THE EFFECT OF DIGOXIN ON ⁸⁶RUBIDIUM UPTAKE BY ERYTHROCYTES FROM MOTHERS AND BABIES

J.G. KELLY, R. McC. McMILLEN & D.G. McDEVITT

Department of Therapeutics and Pharmacology, The Queen's University of Belfast and The Royal Maternity Hospital, Belfast, Northern Ireland.

- 1 The uptake of ⁸⁶rubidium by erythrocytes from mothers and babies has been used as a model system to investigate possible differences in sensitivity to digoxin in the very young.
- 2 While total ⁸⁶rubidium uptake was not significantly different between mothers and babies, the digoxin-sensitive proportion was higher in neonatal erythrocytes. Neonatal cells were less sensitive to digoxin, demonstrated by the requirement for a larger amount of digoxin to inhibit ⁸⁶rubidium uptake and this was accompanied by an increase in numbers of specific erythrocyte binding sites for digoxin.
- 3 These results provide further evidence in support of the hypothesis of decreased sensitivity to digoxin in the very young.

Introduction

The maintenance dose of digoxin administered to adults is generally in the range 2–5 $\mu\text{g kg}^{-1} \text{ day}^{-1}$. This dose is considerably greater in infants and young children however; reference sources suggest doses of the order of 10–25 $\mu\text{g kg}^{-1} \text{ day}^{-1}$ (Nyberg & Wettrell, 1980; Shirkey, 1980). As a result plasma concentrations of digoxin are higher in the very young than in adults (Rogers *et al.*, 1972). Accordingly infants are widely felt to be less sensitive to, or at least more tolerant of, the effects of digoxin than adults (Soyka, 1975). Animal studies tend to support this belief with young dogs (Vargo *et al.*, 1974) and rabbits (Kelliher & Roberts, 1976) showing more tolerance to the toxic effects of digoxin than adult animals.

The mechanisms for this apparent difference in sensitivity could be pharmacokinetic, reflected in differences in the handling of digoxin by the very young, or pharmacodynamic, resulting from a decreased myocardial sensitivity to digoxin in this age group.

Absorption of digoxin is similar in infants and adults (Wettrell & Andersson, 1977). Its elimination rate is slower in premature neonates (Lang & von Bernuth, 1977), but is similar to, or may even exceed that of adults in older infants (Wettrell & Andersson, 1977). Tissue distribution of digoxin in infants, however, differs from that in adults. While the relative distributions are similar in adults and infants, with tissue concentrations in the order ventricular myocardium > kidney > liver > skeletal muscle > plasma

(Andersson *et al.*, 1975), the tissue/plasma concentration ratios are significantly higher in infants. Myocardial uptake of digoxin in infants is twice that of adults and the erythrocyte/plasma concentration ratio in infants is three times that in adults (Gorodischer *et al.*, 1976). This is reflected in larger numbers of specific binding sites for digoxin on erythrocytes from umbilical venous blood (Kearin *et al.*, 1980).

Digoxin binds specifically to Na^+/K^+ ATPase and inhibits its function in a variety of tissues, including erythrocytes. If digoxin produces its main pharmacological effects by occupying a certain fraction of these pump sites, then an increased number of these sites in cells from infants would result in a decreased effect at a given concentration of drug. This would be an explanation for both decreased sensitivity to, and increased tissue binding of, digoxin in infants. Measurement of myocardial Na^+/K^+ ATPase activity in foetal and newborn dogs (Miller & Gilliland, 1972) and lambs (Atwood & Dunkley, 1972) have produced conflicting results with the former workers finding increased activity in newborn animals and the latter finding no change.

As a result of inhibiting Na^+/K^+ ATPase, digoxin inhibits active cellular sodium efflux and potassium uptake. The efficiency of this process can be studied using the kaliomimetic isotope ⁸⁶rubidium. Studies in man have used measurements of erythrocyte ⁸⁶rubidium uptake as a model system for examination of the

cellular effects of digoxin (Aronson *et al.*, 1977). In the present study we have compared the ability of erythrocytes from mothers and neonates to transport ^{86}Rb and have examined the effects of digoxin on this process.

Methods

Subjects

Neonatal blood samples were obtained at delivery from the umbilical cord vein of 14 babies (7M, 7F). Venous blood specimens (15 ml) were obtained from their mothers shortly after birth. Five of the deliveries were Caesarean sections using thiopentone as the anaesthetic agent. Two deliveries were forceps assisted; of these, one mother received an epidural anaesthetic and the other papaveretum. The remaining seven were normal deliveries; two of these mothers received pethidine and the remainder did not receive any drugs.

^{86}Rb uptake by erythrocytes

Erythrocytes were separated by centrifugation and washed three times in a potassium-free Ringer solution (Aronson *et al.*, 1977). Duplicate Erlenmeyer flasks (25 ml) were prepared containing digoxin (0, 15, 30, 60 and 1500 ng). Potassium-free Ringer solution (1 ml) and 0.5 ml washed erythrocytes were added. These mixtures containing 0, 10, 20, 40 and 1000 ng/ml digoxin were incubated, with shaking, for 2 h at 37°C. At the end of this period 0.5 ml of potassium-free Ringer containing 2.3 μg ^{86}Rb (Radiochemical Centre, Amersham, 463 $\mu\text{g}/\text{ml}$, 1 mCi/ml on activity reference date) was added and the incubation continued for a further 1 h. Portions (0.5 ml) of these cell suspensions were then removed and washed three times with potassium-free Ringer solution. After the last wash the supernatant was removed and 50 μl portions of packed cells added to liquid scintillation vials containing 1 ml of isopropanol/toluene (Packard Instruments Ltd), 1:1. After 30 min, 10 ml of a Triton/toluene/PPO based scintillation cocktail was added to each vial and the radioactivity counted in a liquid scintillation counter with an efficiency of 15%. After calculating the specific activity of the ^{86}Rb on the day of counting (decay half-life 18 days), the uptake of rubidium in nmol ml^{-1} cells h^{-1} was estimated in the absence of digoxin and in the presence of digoxin 1000 ng/ml (digoxin insensitive rubidium uptake). From a graph of rubidium uptake (in counts per minute) vs concentration of digoxin the concentration of digoxin required to inhibit digoxin sensitive rubidium uptake by 50% was calculated.

Digoxin binding by erythrocytes

There was sufficient blood in eight of the 14 mother-baby pairs to permit measurement of numbers of specific erythrocyte digoxin binding sites. This assay employed essentially the method of Erdmann & Hasse (1975). Erythrocytes were lysed in distilled water and the membranes were collected after centrifuging at 35,000 g for 30 min (0–4°C). Membranes were resuspended and recentrifuged. The resulting pellet was homogenized in a volume of assay buffer equal to the original volume of blood. Erythrocytes in the whole blood specimens were counted using a haemocytometer. Five samples from each specimen were counted and the mean value used. The estimated coefficient of variation was 4.8%. Aliquots (0.5 ml) of erythrocyte membranes were incubated with tritiated digoxin (10.1 Ci/mmol, New England Nuclear). Concentrations of digoxin (all $\times 10^{-9}$ M) were 2.5, 5, 10, 25, 50, 100. The assay buffer contained 50 mM imidazole/HCl, pH 7.25, 3 mM MgCl_2 and 3 mM imidazole phosphate. The final volume was 1 ml. Assay tubes were incubated for 2 h at 37°C and then centrifuged at 35,000 g (30 min, 0°C) and washed with ice cold buffer (1 ml). Aliquots (0.5 ml) of the pooled supernatant were counted in a liquid scintillation counter to allow calculation of unbound digoxin. The membrane pellets were digested in NaOH and acidified with HCl before counting. The scintillation cocktail was Triton X-100/toluene/PPO based. Samples were corrected for quench by an external standard ratio method. To calculate specific binding, portions of erythrocyte membranes containing the concentrations of tritiated digoxin described above were simultaneously carried through the above procedure in the presence of ouabain 10^{-3} M. Specific binding was calculated for each digoxin concentration as the total amount bound less that remaining in the presence of the saturating concentration of ouabain. Mean specific binding of digoxin was 71% at 2.5×10^{-9} M and fell smoothly to 34% at 100×10^{-9} M. Numbers of binding sites were calculated from Scatchard plots, assuming each site to bind one molecule of digoxin (Erdmann & Hasse, 1975). Results from mothers and babies were compared using the Wilcoxon ranks test for matched pairs.

Results

Figure 1 shows a typical graph of ^{86}Rb uptake vs amount of digoxin added. From these results in each subject, the following were calculated—total ^{86}Rb uptake in nmol ml^{-1} cells h^{-1} ; the amount of ^{86}Rb uptake sensitive to digoxin and the quantity of digoxin which inhibited this by 50%. Total ^{86}Rb uptake by erythrocytes from the mothers ranged from 2.6 to 13.2 nmol ml^{-1} cells h^{-1} with a mean and s.d. of

Figure 1 Effects of increasing amounts of digoxin (ng/ml added) on erythrocyte ^{86}Rb uptake (typical graph from one subject).

$8.6 \pm 3.2 \text{ nmol ml}^{-1} \text{ cells h}^{-1}$. Values in the babies were similar with a range from 2.3 to $16.2 \text{ nmol ml}^{-1} \text{ cells h}^{-1}$ and a mean value of $8.2 \pm 3.6 \text{ nmol ml}^{-1} \text{ cells h}^{-1}$ (Figure 2). The percentage of this uptake which could be inhibited by digoxin was however significantly greater in erythrocytes from the babies (Figure 3). Mean digoxin-sensitive ^{86}Rb uptake was $72.4 \pm 10.9\%$ (range 53.8–88.4) in cells from the mothers; in the babies this was $82.7 \pm 6.9\%$ (range 65.6–91.6).

Figure 2 Uptake of ^{86}Rb (in $\text{nmol ml}^{-1} \text{ cells h}^{-1}$) by erythrocytes from mothers and babies. The means and s.d. are indicated.

This difference was significant ($P < 0.01$). Absolute digoxin-sensitive ^{86}Rb uptake tended to be higher in babies than in their mothers ($7.0 \pm 3.2 \text{ nmol ml}^{-1} \text{ cells h}^{-1}$ in babies; $6.2 \pm 2.6 \text{ nmol ml}^{-1} \text{ cells h}^{-1}$ in mothers) but the difference just failed to reach statistical significance. The amount of added digoxin required to inhibit this uptake by 50% was however significantly larger for the neonatal erythrocytes (Figure 4). This varied from 2.1 to 9.2 ng/ml (mean $6.1 \pm 2.1 \text{ ng/ml}$) in mothers and from 3.3 to 15.4 (mean $7.6 \pm 3.0 \text{ ng/ml}$) in the babies ($P < 0.01$).

In the eight pairs studied neonatal erythrocytes had significantly larger numbers of specific digoxin binding sites than those from the mothers (260 ± 117 and 162 ± 57 sites per cell respectively, $P < 0.05$, Figure 5). The apparent dissociation constant (K_D) for the binding did not appreciably differ between erythrocytes from mothers and babies ($11.7 \pm 3.9 \text{ nM}$ in mothers; $12.4 \pm 4.2 \text{ nM}$ in babies).

There was a significant linear correlation between mothers and babies in respect to total ^{86}Rb uptake (correlation coefficient 0.9, $P < 0.01$), a weaker correlation in respect to the 50% effective concentration of digoxin (correlation coefficient 0.65, $P < 0.05$) and no significant correlation in respect to the percentage of ^{86}Rb uptake which was digoxin-sensitive (correlation coefficient 0.50, $0.1 > P > 0.05$).

Figure 3 Digoxin-sensitive ^{86}Rb uptake (as a percentage of total rubidium uptake) in maternal and neonatal erythrocytes.

There were no differences apparent as a result either of the mothers having received drugs or of delivery by Caesarian section.

Figure 4 Amounts of digoxin (ng/ml added) required to inhibit digoxin-sensitive ^{86}Rb uptake by 50% in maternal and neonatal erythrocytes.

Figure 5 Numbers of specific digoxin binding sites on maternal and neonatal erythrocytes in eight mother-baby pairs.

Discussion

In this study we have used erythrocyte ^{86}Rb uptake as a model system to investigate possible differences in sensitivity to digoxin in the very young. We have demonstrated significant differences between erythrocyte handling of ^{86}Rb in neonates and their mothers. While total ^{86}Rb uptake, in $\text{nmol ml}^{-1} \text{ cells h}^{-1}$, was not significantly different, the proportion of this uptake which was sensitive to digoxin was higher in neonatal erythrocytes. We compared the sensitivity of this system to digoxin in cells from mothers and babies by comparing the amounts of digoxin required to reduce erythrocyte ^{86}Rb uptake by 50%. Neonatal cells were less sensitive to digoxin, demonstrated by the requirement for a larger amount of digoxin to inhibit ^{86}Rb uptake and this was accompanied by an increase in numbers of specific erythrocyte binding sites for digoxin.

Extrapolations from such a peripheral cell model to the heart must, of course, be made with caution. Definitive studies would take two forms. The first would involve a comparison of digoxin binding and measurement of its effects on myocardial tissue from infants and adults. The second would compare cardiac responsiveness to similar concentrations of digoxin in infants and adults using, for example, systolic time interval measurements.

There is evidence, however, to support the relation between our results and the effects of digoxin on the heart. Ford and his colleagues (1979) studied patients in the early phase of digoxin therapy. Unoccupied erythrocyte binding sites were labelled with tritiated digoxin. This allowed estimation of the amount of erythrocyte binding sites unoccupied by the therapeutically administered digoxin (given as $\text{pg } [^3\text{H}]\text{-digoxin bound per } 0.5 \text{ ml cells}$). There was a significant positive correlation between the amount of unoccupied sites and both erythrocyte ^{86}Rb uptake and the rate-correlated systolic time index. Erythrocyte ^{86}Rb uptake also correlated significantly with the rate-corrected systolic time index in these studies.

Recently published animal studies also support extrapolation of our results to the heart. Marsh *et al.* (1981) found that specific myocardial binding of ouabain was significantly higher in young dogs (1–2 weeks and 6–8 weeks old) as compared to adult dogs. Additionally, 6 week old dogs took longer to manifest cardiotoxicity after digoxin administration than did adult dogs. Marsh and colleagues (1981) also measured ouabain-sensitive myocardial ^{86}Rb uptake in dogs and guinea-pigs and found this to be significantly higher in young animals. Values for total ^{86}Rb uptake by myocardium were however, not stated.

One feature of our results is the difference between the proportions of digoxin-sensitive ^{86}Rb up-

take in erythrocytes from mothers and babies. This digoxin-sensitive component represents the contribution of Na⁺K⁺ ATPase to cellular ⁸⁶rubidium uptake. The digoxin insensitive component is made up of other active transport processes and a small diffusion component (Beauge & Adragna, 1971). This digoxin insensitive component accounted for 27% ± 2.8 of total ⁸⁶rubidium uptake in the mothers and 18.7% ± 1.9 in the babies. At present, we cannot tell if this difference extends to other tissues and its physiological significance is unknown.

It may be that the greater specific binding of digoxin and the relative insensitivity to its effects in

cells from the very young are reflections of particular cellular metabolic requirements in the foetus and neonate. Active cation transport is proportional to cellular glycolytic ability (Whittam & Ager, 1965; Parker & Hoffman, 1967) and it has been suggested that increased cellular Na⁺K⁺ ATPase activity is related to the ability of the foetus to withstand hypoxia (Rosen & Sigstrom, 1978).

Irrespective of these speculations, however, our results provide further evidence in support of the hypothesis of decreased sensitivity to digoxin in the very young.

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