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Interactions of amiodarone with digoxin in rats

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1 The influence of oral amiodarone treatment on the blood and tissue concentrations of digoxin was investigated in the anaesthetized rat by use of unlabelled and [^{3}H]-digoxin.

2 Amiodarone diminished the total body clearance and the apparent volume of digoxin distribution by 60%. This reduction was due to a 50% reduction of the hepatobiliary clearance, whereas the renal clearance did not change.

3 Amiodarone treatment increased blood, myocardial and skeletal muscle [^{3}H]-digoxin concentrations by 200% indicating passive equilibration between blood and these tissues, and resulting in unaltered tissue to blood ratios. In contrast, the liver concentration increased by 70% only and the liver to blood ratio therefore decreased under amiodarone treatment.

4 It is concluded that the hepatobiliary elimination of digoxin is decreased in amiodarone-treated rats compared to controls and is responsible for the increased levels of blood and tissue glycoside.

Introduction

Administration of the potent antiarrhythmic drug amiodarone to digoxin-treated patients increases the plasma digoxin concentration (Moysey *et al.*, 1981; Furlanello *et al.*, 1982; Koren *et al.*, 1984; Douste-Blazy *et al.*, 1984; Oetgen *et al.*, 1984). The mechanisms of this change have not been settled and different possibilities have been proposed. Douste-Blazy *et al.* (1984) have suggested that the rise in digoxin plasma concentration may be due to displacement of digoxin from tissues and the suggestion made by others (Nademanee *et al.*, 1984), that digoxin might be displaced from heart tissue by amiodarone is particularly interesting. Increased digoxin plasma concentrations were also attributed to reduction of the digoxin clearance (Koren *et al.*, 1984; Nademanee *et al.*, 1984). It has been claimed that in adults, renal elimination of digoxin increases in parallel to plasma digoxin concentrations (Douste-Blazy *et al.*, 1984) when amiodarone is added to digoxin treatment. However, in children a reduction of renal digoxin clearance was reported (Koren *et al.*, 1984). Recently it has been demonstrated that plasma digoxin concentration also increases in rats (Koren *et al.*, 1983) if digoxin and amiodarone are combined. In the present study the question of the mechanism of the amiodarone/digoxin interaction was therefore investigated in rats by use of [^{3}H]-digoxin with and without unlabelled digoxin.

Methods

Female Wistar rats were treated with amiodarone, added to the food (Cat. No. 835, Nafag, Gossau, Switzerland) in a concentration of 0.86 g amiodarone base per kg. Amiodarone was obtained from Sanofi Pharma AG, Basel, Switzerland, in tablet form (Cordarone) and mixed with the food. Control rats were fed the same food without amiodarone, but containing 3 mg iodine kg^{-1}. As indicated in Figure 2 the number of animals differed in the experimental groups. The duration of amiodarone treatment ranged from 10 to 23 days (mean 12.5 days).

On the last day of each feeding period 10 μCi [^{3}H]-digoxin was injected in the tail vein of all animals except in group 1. Blood was repeatedly aspirated by heart puncture (group 1) or catheterization of the inferior vena cava (groups 2, 3). After 6 h blood was aspirated through the inferior vena cava in all animals. The animals were killed by exsanguination and tissue samples of liver, heart and skeletal muscle were taken for determination of radioactivity. Blood sampling was performed under ether anaesthesia (groups 1, 5, 6) or under sodium-pentobarbitone (groups 2, 3, 4), using an initial intraperitoneal dose of 50 mg kg^{-1} body weight and intravenous maintenance doses of 5 mg kg^{-1}. Rectal temperature was maintained at 37°C by a water perfused device. Oxygen was given through

an intratracheal cannula inserted by tracheostomy. On the day of the [^{3}H]-digoxin experiments the mean weights of the amiodarone and control animals of each group were the same, ranging from 206 to 265 g (amiodarone groups) and 198 to 256 g (controls).

[^{3}H]-digoxin (100 μCi = 5.7 μg digoxin), Code TRK, 433, Batch 34, was purchased from Amersham International plc, Buckinghamshire, England. Nominal radiochemical purity was 97.6%.

The additional experimental conditions were as follows:

Group 1: Blood levels of [^{3}H]-digoxin after single application

On day 10, unlabelled digoxin 0.225 mg kg^{-1} together with [^{3}H]-digoxin 100 μCi kg^{-1} was injected in the tail vein. Blood was sampled 2 min after and 1, 3, 6, 9 and 12 h after injection.

Group 2: Tissue distribution of radioactivity in animals with bile drainage

Sodium chloride (9 g l^{-1}) was infused at a rate of 5.8 ml per h through the inferior vena cava. The common bile duct and the urinary bladder were catheterized and the laparotomy was closed by a suture. As soon as a constant flow of bile and urine was observed [^{3}H]-digoxin was injected in the tail vein. At hourly intervals samples of 0.3 ml blood were taken and hourly quantities of bile and urine were collected for 6 h.

Group 3: Tissue distribution of radioactivity without biliary cannula

The experiment was performed as in group 2 but without biliary drainage, in order to investigate whether bile drainage abolishes the differences in blood radioactivity between amiodarone and control animals.

Group 4

This experiment was performed as a control experiment for groups 2 and 3 without fluid load, biliary drainage and blood sampling.

Group 5: Influence of pretreatment with unlabelled digoxin on radioactivity

Unlabelled digoxin (0.1 mg kg^{-1} body weight daily) was injected s.c. from day 8 to 23. On days 13 and 17, 24 h after the last digoxin injection, 1.0 ml heart blood was aspirated for determination of digoxin plasma concentration by radio immunoassay (Clinical Assay, Travenol AG, Zürich). On day 23 [^{3}H]-digoxin was injected and the animals were killed 6 h later.

Group 6: Blood radioactivity after repeated administration of a mixture of unlabelled and labelled digoxin

Unlabelled digoxin (0.1 mg kg^{-1} per day) together with [^{3}H]-digoxin (50 μCi kg^{-1} daily) was injected s.c. from day 10 to 14. On day 15, [^{3}H]-digoxin was injected into the tail vein and the animals killed 6 h later.

Processing of blood, tissue, bile and urine samples

Blood (100 μl) was digested for 1 h at 60°C in 300 μl ethanol-Protosol (1:1, V/V) (Protosol: New England Nuclear, NEF-935), bleached with 200 μl H_2O_2 (30%) and 15.5 ml scintillation liquid was added (mixture of 15 ml Biofluor, New England Nuclear, NEF-961 and 0.5 ml 0.5 N HCl). Tissue samples were cleared of superficial blood traces by suction with a filter paper, weighed and digested over night at 60°C in Protosol (13.8 ml g^{-1} liver, 12.9 ml g^{-1} heart and 9.8 ml g^{-1} skeletal muscle). Scintillation liquid (10 ml) (Econofluor, New England Nuclear, NEF-941) was then added.

Bile (100 μl) was bleached with 1 drop of H_2O_2 (30%) and incubated for 1 h at 60°C. Scintillation liquid (10 ml) (Aquassure, New England Nuclear, NEF-965) was then added.

To 100 μl urine, 10 ml scintillation liquid (Aquassure) was added.

The radioactivity of blood, tissue, bile and urine was measured in a β-counter (Intertechnique, SL 4000, Liquid Scintillation Counter).

Calculations

Kinetic analysis was performed using non-compartmental techniques (Gibaldi & Pessier, 1982).

AUC = Area under the blood radioactivity/time curve, calculated by the trapezoidal method until the last sampling point and extrapolated to infinity. Extrapolation was done by dividing the radioactivity at the last sampling point by the slope of the log-linear portion of the radioactivity/time plot. This slope (β) was calculated by log-linear regression.

$$t_{\frac{1}{2}} = \text{terminal elimination half-life} = \frac{\log 2}{\beta}$$

$$Cl_B = \text{total body clearance} = \frac{\text{dose injected}}{AUC}$$

$$Vd = \text{apparent volume distribution} = \frac{Cl_B}{\beta}$$

Renal clearance

$$= \frac{\text{cumulative amount of radioactivity in urine (hours 3–6)}}{\text{AUC (hours 3–6)}}$$

Biliary clearance

$$= \frac{\text{cumulative amount of radioactivity in bile (hours 3–6)}}{\text{AUC (hours 3–6)}}$$

Statistics

Results are given as means ± s.d. Student's *t* test (two tailed) for paired and unrelated data was used and $P < 0.05$ was taken as the level of statistical significance.

Results

In Figure 1 the mean log/linear blood radioactivity/time curves of groups 1–3 are plotted. Amiodarone-treated animals showed higher blood radioactivity than controls. Radioactivity dropped rapidly within the first 2–3 h after injection; thereafter an equilibrium was reached. The observation time was therefore limited to 6 h in the other experiments.

Figure 2 shows that 6 h after [^{3}H]-digoxin injection radioactivity was significantly higher in amiodarone-treated animals than in controls. This finding was independent of whether digoxin was given as a tracer dose or together with large amounts of the unlabelled compound.

As shown in Figure 3, amiodarone treatment did not change the ratio of the tissue to blood radioactivity for heart and skeletal muscle but diminished it by values from 33.7% to 56.4% for the liver.

Compared to controls the total body clearance (Cl_B) of radioactivity in amiodarone-treated animals was only 38% of that in controls (group 1, Table 1) and the apparent volume of distribution (Vd) was 41%. As expected from this parallel reduction of Cl_B and Vd $t_{\frac{1}{2}}$ of total radioactivity did not change (group 1, Table 1). In amiodarone-treated animals, the biliary clearance was only 50% of that of controls (Table 1, group 2), $P < 0.01$. There was no statistical difference in renal clearance (group 2), $P > 0.20$. The cumulative excretion of radioactivity in 6 h in amiodarone-treated animals was 73% of that in controls ($P < 0.001$). This was due to a lower biliary excretion (Table 1).

Comparison of blood and tissue radioactivity in groups 2, 3 and 4, reveals that neither bile drainage nor infusion of large amounts of liquid abolish the pronounced differences of blood and tissue radioactivity between animals treated with amiodarone and controls (Figure 1).

Digoxin plasma concentrations were 2.04 ± 1.08 nmol l^{-1} after 5 digoxin injections and 1.88 ± 0.54 nmol l^{-1} after 9 injections in amiodarone-treated animals (group 5). Since there was no difference between these values ($P > 0.45$), it can be assumed that a steady state was approached after 5 injections. In control animals, plasma digoxin levels were $<$ 0.6 nmol l^{-1} on both days, except in 1 animal which had a digoxin level of 0.9 nmol l^{-1} after 5 injections. Thus amiodarone induced a large increase in radioim-

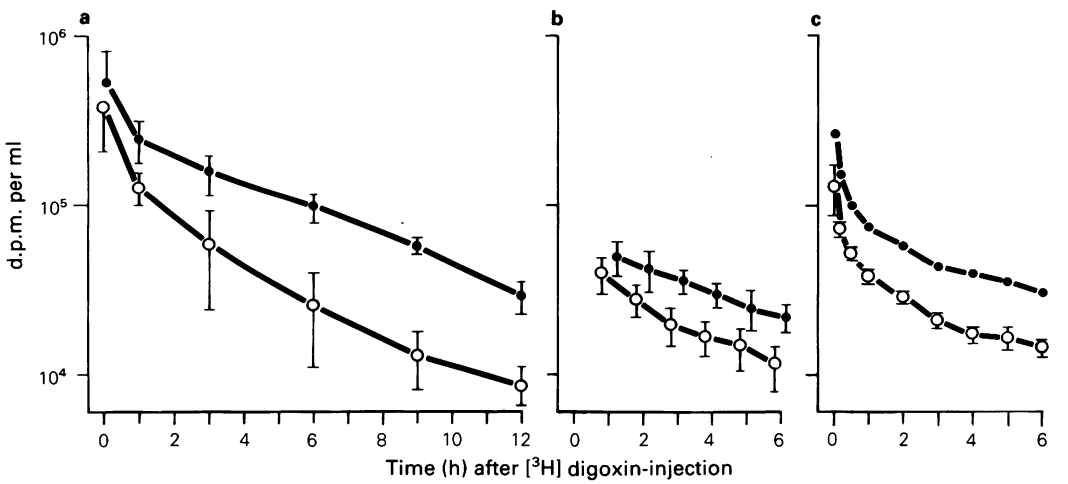

Figure 1 Log blood radioactivity/time curves of amiodarone-treated (●) and control (○) rats. (a) Group 1; (b) group 2; (c) group 3.

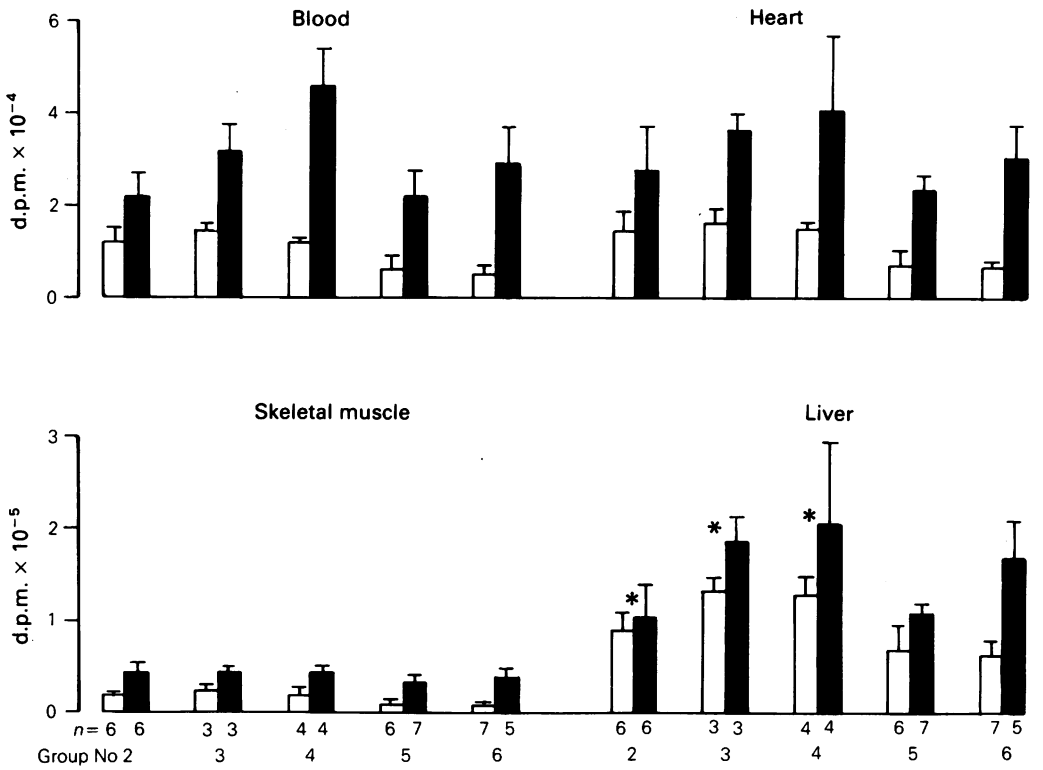

Figure 2 Blood radioactivity per ml and tissue radioactivity per g in rats of groups 2–6, six hours after the last [^{3}H]-digoxin injection. All the differences between amiodarone-treated (■) and control animals (□) were significant ($P < 0.05$), except values marked by asterisks.

munologically determined unlabelled digoxin concentrations, confirming the results obtained by measurement of radioactivity.

Discussion

The present study was performed to investigate the possible mechanisms leading to the increased digoxin concentration which is observed during amiodarone therapy (Moysey *et al.*, 1981; Furlanello *et al.*, 1982; Douste-Blazy *et al.*, 1984; Oetgen *et al.*, 1984). [^{3}H]-digoxin was used since the measurement of digitalis glycoside concentrations after extraction procedures is hampered by the fact that the extractable fractions are quantitatively different for different metabolites and for tissues, blood and body excretions (Aderjan *et al.*, 1979; Shepard *et al.*, 1986). The use of [^{3}H]-digoxin therefore provided more adequate information on the quantitative distribution of [^{3}H]-digoxin and its tritiated metabolites between blood and tissue, but this method cannot distinguish between the parent compound and tritiated metabolites. Similar considerations apply to digoxin radioimmunoassays including the one used in the present study, in that the digoxin antibodies cross-react with such digoxin metabolites which are present in digoxin-treated man and rats (Stoll *et al.*, 1972; Von Bergmann *et al.*, 1972). Our study, therefore, does not provide information on the precise change of the fate of the digoxin molecule in amiodarone-treated animals compared to controls, but nevertheless, provides new information about principal differences in digoxin handling between these groups. In fact, this is the first study which identifies the hepatobiliary system as the site of altered digoxin metabolism and/or transport during amiodarone treatment: it shows that (1) the biliary excretion of radioactivity is decreased and that (2) the liver uptake of radioactivity is lower (as shown by the lower ratio of liver to blood radioactivity) in amiodarone-treated animals.

We consider that the decrease of the biliary excretion of radioactivity in the presence of the same or increased radioactivity in the liver compared to con-

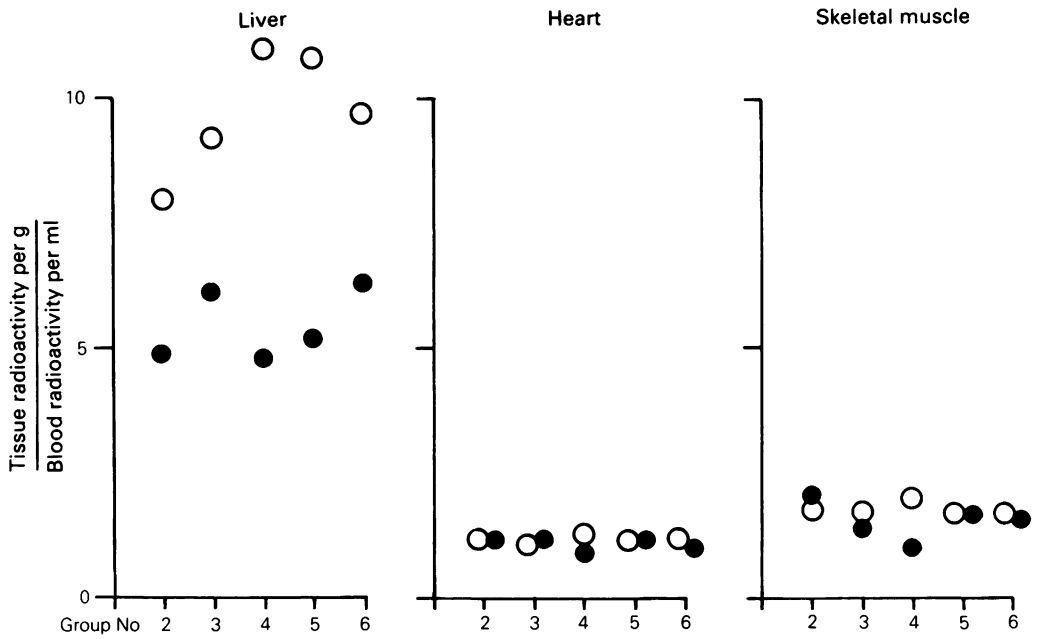

Figure 3 Radioactivity per g tissue/radioactivity per ml blood in the same animals as in Figure 2, 6 h after the last [^{3}H]-digoxin injection. (●) Amiodarone-treated rats; (○) controls.

Table 1 Pharmacokinetic data for groups 1 and 2 (see text for group details)

		Controls	*Amiodarone-treated*	
	n	4	4	*P*
	Body clearance (ml h^{-1}. kg)	371 ± 88	140 ± 26	<0.02
Group 1 (Repeated heart puncture)	Apparent volume of distribution (ml kg^{-1})	4281 ± 1451	1748 ± 409	<0.05
	Blood half-life of radioactivity (h)	3.4 ± 0.5	3.9 ± 0.8	>0.35
	n	6	6	*P*
	Biliary clearance (ml h^{-1}. kg)	165 ± 53	75 ± 16	<0.01
	Renal clearance (ml h^{-1}. kg)	80 ± 36	57 ± 26	>0.20
	Biliary + renal clearance (ml h^{-1}. kg)	245 ± 68	131 ± 33	<0.01
Group 2 (Bile drainage)	Cumulative excreted radioactivity in the first 6 h (% of injected dose)			
	: in bile	33.9 ± 4.5	20.8 ± 2.3	<0.001
	: in urine	8.9 ± 4.6	10.5 ± 2.3	>0.5
	: in bile + urine	42.8 ± 3.2	31.3 ± 2.9	<0.001

trols (Figure 2) must be due to a decrease of the hepatobiliary transport and/or hepatic digoxin metabolism. The concept of altered drug metabolism is supported by recently described results which indicate that liver cell function is depressed during amiodarone treatment (Grech-Bélanger, 1984; Pirovino *et al.*, 1986), cases of severe liver damage sometimes with fatal outcome in patients under-going amiodarone treatment have been described (Yagupsky, 1985; Rumessen, 1986). The only reason for the decrease of the apparent volume of [^{3}H]-digoxin distribution that we could identify was the altered hepatobiliary handling of [^{3}H]-digoxin.

Other mechanisms which could contribute to a decrease of the V_d of [^{3}H]-digoxin, i.e. a decrease of the renal clearance (as observed in 3 children by Koren *et al.*, 1984) or a decreased uptake of peripheral tissues, such as heart and skeletal muscle, could not be identified. In fact, the radioactivity in heart and skeletal muscle increased by the same factor of 2 to 3 as in blood (Figures 2 and 3), indicating passive equilibration between blood and tissues at all times and the absence of any interference of amiodarone with this process. This finding is in line with earlier observations by Koren *et al.* (1983) for rat diaphragm and heart. The finding of a 2 to 3 fold increase in radioactivity in the blood of amiodarone-treated animals is confirmed by RIA in rats given unlabelled digoxin and is further supported by results from another study (Koren *et al.*, 1983).

We conclude that the increase of serum digoxin levels in the rat induced by amiodarone results from a reduction of the total body clearance, the latter being due to a 50% reduction of the biliary clearance. Subsequently, blood levels of digoxin increase dramatically, and so do tissue concentrations, since digoxin passively equilibrates with peripheral tissues. Recently a decrease of 32% of the non-renal clearance of digoxin in amiodarone-treated normal subjects has been reported (Fenster *et al.*, 1985), and this is compatible with the assumption of a diminished hepatobiliary elimination in man. The data suggest that combined therapy with digoxin and amiodarone enhances digitalis toxicity by increasing the drug concentration in the heart *pari passu* with that in the blood. It is conceivable that the pharmacokinetic data of drugs with preferential hepatobiliary metabolism may change similarly when combined with amiodarone (Greche-Bélanger, 1984; Pirovino *et al.*, 1986).

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References

ADERJAN, R., DOSTER, S., PETRI, H. & SCHMIDT, G. (1979). Herzglykoside und Metaboliten – Probleme der Wiederfindung in Gewebsextrakten. Abtrennung sichtbarer Substanzflecke im Nanogrammbereich mittels HPTLC. *Z. Rechtsmed.*, **83**, 201–207.

DOUSTE-BLAZY, P.H., MONTASTRUC, J.L., BONNET, B., AURIOL, P., CONTE, D. & BERNADET, P. (1984). Influence of amiodarone on plasma and urine digoxin concentrations. *Lancet*, **i**, 905.

FENSTER, P.E., WHITE, N.W. & HANSON, C.D. (1985). Pharmacokinetic evaluation of the digoxin-amiodarone interaction. *J. Am Coll. Cardiol.*, **5**, 108–111.

FURLANELLO, F., INAMA, G., FERRARI, M., PADRINI, R., PIOVAN, M., GUARNERIO, M., VEGARA, G., DEL FAVERO, A., DAL FORNO, P. & DISERTORI, M. (1982). Amiodarone and amiodarone plus digitalis in the treatment of paroxysmal supraventricular reciprocating tachyarrhythmias. *Pharmac. Res. Comm.*, **14**, 731–737.

GIBALDI, M. & PESSIER, D. (1982). *Pharmacokinetics*. p. 212, New York, Basel: M. Dekker.

GRECH-BÉLANGER, O. (1984). Depressive effect of amiodarone on hepatic drug metabolism in the rat. *Res. Commun. Chem. Path. Pharmac.*, **44**, 15–30.

KOREN, G., HESSLEIN, P.S. & MACLEOD, S.M. (1984). Digoxin toxicity associated with amiodarone therapy in children. *J. Pediatr.*, **104**, 467–470.

KOREN, G., SOLDIN, S. & MACLEOD, S.M. (1983). Digoxin-amiodarone interaction: in vivo and in vitro studies in rats. *Can. J. Physiol. Pharmac.*, **61**, 1483–1486.

MOYSEY, J.O., JAGGARAO, N.S.V., GRUNDY, E.N. & CHAMBERLAIN, D.A. (1981). Amiodarone increases plasma digoxin concentrations. *Br. med. J.*, **282**, 272.

NADEMANEE, K., KANNAN, R., HENDRICKSON, J.A., OOKHTENS, M., KAY, I. & SINGH, B. (1984). Amiodarone-digoxin interaction: clinical significance, time course of development, potential pharmacokinetic mechanisms and therapeutic implications. *J. Am. Coll. Cardiol.*, **4**, 111–116.

OETGEN, W.J., SOBOL, S.M., TRI, T.B., HEYDORN, W.H., DARIA, J.E. & RAKITA, L. (1984). Amiodarone-digoxin interaction: clinical and experimental observations. *Chest*, **86**, 75–79.

PIROVINO, M., MUELLER, O., HONEGGER, U.E., REICHEN, J., ZYSSET, T., STAEUBLI, M. & STUDER, H. (1986). Amiodarone: A model drug to study the interrelation between altered hepatocellular structure and function. *J. Hepatol.*, **3**, Suppl. 1, 63, Abstract.

RUMESSEN, J.J. (1986). Hepatotoxicity of amiodarone. *Acta Med. Scand.*, **219**, 235–239.

SHEPARD, T.A., HUI, J. & CHANDRASEKARAN, A. (1986). Digoxin and metabolites in urine and feces: a fluorescence derivatisation-high performance liquid chromatographic technique. *J. Chromatogr.*, **380**, 89–98.

STOLL, R.G., CHRISTENSEN, M.S., SAKMAR, E. & WAGNER,

J.G. (1972). The specificity of the digoxin radioimmunoassay procedure. *Res. Commun. Chem. Path. Pharmac.*, **4**, 503–510.

VON BERGMANN, K., ABSHAGEN, U. & RIETBROCK, N. (1972). Quantitative analysis of digoxin, 4'''-acetyldigoxin and 4'''-methyldigoxin and their metabolites in bile and urine of rats. *Naunyn-Schmiedebergs Arch. Pharmac.*, **273**, 154–167.

YAGUPSKY, P., GAZALA, E., SOFER, S., MAOR, E. & ABARBANEL, J. (1985). Fatal hepatic failure and encephalopathy associated with amiodarone therapy. *J. Pediatrics*, **107**, 967–970.

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