Enhanced Sensitivity to Digoxin in Dystrophic Mice

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Summary

We estimated the effect of digoxin on the myocardial potassium content and the action potentials of the left ventricular papillary muscles in dystrophic mice (C57BL/6J CL dy·dy). All of 10 dystrophic mice died following ip injection of digoxin at a dose of one quarter of the iv LD50 for normal mice. The myocardial digoxin concentration and the myocardial potassium content in dystrophic mice were similar to those in normal mice before and 60 min after the ip injection of digoxin.

The action potential durations (APD) in dystrophic mice were significantly longer than those in normal mice. Perfusion of digoxin (2 µg/ml) for 30 min reduced the APD significantly and induced arrhythmias in dystrophic mice, but it did not bring about any significant change in normal mice.

These data suggest that dystrophic mice have increased sensitivity to digitalis. This hypersensitivity to digitalis is not due to increased myocardial digoxin uptake or decreased myocardial potassium content.

Additional Indexing Words:
Dystrophic mice       Myocardial digoxin       Hypersensitivity
Action potential      Cardiomyopathy

Patients with progressive muscular dystrophy (PMD), which is often complicated by cardiomyopathy, usually develop congestive heart failure for which a standard medical regimen of digitalis is obviously indicated. It is also recognized that the myopathic heart including that caused by PMD is sensitive to even small doses of digitalis. In order to clarify the mechanism of the hypersensitivity to digitalis in patients with PMD, we estimated the
myocardial digoxin uptake and the effect of digoxin on myocardial potassium content and the action potentials of the left ventricular papillary muscle in dystrophic mice.

Methods

Eight-week-old homogenous normal and dystrophic mice of the C57BL/6JCLdy•dy strain of either sex were used in this study.

Experiment I

Tolerance to digoxin was examined by an intraperitoneal (ip) injection of digoxin at a dose of 5 mg/Kg (a quarter of the iv LD50) in 10 normal and 10 dystrophic mice, after which the survival rate in each group was observed.

Experiment II

The potassium content of the left ventricular muscle, before and 60 min after an ip injection of digoxin (5 mg/Kg), was determined in 8 normal and 8 dystrophic mice.

Experiment III

The digoxin concentrations in the left ventricular muscle, 60 min after an ip injection of digoxin (5 mg/Kg), were measured in 10 normal and 10 dystrophic mice.

Experiment IV

The effect of digoxin (2 μg/ml) on the action potentials of the left ventricular papillary muscles was examined in 10 normal and 10 dystrophic preparations. Mice were anesthetized with sodium pentobarbital, 30 mg/Kg ip injection. The heart was quickly removed and dissected in oxygenated Tyrode's solution and the left ventricular papillary muscles were excised. The preparations were mounted in a 2 ml volume tissue chamber perfused with Tyrode's solution, gassed with 95% O₂ and 5% CO₂, at a constant flow rate of 1.8 ml/min using a micro-tube pump (Atto, SJ-1211). The temperature of the chamber was maintained at 36–37°C. The Tyrode's solution had the following composition (mM): NaCl 137, KCl 2.7, CaCl₂ 1.8, MgCl₂ 1.0, NaHCO₃ 11.9, NaH₂PO₄ 0.42, glucose 5.5, pH 7.4. Rectangular pulsed currents 2 msec in duration and twice diastolic threshold in intensity were delivered at a cycle length of 200 msec through bipolar silver wires. The transmembrane potentials were measured by means of microelectrode penetrations of second layer cells of the endocardial surface. Approximately 10 penetrations were done in each preparation for the recording of action potentials. All microelectrodes were filled with 3M KCl and had a resistance of 10–30 MΩ. The microelectrode was coupled to the input of an amplifier with neutralized input capacity, and the recorded potential was displayed on
Vol. 25 No. 5 ENHANCED SENSITIVITY TO DIGOXIN IN DYSTROPHIC MICE 767

an oscilloscope. The maximal rate of depolarization (dV/dt) was obtained by an electronic differentiator and was displayed on the other beam of the oscilloscope. Each preparation was driven for 30 min to ensure equilibration. After obtaining control recordings, the preparations were superfused with Tyrode's solution containing digoxin (2 µg/ml) for 30 min. The duration of the plateau was measured at the 50% and 80% level of full repolarization.

The myocardial potassium was extracted with 1.5 mol·liter⁻¹ HNO₃ overnight and its level determined on a flame photometer in duplicate using lithium as an internal standard. Myocardial digoxin was extracted with absolute ethanol and measured using a radioimmunoassay kit labelled with ¹²⁵I. Values are expressed as mean±standard deviation. Statistical analysis was performed with Student's t-test.

RESULTS

1. Tolerance to digoxin

While no normal mouse died following ip injection of digoxin (5 mg/kg), all of 10 dystrophic mice died 60 min or later after ip injection of the same dose of digoxin.

2. Potassium content of the left ventricular muscle

The average values of myocardial potassium before the injection of digoxin for normal and dystrophic mice were 344±12 mEq/kg FFS and 340±11 mEq/kg FFS, respectively, and the difference was not statistically significant. The average values of myocardial potassium 60 min after the injection of digoxin (5 mg/Kg) for normal and dystrophic mice were 319±15 mEq/Kg FFS and 312±15 mEq/Kg FFS, respectively. Thus, there was no difference in the effect of digoxin on myocardial potassium level between normal and dystrophic mice (Fig. 1).

3. Myocardial digoxin level

The average values of myocardial digoxin 60 min after the ip injection of digoxin (5 mg/Kg) for normal and dystrophic mice were 261.2±54.9 ng/10⁻¹ g and 259.4±86.4 ng/10⁻¹ g, respectively. The difference was not significant.

4. Transmembrane potential characteristics

The following parameters are shown in Table I: resting potential (RP), overshoot (OS), action potential amplitude (AP), maximal rate of depolarization (dV/dt) and 50% and 80% action potential duration (APD50 and APD80), all of which were measured before and after perfusion of digoxin.

Before perfusion of digoxin, the RP, OS, AP and dV/dt of dystrophic
mice were significantly lower than those of normal mice and the APD50 and APD80 of dystrophic mice were significantly longer than those of normal mice (p<0.001). Although perfusion of digoxin did not bring about any significant change in the RP, OS, AP, dV/dt, APD50 and APD80 of normal mice, the perfusion significantly reduced the APD50 and APD80 of dystrophic mice (p<0.001). Fig. 2 shows representative recordings of action potentials in normal and dystrophic mice. Furthermore, the superfusion of digoxin induced arrhythmias in 7 of 10 preparations of dystrophic mice although no arrhythmia was induced by digoxin in normal mice (Fig. 3). The average values of myocardial digoxin 30 min after superfusion of Tyrode's solution containing digoxin (2 μg/ml) for normal and dystrophic mice were 119.0±24.2 ng/10^-1 g and 122.5±21.3 ng/10^-1 g, respectively and the difference was not significant.
Table I. Effect of Digoxin on Action Potential of the Left Ventricular Papillary Muscle in Normal and Dystrophic Mice

<table>
<thead>
<tr>
<th></th>
<th>Rp (mV)</th>
<th>Os (mV)</th>
<th>Ap (mV)</th>
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<tbody>
<tr>
<td></td>
<td>normal</td>
<td>dystrophy</td>
<td>normal</td>
</tr>
<tr>
<td>Control</td>
<td>81±5</td>
<td>75±4</td>
<td>26±4</td>
</tr>
<tr>
<td></td>
<td>(n=100)</td>
<td>(n=97)</td>
<td>(n=100)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>81±5</td>
<td>75±5</td>
<td>25±4</td>
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<td></td>
<td>(n=101)</td>
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<tr>
<td>p</td>
<td>NS</td>
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<td>NS</td>
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</table>

Values in this table are expressed as mean±standard deviation.
Rp=resting potential; Os=overshoot; Ap=action potential amplitude; APD_{50}=50% duration of action potential; APD_{80}=80% duration of action potential; dV/dt=maximal rate of depolarization; NS=not significant.
×: p<0.001.

DISCUSSION

It is well recognized that patients with primary and secondary cardiomyopathy are sensitive to digitalis. In order to clarify the mechanism of hypersensitivity to digitalis, it seems essential to estimate myocardial digoxin uptake, myocardial potassium content, myocardial Na-K ATPase activity.

A dystrophic mouse is an animal model of human muscular dystrophy and the myocardium of this animal has been reported to be involved. In order to clarify the hypersensitivity to digitalis in patients with PMD, we used dystrophic mice. All dystrophic mice died following ip injection of digoxin at a dose of one quarter of the iv LD50 for normal mice. We were able to obtain ECG recordings during the terminal event in two dystrophic mice. Both of them showed ventricular fibrillation. The myocardial digoxin uptake of dystrophic mice, both in vivo and in vitro, was similar to that of normal mice. It is concluded therefore, that the higher myocardial digoxin uptake was not responsible for the enhanced sensitivity to digoxin seen in dystrophic mice.
Fig. 2. The effect of digoxin on the action potentials of left ventricular papillary muscles in normal and dystrophic mice. Note the apparent prolongation in action potential duration (APD) of a dystrophic mouse. The APD of a dystrophic mouse shows a decrease following perfusion of digoxin, though that of a normal mouse shows no such change. The upper trace shows transmembrane potential and lower one maximal rate of depolarization. Bars (−) indicate zero line.

It is well recognized that a large dose of digitalis inhibits Na-K ATPase and reduces the level of myocardial potassium. If the level of myocardial potassium in dystrophic mice is reduced compared to that in normal mice or is reduced more than in normal mice by the same dose of digoxin, it may play an important role in the enhanced sensitivity to digoxin seen in dystrophic mice. However, the myocardial potassium levels in dystrophic mice before and 60 min after the ip injection of digoxin were similar to those in normal mice. While there was no difference in the myocardial potassium content between dystrophic and normal mice, the RP of the dystrophic cell was significantly lower than that of the normal cell. As the basis of the RP is understood in terms of the electrochemical gradient for potassium that exists across the sarcolemma, the lower RP of the dystrophic cell may be explained by either a decreased outward current or an increased inward background current. Rossner et al performed an electrophysiological study on the cardiac papillary muscle of the cardiomyopathic Syrian hamster (BIO 14.6) and found that the plateau of the action potential of the myopathic cell was longer than that of the normal cell. In our study, the plateau of the action potential of
the dystrophic cell was also significantly longer than that of the normal cell. This finding could be explained by the presence of either an increased slow inward current, a decreased late outward current or both. In any case, it is clear that the membrane permeabilities of the myocardial cells are abnormal in dystrophic mice. The most interesting finding of our study was that the same level of myocardial digoxin reduced the plateau of the action potentials and induced arrhythmias in dystrophic mice, in spite of little effect on the action potentials of normal cells.

It is well known that digitalis inhibits Na-K ATPase. As a result, intracellular sodium ([Na$_i$]) increases, which in turn cause an increase in intracellular calcium ([Ca$_i$]) via the Na-Ca exchange system. This increase in [Ca$_i$] causes an increase in K$^+$ permeability and increased outward current during plateau and results in a decrease in APD.$^{12}$ With toxic concentration of digitalis, [Ca$_i$] is further increased. It seems that the augmented [Ca$_i$] may cause a transient oscillatory change in [Ca$_i$] and may result in oscillatory after-potentials (OAP)$^{13-15}$ which seems to be an important mechanism for the induction of arrhythmias by digitalis intoxication.$^{16}$

Because the [Ca$_i$] of the myopathic cell is reported to be increased in dystrophic mice$^{8}$ as well as in the cardiomyopathic Syrian hamster,$^{17}$ OAP may easily be induced by digoxin in dystrophic mice. Further studies should
be done to confirm this possibility.

In conclusion, dystrophic mice have an enhanced sensitivity to digoxin which is not attributable to increased myocardial digoxin or decreased myocardial potassium.

REFERENCES