The Physiologic Effects of Digoxin Under Steady-state Drug Conditions in Newborn and Adult Sheep

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SUMMARY The physiologic response to the chronic administration of digoxin was studied in 12 adult and 13 newborn sheep. Vascular pressures, cardiac output, isovolumic contraction phase indexes and systolic time intervals were measured before and after 2 weeks of digoxin therapy. Physiologic measurements were correlated with drug levels in plasma and myocardium. Resting myocardial function in newborns exceeded that in ewes. In ewes, the heart rate decreased from 98 to 74 beats/min, the prejection period (PEP) decreased from 76 to 57 msec, the ratio of PEP to left ventricular ejection time (LVET) decreased from 0.323 to 0.223 and dP/dt max increased from 2415 to 3460 mm Hg · sec⁻¹ as plasma concentrations of digoxin increased to a mean of 1.8 ng/ml. Although the final steady-state plasma concentration of digoxin in newborn lambs averaged 1.7 ng/ml, cardiac output, PEP, PEP/LVET and dP/dt max did not change significantly from baseline values. These studies suggest that developmental differences in the physiologic response to digoxin are due either to a limited capacity for improvement in myocardial contractility shortly after birth or to an age-related difference in the effect of digoxin on myocardial tissue.

FULL-TERM newborn infants are treated routinely with higher doses of digoxin per kilogram of body weight than adults. The reasons for this age-related variation in drug use are not established. Most studies of the pharmacokinetics of digoxin have shown variable and small differences in drug half-life, drug clearance and drug distribution between mature and immature subjects. The metabolism of digoxin and its binding to serum protein does not vary with age. Although studies in several animal species and man establish that immature subjects tolerate digoxin better than mature subjects, the use of higher drug doses in young patients is controversial. Age-related differences in the inotropic effect of digoxin have been demonstrated in some studies but not in others.

We developed a model of drug administration to newborn and adult sheep to study age-related differences in drug effect. Physiologic studies were performed only under steady-state drug conditions, and pharmacokinetic studies were performed concurrently so that drug handling and distribution could be related to drug effect.

Methods

Animal Preparation

Twelve mixed-breed ewes and 13 newborn lambs were studied. Ewes were operated on after a 48-hour fast. Newborns underwent surgery in the first 4 days of life. Halothane was administered via controlled ventilation for general anesthesia; local anesthesia was achieved by 1% xylocaine infiltration subcutaneously at incision sites. Polyvinyl catheters (0.050 inch i.d.; 0.090 inch o.d.) were positioned in the right atrium via the jugular vein and in either the ascending aorta via the carotid artery (ewes) or in the descending aorta (newborns). A #5F catheter-tip manometer (Millar Instruments) was positioned retrogradely in the left ventricle via the left carotid artery. Three silver-tipped electrocardiographic electrodes were sewn subcutaneously to the chest wall. Catheters and cables were tunneled subcutaneously to the left flank and encased in a nylon mesh pouch sewn to the skin. A Foley catheter (#8F in newborns and #14F in ewes) was placed in the urinary bladder either retrogradely (females) or by a suprapubic dissection (males). The catheter was tethered to the left hind limb with nylon sutures. The animals were treated intramuscularly for the first 5 postoperative days with penicillin G, 50,000 U/kg/day, and streptomycin, 15 mg/kg/day. Studies were performed 48 hours or more after surgery.

Measurements were made on two consecutive days 4–8 days postoperatively (baseline) and again after two courses of digoxin therapy, 12–14 days postoperatively and 18–22 days postoperatively. Studies were performed while animals rested unrestrained in their cages.

Digoxin Therapy

Digoxin therapy was begun after baseline physiologic studies and bolus drug administration studies had been completed. Four ewes and five newborns were studied sequentially for 3 weeks without therapy as control groups. Animals were loaded with i.v. digoxin (125 µg/kg in newborns and 75 µg/kg in ewes) in three divided doses. Maintenance i.v. therapy was started 24 hours after the institution of loading therapy and continued for 4–6 days before repeat physiologic measurements. Maintenance doses were divided and given twice per day (10–30 µg/kg/24 hours).
hours in ewes and 15–50 μg/kg/24 hours in newborns). When physiologic measurements were made, blood samples were obtained for sodium, potassium, calcium, albumin, creatinine, hematocrit and arterial blood gases. Blood samples for digoxin determination were obtained 6 and 8 hours after the morning maintenance dose. Samples were analyzed by radioimmunoassay with tritiated tracer and the results were averaged. The coefficient of variation of the assay was 6% at 1 ng/ml and 13% at 3 ng/ml. In addition, urine samples were collected for 6–8 hours during study days; an aliquot of the urine was analyzed subsequently for digoxin and creatinine concentration.

After completion of studies, the maintenance dose of digoxin was increased (15–40 μg/kg/24 hours in ewes and 25–75 μg/kg/24 hours in newborns) and continued for an additional 4–6 days. Physiologic studies and blood and urine sampling procedures were repeated on two consecutive days. Animals were then sacrificed by pentobarbital overdose. Tissue samples were obtained for analysis of digoxin content in left ventricular (LV) myocardium, skeletal muscle, midbrain, liver, kidney, bone, skin and small intestine. Digoxin concentrations in tissue were determined using a corrected extraction technique. Drug recovery from tissue was 70–94% (average 87%). Digoxin content was expressed both as a concentration (ng/g wet weight − ng/g wet weight) and as the ratio of tissue concentration to the steady-state concentration of digoxin in plasma before sacrifice. Plasma and tissue concentrations of digoxin at steady state were related to physiologic measurements made during the study period.

Physiologic Measurements

Physiologic data were recorded with a direct-writing recorder (Beckman Instruments, R-6111) at paper speeds of 10, 25, 50, 100 and 200 mm/sec. Arterial blood samples for determination of hematocrit, pH, P02 and Pco2 (Instrumentation Laboratories Model 113-04) were obtained on each study day. Blood samples were also obtained for analysis of calcium by the cresolphthalein complexone method, sodium and potassium by flame photometry and albumin by a dye-binding technique. Intravascular pressures were recorded with Statham P23Db strain gauges. The ECG was recorded with an AC/DC coupler (Beckman 9806-A). LV pressure was recorded with the Millar catheter and used to generate its first derivative with respect to time (LV dP/dt) with a differentiating circuit (Beckman 9879) calibrated internally and verified by application of a triangular wave form. The LV dP/dt maximum value was noted directly from the tracing; an LV dP/dt value normalized to a developed intraventricular pressure of 40 mm Hg was calculated and expressed as Vce dp 40. The rationale for including this estimate of myocardial function has been presented in detail elsewhere. Myocardial performance was also assessed by measurement of the systolic time intervals: prejection period (PEP) and LV ejection time (LVET). End-diastolic and dicrotic notch levels of aortic pressure were interpreted as the opening and closing points of the aortic valve. These pressures were marked on the LV pressure tracing and, in conjunction with the ECG, used to measure the PEP and LVET. Measurements were made on four consecutive complexes at a paper speed of 200 mm/sec and the results were averaged. The PEP/LVET ratio was computed from the averaged PEP and LVET measurements. Heart rate was recorded with a cardiotachometer (Beckman 9857).

Cardiac output was measured using indocyanine green dye and an Electronics for Medicine #DCCO-04 densitometer/output computer with internal standardization (Lyons Medical Instrument Corporation). Four successive output determinations were made on each study day and the results were averaged. Systemic vascular resistance was calculated from measurements of cardiac output and mean aortic and right atrial pressures. A schematic flow diagram of the study design is shown in figure 1.

ECG tracings were used to measure the PR interval, calculate the PEP and document toxicity to digoxin. Digoxin toxicity was defined as more than five premature ventricular complexes per minute of recording, nonsinus rhythm or atrioventricular block.

Statistics

Baseline measurements in ewes and newborns were compared by unpaired t test. Physiologic measurements in pretreatment, control and treated animals were assessed by a one-way analysis of variance for unequal sample sizes. The studentized range test was used to identify which means differed significantly. The relationship between the PEP/LVET ratio and both the plasma and myocardial concentrations of digoxin was examined using analysis of variance.
Results

The study animals were in sound physiologic state at the time of study. Blood chemistries, weight, arterial blood gases and hematocrit were normal. Abnormalities of cardiac rhythm or conduction were not noted in any animal before digoxin therapy. With the exception of weight, no significant differences existed between ewes and newborn lambs.

The physiologic response to digoxin is detailed in table 1. Satisfactory studies were obtained during 21 of 24 steady-state plateaus in ewes and during 21 of 26 steady-state plateaus in newborn lambs. Unsatisfactory conditions resulted from catheter dysfunction or dislodgement and drug toxicity, which prevented measurement of physiologic data.

Control and baseline measurements did not differ (table 1). Before digoxin therapy, all measurements except mean right atrial pressure, LV end-diastolic pressure and the PEP/LVET ratio differed between ewes and newborn lambs. The differences are detailed in table 1 and have been discussed elsewhere.21

Response to Digoxin in Ewes

Digoxin caused a decrease in heart rate when plasma concentrations exceeded 1.2 ng/ml. Right atrial, aortic and LV end-diastolic pressures did not change. Although cardiac output fell and systemic vascular resistance rose, these trends were not statistically significant. PEP decreased progressively, from a mean baseline value of 76 msec to 57 msec with plasma digoxin concentrations > 1.2 ng/ml. Although LVET did not change, the PEP/LVET ratio decreased significantly and progressively as concentrations of digoxin in plasma and myocardium rose (figs. 2 and 3). The dP/dt max and VCE dp 40 increased significantly after digoxin administration. The
PR interval was not prolonged significantly, although the mean interval with digoxin plasma concentrations ≥ 1.2 ng/ml (153 msec) was higher than baseline or control values (fig. 4). Six of 12 ewes showed signs of toxicity to digoxin at plasma concentrations of 0.9–2.7 ng/ml (table 2).

Response to Digoxin in Newborn Lambs

Heart rate decreased progressively with digoxin therapy. Variations in aortic, right atrial and LV end-diastolic pressure were not significant. Cardiac output did not change. Although systemic vascular resistance increased relative to baseline values with plasma digoxin concentrations ≥ 1.2 ng/ml, the value was not significantly different from the control level. The indexes of myocardial contractility did not change with digoxin therapy; however, the levels of dP/dt max and VCE dp 40 greatly exceeded control levels in ewes. The decreasing trend of PEP/LVET was due entirely to prolongation of LVET; PEP remained virtually constant in all groups. No significant change in PEP/LVET was shown in relation to either plasma (fig. 2) or myocardial (fig. 3) digoxin concentrations. The PR interval lengthened progressively as plasma levels of digoxin increased (fig. 4). Two of 13 newborn

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**Figure 2.** Changes in the ratio of pre-ejection period to left ventricular ejection time (PEP/LVET) in newborn lambs and ewes related to the concentration of digoxin in plasma.

**Figure 3.** Changes in the ratio of pre-ejection period to left ventricular ejection time (PEP/LVET) in newborn lambs and ewes related to the concentration of digoxin in left ventricular myocardium.

**Figure 4.** Percent change in PR interval in newborn lambs and ewes related to the concentration of digoxin in plasma.
lambs showed signs of toxicity to digoxin at plasma concentrations of 3.1–4.3 ng/ml (table 2).

**Discussion**

The relationship of age and maturity to the physiologic effects of digoxin are controversial. Although increased drug tolerance in the young has been established by studies in both animals and man, a relationship between the inotropic effect of digoxin and age has not been established with certainty.

Interpretation of clinical studies in man is complicated by the varying clinical status of subjects, the small numbers of patients studied and the difficulty in establishing steady-state drug conditions before making physiologic measurements. The animal model used in this study was designed to avoid these pitfalls and to facilitate correlation of the pharmacokinetics of digoxin with its physiologic effects in mature and immature subjects.

The baseline measurements show that the levels of resting cardiac function in newborn lambs and ewes are not comparable. Cardiac output normalized to body weight, heart rate and myocardial contractility (as estimated by dP/dt max and V<sub>CE</sub> dp 40) are higher in newborn lambs than in the ewes (table 1). Systemic arterial blood pressure and systemic vascular resistance are lower in lambs than in ewes. These data emphasize the extraordinary level of myocardial performance in the neonatal period. The PEP/LVET ratio was the single index of cardiac function found to be comparable in sheep at the extremes of age, a finding in agreement with previous studies.

The response of physiologic variables to digoxin therapy also differed between the newborn lambs and ewes. Although some age-related differences exist in the total body and renal clearance of digoxin, tissue-to-plasma digoxin ratios do not vary with age. Nevertheless, at plasma concentrations considered therapeutic in man, digoxin had no significant effect on PEP, PEP/LVET, dP/dt max, V<sub>CE</sub> dp 40 or cardiac output in lambs. In ewes, the PEP/LVET decreased progressively with digoxin administration, and dP/dt max, V<sub>CE</sub> dp 40 and PEP differed after treatment from control values. In both ewes and lambs, heart rate and atrioventricular conduction slowed after digoxin administration (fig. 4), although the relationship appeared more linear in the newborn than in the ewe.

The lack of change in the measured variables in lambs need not reflect a state of drug resistance. The animals used for study were normal newborn lambs with normal cardiovascular function. Because the resting level of cardiac performance after birth greatly exceeds that seen in adults, a plausible explanation for the lack of response to digoxin may be a limited capacity to improve myocardial function in healthy newborn subjects.

The decrease in heart rate and the prolongation in LVET after digoxin administration was small in lambs. Moreover, because the PEP is short, the electromechanical delay before isovolumic contraction is fixed in the absence of conduction abnormalities and the dP/dt max is high before digoxin therapy, the potential for reduction in the PEP/LVET ratio is small in newborn subjects with normal myocardial function. The ability of digoxin to improve indexes of myocardial contractility in immature animals with compromised myocardial function has not been studied.

The results of these experiments are supported by clinical studies in man. Hoeschen and Cuddy showed a progressive decrease in the LVET with increasing serum concentrations of digoxin in 21 adult patients with compromised myocardial function. Weissler and Schoenfeld and Weissler et al. showed that cardiac glycosides reduce both the PEP and LVET in adult patients with cardiac failure and that the PEP/LVET ratio also decreases. Carliner et al. also showed a progressive change in systolic time intervals toward normal levels as the serum concentration of digoxin increased in eight patients with compensated congestive heart failure. From a mean of 0.367 before therapy, PEP/LVET decreased to 0.341 when the mean plasma digoxin concentration was 0.5 ng/ml and to 0.316 when the mean plasma digoxin concentration rose to 0.9 ng/ml.

Studies in infants and children establish an effect of digoxin on indexes of myocardial function, although

### TABLE 2. Toxicity to Digoxin

<table>
<thead>
<tr>
<th>Animal</th>
<th>Plasma digoxin concentration (ng/ml)</th>
<th>Myocardial digoxin concentration (ng/g wet weight)</th>
<th>Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewe 270</td>
<td>1.2</td>
<td>101</td>
<td>Intermittent 3° AV block</td>
</tr>
<tr>
<td>Ewe 264</td>
<td>2.2</td>
<td>310</td>
<td>Ventricular premature complexes</td>
</tr>
<tr>
<td>Ewe 95</td>
<td>0.9</td>
<td>166</td>
<td>3° AV block</td>
</tr>
<tr>
<td>Ewe 285</td>
<td>1.1</td>
<td>114</td>
<td>Junctional tachycardia</td>
</tr>
<tr>
<td>Ewe 008</td>
<td>2.7</td>
<td>280</td>
<td>Ventricular premature complexes</td>
</tr>
<tr>
<td>Ewe 009</td>
<td>1.4</td>
<td>111</td>
<td>Ventricular premature complexes</td>
</tr>
<tr>
<td>Newborn B</td>
<td>4.3</td>
<td>409</td>
<td>3° AV block</td>
</tr>
<tr>
<td>Newborn T</td>
<td>3.1</td>
<td>300</td>
<td>3° AV block</td>
</tr>
</tbody>
</table>

Abbreviation: AV = atrioventricular.
the dose-response relationship and the progression of change with increasing concentrations of digoxin in plasma are less clear than in adults.

Levy et al.39 showed shortening of the PEP and PEP/LVET ratio in 27 normal newborns and 10 infants with congestive heart failure 4 hours after administration of digoxin, 30 μg/kg. LVET did not change significantly. Systolic time intervals did not change further after the administration of additional amounts of digoxin. Park et al.40 described changes in systolic time intervals for a variety of congenital cardiac defects, before and after digoxin therapy. Eleven patients with left-to-right shunts at the ventricular level (ventricular septal defect) and five infants with primary myocardial disease had abnormal systolic time intervals before therapy. The PEP/LVET ratio normalized in four of six patients with ventricular septal defect and in three of five with myocardial disease after digoxin treatment. Three infants with patent ductus arteriosus and congestive heart failure showed a response in systolic time intervals to treatment with digoxin; neither the PEP nor the LVET, however, was abnormal before therapy.

Pinsky et al.41 studied the effects of two dosage regimens of digoxin on echocardiographically determined systolic time intervals in 13 premature infants with patent ductus arteriosus. In seven patients on the higher dosage schedule, the mean serum digoxin concentration was 3.5 ng/ml; LVET decreased 9.3% and PEP decreased 18.8%. In six infants who received lower doses of digoxin, the mean serum digoxin concentration was 1.73 ng/ml; LVET decreased 10.3% and PEP decreased 19%. Absolute values for PEP and LVET and changes in the PEP/LVET ratio were not given. If the PEP were 50 msec before therapy, the interval change after digoxin would be approximately 10 msec.

Tolerance of the immature organism to the toxic effects of digoxin is established in most animal species and in man. Several investigators suggest that the inotropic effect of digoxin is also related to age. The explanation for this developmental variation in physiologic effect remains unsettled by these studies. Digoxin (and inotropic agents in general) may not be able to improve substantially the contractile state of the normally functioning neonatal heart. The cardiac output and rate of LV pressure development in the newborn are extraordinary, and greatly exceed those in adults. The brief duration of the isovolumic contraction and PEP in the neonatal subject42 underlines the limitation of the unimpaired, immature myocardium to contract more vigorously after inotropic intervention (fig. 5). Because congenital cardiac disease is not always associated with impaired myocardial function, inotropic agents such as digoxin may be inappropriate drugs for improving the hemodynamic status of some patients. The studies reported here in no way argue against the use or effectiveness of cardiac glycosides in subjects with compromised myocardial function, regardless of age.

Alternatively, the immature and mature myocardium may respond differently to digoxin. Sodium-potassium ATP-ase activity or the ability of digoxin to inhibit monovalent cation transport may vary with age and account for developmental differences in the physiologic effects of digoxin.44, 45 Future studies must pursue developmental differences in the cellular effects of digoxin. Those studies may determine whether "resistance" to digoxin in the young is due to a decreased drug effect on the myocardial cell membrane or to the limited capacity of the performance, immature cardiovascular system to improve its function after inotropic intervention.

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