feeding (MR: 1.39 ± 0.4 ; *P* < 0.01), whereas hydroxylation remained unchanged in SD control rats. In both strains, amiodarone treatment was associated with a lowering of the MR – equivalent to faster hydroxylation, from 0.14 ± 0.06 to 0.096 ± 0.01 (*P* < 0.05) in SD rats, and from 0.88 ± 0.2 to 0.69 ± 0.17 in DA rats (*P* < 0.05). The effect of chronic amiodarone treatment on hydroxylation in DA rats was most evident when the MR was compared at the end of

Figure 1 Metabolic ratios (MR) of debrisoquine to 4-OH-debrisoquine before and after the 5 months treatment period. Chronic amiodarone feeding of both rat strains is associated with a significant decrease in MR indicating faster hydroxylation of debrisoquine. Note the marked difference in MR between control and amiodarone-fed Dark-Agouti rats at the end of the feeding period ($P < 0.002$). Solid lines: Sprague-Dawley rats; broken lines: Dark Agouti rats.

the treatment period, being 1.39 ± 0.40 in DA rats fed the control diet vs. 0.69 ± 0.17 in amiodarone-fed DA rats ($P < 0.002$) (Figure 1).

Liver and body weights at the end of treatment

Body weight of the SD rats was lower in the amiodarone-treated group (283.3 ± 12.7 g) than in the controls (341.2 ± 25.4 g), but liver weight/body weight ratio remained unchanged. In DA rats, the mean body weight of amiodarone-treated animals was inferior to that of controls ($P < 0.001$); liver weight of treated animals was higher ($P < 0.05$), resulting in a significant increase in liver/body weight ratio after amiodarone treatment ($P < 0.002$; Figure 2).

Light and electron microscopy

No morphological differences were observed between the livers of control and amiodarone-treated SD rats either by light or electron microscopy. Generally, the hepatocytes of DA rats had more smooth endoplasmic reticulum (SER) than those of the SD rats, and in the amiodarone-treated DA rats there was considerably more SER than in the DA controls.

Figure 2 Liver to body weight ratios ($\times 100$) after 5 months of treatment. Dark Agouti rats (b) show a significant increase in this ratio ($P < 0.002$); no change is seen in Sprague-Dawley rats (a). Open columns: control rats; hatched columns: amiodarone-fed rats.

Moreover, the livers of amiodarone-treated DA rats revealed characteristic cytoplasmic inclusions (dense bodies) similar to those described in the amiodarone-treated guinea-pigs (Pirovino *et al.*, 1988).

These inclusions were found in hepatocytes, Kupffer cells, fat storing cells and, especially, in biliary epithelial cells (Figures 3 and 4). Occasionally, dense bodies were found in hepatic nerve axons. Dense bodies were of different forms and structures, some lamellated, some containing densely packed membranes as a fine meshwork or in the form of fingerprint-like myelin figures. Lobular architecture was preserved in all animals, and there were no signs of inflammation or of an increase in fibrous tissue.

Amiodarone and desethyl-amiodarone concentrations in plasma and liver

Amiodarone and DEA concentrations in plasma and liver homogenates after 5 months of treatment are given in Figure 5. Mean plasma amiodarone concentrations were $1.0 \pm 0.4 \mu\text{mol l}^{-1}$ in the SD rats, whereas mean DEA plasma concentrations were $0.1 \mu\text{mol l}^{-1}$, resulting in a plasma amiodarone/DEA ratio of 9.5 ± 2.2 ($n = 5$). Concentrations were significantly higher in amiodarone-treated DA rats than in drug-treated SD rats. In plasma, amiodarone concentrations averaged 3.8 ± 0.5 ($P < 0.001$), DEA concentrations 1.4 ± 0.15 ($P < 0.001$), with an amiodarone/DEA ratio of 2.8 ± 0.6 ($P < 0.001$). Liver tissue concentrations of amiodarone were $3.7 \pm 1.7 \text{ nmol g}^{-1}$ wet wt. of liver in SD rats and 26.2 ± 7.7 in DA rats ($P < 0.001$). DEA levels were 1.5 ± 1.2 (SD rats) vs. $51.9 \pm 11.9 \text{ nmol g}^{-1}$ (DA rats, $P < 0.001$). Liver amiodarone/DEA ratio in DA rats was the opposite of that

Figure 3 Dark Agouti rats after 5 months of amiodarone treatment: part of an intrahepatic bile duct with numerous dense lamellated bodies within the epithelial cells. Magnification 5200 $\times$. Horizontal bar represents 2 μm . Inset: one of the lamellated bodies marked with an asterisk at higher magnification (23,400 $\times$). Horizontal bar represents 0.5 μm .

Figure 4 Dark Agouti rats after 5 months of amiodarone treatment: lamellated body from a bile duct cell showing a fine meshwork and an amorphous matrix. Magnification 65,000 $\times$. Bar represents 0.2 μ m.

found in SD rats, averaging 0.5 ± 0.06 , compared to 2.5 ± 1.0 in SD rats ($n = 5$).

Phospholipids in liver

Total phospholipid content of liver homogenate as measured by fluorimetric assay with DPH and by malachite green did not reveal any significant differences between controls and amiodarone-treated animals in either strain.

Microsomal enzymes

Total, uncomplexed and complexed cytochrome P-450 as well as hepatic NADPH cytochrome c reductase were measured at the end of the treatment period in all animals (Table 1). In SD rats, there were no changes in either enzyme, and there was no complex formation. In amiodarone-treated DA rats, a substantial amount of complexed cytochrome P-450 was found ($P < 0.001$), as directly measured by its absorption around 455 nm and confirmed by the difference between total cytochrome P-450 (after addition of potassium ferricyanide) and uncomplexed cytochrome P-450 (Table 1). At the same time, there was evidence for induction of both NADPH-cytochrome

Figure 5 Amiodarone and desethyl-amiodarone (DEA) concentrations in (a) plasma (nmol ml^{-1}) and in (b) liver homogenate (nmol g^{-1} wet wt. of liver). Amiodarone and DEA concentrations are higher both in plasma and liver homogenate of Dark Agouti (DA) rats than of Sprague-Dawley (SD) rats. In DA rats, the hepatic amiodarone/DEA ratio is < 1 as opposed to the findings in SD rats. Open columns: SD rats; hatched columns: DA rats.

c reductase ($P < 0.001$) and total cytochrome P-450 ($P < 0.001$) in the treated DA rats (Table 1).

Discussion

We have found major differences in the hepatic effects of chronic amiodarone treatment in the two different rat strains studied. Female Dark Agouti rats, with genetic debrisoquine hydroxylation deficiency, have revealed higher concentrations of amiodarone and DEA in plasma and liver, a reversal of the amiodarone/DEA ratio in the liver, ultrastructural changes suggesting hepatic phospholipidosis and evidence of liver

Table 1 NADPH-cytochrome c reductase and cytochrome P-450 activities after a 5 month experimental period

	<i>NADPH-cytochrome c</i> <i>reductase activity</i> (nmol min ⁻¹ mg ⁻¹ micr. protein)	<i>Cytochrome P-450</i> <i>Uncomplexed</i> <i>Complexed</i> <i>Total</i> (nmol mg ⁻¹ micr. protein)		
<i>SD rats</i>				
Control	39 ± 3	0.38 ± 0.08	0	0.38 ± 0.07
Amiodarone	41 ± 4	0.40 ± 0.07	0	0.40 ± 0.06
<i>DA rats</i>				
Control	49 ± 8	0.30 ± 0.04	0	0.30 ± 0.03
Amiodarone	82 ± 8*	0.65 ± 0.01	0.05 ± 0.04	0.72 ± 0.12*

Uncomplexed cytochrome P-450 was measured as the CO-difference spectrum of dithionite-reduced microsomes (micr.). Total cytochrome P-450 was similarly measured, but after first adding $50 \mu\text{M}$ potassium ferricyanide to the microsomes (to disrupt the cytochrome P-450 Fe^{2+} -metabolite complex). Complexed cytochrome P-450 was measured by its absorption around 455 nm, after adding $50 \mu\text{M}$ potassium ferricyanide to the reference cuvette only.

* Significantly different from that in control rats, $P < 0.001$.

enzyme induction. The fact that morphological lesions were recognizable in DA but not in SD rats supports our previous finding in the guinea-pig that hepatic phospholipidosis as assessed ultrastructurally is related to the extent of hepatic drug accumulation (Pirovino *et al.*, 1988).

However, in contrast to our previous findings in guinea-pigs phospholipidosis was not quantifiable biochemically in terms of increased hepatic phospholipid content. This is in agreement with the relatively low amiodarone and DEA contents of the liver homogenates compared to that of the guinea-pig where concentrations up to 100 times higher than in SD rats were associated with ultrastructural markers of phospholipidosis. Since the extent of phospholipidosis is thought to depend on the degree of drug-phospholipid complex formation and thus on the availability of trapped drug in the hepatocytic lysosomes, it is understandable that low drug storage is associated with only a slight phospholipidosis.

Our results do not provide firm evidence for a direct and causal relationship between the hydroxylator status and the differences seen in the two rat strains. Phenotyping has indeed shown that DA rats are much slower hydroxylators than SD rats and that the defect in hydroxylation increases with age. Alterations in amiodarone and DEA metabolism as evidenced by the increase in amiodarone and DEA plasma and hepatic tissue concentrations could be linked to the defect in debrisoquine hydroxylation, as has been shown for debrisoquine and many other co-segregating substrates including the demethylation of dextrometorphan (Küpfer & Preisig, 1983; Küpfer *et al.*, 1986). In addition, altered metabolic pathways, delayed drug elimination, differences in tissue binding and other pharmacokinetic characteristics such as hepatic uptake of drugs (Reichen *et al.*, 1986) might be at the origin of our observations in the DA rat. However, the fact that drug accumulation and metabolism are so different in the two rat strains studied,

points to a possible influence of pharmacogenetic factors on the susceptibility to amiodarone side effects in man. This has been demonstrated for drugs such as perhexiline (Shah *et al.*, 1982; Morgan *et al.*, 1984; Meier *et al.*, 1986).

Chronic amiodarone treatment led to hepatic enzyme induction in DA rats. This was shown by the significantly increased liver to body weight ratios, by an increase in smooth endoplasmic reticulum demonstrated morphologically, by the induction of hydroxylation as shown by a lower metabolic ratio of debrisoquine to 4-OH-debrisoquine, and by net increases in total cytochrome P-450 and in NADPH cytochrome c reductase activities. In addition, there was formation of a cytochrome P-450-metabolite complex in DA rats, whereas no formation of this complex and no enzyme induction was detected in SD rats. Formation of the cytochrome P-450Fe(II)-amiodarone metabolite complex apparently involves glucocorticoid-inducible cytochrome P-450 isoenzymes (Larrey *et al.*, 1986). Conceivably, decreased metabolism of amiodarone and DEA by the isoenzyme metabolizing debrisoquine might explain greater formation of the complex by glucocorticoid-inducible isoenzymes in DA rats.

In conclusion, the hepatic effects of chronic amiodarone treatment were markedly different in the 2 rat strains studied. Poor hydroxylation, or possibly associated anomalies of drug metabolism, favoured hepatic accumulation of amiodarone and even more of its metabolite DEA; this was accompanied by hepatic enzyme induction and drug-cytochrome P-450-metabolite complex formation. Studies in man with deficient debrisoquine hydroxylation are needed to see whether this type of genetically determined metabolic defect is associated with an increased susceptibility to amiodarone side effects.

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References

- AL-DABBAGH, S.G., IDLE, J.R. & SMITH, R.L. (1981). Animal modelling of human polymorphic drug oxidation: the metabolism of debrisoquine and phenacetin in rat inbred strains. *J. Pharm. Pharmacol.*, **33**, 161–164.
- ALVÁN, G., GRIND, M., GRAFFNER, C. & SJÖQVIST, F. (1984). Relationship of N-demethylation of amiflamine and its metabolite to debrisoquine hydroxylation polymorphism. *Clin. Pharmacol. Ther.*, **36**, 515–519.
- BABANY, G., MALLAT, A., ZAFRANI, E.S., GIRARDIN, M.F., CARCONE, B. & DHUMEAUX, D. (1986). Chronic liver disease after low daily doses of amiodarone. *J. Hepatol.*, **3**, 228–232.
- ERDTMANSKY, P. & GOEHL, T.J. (1975). Gas-liquid chromatographic electron capture determination of some monosubstituted guanido-containing drugs. *Analyt. Chem.*, **47**, 750–752.
- HEATH, M.F., COSTA-JUSSÀ, F.R., JACOBS, J.M. & JACOBSON, W. (1985). The induction of pulmonary phospholipidosis and the inhibition of lysosomal phospholipases by amiodarone. *Br. J. Exp. Pathol.*, **66**, 391–397.
- HOSTETLER, K.Y., REASOR, M.J., WALKER, E.R., YAZAKI, P.J. & FRAZEE, B.W. (1986). Role of phospholipase A inhibition in amiodarone pulmonary toxicity in rats. *Biochem. Biophys. Acta*, **875**, 400–405.
- HOSTETLER, K.Y. & MATSUZAWA, Y. (1981). Studies on the mechanism of drug-induced lipidosis. Cationic amphiphilic drug inhibition of lysosomal phospholipases A and C. *Biochem. Pharmacol.*, **30**, 1121–1126.
- JOUANÉL, P., MOTTA, C., DELATTRE, J. & DASTUGUE, B. (1980). A rapid and sensitive fluorometric assay of serum phospholipids. *Clin. Chim. Acta*, **105**, 173–181.
- KÜPFER, A. & PREISIG, R. (1983). Inherited defects of hepatic drug metabolism. *Semin. Liver Dis.*, **3**, 341–354.
- KÜPFER, A., SCHMID, B. & PFAFF, G. (1986). Pharmacogenetics of dextrometorphan O-demethylation in man. *Xenobiotica*, **16**, 421–433.
- LARREY, D., TINEL, M., LETTERON, P., GENEVE, J., DESCATOIRE, V. & PESSAYRE, D. (1986). Formation of an inactive cytochrome P-450 Fe(II)-metabolite complex after administration of amiodarone in rats, mice and hamsters. *Biochem. Pharmacol.*, **35**, 2213–2220.
- LIM, P.D., TEWBY, P.N., STOREY, G.C.A. & HOLT, D.W. (1984). Neuropathy and fatal hepatitis in a patient receiving amiodarone. *Br. Med. J.*, **288**, 1638–1639.
- LUELLMANN, H., LUELLMANN-RAUCH, R. & WASSERMANN, D. (1978). Lipidosis induced by amphiphilic cationic drugs. *Biochem. Pharmacol.*, **27**, 1103–1108.
- MASON, J.W. (1987). Amiodarone. *N. Engl. J. Med.*, **316**, 455–466.
- MEIER, C., WAHLÄNDER, A., HESS, C. & PREISIG, R. (1986). Perhexiline-induced lipidosis in the Dark Agouti rat: an animal model of genetically determined neurotoxicity. *Brain*, **109**, 649–660.
- MORGAN, M.Y., RESHEF, R., SHAH, R.R., OATES, N.S., SMITH, R.L. & SHERLOCK, S. (1984). Impaired oxidation of debrisoquine in patients with perhexiline liver injury. *Gut*, **25**, 1057–1064.
- PESSAYRE, D., BICHARA, M., FELDMANN, G., DEGOTT, C., PÔTET, F. & BENHAMOU, J.P. (1979). Perhexiline maleate-induced cirrhosis. *Gastroenterology*, **76**, 170–177.
- PIROVINO, M., MÜLLER, O., ZYSSET, T. & HONEGGER, U. (1988). Amiodarone-induced hepatic phospholipidosis: Correlation of morphological and biochemical findings in an animal model. *Hepatology*, **8**, 591–598.
- POUCELL, S., IRETON, J., VALENCIA-MAYORAL, P., DOWNAR, E., LARRATT, L., PATTERSON, J., BLENDIS, L. & PHILLIPS, M.J. (1984). Amiodarone-associated phospholipidosis and fibrosis of the liver. *Gastroenterology*, **86**, 926–936.
- REICHEN, J., KRÄHENBÜHL, S., KÜPFER, A., SÄGESSER, H. & KARLAGANIS, G. (1986). Defective bile acid transport in an animal model of defective debrisoquine hydroxylation. *Biochem. Pharmacol.*, **35**, 753–759.
- RIGAS, B., ROSENFELD, L.E., BARWICK, K.W., ENRIQUEZ, R., HELZBERG, J., BATSFORD, W.P., JOSEPHSON, E. & RIELY, C.A. (1986). Amiodarone hepatotoxicity. A clinicopathologic study of five patients. *Ann. Intern. Med.*, **104**, 348–351.

- SHAH, R.R., OATES, N.S., IDLE, J.R., SMITH, R.L. & LOCKHART, J.D.F. (1982). Impaired oxidation of debrisoquine in patients with perhexiline neuropathy. *Br. Med. J.*, **284**, 295–299.
- SIMON, J.B., MANLEY, P.N., BRIEN, J.F. & ARMSTRONG, P.W. (1984). Amiodarone hepatotoxicity simulating alcoholic liver disease. *N. Engl. J. Med.*, **311**, 167–172.
- STÄUBLI, M., BIRCHER, J., GALEAZZI, R.L., REMUND, H. & STUDER, H. (1983). Serum concentrations of amiodarone during long-term therapy. Relation to dose, efficacy and toxicity. *Eur. J. Clin. Pharmacol.*, **24**, 485–494.
- VAN VELDHoven, P.P. & MANNAERTS, G.P. (1987). Inorganic and organic phosphate measurements in the nanomolar range. *Annal. Biochem.*, **161**, 45–48.

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