Age-Related Differences in Tolerance to Digoxin

Lower Digoxin Concentration in Myocardial Microsomal Fraction in Infant Rats

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SUMMARY

To clarify the mechanism responsible for age-related differences in tolerance to digitalis, we studied the potassium contents of serum and myocardium and the digoxin concentrations of serum, myocardium, and myocardial microsomal fraction in infant and adult rats. While serum potassium concentration was the same in both infant and adult rats, the potassium content of myocardium found in the infant rats was significantly higher than that in the adult ones. Digoxin concentration of serum in infant rats was lower than that in the adult ones after a bolus injection of the same dose of digoxin per kilogram of body weight was given. Digoxin concentration of microsomal fraction, obtained 1 hour after the administration of 0.2 mg/Kg of digoxin in infant rats, was lower than that obtained 1 hour after the administration of 0.1 mg/Kg of digoxin in adult ones even though the digoxin concentrations of serum and myocardium in infant rats were significantly higher than those obtained in adult ones. Thus, less sensitivity to digitalis found in infant rats may be attributable to the higher potassium content of myocardium and the lower digoxin concentration of microsomal fraction.

Additional Indexing Words:
Digoxin  Potassium  Microsomal fraction  Tolerance to digitalis

Clinical experiences suggest that infants are less sensitive to digitalis and that they require more digitalis per kilogram of body weight than adult patients to achieve similar serum levels.\(^1^\)-\(^3^\) Some experimental studies in animals and humans have confirmed that there are no differences in absorption, metabolism, and excretion of digitalis between infants and adults.\(^4^\),\(^5^\) But as reported by Glantz et al,\(^6^\) mature subjects showed higher levels of digitalis in serum and in myocardium for the same dose of...
digitalis per kilogram of body weight and one could account for this difference by the fact that young subjects had larger plasma volume and interstitial fluid space than did adult ones. Berman et al.\(^7\) also reported that levels of digoxin in plasma and tissue differed between fetal and adult sheep, while at the same time the tissue-plasma ratios were similar. They also studied the relationship of pre-ejection period-left ventricular ejection time ratio (PEP/ET) and the prolongation of the PR interval to concentrations of digoxin in plasma taken from fetuses and ewes and concluded that age-related differences in inotropic and toxic effects of digoxin were related to differences in response to the drug rather than drug kinetics. They suggested that these age-related variation might be due to differences in sensitivity of the target organs to the drug such as variety of myocardial receptor affinity for digitalis or myocardial receptor density. In order to clarify one aspect of this unsolved problem, we studied potassium concentrations in serum and myocardium, digoxin concentrations in serum, myocardium, and especially in microsomal fraction which might contain cardiac digitalis receptors in both infant and adults rats.

**METHODS**

Three-week old infant Wistar rats weighing 50–60 Gm and 8-week old adult ones weighing 200–330 Gm were used in this study.

Experiment 1; Tolerance to digoxin was examined in 10 infant and 10 adult rats by intraperitoneal injection of large dose of digoxin (5 mg/Kg). The electrocardiograms were recorded before and after the digoxin injection in some of these animals. Survival rate in each group was observed.

Experiment 2; Potassium concentrations in serum and myocardium and water content in myocardium were determined in 8 infant and 8 adult rats in control condition without any administration of digoxin.

Experiment 3; Digoxin concentration in serum and myocardium was determined after injection of digoxin. Digoxin of 0.1 mg/Kg was injected intraperitoneally. They were decapitized 30, 60, 90, 120, and 150 min after injection and the digoxin concentration in the serum was determined. Sixty min after injection, the digoxin concentration of myocardium was also determined in 8 infant and 13 adult rats. In addition, digoxin of 0.2 mg/Kg was administered intraperitoneally and the digoxin concentration in the serum and myocardium was measured 60 min after the injection in 9 infant rats.

Experiments 4; Digoxin concentration was determined in the microsomal fraction of myocardium after injection of digoxin. Digoxin of 0.2 mg/Kg and 0.1 mg/Kg was injected intraperitoneally in 27 infant and 18 adult rats, respectively. They were decapitized 60 min after the injection. Their hearts were used for determination of digoxin concentrations in microsomal fraction. Because the hearts were not large enough to obtain a sample for the measurement of microsomal fraction, every sample was composed of 3 hearts in infant rats and 2 hearts in adult ones. Digoxin concentration in microsomal fraction was determined in 9 samples in each group.
Biochemical analysis of plasma and tissue:

Potassium levels in serum were determined by flame photometry. Potassium concentrations in myocardium were measured by modified Graham's method.\(^8\)

After visible fat was removed, the heart muscle, weighing 60–200 mg, was weighed in dry, dust-free and preweighed glasses. The water content of the sample was determined by weighing the glass and sample after drying at 90°C under vacuum for 6 hours. The dry muscles were extracted with 1.5 M HNO\(_3\) for overnight. Potassium levels were determined on a flame photometer in duplicate using lithium as internal standard. Myocardial digoxin was extracted as follows:\(^9\) Tissue samples, weighing 60–150 mg, were homogenized in absolute ethanol for 30 sec with Labor-Dispergiergerät X1020 (LADO, West Germany). The homogenates were centrifuged at 1000×g for 10 min and supernatants were removed. The precipitates were twice re-extracted in additional absolute ethanol. The combined supernatants were evaporated to dryness, and resuspended in Tris-buffer (pH 7.4) containing 5% bovine serum albumin. After injection of \(^{125}\)I digoxin to adult rats, 94±3% of myocardial radioactivity was extracted by this method. The microsomal fraction was extracted as follows:\(^10\) the cardiac muscles, weighing 1.5 to 2 Gm, were sliced and homogenized in sucrose-EDTA (ethylen diamine tetra-acetic acid) solution (0.33 M sucrose: 0.001 M EDTA). Homogenization was carried out in an ice bath for 2 min with Labor-Dispergiergerät X1020 (LADO, West Germany), followed by centrifugation at 4°C at 166,000×g for 1 hour. The particulate pellet was resuspended in 10 volumes of cold sucrose EDTA solution. The solution was centrifuged at 450×g for 10 min, then at 12,000×g for 15 min. The microsomal fraction was separated by centrifuging the final supernatants at 166,000×g for 1 hour. Fig. 1 shows an electron micrograph of microsomal fraction extracted by this method. The microsomal pellet was suspended with a known amount of pure water using Vortexmixer. A known aliquot was taken for

Fig. 1. The electron micrograph of the microsomal fraction of the heart muscle extracted for the determination of the digoxin concentration. Magnification, ×20,000.
protein determination by Lowry's method.\textsuperscript{11}) Other representative samples were evaporated to dryness using vacuum evaporator and resuspended in Tris-buffer (pH 7.4) containing 5% bovine serum albumin. Digoxin concentrations were measured by using radioimmunoassay kit labelled by $^{125}$I (Abbott Laboratory). Assays were performed in duplicate. Preliminary experiments were performed and the variation between repeated determinations of digoxin in serum and Tris-buffer containing 5% bovine serum albumin was less than 5%.

\textit{Statistical analysis:}

Student's $t$-test was used for statistical analysis.

\section*{Results}

1. Tolerance to digoxin

While no infant rat died by intraperitoneal injection of digoxin of 5 mg/Kg, 8 out of 10 adult rats died by injection of the same dose of digoxin. A series of electrocardiograms, taken from a rat which died after injection of digoxin, are shown in Fig. 2. In this case, sinus bradycardia and the pro-
longation of the PR interval were observed at first and then the QRS intervals were prolonged.

2. Potassium levels in serum and myocardium

While there was no difference between the serum potassium concentrations of infant and adult rats (3.9±0.4 mEq/L in infant rats and 3.9±0.4 mEq/L in adult ones), the potassium level of myocardium in infant rats (392±10 mEq/dry weight Kg) was significantly higher than that in the adult ones (367±18 mEq/dry weight) p<0.01. The water content of myocardium was 78±1% in both infant and adult rats (Table 1).

3. Digoxin concentration in serum and myocardium

Fig. 3 shows the change of serum digoxin concentrations after intraperitoneal administration of 0.1 mg/Kg of digoxin in infant and adult rats. The averages of serum digoxin concentration in the adult rats were always higher than those in the infant rats (Table II). But both of the curves showed almost the same shape. Serum digoxin concentration, digoxin concentration in myocardium and myocardium-serum ratios 1 hour after intraperitoneal administration of 0.1 mg/Kg of digoxin in both infant and adult rats are

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<tr>
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<th>Infant rats</th>
<th>Adult rats</th>
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<tr>
<td></td>
<td>n Mean±SD</td>
<td>n Mean±SD</td>
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<tr>
<td>Serum potassium concentration (mEq/L)</td>
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<td>8 3.9±0.4</td>
<td>NS</td>
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<tr>
<td>Potassium level of myocardium (mEq/Kg)</td>
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<td>8 367±18</td>
<td>&lt;0.01</td>
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<td>Water content (%)</td>
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<td>8 78±1</td>
<td>NS</td>
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NS=not significant; SD=standard deviation.

Fig. 3. After a dose of 0.1 mg/Kg of digoxin, the average value of the serum digoxin concentration in adult rats remains higher than that in infant rats, although both curves have almost the same shape.
Table II.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Serum Digoxin Concentration (ng/ml)</th>
<th>Adult rats</th>
<th>P</th>
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<tr>
<td></td>
<td>Infant rats</td>
<td>Adult rats</td>
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<td></td>
<td>n</td>
<td>Mean±SD</td>
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<td>60</td>
<td>8</td>
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<tr>
<td>90</td>
<td>4</td>
<td>6.5±0.6</td>
<td>5</td>
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<tr>
<td>120</td>
<td>6</td>
<td>4.4±0.3</td>
<td>6</td>
</tr>
<tr>
<td>150</td>
<td>4</td>
<td>3.7±1.6</td>
<td>5</td>
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</tbody>
</table>

NS=not significant; SD=standard deviation.

Fig. 4. The digoxin concentrations in serum and myocardium in infant rats are significantly lower than those in adult ones, but the myocardium-serum ratios are the same in both infant and adult rats.

shown in Fig. 4. The serum digoxin concentration in infant (7.7±1.1 ng/ml) was significantly lower than that (10.2±1.6 ng/ml) in adult. The digoxin concentration in myocardium in infant rats (28.2±7.0 ng/Gm) was also significantly lower than that (39.8±8.8 ng/Gm) in adult rats (p<0.01), but the myocardium-serum ratios of infant and adult rats were not different. The serum digoxin concentration (19.9±4.1 ng/ml) and the digoxin concentration in myocardium (63.4±7.8 ng/Gm), obtained 1 hour after administration of 0.2 mg/Kg of digoxin in the infant rats, were significantly higher than those obtained 1 hour after the administration of digoxin of 0.1 mg/Kg.
in the adult rats. But there was still no significant difference in the myocardium-serum ratios of the infant and adult rats (Fig. 5).

4. Digoxin concentration in microsomal fraction of myocardium

In order to obtain almost the same levels of serum and myocardial digoxin concentrations as those in adult rats given 0.1 mg/Kg of digoxin,
double amount of digoxin (0.2 mg/Kg) were administered to the infant rats. However, the digoxin concentrations in the serum and myocardium of the infant rats to which 0.2 mg/Kg of digoxin had been given, were significantly higher than those of adult ones given 0.1 mg/Kg of digoxin. The average of digoxin concentration in microsomal fraction of myocardium in the infant rats (0.4±0.2 ng/mg protein) was lower than that in the adult one (0.5±0.2 ng/mg protein), although it was not significant (Fig. 6).

DISCUSSION

In this study, it was clearly demonstrated that infant rats had more tolerance to digoxin of the same dose per kilogram of body weight. In infant rats, serum and myocardial digoxin concentrations were lower than those in adult rats, however, the pharmacokinetic curves showed almost the same shape as adult rats when the same dose of digoxin per kilogram was administered. These results were consistent with those previously reported by Glantz et al. In addition, digoxin concentrations in microsomal fraction, which might contain digitalis receptors, showed almost the same level in both infant and adult rats in spite of higher myocardial digoxin concentration in infant rats. This fact seems very interesting when we consider Berman’s and Atwood’s results. Berman reported that age-related differences in inotropic and arrhythmogenic effects of digoxin existed and were related to differences in drug response rather than drug kinetics. Atwood et al reported that there was no significant difference in the effects of oubain on Na-K ATP-ase activity between fetal and adult sheep. Accordingly, lower concentrations of digoxin in microsomal fraction may be partly responsible for increased tolerance to digoxin in infant rats.

Another important factor which may contribute variations of digitalis effect is potassium. There are 2 opposite opinions concerning digitalis effect on Na-K ATP-ase, one is inhibition and the other is stimulation. However, it seems well recognized that a toxic dose of digitalis inhibits Na-K ATP-ase and reduces potassium level of myocardium. Higher myocardial potassium concentration in infants may have a potential effect to prevent a decrease in the myocardial potassium in digitalis intoxication.

In conclusion, higher tolerance to digitalis administration in an infant rat may be attributable to several factors including 1) lower digoxin concentration in serum and myocardium when a bolus injection of the same dose of digoxin per kilogram of body weight is given 2) almost the same digoxin concentration in microsomal fraction as adult rats even though a higher digoxin concentration in serum and myocardium is present, and 3) the higher
potassium level in myocardium.

REFERENCES