Tissue extraction of amiodarone and *N*-desethylamiodarone in man after a single oral dose

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1 The hepatic extraction of amiodarone and *N*-desethylamiodarone has been investigated in seven patients following catheterization of the portal and hepatic veins under general anaesthesia. Amiodarone (600 mg) was administered orally 4 h before regional blood sampling.

2 Concentrations of amiodarone and *N*-desethylamiodarone, determined by h.p.l.c., were about twice as high in the portal vein compared with those in the hepatic vein, the calculated hepatic extraction ratios of both compounds being 0.39 ± 0.07 and 0.34 ± 0.03 , respectively.

3 The presence of *N*-desethylamiodarone in the portal vein in higher concentrations than in the hepatic vein strongly suggests that *N*-dealkylation of amiodarone occurs in the gut wall or lumen, a finding which might account for the low and highly variable intersubject amiodarone bioavailability.

Keywords amiodarone *N*-desethylamiodarone hepatic extraction

Introduction

Although amiodarone is a highly lipid-soluble drug, its absolute oral bioavailability is incomplete and shows a wide intersubject variability (Holt *et al.*, 1983; Tucker *et al.*, 1984). Estimation of the hepatic extraction of amiodarone from intravenous data suggests that this limited bioavailability of the drug cannot be accounted for solely by hepatic first-pass metabolism but might rather be attributed either to an incomplete absorption across the gastrointestinal tract or to a first-pass metabolism in the intestinal mucosa or gut wall. The present study, involving measurements of amiodarone and its metabolite (*N*-desethylamiodarone) in the portal and hepatic veins, as well as in a peripheral artery and a peripheral vein, was undertaken to investigate these problems further by allowing direct calculation of organ extraction ratios of these compounds in man.

Methods

Patients

Seven patients (two male and five female, age 28–43 years, weight 56–72 kg) free of hepatocellular dysfunction and porto-systemic shunts took part in the study. Five women and one man had a single insulinoma and one man had the Zollinger-Ellison syndrome (ZES) previously diagnosed from clinical and biological follow-up. All of these patients underwent successively an arteriography and a pancreatic phlebography in an attempt to localize the endocrine tumour by using selective pancreatic venous sampling for hormonal radioimmunoassay determinations as described previously (Roche *et al.*, 1982). Informed consent was obtained from all patients for this investigation, which was approved by the hospital ethics committee.

The patients were anaesthetized with althesin, a non hepatic microsomal enzyme inducing general anaesthetic drug (Sear & McGivan, 1980) ($0.07 \text{ ml kg}^{-1} \text{ h}^{-1}$ after a loading dose of 0.07 ml/kg) and were ventilated artificially with a mixture of nitrous oxide (60%) and oxygen (40%). Phenoperidine and pancuronium bromide were given at regular intervals (total dose: $4.5 \pm 0.03 \text{ mg}$ and $5.0 \pm 0.5 \text{ mg}$, respectively). After arteriography, the catheter remained located either in the left hypogastric artery (four patients) or in the femoral artery (three patients) for further peripheral arterial blood sampling (PA). Pancreatic phlebography was performed by percutaneous transhepatic catheterization (5-Fr Cook catheter, one side hole) as described previously (Reichardt & Ingemansson, 1980; Roche *et al.*, 1982). Blood samples were always drawn from the end of the superior mesenteric vein (SMV) just below the origin of the portal trunk. An additional catheter was located in the main right hepatic vein (HV) through the femoral vein and a Vygon 16F catheter was positioned in a dorsal vein of the foot for peripheral venous sampling (PV). By this procedure, four blood samples could be collected simultaneously in less than 30 s from SMV, HV, PA and PV vessels. The entire catheterization study lasted an average of 160 min. In patients with insulinomas, carbohydrate intravenous supplementation was stopped 2 h before the study and replaced by Ringer solution. Capillary blood sugar level was checked regularly. The electrocardiogram and the arterial pressure (sphygmomanometry) were monitored at regular intervals.

All patients with insulinomas were drug-free and cimetidine was withdrawn 1 month before the study in the patient with ZES.

Protocol design

Four hours before the pancreatic phlebography and 2 h before general anaesthesia, each patient was given an oral dose of 600 mg of amiodarone. Then, three series of blood sampling (collected within less than 30 s from SMV, HV, PA and PV) were performed in each patient at 30 min intervals. Blood samples (5 ml on dry heparin) were centrifuged immediately and the plasmas were separated for storage at -20°C until analysis which was always performed within 72 h.

Amiodarone assay

Amiodarone, *N*-desethylamiodarone and an internal standard (chlorpromazine, Rhône-

Poulenc) were measured by an h.p.l.c. technique using a modification of previously described methods (Flanagan *et al.*, 1980, 1981; Storey *et al.*, 1982). For the analysis, 20 μl internal standard (1 μg) in methanol, 500 μl plasma, 500 μl acetate buffer (pH 5.0) and 2 ml diethylether were mixed on a horizontal shaker for 30 s. After centrifugation (5 min, 2500 rev/min), the ether phase was transferred to another vial and evaporated under a gentle stream of nitrogen at 60°C . The residue was dissolved in 100 μl of mobile phase (100 ml of methanol: *tert*.-butyl-methylether:trimethylpentane (80:10:10) added to 100 μl of a 25% aqueous solution of potassium bromide) and 20 μl were injected into the chromatograph (Lirec h.p.l.c. apparatus) equipped with a Lirec A-801 pump and a Microporasil (Waters n° 27477) $30 \times 0.4 \text{ cm}$ steel column coupled to a Waters 480 UV detector (240 nm). Flow rate was 1.0 ml/min, the pressure was approximately 1000 psi and the column temperature was ambient.

Calibration was done by diluting known amounts of a methanol solution of amiodarone and *N*-desethylamiodarone with drug-free plasma to which was also added the internal standard at a concentration of 50 $\mu\text{g/ml}$. The ratios of retention times of amiodarone and *N*-desethylamiodarone to that of the internal standard were 0.62 and 0.47, respectively. Standard curves for amiodarone and *N*-desethylamiodarone were linear over the concentration range of 0.1 to 10 $\mu\text{g/ml}$ and the sensitivity limit of the assay was 0.05 $\mu\text{g/ml}$ for both compounds. Extraction efficiency was 80–85% for amiodarone and *N*-desethylamiodarone and 75–82% for the internal standard. Within and between-assay variability was < 10% for both amiodarone and *N*-desethylamiodarone.

Calculations

Considering the dual blood supply of the liver, assuming that the SMV and hepatic artery contribute respectively for $\frac{2}{3}$ and $\frac{1}{3}$ of this blood supply (Ohnhaus, 1979) and postulating that hepatic artery and peripheral artery (PA) amiodarone and *N*-desethylamiodarone plasma concentrations are identical, the hepatic extraction ratio for both compounds was calculated as:

$$E_H = \frac{(\text{SMV} \times \frac{2}{3} + \text{PA} \times \frac{1}{3}) - \text{HV}}{(\text{SMV} \times \frac{2}{3} + \text{PA} \times \frac{1}{3})}$$

The peripheral extraction ratio was calculated as

$$E_p = \frac{\text{PA} - \text{PV}}{\text{PA}}$$

The data are presented as means $\pm$ s.e. mean. Student's *t*-test was used for statistical analysis of the data.

Results

Since amiodarone and *N*-desethylamiodarone concentrations in the three different plasma samples obtained from the same sampling site were almost identical in each patient during the period from 4 to 5 h after oral administration of amiodarone, all of these values were pooled.

Figure 1 shows the plasma concentrations of amiodarone and *N*-desethylamiodarone measured at the four sampling sites. Amiodarone and *N*-desethylamiodarone plasma concentrations are significantly higher (a) in SMV than in HV, the calculated hepatic extraction ratios being respectively 0.39 ± 0.07 and 0.34 ± 0.03 , and (b) in SMV than in PA. Figure 1 also shows that there was no difference between amiodarone and *N*-desethylamiodarone plasma concentrations in PA and PV, calculated peri-

pheral extraction ratios being respectively 0.04 ± 0.05 and 0.01 ± 0.06 . Finally, the *N*-desethylamiodarone/amiodarone plasma levels ratios were respectively 0.14 ± 0.07 in SMV, 0.14 ± 0.09 in HV, 0.14 ± 0.06 in PA and 0.13 ± 0.05 in PV, there being thus no significant difference between the values obtained from the different blood sampling sites.

Discussion

In this study, the calculated hepatic extraction ratio of amiodarone was 0.39 ± 0.07 . This value is clearly higher than that (0.13) previously estimated from systemic clearance experiments (Holt *et al.*, 1983). However, it must be kept in mind that our figure reflects an instantaneous measurement performed 4 h after a single oral administration of amiodarone (i.e. not at steady state) and not a time average value which determines bioavailability. With these limitations, and inasmuch as one can talk about 'first-pass effect' 4 h after drug intake, it is difficult to

Figure 1 Plasma concentrations of amiodarone (□) and *N*-desethylamiodarone (■) in the different sampling sites 4 h after oral administration of 600 mg of amiodarone.

determine whether the calculated hepatic extraction ratio of amiodarone (0.39 ± 0.07) reflects uptake or metabolism or both. Uptake is supported by the post-mortem data demonstrating extensive accumulation of amiodarone in liver samples taken after sudden death in patients on long term therapy with this drug (Storey *et al.*, 1982; Haffajee *et al.*, 1983; Holt *et al.*, 1983; Maggioni *et al.*, 1983). However, although a similar plasma ratio of metabolite to parent drug in SMV and HV was found in this study, this finding does not rule out the hypothesis of an extensive hepatic production of *N*-desethylamiodarone which could then undergo an early instantaneous and large hepatic uptake. However, that high concentrations of *N*-desethylamiodarone were found in liver samples taken post-mortem (Storey *et al.*, 1982; Haffajee *et al.*, 1983; Holt *et al.*, 1983; Maggioni *et al.*, 1983) cannot be considered as a proof of this hypothesis, since this tissue accumulation could also result from the first-pass hepatic extraction of the *N*-desethylamiodarone present in the SMV, which was also found to be high (extraction ratio: 0.34 ± 0.03).

The other important finding in this study was the presence of *N*-desethylamiodarone in the SMV in concentrations significantly higher than in (a) the hepatic vein and (b) the peripheral artery. These data strongly suggest that orally administered amiodarone is extensively metabolized through *N*-dealkylation during its first-pass in the gut lumen or across the intestinal mucosal cells, as is almost the case with other

drugs such as imipramine and flurazepam (Dencker *et al.*, 1976; Mahon *et al.*, 1977). Such a metabolic process could partially explain the apparent discrepancy between the low and highly variable intersubject bioavailability of amiodarone and its high lipid-soluble properties (Holt *et al.*, 1983). That higher concentrations of *N*-desethylamiodarone in SMV than in HV and PA were found in this study could also be accounted for by enterohepatic recirculation of the compound which is present in the bile (Maggioni *et al.*, 1983). However, this hypothesis is unlikely to contribute largely to the phenomenon since it would imply huge biliary concentrations of the compound. Further investigations, involving radiolabelled amiodarone administration or comparison of intravenous and oral administration of amiodarone, are needed to further clarify the problem.

Finally, neither amiodarone nor *N*-desethylamiodarone were significantly extracted between arterial and venous blood sampling sites. This finding contrasts with the large tissue accumulation of both compounds reported on chronic amiodarone therapy. Besides the fact that in our study amiodarone was administered only once, the difference might also be explained by blood shunting consequent upon peripheral vasodilation produced by anaesthesia and/or by radiography contrast media and/or by the vasoactive peptides possibly released by the tumours.

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