COMPARATIVE PHARMACOKINETICS AND PHARMACODYNAMICS OF CARDIAC GLYCOSIDES

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- 1 The pharmacokinetics and pharmacodynamics of ouabain, digoxin and β -methyl digoxin (medigoxin) have been investigated in a crossover study in four normal healthy volunteers.
- 2 Pharmacokinetics were studied using [^3H]-labelled glycosides and the shortening of the left ventricular ejection time (LVET) was used as a measure of the effect of the drugs. A graded exercise protocol was used to correct for the effects of heart rate on LVET.
- 3 In three of the four subjects, both digoxin and β -methyl digoxin produced a shortening in the LVET, but no such change could be detected with ouabain in any of the four subjects.
- 4 There was a good linear correlation between the shortening of the LVET and the amounts of digoxin or β -methyl digoxin present in the body tissues.
- 5 One subject who showed no drug-related LVET shortening had greatly enhanced clearances of all three drugs studied.

Introduction

Although there have been a large number of studies of the pharmacokinetics of digoxin in recent years, including compartmental modelling (Kramer, Lewis, Cobb, Forester, Visconti, Wanke, Boxenbaum & Reuning, 1974; Sumner, Russell & Whiting, 1976; Harrison & Gibaldi, 1977) and a number of studies of the pharmacodynamic effects of digoxin as measured by non-invasive techniques (Shapiro, Narahara & Taubert, 1970; Das, Talmers & Weissler, 1977; Dobbs, Kenyon & Dobbs, 1977) there have been very few attempts to validate compartmental models by simultaneous measurement of left ventricular function.

Reuning, Sams & Notari (1973) fitted published plasma or blood level data to a two compartment model, and compared the predicted tissue compartment levels with changes in the left ventricular ejection time (LVET) measured by Shapiro *et al.* (1970) in a group of normal subjects. The authors demonstrated a good linear correlation between change in LVET and the fraction of the drug in the tissue compartment. In a more recent study, Hinderling & Garrett (1977) found that a significant linear correlation existed between change in LVET

and amount of β -methyl digoxin (medigoxin) in its 'moderately deep' tissue compartment, and of digoxin in its 'shallower' compartment. Kramer, Kolibash, Lewis, Bathala, Visconti & Reuning (1979) measured changes in electromechanical systole (QS_2) after administration of digoxin and found that these could be related to the amount of drug in the 'deep' peripheral compartment by a Langmuir-type equation.

All these studies employed measurement of systolic time intervals (STIs) in resting subjects, with corrections for heart rate using the relationships described by Weissler, Harris & Schoernfeld (1968). However, results obtained in this laboratory from experiments adhering to a similar resting, supine protocol, indicated that the effects measured following intravenous doses of several cardiac glycosides could be reproduced following placebo injection (Kelman, Sumner, Lawrence & Whiting, 1978). This was interpreted as indicating that heart rate corrections derived from data obtained in a large group of normal subjects were inappropriate when applied to individual subjects. Thus it became essential to derive the STI v heart rate relationship for

each subject, and for this purpose a protocol involving graded exercise was adopted in order to produce a suitable range of heart rates.

In this paper we present results from a crossover study of the pharmacokinetics and pharmacodynamics of ouabain, digoxin and β -methyl digoxin.

Methods

Pharmacokinetics

The subjects used were four healthy male volunteers aged between 20 and 35 years. Each was given a thorough medical examination, which included the recording of exercise ECG, prior to the experiment. Written informed consent was obtained and the experiment was approved by the Hospital Research and Ethical Committee.

The study took the form of a 4×4 Latin square design, in which each subject received each of the three drugs and a placebo at intervals of two weeks. On the day of the study subjects received a standard light breakfast at 07.00 h, a standard light lunch at 14.00 h and a hot evening meal at 20.30 h. A cannula was inserted into the antecubital vein of one arm and the drug administered at 08.30 h by intravenous injection into an antecubital vein of the other arm, the subject being supine.

The drug preparations injected were as follows:

Ouabain; 100 μ Ci [3 H]-ouabain (New England Nuclear, Specific Activity 12 Ci/mmol) in sterile water, followed by 0.5 mg ouabain in ethanol and propylene glycol made up to 20 ml with saline and injected over 5 min.

Digoxin; 100 μ Ci 125 I-digoxin (New England Nuclear, Specific Activity 17.5 Ci/mmol) in sterile water and ethanol (9:1), followed by 1.0 mg digoxin ('Lanoxin', Burroughs Wellcome) made up to 20 ml with saline and injected over 5 min.

β -methyl digoxin; 380 μ Ci 125 I- β -methyl digoxin (Boehringer Mannheim GmbH), equivalent to 1 mg (specific activity 0.27 Ci/mmol) made up to 20 ml with saline and injected over 5 min.

Placebo; 30 μ Ci 51 Cr EDTA (Radiochemical Centre), for the measurement of glomerular filtration rate followed by 4 ml of ethanol and propylene glycol made up to 20 ml with saline and injected over 5 min.

Sampling and collection

Blood samples (7 ml in lithium heparin, 3 ml in plain glass tubes) were taken at 2, 5, 10, 20, 30 and 40 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 h and then at 24, 32, 48, 72 and 96 h after injection. Blood collected in lithium heparin tubes was centrifuged

and the plasma separated and stored at -20°C . Blood in plain tubes was allowed to clot and then centrifuged, the serum separated and stored at -20°C .

Urine collections were taken over the following time periods; 0–12 h, 12–24 h, and then at 24 h intervals for 1 week following administration of the drug. Aliquots from each urine collection were stored at -20°C .

Twenty-four hour faecal collections were also made for 1 week following administration of the drug. Faecal collections were weighed and homogenized with water, and aliquots stored at -20°C .

Assay of radioactivity in plasma and urine

Aliquots of plasma (1 ml) and urine (1 ml) were mixed with 10 ml NE 260 liquid scintillator (Nuclear Enterprises, Edinburgh) and counted on a Packard TriCarb Liquid Scintillation Counter for a time sufficient to accumulate 5000 counts, giving a statistical error of 1.4%. The chloroform extractable radioactivity of plasma and urine was also measured.

The activity of stool aliquots was assayed after combustion in an Intertechnique Model IN2401 sample oxidiser.

Assay of total drug concentration in serum

Serum obtained following administration of digoxin and β -methyl digoxin was analyzed for total digoxin by radioimmunoassay using [125 I]-digoxin (Wellcome Reagents) and a sheep anti-digoxin serum. Separation of free and bound fractions was carried out using a second antibody (Donkey antisheep/goat, Wellcome Reagents).

Thin layer chromatography

Aliquots of all urine samples collected after administration of ouabain were applied to thin layer chromatography plates (Merck Silica Gel Coated Plates, thickness 0.25 mm). An aliquot of the injected solution was also applied. The solvent system used was chloroform: methanol: water (65:30:5). After drying, the silica gel was scraped off in 1 cm strips and suspended in a gel formed by mixing 4 ml water with 10 ml Instagel (Packard).

Pharmacodynamics

Pharmacodynamics of the drugs were assessed using measurements of STIs involving the simultaneous recording of ECG, phonocardiogram and carotid artery pressure pulse (Weissler *et al.*, 1968). Using a Devices chart recorder run at a paper speed of 100 mm/s, recordings were made at 30 min intervals

up to 6 h following drug or placebo injection, and hourly from 6h to 12h. A recording was also made at 24h. At the time of each observation, the subject was required to exercise on a bicycle ergometer for 1 min at each of six levels, corresponding to work rates of 50, 75, 90, 100, 125 and 150 Watts. An additional 1 min period of exercise at 180 Watts was used to complete the pre-dose observations. STIs were recorded for five consecutive heart beats immediately after each exercise level; a heart rate range of approximately 50 beats/min was thus obtained for each subject. The data obtained from the pre-dose session, carried out at 08.15h on the day of the trial, were used to obtain the STI ν heart rate relationship for each subject. The STIs subsequently measured on that day were compared with the values expected from this STI ν heart rate relationship to obtain their deviation from the pre-dose, control data. The mean deviation was then calculated for each recording session.

Curve fitting and compartmental modelling

All plasma ^3H —concentration curves were fitted to a sum of three exponential functions using a generalized least squares fitting programme based on the NAG group of subroutines and run on the NUMAC IBM 360/370 computer. Rate constants of the three compartment model were obtained using standard equations (Gibaldi & Perrier, 1975).

Results

Pharmacokinetics

Ouabain Clearances and final half lives of [^3H]—ouabain for the four subjects are shown in Table 1. Mean ($\pm$ s.d.) 7 day urinary recovery was $(41 \pm 8)\%$ dose, and mean 7 day faecal recovery was $(27 \pm 5)\%$ dose, a urine/faecal ratio of 1.6 ± 0.6 .

The ouabain recoveries obtained by thin layer chromatography are shown in Table 2.

Digoxin The mean plasma ^3H concentration for the four subjects following administration of 12α -[^3H]—digoxin is shown in Figure 1. Also shown are the chloroform extractable ^3H concentrations and the digoxin concentration as measured by radioimmunoassay. Owing to large statistical errors, the chloroform extractable ^3H concentration after 24h could not be measured reliably. However, on the basis of results up to 12h it can be seen that the chloroform extractable ^3H concentration is essentially the same as the concentration measured by radioimmunoassay, although both are on average about 20% less than the total ^3H concentration, with the discrepancy being less at earlier times. The clearances and half lives based on total label for the

four subjects are shown in Table 3; for comparison, the mean clearance based on radioimmunoassayable concentrations is also shown in the table.

Mean 7 day urinary recovery was $(62 \pm 7)\%$ dose, mean 7 day faecal recovery was $(10 \pm 3)\%$ dose, a urine/faecal ratio of 6.7 ± 1.9 .

β -methyl digoxin The mean plasma ^3H concentration for the four subjects following administration of 12α - ^3H - β -methyl digoxin is shown in Figure 2. Also shown are the concentrations as measured by radioimmunoassay. The chloroform extractable ^3H concentration is not shown separately, as in all cases the chloroform extractable concentration was more than 95% of the total ^3H concentration. The concentration as measured by radioimmunoassay was on average only 56% of the total ^3H concentration; as with digoxin, the discrepancy was less at earlier times and was a maximum 1–2 h after injection. The clearances and half lives based on total label for the four subjects are shown in Table 4; for comparison the mean clearance based on radioimmunoassayable concentrations is also shown in the table. Mean 7 day urinary recovery was $(77 \pm 7)\%$ dose, mean 7 day faecal recovery was $(18 \pm 3)\%$, a urine/faecal ratio of 4.4 ± 0.9 .

Pharmacodynamics

Heart rates obtained at each exercise level and systolic and diastolic blood pressures measured before and after each exercise period showed no significant changes during the course of the day.

The STI most commonly used to investigate inotropic effect, the left ventricular ejection time (LVET), was the STI of principal interest in this study, and only results which reflect changes in LVET will be presented.

Individual LVET data obtained from the pre-dose exercise sessions were satisfactorily described by a linear relationship of the form:

$$\text{LVET} = A - B \cdot (\text{HR})$$

The value of ΔLVET , where

$$\Delta\text{LVET} = (\text{LVET})_{\text{measured}} - A + B \cdot (\text{HR})$$

Table 1 [^3H]—ouabain: clearances and half-lives

Subject	Renal (ml/min)	Extra- renal (ml/min)	GFR	Final half-life (h)
MM	61	70	101	79.7
DG	107	36	126	80.4
RW	64	55	93	79.5
SB	71	47	107	96.4
Mean	76	52	107	84.0

Figure 1 ■ Mean ^3H concentration in plasma for all four subjects following administration of ^3H -digoxin. ● mean chloroform extractable ^3H concentration in plasma. ○ Mean digoxin concentration as measured by radioimmunoassay.

Figure 2 ■ Mean ^3H concentration in plasma for all four subjects following administration of ^3H - β -methyl digoxin. ● mean chloroform extractable ^3H concentration in plasma. ○ Mean digoxin concentration as measured by radioimmunoassay.

was calculated for each exercise level and the mean ΔLVET was then obtained for each recording session.

The results obtained for subject RW are shown in Figure 3. The values of ΔLVET following digoxin and β -methyl digoxin were similar up to about 6h, and showed a maximum shortening of about 35ms between 2 and 5h. After ouabain, maximum LVET shortening occurred at about 1h, but the LVET rapidly returned to values obtained following placebo administration. It is possible, however, that the present technique was not sufficiently sensitive to resolve effects of short duration which occurred almost immediately after ouabain administration. A marked shortening of the LVET also occurred following placebo. This was of the order of 15 ms and remained fairly constant throughout the course of the experiment. It could be attributed to the subject's adaptation to a rigorous protocol.

The time course of LVET shortening produced by digoxin and β -methyl digoxin in subjects SB and MM was similar to that for RW, but the drug effects were of smaller magnitude. Again, placebo injections produced shortening of the LVET by about 10 ms,

and this was fairly constant throughout most of the experiment. However, in SB and MM, ouabain had less effect on LVET than placebo in the 3 to 10h post-injection period, but it is not certain whether this was attributable to a negative inotropic effect produced by ouabain over this period, or merely reflected variations in the response of individual subjects.

The changes in LVET produced in subject DG were quite different to those obtained in other subjects: DG showed no positive inotropic response to any of the drugs under investigation. This subject showed much higher renal and extra-renal clearances of all three drugs.

Figure 4 illustrates the mean data obtained from the four subjects and displays the main features presented by RW, SB and MM. It is evident that both digoxin and β -methyl digoxin produce their maximum positive inotropic effects between 2 and 6h following an intravenous injection with corresponding ΔLVET half-lives of $29.8 \pm 9.5\text{h}$ and $38.1 \pm 17.6\text{h}$ respectively (mean $\pm 95\%$ confidence limit).

Table 2 Recovery of ^3H ouabain using thin layer chromatography

Subject	% Recovery	
	Standard	Urine
RW	71	82 ± 13
SB	71	73 ± 6
DG	74	70 ± 20
MM	77	78 ± 12
Mean	73 ± 3	75 ± 4

% Recovery is defined as
$$\frac{\text{Activity in ouabain peak} \times 100}{\text{Activity applied to TLC plate}}$$

Table 3 ^3H -digoxin: clearances and half-lives

Subject	Renal clearance	Extra-renal clearance	GFR	Final half-life (h)
	(ml/min)	(ml/min)		
MM	130	57	101	32.7
DG	177	140	126	29.7
RW	109	60	93	51.3
SB	133	68	107	37.3
Mean (Total ^3H)	137	81	107	37.8
Mean (RIA)	154	82

Figure 3 Time course of LVET shortening for subject RW. ● placebo, ○ digoxin, ▽ β -methyl digoxin, □ ouabain.

Figure 4 LVET shortening; Mean results for the four subjects. ● placebo, ○ digoxin, ▽ β -methyl digoxin, □ ouabain.

Compartmental modelling

The results of fitting the changes in LVET after digoxin to the model shown in Figure 5 are given in Table 5. In this model the 'deep' and 'shallow' compartments both represent tissue spaces and are so called because the rate constant for transfer of drug out of the 'shallow' compartment is an order of magnitude higher than the rate constant for transfer out of the 'deep' compartment. This model has been discussed in more detail in a previous publication (Sumner *et al.*, 1976). Table 5 gives the correlation coefficients between changes in LVET and predicted amounts of digoxin in the 'deep' and 'shallow' compartments separately and together. In general the highest correlation coefficients are obtained when the 'deep' and 'shallow' compartments are combined.

Figure 6 shows the change in LVET after digoxin plotted against the amount of drug in the combined 'deep' and 'shallow' compartments (i.e. the body less the central compartment); the best straight line fit is also indicated. The variation with time of the amount of drug in the combined 'deep' and 'shallow' compartments is shown as the solid curve in Figure 7a.

Similar data for β -methyl digoxin are given in Table 6 and Figures 8 and 7b. The results for β -methyl digoxin are not as clear cut as for digoxin, but, at least as far as the mean data are concerned, the highest correlation coefficient obtained is that between the change in LVET and the amount of drug in the 'deep' and 'shallow' compartments combined.

All the above correlation coefficients assume a linear relationship between LVET and the amount of drug in a compartment. Correlations between LVET and the logarithm of amount of drug were investigated, but were not found to be as significant as linear relations.

Discussion

Ouabain

Owing to the lack of inotropic effect observed following ouabain administration in this study, no compartmental modelling has been carried out. However, two features are worthy of note. The first is the very long plasma half-life, 84 ± 8 h. Previous work on ouabain kinetics is scarce, but Lahrtz, Reinold & van Zwieten (1969) found a half-life of approximately 50 h, and Selden & Smith (1972), a half-life of only 22 h, both in groups of normal volunteers. Selden & Smith (1972) also found the 'physiological half-life' to be approximately 21 h, but this was based on STI measurements at rest using Weissler's *et al.* (1968) corrections, a technique which the present authors have questioned previously (Kelman *et al.*, 1978). One point that may be significant is that both Lahrtz *et al.* (1969) and Selden & Smith (1972) sampled only up to 48 h and this may have given a falsely low reading of the half life. An alternative explanation is that some of the ^3H in plasma is present in a form other than ouabain,

Table 4 [^3H]- β -methyl digoxin: clearances and half-lives

Subject	Renal clearance (ml/min)	Extra-renal clearance (ml/min)	GFR	Final half-life (h)
MM	86	—	101	83.2
DG	115	37	126	37.3
RW	74	16	93	55.0
SB	79	21	107	48.8
Mean				
(Total ^3H)	89	25	107	56.1
Mean (RIA)	139	28

Figure 5 Three compartment model used for fitting data in his study. The central compartment probably consists of the plasma and extra-cellular fluid; the 'shallow' and 'deep' compartments both represent body tissues.

perhaps as a metabolite, or as a result of ^3H exchange, or possibly an impurity present in the original dose. A metabolite seems highly unlikely (Cox, Roxburgh & Wright, 1974).

Regarding the other two possibilities, Table 2 indicates that the recovery of $[^3\text{H}]$ -ouabain from urine as measured by thin layer chromatography is essentially the same as that from the standard. Moreover, there is no trend towards a lower recovery at later times, indicating that the majority of the plasma activity is $[^3\text{H}]$ -ouabain.

The second feature of interest in the ouabain data is the relative importance of renal and non-renal excretion. The ratio of renal to non-renal clearance (see Table 1) is 1.46 , in agreement with the urine/faecal excretion ratio of 1.6 ± 0.6 , although both figures conceal a wide variation. By comparison, Selden & Smith (1972) obtained a urine/faecal excretion ratio of 1.4 ; however, Lahrtz *et al.* (1969) did not find any significant non-renal excretion.

Digoxin

With the exception of subject DG, the values of half-life, renal and non-renal clearance agree well with those found previously (Koup, Greenblatt, Jusko, Smith & Koch-Weser, 1975; Rietbrock, Guggenmos, Kuhlmann & Hess 1976; Sumner *et al.*, 1976).

Although the kinetic data can be fitted to a three compartment model (Kramer *et al.*, 1974; Sumner *et al.*, 1976), Table 5 shows that a better correlation is obtained between the change in LVET and the amount of drug in the combined tissue compartments, i.e. the amount of drug in the body less that in the central compartment. This approach is advocated by Wagner, who has recently criticised the use of compartmental models in pharmacokinetics (Wagner, 1976).

There are at least two further, more sophisticated approaches to the problems of correlating pharmacokinetics and pharmacodynamics. The effect

Table 5 Correlation coefficients between decrease in LVET and amount of digoxin in the tissue compartments

Subject	'Shallow' compartment	'Deep' compartment	'Shallow' and 'Deep' combined
MM	0.29 (NS)	0.58 ($P < 0.05$)	0.80 ($P < 0.01$)
RW	-0.07 (NS)	0.62 ($P < 0.05$)	0.92 ($P < 0.01$)
SB	0.41 (NS)	0.04 (NS)	0.36 (NS)
Mean	0.25 (NS)	0.57 ($P < 0.05$)	0.90 ($P < 0.01$)

Note: Subject DG is not listed individually but is included in the mean data.

Table 6 Correlation coefficients between decrease in LVET and amount of β -methyl digoxin in the tissue compartments

Subject	'Shallow' compartment	'Deep' compartment	'Shallow' and 'Deep' combined
MM	0.53 ($P < 0.05$)	-0.24 (NS)	0.18 (NS)
RW	0.68 ($P < 0.01$)	0.13 (NS)	0.66 ($P < 0.01$)
SB	-0.05 (NS)	0.47 (NS)	0.46 (NS)
Mean	0.47 (NS)	0.21 (NS)	0.80 ($P < 0.01$)

Note: Subject DG is not listed individually but is included in the mean data.

Figure 6 Decrease in LVET (mean of four subjects) plotted against the percentage of digoxin in the tissues. The - - - - - line is the best least squares fit.

can be expressed as a function of the amount of drug in more than one compartment, or alternatively the 'Effect Model' of Sheiner, Stanski, Vozeh, Miller & Ham (1979) may be used. These alternative approaches are being explored and will form the subject of a future communication.

All the correlation coefficients shown in Table 5 are of course based on the assumption of a linear relationship between LVET and drug concentration, and it can be seen from Figure 6 that the data are certainly not inconsistent with a linear relationship. There are some theoretical grounds for thinking that the drug effect is proportional to the logarithm of the concentration; however, when such a relation was assumed, it invariably gave lower correlation coefficients. This was true regardless of whether the 'deep' and 'shallow' compartments were considered separately or together.

Subject DG is of particular interest as both his renal and extrarenal clearances were substantially higher than average, and he did not show any positive inotropic effect after administration of digoxin, as measured by the STI technique. It is possible that this lack of response was related to rapid metabolism of digoxin into cardioinactive forms.

It is of interest that the fraction of ^3H activity extractable in chloroform is comparable with the concentration measured by radioimmunoassay. It is now well established that RIA methods measure some metabolites of digoxin as well as digoxin itself (Stoll, Christensen, Sakmar, & Wagner, 1972).

High cross reactivities to mono- and bis-digitoxides and digoxigenin were found in the assay used in this study, but very low cross reactivities to

dihydrodigoxin and epidigoxigenin. However, all these metabolites were found to be extractable into chloroform with extraction efficiencies greater than 95%; the water soluble fraction (which can be as high as 20% or even more) must therefore consist of conjugation products. Metabolism of digoxin has been said to occur to only a small extent (Doherty, 1973), although Clark & Kalman (1974) have recently reported that dihydrodigoxin can be an important metabolite, and is probably cardioinactive. The chloroform extractable fraction is sometimes held to be the cardioactive fraction, although there is no direct evidence of this in man. Clearly, if a significant part of the total ^3H activity is cardioinactive, it will not be legitimate to correlate predicted amounts of

Figure 7 a) Decrease in LVET with time after injection of digoxin: mean of four subjects. The solid curve represents the amount of digoxin in the tissues, normalised using the linear relationship shown in **Figure 6 b)** Decrease in LVET with time after injection of β -methyl digoxin: mean of four subjects. The solid curve represents the amount of β -methyl digoxin in the tissues, normalised using the linear relationship shown in **Figure 8**.

Figure 8 Decrease in LVET (mean of four subjects) plotted against the percentage of β -methyl digoxin in the tissues. The - - - - line is the best least squares fit.

drug in the tissue obtained from the total ^3H curve with changes in left ventricular function. To investigate whether this is important we have correlated LVET with tissue concentrations as predicted from the RIA curve. The correlation is very similar, in fact slightly better, indicating that RIA is a better measure of cardioactivity.

References

- BOERNER, D., OLCAY, A., SCHAUMANN, W. & WEISS, W. (1976). Absorption of β -methyl-digoxin determined after a single dose and under steady state conditions. *Eur. J. clin. Pharmac.*, **9**, 307–314.
- CLARK, D.R. & KALMAN, S.M. (1974). Dihydrodigoxin: a common metabolite of digoxin in man. *Drug. Metab. Dispos.*, **2**, 148–150.
- COX, E., ROXBURGH, G. & WRIGHT, S.E. (1959). The metabolism of ouabain in the rat. *J. Pharm. Pharmac.*, **11**, 535–539.
- DAS, G., TALMERS, F.N. & WEISSLER, A.M. (1977). Comparative pharmacodynamics of β -methyl digoxin and digoxin in man. *Clin. Pharmac. Ther.*, **22**, 280–285.
- DOBBS, S.M., KENYON, W.I. & DOBBS, R.J. (1977). Maintenance digoxin after an episode of heart failure: placebo-controlled trial on outpatients. *Br. med. J.*, **1**, 749–752.
- DOHERTY, J.E. (1973). Digitalis Glycosides: pharmacokinetics and their clinical implications. *Ann. intern. Med.*, **79**, 229–238.
- GARRETT, E.R. & HINDERLING, P.H. (1977). Pharmacokinetics of β -methyl digoxin in healthy humans IV: Comparisons of radioimmunoassays, total radioactivity and specific assays of β -methyl digoxin and digoxin in plasma. *J. pharm. Sci.*, **66**, 806–810.
- GIBALDI, M. & PERRIER, D. (1975). *Pharmacokinetics*, p.89. New York: Marcel Dekker, Inc.
- HARRISON, L.I. & GIBALDI, M. (1977). Physiologically based pharmacokinetic model for digoxin disposition in dogs and its preliminary application to humans. *J. pharm. Sci.*, **66**, 1679–1683.
- HINDERLING, P.H. & GARRETT, E.R. (1977). Pharmacokinetics of β -methyl digoxin in healthy humans III: Pharmacodynamic correlations. *J. pharm. Sci.*, **66**, 326–329.
- KELMAN, A.W., SUMNER, D.J., LAWRENCE, J.R. & WHITING, B. (1978). Measurement of systolic time intervals. *Br. J. clin. Pharmac.*, **6**, 549–550.
- 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. Pharmacokin. Biopharm.*, **3**, 181–191.
- KRAMER, W.G., KOLIBASH, A.J., LEWIS, R.P., BATHALA, M.S., VISCONTI, J.A. & REUNING, R.H. (1979). Pharmacokinetics of digoxin: Relationship between response intensity and predicted compartmental drug levels in man. *J. Pharmacokin. Biopharm.*, **7**, 47–61.
- KRAMER, W.G., LEWIS, R.P., COBB, T.C., FORESTER, W.F. JR., VISCONTI, J.A., WANKE, L.A., BOXENBAUM, H.G. & REUNING, R.H. (1974). Pharmacokinetics of digoxin:

β -methyl digoxin

The values found for half-life and renal and extra-renal clearances in this study are broadly in agreement with those found by other workers (Kramer & Scheler, 1972; Rietbrock, Abshagen, Bergmann & Rennekamp, 1975; Boerner, Olcay, Schaumann & Weiss, 1976; Rietbrock *et al.*, 1976).

The points made about compartmental modelling for digoxin apply equally to β -methyl digoxin. As with digoxin, subject DG had higher than average clearances and showed no positive inotropic effect.

The fraction of plasma ^3H activity extractable in chloroform was greater than 95%, suggesting that the ^3H and RIA curves should be comparable. However, the RIA concentration diverges markedly from the ^3H concentration, the discrepancy being as much as 50% in some cases. A similar effect has been described by Garrett & Hinderling (1977). β -methyl digoxin is broken down initially to digoxin and Garrett & Hinderling (1977) have suggested that the presence of one glycoside may modify the response of the assay to the other glycoside.

This study shows the importance of devising a protocol which allows the determination of *individual* STI v heart rate relationships; the ability to discriminate between drug and placebo effects demonstrates the validity of this approach. For digoxin and β -methyl digoxin it has been possible to relate the pharmacological response to the amount of drug present in the tissue compartments of the body as predicted from pharmacokinetic principles.

- Comparison of a two- and a three- compartment model in man. *J. Pharmacokin. Biopharm.*, **2**, 299–312.
- KRAMER, P. & SCHELER, F. (1972). Renale eliminationskinetik verschiedener herzglykoside. *Dtsch. Med. Wochenschr.*, **97**, 1485.
- LAHRTZ, H.G., REINOLD, H.M. & VAN ZWIETEN, P.A., (1969). Serum concentration and urinary excretion of [³H]-ouabain in patients suffering from liver or kidney diseases. *Pharmacol. Clin.*, **1**, 114–118.
- 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.*, **13**, 127–141.
- RIETBROCK, N., ABSHAGEN, U., BERGMANN, K. & RENNEKAMP, H.: (1975). Disposition of β -methyl-digoxin in man. *Eur. J. clin. Pharmac.*, **9**, 105–114.
- REITBROCK, N. GUGGENMOS, J., KUHLMANN, J. & HESS, U. (1976). Bioavailability and pharmacokinetics of β -methyl digoxin after multiple oral and intravenous doses. *Eur. J. clin. Pharmac.*, **9**, 373–379.
- SELDEN, R. & SMITH, T.W., (1972). Ouabain pharmacokinetics in dog and man. *Circulation*, **45**, 1176–1182.
- 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.
- SHEINER, L.B., STANSKI, D.R., VOZEH, S., MILLER, R.D. & HAM, J. (1979). Simultaneous modelling of pharmacokinetics and pharmacodynamics: Application to d-tubocurarine. *Clin. Pharmac. Ther.*, **25**, 358–371.
- 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.
- SUMNER, D.J., RUSSELL, A.J. & WHITING, B. (1976). Digoxin pharmacokinetics: Multicompartmental analysis and its clinical implications. *Br. J. clin. Pharmac.*, **3**, 221–229.
- WAGNER, J. (1976). Linear pharmacokinetic equations allowing direct calculation of many needed pharmacokinetic parameters from the coefficients and exponents of polyexponential equations which have been fitted to the data. *J. Pharmacokin. Biopharm.*, **4**, 443–467.
- WEISSLER, A.M., HARRIS, W.S., SCHOENFELD, C.D. (1968) Systolic time intervals in heart failure in man. *Circulation*, **37**, 149–159.

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