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Evaluation of Efficacy of Mineral Oil, Charcoal, and Smectite in a Rat Model of Equine Cantharidin Toxicosis

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Background: The efficacy of orally administered therapeutics for the treatment of cantharidin intoxication has not been evaluated in controlled studies.

Objective: To develop a model of acute cantharidin intoxication in laboratory rats and to evaluate in this model the relative efficacy of 3 gastrointestinal therapies used to treat equine cantharidin toxicosis.

Animals: Sixty-four male Sprague-Dawley rats.

Methods: A blinded, randomized, controlled study was performed on rats surgically implanted with telemetry transmitters for evaluating heart rate, locomotor activity, and body temperature. Orogastric administration of cantharidin was performed within 15 seconds before administration of mineral oil, activated charcoal, or smectite. Negative control groups received therapeutic agents alone. Urine was collected for cantharidin analysis. Rats were sacrificed 24 hours after intoxication, and tissues were collected for histopathologic evaluation. Data analysis included ANOVA procedures and contingency tables.

Results: Six of 8 cantharidin-intoxicated rats treated with mineral oil died; bradycardia and hypothermia developed in the animals of this group 0–8 hours after intoxication. Rats treated with mineral oil had higher urine cantharidin concentrations than rats receiving cantharidin alone or with smectite ($P = .04$). The most severe hypothermia (30.6°C ± 1.0) developed in rats administered mineral oil at 4–8 hours after intoxication, whereas those treated with charcoal (35.2°C ± 0.8) had mean body temperatures higher than all other treatment groups ($P = .03$). Survival times in the charcoal ($P = .16$) and smectite ($P = .12$) treatment groups were not statistically different from negative controls.

Conclusions and Clinical Importance: Mineral oil is often used in the treatment of equine cantharidin toxicosis. Our findings suggest that mineral oil increases cantharidin absorption, worsening morbidity and fatality in rats.

Key words: Biosponge; Charcoal; Mineral Oil; Smectite.

Cantharidin toxicosis was first reported in humans in 1921.[1] Human morbidity and fatality have occurred because of accidental or deliberate exposure for medicinal purposes and as an alleged aphrodisiac.[2–5] The toxicosis occurs in a variety of other species, including rabbits, dogs, cats, emus, chickens, sheep, goats, and cattle. Horses, however, appear to be the most commonly affected species, and most often toxicosis is associated with the consumption of alfalfa hay contaminated with blister beetles.[6–11]

Cantharidin is a potent acantholytic vesicant present in the hemolymph of various species of blister beetles.[12–15] Although cantharidin toxicosis in horses occurs most frequently in the southwestern states, outbreaks are also reported throughout the Midwest.[16] Furthermore, transported hay can be a source of cantharidin exposure in areas where the beetles are not endemic.[17]

Abbreviations:

BSC	cantharidin plus smectite group
CAN	cantharidin only group
CHC	cantharidin plus charcoal group
DMSO	dimethyl sulfoxide
GC-MS	gas chromatography-mass spectrometry
GI	gastrointestinal
MOC	cantharidin plus mineral oil group

Many of the clinical signs associated with cantharidin toxicosis in horses are related to local irritation of the gastrointestinal (GI) mucosa after ingestion.[18] Cantharidin absorbed through the gastrointestinal tract produces systemic effects and is excreted in the urine resulting in irritation of the urinary bladder and dysuria.[19] In horses, clinical signs can manifest as severe colic, enterocolitis, synchronous diaphragmatic flutter, polydipsia, pollakiuria, pyrexia, tachycardia, and tachypnea.[18,19] Common clinicopathologic changes include profound hypocalcemia, hypomagnesemia, microscopic hematuria, and hyposthenuria in the face of dehydration. The presumptive clinical diagnosis is based on exposure risk coupled with clinical signs and clinicopathologic data, and confirmed by demonstration of toxin via GC-MS in either urine or intestinal contents.[6]

Treatment for cantharidin toxicosis is aimed at maintaining fluid and electrolyte homeostasis while minimizing intestinal irritation and cantharidin absorption.[20] Attempts to achieve the latter are through the use of gastrointestinal therapeutics. Mineral oil is used commonly to treat equine colic, and many publications recommend the use of mineral oil or activated charcoal

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as agents to decrease transit time through the gastrointestinal tract and adsorb cantharidin, respectively.[18,20–22] Biosponge, a di-tri-octahedral smectite, has been used to adsorb clostridial enterotoxins,[23,24] and could be a viable adsorbent for cantharidin as well.

The purpose of this study was to utilize a model of acute cantharidin toxicosis in rats to determine the relative efficacy of mineral oil, activated charcoal, and smectite to ameliorate the clinical signs and lesions induced by cantharidin exposure.

Materials and Methods

All procedures involving animals were performed according to protocols approved by Oklahoma State University Institutional Animal Care and Use Committee. Eight groups of 8 male Sprague-Dawley rats, weighing on average 363 g at toxin administration, were purchased from Harlan Sprague Dawley Inc.[a] The rats were allowed ad libitum access to feed (PMI Laboratory Rodent Diet 5001)[b] and tap water, and maintained under a 12 hours : 12 hours light:dark cycle, with the light phase starting at 0700 hours. Commercial shoebox caging was utilized for initial housing before surgery and for surgical recovery. Twenty-four hours before cantharidin administration, the rats were placed in metabolic cages (Metabolic System—hanging)[c] and maintained throughout the study in an isolated room (ambient temperature 71.5°F) within the AAALAC-accredited Oklahoma State University Animal Resources facility.

Surgical Procedure for Telemetric Transmitter Implantation

Rats were anesthetized with an intraperitoneal administration of a ketamine (Ketathesia)[d] and xylazine [Anased][e] mixture (10 : 1 ratio; 0.44–0.66 mL/kg body weight). After aseptic surgical preparation, a telemetric transmitter (CTA-F40)[f] was placed SC along the dorsal midline over the scapulae. All rats were given 7–10 days to recover from surgery before starting the study. One of the 64 rats died from surgical complications 4 days after surgery; therefore, the group receiving DMSO and saline had only 7 rats.

Treatments

Rats were randomly divided into groups of 8 for placement into the various treatment groups (Table 1). The dose of crystalline cantharidin was determined from pilot studies by the up-and-down method of Bruce.[25] Crystalline cantharidin[g] was administered per os at 6.9 mg/kg. Cantharidin was dissolved in DMSO (DOMOSO)[h] while in a heated (80°F) agitator. After each rat dose was calculated, the solution was diluted into a 5% solution using 0.9% NaCl while setting in a 50°F water bath. Gastrointestinal medications were administered at 0800 hours with the following doses: activated charcoal (Toxiban)[i] 1.008 g/kg per os; mineral oil (Vetone)[j] 8.4 mL/kg per os; smectite[k] 3.024 g/kg per os. Doses of gastrointestinal medications were extrapolated from those typically administered to a 450-kg horse. Treatments were administered within 15 seconds of cantharidin via the same orogastric needle.

Table 1. Experimental groups.

Cantharidin (Treatment Groups)
Group 1—(MOC) Mineral oil, cantharidin, vehicle (DMSO), saline
Group 2—(CHC) Activated charcoal, cantharidin, vehicle, saline
Group 3—(BSC) Biosponge, cantharidin, vehicle, saline
Group 4—(CAN) (no treatment), cantharidin, vehicle, saline
No Cantharidin (Negative Control Groups)
Group 5—Mineral oil, vehicle, saline
Group 6—Activated charcoal, vehicle, saline
Group 7—Biosponge, vehicle, saline
Group 8—Vehicle, saline

Sampling Procedures

After the surgical recovery period, rats were placed individually in metabolic cages. Baseline heart rate, body temperature, and activity level were recorded for a 24-hour period. Immediately after baseline recording, treatments were administered by orogastric gavage and heart rate; body temperature and activity level were recorded continuously for an additional 24 hours. All telemetric data were acquired and analyzed by DSI software (Dataquest A.R.T. TM version 2.0).[f] Urine production and water intake were measured, and urine was collected over the 24-hour period. Rats that remained alive after the 24-hour data collection period were euthanized by CO_2 asphyxiation and necropsy was performed immediately. Rats that died within the 24-hour data collection period were necropsied within 30 minutes of death. Heart, lung, liver, stomach, small and large intestine, kidney, and urinary bladder were collected for histopathologic evaluation. Tissues were fixed in 10% buffered formalin, imbedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and evaluated microscopically. Each rat was given a score to indicate the presence of lesions found on histopathologic evaluation of tissues collected (1 = no lesion; 2 = lesion).

Urine Cantharidin Analysis

Urine samples were analyzed for cantharidin using GC-MS. Urine was acidified, extracted with toluene, and 1 μL of the toluene extract was subjected to GC-MS analysis.[4,6,26]

Data Analysis

SAS Version 9.2[l] was used for all statistical analyses. The response variables of each rat were recorded every 5 seconds during the 24 hours before and 24 hours after cantharidin dosing. Data for each response variable were then averaged over consecutive 4-hour time periods. The mean response variables and mean urine cantharidin concentration were analyzed for statistical significance by analysis of variance procedures. A 3-factor factorial with repeated measures model was utilized, with cantharidin (present or absent) and treatment as the main unit factor, and time as the repeated factor. An autoregressive with period 1 covariance structure was used to model the within-rat covariance. Simple effects of treatment and cantharidin were analyzed at specified levels of the other factors. Contingency tables and Fisher's Exact Tests were used to compare histology results with cantharidin levels. A significance level of .05 was used to determine statistical significance.

Results

Temperature

In the first 12 hours after treatment, rats exposed to cantharidin alone ($P = .02$) and cantharidin plus mineral oil ($P < .01$) had significantly lower body

temperatures than negative controls (Fig 1). Body temperatures in rats exposed to cantharidin plus smectite ($P = .21$) and cantharidin plus charcoal ($P = .44$) were similar to negative controls 0–4 hours after intoxication.

Rats exposed to cantharidin plus mineral oil developed the most severe hypothermia (30.6°C ± 1.0) of any groups during the study at 4–8 hours after intoxication. In sharp contrast, during this same time period, the cantharidin plus charcoal group (35.2°C ± 0.8) had body temperatures higher than all other cantharidin treatment groups ($P < .03$). The body temperatures of rats exposed to cantharidin alone (33.6°C ± 0.8) and cantharidin plus smectite (33.7°C ± 0.8) were not significantly different from each other in the same time period ($P = .31$).

By the 8- to 12-hour time period, the temperatures in all treatment groups were significantly lower than negative controls ($P < .01$). Rats exposed to cantharidin plus smectite (33.5°C ± 1.3) had significantly lower body temperature compared with rats exposed to cantharidin plus charcoal (34.9°C ± 1.3) at this time period ($P = .04$).

Temperatures in rats exposed to cantharidin plus charcoal were statistically similar to negative controls for the remaining time periods (12–24 hours), whereas temperatures in rats exposed to cantharidin alone and cantharidin plus smectite remained lower than those in the negative control groups ($P < .01$). The 2 rats exposed to cantharidin plus mineral oil that survived past 12 hours appeared to improve as body temperatures were comparable to negative controls for the remaining study period (16–24 hours).

Heart Rate

Heart rate in the rats that received cantharidin alone and with charcoal or smectite was statistically similar to negative controls throughout all time periods. The mineral oil group had a significantly lower heart rate than the cantharidin plus charcoal group at the 0- to 4-hour time period ($P = .05$), and both the groups exposed to cantharidin plus smectite and cantharidin plus charcoal in the 4- to 8-hour time period ($P < .01$). Heart rates of rats exposed to cantharidin plus mineral oil were significantly lower than negative controls for the 4- to 8-hour time period ($P = .01$), but similar in all other time periods. Heart rates in the cantharidin plus mineral oil group were similar to heart rates in the cantharidin alone group during the 4- to 8-hour time period ($P = .11$).

Locomotor Activity

Rats given cantharidin alone had significantly lower activity levels 8–20 hours after intoxication when compared with negative controls ($P = .01$). There was no significant difference in the mean locomotor activity between groups at any other time points.

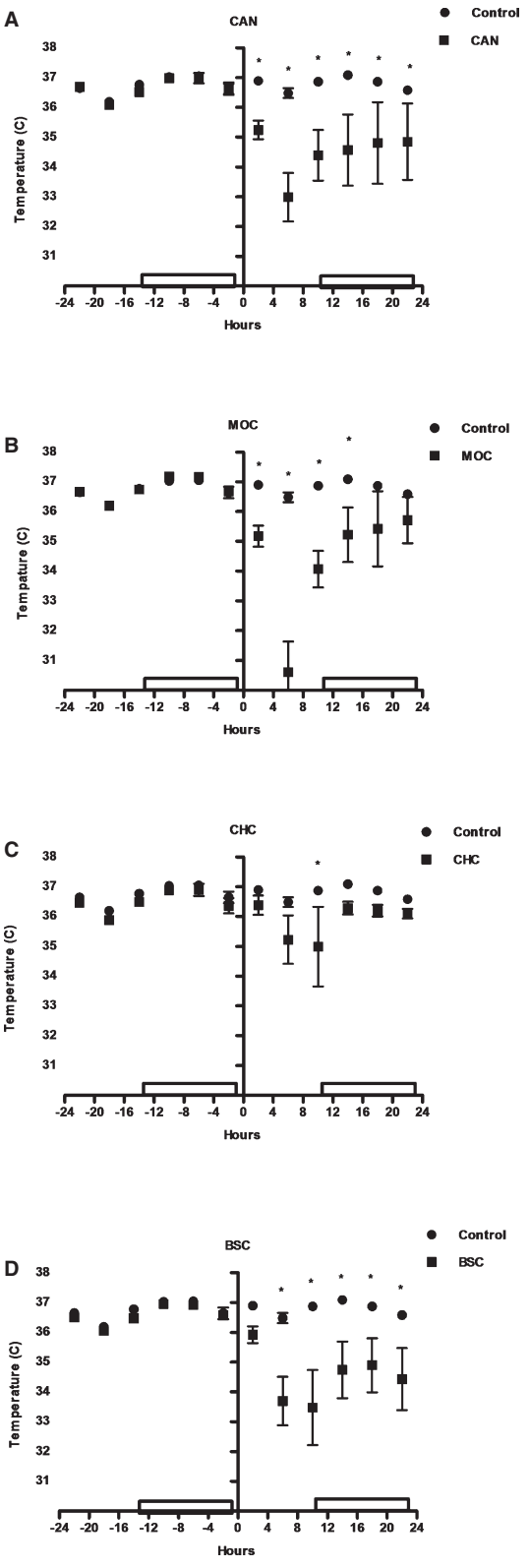

Fig 1. Time course of changes in body temperature (mean ± SE) after oral gavage exposure to **(A)** cantharidin only (CAN), **(B)** cantharidin plus mineral oil (MOC), **(C)** cantharidin plus charcoal (CHC), and **(D)** cantharidin plus smectite (BCS). On the *x*-axis, 0 represents the time of dosing. The open boxes on the *x*-axis represent the dark/night phase. (*$P < .05$)

Urine Cantharidin Analysis

Cantharidin was detected in urine samples from all rats exposed to cantharidin. The urine cantharidin concentration was significantly higher in the intoxicated group treated with mineral oil compared with the smectite and cantharidin alone groups ($P = .04$). Urine cantharidin concentrations in the cantharidin plus charcoal group were statistically similar to the concentration in all treatment groups (Fig 2).

Water Intake and Urine Production

Urine volume was significantly reduced in rats given cantharidin alone (9.3 mL versus 16.0 mL, $P < .02$). Urine volume was also significantly reduced in rats given cantharidin and either charcoal (11.9 mL versus 18.0 mL, $P > .04$) or mineral oil (5.1 mL versus 15.9 mL, $P > .01$). Urine volume was not significantly decreased in rats exposed to cantharidin plus smectite. All cantharidin exposed treatment groups had a significantly lower water intake compared with the negative controls ($P < .01$).

Histology

Most rats of each treatment group receiving cantharidin had significant microscopic lesions compared with control groups (smectite–5/8 rats, $P = .03$; charcoal–7/8 rats, cantharidin–7/8 rats, and mineral oil–8/8 rats, $P = .01$; controls–0/8 rats). There was no significant difference between lesion scores when treatment groups were compared ($P = .34$). Lesions in rats receiving cantharidin included acantholysis of the nonglandular epithelium of the stomach and dilatation of the proximal convoluted tubules, which often contained increased amounts of intraluminal eosinophilic proteinaceous fluid.

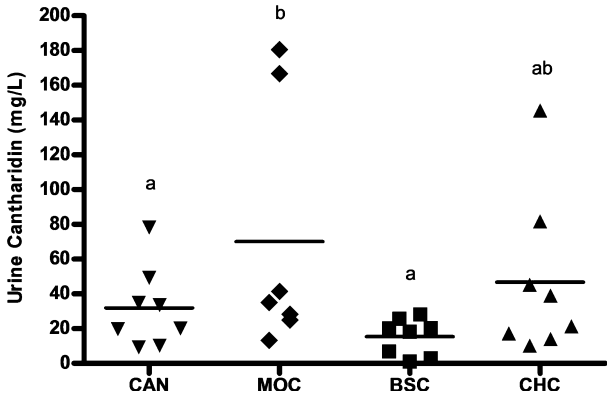

Fig 2. Urine cantharidin concentrations (mean) of rats dosed with cantharidin alone (CAN) or cantharidin followed by mineral oil (MOC), smectite (BSC), or activated charcoal (CHC). Lower case letters indicate significance. ($P < .05$)

Survival Time and Fatality Rate

Six of 8 rats in the cantharidin plus mineral oil group died. The fatality rate of the remaining treatment groups was 3/8 for cantharidin alone and 2/8 for cantharidin plus smectite and cantharidin plus activated charcoal. In each instance where death occurred, rats in the cantharidin plus mineral oil group died in a shorter interval compared with rats in other groups. The rats in the cantharidin plus mineral oil group had a significantly lower survival time than rats in all other treatment and negative control groups ($P < .01$) (Table 2). Rats in the cantharidin only group had a lower survival time compared with negative controls ($P = .01$). The survival time in the cantharidin plus smectite and cantharidin plus charcoal groups were not significantly different from each other or from negative controls.

Discussion

This study demonstrates that treating rats with mineral oil increases the fatality rate associated with cantharidin toxicosis. Mineral oil–treated cantharidin-intoxicated rats also had increased urine concentrations of cantharidin and developed the most severe hypothermia. Bradycardia was also more profound in the mineral oil–treated rats compared with treatment with activated charcoal and smectite during the first 8 hours of intoxication. Hypothermia and bradycardia were unexpected findings in this model, in light of clinical findings in horses with cantharidin toxicosis.

In contrast to natural and experimental cantharidin intoxication in horses that typically develop mild pyrexia, rats developed hypothermia in our cantharidin toxicosis model.[19] The pyrexia that frequently accompanies cantharidin toxicosis in horses might be attributable to the absorption of bacterial endotoxin through damaged colonic mucosa.[27] Although horses typically develop an early febrile response to cantharidin toxicosis, hypothermia can occur in moribund cases showing signs of hypovolemic and endotoxic shock (personal experience) and thus our findings in rats may merely reflect the lethal dosage of cantharidin that was administered.

Table 2. Comparison of survival time (hours) in all groups. Lethality was only significantly different from the cantharidin alone group in rats given mineral oil ($P = .01$).

	Cantharidin		
	Y	N	*P* Value*
No treatment	18.13 hours ± 2.88[a]	24 hours	.0158
Mineral oil	12.38 hours ± 2.58[b]	24 hours	<.0001
Activated charcoal	20.63 hours ± 2.26[a]	24 hours	.1573
Biosponge	20.25 hours ± 2.46[a]	24 hours	.1169

*P value = Y versus N. Y = group administered cantharidin. Lower case letters indicate significance ($P < .05$).

Because mineral oil is a cathartic, and cantharidin is lipid soluble, it was expected that cantharidin would dissolve in mineral oil and be eliminated in the feces. Mineral oil is not well absorbed, but its absorption has been shown to increase when emulsified.[28] Fat soluble substances such as aliphatic hydrocarbons and vitamins A, D, E, and K have reduced intestinal absorption when mineral oil is administered.[29] Other fat soluble substances such as chlorinated hydrocarbons (lindane) have increased intestinal absorption when mineral oil is administered.[30] The more profound early hypothermia, bradycardia, and increased urine concentrations of cantharidin noted in rats treated with mineral oil suggest that mineral oil enhances cantharidin absorption in rats.

The development of bradycardia in this model was unexpected because horses with cantharidin toxicosis typically exhibit tachycardia.[18,19] The tachycardia that develops in equine toxicosis is potentially caused by multiple factors including pain, endotoxemia, volume depletion, acidosis, and hypocalcemia.[7,21,27] Furthermore, direct myocardial damage has also been documented in clinical cases of cantharidin toxicosis in horses.[27,31] We did not note evidence of gross myocardial damage in the rats in this study. The profound bradycardia in the cantharidin plus mineral oil group might be caused by the severe hypothermia that developed when animals became moribund.

Locomotor activity data showed few significant variations among treatment groups. This was likely attributable to the rat's circadian rhythm. Rats are nocturnal scavengers and relatively inactive during the light hours.[32] Cantharidin was administered during the low-activity period. Rats receiving cantharidin may have demonstrated different locomotor activity if the treatment had been initiated at the beginning of the dark phase during a period of high nocturnal activity.

Our data suggest that mineral oil enhances the absorption of cantharidin when administered in close proximity to the toxin. It remains unclear why the urinary concentrations of cantharidin were similar between the mineral oil and charcoal groups, in light of the physiologic improvement in the intoxicated group treated with charcoal. Considering that the urine concentration of cantharidin was lowest in the smectite-treated intoxicated rats, and the similar mortality rate in the smectite- and charcoal-treated groups, a larger sample size might have provided additional evidence of the potential efficacy of these 2 adsorbents.

The timing of treatment in this model is unlikely to be clinically relevant in horses exposed to cantharidin. This model was used merely to determine which therapeutic agent would reduce absorbtion of cantharidin in vivo and ameliorate the toxicosis. Horses that have consumed blister beetles are rarely noticed until clinical signs are apparent. This can be several hours after ingestion, and the majority of cantharidin might have already been absorbed.

In conclusion, our data suggest that mineral oil is contraindicated for the treatment of cantharidin toxicosis. Although we found limited evidence that either activated charcoal or smectite significantly decreases cantharidin absorption, changes in body temperature and heart rate suggest that these other adsorbents better ameliorate the pathophysiologic effects of cantharidin intoxication in rats. The results of this study provide rationale to treat cantharidin toxicosis in horses with activated charcoal. Further studies evaluating the efficacy of different doses of charcoal and smectite, as well as other medications, to treat cantharidin toxicosis are warranted.

Footnotes

[a] Harlan Sprague Dawley, Inc, Indianapolis, IN
[b] PMI Feeds, Richmond, IN
[c] Lab Products, Inc, Seaford, IA
[d] Bioniche Teoranta-Inverin, Co, Galway, Ireland
[e] Lloyd Laboratories, Shenandoah, IA
[f] DSI, St Paul, MN
[g] Sigma Aldrich, Inc, St Louis, MO
[h] Fort Dodge Animal Health, Fort Dodge, IA
[i] Lloyd Inc
[j] Meridian, Meridian, ID
[k] Biosponge; Platinum Performance, Inc, Buellton, CA
[l] SAS Institute, Cary, NC

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Conflict of Interest Declaration: Authors disclose no conflict of interest.

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