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TRANSIENT CHANGES IN PLASMA DIGOXIN CONCENTRATION AND THE DEVELOPMENT OF CARDIOTOXICITY

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1 Eight dogs were given two infusions of digoxin 0.1 mg/kg, one over 9 min and the other one over 90 min in a randomized sequence, allowing at least 12 days between each experiment.

2 Digoxin plasma profiles reflected the rate of digoxin infusion, the peak concentration of drug attained at the end of each infusion being considerably higher but more transient after the 9 min than after the 90 min transfusion.

3 Digoxin reduced the amount of acetylstrophanthidin required to produce electrocardiographic evidence of cardiotoxicity. This increase in cardiac sensitivity at 150 and at 360 min after the start of the digoxin infusion was independent of rate of infusion.

4 These results suggest that the development of cardiotoxicity is dependent upon the quantity of digoxin delivered into the systemic circulation regardless of the plasma concentration.

5 By inference, cardiotoxicity is related solely to the amount and not the rate of absorption from a given dose of digoxin.

Introduction

In contrast to most drugs, transient changes in plasma digoxin concentration are not associated with concurrent changes in pharmacological effects. The effects of a single dose of digoxin on the heart have been shown to be delayed and to be maximal at a time when plasma levels are diminishing. This relationship has been demonstrated for both chronotropic and inotropic effects in man (Ganz, Fujimari, Penna, Greiner & Gold, 1957; Shapiro, Narahara & Taubert, 1970) and for cardiotoxic effects in dogs (Chapple, Hughes & Johnson, 1976). However it seems possible that the extent of digoxin cardiotoxicity could be quantitatively related to the preceding peak plasma level, if drug binding to cardiac receptor sites were related in intensity or extent to the peak plasma level of digoxin. The studies described here were designed to examine this possibility.

Methods

Experiments were performed on eight unanaesthetized mongrel dogs, of either sex, initially weighing 11.5–19.0 kg. The cardiac tolerance to acetylstrophanthidin was assessed in each animal by the recognition of well-defined changes in the electrocardiogram (ECG) during infusion of a 100 μg/ml solution in 0.9% w/v NaCl solution (saline) and 7% ethanol at a rate of 0.095 mg/min, as described previously (Chapple *et al.*, 1976). The amount of acetylstrophanthidin required to induce cardiotoxicity, and the time required for the restoration of sinus rhythm were determined. The tolerance to acetylstrophanthidin was assessed on two occasions in each study, namely at 150 min (ASI) and 360 min (ASII) after either the beginning of a control infusion of saline over 9 or 90 min 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 two studies with intravenous doses of digoxin 0.1 mg/kg infused over either 9 or 90 min using a randomized sequence of administration and allowing at least 12 days between each experiment. Digoxin stock solution containing 1 mg/ml in 70% alcohol was diluted just before administration and the diluted solution contained 1.4 to 14% ethanol. Each dog was weighed at the beginning of each experiment. Two control experiments with acetylstrophanthidin alone were

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carried out on the day before a digoxin experiment. Plasma samples were collected for determination of digoxin at 0, 5, 10, 15, 20, 30, 60, 90, 120, 150 and 360 min, and for serum potassium, calcium, bicarbonate and urea at 0 and 6 h after the administration of digoxin. Plasma levels of digoxin were determined by radioimmunoassay using methods already described (Chapple *et al.*, 1976).

The significance of the results was tested by analysis of variance and *t* test where appropriate, recovery times being transformed into logarithms prior to analysis. The plasma digoxin levels were fitted to both a two- and a three-compartment theoretical model.

Results

The ECG changes observed after infusion of acetylstrophanthidin showed some variation between occasions for individual dogs but the end point was usually ventricular tachycardia.

The dose of acetylstrophanthidin per kg of body weight required to induce cardiotixicity is shown in Table 1. There was no significant difference between the two control infusion times but acetylstrophanthidin tolerance tended to be slightly lower for ASII than for ASI. Digoxin infusion reduced the amount of acetylstrophanthidin required to cause cardiotoxicity ($P < 0.01$) and this appeared more obvious for ASI. However, the reduction in tolerance was independent of the rate of infusion of digoxin.

The time required for the ECG to return to normal after acetylstrophanthidin-induced toxicity was not significantly different for the two control infusion times. In the digoxin-treated dogs, recovery from cardiotoxic effects of acetylstrophanthidin was significantly delayed and was more apparent for ASI (Table 2). Recovery time was not significantly affected by different infusion rates of digoxin.

In most control experiments vomiting occurred after acetylstrophanthidin, there being little difference between infusion times or between ASI and ASII for the time to first vomit or for frequency of vomiting. Digoxin also caused the dogs to vomit, there being no difference between the 9 min or 90 min infusion for frequency of vomiting. Perhaps not surprisingly the time from the start of the digoxin infusion to the first vomit was significantly greater for the 90 min infusion (9 min infusion, 25 ± 5.9 min; 90 min infusion, 72 ± 7.2 min; $P < 0.001$). After digoxin infusion, acetylstrophanthidin caused more rapid onset of vomiting but decreased the frequency of such episodes.

The peak plasma concentration of digoxin varied for the two infusion rates (Figure 1). The 9 min infusion gave a mean peak plasma concentration of 161.7 ng/ml, while the administration over 90 min produced a mean peak of 52.5 ng/ml. The area under the concentration-time curve for the 9 min infusion was significantly greater than that for the 90 min infusion ($P < 0.05$).

Acetylstrophanthidin sensitivity was calculated as the difference between the amounts of acetylstrophanthidin required to induce cardiotoxicity in control and post-digoxin studies. These calculations were carried out from the results for comparable infusion times (9 or 90 min) and for comparable acetylstrophanthidin infusions (ASI or II) for individual dogs. Comparison of the results

Table 1 Effects in unanaesthetized dogs of digoxin (0.1 mg/kg) infused intravenously over 9 or 90 min on the intravenous dose of acetylstrophanthidin (μg/kg) required to induce cardiotoxicity

Infusion time (min)	*Acetylstrophanthidin dose (μg/kg)* I	II
Control 9 min	114 ± 12.6	98 ± 10.7
Digoxin 9 min	24 ± 4.0***	29 ± 3.1***
Control 90 min	107 ± 11.6	103 ± 11.4
Digoxin 90 min	27 ± 4.0***	37 ± 4.7***

Columns I and II represent tolerance testing at 150 and 360 min respectively after digoxin administration. Mean values ± s.e. for groups of eight dogs are shown.
Asterisks denote differences between control and treatment values significant at the 0.1% (***) level.

Table 2 Effects in unanaesthetized dogs of digoxin (0.1 mg/kg) infused intravenously over either 9 or 90 min on the times to recovery from acetylstrophanthidin-induced toxicity

Infusion time (min)	*Recovery from acetylstrophanthidin (min)* I	II
Control 9 min	13 ± 1.5	12 ± 1.6
Digoxin 9 min	52 ± 10.2***	44 ± 11.1***
Control 90 min	19 ± 6.6	20 ± 4.2
Digoxin 90 min	61 ± 15.3***	37 ± 4.9*

Columns I and II represent tolerance testing at 150 and 360 min respectively after digoxin dosing. Mean values ± s.e. for groups of eight dogs are quoted.
Asterisks denote differences between control and treatment values significant at the 5% (*) and 0.1% (***) levels.

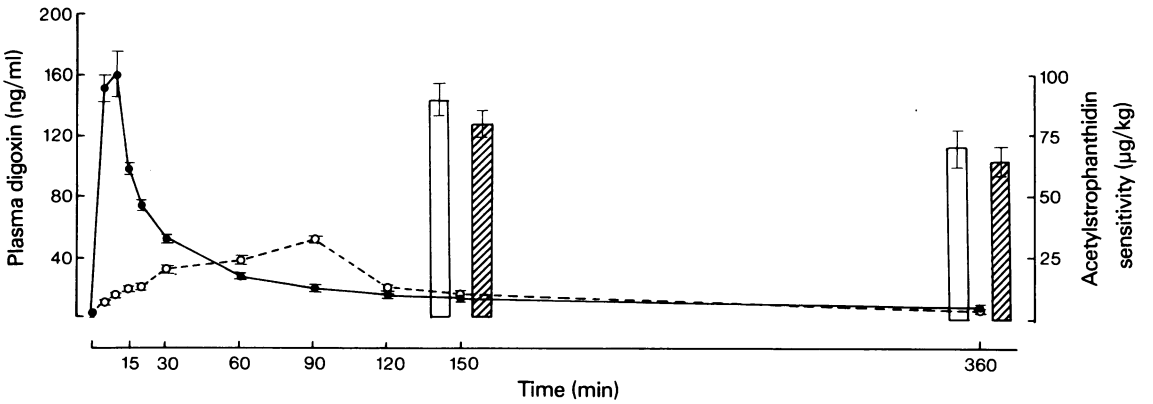

Figure 1 Comparison of mean plasma digoxin concentration (curves: (●) for 9 min and (○) for 90 min infusion) and mean acetylstrophanthidin sensitivity (columns: open for 9 min and hatched for 90 min infusion) for two infusion rates of digoxin administered to unanaesthetized dogs. Bars represent standard error of the mean.

obtained with differing infusion times showed that acetylstrophanthidin sensitivity was clearly independent of the profiles of plasma digoxin concentration (Figure 1).

On the assumption that the persistence of sensitivity to acetylstrophanthidin might be due to the sequestration of digoxin at its site of action, attempts were made to establish a model that would demonstrate this effect. It was found that a three-compartment model (one central compartment and two side compartments) gave a better fit to the observed plasma levels (identified as the central compartment) than did a two-compartment system. In both cases it was supposed that no limitations of space would apply, and that transfer rates could be expressed as simple proportions transferred per minute. Allowance was made for elimination to occur independently from each compartment.

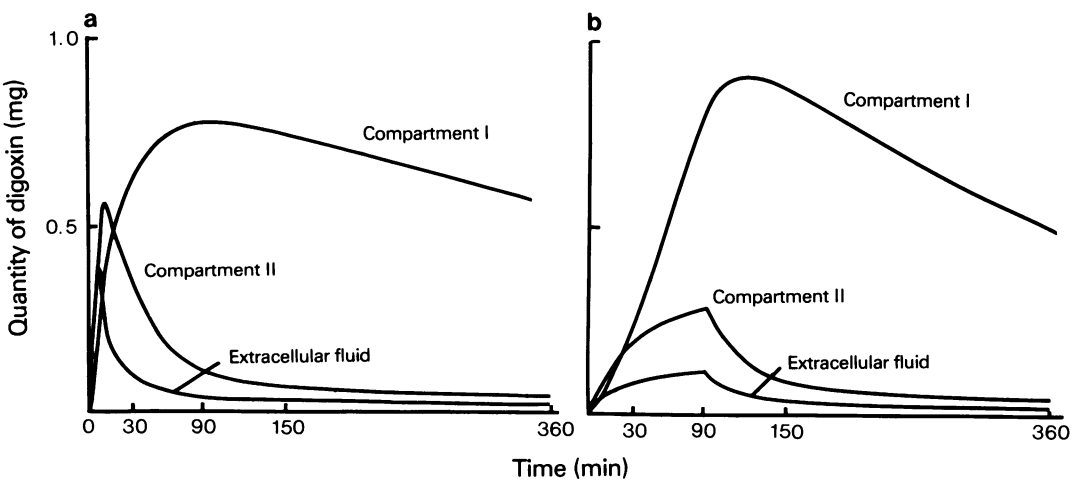

Figure 2 Changes over 6 h in relative quantities of digoxin in a three-compartment open model, following two rates of intravenous administration, (a) 9 min infusion, (b) 90 min infusion.

The mean parameters for the 9 and 90 min infusions were separately estimated and are shown in Table 3.

Table 3 Mean parameters for the 9 and 90 min infusions of digoxin assuming a three-compartment model

	9 min	*90 min*
Extracellular fluid (e.f.) to Compartment I	0.1195	0.1972
e.f. to Compartment II	0.2144	0.3769
e.f. to waste	0.0529	0.0315
Compartment I to e.f.	0.0047	0.0050
Compartment I to waste	0.0001	0.0022
Compartment II to e.f.	0.0871	0.1409
Compartment II to waste	0.0001	0.0023

Using these mean parameters the simulated results shown in Figure 2 were obtained. There was no significant change in serum calcium, bicarbonate, potassium or urea during any experiment. The individual maximal fall in serum potassium from initial to 6 h blood sample was 0.6 mmol/litre.

Discussion

Rapid intravenous administration or ingestion of high bioavailability oral preparations of digoxin produce transient high plasma concentrations and it has been suggested that this might be associated with increased risk of toxicity (Harter, Skelly & Steers, 1974; Reissell, Manninen, Ojala & Karjalainen, 1974). Our previous work suggested that there was no temporal relationship between transiently high concentrations and increased tendency to cardiac toxicity (Chapple *et al.*, 1976). However, it was still necessary to assess any effect of such plasma concentration changes upon the delayed cardiac effects. In the present study a marked difference in the profiles of plasma digoxin concentration was produced by different rates of infusion of the same dose of digoxin. This difference was not associated with any alteration in delayed cardiotoxicity, whether assessed by cardiac sensitivity to acetylstrophanthidin or by the time for subsequent recovery to sinus rhythm. As in previous studies (Chapple *et al.*, 1976), it was noted that recovery time was the less sensitive index of cardiotoxicity. It appears that the tendency to cardiotoxicity is determined solely by the quantity of digoxin entering into the circulation and is unrelated to rate of entry. By inference, neither the speed of intravenous injection nor the rate of intestinal absorption affect the risk of important cardiac toxicity.

Although equal doses of digoxin were injected, the area under the plasma concentration curve was significantly greater after the rapid infusion possibly because a period of 9 min limited the distribution of digoxin from plasma to tissues. Nevertheless, rapid injection of digoxin failed to enhance the tendency to cardiotoxicity despite much higher peak concentrations and the determination of a greater mean area under the plasma concentration curve.

The lack of correlation between plasma digoxin concentration and cardiac effects might be explained by a different time course for the concentration in the plasma and at receptor sites. In man, at least two compartments are required in any pharmacokinetic model to predict adequately the profile of plasma digoxin concentration over a period of several hours after injection (Reuning, Sams & Notari, 1973; Rabkin & Grupp, 1975). Extracellular fluid may represent the central compartment as the calculated time course of fluctuations in its digoxin content follows that in plasma and digoxin distributes freely between plasma and extracellular fluid. The single peripheral compartment in a two-compartment open model contains a much greater percentage of the administered digoxin and the time at which the maximum quantity occurs is delayed in relation to the peak plasma level. If cardiac receptor sites formed part of this peripheral compartment, the delay in peak effect might simply reflect the more gradual increase in the quantity of digoxin bound to receptors. Several authors have claimed that predictions of the observed time sequence of plasma concentration are substantially improved using a three-compartment open model (Kramer, Lewis, Cobb, Forester, Visconti, Wanke, Boxenbaum & Reuning, 1974; Koup, Greenblatt, Jusko, Smith & Koch-Weser, 1975; Sumner, Russell & Whiting, 1976). In four human subjects, Sumner *et al.* (1976) described two larger peripheral compartments with marked differences in rate of loss but with similar delays in attainment of maximal digoxin content. In the present study in dogs a slightly modified form of the three-compartment model produced interestingly different conclusions (Figure 2) concerning the time course of digoxin content in the three compartments. In the larger peripheral compartment the estimated digoxin content would be consistent with a delayed peak with slow decline over 6 hours. However, the other peripheral compartment should contain considerably less digoxin and its time course seemed only moderately delayed in relation to that of the central compartment. In dogs it is not possible to explain the delay in peak pharmacological effects in relation to the pharmacokinetics of digoxin, unless cardiac receptor sites form part of the larger peripheral compartment. Unfortunately, studies of total myocardial concentration are unlikely to define the pharmacokinetics of digoxin at receptor sites as most myocardial digoxin is bound non-specifically (Lüllmann & Van Zwieten,

1969) and the latter may show different pharmacokinetics from digoxin bound to receptor sites (Marks, 1972). Our findings are compatible with the hypothesis that maximal specific binding of digoxin at receptor sites develops over a period of an hour or more.

Since dogs absorb and eliminate digoxin at rates comparable to those found in man (Barr, Smith, Klein, Hagemeijer & Lown, 1972) it may be possible to derive clinically relevant information from these experiments. If so, it can be concluded that the qualitative differences in plasma concentration profiles seen with the administration of oral preparations of high bioavailability are not associated with variation in cardiac response. The greater effect produced by such preparations is due to an increased amount of absorption.

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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.

CHAPPLE, D.J., HUGHES, R. & JOHNSON, B.F. (1976). The relationship between cardiotoxicity and plasma digoxin concentration in conscious dogs. *Br. J. Pharmac.*, **57**, 23–27.

GANZ, A., FUJIMORI, H., PENNA, M., GRENIER, T. & GOLD, H. (1957). Intramuscular administration of digoxin in propylene glycol. *Proc. Soc. exp. Biol. Med.*, **95**, 349–350.

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

KOUP, J.R., GREENBLATT, D.J., JUSKO, W.J., SMITH, T.W. & KOCH-WESER, J. (1975). Pharmacokinetics of digoxin in normal subjects after intravenous bolus and infusion doses. *J. Pharmacokinet. Biopharm.*, **3**, 181–192.

KRAMER, W.G., LEWIS, R.P., COBB, T.C., FORESTER, W.F., VISCONTI, J.A., WANKE, L.E., BOXENBAUM, H.G. & REUNING, R.H. (1974). Pharmacokinetics of digoxin: comparison of a two- and a three-compartment model in man. *J. Pharmacokinet. Biopharm.*, **2**, 299–312.

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

MARKS, B.H. (1972). Factors that affect the accumulation of digitalis glycosides by the heart. *Basic and Clinical Pharmacology of Digitalis*, ed. Marks, B.H. & Weissler, A.M., pp. 69–73. Springfield: Thomas.

RABKIN, S.W. & GRUPP, G. (1975). A two compartment open model for digoxin pharmacokinetics in patients receiving a wide range of digoxin doses. *Acta Cardiol.*, **30**, 343–351.

REISSELL, P., MANNINEN, V., OJALA, K. & KARJALAINEN, J. (1974). Non-equivalence of digoxin tablets. *Ann. clin. Res.*, **6**, Suppl. 11: 4–8.

REUNING, R.H., SAMS, R.A. & NOTARI, R.E. (1973). Role of pharmacokinetics in drug dosage adjustment. 1. Pharmacologic effect kinetics, and apparent volume of distribution of digoxin. *J. Clin. Pharmac. New Drugs*, **13**, 127–141.

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.

SUMNER, D.J., RUSSELL, A.J. & WHITING, B. (1976). Digoxin pharmacokinetics: multicompartmental analysis and its clinical implications. *Br. J. clin. Pharmac.*, **3**, 221–229.

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