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Systemic Effects of a Polyethylene Polymer-Based Obstetrical Lubricant in the Peritoneal Cavity of the Horse

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A polyethylene polymer-based obstetrical lubricant [a] has been used to facilitate successful delivery of numerous foals. However, this study has demonstrated that the polyethylene polymer is rapidly absorbed from the peritoneal cavity and that it causes an acute, fatal toxicity. Thus, extreme caution should be employed by surgeons if a mare requiring cesarean section has had large volumes of polyethylene polymer-based lubricant infused into the uterus.

1. Introduction

Although uncommon, equine dystocia is considered to be an emergency situation with potentially fatal consequences for both the fetus and the mare. Limb and/or head and neck malpostures are the major cause of obstetrical difficulties. The foal's long extremities can make obstetrical complications especially difficult to correct. The mare's reproductive tract is extremely sensitive to manipulations, and development of scar tissue can have an adverse effect on future fertility. Instillation of copious volumes of obstetrical lubricant is advocated to protect these delicate tissues. It also distends the uterus, and thus, provides additional space to manipulate the fetus. If attempts at mutation are not successful, an experienced clinician may be able to resolve the problem with a 1 - 2 cut fetotomy. However, if a uterine laceration is present or if a cesarean section becomes necessary, the lubricant may enter the peritoneal cavity. A common obstetrical lubricant consists of a polyethylene polymer (PEP; 25% w/w) in a dispersing agent base [a] that, when mixed with water to the appropriate consistency, forms a 1 - 2% (w/v) solution. The effects of this lubricant on the peritoneal cavity and the potential for toxicity are not known.

Thus, some surgeons express concern about the prior use of large volumes of liquid obstetrical lubricant. However, obstetricians would counter that if the volume is limited, far more mares will require surgery. Equine surgeons continue to use assorted therapeutic agents for which anti-adhesive claims have been made. However, to date, no substance has gained general acceptance [1]. One such substance is carboxymethylcellulose sodium. A 1% solution of carboxymethylcellulose sodium has been used in human and equine abdominal surgery, but its anti-adhesive efficacy has produced equivocal results [2-4]. Pure carboxymethylcellulose sodium powder can be purchased for surgical use. The commercially prepared carboxymethylcellulose-based veterinary lubricants are primarily intended to facilitate palpation of the viscera per rectum. Some also contain propylene glycol. These "palpation lubes" generally contain methyl and propyl parahydroxybenzoate as preservatives. Some veterinarians add other antiseptic solutions. Thus, these commercially prepared carboxymethylcellulose sodium formulations should not be assumed to be inert if they were to contaminate the peritoneal cavity.

This project was designed to simulate the accidental contamination of the peritoneal cavity during surgical manipulation (~1.0 l) during a cesarean section. The objective was to determine what effects, if any, a PEP-based obstetrical lubricant may have on the equine peritoneal cavity. We hypothesized that a mild, transient peritonitis would result, no fibrin deposition would occur, and no systemic effects would occur. Equine peritonitis is a serious disease that, if not treated early and actively, can be fatal. The fibrin that is deposited on damaged or irritated tissues often results in adhesions that can lead to abdominal pain and intestinal blockage. The proposal entailed serial abdominocentesis of horses and then euthanasia at 2 wk to investigate the possibility of adhesions. The Ohio State University Institutional Laboratory Animal Care and Use Committee (ILACUC) required that a preliminary study be conducted on rodents.

2. Materials and Methods

Rat Experiment 1

Part A

Abdominocentesis and intra-peritoneal injections were performed on anesthetized rats (250 - 300 g). A 1-in, 22-gauge IV catheter was inserted into the lower right quadrant of the abdomen, and 5 ml of warm normal saline was injected. The catheter was capped and left in place while the rat was gently rocked for 1 min to ensure uniform distribution of saline throughout the abdominal cavity. A baseline peritoneal fluid sample was then collected by gravity flow into an ethylenediamine tetra-acetic acid (EDTA) vial. The PEP-based lubricant solution (2% w/v) was prepared to approximate the concentration that would be mixed in a 9-l bucket for use in an equine dystocia. Assuming that 1 l of lubricant may contaminate the peritoneal cavity of a mare (1000 ml/500 kg), a 2 ml/kg volume of 2.0% (w/v) PEP-based lubricant was infused into the rat peritoneal cavity (n = 6). Sterile water was used as a control (n = 4). Rats were to be euthanized in a CO₂ chamber at 72 h, and a necropsy performed to evaluate gross and histologic evidence of peritonitis. Blood samples for complete blood count (CBC) and chemistry were collected by direct cardiac stick immediately after euthanasia.

Part B

The peritoneal infusions were repeated using a 1.25% (w/v) PEP-based lubricant following the manufacturer's mixing instructions. Rats were monitored on an hourly basis.

Rat Experiment 2

The commercial lubricant contains 25% (w/v) PEP and 75% (w/v) dispersing agent. The manufacturer responded to the preliminary findings by providing pure samples of the PEP and dispersing agent (sucrose). Solutions were prepared based on the component ratios (1:3) and the manufacturer's mixing instructions on the label (1.25% PEP-based lubricant solution). Rats were infused with 2 ml/kg of either a 0.31% (w/v) PEP solution or a 0.94% (w/v) sucrose solution. Rats were monitored on an hourly basis and then euthanized at 5 h.

Rat Experiment 3

Additional rats were divided into a 0.94% sucrose group (n = 6), a 0.31% pure PEP group (n = 6), and a 0.15% pure PEP group (n = 6). One rat from each PEP group was euthanized at 1, 2, 3, and 4 h after injection, and the remaining two rats were euthanized at 5 h after infusion. The sucrose rats were euthanized at 24 h (n = 2) and 72 h (n = 4).

Horse

Based on the apparent - and unexpected - PEP toxicity that was evident in the rats, the Ohio State University ILACUC review panel approved a limited study in four horses. The left paralumbar fossa was surgically prepared, and a 22-fr thoracic drain was inserted into the peritoneal cavity. One liter of sterile water containing either 20 g of PEP-based lubricant (2%), 10 g PEP-based lubricant (1%), 2.5 g PEP (0.25%), or 7.5 g sucrose (0.75%) was infused into the peritoneal cavity in each horse. Horses were prepared for electrocardiograms and fitted with a facial artery catheter for measurement of arterial blood pressure. Horses were serially monitored by physical examination, CBC, abdominocentesis, serum chemistry, hemostasis screen, and urinalysis.

Nuclear Magnetic Resonance Analysis

Samples of the commercial PEP-based lubricant powder as well as samples of the PEP base and the sucrose dispersing agent were submitted to the Nuclear Magnetic Resonance (NMR) laboratory at the Ohio State University Chemical Instrument Center for analysis to determine the level of purity.

3. Results

Rat Experiment 1

Part A

Within 12 h (overnight) of injection of the 2.0% (w/v) PEP-based lubricant, the treatment rats were found dead, with blood-stained litter in their cages. Necropsy revealed dark congested kidneys. The ureters and bladder were distended with dark red urine. The control rats were bright and alert, and at necropsy, showed no evidence of peritonitis or pathology of the urinary tract.

Part B

Two hours after infusion, the treatment rats began urinating "blood" and displayed progressive weakness that initially affected the hind limbs. Within 12 h of infusion, all treatment rats (n = 6) were dead, with blood-stained litter in their cages. Necropsy findings were identical to that observed in the 2.0% rats. Histologic examination of both treatment groups revealed identical lesions - marked accumulation of eosinophilic granular material in the bladder, kidneys, and spleen.

Rat Experiment 2

The PEP rats (n = 2) displayed hind limb weakness within 2 h. Dark red urine was noted within 3 h, and rats were

euthanized at 5 h after infusion. At necropsy, the findings were identical to those previously observed in the lubricant-treatment rats. The sucrose rats remained bright and alert.

Rat Experiment 3

The sucrose rats remained bright and alert, and no histologic or gross abnormalities were detected. No gross or histologic abnormalities were seen in the PEP rats at 1 h. At 2 and 3 h after infusion, both PEP concentrations (3.1 and 1.5 mg/ml) had caused a mild accumulation of eosinophilic granular material in the bladder, and the urine was slightly blood-tinged. At 4 and 5 h after infusion, the kidneys were dark and congested, and the ureters and bladder contained dark red urine. Both PEP concentrations caused a marked deposition of eosinophilic granular material in the bladder, kidneys, and red pulp of the spleen. Acute tubular degeneration and necrosis was apparent in kidney sections.

Rats infused with PEP showed a significant elevation in several blood chemistry values creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urine nitrogen (BUN), creatinine, and K^+ . The elevated CK may be indicative of muscle breakdown and could explain the progressive weakness; however, a high hemolytic index can affect the analyzer's ability to accurately interpret this value. Elevated liver enzymes, BUN, and creatinine are indicative of early hepatic and renal failure. However, it was suspected that the acute deaths may have been associated with hyperkalemia-induced cardiac arrhythmia. The packed-cell volume (PCV) of all PEP-infused rats was normal, but the total nucleated cell count was elevated.

Horse

One horse served as its own control; initially, it received 7.5 g sucrose IP and 2.5 g PEP IP 7 days later. The horse developed a suppurative peritonitis at 24 h after sucrose infusion, but the peritoneal fluid values had returned to baseline by 96 h. Arterial blood pressure and ECG tracings remained normal for 24 h. All physical parameters, serum chemistry, CBC, and hemostasis screen remained normal in this horse over the 7-day period after sucrose infusion.

Horses that received 10 or 20 g of PEP-based lubricant or 2.5 g PEP IP either died ($n = 2$) or developed clinical signs severe enough to warrant euthanasia ($n = 2$) between 3 min and 15 h after infusion. Horses became agitated, pawed, circled the stall, head pressed, star-gazed, and showed evidence of abdominal discomfort. These signs were only transiently responsive to medication with flunixin meglumine or xylazine. Horses were euthanized if signs of pain recurred after two doses of analgesics. Horses became tachycardic and had injected mucous membranes. Systolic, diastolic, and mean arterial blood pressures were elevated in all horses after peritoneal infusion of the PEP-based lubricant or the pure PEP solution. These remained elevated until death or euthanasia. Serum fibrinogen, one stage prothrombin time, and activated partial tissue thromboplastin times were unaffected by IP infusion of PEP-based lubricant or pure PEP solution. The serum remained negative for the presence of fibrinogen degradation products at 1:5 and 1:25 in all horses. Intraperitoneal infusion of PEP-based lubricant or pure PEP solution caused alteration in serum chemistry values. Horses became azotemic (creatinine, 15.2 mg/dl) and hyperosmolar (293 mOsm/kg) with an increased anion gap (39 mEq/l). Total protein, albumin, and globulins were decreased. No significant changes were noted in CBCs. The serum had a markedly elevated hemolytic index. Urine was green to black in color, and urinalysis was positive for blood and protein.

Histopathologic examination revealed no significant lesions in the heart, lung, liver, spleen, intestinal tract, central nervous system (cortex, mesencephalon, myelencephalon, and cerebrum), or skeletal muscle. The renal tubules were diffusely distended with large amounts of eosinophilic granular material and lesser amounts of amorphous eosinophilic material. Widespread minimal acute tubular epithelial cell degeneration was present. Lymphatics in the diaphragm of the horse that died acutely (3 min) were distended by amorphous eosinophilic material, and all viscera were diffusely congested.

NMR

The 1H -NMR of the PEP showed a sharp singlet at 3.64 ppm and a second singlet at 4.68 ppm. The former is expected for pure poly (ethylene oxide), and the latter was indicative of the solvent, deuterium oxide, in which the sample had been dissolved. The ^{13}C -NMR of the PEP showed the expected shift at 69.61 ppm for poly (ethylene oxide). No other carbons were present. The PEP-based lubricant powder clearly showed the presence of poly (ethylene oxide) and sucrose. The ^{13}C -NMR very clearly showed all the carbons from the sucrose and from the polymer, again, with no indication of impurities.

4. Discussion

The presence of pink serum, black urine, and eosinophilic material in the renal tubules are indicative of hemolysis. Specific tests were not performed to distinguish hemoglobinuria from myoglobinuria. The presence of a normal creatinine kinase and the absence of skeletal muscle lesions on histopathology are suggestive of hemoglobinuria. There is no satisfactory explanation for the one acute death (2% PEP-based lubricant w/v) observed. Necropsy confirmed that the peritoneal infusate was into the peritoneal cavity in all four horses and that no other structures were damaged. The cause of the colic, agitation, and neural signs can not be explained by serum chemistry or CBCs and necropsy findings. We considered the possibility of a toxic contaminant, but the NMR analyses of all samples were very clean with no apparent impurities being detected.

This PEP-based lubricant has proven to be safe and effective for intra-uterine obstetrical application throughout many years of use in our veterinary hospital. However, the results of this study demonstrate that peritoneal contamination with an amount as small as 2.5 g pure PEP powder is toxic in horses. This equates to contamination of the peritoneal cavity with

1.0 l of a 1% (w/v) solution of the commercial PEP-based lubricant. The presence of a uterine laceration can be confirmed by abdominocentesis [5,6]. If this PEP-based lubricant has been infused into the uterus and then a caesarean section becomes necessary, extreme caution should be exercised to prevent any spillage into the peritoneal cavity. Human safety issues (powder aspiration) during preparation of the liquid lubricant are currently being investigated in our laboratory.

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Footnote

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