Br. J. Pharmac. (1976), **57**, 23–27

THE RELATIONSHIP BETWEEN CARDIOTOXICITY AND PLASMA DIGOXIN CONCENTRATION IN CONSCIOUS DOGS

D.J. CHAPPLE, R. HUGHES & B.F. JOHNSON

Departments of Pharmacology and Clinical Pharmacology, The Wellcome Research Laboratories, Beckenham, Kent

1 The tendency of a given oral dose of digoxin to induce cardiac dysrhythmia was determined indirectly at various times after its administration to eight conscious dogs by measurement of the intravenous dose of acetylstrophanthidin necessary to induce toxic changes in the ECG. Acetylstrophanthidin was used because its rapid elimination from the body permitted estimates to be made 45, 180 and 360 min after digoxin administration.

2 Each dog underwent four studies in which doses of 0.05, 0.1, 0.2 and 0.4 mg/kg digoxin were used in a randomized sequence allowing at least ten days between each dose.

3 Digoxin reduced the amount of acetylstrophanthidin required to cause toxic changes in the ECG; this increase in cardiac sensitivity was dose-dependent.

4 There was no correlation between plasma levels of digoxin and the tendency to dysrhythmia, since peak plasma concentrations of digoxin were reached at about 60 min after dosing whereas maximal sensitivity to acetylstrophanthidin was found 3 to 6 h after administration of digoxin.

5 These results suggest that there is little or no increased risk of cardiotoxicity during periods of transient increase in plasma levels of digoxin.

Introduction

Some oral digoxin preparations of high bioavailability produce relatively high peak plasma concentrations and it has been suggested that this could be associated with an increased incidence of toxicity in man (Harter, Skelly & Steers, 1974; Reissell, Manninen, Ojala & Karjalainen, 1974). In fact nausea can occur within 2 h of an oral dose of digoxin, i.e. at about the time of peak plasma concentration (Redfors, 1972; Falch, Teien, Bjerkelund, 1973). However, maximal positive inotropic and chronotropic effects occur several hours after oral or intravenous digoxin administration to man, presumably after tissue-plasma equilibrium has been established (Ganz, Fujimori, Penna, Greiner & Gold, 1957; Shapiro, Narahara & Taubert, 1970).

The aim of this study was to determine whether susceptibility to cardiac dysrhythmias or conduction disturbances was maximal at the time of peak plasma levels of digoxin. Toxic effects of digoxin on the heart were determined indirectly at various times after oral administration to conscious dogs by assessing the intravenous dose of acetylstrophanthidin needed to induce characteristic electrocardiographic changes.

Methods

Studies were performed on eight unanaesthetized mongrel dogs, of either sex, initially weighing 10.4–17.4 kg, and trained to lie quietly throughout the experiment. The cardiac tolerance to acetylstrophanthidin was determined in each animal by the infusion of a 100 μg/ml solution in 0.9% w/v NaCl solution (saline) and 6% ethanol via a 'Bardic 1-Catheter' (C.R. Bard International Ltd) inserted into either a cephalic or saphenous vein. The rate of infusion was maintained at 95 μg/min by means of a continuous slow injector (C.F. Palmer Ltd). The ECG (lead II) was monitored continuously and the amount of acetylstrophanthidin required to induce cardiotoxicity determined.

It was accepted that cardiotoxicity had developed when one or more of these ECG changes occurred: (a) multifocal ectopics; (b) ventricular ectopic beats occurring with an equal or greater frequency than normal complexes; (c) supraventricular or ventricular tachycardia, or (d) complete heart block. When cardiotoxicity was established the infusion was stopped and the time noted for the restoration of sinus

rhythm. Tolerance to acetylstrophanthidin was determined on three occasions in each study, namely at 45 (ASI), 180 (ASII) and 360 min (ASIII) after either the beginning of a control experiment or the administration of digoxin. Hartmann's Solution (Ringer-lactate) was infused at 1 drop/s for 15 min after each acetylstrophanthidin test to replace fluid losses from vomiting.

Each dog underwent four studies with oral doses of 0.05, 0.1, 0.2 and 0.4 mg/kg digoxin using a randomized sequence of administration; at least 10 days was allowed between each experiment. Digoxin stock solution containing 5 mg/ml in 70% alcohol was diluted just before administration and the diluted solution contained 1.4 to 14% ethanol. The digoxin was administered to each dog by a catheter inserted into the oesophagus and was washed in with 20 ml of water. Each dog was weighed at the beginning of each experiment. At least two control experiments with acetylstrophanthidin alone were carried out, including one at the beginning of the study, and one before the third or fourth dose of digoxin. A second 'Bardic 1-Catheter' was inserted into the contralateral cephalic or saphenous vein for sampling of blood at 0, 15, 30, 45, 60, 90, 120 and 360 min after the administration of digoxin. The plasma levels of digoxin were measured by a radioimmunoassay technique using the Lanoxitest-gamma kit (Wellcome Reagents Ltd) with an iodinated tyrosine derivative of digoxin as the tracer, but with the modification that standards were prepared with pooled dog serum. Serum potassium, calcium and urea were determined 6 h after the administration of digoxin. The results were subjected to analysis of variance, recovery times being transformed into logarithms prior to analysis.

Results

Changes in the ECG observed after infusion of acetylstrophanthidin and accepted as evidence of cardiotoxicity varied between occasions for individual dogs. This variation was seen on different days, or occasionally on the same day with different ECG changes for ASI, II or III. One dog died at ASII after receiving the 0.2 mg/kg dose of digoxin. In this dog the infusion was stopped when the desired ECG changes occurred, but the heart went into irreversible ventricular fibrillation.

Table 1 Effects in unanaesthetized dogs of oral doses of digoxin on the intravenous dose of acetylstrophanthidin (μg/kg) required to induce cardiotoxicity

Digoxin dose (mg/kg)	*Acetylstrophanthidin dose (μg/kg)* I	II	III
Control	135 ± 11.1	116 ± 9.2	130 ± 9.3
0.05	133 ± 15.7	97 ± 10.4	117 ± 16.9
0.1	113 ± 13.4	89 ± 11.6	107 ± 18.9
0.2	81 ± 15.5*	55 ± 11.3**	57 ± 15.8**
0.4	80 ± 16.0*	42 ± 6.1**	40 ± 5.3**

Columns I, II and III represent tolerance testing at 45, 180 and 360 min after digoxin dosing.
Mean values ± s.e. for groups of eight dogs (0.05 and 0.1 mg/kg) and seven dogs (0.2 and 0.4 mg/kg). Asterisks denote significant differences between control and treatment values at the 5% (*) and 1% (**) levels respectively.

Table 2 Effects in unanaesthetized dogs of oral doses of digoxin on the times to recovery from acetylstrophanthidin-induced toxicity

Digoxin dose (mg/kg)	*Recovery from acetylstrophanthidin (min)* I	II	III
Control	10.2 ± 1.3	9.9 ± 1.1	8.6 ± 1.0
0.05	8.6 ± 1.6	18.1 ± 7.2	10.3 ± 1.9
0.1	15.4 ± 4.1	15.4 ± 4.3	16.9 ± 5.9
0.2	13.5 ± 3.2	16.9 ± 4.3*	17.6 ± 5.0**
0.4	15.1 ± 3.7	19.9 ± 3.7**	25.1 ± 6.4**

Columns I, II and III represent tolerance testing at 45, 180 and 360 min after digoxin dosing.
Mean values ± s.e. for groups of eight dogs (0.05 and 0.1 mg/kg), six dogs (0.2 mg/kg) and seven dogs (0.4 mg/kg). Asterisks denote differences between control and treatment values significant at the 5%(*) and 1% (**) levels.

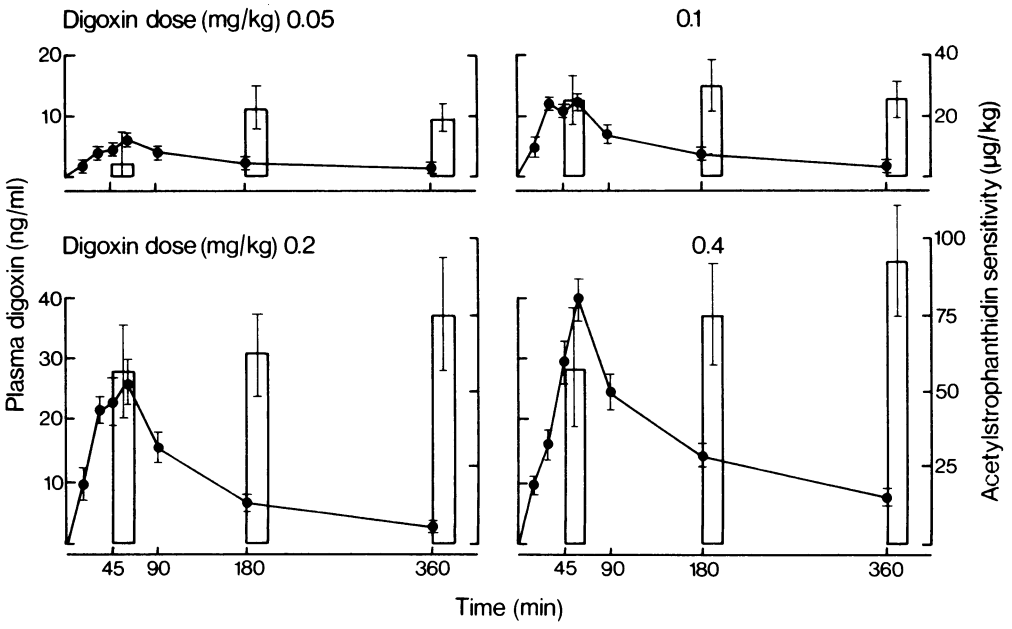

Figure 1 Comparisons of mean plasma digoxin concentration and mean acetylstrophanthidin sensitivity (see text) for four oral doses of digoxin administered to unanaesthetized dogs. The plasma digoxin levels are shown by the curves whilst acetylstrophanthidin sensitivity is represented by the columns. Vertical lines represent s.e. mean. The mean plasma digoxin concentrations are taken from Table 3.

The amount of acetylstrophanthidin per kg of body weight that was required to produce cardiotoxicity is shown in Table 1. The control doses of acetylstrophanthidin were calculated for each administration (ASI, II or III) for each dog and these doses varied more between dogs than between each administration. However, a small but significant reduction in acetylstrophanthidin tolerance was noted for ASII. Retching and vomiting usually occurred before cardiotoxicity, but the associated electrocardiographic changes were normally of short duration.

Treatment with digoxin reduced the amount of acetylstrophanthidin required to cause cardiotoxicity and this increased susceptibility to toxicity was dose-dependent (Table 1). At the lower dose levels of 0.05 and 0.1 mg/kg digoxin, increased susceptibility was most obvious at ASII, though not significant. At the two higher dose levels of 0.2 and 0.4 mg/kg of digoxin, reduced acetylstrophanthidin tolerance was maximal at ASII, persisted to ASIII, and differed significantly from the control experiments.

The time required for the ECG to return to normal after treatment with acetylstrophanthidin differed between dogs in the control experiments, but there was no significant difference between each administration of acetylstrophanthidin (ASI, ASII, ASIII) in individual dogs (Table 2). In one dog which received 0.2 mg/kg digoxin, acetylstrophanthidin-induced toxicity was abnormally prolonged; ectopic beats persisted for 130 min after ASI and 165 min after ASII, and ventricular tachycardia persisted for 48 h after ASIII. These data were excluded from analysis.

Table 3 Plasma digoxin concentration (ng/ml) after oral administration of digoxin

Digoxin dose	*Time (min) after administration*							
(mg/kg)	*0*	*15*	*30*	*45*	*60*	*90*	*180*	*360*
0.05	0	1.8±0.7	3.8±0.9	4.4±0.7	5.8±1.3	3.8±0.8	1.9±0.8	0.7±0.2
0.1	0	4.7±1.8	11.6±1.2	10.4±1.0	11.9±1.5	6.5±1.7	3.3±0.7	1.5±0.4
0.2	0	9.6±3.5	22.0±3.5	23.1±5.4	26.2±5.6	15.5±3.5	6.7±1.9	2.7±0.8
0.4	0	9.9±2.2	16.3±3.5	29.9±4.6	40.5±4.7	25.0±4.2	14.4±2.5	7.3±1.6

Mean values ± s.e. for group of eight dogs are shown for various times after dosing.

Recovery from acetylstrophanthidin-induced toxicity tended to be prolonged with increasing dosage of digoxin. This prolongation was least obvious at ASI and the recovery times were only significantly greater than the controls at ASII and ASIII after the two higher doses of 0.2 and 0.4 mg/kg digoxin.

The peak plasma concentration of digoxin was reached at about 60 min after each dose and then declined steadily (Table 3). In these dogs, vomiting did not occur until the first infusion of acetylstrophanthidin which was started 45 min after administration of digoxin. The occurrence of vomiting after the first infusion of acetylstrophanthidin depended on the dose of digoxin administered, i.e. for 0.05, 0.1, 0.2 and 0.4 mg/kg digoxin the mean times for the onset of vomiting from starting the infusion of acetylstrophanthidin were 15, 11, 7 and 6 min respectively.

Acetylstrophanthidin sensitivity was calculated as the difference between the amounts of acetylstrophanthidin required to induce cardiotoxicity in the digoxin and the control studies. The calculations were carried out from the results for comparable acetylstrophanthidin infusions (ASI, II or III) for individual dogs. Comparison of the plasma levels of digoxin and acetylstrophanthidin sensitivity showed that there was no correlation between these two variables (Figure 1). This was most evident after the 0.2 and 0.4 mg/kg doses of digoxin.

There was no significant change in serum calcium, potassium or urea during any experiment. The individual maximal fall in serum potassium from initial to 6 h blood sample was 0.2 mEq/litre.

Discussion

The absorption of digoxin occurs rapidly from the upper part of the gastrointestinal tract; peak plasma concentrations usually appearing after about 1 h in man (White, Chamberlain, Howard & Smith, 1971). Interest in peak concentrations arose from the observation that tablets of higher bioavailability produced an earlier and more prominent peak concentration (Johnson, Fowle, Lader, Fox & Munro-Faure, 1973). Concern was expressed that such high concentrations, although transient, might produce cardiotoxicity (Falch *et al.*, 1973; Harter *et al.*, 1974; Reissel *et al.*, 1974).

One method devised to detect the early stages of developing cardiotoxicity used acetylstrophanthidin (Lown & Levine, 1954). The effects of this synthetic cardiac glycoside are additive to those of digoxin at myocardial receptors, so that the quantity which must be infused before ECG signs of cardiotoxicity occur may be used as an index of the tendency to digoxin toxicity. The value of acetylstrophanthidin is that its additive cardiac effects are rapid in onset and highly transient, its plasma elimination half-life being 1.2 h in dogs and 2.3 h in man (Selden, Klein & Smith, 1973). This is much shorter than that of any other digitalis analogue and the decay of its pharmacological effect is even more rapid (Klein, Nejad, Lown, Hagemeijer & Barr, 1971). This relatively rapid rate of elimination allows repeated estimates of acetylstrophanthidin tolerance. Control experiments showed no important change in acetylstrophanthidin tolerance between the first and third infusions, nor was there any increase in the time for recovery of sinus rhythm.

In this work some aspects of the cardiotoxicity associated with the infusion of acetylstrophanthidin differed from those reported by others. Ventricular tachycardia was not the only dysrhythmia, as has been suggested (Barr, Smith, Klein, Hagemeijer & Lown, 1972; Kumar, Hood, Gilmour & Abelmann, 1972). Indeed Cope, Hopkins & Taylor (1973) also showed that the nature of the tachycardia or conduction block may vary. Higher doses of acetylstrophanthidin were required to produce cardiotoxicity in our studies than in those noted above, possibly because our dogs were unmedicated whereas anaesthetized or sedated dogs were used by others. Furthermore the infusion rates of acetylstrophanthidin varied in these previous studies and this must be of great importance in view of its rapid elimination.

In dogs, Barr *et al.* (1972) showed that ventricular tachycardia frequently developed spontaneously after intravenous administration of 0.1 mg/kg digoxin and persisted for between 0.5 to 3 h; also that acetylstrophanthidin tolerance was somewhat greater at 6 than at 2 hours. These results suggest that digoxin accumulates at myocardial receptor sites over periods of 1 to 2 h after injection and that deterioration in its activity can be detected by 6 hours. In the present study, ventricular tachycardia did not develop spontaneously after digoxin given orally, but it was readily induced by acetylstrophanthidin, particularly after the 0.2 and 0.4 mg/kg doses of digoxin, i.e. the susceptibility to acetylstrophanthidin was dependent upon the magnitude of the preceding dose of digoxin. However, at all doses of digoxin, acetylstrophanthidin sensitivity was lowest 45 min after oral administration of digoxin, although at this time plasma concentrations were at or near their maximum. Cardiac toxicity was always more easily induced at either 3 or 6 h after dosing with digoxin and there was a suggestion that maximal sensitivity might occur later with higher doses (Figure 1). It is clear that the greater tendency to cardiotoxicity occurs not at maximum plasma concentration of digoxin but some hours later. It is possible that this delay may be somewhat longer after oral than after intravenous administration. Continuing intestinal absorption might lead to the cardiac effects being more prolonged after oral administration, and this may account for acetyl-

strophanthidin sensitivity being greatest 6 h after the two larger doses of 0.2 and 0.4 mg/kg digoxin. The time for recovery to sinus rhythm was a less sensitive index of cardiotoxicity than the dose of acetylstrophanthidin required to induce toxicity. However, the same general trend was noted, as recovery from acetylstrophanthidin was more delayed when given 3 to 6 h after the higher doses of digoxin.

Acetylstrophanthidin induced vomiting but electrolytes were appropriately replaced by Hartmann's solution, since serum potassium, calcium and urea were not significantly altered during the studies. Although a change in serum electrolytes can alter the sensitivity of the heart to cardiac glycosides, this was unlikely in these experiments.

Dogs absorb digoxin well, and respond to and eliminate it at rates comparable to those of man (Barr *et al.*, 1972). They may also have similar processes of tissue-plasma kinetic equilibrium. With this assumption, the reported studies suggest that cardiotoxicity will not be temporally related to peak plasma concentrations in man. The rate of myocardial uptake of digoxin in dogs is linearly related to its plasma concentration (Roberge, Marcus, Kapadia & Lown, 1970). It may be that some part of the greater effectiveness of high bioavailability preparations is related to the exaggerated peak plasma concentrations. However, only a fraction of digoxin taken up by the myocardium is pharmacologically effective (Lüllman & Van Zwieten, 1969) and the relation of the plasma concentration of digoxin and this active myocardial fraction is unknown. It is also unknown whether the qualitative difference in plasma concentration profile resulting from different oral digoxin preparations is associated with variation in cardiac response. However, it is clear that cardiac response to digoxin is not transient but is delayed and prolonged.

Acetylstrophanthidin was kindly supplied by Dr Colin Speirs of Eli Lilly Co., Windlesham. We also wish to thank Miss Madeline Brockbanks for performing the digoxin determinations and Mr A.G.S. Merrivale for technical assistance.

References

BARR, I., SMITH, T.W., KLEIN, M.D., HAGEMEIJER, F. & LOWN, B. (1972). Correlation of the electrophysiologic action of digoxin with serum digoxin concentration. *J. Pharmac. exp. Ther.*, **180**, 710–722.

COPE, G.D., HOPKINS, B.E. & TAYLOR, R.R. (1973). Effect of chronic circulatory volume overload on digitalis intoxication. *Cardiovasc. Res.*, **7**, 638–641.

FALCH, D., TEIEN, A. & BJERKELUND, C.J. (1973). Comparative study of the absorption, plasma levels and urinary excretion of the 'new' and the 'old' Lanoxin. *Br. med. J.*, **1**, 695–697.

GANZ, A., FUJIMORI, H., PENNA, M., GREINER, T. & GOLD, H. (1957). Intramuscular administration of digoxin in propylene glycol. *Proc. Soc. exp. Biol. Med.*, **95**, 349–353.

HARTER, J.G., SKELLY, J.P. & STEERS, A.W. (1974). Digoxin—the regulatory viewpoint. *Circulation*, **49**, 395–398.

JOHNSON, B.F., FOWLE, A.S.E., LADER, S., FOX, J. & MUNRO-FAURE, A.D. (1973). Biological availability of digoxin from Lanoxin produced in the United Kingdom. *Br. med. J.*, **4**, 323–326.

KLEIN, M., NEJAD, N.S., LOWN, B., HAGEMEIJER, F. & BARR, I. (1971). Correlation of the electrical and mechanical changes in the dog heart during progressive digitalisation. *Circulation Res.*, **29**, 635–645.

KUMAR, R., HOOD, W.B., Jnr., GILMOUR, D.P. & ABELMANN, W.H. (1972). Effects of acetylstrophanthidin on cardiovascular function in intact, conscious dogs. *J. Pharmac. exp. Ther.*, **180**, 24–30.

LOWN, B. & LEVINE, S.A. (1954). Current concepts in digitalis therapy. *New Engl. J. Med.*, **250**, 866–874.

LÜLLMANN, H. & VAN ZWIETEN, P.A. (1969). The kinetic behaviour of cardiac glycosides *in vivo*, measured by isotope techniques. *J. Pharm. Pharmac.*, **21**, 1–8.

REDFORS, A. (1972). Plasma digoxin concentration—its relation to digoxin dosage and clinical effects in patients with atrial fibrillation. *Br. Heart J.*, **34**, 383–391.

REISSELL, P., MANNINEN, V., OJALA, K. & KARJALAINEN, J. (1974). Non-equivalence of digoxin tablets. *Ann. Clin. Res.*, **6**, 4–8.

ROBERGE, G., MARCUS, F.I., KAPADIA, G.G. & LOWN, B. (1970). Digitalisation of the myocardium in the intact animal by direct coronary arterial drug administration. II. Relation of the rate of administration to myocardial concentration and toxicity. *Am. J. Cardiol.*, **26**, 365–368.

SELDEN, R., KLEIN, M.D. & SMITH, T.W. (1973). Plasma concentration and urinary excretion kinetics of acetylstrophanthidin. *Circulation*, **47**, 744–751.

SHAPIRO, W., NARAHARA, K. & TAUBERT, K. (1970). Relationship of plasma digitoxin and digoxin to cardiac response following intravenous digitalization in man. *Circulation*, **42**, 1065–1072.

WHITE, R.J., CHAMBERLAIN, D.A., HOWARD, M. & SMITH, T.W. (1971). Plasma concentrations of digoxin after oral administration in the fasting and postprandial state. *Br. med. J.*, **1**, 380–381.

(Received August 21, 1975)