Augmentation of Myocardial Digoxin Concentration in Hemorrhagic Shock


SUMMARY

The effect of the shock state on myocardial digoxin uptake and plasma digoxin levels was examined in unanesthetized dogs following hemorrhage. Five minutes after intravenous administration of tritiated digoxin the myocardial digoxin content in animals with shock was greater than in normal animals in both left ventricle (LV) (165 ± 15 (sn) ng/g vs 130 ± 26 ng/g, P < 0.02) and right ventricle (RV) (142 ± 13 ng/g vs 111 ± 22 ng/g, P < 0.02) as was the plasma digoxin concentration (61.6 ± 11.8 ng/ml vs 43.3 ± 4.6 ng/ml, P < 0.02). After one hour, in another group of dogs, the difference in myocardial concentration of digoxin between test and normal groups was even greater (LV: 213 ± 26 ng/g vs 133 ± 13 ng/g, P < 0.001; RV: 171 ± 9 ng/g vs 111 ± 8 ng/g, P < 0.001) despite lower plasma digoxin concentration in the test group (12.9 ± 2.9 ng/ml vs 17.3 ± 2.5 ng/ml, P < 0.05). Diminished peripheral blood flow, peripheral digoxin delivery and uptake were probably responsible for the early difference in plasma digoxin levels. Resultant greater plasma concentrations of digoxin presented to the myocardium in the early phase, coupled with relative preservation of myocardial blood flow, may explain the greater myocardial uptake in animals with shock although myocardial mechanical factors may also be implicated. Augmented uptake of digoxin by the myocardium in canine hemorrhagic shock may be relevant to the altered susceptibility to glycoside action in clinical shock syndromes.

Additional Indexing Words:
Glycosides
Digitalis

While the role of digitalis glycosides in the treatment of subjects with various shock syndromes has recently been questioned, the glycosides are nevertheless often used in the management of cardiogenic and other types of shock. One determinant of the myocardial action of cardiac glycosides is their myocardial concentration as shown, for example, by the relation between myocardial content of a glycoside and the magnitude of the inotropic response induced. The myocardial content of a glycoside is also undoubtedly important in the pathogenesis of digitalis cardiotoxicity, although other factors such as the state of the myocardium and the ionic milieu are probably equally so. The last of these, in particular the extracellular concentration of potassium and of sodium also influences glycoside uptake by the myocardium. Despite recent interest in factors affecting myocardial glycoside uptake and the frequent use of digitalis in the shock syndrome, it is not known whether the shock state influences myocardial uptake of digitalis glycosides. We have recently found (unpublished observations) that anesthesia and thoracotomy result in increased myocardial digoxin concentration in the dog, and this finding prompted us to examine the influence of shock on myocardial digoxin uptake. Hemorrhage was used to produce a moderate hypotensive state in unanesthetized dogs. We then compared the myocardial content of digoxin five minutes and one hour after intravenous administration with that in normal animals.

Methods

Mongrel dogs (7.7 to 14.3 kg) lay on their side placated by an attendant. A femoral artery was exposed using lidocaine 1.0% for local anesthesia. A fine polyethylene cannula (22 gauge) was passed through a trocar into the artery and left indwelling without occluding the artery. Alternatively a small side branch was cannulated. Arterial pressure was measured using a Statham P23 AA transducer and was recorded on a multichannel Hewlett Packard direct writing recorder.

The animals were bled from a cannula placed in the superior vena cava through an external jugular vein. Those subsequently studied five minutes after the administration of digoxin were bled of 30 ± 10 (sd) ml/kg over a period of 15 to 35 min and those studied one hour after digoxin were bled of 32 ± 3 ml/kg over 12 to 21 min. Blood pressure of the former group was reduced from an average of 155/102 mm Hg to 71/46 mm Hg and that of the latter group from

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158/115 mm Hg to 70/45 mm Hg measured 10 min after the completion of bleeding and immediately before digoxin administration (table 1).

$12 \alpha - ^3H$ digoxin (0.05 mg/kg body wt; specific activity 120 $\mu$Ci/mg digoxin) (New England Nuclear Corp., Boston Mass.), shown to be radiochemically homogeneous by thin layer chromatography and radioscanning, was given intravenously over one minute. The myocardium was sampled either 5 or 60 min later, following rapid excision of the heart under intravenous pentobarbital anesthesia. Duplicate samples (0.4–1.0 g) were taken from anterolateral free wall of left ventricle, free wall of right ventricle, left atrium, and from hind limb skeletal muscle. As previously described the samples were blotted dry, weighed, homogenized in 8.0 ml of 50% ethanol in water, extracted with the addition of a further 80 ml of absolute ethanol containing 2.0 mg of unlabelled carrier digoxin, and centrifuged at 2100 g for 40 to 60 min. The supernatant was vacuum dried. The residue after drying was redissolved in a mixture of 5.0 ml of absolute ethanol and 45 ml of scintillant, the latter containing 4.5 g of 2,5-diphenyloxazole (PPO), and 0.09 g of 1.4-bis-2-(4-methyl-5-phenyloxazolyl) benzene (M2 POPOP) and toluol to 1 liter. Paired 20 ml aliquots were counted in a Nuclear Chicago Liquid Scintillation Counter. Quenching was corrected by External Standards Ratio Method and results are expressed as ng digoxin/g wet weight of myocardium. The recovery of tritiated digoxin added to myocardial samples in known amounts was 91–93% using these methods, as previously described.* Following in vivo digoxin administration, estimation of myocardial digoxin was 91 ± 2% of that using a method involving solubilization of myocardium (Soluene, Packard). Since myocardial digoxin is referred to wet weight of myocardium the results could differ from those referred to dry weight if there was a fluid shift from, or to, the myocardium as a result of hemorrhage. To account for any but a trivial part of the 50% or so alteration in digoxin content of the myocardium associated with shock, a massive fluid shift would be required. That this did not occur is apparent from the similarity of myocardial to body weight ratios in shocked and normal animals.

Plasma digoxin was prepared for counting by the addition of 0.2 ml of plasma to 20 ml of scintillant mixture, the latter containing 4.5 g of 2,5-diphenyloxazole (PPO), 0.09 g of 1.4-bis-2-(4-methyl-5-phenyloxazolyl) benzene (M2 POPOP), 300 ml "Triton X - 100" and toluol to 1 liter.

Six animals with shock were studied at each of the two time intervals after digoxin administration. Their results were compared with those of six normal animals studied at the same time intervals but without preceding hemorrhage or measurement of arterial pressure. The mean weight of animals with shock (10.5 ± 1.7 kg) was not different from normal controls (11.0 ± 1.4 kg).

The significance of difference between group means was evaluated by Student's $t$-test.

**Results**

Table 1 presents blood pressures at various times in the animals subjected to hemorrhage, as well as heart rates and some biochemical parameters of the shocked and normal animals. Heart rate in both five minute and one hour shock groups was significantly higher than in their respective control groups. A mild metabolic acidosis with respiratory compensation was present in the animals with shock. Plasma potassium

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**Table 1**

<table>
<thead>
<tr>
<th>Parameters Defining the Shock and Normal Control Groups</th>
<th>Before bleeding</th>
<th>After bleeding</th>
<th>Arterial pressure</th>
<th>pH</th>
<th>Hemoglobin (g/L)</th>
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<tbody>
<tr>
<td>Volume bleed mL</td>
<td>30 ± 10</td>
<td>155/102 ± 19/4</td>
<td>100</td>
<td>71/40 ± 6/4</td>
<td>84/54 ± 14/7</td>
</tr>
<tr>
<td>5-minute study groups</td>
<td>Shock (6)</td>
<td>Normal (6)</td>
<td>Shock (6)</td>
<td>Normal (6)</td>
<td></td>
</tr>
<tr>
<td>Six-minute study groups</td>
<td>32 ± 3</td>
<td>183/115 ± 28/26</td>
<td>60 ± 24</td>
<td>70/45 ± 6/6</td>
<td>70/50 ± 6/6</td>
</tr>
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</table>

Values are means ± 1 so.

* $P < 0.005$; ** $P < 0.002$; $P < 0.001$; $\delta = P < 0.005$.
immediately before sacrifice was significantly lower in the one hour test group of animals than in their control group, the reason for which is unclear. There were no differences in plasma sodium, chloride, bicarbonate or creatinine, which are not included in the table.

Myocardial, skeletal muscle and plasma digoxin concentrations are presented in table 2.

Five minutes after digoxin administration there was a significantly greater concentration of digoxin in the left and right ventricular myocardium and in the left atrium of animals with shock than in normal animals. At this time the plasma digoxin level was also higher than in normal animals, but the skeletal muscle concentrations were not significantly different in the two groups.

Sixty minutes after digoxin administration, the myocardial digoxin content in normal animals was similar to that five minutes after administration, but the myocardial content of the animals with shock was significantly greater than it was after five minutes. Thus the difference between the myocardial content of digoxin in shocked and normal animals was even greater sixty minutes after administration than it was after five minutes, indicating a 50 to 60% increase in myocardial digoxin uptake in shock. This was so despite a significantly lower plasma level in shocked than in normal animals one hour after digoxin administration. The myocardial to plasma digoxin concentration ratio at this time was significantly greater in shocked animals. Skeletal muscle digoxin content was significantly lower while the ratio of myocardial to skeletal muscle digoxin concentration was significantly greater.

Discussion

The moderately severe hypotensive state induced by hemorrhage resulted in greater myocardial concentration of digoxin following intravenous administration of the glycoside. In animals with hypotension, myocardial digoxin content was approximately 25% greater than normal five minutes after administration and 50 to 60% greater after one hour. At the latter time, the plasma digoxin concentration was, in contrast, less in the hypotensive animals, so that there was a dissociation between plasma and myocardial levels.

Although myocardial digoxin content was greater in hypotensive animals at both times studied there was a delay in reaching maximum content which was not found in normal animals. The results in our normal animals may appear somewhat surprising in view of the results of other workers. Doherty and Perkins found that the myocardium of anesthetized dogs contained considerably less digoxin 30 minutes after intravenous administration than it did after one hour. Deutscher et al. found only half the maximum myocardial concentration of digoxin after five minutes in open chest anesthetized dogs, although the concentration was near maximum by 15 minutes. We have confirmed the delay in attainment of the one hour myocardial digoxin level in anesthetized open chest dogs and also have found that absolute myocardial concentration is greater than in unanesthetized animals. That is, the effect of anesthesia and thoracotomy seems to be similar to that we are reporting here and we attribute this to the altered hemodynamic conditions produced by these interventions. The observation of early attainment of maximum myocardial concentration in the normal animals does not conflict with the well known delay in onset of digoxin's inotropic effect; Deutscher et al. have also found a time lag between myocardial concentration and onset of inotropic effect.

The relevance of the unanesthetized state to the study of myocardial digoxin uptake has just been stressed. Other workers who have studied the dis-

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<td>Myocardial, Skeletal Muscle and Plasma Digoxin Content</td>
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<td>Five-minute study groups</td>
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<td>Shock (6)</td>
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<td>Normal (6)</td>
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<td>Significance of difference between groups, P</td>
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<td>Sixty-minute study groups</td>
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<td>Shock (6)</td>
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<td>Normal (6)</td>
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<td>Significance of difference between groups, P</td>
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Abbreviations: LV = left ventricle; RV = right ventricle; LA = left atrium; SK = skeletal muscle; P = plasma; wt = weight.

*Values are means ± 1 SD.
ordered physiology of shock also have emphasized the importance of studying the awake unanesthetized animal if possible. Only relatively mild hemorrhagic shock can be studied in this way without the animal becoming unduly distressed, but, in view of this, the effect on myocardial digoxin uptake is all the more impressive. The avoidance of anesthesia or sedation clearly sets a limit on documentation of the coexisting hemodynamic abnormalities. For example, we did not measure myocardial, renal or other organ blood flow, cardiac output, or urinary excretion of digoxin. The latter amounts, at the most, to only 5 to 10% of an intravenous dose of digoxin within the first hour, and change in this fraction could not have critically influenced the results. Hemodynamic changes occurring with regimes of progressive hemorrhage similar to that used here have been fairly well documented in both anesthetized and unanesthetized animals, and we did not feel it necessary to repeat these measurements. Such hemorrhage is associated with decreases in cardiac output and peripheral and myocardial blood flow. Myocardial blood flow is, however, relatively well maintained compared to the average systemic blood flow.

The explanation of our results, based upon the above, may be the following: The greater plasma digoxin level in hypotensive than in normal animals five minutes after digoxin administration suggests that uptake of digoxin by peripheral tissues generally is limited by diminished peripheral blood flow. This is supported by the finding of lower skeletal muscle digoxin content than normal. Greater myocardial uptake is consistent with relative preservation of myocardial perfusion coupled with a greater concentration of digoxin in the myocardial perfusate. Concerning the latter point, myocardial uptake of digoxin varies directly with the concentration of digoxin perfusing the myocardium when digoxin is administered by intracoronary infusion and the uptake of ouabain by the perfused heart in vivo depends upon the concentration of ouabain in the perfusate.

It is, however, difficult to explain solely on this basis the further increase in myocardial digoxin concentration between five minutes and one hour in the hypotensive animals, when plasma digoxin was not greater, but in fact less than normal. Myocardial mechanical factors may have influenced myocardial digoxin uptake. For example, the rate of onset of the inotropic effect of cardiac glycosides in isolated cardiac muscle preparations varies directly with the contraction frequency. Although there is some evidence from studies that contraction frequency does not influence myocardial glycoside binding, we have made preliminary observations in the intact animal which suggest that myocardial uptake of digoxin is influenced by heart rate. We consider that changes in those electrolytes known to influence the myocardial uptake of digitalis glycosides can be dismissed as unimportant. Although the level of extracellular potassium was lower than control in our one hour test group, the difference was quite small. Even marked hypokalemia may not increase myocardial digoxin uptake in vivo. Extracellular sodium concentration, a known determinant of uptake, was not different between the groups.

While myocardial and other factors which determine glycoside uptake clearly require further elucidation, the present experiments were not designed to study the individual determinants of digoxin uptake by the myocardium, but rather to characterize the effect of a clinically important syndrome on the myocardial uptake of digoxin. The main point is that one hour after intravenous administration, at a time when the myocardial effects of digoxin are usually approaching their maximum, moderate shock markedly augments myocardial uptake of digoxin. The myocardial concentration is greater relative to the dose administered and to plasma and skeletal muscle concentration. Clearly the plasma glycoside level does not reflect the myocardial level under these conditions.

It was also outside the scope of the study to examine the myocardial subcellular distribution of digoxin or the relation between digoxin specifically bound to membrane Na+, K+, ATPase and any nonspecifically-bound glycoside. That our observation is relevant to the myocardial effects of digoxin in hemorrhagic shock is supported by the report of increased sensitivity to the arrhythmogenic effect of digoxin in the dog subjected to hemorrhage. We believe that the results are likely to be relevant to other shock syndromes with similarities in disordered circulatory physiology, such as cardiogenic shock, and that the results demonstrate a rationale for the use of moderate dosage regimes of cardiac glycosides in the presence of the shock syndrome.

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