4-Chloro-m-cresol Triggers Malignant Hyperthermia in Susceptible Swine at Doses Greatly Exceeding Those Found in Drug Preparations

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Background: Chlorocresols are used as preservatives in numerous commercial drugs that have been shown to induce myoplasmic Ca^{2+} release; the most potent isoform is 4-chloro-*m*-cresol. The aims of this study were to (1) examine the *in vivo* effects of 4-chloro-*m*-cresol on swine susceptible to malignant hyperthermia and (2) contrast *in vivo* versus *in vitro* dose–response curves.

Methods: Susceptible swine (weight: 38.5 kg ± 3.55 kg) were anesthetized and monitored for variations in physiological responses, including end-tidal CO_2, heart rate, blood pressure, blood chemistry, and temperatures. In the first animals studied, 4-chloro-*m*-cresol, at equivalent cumulative doses of 0.14, 0.28, 0.57, 1.14, 2.27, 4.54, and 9.08 mg/kg ($n = 3$; 12.5, 25, 50, 100, 200, 400, and 800 μmol) were administered, and in a second group, larger doses were used: 1.14, 3.41, 7.95, 17.04 ($n = 4$), and/or 35.22 ($n = 1$) mg/kg (100, 300, 700, 1,500, and/or 3,100 μmol). For comparison, *in vitro* rectus abdominis muscle preparations obtained from normal and susceptible swine were exposed to 4-chloro-*m*-cresol, at cumulative concentrations of 6.25, 12.5, 25, 50, 100, 200, 400, 800, and 1,600 μmol; standard caffeine and halothane contracture testing was also performed.

Results: Episodes of malignant hyperthermia were not triggered in response to administration of low doses of 4-chloro-*m*-cresol, but transient cardiovascular reactions (*e.g.*, tachycardia, arrhythmias, and hypotension) were observed. Subsequently, episodes in these animals were triggered when halothane (0.87; 1 MAC) and succinylcholine (2 mg/kg) were given. Animals administered the higher doses of 4-chloro-*m*-cresol all had fulminant episodes of malignant hyperthermia that were fatal, when equivalent cumulative concentrations were greater than 1,500 μmol. The levels of 4-chloro-*m*-cresol in the plasma rapidly decreased: *e.g.*, 5 min postadministration of the 1,500-μmol dose, the mean plasma level was only 52 ± 18 μmol ($n = 4$). Hemolysis was detected following 4-chloro-*m*-cresol administration at concentrations > 200 μmol. *In vitro*, muscle from susceptible animals elicited contractures > 200 mg at 50-μmol bath concentrations of 4-chloro-*m*-cresol ($n = 29$), whereas normal muscle did not elicit such contractures until bath concentrations were > 800 μmol ($n = 10$).

Conclusions: 4-Chloro-*m*-cresol is a trigger of malignant hyperthermia in susceptible swine, but only when serum concentrations are far above those likely to be encountered in humans. A relatively low concentration of 4-chloro-*m*-cresol, 50 μmol, is sufficient to activate sarcoplasmic $[Ca^{+2}]$ release *in vitro* (*e.g.*, contractures); this same bolus dose administered *in vivo* (0.57 mg/kg) has minimal effects due to the rapid decrease in its plasma levels. (Key words: Chlorocresols; dose–response curves; hemolysis; *in vitro* contracture testing; systemic responses.)

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IN a recent study, chlorocresols were shown to be potent and specific activators of the skeletal muscle ryanodine receptor.[1-3] Not all of the isoforms of cresols are considered to have the same potential for inducing Ca^{2+} release from intracellular stores.[1] Specifically, it was shown that 4-chloro-*m*-cresol was most effective in producing such responses.[1] Chlorocresol derivatives are often used as preservatives in numerous drug preparations, including steroid creams, asthma inhalations, insulins, and succinylcholine (some manufacturers).[2,4-14] *In vitro*, these substances have been shown to elicit contractures in muscles obtained from patients susceptible to malignant hyperthermia.[2,15-19] Thus, it has been speculated that the presence of chlorocresol in commercial preparations of succinylcholine, if they are

administered to individuals susceptible to malignant hyperthermia, is the underlying cause for the initiation of an anesthetic-induced event.[2] However, the relative concentrations of chlorocresols in most drug preparations can be considered to be low. For example, in Midarine (Wellcome, Pomezia, Italy), one of the succinylcholine preparations in which this preservative is found, the concentration is only 0.1%.[2]

The purpose of this study was to examine the *in vivo* effects of 4-chloro-*m*-cresol and its possible role in the triggering of malignant hyperthermia in susceptible swine, and thus to determine the clinical significance (or relevance) of the concentrations of 4-chloro-*m*-cresol in current market preparations. Specifically, experiments were designed to determine a relative dose–response curve. In addition, *in vitro* contracture testing was performed to determine the effective concentrations of 4-chloro-*m*-cresol needed to induce skeletal muscle contractures. The results of these experiments provide new insights as to the plasma concentrations of 4-chloro-*m*-cresol needed to induce episodes of malignant hyperthermia in susceptible swine. In addition, provided by this study is new information as to the pharmacologic effects of this agent on overall systemic physiology.

Materials and Methods

After approval from the Institutional Animal Care and Use Committee of the University of Minnesota, the following experiments were conducted on pure-bred Pietrain swine with the a mutation of arginine 615 in the ryanodine receptor (University of Minnesota Rosemount Animal Facility).[20] Susceptibility to malignant hyperthermia was verified by *in vitro* contracture testing (according to the protocol recommended by the North American Malignant Hyperthermia Registry).

The animals (weight: 38.5 kg ± 3.6 kg) were fasted (12–14 h) and subsequently anesthetized with a mixture of intramuscular tiletamine and zolazepam (Telazol, Fort Dodge, IA) and xylazine (TranquiVed, Vedco, St. Joseph, MO), 0.25 mg/kg of each; intubated; and mechanically ventilated. While probes were positioned for invasive monitoring, the animals were anesthetized with 70% N_2O and thiopental. Paralysis was achieved during induction with a pancuronium bolus (6 ± 2 mg) followed by a continuous infusion during surgery of 30 ± 12 mg. Mechanical ventilation was adjusted to maintain ET_{CO_2} at 40 ± 2 mmHg (Nellcor gas analyzer, Hayward, CA). The following physiological parameters were monitored

throughout the studies: end-tidal CO_2, heart rate, arterial blood pressure, blood chemistry (arterial oxygen [Pa_{O_2}] and carbon dioxide [Pa_{CO_2}] tensions, pH, and K^+, Na^+, and HCO_3 levels), and temperatures (esophageal, rectal, vastus lateralis, liver, kidney, masseter, and tympanic).[21]

During all surgical procedures, the animals were maintained at esophageal temperatures of 38.2 ± 0.6°C. This was accomplished utilizing forced-air warming (Bair Hugger, Model 500, Augustine Medical, Eden Prairie, MN).[21] The rates and volumes of the inspired gases, which were not warmed or humidified, were not adjusted from the beginning of the control period or throughout the rest of the study. The arterial blood Pa_{O_2}, Pa_{CO_2}, pH, and K^+, Na^+, HCO_3, and hematocrit levels were determined from fresh arterial samples using a blood gas analyzer with temperature compensation (Model BGE 1304, Instrumentation Laboratories, Lexington, MA).

A cannula was inserted into either the brachial or femoral artery for pressure monitoring using a SpaceLabs model 1020 (SpaceLabs, Chatsworth, CA) and blood sampling. The ear vein catheter (18-gauge Jelco, Johnson and Johnson Medical, Arlington, TX) served as the primary route for intravenous fluid administration (780 ± 160 ml/h of Ringer's lactate solution). The heart rate was recorded using a five-lead electrocardiogram (SpaceLabs model 1020). Temperatures were recorded in the distal esophagus and rectum using Mon-a-Therm probes (Mallinckrodt Anesthesia Products, St. Louis, MO).[21] The electronic thermocouples were connected to a calibrated Iso-Thermex thermometer (Columbus Instruments, Columbus, OH), accurate to 0.1°C. All data were collected using LabVIEW software (National Instruments, Austin, TX) on a Macintosh computer (Apple Computer, Cupertino, CA).

4-Chloro-m-cresol Preparation and Administration

Following the collection of the last set of control measurements (for a minimum of 30 min), 4-chloro-*m*-cresol (Pfaltz and Bauer, Waterbury, CT) was administered. The 4-chloro-*m*-cresol was prepared in normal saline to a concentration of 5 mg/ml. This initial preparation required warming to approximately 50°C with constant stirring to facilitate dissolution. It was then allowed to cool to room temperature. To minimize acute cardiovascular effects, the 4-chloro-*m*-cresol solution was injected gradually (over a 1- to 10-min period, depending on the concentration; see fig. 1).

The animals were divided into two groups to which different doses of 4-chloro-*m*-cresol were given intra-
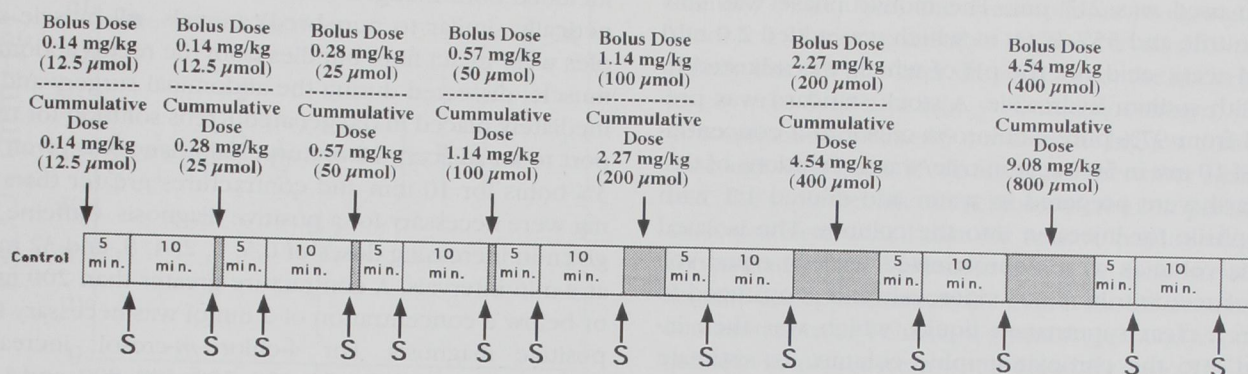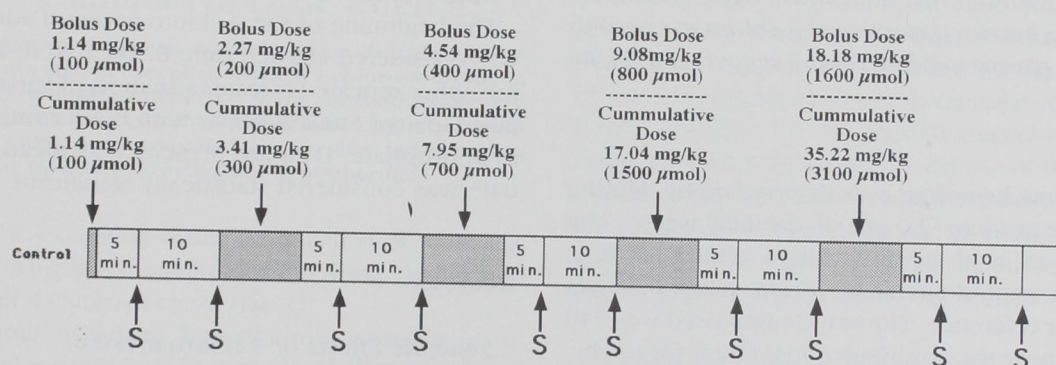
Fig. 1. Schematic diagrams of the experimental protocols employed in the two groups of animals investigated. Control data were collected for a minimum of 30 min. The first group of animals investigated in this study were administered low bolus doses of 4-chloro-m-cresol, and in the second group of animals larger doses were used. Provided are the bolus concentrations in μM and mg/kg concentrations and the equivalent cumulative dose values. The shaded areas indicate the times during which the bolus doses were administered. The larger bolus doses were administered over a 10-min period to minimize associated cardiovascular instability induced by the 4-chloro-m-cresol administration. The times at which blood samples were withdrawn (S) are indicated below each timeline.

venously (see fig. 1). Group 1, the low-dose group, received 4-chloro-m-cresol boluses of 0.14, 0.14, 0.28, 0.57, 1.14, 2.27, and 4.54 mg/kg (12.5, 12.5, 25, 50, 100, 200, and 400 μmol; n = 3), and group 2, the high-dose group, received boluses of 1.14, 2.27, 4.54, 9.08 (n = 4), and/or 18.18 (n = 1) mg/kg (100, 200, 400, 800, and/or 1,600 μmol). These administered boluses resulted in equivalent cumulative doses of 0.14, 0.28, 0.57, 1.14, 2.27, 4.54, 9.08 mg/kg (12.5, 25, 50, 100, 200, 400, 800 μmol) in the first group and 1.14, 3.41, 7.95, 17.04, 35.22 mg/kg (100, 300, 700, 1,500, 3,100 μmol) in the second group. These concentrations were derived using an estimated blood volume of 80 ml/kg. If an animal did not trigger an episode of malignant hyperthermia by the end of its

predesignated concentration run, halothane (0.87%) and succinylcholine (2 mg/kg) were administered, and in all such cases, episodes of malignant hyperthermia were initiated.

Plasma Levels of 4-chloro-m-cresol

Plasma levels of 4-chloro-m-cresol were determined using high-performance liquid chromatography. The system consisted of a Spectra-Physics (San Jose, CA) model SP8810 pump; a Rheodyne (Cotati, CA) model 7010 injection valve with a 20-μl sample loop; an Inertsil ODS-2 250 × 4.6-mm, 5-μm column (MetaCham Technologies, Torrance, CA); and a Waters Chromatography (Milford, MA) model 481 spectrophotometer. The chromatographs were acquired and calculated using a Waters

Baseline (810) chromatography workstation. The wavelength used was 217 nm. The mobile phase was 45% acetonitrile and 55% H_2O, to which was added 2.0 ml/l glacial acetic acid and the pH of which was adjusted to 8.0 with sodium hydroxide. A stock standard was prepared from 97% pure 4-chloro-*m*-cresol to a concentration of 10 mm in 50% acetonitrile/water. Dilutions of this standard were prepared in water and diluted 1:1 with acetonitrile for injection into the column. The isolated plasma volumes were deproteinized with an equal volume of acetonitrile mixed vigorously and centrifuged to obtain a clear supernatant liquid, which was then injected into the chromatographic column. To estimate the concentrations of 4-chloro-*m*-cresol, the appropriate peak spectral areas associated with this agent in the plasma samples were subsequently compared with peak areas obtained for known standards. Drug recoveries done by adding known quantities of 4-chloro-*m*-cresol to blank porcine plasma yielded a mean recovery of 94.9% (n = 8).

Hemolysis

Percent plasma hemolysis was determined by diluting 200 μl of plasma into 2.0 ml of distilled water. The absorbance was read in a Beckman (Fullerton, CA) model 24 spectrophotometer in 1.0-cm quartz cuvettes against a water reference. The wavelength used was 540 nm, which is near the absorbance maximum for oxyhemoglobin. Reference standards were prepared by diluting 100 μl of porcine whole blood into 20.0 ml of distilled water or 0.005% saponin in water, which caused complete hemolysis (Sigma Chemical, St. Louis, MO). Blood of several different hematocrits was used and a reference standard curve of 100% hemolysis was obtained using a linear regression. The absorbance of the reference blood was adjusted to compensate for the lower dilution of the unknown plasma. The standard curves for both water and saponin were virtually identical; therefore they were combined to yield a single reference line with a correlation coefficient of 0.991. Percent hemolysis was determined by dividing the absorbance value of the unknown plasma by the absorbance value of 100% hemolyzed whole blood of the same hematocrit and multiplying by 100.

In Vitro *Contracture Testing*

In vitro testing was performed using rectus abdominis muscle and the protocol recommended by the North American Malignant Hyperthermia Registry on equipment previously described.[22,23] Additional muscle samples were obtained from normal swine (n= 4), which included both mongrel and "near" Pietrain (90–95% genetically similar to pure-bred) animals. All muscle samples were intact fiber bundles from the rectus abdominis muscle, dissected during the abdominal surgery and immediately placed in oxygenated Krebs solution for transport to an adjacent laboratory. Halothane was given as a 3% bolus for 10 min and contractures greater than 700 mg were necessary for a positive diagnosis. Caffeine was given in increasing doses of 0.5, 1, 2, 4, 8, and 32 mmol at 4-min intervals. A contracture greater than 200 mg at or below a concentration of 2 mmol was necessary for a positive diagnosis. For 4-chloro-*m*-cresol, increasing doses of 6.25, 12.5, 25, 50, 100, 200, 400, 800, and 1,600 μmol were given at 4-min intervals.

Data Analysis

The beginning of the 4-chloro-*m*-cresol administration was considered elapsed time 0. Data were analyzed using either repeated-measures analysis of variance or two-tailed, paired Student t tests with Bonferroni corrections as appropriate. Data are presented as mean $\pm$ SD; $P < 0.05$ was considered statistically significant.

Results

Systemic Effects of 4-chloro-m-cresol

Episodes of malignant hyperthermia were not triggered in response to administration of low doses of 4-chloro-*m*-cresol, but transient cardiovascular reactions (*e.g.*, tachycardia, arrhythmias, and hypotension) were observed (fig. 2). Subsequently, episodes in these animals were triggered when halothane (1 MAC) and succinylcholine (2 mg/kg) were given. However, animals administered the higher doses of 4-chloro-*m*-cresol all elicited fulminant episodes of malignant hyperthermia, when equivalent cumulative concentrations were 7.95 mg/kg (700 μmol, n = 3) or in one case 17.04 mg/kg (1,500 μmol; fig. 2).

The severity of the transient cardiovascular responses (tachycardia, arrhythmias, and hypotension) increased with increasing concentrations of 4-chloro-*m*-cresol. To minimize such acute cardiovascular effects, the 4-chloro-*m*-cresol solution was injected gradually (over a 1- to 10-min period, depending on the concentration; see fig. 1). Even then, in some cases, the mean arterial pressures dropped below 60 mmHg during the infusion of bolus doses greater than 400 μM. Interestingly, all hemodynamic values returned to baseline within 5 min after completion of injec-
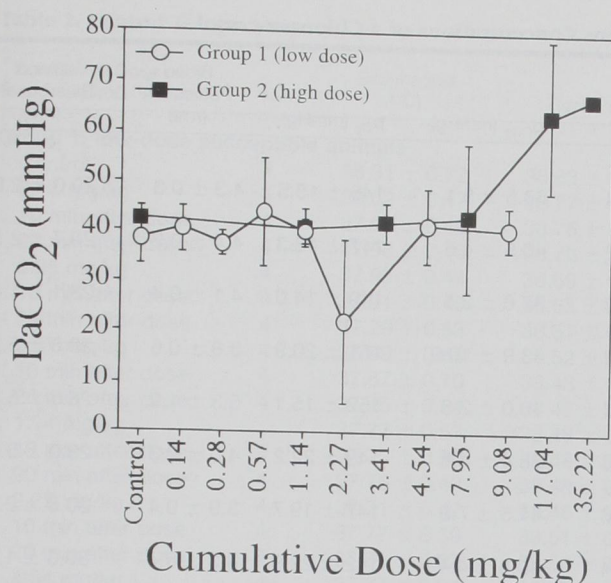
Fig. 2. Changes in the mean arterial carbon dioxide tension measured 5 min after consecutive doses of 4-chloro-m-cresol. In the group 1 (low dose) animals (n = 3) the concentrations were stable throughout the 4-chloro-m-cresol infusions, whereas in the group 2 (high dose) animals (n = 4), they began to rise because of the initiation of malignant hyperthermia.

tion, with the exception being the initiation of fulminant episodes of malignant hyperthermia following exposure to high levels of 4-chloro-m-cresol (fig. 2).

The initiated malignant hyperthermia episodes in all cases resulted in death (n = 7). Arterial pH, Pa_{O_2}, Pa_{CO_2}, and K^+ level showed no statistical significant changes until 5 min into the fulminant malignant hyperthermia episode. The time in which it took the end-tidal CO_2 to reach 70 mmHg, from initial triggering, also showed no significant difference between the two groups of animals, even though the administration of halothane and succinylcholine was required for initiation in the group 1 animals (fig. 3). Proliferation rates determined for changes in Pa_{CO_2} were similar between the two groups of animals (fig. 3). The Pa_{CO_2} levels, for both groups, stayed well within control values until cumulative doses of 4-chloro-m-cresol exceeded 17.04 mg/kg (1,500 μmol). Nevertheless, in all animals once an episode of malignant hyperthermia was initiated the Pa_{CO_2} rose dramatically (see table 1). Rises in core temperature were observed for the high-dose animals in which the 4-chloro-m-cresol did initiate episodes of malignant hyperthermia (table 2).

Plasma Levels of 4-chloro-m-cresol and Hemolysis

Concentrations of 4-chloro-m-cresol in the blood rapidly decreased (table 1). For example, 5 min after administra-

tion of the 7.95-mg/kg (700-μmol) dose in the group 2 animals, (high dose) the mean plasma level was 19.31 μmol $\pm$ 4.35 (n = 4; see table 1). These levels decreased so rapidly that, 15 min after the same dose, the mean plasma levels were 9.49 $\pm$ 4.8 (n = 4; data not shown). Plasma concentrations obtained 5 min after the highest cumulative dose and subsequent levels measured thereafter again illustrate the rapid decline of 4-chloro-m-cresol in plasma (fig. 4). It was determined that the relative half-life of plasma concentrations of 4-chloro-m-cresol in these susceptible animals was approximately 20 min. It should be noted that concentrations of 4-chloro-m-cresol were found to be maintained in incubated fresh whole blood for up to 3 h and that no degradation within the blood was identified.

Visible hemolysis (*i.e.*, detectable to the eye) was observed in plasma samples following 4-chloro-m-cresol concentrations > 3.41 mg/kg (300 μmol). However, spectrophotometric analysis detected that significant hemolysis had occurred even following the administration of the 1.14-mg/kg (100-μmol) cumulative dose (0.07 $\pm$ 0.01%; see table 1). The animals administered the lower doses elicited a maximum hemolysis of 0.98 $\pm$ 0.23% 5 min after the maximum cumulative dose of 4-chloro-m-cresol was given; *i.e.*, 9.08 mg/kg (800 μmol). The hemolysis was even greater in the high-dose group, which provided a maximum hemolysis of 3.26 $\pm$ 0.90% 5 min
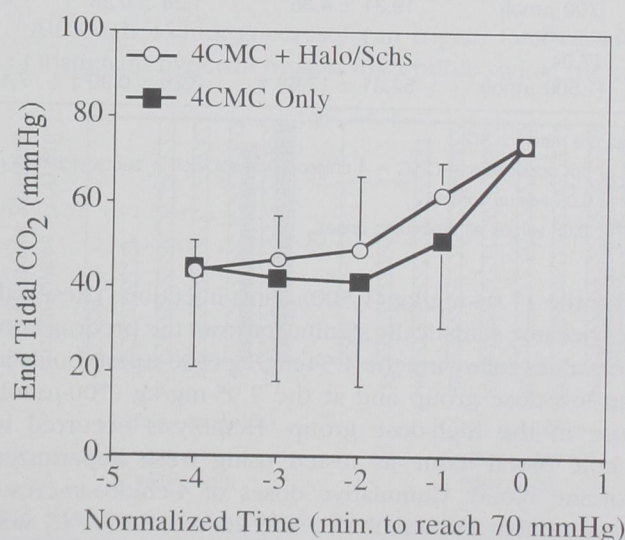
Fig. 3. A comparison of the rate of rise of end-tidal CO_2 between the high- and low-dose swine following the initiation of episodes of malignant hyperthermia. The low-dose animals were triggered with halothane (0.87%) and succinylcholine (2 mg/kg), whereas the high-dose animals triggered with or during 4-chloro-m-cresol infusions at cumulative doses greater than 17.04 mg/kg (1,500 μmol). End-tidal CO_2 was normalized to 70 mmHg at time 0. Values represent means $\pm$ SD.

Table 1. Changes in Blood Chemistry after the Administration of Various Concentrations of 4-Chloro-*m*-cresol

Cumulative Dose of 4-CmC (mg/kg)	Plasma Concentration of 4-CmC (μM)	Hemolysis (%)	pH	p_{CO_2} (mmHg)	p_{O_2} (mmHg)	Blood Potassium (mM)	Blood Bicarbonate (mM)
Group 1: low-dose susceptible animals (n = 3)							
Control	0	0	7.48 ± 0.03	38.5 ± 5.1	145 ± 13.5	4.3 ± 0.3	29.0 ± 2.9
5 min after 0.14 (12.5 μmol)	0	0	7.47 ± 0.02	40.5 ± 4.6	147 ± 18.3	4.4 ± 0.2	29.7 ± 2.5
5 min after 0.28 (25 μmol)	0	0	7.49 ± 0.03	37.6 ± 2.5	149 ± 14.0	4.1 ± 0.4	29.1 ± 1.4
5 min after 0.57 (50 μmol)	0.35 ± 0.33	0	7.42 ± 0.52	43.9 ± 10.9	151 ± 20.9	3.8 ± 0.6	28.8 ± 5.2
5 min after 1.14 (100 μmol)	1.18 ± 0.24	0.06 ± 0.06	7.45 ± 0.07	40.0 ± 2.6	152 ± 15.1	5.3 ± 1.9	28.3 ± 5.3
5 min after 2.27 (200 μmol)	3.58 ± 0.23*	0.23 ± 0.13	7.45 ± 0.08	38.4 ± 4.8	149 ± 22.2	4.0 ± 0.3	28.0 ± 5.2
5 min after 4.54 (400 μmol)	9.41 ± 1.62*†	0.57 ± 0.06*†	7.48 ± 0.06	41.5 ± 7.5	147 ± 19.7	3.9 ± 0.4	30.8 ± 2.2
5 min after 9.08 (800 μmol)	20.97 ± 1.73*†	0.98 ± 0.23*†	7.48 ± 0.04	40.8 ± 4.4	142 ± 22.9	4.3 ± 0.4	30.3 ± 1.5
5 min after halothane, 1 MAC and Sch 2 mg/kg	NA	NA	7.06 ± 0.07*†	85.8 ± 7.8*†	174 ± 188	6.6 ± 0.8*	25.1 ± 5.9
Group 2: high-dose susceptible animals (n = 4)							
Control	0	0	7.47 ± 0.02	42.3 ± 2.6	139 ± 13.9	4.4 ± 0.15	30.9 ± 1.4
5 min after 1.14 (100 μmol)	2.73 ± 2.08	0.07 ± 0.01	7.47 ± 0.03	40.8 ± 3.7	140 ± 17.6	4.2 ± 0.31	29.8 ± 3.9
5 min after 3.41 (300 μmol)	6.44 ± 3.66	0.41 ± 0.19	7.46 ± 0.04	42.0 ± 4.0	136 ± 24.6	4.1 ± 0.10	30.3 ± 0.6
5 min after 7.95 (700 μmol)	19.31 ± 4.35*	1.28 ± 0.38*	7.43 ± 0.12	46.5 ± 11.6	135 ± 24.7	5.1 ± 1.30	28.2 ± 4.8
5 min after 17.04 (1,500 μmol)	52.31 ± 17.68*†	3.26 ± 0.90*†	7.11 ± 0.29*†	63.6 ± 15.2*	107 ± 42.2	6.5 ± 1.18*	22.1 ± 8.2*

Data are mean ± SD.

NA = not applicable; 4-CmC = 4-chloro-*m*-cresol; Sch = succinylcholine chloride.

* $P < 0.05$ *versus* control.

† $P < 0.05$ *versus* all preceding doses.

after the 17.04-mg/kg (1,500-μmol) injection. These values became statistically significant from the predrug control values following the 4.54-mg/kg (400-μmol) bolus in the low-dose group and at the 7.95-mg/kg (700-μmol) dose in the high-dose group. Hemolysis occurred in whole blood alone as tested using fresh heparinized porcine blood: Cumulative doses of 4-chloro-*m*-cresol were added and incubated for 20 min at 37°C, and estimated hemolysis was found to be only slightly higher than in corresponding *in vivo* samples.

In Vitro *Contracture Testing and Responses to 4-chloro-*m-*cresol*

All muscle bundles obtained from the swine susceptible to malignant hyperthermia elicited positive *in vitro* contracture tests to both halothane and caffeine. Figure 5 shows the *in vitro* comparison test of susceptible and normal bundles to caffeine and 4-chloro-*m*-cresol. All muscle bundles produced contractures ≥ 200 mg at 2 mmol caffeine, compared with 8 mmol in normal bundles (fig. 5). With 4-chloro-*m*-cresol, 100% of the susceptible bundles produced contractures ≥ 200 mg at 50 μmol, compared with 1,600 μmol in normal bundles (fig. 5). More specifically, 20 of 24 bundles exposed to 4-chloro-*m*-cresol produced contractures ≥ 200 mg at bath concentrations < 50 μmol. In terms of diagnostic differentiation, caffeine provides a 4- to 16-fold concentration difference, and 4-chloro-*m*-cresol provides at least a 32-fold diagnostic

Table 2. Central Temperatures

Cumulative Dose of 4-CmC	N	Esophageal (°C)	Rectal (°C)
Group 1: low-dose susceptible animals			
Control	4	38.31 ± 0.72	38.83 ± 0.50
0.14 mg/kg	4	38.02 ± 0.47	38.77 ± 0.53
10 min after dose	4	37.97 ± 0.46	38.76 ± 0.57
20 min after dose	4	37.98 ± 0.45	38.70 ± 0.59
0.28 mg/kg	4	37.93 ± 0.44	38.69 ± 0.62
10 min after dose	4	37.91 ± 0.45	38.65 ± 0.65
20 min after dose	4	37.76 ± 0.53	38.57 ± 0.71
0.57 mg/kg	4	37.69 ± 0.63	38.52 ± 0.72
10 min after dose	4	37.67 ± 0.70	38.43 ± 0.86
20 min after dose	4	37.75 ± 0.54	38.47 ± 0.79
1.14 mg/kg		37.72 ± 0.57	38.48 ± 0.79
10 min after dose	4	37.75 ± 0.50	38.48 ± 0.73
20 min after dose	4	37.76 ± 0.42	38.48 ± 0.62
2.27 mg/kg	4	37.71 ± 0.42	38.48 ± 0.62
10 min after dose	4	37.77 ± 0.39	38.51 ± 0.57
20 min after dose	4	37.81 ± 0.32	38.54 ± 0.53
4.54 mg/kg	4	37.77 ± 0.34	38.53 ± 0.51
10 min after dose	4	37.77 ± 0.31	38.56 ± 0.52
20 min after dose	4	37.76 ± 0.26	38.54 ± 0.42
9.08 mg/kg	4	37.73 ± 0.29	38.54 ± 0.45
10 min after dose	4	37.71 ± 0.31	38.51 ± 0.43
20 min after dose	4	37.77 ± 0.33	38.56 ± 0.47
30 min after dose	3	37.77 ± 0.46	38.64 ± 0.54
40 min after dose	1	38.12	38.52
Group 2: high-dose susceptible animals			
Control	4	38.51 ± 0.38	39.16 ± 0.36
1.14 mg/kg	4	38.44 ± 0.32	39.05 ± 0.35
10 min after dose	4	38.48 ± 0.31	38.99 ± 0.35
20 min after dose	4	38.53 ± 0.32	39.01 ± 0.36
3.41 mg/kg	4	38.47 ± 0.35	39.01 ± 0.38
10 min after dose	4	38.49 ± 0.28	38.97 ± 0.41
20 min after dose	4	38.57 ± 0.31	38.98 ± 0.42
7.95 mg/kg	4	38.61 ± 0.35	39.04 ± 0.37
10 min after dose	4	38.63 ± 0.34	39.13 ± 0.57
20 min after dose	4	38.72 ± 0.44	39.21 ± 0.68
17.04 mg/kg	4	39.11 ± 1.20	39.48 ± 1.04
10 min after dose	4	39.55 ± 1.32	39.70 ± 1.34
20 min after dose	3	39.91 ± 1.15	39.75 ± 1.55
30 min after dose	2	41.25 ± 1.22	40.05 ± 2.84
40 min after dose	1	41.06	38.32
50 min after dose	1	41.77	38.46
60 min after dose	1	42.49	38.63
35.22 mg/kg	1	38.81	39.22
10 min after dose	1	39.69	39.31
20 min after dose	1	41.47	39.93
30 min after dose	1	42.1	40.49
40 min after dose	1	42.42	40.89

Data are mean ± SD.

4-CmC = 4-chloro-m-cresol.

concentration difference. Three percent halothane contracture testing was also performed (data not shown). During exposure to 3% halothane bath concentration, all the muscle bundles from the suscepti-
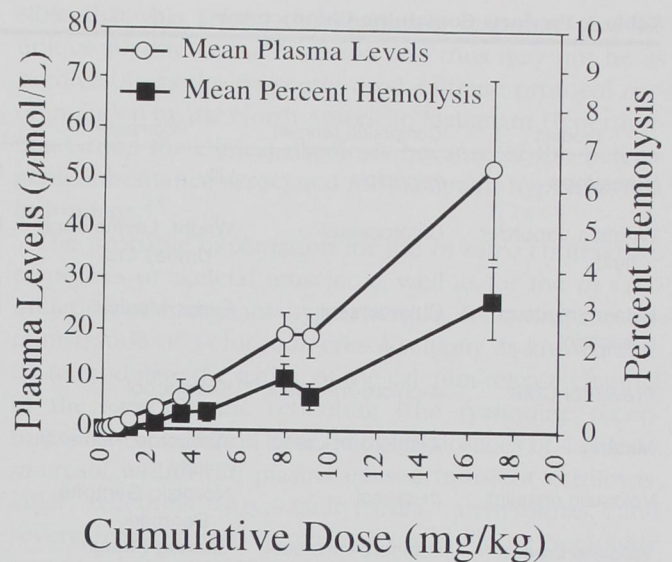
Fig. 4. Mean ± SD plasma levels of 4-chloro-*m*-cresol and percent hemolysis measured 5 min after consecutive doses of 4-chloro-*m*-cresol. The cumulative dose was calculated using an assumed blood volume of 80 ml/kg of body weight. Data shown were obtained from both the low- and high-dose groups.

ble swine (n = 33) and none of the normal bundles (n = 10) had contractures > 700 mg.

Discussion

Although 4-chloro-*m*-cresol can trigger fatal episodes of malignant hyperthermia in susceptible swine, the con-
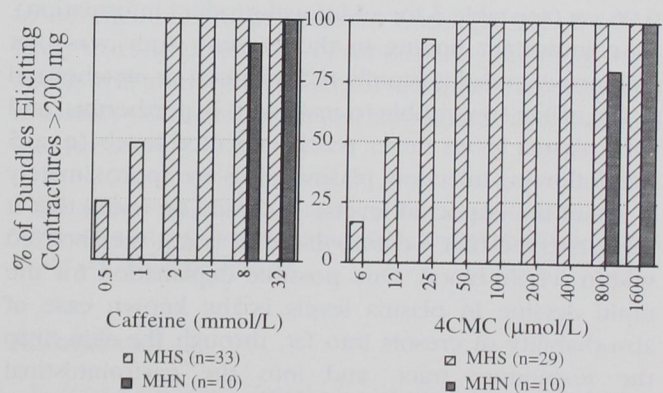

Fig. 5. An *in vitro* comparison of malignant hyperthermia susceptible and nonsusceptible muscle bundles (intact fiber bundles from the rectus abdominis muscle) to graded doses of either caffeine or 4-chloro-*m*-cresol. Contractures of greater than or equal to 200 mg of force were considered a positive response. The graded caffeine contracture test provided a 4- to 16-fold concentration difference between susceptible and normal muscle bundles, whereas the 4-chloro-*m*-cresol contracture responses provided a 32-fold diagnostic concentration difference.

Table 3. Products Containing Chlorocresols

Product	Compound (isomer)	Company	Reactions Noted	Concentration in the Preparation (%)	Effective Concentration (mg/kg)	Reference
Humatrope	*m*-cresol	Lilly	Myalgia, elevated CK activity	0.3	0.18	Bach *et al.* (1992)
Wright's Vaporizer fluid	Chlorocresol	Wright, Layman and Umney Ltd.	Death, seizures (accidental ingestion)	10.0	250	Wiseman *et al.* (1980)
Pularin (mucous heparin)	Chlorocresol	Evans Medical	Acute and severe pain of injection site	0.15	Variable	Ainley *et al.* (1977)
Preventol CMK	4-chloro-*m*-cresol	Krampocol	Contact dermatitis	Unknown	Variable	Dooms-Goossens *et al.* (1981)
Midarin	4-chloro-*m*-cresol	Wellcome, Pomezia, Italy	*In vitro* muscle contracture	0.1	0.1	Tegazzin *et al.* (1996)
Velosulin (insulin)	*m*-cresol	Nordisk, Gentofte, Denmark	Inflammation at infusion site	0.27	Variable	van Faassen *et al.* (1990)
Valisone cream	4-chloro-*m*-cresol	Shering Plough	Contact dermatitis	0.01–0.1 Base	Variable	Lewis and Emmett (1987)

CK = creatine kinase.

centrations necessary should be considered nonpharmacologic. It is highly unlikely that an episode of malignant hyperthermia would occur through exposure to 4-chloro-*m*-cresol under normal clinical circumstances. For example, concentrations found in one available preparation of succinylcholine, Midarin (Wellcome, Pomezia, Italy), are low, and if one administered a 2-mg/kg dose of either a 20- or 50-mg/ml preparation, estimated blood concentrations would range between 0.01 and 0.06 μM (see table 3 for additional product information).

An important finding in the present study was that 4-chloro-*m*-cresol is rapidly redistributed or metabolized in the swine susceptible to malignant hyperthermia, and thus plasma levels never reach expected levels (*e.g.*, 5 min following injection, plasma levels are approximately 2–4% of the expected levels). It should be noted that it was observed that 4-chloro-*m*-cresol is not metabolized within whole blood. One possible explanation for the rapid decline in plasma levels is the known ease of absorbability of cresols into fat, through the skin, into the respiratory tract, and into the gastrointestinal tract.[5,7,8] Although the rate and extent of absorption of cresols have not been studied specifically, studies have shown that both gastrointestinal and dermal absorption are rapid and extensive.[8] Cresols are also distributed in all the major organs.[5,7,8] The primary metabolic pathway for cresols is conjugation with glucuronic acid and inorganic sulfate. Minor metabolic pathways for cresols include hydroxylation of the benzene ring and side-chain

oxidation. The main route of elimination of cresols from the body is renal elimination in the form of conjugates.[8]

Other forms of cresols have also been suspected to be reactive, but previous work has shown that the meta form of chlorocresol is the most potent for inducing the release of intracellular Ca^{2+}.[1] The *in vivo* bolus doses of 4-chloro-*m*-cresol required to initiate episodes of malignant hyperthermia in susceptible swine were found to be extremely high (> 9.08 mg/kg or 800 μmol), and it was noted that such concentrations also cause cardiovascular aberrations and were cytotoxic for red blood cells (*i.e.*, resulting in significant hemolysis). Such a hemolytic response is normally not observed following the administration of other known triggering agents: *i.e.*, volatile anesthetic agents or succinylcholine administered at concentrations that will elicit episodes of malignant hyperthermia in such animals. It was noteworthy that the measured plasma levels at which episodes of malignant hyperthermia were induced were similar to those that elicit skeletal muscle contracture responses *in vitro*. More specifically, the 4-chloro-*m*-cresol levels were approximately 50 μmol for contracture responses, > 200 mg for muscle bundles obtained from the susceptible animals. This compares well with the *in vivo* plasma levels (52 μmol) obtained 5 min after the administration of the 17.04 mg/kg (1,500 μmol) bolus (table 1). Therefore, it is unlikely that the clinical use of substances containing a chlorocresol agent as a preservative will initiate an episode of malignant hyperthermia in a

susceptible individual, because the ultimate circulating plasma levels would be extremely low. Hence, much like caffeine, patients susceptible to malignant hyperthermia should readily tolerate low levels of these agents within their bodies.

It is well established that succinylcholine is a trigger of episodes of malignant hyperthermia in susceptible swine.[26,27] The underlying mechanism by which succinylcholine initiates episodes of malignant hyperthermia remains unclear. Nevertheless, even in those preparations of succinylcholine that use chlorocresols as preservatives it is unlikely that the low concentrations of these agents would have an important contribution as to the onset or proliferation of a subsequently initiated episode. As with other known triggers of malignant hyperthermia episodes in susceptible swine, once an event was initiated by high *in vivo* concentration of 4-chloro-*m*-cresol the proliferation rate of the event was rapid and similar to those previously reported for animals with core temperatures of approximately 38°C.[21,24-26]

In previous reports it has been noted that unless certain experimental and physiologic criteria are not controlled during studies performed to initiate episodes of malignant hyperthermia in susceptible swine, such events will not proliferate.[21,24-26] Specifically, it has been shown that hypotension minimized the ability to trigger episodes with either succinylcholine or volatile anesthetics. Similarly, it was recently reported by our laboratory that the core temperature of the animal at the time of exposure to triggering agents is vital to control.[21] It should be noted that in the present experiments all animals maintained a high mean arterial pressure, and their core temperatures were maintained at or slightly above the normal core temperature in swine, 38°C. Such parameters were controlled to insure that the potential triggering response following various bolus concentrations of 4-chloro-*m*-cresol would not be attenuated.

The *in vitro* 4-chloro-*m*-cresol responses of the isolated skeletal muscle bundles from the susceptible animals were clearly different from normal muscle, and these results were consistent with previous findings for human muscle.[15-19] From a potential diagnostic standpoint, it is interesting to consider that caffeine contracture testing provided a 4- to 16-fold concentration difference, and 4-chloro-*m*-cresol provided at least a 32-fold diagnostic concentration difference between normal and susceptible responses. Thus, the use of a dose–response, 4-chloro-*m*-cresol contracture test may be more reliable to administer than the caffeine test: *e.g.*, this may decrease center-to-center variability. Nevertheless, it is possible that this strong differentiating response may be unique for the swine mutation and thus may not be as predictable as the presently used caffeine protocol recommended by the North American Malignant Hyperthermia Group for clinical diagnosis because of the heterogenic inheritance associated for malignant hyperthermia in humans.[23]

The probable explanation for the *in vitro* contracture responses of skeletal muscle, as well as for the *in vivo* initiation of malignant hyperthermia, following the administration of 4-chloro-*m*-cresol is likely its known ability to modulate the gating of the calcium-release channel of the sarcoplasmic reticulum (the ryanodine receptor).[1-3] It is described here that high dosages of 4-chloro-*m*-cresol within the plasma caused transient cardiovascular reactions (*e.g.*, tachycardia, arrhythmias, and severe hypotension) as well as cytotoxic or hemolytic responses. As this agent circulates through the cardiovascular system, it likely causes abnormal calcium regulation in cardiac tissue, vascular smooth muscle, and blood cells. Fortunately, the half-life of this agent is short, so its effects are relatively transient, except for the hemolysis that was noted to accumulate as larger bolus doses were administered.

It is concluded that 4-chloro-*m*-cresol will trigger episodes of malignant hyperthermia in susceptible swine when serum concentrations are sustained at critical levels that activate skeletal muscle intracellular Ca^{+2} release. However, the serum levels of 4-chloro-*m*-cresol needed to initiate such events are far above those likely to be encountered in humans. Consequently, it is unlikely that the clinical use of substances containing these agents will initiate an episode of malignant hyperthermia in a susceptible individual and thus could be considered safe for use.

References

1. Zorzato F, Scutari E, Tegazzin V, Clementi E, Treves S: Chlorocresol: An activator of ryanodine receptor-mediated Ca^{2+} release. Mol Pharmacol 1993; 44:1192-201

2. Tegazzin V, Scutari E, Treves S, Zorzato F: Chlorocresol, an additive to commercial succinylcholine, induces contracture of human malignant hyperthermia-susceptible muscles via activation of the ryanodine receptor Ca^{2+} channel. ANESTHESIOLOGY 1996; 84:1380-5

3. Herrmann-Frank A, Richter M, Sarközi S, Mohr U, Lehmann-Horn F: 4-chloro-*m*-cresol, a potent and specific activator of the skeletal muscle ryanodine receptor. Biochim Biophy Acta 1996; 1289:31-40

4. Abdelaziz AA, el-Nakeeb MA: Sporicidal activity of local anaesthetics and their binary combinations with preservatives: J Clin Pharm Therap, 1988; 13:249–56

5. Ainley EJ, Mackie IG, Macarthur D: Adverse reaction to chlorocresol-preserved heparin (letter). Lancet 1977; 1:705

6. Bach MA, Blum DM, Rose SR, Charnas LR: Myalgia and elevated creatine kinase activity associated with subcutaneous injections of diluent. J Pediatr 1992; 121:650–1

7. Bray JG Jr: "Heparin allergy" and cardiopulmonary bypass (letter). J Cardiothorasc Vasc Anesth 1993; 7:505–6

8. Dooms-Goossens A, Degreef H, Vanhee J, Kerkhofs L, Chrispeels MT: Chlorocresol and chloracetamide: Allergens in medications, glues, and cosmetics. Contact Derm 1981; 7:51–2

9. Jaschonek K Faul C, Daiss W, Weisenberger H: Effects of heparin on prostacyclin binding and platelet adenylate cyclase activity stimulated by iloprost and forskolin. Prostagland Leuko Med 1996; 24:199–206

10. Lewis PG, Emmett EA: Irritant dermatitis from tributyl tin oxide and contact allergy from chlorocresol. Contact Derm 1987; 17:129–32

11. Sim AT, White MD, Denborough MA: The effect of oxytocin on porcine malignant hyperpyrexia susceptible skeletal muscle. Clin Exp Pharm Physiol 1987; 14:605–10

12. van Faassen I, Razenberg PP, Simoons-Smit AM, van der Veen EA: Carriage of *Staphylococcus aureus* and inflamed infusion sites with insulin-pump therapy. Diabet Care 1989; 12:153–5

13. van Faassen I, Verweij-van Vught AM, Lomecky-Janousek MZ, Razenberg PP, van der Veen EA: Preservatives in insulin preparations impair leukocyte function: In vitro study. Diabetes Care 1990; 13:71–4

14. Wiseman HM, Turner WM, Volans GN: Acute poisoning due to Wright's vaporizing fluid. Postgrad Med J 1980; 56:166–8

15. Ben-Abraham R, Krivosic-Horber RM, Haudcoeur G, Perel A, Adnet PJ: Effect of chlorocresol vs caffeine on muscle contracture in malignant hyperthermia susceptible patients. Harefuah 1997; 132:839–41

16. Gilly H, Musat I, Fricker R, Bittner RE, Steinbereithner K, Kress HG: Classification of malignant hyperthermia-equivocal patients by 4-chloro-M-cresol. Anesth Analg 1997; 85:149–51

17. Hermann-Frank A, Richter M, Lehmann-Horn F: 4-Chloro-*m*-cresol: A specific tool to distinguish between malignant hyperthermia-susceptible and normal muscle. Biochem Pharmacol 1996; 52:149–55

18. Ørding H, Glahn K, jGardi T, Fagerlund T, Bendixen D: 4-Chloro-*m*-cresol test: A possible supplementary test for diagnosis of malignant hyperthermia susceptibility. Acta Anaesthesiol Scand 1997; 41:967–72

19. Wappler F, Scholz J, von Richrhofen V, Fiege M, Steinfath M Schulte am Esch J: 4-chloro-*m*-cresol-induced contractures of skeletal muscle specimen from patients at risk for malignant hyperthermia. Anasthesiol Intensivmed Notfallmed Schmezther 1997; 32:541–8

20. Gillard EF, Otsu K, Fujii J, Khanna K, De Leon S, Derdemezi J, Britt BA, Duff CL, Worton G, Maclennan DH: A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. Genomics 1991; 11:751–5

21. Iaizzo PA, Kehler CH, Zink RS, Belani KG, Sessler DI: Thermal response in acute porcine malignant hyperthermia. Anesth Analg 1996; 80:803–9

22. Iaizzo PA, Wedel DJ, Gallagher WJ: In vitro contracture testing for the determination of susceptibility to malignant hyperthermia: A methodological update. Mayo Clin Proc 1991; 66:998–1004

23. Larach MG, the North American Malignant Hyperthermia Group: Standardization of the caffeine halothane muscle contracture test. Anesth Analg 1989; 69:511–5

24. Iaizzo PA, Wedel DJ: Episodes of malignant hyperthermia induced by the depolarizing muscle relaxant succinylcholine. Anesth Analg 1994; 79:143–51

25. Wedel DJ, Iaizzo PA, Milde JH: Desflurane is a trigger of malignant hyperthermia in susceptible swine. ANESTHESIOLOGY 1991; 74:508–12

26. Wedel DJ, Gammel SA, Milde JH, Iaizzo PA: Delayed onset of malignant hyperthermia induced by isoflurane and desflurane compared to halothane in susceptible swine. ANESTHESIOLOGY 1993; 78:1138–44

27. Galloway GJ, Denborough MA: Suxamethonium chloride and malignant hyperthermia. Br J Anaesth 1986; 58:447–50