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Effect of dantrolene sodium on contractility of isolated human uterine muscle

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SUMMARY. **The administration of intravenous dantrolene in a parturient susceptible to malignant hyperthermia has been associated with post partum uterine atony. We examined the effect of dantrolene sodium for injection (Dantrium Intravenous) on spontaneous contractility of uterine smooth muscle from women in term pregnancy in an isolated preparation. Dantrolene sodium for injection at 5 μg/ml and 10 μg/ml had no effect on the spontaneous contractility of the uterine muscle preparations. At a cumulative concentration of 20 μg/ml, a mild depression (16 ± 14%) in the frequency of spontaneous contractions was noted. However, a similar depression in the muscle preparations treated with mannitol suggests that the depression observed with the dantrolene was likely due to the mannitol that was included in the dantrolene formulation rather than to dantrolene sodium itself. We conclude that dantrolene sodium has no effect on the spontaneous contractility of uterine smooth muscle. The depression of uterine muscle activity observed with dantrolene for injection appears attributable to the mannitol.**

INTRODUCTION

Malignant hyperthermia (MH) is a syndrome of fulminant hypermetabolism of skeletal muscle in reaction to anesthetic drugs. The syndrome is often fatal.[1]

Intravenous dantrolene is effective in the treatment of MH and also has been recommended for the prophylaxis of MH in a susceptible patient before anesthesia.[2-4] However, the administration of intravenous dantrolene in a parturient susceptible to MH has been of concern for post partum uterine atony leading to excessive blood loss[5] and potential adverse neonatal effects.[6]

In isolated muscle preparations of guinea-pig uterus, dantrolene suppressed the activity of oxytocin-induced contractions.[7] Furthermore, the relaxation effects of dantrolene sodium on cardiac muscle,[8] vascular,[9] and intestinal smooth muscle[10] have been observed in laboratory animals. Nevertheless, the effect of dantrolene sodium on the contractility of human uterine smooth muscle has not been investigated.

The purpose of this study was to evaluate the effect of dantrolene sodium for injection (Dantrium Intravenous, Proctor & Gamble Pharmaceuticals) on spontaneous contractions in isolated uterine muscle preparations from pregnant women.

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METHODS

The protocol was approved by the institutional review board for research involving human subjects. Informed consent was obtained from 8 healthy patients in term pregnancy.

A small segment of myometrium was excised from the incisional surface of the uterus during elective cesarean section under epidural anesthesia following delivery of the infant and the placenta. The myometrial segment was placed in a cooled tissue container, and was immediately sent to the laboratory for preparation.

The longitudinal segments (measuring approximately 3 × 10 mm) of the uterine muscle were vertically suspended between hooks in a 15 ml tissue bath containing Krebs solution. The Krebs solution had the following composition (in millimoles per liter): NaCl: 118.2, KCl: 4.7, $CaCl_2$: 2.5, $MgSO_4 \cdot 7H_2O$: 1.2, $NaHCO_3$: 25, $NaH_2PO_4 \cdot H_2O$: 1.2, and glucose: 11. The bath solution was continuously aerated with a mixture of 5% CO_2 and 95% O_2 at 37°C and had a pH of 7.4 ± 0.1. The resting tension of the muscle strips was gradually adjusted to between 0.2 g and 0.4 g until the baseline was free of drift. The bath solution was flushed every 15 min.

When spontaneous contractions became regular in frequency and uniform in amplitude (normally within 2 hours), reconstituted dantrolene sodium (dantrolene solution) was directly added to the bath in a

cumulative manner to achieve final concentrations of 5 μg/ml, 10 μg/ml and 20 μg/ml of dantrolene sodium, allowing 15–20 min at each concentration. (In our pilot study, the depression in uterine muscle activity occurred within 10 min after the treatment with the drug and the contractility tended to return to its previous level in 20 min.) The resting tension, amplitude, and frequency of the muscle contractions were recorded following the control measurement. Frequency was measured by the number of contractions per 20 min.

Amplitude in grams (peak contractile tension minus resting tension) of the myometrial strips was measured by a force displacement transducer (BGG-25 GMS, Kulite Semiconductor), which was connected to the upper hook of the muscle strip, and was recorded with the Scope program of MacLab data recording system (Analog Digital Instruments USA). The control amplitude and frequency were taken to represent 100% and subsequent responses to the drug were expressed as a percentage of control. Depression (%) was calculated by the percentage decrease in amplitude or frequency of the contraction. When a decrease in amplitude or frequency of the contraction was observed, the tissue bath solution was replaced with the Krebs solution and further observation was made to confirm that each preparation returned toward the control level.

The dantrolene solution was freshly reconstituted by adding 19 ml of distilled water to a vial of Dantrium Intravenous (Proctor & Gamble Pharmaceuticals). The vial contained 20 mg of dantrolene sodium with 3000 mg of mannitol in 20 ml. The final concentration was dantrolene sodium 1 mg/ml and mannitol 150 mg/ml. The volume of dantrolene solution added to the bath ranged from 0.075 ml to 0.3 ml; thus, these volumes resulted in dantrolene sodium concentrations from 5 μg/ml to 20 μg/ml and mannitol from 0.75 mg/ml (4×10^{-3} M) to 3 mg/ml (1.6×10^{-2} M) in the 15 ml bath.

Because of the inclusion of mannitol in the dantrolene solution the effects of mannitol on the muscle preparations were examined in a second phase of the study. In additional muscle preparations (or when dantrolene was washed from the bath solution), mannitol (Mannitol Injection, USP, 25%, Lyphomed) was added to the bath, in a cumulative manner. The final concentrations of mannitol in the bath were the same as above: 0.75 mg/ml, 1.5 mg/ml, and 3 mg/ml. Data (resting tension, amplitude and frequency of the contractions) were recorded.

Data (resting tension, amplitude, and frequency) from the muscle contractions treated with three concentrations of dantrolene, and data from the mannitol-treated contractions were compared by a repeated measures analysis of variance (ANOVA) followed by a Dunnett *t*-test. The depression (%) in frequency observed by the addition of dantrolene (compared to the control) was compared with the depression in frequency produced with mannitol by a paired *t*-test to determine a significance of differences. $P<0.05$ was considered statistically significant.

RESULTS

The mean values of the resting tensions, amplitudes and frequencies of the muscle contractions treated with cumulative concentrations of dantrolene sodium for injection in eight muscle preparations are shown in Table 1. Dantrolene sodium for injection at concentrations of 5 μg/ml and 10 μg/ml had no significant effect on either the amplitude or frequency of spontaneous contractions of the uterine smooth muscle preparations. At a cumulative concentration of 20 μg/ml a mild decrease in the mean contraction frequency compared to the control was observed ($P<0.01$). The calculated depression (%) was 16 ± 14% (mean ± SD). The frequency depression returned to the control frequency after the bath solution had been replaced; two muscle preparations which had not returned to the control were replaced by additional muscle preparations.

The mean values of the resting tensions, amplitudes and frequencies of the muscle contractions

Table 1. Values (means ± SD, $n = 8$) of resting tension, amplitude and frequency in the spontaneous contractions of uterine muscle treated with dantrolene solution

	Control	Dantrolene		
		5 μg/ml	10 μg/ml	20 μg/ml
Resting tension (grams)	0.27 ± 0.15	0.26 ± 0.15	0.27 ± 0.16	0.26 ± 0.22
Amplitude (grams)	1.40 ± 0.74	1.42 ± 0.76	1.44 ± 0.78	1.47 ± 0.79
Frequency (contractions /20 min)	2.70 ± 1.20	2.68 ± 1.00	2.53 ± 1.08	2.17 ± 0.66*

* Significantly different from control, $P<0.01$.

Table 2. Values (means ± SD, n = 8) of resting tension, amplitude and frequency in the spontaneous contractions of uterine muscle treated with mannitol

	Control	Mannitol		
		0.75 mg/ml	1.5 mg/ml	3.0 mg/ml
Resting tension (grams)	0.50 ± 0.10	0.50 ± 0.08	0.51 ± 0.09	0.45 ± 0.12
Amplitude (grams)	1.09 ± 0.52	1.14 ± 0.58	1.17 ± 0.57	1.13 ± 0.56
Frequency (contractions /20 min)	2.94 ± 1.56	2.82 ± 1.24	2.82 ± 1.24	2.53 ± 1.10*

* Significantly different from control, $P<0.01$.

treated with mannitol alone are shown in Table 2. Mannitol at concentrations of 0.75 mg/ml and 1.5 mg/ml had no effect on the spontaneous contractions of the uterine muscle preparations. Mannitol at a cumulative concentration of 3 mg/ml produced a similar decrease in the mean frequency compared to the control ($P<0.01$), with the calculated depression of 12 ± 7%. However, the depression of the contraction frequency produced by the addition of dantrolene solution was not statistically different from the that caused by mannitol alone ($P = 0.48$). The effects of dantrolene and mannitol on uterine muscle contractions in a single preparation are shown in the Figure.

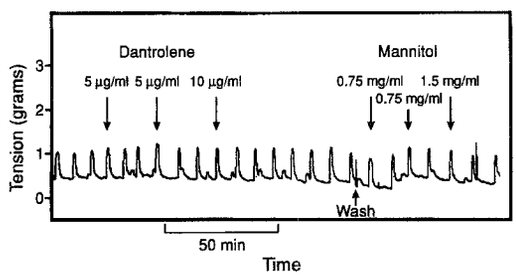

Fig. An experimental trace of uterine muscle contractions is shown. In a single preparation, uterine muscle was treated with dantrolene and mannitol in a cumulative manner.

DISCUSSION

Although the incidence of MH in pregnant patients is low, fulminant episodes have been reported.[11-13] In parturients susceptible to MH, the stress of labor and delivery and the possible need for general anesthesia for cesarean delivery are potential triggering factors.

The pathogenesis of MH appears to involve an abnormal Ca^{2+} release channel (the ryanodine receptor) of the skeletal muscle sarcoplasmic reticulum[14] which, in response to triggering agents, releases excessive calcium into the myoplasm from the sarcoplasmic reticulum. The increase in myoplasmic free calcium induces contractions of skeletal muscle, hypercatabolism with hyperthermia, respiratory and metabolic acidosis, elevation of creatine phosphokinase and serum electrolyte abnormalities. Early diagnosis and prompt treatment are the keys to the treatment of MH crisis.

The treatment of MH includes a number of therapeutic measures including the administration of dantrolene sodium. Dantrolene sodium is thought to reduce myoplasmic free Ca^{2+} by preventing Ca^{2+} release from the sarcoplasmic reticulum in patients susceptible to malignant hyperthermia.[15]

Although a therapeutic serum concentration of dantrolene (>3.0 µg/ml) is most reliably achieved by a single dose of intravenous dantrolene 2.5 mg/kg,[16] repeated doses up to a total of 10 mg/kg may be necessary to treat an acute MH episode.[17] In the present study, dantrolene sodium concentrations from 5 µg/ml to 20 µg/ml were chosen for evaluation, based on the clinical dose of intravenous dantrolene sodium. The same dose of intravenous dantrolene is recommended for MH in a parturient. Pregnancy does not alter the pharmacokinetics of intravenous dantrolene in spite of blood volume expansion and increase in body fat content during pregnancy.[18]

Dantrolene is not advocated prophylactically in parturients susceptible to MH because of potential maternal (muscle weakness, drowsiness and post partum uterine atony) and neonatal (muscle weakness) side effects.[6, 19, 20] Post partum uterine atony has been reported in a parturient treated with intravenous dantrolene[5] although the effects of dantrolene on hemodynamic parameters[21] and bladder contractility[22] are insignificant. Dantrolene readily crosses the placenta. The fetal/maternal ratio of serum concentrations was found to be 0.48.[18] Its safety to the fetus and neonate has not been determined. However, dantrolene (36 vials of intravenous dantrolene) should be immediately available in the labor and delivery suite.

Dantrolene is a weak acid with a pKa of about 7.5,[23] but even its sodium salt is relatively insoluble in water. A solvent vehicle such as mannitol, glycerol or lecithin is required to make solution for injection. Dantrium Intravenous (Proctor & Gamble Pharmaceuticals) is the only intravenous formulation available commercially. Each vial contains 20 mg of dantrolene sodium with 3000 mg of mannitol to

improve its solubility[24] and sodium hydroxide to raise the pH of the solution. An intravenous dantrolene formulation which uses lecithin-coated microcrystals of dantrolene sodium has been shown to be effective in limb muscle twitch depression in swine, [25] but is not commercially available yet.

Mannitol produces a dose-dependent inhibitory effect on vascular smooth muscle in animals.[26, 27] On dog arteries, 31% relaxation with mannitol 2.5×10^{-2} M (4.5 mg/ml) was observed.[26] Mannitol may cause an activation of the sodium pump and scavenge radicals in the cell membrane with subsequent relaxation of smooth muscle cells.[28] The present study suggests that mannitol may inhibit uterine smooth muscle activity. It is conceivable that the amount of mannitol administered when using intravenous dantrolene may cause uterine relaxation. Further research should clarify the clinical significance of this effect, and whether it is reversible by oxytocin.

In conclusion, dantrolene sodium has no effect on spontaneous contractility in an isolated uterine smooth muscle preparation from pregnant women. If uterine muscle relaxation is observed with intravenous dantrolene administration, the relaxation is most likely attributable to the mannitol in the intravenous formulation.

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