RESEARCH REPORTS

Effect of Vehicle on the Nasal Absorption of Epinephrine During Cardiopulmonary Resuscitation

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Study Objectives. We have shown in previous studies that epinephrine administered intranasally is a feasible route of administration during cardiopulmonary resuscitation (CPR). To promote the absorption of epinephrine we administered phentolamine prior to epinephrine and used a bile salt as a vehicle to dissolve the epinephrine. The purpose of this study was to compare the effect of two different vehicles (bile salt vs surfactant) in promoting the absorption of nasally administered epinephrine during CPR and to determine their effects on the nasal mucosa.

Study Design. A randomized, blinded study.

Setting. A controlled laboratory environment.

Subjects. Eleven mongrel dogs.

Interventions. Each dog underwent 3 minutes of unassisted ventricular fibrillation (VF) followed by 7 minutes of VF with CPR. Five minutes after the start of VF, 10 dogs received intranasal phentolamine 0.25 mg/kg/nostril followed 1 minute later by intranasal epinephrine 7.5 mg/kg/nostril. The epinephrine was dissolved in a randomly assigned vehicle consisting of either taurodeoxycholic acid (group A, bile salt) or polyoxyethylene-9-lauryl ether (group B, surfactant). One animal acted as a control and received 0.9% sodium chloride nasally.

Measurements and Main Results. Data from eight dogs (one control) were included for analysis. Histology of the nasal cavity demonstrated severe multifocal erosion and ulceration of the respiratory epithelium for groups A and B compared with the control. The severity was similar between the two groups. In addition, no significant differences in plasma epinephrine concentrations or blood pressure responses were seen between the groups.

Conclusion. Based on histology, polyoxyethylene-9-lauryl ether offered no advantage over taurodeoxycholic acid in its effect on the nasal mucosa. The data available for changes in epinephrine concentration and pressure also suggest no difference between the two vehicles in promoting the absorption of epinephrine during CPR in an animal model.
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Rapid administration of epinephrine by intravenous or endotracheal routes during out-of-hospital cardiac arrest may be delayed due to technical difficulties in endotracheal intubation or intravenous catheter injection. In an attempt to develop an alternate route of administration in which rapid administration of epinephrine is possible without requiring technical skills, we

evaluated the nasal route of drug administration during cardiac arrest.[1, 2] Previously we showed that nasal administration of epinephrine was comparable to standard-dose, intravenous epinephrine during ventricular fibrillation (VF) and cardiopulmonary resuscitation (CPR) in a canine model.[1]

In this study we administered phentolamine 5 mg/nostril 1 minute prior to epinephrine administration 14 mg/nostril to reduce nasal mucosa vasoconstriction and thereby promote absorption. To define the optimum dose combination of these two drugs, we recently completed a dose-ranging study.[2] Results suggest that 0.25 mg/kg/nostril of phentolamine followed by 7.5 mg/kg/nostril of epinephrine provided the greatest increase in plasma epinephrine concentrations and blood pressures among the doses evaluated. In both studies, a bile salt (taurodeoxycholic acid) was added to the epinephrine solution to promote further absorption. Pilot data from our laboratory suggested that bile salts improved the response to nasally administered epinephrine.[1] Other agents such as surfactants, however, may be superior in promoting epinephrine absorption. A recent study comparing bile salts to surfactants suggests that surfactants may be better promoters.[3] In addition, the effect of these agents on the nasal mucosa in this model is unknown. Therefore, this study was performed to compare the effect of bile salt versus surfactant in promoting the absorption of epinephrine, and to assess the effects of the two agents on the nasal mucosa during VF and CPR in a canine model.

Methods

Animal Preparation

The study was approved by the University of Michigan Unit for Laboratory Animal Medicine. Ten mongrel dogs, each weighing 20.2 ± 3 kg, were evaluated in a randomized, blinded study comparing the effects of two different vehicles on

nasal mucosa and epinephrine absorption. A random numbers table was used for randomization. One additional animal served as a control, who received normal saline administered nasally.

Intravenous pentobarbital 25 mg/kg was used to induce anesthesia, which was maintained with intravenous pentobarbital 5mg/kg administered as needed. However, pentobarbital was not administered for at least 30 minutes prior to experimentation. After induction, each animal was placed in a supine position on a surgical table with a thermoblanket. A cuffed endotracheal tube was inserted following intubation. Ventilation was started using an Ohio Anesthesia Ventilator (Ohio Medical Products, Madison, WI); the initial tidal volume was 10–15 ml/kg with a respiratory rate of 15 breaths/minute. After tracheal intubation, the femoral arteries and vein were surgically exposed along with the right jugular vein.' A 6-F pigtailed catheter was placed in the aortic arch and right ventricle for aortic pressure measurements and induction of VF, respectively. Another 6-F catheter was placed into the right atrium for measurement of right atrial pressures. The position of the catheters was determined by pressure measurements and waveforms and, if necessary, confirmed at the end of the study by necropsy. All dogs received a heparin 150-unit/kg intravenous bolus dose following catheter placement to help maintain catheter patency.

Pressures from the aorta and right atrium were measured and recorded throughout the study. The pressures were measured with a calibrated Gould P23 transducer and recorded with a Gould eight-channel RS 3800 graphic recorder (Gould Inc., Cleveland, OH). A single-lead electrocardiogram was also recorded by the RS 3800 graphic recorder.

Before the start of the study, arterial blood gases were stabilized by altering the respiration rate or tidal volume to achieve and maintain an arterial pH of 7.4 ± 0.1 and a partial pressure of carbon dioxide (pCO_2) of 40 ± 10 mm Hg. Arterial blood gas measurements were taken at baseline prior to the start of VF, again following a 20-minute stabilization period, and 5 minutes after the start of CPR. All blood gas samples were placed on ice and analyzed using a Radiometer ABL2 (Acid-Base Laboratory, Cleveland, OH).

Experimental Procedure

Following induction of anesthesia, insertion of

monitoring catheters, and obtainment of baseline measurements, VF was induced by a 24-mA, 60-Hz electric current through a pacing wire placed in the right ventricle. After 3 minutes of unassisted VF, CPR was started and maintained for 7 minutes. A pneumatic chest compression device (Thumper; Michigan Instruments, Grand Rapids, MI), set at a chest compression rate of 80/minute with a chest compression duration of 0.5 second, was used to perform CPR. Following every fifth compression, diastole was prolonged by 0.5 second, and the lungs were inflated to an inspiratory pressure of approximately 15 cm of H_2O with 100% oxygen by a synchronized, pressure-limited ventilator (Thumper). The compression force was set initially to produce a stable coronary perfusion pressure gradient (aortic–right atrial mid-diastolic pressure) during the relaxation phase between 10 and 20 mm Hg. This same compression force was maintained throughout the study period.

After 2 minutes of CPR (a total of 5 minutes of VF), phentolamine 0.25 mg/kg/nostril was administered nasally followed 1 minute later by epinephrine 7.5 mg/kg/nostril. The vehicle in which epinephrine was dissolved (bile salt vs surfactant) was assigned in a randomized and blinded manner. The dose of phentolamine and epinephrine used was based on our previous dose-ranging study.[2]

After 7 minutes of CPR (a total of 10 minutes of VF), defibrillation with 7 J/kg of energy was attempted (Model 604-A; Mennen/Greatbach Electronics Inc., Clarence, NY). If VF did not terminate, CPR was continued with additional defibrillation attempts using 10 J/kg every 30 seconds, if necessary, up to 10 minutes from the first defibrillation. Resuscitation was defined as the attainment of an organized cardiac rhythm with a systolic blood pressure of 60 mm Hg or more for at least 2 minutes within a 12-minute period from the first defibrillation.

Arterial plasma epinephrine concentrations were obtained prior to the administration of epinephrine and every minute thereafter until the first defibrillation attempt. Samples were collected and placed into chilled heparinized tubes and centrifuged immediately. All samples were stored at -70°C until assayed. Epinephrine concentrations were determined by high-performance liquid chromatography with electrochemical detection.[4] Dihydroxy-benzylamine (Sigma Chemical Company, St. Louis, MO) was added to each sample as the internal standard. The interday coefficient of variation for epinephrine was 6.8%.

At the end of the study, each dog was euthanized with potassium chloride to effect, and two animals from each group were evaluated for changes in the histology of the nasal mucosa. Specifically, the maxilla and nasal cavity were removed, fixed in 10% buffered formalin, and decalcified with Decalcifying Solution (Baxter, McGraw Hill, IL). Four representative cross-sections of the nasal cavity were made, processed, embedded in paraffin, sectioned at 5 μ, stained with hematoxylin and eosin, and evaluated by a veterinary pathologist.

Animals were excluded from analysis if the position of the Thumper piston could not be maintained on the chest as determined by the blinded investigator performing CPR or if there was fluctuation in coronary perfusion pressure resulting from a change in Thumper position. In addition, animals were excluded if a minimum coronary perfusion pressure of 5 mm Hg could not be obtained before epinephrine administration. The exclusion criteria were required because inclusion of these animals with variable or inadequate coronary perfusion pressure caused by poor chest configuration or CPR technique would potentially bias the data since the end points of the study include changes in pressure and survival.

Drug Preparation and Administration

Phentolamine mesylate powder was dissolved in 0.9% sodium chloride so that 0.95 ml would deliver the required amount of drug. Racemic epinephrine hydrochloride powder was dissolved in either a 1% taurodeoxycholic acid solution (group A, bile salt) or a 1% polyoxyethylene-9-lauryl ether solution (group B, surfactant), depending on randomization, so that 0.95 ml would deliver the required amount of drug. Taurodeoxycholic acid and the polyoxyethylene-9-lauryl ether were dissolved in 0.9% sodium chloride with HEPES 0.5% added as a buffer (pH 7.4). All chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Employing a previously described technique, the drug was administered intranasally by inserting a 21-gauge intravenous catheter without the needle (Angiocath; Deseret Medical, Inc., Sandy, UT) into each nostril and attaching a 1-ml syringe.[1,2,5] To help facilitate dispersion of drug onto the nasal mucosa, the catheter tip was ligated, and six holes were made within a 1.5-cm space from the tip using a 26-gauge needle.

Table 1. Arterial Blood Gas Values (mm Hg) at Baseline and at 5 Minutes of CPR

	pH	pCO_2	pO_2
Baseline			
Group A	7.35 ± 0.02	37 ± 6	610 ± 28
Group B	7.39 ± 0.03	36 ± 4	590 ± 68
CPR			
Group A	7.46 ± 0.09	19.9 ± 4.8	442 ± 121
Group B	7.47 ± 0.08	22.8 ± 1.8	524 ± 49

Data Analysis

Histological evaluation and measurement of pressure waveforms (average of five readings/measurement) were determined by a blinded investigator. Statistical comparison of the treatment groups was performed using Student's t test with Bonferroni's correction, and Fisher's exact test where appropriate. Data are reported as mean and standard deviation.

Results

Ten dogs were randomized, and of these, eight (one control) were included for analysis. Two dogs from group A (bile salt) and one dog from group B (surfactant) were excluded from analysis because of poor chest configuration and CPR technique as defined in the previous section. Arterial blood gas results at baseline and during CPR were similar between the two groups and are listed in Table 1.

Normal histology of nasal turbinate as seen in the control dog (Figure 1) demonstrates normal ciliated columnar epithelial cells and intact basement membrane. The columnar epithelial cells are attached to the basement membrane and closely packed. Compared with the control dog (Figure 1), the nasal mucosa for both group A (Figure 2) and group B (Figure 3) dogs demonstrates severe multifocal erosion of the nasal epithelium. The columnar epithelial cells show significant disruption and deciliation with only remnants of the cells remaining. No intact epithelial cells are observed. Ulceration of the basement membrane is also seen in Figure 3. These effects were noted especially in the turbinates projecting into the nasal cavity. By visual inspection, the severity of damage between group A and B was similar.

Both group A and group B demonstrated increases in plasma epinephrine concentrations over time with no significant differences (p>0.25) between the two groups even though group A had over 2 times the observed concentrations of epinephrine (Table 2). In addition, when the data were adjusted for baseline differences (analyzed for percentage change) at the time of
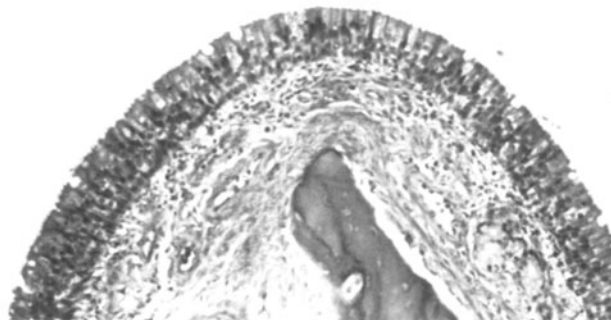

Figure 1. Normal turbinate mucosa from control dog (x 470).
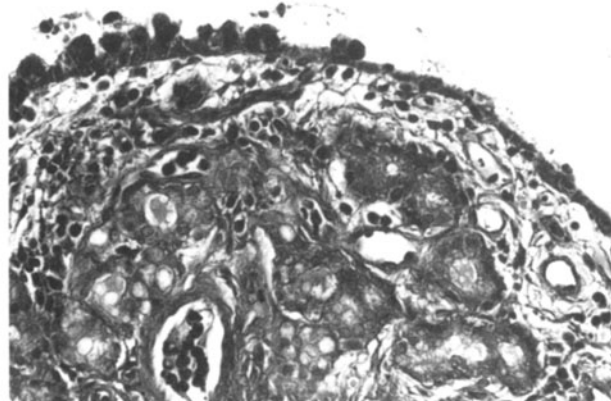

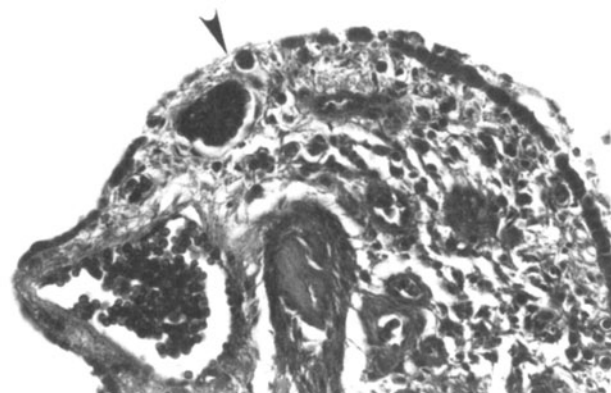Figure 2. Severe erosion of turbinate mucosa from Group A dog (x 470).

Figure 3. Severe erosion of turbinate mucosa from Group B dog (x 470). Note the focus of ulceration (arrowhead).

Table 2. Mean Plasma Epinephrine Concentration (pg/ml) during CPR

	Before Epinephrine Administration	Minutes After Epinephrine Administration			
		1	2	3	4
Group A	5905 ± 1259	11,936 ± 7823	129,364 ± 159,566	314,153 ± 352,515	397,486 ± 424,367
Group B	12,456 ± 9,764	15,320 ± 6067	47,427 ± 61,174	78,373 ± 96,484	179,379 ± 257,550

Table 3. Mean Pressures (mm Hg) Before and During CPR

	Baseline	At Time of Phentolamine Administration	Epinephrine Administration Time (min)				
			0	1	2	3	4
Group A							
AOS	120 ± 15	42 ± 21	52.7 ± 22	54 ± 24	54 ± 24	51.4 ± 22	49.4 ± 20
AOD	90 ± 9	16.5 ± 5	20.1 ± 8.2	21 ± 8.5	20 ± 11	19.3 ± 10	19.3 ± 10
CPP	88 ± 8	15.3 ± 5	15.4 ± 3	17.3 ± 3.5	16.6 ± 7	15.9 ± 6	16.3 ± 7
Group B							
AOS	115 ± 21	49.1 ± 12	51.3 ± 15	53.8 ± 16	54.9 ± 18	55.3 ± 16	51.8 ± 19
AOD	81 ± 16	26 ± 7	26.7 ± 8	28.1 ± 9	28.8 ± 11	27.9 ± 12	27.3 ± 13
CPP	78 ± 14	18.3 ± 6	18.5 ± 7	20.3 ± 10	20.6 ± 13	20.4 ± 14	21 ± 15

AOS = aortic systolic pressure; AOD = aortic diastolic pressure; CPP = coronary perfusion pressure.

epinephrine administration, no significant differences were seen at any time point. The time to maximum change in epinephrine concentrations for each dog in each group occurred at the last time point.

Mean pressures over time, at baseline and during CPR, are shown in Table 3. No significant differences were seen at any time point. In addition, when the data were adjusted for baseline differences (analyzed for percentage change) at the time of epinephrine administration, no significant differences were seen at any time point (p>0.33). Overall, 2 of 3 animals in group A and 2 of 4 animals in group B had an increase in pressure during the study. The peak change in coronary perfusion pressure in these animals was similar between the two groups, 33% change in group A versus 38% change in group B. Resuscitation rate favored group B; 3 of 4 of the group B animals were resuscitated and 0 of 3 in group A (p=0.14).

Discussion

The results of this study demonstrated that nasal administration of epinephrine, regardless of the vehicle used (bile salt versus surfactant), caused significant cellular disruption of the nasal mucosa. In addition, both vehicles increased plasma epinephrine concentrations and blood pressures to a similar degree. These limited data suggest that the effects of polyoxyethylene-9-lauryl ether are similar to and offer no advantage

over taurodeoxycholic acid in enhancing the absorption of nasally administered epinephrine during CPR.

Our previous studies showed that the nasal administration of epinephrine during an emergency situation may be feasible; however, the effect of this route of administration on the nasal mucosa was unknown.[1, 2] This study demonstrated that nasal administration of epinephrine, regardless of the vehicle used, caused significant cellular damage including loss of the epithelial layer and disruption of the basement membrane. These results appear to be similar to a previous rat study in which these vehicles were evaluated.[3] The mechanism by which bile salts and surfactants work as promoters is unknown, but may include cellular disruption such as formation of temporary membrane pores, relaxation of the tight junctions between cells, and actual destruction of cells.[3, 6] The data from this study strongly suggest that taurodeoxycholic acid and polyoxyethylene-9-lauryl ether promote the absorption of epinephrine in this model by altering, in part, the integrity of the cellular structures of the nasal mucosa.

The long-term morbidity that may occur from the administration of intranasal epinephrine with either vehicle is unknown. However, it is interesting to note that polyoxyethylene-9-lauryl ether (0.1-1%) has been used to promote the absorption of intranasal insulin in humans

without significant adverse effects.[7] The reason for the limited adverse effects observed may relate to the model used. In the previous study in humans, the insulin and vehicle were allowed to be "washed" away by normal physiological processes that may have limited cellular disruption of the nasal mucosa.[3, 7] In contrast, the drug and vehicle in this current study were administered in a low flow state followed by euthanasia and removal of the nasal mucosa over a 30- to 60-minute time period. The damage to the nasal mucosa may potentially be less severe if spontaneous circulation is returned, allowing removal of the drug and vehicle by normal physiological processes or if the nasal passage is "washed" with normal saline. Finally, the degree of morbidity associated with intranasal administration of epinephrine may be justified if a decrease in mortality can be achieved with this method.

Each group demonstrated increased plasma epinephrine concentrations from baseline, with group A demonstrating the greatest elevations. This difference was not statistically significant ($p > 0.25$) and may be due to the high degree of variability in epinephrine levels observed in the study leading to a type II error. To detect a difference between groups at the last time point, approximately 60 animals would need to be studied ($\alpha = 0.05$, $\beta = 0.2$). The high degree of variability can be accounted for by one animal in each group that demonstrated tremendous increases in epinephrine concentrations from baseline (881,850 pg/ml and 557,376 pg/ml for groups A and B, respectively). When these animals are excluded from analysis, group A still demonstrates the highest increase in epinephrine concentrations (153,024 ± 40,071 pg/ml vs 50,978 ± 24,035 pg/ml, $p = 0.094$). These elevations in epinephrine concentrations observed are probably due to the absorption of epinephrine from the nasal mucosa since in our previous study epinephrine concentrations increased by only 35,000 pg/ml in the group with the smallest amount of epinephrine administered (0.075 mg/kg).[2]

As with the epinephrine concentration results, both groups demonstrated similar changes in blood pressures with no clinical or statistical difference between the two groups ($p > 0.22$). Overall, neither group demonstrated impressive increases in blood pressures as had been shown in previous studies,[1, 2] due perhaps to the limited number of animals studied. However, individual animals did show increases in peak pressures as

pointed out in the results section.

The obvious limitation of this study is the low number of animals studied, which does not allow us to evaluate definitively the effects of these two vehicles on changes in epinephrine concentrations or pressure. However, based on our experience in this area, if polyoxyethylene-9-lauryl ether were going to increase epinephrine absorption to a greater degree compared with taurodeoxycholic acid, a clear trend or even statistical significance would have been seen with the number of animals studied. In fact, excluding the one outlier from each group as discussed above, a statistical difference was nearly observed, $p = 0.094$, suggesting that taurodeoxycholic acid enhanced the absorption of epinephrine more than polyoxyethylene-9-lauryl ether. Furthermore, a power analysis on these data suggests that less than four animals in each group would need to be studied to demonstrate statistical difference between the two groups. Based on these findings and our experience, we believe that polyoxyethylene-9-lauryl ether does not enhance the nasal absorption of epinephrine to a greater degree than taurodeoxycholic acid.

Despite the apparent limitation on evaluating epinephrine concentrations, this study does allow for the evaluation of histological effects on the nasal mucosa, a primary purpose of the study. One limitation on the histological evaluation is the time frame between the end of the study and the removal of the nasal mucosa. Less damage to the nasal mucosa may have occurred if dissection time were faster or if the nasal cavity were washed with normal saline. Further studies would be needed to determine the effect of time and washing on the degree of damage to the nasal mucosa. In addition, it would be interesting to determine the effect of epinephrine alone on the nasal mucosa to assess its ability to affect the nasal mucosa. Theoretically, epinephrine, with its potent vasoconstricting effects along with underlying hypoperfusion during CPR, may cause significant damage to the nasal mucosa. Another potential limitation of this study, which we have addressed in previous articles,[1, 2] is the use of an animal model to study nasal drug absorption.

In conclusion, this study demonstrated that both taurodeoxycholic acid and polyoxyethylene-9-lauryl ether caused significant damage to the nasal mucosa with no major visual difference between the two agents. In addition, limited data suggest that the effectiveness of each agent in promoting the absorption of epinephrine as

determined by plasma epinephrine concentrations and pressure changes are similar.

Acknowledgment

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