

## Cardiovascular Effects and Placental Passage of Dantrolene in the Maternal-fetal Sheep Model

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Using the chronic maternal-fetal sheep preparation, nine pregnant ewes were studied to determine the effects of intravenous dantrolene sodium on maternal and fetal physiology, with particular reference to its placental passage, and its effects on uterine blood flow and uterine tone. Two doses of dantrolene sodium were studied: 1.2 mg/kg and 2.4 mg/kg. After 2.4 mg/kg, maternal cardiac output increased 29% ( $P < 0.05$ ) after 1 min and returned to normal after 30 min. Maternal mean arterial pressure increased 13% after 1 min and remained significantly elevated ( $P < 0.01$ ) for 3 h. No significant changes ( $P > 0.05$ ) were observed in maternal heart rate, uterine artery blood flow, or central venous pressure. Maternal arterial pH declined from 7.42 to 7.39 ( $P < 0.01$ ) after 1 min and returned to baseline values after 10 min. Fetal heart rate decreased 24% ( $P < 0.01$ ) after 3 min and returned to normal after 10 min; the mean fetal arterial pressure remained unchanged ( $P > 0.05$ ). Fetal arterial pH declined from 7.29 to 7.27 ( $P < 0.05$ ) after 1 min and remained significantly decreased for 120 min. Similar changes of lesser magnitude and shorter duration were seen following the 1.2 mg/kg dose. Maternal levels of dantrolene were less than 3  $\mu\text{g/ml}$ . Although an equilibrium between maternal and fetal plasma dantrolene concentrations was apparent at 5 min, the fetal levels of dantrolene were approximately 10% of the mother's. The results indicate that the administration of intravenous dantrolene at 1.2 mg/kg or 2.4 mg/kg has no clinically significant adverse effect on mother or fetus in the sheep model. (Key words: Acid-base equilibrium: fetus. Blood pressure, drug effects: Dantrolene. Malignant hyperthermia, treatment: Dantrolene. Pharmacokinetics: Dantrolene. Pregnancy placental transfer: Dantrolene.)

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THE EFFICACY OF DANTROLENE in the therapy of malignant hyperthermia (MH) crises was first observed by Harrison in 1975, when he noted that it relieved halothane-induced MH reactions in susceptible Landrace swine.<sup>1</sup> In 1982, a multi-center prospective study demonstrated that dantrolene at 2.5 mg/kg was effective in reversing MH and significantly decreasing mortality.<sup>2,3</sup>

In addition to its effects on skeletal muscle, recent *in vitro* work has shown that dantrolene alters the function of both smooth muscle and cardiac muscle.<sup>4</sup> In humans, as in animals, no appreciable alteration in cardiovascular or respiratory function has been noted.<sup>5</sup> During pregnancy, the safety of dantrolene has yet to be established. In one case report, two parturients with malignant hyperthermia susceptibility were given oral dantrolene prior to general anesthesia for cesarean section, and no adverse effects on mother or infant were demonstrated, although dantrolene was observed to readily cross the placenta,<sup>6</sup> as indicated by dantrolene levels in the cord venous blood.

In the present study using pregnant ewes, the effects of two doses of intravenous dantrolene (1.2 mg/kg and 2.4 mg/kg) on maternal and fetal cardiovascular dynamics, and acid-base status, were investigated. In addition, the kinetics and distribution of dantrolene were also evaluated.

### Materials and Methods

Nine pregnant ewes near term (mean gestational ages 135-140 days) were studied. The study was approved by the Animal Care Research Committee. Each ewe was administered halothane (4.5%), oxygen (35%), and nitrous oxide (60%) until apneic. An endotracheal tube was inserted, and the ewe was maintained on halothane (0.5-3.0%), nitrous oxide (60%), and oxygen (35%). A Swan-Ganz® catheter was inserted into the pulmonary artery *via* the right jugular vein, and cannulae (polyvinyl Fr. 8) were placed in a maternal femoral vein and artery. A hysterotomy *via* a mid-line abdominal incision was then performed, allowing exposure of fetal head and neck. Cannulae (polyvinyl Fr. 8) were secured in a fetal carotid artery and jugular vein. A catheter was inserted into the uterine cavity for measurement of amniotic fluid pressure. The fetal head was then reinserted and the uterus closed. A pre-calibrated electromagnetic flow probe (Carolina Medical, King, NC) was secured

around a main branch of a uterine artery for measurement of uterine blood flow. All catheters were then brought out to a pocket on the ewe's right side.

The sheep were allowed to recover from the operation for at least 24 h before the experiment began. A number of earlier studies demonstrated that the various physiological parameters were essentially normal 24 h after the operation; animals appear to be without pain, and exhibited normal eating and drinking of water. The experiment was performed with the alert sheep standing in a cage in a suspended loose mesh sling. The following variables were recorded on a model R-411 Beckman dynograph eight-channel recorder: maternal and fetal arterial pressures; maternal and fetal heart rates; maternal pulmonary artery, central venous, and intra-amniotic pressure; and uterine blood flow. Pressures were measured with Hewlett-Packard and Cobe pressure transducers. Uterine blood flow was recorded with a Carolina Medical Electronics square wave electromagnetic flowmeter. Cardiac output was measured by the thermo-dilution technique and integrated with an Edward's Cardiac Output Computer. Maternal stroke volume and systemic vascular resistance (SVR) were calculated using standard formulae.

Maternal temperature was monitored and remained between 38° and 39° C. Arterial blood gases were measured with a Radiometer blood gas analyzer BMS-3, and were corrected to actual temperature of the sheep.

A control period of 30 min was observed, during which vital signs were stable (table 1). Following the control period, dantrolene sodium (Dantrium®, Norwich-Eaton Pharmaceuticals, Norwich, NY) in a dose of 1.2 mg/kg was injected. Dantrolene was prepared by adding 15 ml of sterile water to a vial containing dantrolene 20 mg, mannitol 3 g, and sufficient sodium hydroxide to yield a pH of approximately 9.5. An adequate amount of drug was dissolved in this manner and administered slowly (2–3 min.) intravenously through the external jugular vein, which was flushed with 0.9% normal saline solution. The commercial dantrolene preparation contains 3.0 grams of mannitol/20 mg dantrolene. As far as could be ascertained, the dose of mannitol administered does not affect the various physiological parameters. Although there were 21 3.0-ml samples of blood taken from the fetus over a 6-h period, there appeared to be no deleterious effect on the fetus due to the loss of this volume of blood. Cardiovascular data sets were obtained at 1, 3, 5, 10, 15, 30, 45, and 60 min after the injection and then every 30 min, until the end of 3 h. Concomitantly, maternal and fetal arterial blood was sampled for blood gas and dantrolene levels. After this 3-h period, the experiment was repeated using dantrolene sodium 2.4 mg/kg. The cardiovascular data sets and blood samples were collected at the

TABLE 1. Maternal and Fetal Cardiovascular Data and Blood-gas Tension during Control Period

Cardiovascular Data	Maternal	Fetal
Heart rate (beats/min)	110 ± 6.0	174 ± 5.0
Mean arterial pressure (mmHg)	88 ± 2.8	51 ± 1.9
Cardiac output (l/min)	7.29 ± 0.65	—
Systemic vascular resistance (dynes · sec · cm <sup>-5</sup> )	1041 ± 120	—
Uterine blood flow (ml/min)	460 ± 60	—
Uterine tone (mmHg)	4 ± 1.5	—
Blood gas values		
pH	7.44 ± 0.01	7.33 ± 0.01
Paco <sub>2</sub> (mmHg)	34 ± 1	48 ± 1.6
PaO <sub>2</sub> (mmHg)	101 ± 4	20 ± 2.2

Data given as mean ± SEM.

same time intervals as stated for the 1.2 mg/kg dose. The results were analyzed by the analysis of variance, and Tukey's *t* test (*P* < 0.05 was considered significant).

Plasma dantrolene levels were determined by using a modified reverse phase liquid chromatography, as described by Katugi *et al.*<sup>7</sup> The HPLC system consisted of a Laboratory Data Control (LDC) Constrametric II® high-performance liquid chromatograph equipped with LDC spectromonitor III variable wavelength detector operated at 375 nm. The reverse phase HPLC column (30 cm × 3.9 mm) was packed with  $\mu$  Bondapak® C18 (Waters, Chromatography Division, Milford, MA), and extracted plasma samples were introduced using a syringe loading sample injector (Rheodyne model 7125). The eluant was 50% acetonitrile in 20 mM glycine buffer adjusted to pH 3.5 with 1.0 M HCl. A flow rate of 1.0 ml/min was utilized.

Plasma samples (0.5 ml maternal, 1.0 ml fetal) were placed in stoppered test tubes, and a sufficient volume of 0.4 M acetate buffer (pH 4.0) was added to yield a final volume of 5.0 ml. The tubes were mixed and allowed to sit for at least 1 min. Five milliliters of HPLC reagent grade chloroform was then added to each test tube. The tubes were gently shaken for 10 min, followed by centrifugation (1500 × *g*) for 10 min.

An aliquot of the chloroform layer (1.0 ml maternal, 2.0 ml fetal) was then transferred to a clean, small tube. The chloroform was evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 100  $\mu$ l of the mobile phase (acetonitrile-glycine buffer), and 10  $\mu$ l was injected into the HPLC system.

Standard curves for dantrolene were prepared by adding dantrolene to the mobile phase in varying concentrations. The elution of dantrolene was found to be 5.1 min. The efficiency of the extraction procedure was determined by adding known amounts of dantrolene to drug-free plasma followed by extraction. Repeated recovery experiments indicated that 90% ± 5.0 of the dantrolene was extracted from the plasma. All values

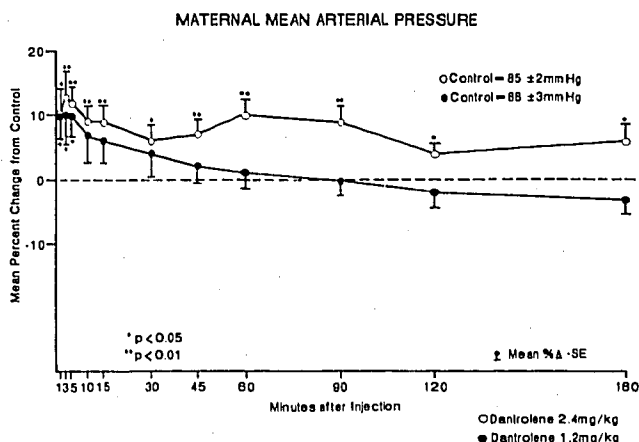


FIG. 1. Maternal mean arterial pressure. A significant increase ( $P < 0.05$ ) occurred rapidly with both doses, but persisted only with the higher dose.

were corrected for 90% recovery. The accuracy of the procedure was confirmed by running standard curves on repeated occasions. No internal standard was used in the procedure.

After analyzing drug levels, the logs of the concentrations representing the times 45–180 min after each injection were subjected to a linear regression analysis.

## Results

### MATERNAL EFFECTS

**Cardiovascular and Acid-base Status.** Maternal cardiac output increased significantly ( $P < 0.01$ ) following dantrolene administration in a dose-related fashion. A peak mean per cent increase of 19% was observed during the first 15 min following administration of dantrolene at 1.2 mg/kg, compared to a peak mean per cent increase of 29% following dantrolene at 2.4 mg/kg. The cardiac

output returned to normal after 30 min with both doses.

Following administration of dantrolene at 1.2 mg/kg, maternal mean blood pressure rose 10% ( $P < 0.05$ ) after 1 min and remained significantly elevated for only 10 min. Following administration of dantrolene at 2.4 mg/kg, the maternal mean blood pressure increased 13% ( $P < 0.05$ ) after 1 min and remained elevated for 3 h (fig. 1). No significant change ( $P > 0.05$ ) was observed in maternal heart rate or central venous pressure at either dose. Systemic vascular resistance decreased 10% ( $P < 0.05$ ) for 10 min following administration of dantrolene at 1.2 mg/kg, but no significant change ( $P > 0.05$ ) was noted after the 2.4-mg/kg dose.

Maternal systolic pulmonary arterial pressure (PAP) increased significantly following administration of dantrolene, and the magnitude of the effect was dose-related. At 1.2 mg/kg, systolic pulmonary arterial pressure increased 16% ( $P < 0.05$ ) after 1 min, and remained significantly elevated up to 10 min, while, with 2.4 mg/kg, systolic PAP increased 33% ( $P < 0.05$ ), and remained elevated up to 30 min after the injection. Pulmonary vascular resistance (PVR) was not affected in a consistent manner.

Administration of dantrolene at 1.2 mg/kg caused no significant change ( $P > 0.05$ ) in pH,  $P_{aCO_2}$ , or  $P_{aO_2}$ . At 2.4 mg/kg, maternal pH declined from 7.42 to 7.39 ( $P < 0.01$ ) after 1 min, and returned to baseline values after 10 min;  $P_{aCO_2}$  increased 11% above baseline values (32 to 36 mmHg) after 1 min ( $P < 0.05$ ) and remained in this range for 2 h;  $P_{aO_2}$  did not change ( $P > 0.05$ ).

After dosing, there was a rapid decay phase seen for the first 30 min, followed by a slower elimination phase. The terminal slopes of dantrolene concentrations represented half-lives of 8.7 h and 7.5 h for the 1.2-mg/kg and 2.4-mg/kg doses, respectively.

The fetal/maternal dantrolene concentration ratios were low, but equilibrium between the mother and fetus was rapidly attained. Beginning with the points taken 5 min after injection, the ratios were  $13.33 \pm 2.25$  (mean  $\pm$  SD) for the 1.2-mg/kg dose, and  $10.95 \pm 2.00$  for the 2.4-mg/kg dose. Although these ratios differed at the  $P = 0.022$  level, the small difference is likely of little biological significance (table 2).

**Uterine Blood Flow.** Following administration of dantrolene at 1.2 mg/kg, uterine artery blood flow decreased ( $P < 0.01$ ) at 1 and 5 min to 81 and 85% of control values, respectively, but returned to normal after 10 min. Uterine artery blood flow was not decreased ( $P > 0.05$ ) following dantrolene at 2.4 mg/kg (fig. 2).

**Intrauterine Pressure.** Neither doses of dantrolene produced significant alteration in uterine tone ( $P > 0.05$ ).

TABLE 2. Maternal Artery and Fetal Artery Dantrolene Levels\*

Time (min)	Dose: 1.2 mg/kg			Dose: 2.4 mg/kg		
	Maternal $\mu\text{g/ml}$	Fetal $\mu\text{g/ml}$	M/F	Maternal $\mu\text{g/ml}$	Fetal $\mu\text{g/ml}$	M/F
1	$1.27 \pm 0.04$	$0.04 \pm 0.02$	31.75	$2.23 \pm 0.12$	$0.33 \pm 0.07$	6.76
5	$1.05 \pm 0.01$	$0.07 \pm 0.02$	15.00	$2.26 \pm 0.29$	$0.20 \pm 0.05$	11.30
10	$1.03 \pm 0.07$	$0.08 \pm 0.02$	12.88	$2.03 \pm 0.24$	$0.20 \pm 0.04$	10.15
15	$0.95 \pm 0.12$	$0.09 \pm 0.02$	10.56	$1.80 \pm 0.18$	$0.17 \pm 0.04$	10.58
30	$0.94 \pm 0.09$	$0.09 \pm 0.02$	10.44	$1.56 \pm 0.17$	$0.12 \pm 0.03$	13.00
45	$0.71 \pm 0.06$	$0.05 \pm 0.02$	14.20	$1.41 \pm 0.13$	$0.15 \pm 0.04$	9.40
60	$0.94 \pm 0.04$	$0.06 \pm 0.02$	15.67	$1.50 \pm 0.14$	$0.16 \pm 0.04$	9.38
90	$0.66 \pm 0.04$	$0.05 \pm 0.02$	13.20	$1.44 \pm 0.10$	$0.17 \pm 0.04$	8.47
120	$0.86 \pm 0.04$	$0.05 \pm 0.01$	17.20	$1.30 \pm 0.10$	$0.11 \pm 0.02$	11.82
150	$0.66 \pm 0.05$	$0.06 \pm 0.02$	11.00	$1.22 \pm 0.10$	$0.12 \pm 0.02$	10.17
180	$0.66 \pm 0.08$	$0.05 \pm 0.01$	13.20	$1.22 \pm 0.16$	$0.08 \pm 0.02$	15.25

\* Numbers represent the mean  $\pm$  SEM of at least eight sheep.

### FETAL EFFECTS

Fetal heart rate (FHR) decreased significantly in response to dantrolene administration at both doses (fig. 3). Following dantrolene at 1.2 mg/kg, FHR decreased 13% after 3 min ( $P < 0.05$ ), and returned to normal after 10 min. Following dantrolene at 2.4 mg/kg, FHR decreased 24% after 3 min ( $P < 0.05$ ), and again returned to normal after 10 min. There was no significant change in fetal mean blood pressure at either dose ( $P > 0.05$ ). Fetal pH declined from 7.33 to 7.31 ( $P < 0.05$ ) after 1 min, and remained at this level for 3 h, following 1.2 mg/kg of dantrolene sodium; however, no change in  $\text{PaCO}_2$  or  $\text{PaO}_2$  was observed. At 2.4 mg/kg, fetal pH declined from 7.29 to 7.27 ( $P < 0.05$ ) after 1 min, and remained at this level for 120 min. The  $\text{PaCO}_2$  increased 9% after 15 min ( $P < 0.05$ ), and remained at this level for the 3-h duration of the study. The  $\text{PaO}_2$  declined 14% after 5 min (22 to 19 mmHg) ( $P < 0.05$ ), and returned to control values within 30 min.

### Discussion

The results of this study indicate that the intravenous administration of dantrolene in a dose of 1.2 or 2.4 mg/kg was not followed by deleterious alterations in maternal cardiovascular function. The dose of 2.4 mg/kg was selected as approximating the dose usually recommended in humans. Flewelling *et al.* observed that, in humans administered 2.4 mg/kg of dantrolene, there was no significant effect on heart rate, mean arterial pressure, or end-tidal  $\text{CO}_2$ .<sup>8</sup> In the present study, the only lasting maternal changes observed were that of mild elevation of maternal blood pressure and  $\text{PaCO}_2$ . No significant change in maternal heart rate or uterine artery blood flow was observed.

The absence of deleterious cardiovascular effects was demonstrated by Ellis, who administered up to 30 mg/kg of dantrolene intravenously without appreciable effect on cardiovascular or respiratory function. Ellis did observe a decrease in respiratory volume compensated for by an increase in respiratory rate.<sup>5</sup>

That a species specific effect may be present is indicated by the finding that dogs given 10 mg/kg of dantrolene demonstrated an increased blood pressure and decreased cardiac index by increasing systemic vascular resistance.<sup>9</sup> Furthermore, the pregnant state may modify the physiologic response to the drug. Fetal status was not adversely affected in any clinically significant fashion. Some concern is raised by the fact that fetal heart rate declined by 13% and 24% at 3 min in response to dantrolene administration at 1.2 mg/kg and 2.4 mg/kg, respectively. However, heart rate in both instances returned to baseline range after 10 min. An explanation for the decrease in fetal heart rate is not apparent. Uterine artery blood flow decreased signifi-

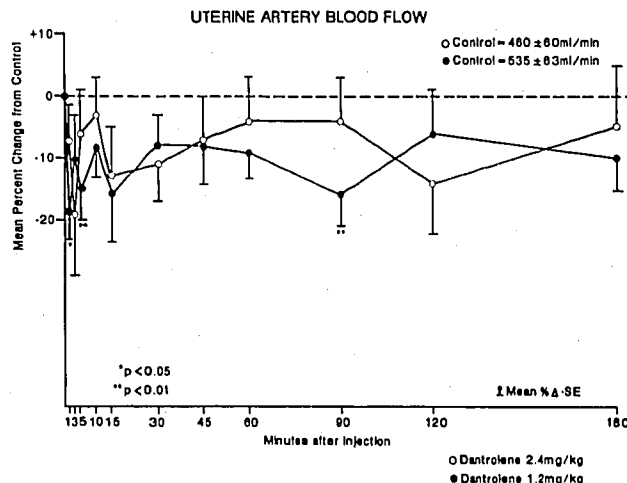


FIG. 2. Uterine artery blood flow. The decrease in uterine artery blood flow was significant at 1 and 5 min ( $P < 0.05$ ) following dantrolene 1.2 mg/kg.

cantly but transiently following dantrolene at 1.2 mg/kg (but not following 2.4 mg/kg), and this decrease in uterine artery blood flow might account for the transient decrease in fetal heart rate. It is difficult to reconcile the absence of the decreased effect of the higher dose on uterine artery blood flow. The fetal  $\text{PaCO}_2$  was elevated slightly after 2.4 mg/kg, but not after 1.2 mg/kg, reflecting increased maternal  $\text{PaCO}_2$  values that occurred exclusively after the higher dose.

The observed half-life for dantrolene in these experiments, about 8 h, is similar to that reported in humans, 9 h.<sup>10</sup> Dantrolene is known to bind to albumin with affinity constants between  $1.7 \times 10^4$  and  $1.5 \times 10^6 \text{ M}^{-1}$ .<sup>11,12</sup> This could lead to more than 99% of dantrolene being associated onto plasma proteins. Albumin concentration in the 125-day fetal sheep is 53% of that in the mother.<sup>13</sup> This would imply that the percent bound in the fetus is less than in the mother, such

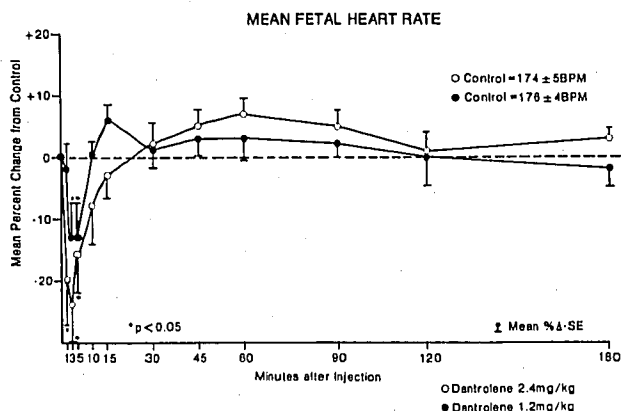


FIG. 3. Fetal heart rate. There was a significant but transient decrease in FHR at 1, 3, and 5 min following dantrolene at 1.2 mg/kg and 2.5 mg.

that low fetal dantrolene concentrations would be expected.

This is the most likely explanation for our results. However, since the free drug concentrations should be equal in mother and fetus, the low total fetal concentration *per se* does not indicate a low probability of fetal effect. Our failure to observe any significant physiological effect is the more definitive contribution of this work.

The rapid attainment of equilibrium between the fetus and the mother supports the idea that placental transfer of dantrolene is not intrinsically poor, but, rather, is limited by the availability of free dantrolene in maternal plasma. A first-pass effect in the fetus could not explain the 12-fold difference between maternal and fetal drug concentrations.

Morison presented data for two pairs of maternal/umbilical cord dantrolene concentration ratios.<sup>6</sup> These ratios, 3.5 and 1.9, are very much higher than observed. There is no obvious explanation for this discrepancy.

In conclusion, this study indicates that dantrolene sodium administered intravenously to gravid sheep has limited and slight effects on maternal and fetal cardiovascular and acid-base parameters in the chronic maternal-fetal sheep model.

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