CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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PHARMACOLOGY REVIEW(S)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number:	NDA 205-029
Supporting document/s:	0000
Applicant's letter date:	December 4, 2012
CDER stamp date:	December 4, 2012
Product:	Epinephrine (HCL) Injection USP, 1:1000 (1mg/mL)
Indication:	Treatment of increasing systemic arterial blood pressure in acute hypotensive states associated with septic shock
Applicant:	Belcher Pharmaceuticals, LLC (Belcher)
Review Division:	Cardiovascular and Renal Products
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1 EXECUTIVE SUMMARY

1.1 INTRODUCTION AND CLINICAL RATIONALE

Belcher Pharmaceuticals, LLC, proposes that epinephrine USP, 1:1000 (1 mg/mL) Injection (0.05 to 2.0 μg/kg/min) will provide hemodynamic support for increasing systemic arterial blood pressure to achieve a desired MAP ≥ 70 mmHg in acute hypotensive states associated with septic shock.

This submission is primarily based on published literature [i.e., a 505(b)(2) application] and safety information from the listed reference drug Twinject (NDA 020800; May 30, 2003). In addition, there are several (almost 39) approved epinephrine drug formulations currently being marketed as 1 mg/mL, (1:1000), 0.1 mg/mL (1:10,000), and 0.5 mg/mL (1:2000) solutions.

Epinephrine, a sympathomimetic (adrenergic) drug has been in the market for over 50 years in treating hypotension associated with septic shock. The mechanism to increase blood pressure is due to the activation of α and β-adrenergic receptors affecting myocardial stimulation leading to increased ventricular contraction (positive inotropic action) and heart rate (positive chronotropic action), and vasoconstriction in many vascular beds, including veins.

1.2 BRIEF SUMMARY OF NONCLINICAL FINDINGS

Results from nonclinical septic model studies have shown that administration of epinephrine has significantly improved the mean arterial pressure (MAP) and myocardial performance in a dose dependent manner, by increasing contractility, stroke volume, and cardiac output. However, nonclinical data are limited in reproducing the severe sepsis seen in humans.

Metabolic effects such as hyperlactemia, hyperglycemia and hypokalemia, decreased mesenteric, coronary and renal conductance were associated with epinephrine treatment (Levy 2003).

Data from NTP studies (1990) have shown an equivocal response of epinephrine when tested in *Salmonella typhimurium* strain TA100 in the absence of metabolic activation system (S-9) and negative in the presence of metabolic activation (S9).

The data from published reports suggest that epinephrine was not carcinogenic in 2-year rat studies. However, studies were considered inadequate (NTP, Report 380, 1990), in that doses were too low to have an adequate systemic challenge from the drug compound.

Epinephrine has been shown to interfere with ovum implantation and fetus survival in rabbits (Auletta, 1971). Developmental effects have been observed in rabbits at a subcutaneous dose of 1.2 mg/kg, in mice at a subcutaneous dose of 1 mg/kg, and in hamsters at a subcutaneous dose of 0.5 mg/kg. Hemorrhages, edema and necrosis of distal extremities were observed when 5-50 μg of adrenalin was injected directly into rabbit fetuses at 18 to 22 days of gestational age (Shepard, 1986).

In addition to this, implantation loss, incidence of arrested fetuses and gastroschisis were observed in epinephrine-treated Dutch-Belted rabbits, showing a teratogenic potential of epinephrine.

1.3 RECOMMENDATIONS

1.3.1 Approvability

Approvable

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

8.1 Pregnancy
The sponsor did not address the loss in ovum implantation, fetus survival and teratogenic potential (gastroschisis in one fetus) of epinephrine in rabbits (Auletta, 1971). Epinephrine has been shown to affect the ovum transport and the motility of the rabbit oviduct (Longley et al 1968) and impair implantation in rats (Crist & Hulka, 1970). A decreased number of implantations and fetuses were observed in rabbits treated with epinephrine on Days 6 to 7 and 7 to 9 ($P<0.01$ and $P<0.01$ respectively). In addition to this, epinephrine has been shown to interfere with ovum implantation and fetus survival in rabbits and having a potential teratogenic activity as gastroschisis in one fetus (Table 10) at Day 6/7 (Auletta, 1971) and abnormally absent aortic arches related to dysrhythmogenesis (Rajala et al, 1988).

Based on the developmental effects of epinephrine, the Sponsor's version of 8.1 Pregnancy section as cited below:

Pregnancy Category C:
Epinephrine has been shown to have developmental effects in rabbits at a subcutaneous dose of 1.2 mg/kg (approximately 30 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis), in mice at a subcutaneous dose of 1 mg/kg (approximately 7 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis), and in hamsters at a subcutaneous dose of 0.5 mg/kg (approximately 5 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis). These effects were not seen in mice at a subcutaneous dose of 0.5 mg/kg (approximately 3 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis). Although there are no adequate and well-controlled studies in pregnant women, epinephrine crosses the placenta (but not the blood-brain barrier) and could lead to fetal anoxia, spontaneous abortion or both. [redacted] (b) (4)

Should be revised as:

Pregnancy Category C:
Epinephrine has been shown to have developmental effects in rabbits at a subcutaneous dose of 1.2 mg/kg (approximately 30 times the maximum recommended daily subcutaneous or

intramuscular dose on a mg/m2 basis), in mice at a subcutaneous dose of 1 mg/kg (approximately 7 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis), and in hamsters at a subcutaneous dose of 0.5 mg/kg (approximately 5 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis). These effects were not seen in mice at a subcutaneous dose of 0.5 mg/kg (approximately 3 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis). In rabbits, epinephrine interferes with ovum implantation and fetus survival. Although there are no adequate and well-controlled studies in pregnant women, epinephrine crosses the placenta (but not the blood-brain barrier) and could lead to fetal anoxia, spontaneous abortion or both. Therefore, epinephrine should be used in pregnancy only if considered essential by the physician and the potential benefit justifies the potential risk to the fetus.

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Based on the data from mutagenicity and carcinogenicity studies (# page 40) the first paragraph of the Sponsor's version of 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility section as cited below:

[redacted] (b) (4)

Should be revised as:

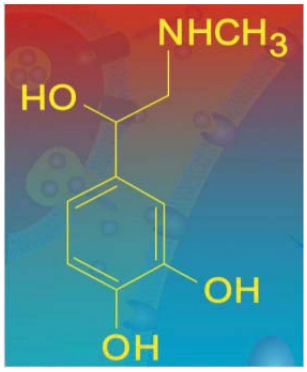
Epinephrine has been shown to have an equivocal response when tested in the bacterial reverse mutation (Ames) test. No carcinogenic effects of epinephrine were observed in rats or mice when exposed by the inhalation route. However, systemic exposures were considered inadequate. There are no data available from either animal or human studies regarding effects of epinephrine on fertility.

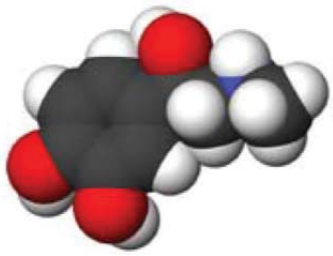
2 Drug Information

2.1 Drug

EPIVAS	Epinephrine Injection, USP 1:1000) 1 mg/mL
CAS Registry Number (Optional)	51-43-4
Generic Name	Epinephrine (Adrenaline)
Chemical Name	1, 2-Benzenediol, 4-[1-hydroxy-2-(methylamino) ethyl]-, (R)-(-)-1-(3, 4-Dihydroxyphenyl)-2-(methylamino)-ethanol
Other name	1-hydroxy-2 (methylamino) ethyl]-1,2 benzenediol
Molecular Formula	$C_9H_{13}NO_3$
Molecular Weight	183.2044

Structure or Biochemical Description





Systematic (IUPAC) name

(R)-4-(1-hydroxy-2-(methylamino)ethyl)benzene-1,2-diol

Figure 1: Epinephrine structure and Space filling model (Wikipedia)

Pharmacologic Class	Sympathomimetic Catecholamine

2.2 Relevant NDAs.

Adrenalin (N-204200), Septocane (N-022010, N-020971), Intocaine (N-020530), Twinject (N-020800), Auvi-Q(N-201739), Xylocaine (N-021381, N-006488), Epipen (N-019430), Orabloc (N-022466), Citanest (N-021383), Levophed (N-007513), Lidosite (N-021504).

2.3 Drug Formulation

Refer to [redacted] DMF No. [redacted] for all Active Ingredient Manufacturer (AIM) information concerning manufacture of the drug substance.

Material	Function	Quantity mg/ml	Quantity mg/1ml ampoule	Reference
Epinephrine Base	Drug Substance	[redacted]	[redacted]	USP
Sodium Chloride	Tonicity Agent			USP
[redacted]				USP
Hydrochloric Acid [redacted]	pH Adjuster			USP
Water for Injection	[redacted]			USP
[redacted]				USP
[redacted]				

Specification:

Ingredients are in compliance with USP monograph.

Test	Specification	References
Appearance	White or almost white powder	Visual
Epinephrine Identification (Colorimetric test)	Complies	USP
Optical rotation (on dried substance)	[redacted]	USP
Loss on drying		USP
Epinephrine Assay (Titration)		USP
Impurities		
[redacted]		USP
		USP
		USP
Residual Solvents (GC)		
[redacted]		In-house Method
		USP

2.4 Comments on Novel Excipients

All excipients are in compliance with pharmacopoeial monograph requirements as below:

Excipient	Pharmacopoeial reference
Sodium chloride	USP
Hydrochloric acid	USP
Water for injection	USP
(b) (4)	USP

2.5 Comments on Impurities/Degradants of Concern

Safety in regard to epinephrine impurities (b) (4) (b) (4), and (b) (4) is in accordance to approved listed drug (Twinject).

Impurities		
(b) (4)		USP
		USP
		USP
Residual Solvents (GC)		
(b) (4)		In-house Method
		USP

Listed Drug	How supplied/ route of administration	Indications	Dosage
Twinject (0.3 mg epinephrine/mL) Shionogi Inc distributed by Greenstone LLC (label date 1/2010 NDA 020800; Approval 5/2003	Single use auto-injector for subcutaneous or intramuscular injection	Epinephrine auto-injector is indicated in the emergency treatment of severe allergic reactions (Type I).	The dosage is 0.3 mg delivered in1 mL and is for use by patients who weigh 30 kg (approx. 65 pounds) or greater.

2.6 Proposed Clinical Population and Dosing Regimen

Epinephrine injection USP, 1:1000 (1 mg/mL) is proposed for intravenous infusion in increasing the systemic arterial blood pressure in acute hypotensive states associated with septic shock. The dosing regimen for intravenous administration of epinephrine is 0.05 to 2.0 µg/kg/min in hypotension associated septic shock, and is titrated to achieve a desired mean arterial pressure (MAP) of ≥70 mmHg. A periodic adjustment of dosage (b) (4) in increments, such as by 0.05 to 0.2 µg/kg/min, is necessary to achieve the desired goal. Epinephrine should be titrated closely and the minimum dose should be used as required. Once the hemodynamics is stabilized for several hours, the dosage may be weaned down incrementally over time.

2.7 Regulatory Background

Belcher Pharmaceuticals, LLC, submitted a pre-IND meeting package (PIND (b) (4)) for intravenous administration of epinephrine (b) (4) Based on the information in its PIND package (dated Dec. 4, 2012), Belcher proposed epinephrine USP, 1:1000 (1 mg/mL) Injection (0.05 to 2.0 µg/kg/min) to provide hemodynamic support for increasing systemic arterial blood pressure (to achieve a desired M AP ≥ 70 mmHg) in acute hypotensive states associated with septic shock. This current NDA submission is primarily based on published literature [i.e., a 505(b)(2) application)] and safety information from the listed reference drug Twinject (NDA 020800; May 30, 2003).

Listed Drug	How supplied/ route of administration	Indications	Dosage
Twinject (0.3 mg epinephrine/mL) Shionogi Inc distributed by Greenstone LLC (label date 1/2010 NDA 020800; Approval 5/2003	Single use auto-injector for subcutaneous or intramuscular injection	Epinephrine auto-injector is indicated in the emergency treatment of severe allergic reactions (Type I).	The dosage is 0.3 mg delivered in1 mL and is for use by patients who weigh 30 kg (approx. 65 pounds) or greater.

In addition, there are several (almost 39) approved epinephrine drug formulations currently being marketed as 1 mg/mL, (1:1000), 0.1 mg/mL (1:10,000), and 0.5 mg/mL (1:2000) solutions for intramuscular or subcutaneous administration for the treatment of anaphylactic shock (EpiPen and Twinject), and anesthetic combination products that contain epinephrine at low doses (such as Septocaine; Octocaine; Xylocaine with epinephrine).

3. STUDIES SUBMITTED

3.1 Studies Reviewed

Levy et al. (2003). Effects of epinephrine and norepinephrine on hemodynamics, oxidative metabolism, and organ energetics in endotoxemic rats
Minneci et al. (2004). Differing effects of epinephrine, norepinephrine, and vasopressin on survival in a canine model of septic shock (in vivo study in dogs)

Myburgh et al, (2006). An appraisal of selection and use of catecholamines in septic shock - old becomes new again (in vitro study using a sheep preparation)
Di Giantomasso (2005). The hemodynamic and metabolic effects of epinephrine in experimental hyperdynamic septic shock (in vivo study in sheep)
ASTM Hydrolysis (ASTM F 756-08)

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

NDA 020800 (Twinject)

4. PHARMACOLOGY

4.1 Primary Pharmacology

Biosynthesis: Epinephrine (also known as adrenaline) is a hormone belonging to group of catecholamines and synthesized in adrenals from the amino acid tyrosine under the control of CNS in response to a physiological stress (Fig. 2). Once the dopamine and norepinephrine are synthesized, norepinephrine is methylated and finally converted to epinephrine by phenylethanolamine N-methyltransferase (PNMT) in the cytosol of adrenergic neurons using the *S*-adenosylmethionine (SAMe) as methyl donor. Circulating epinephrine is rapidly inactivated by catechol O-methyltransferase (COMT) and monoamine oxidase (MAO) that is highly present in the liver.

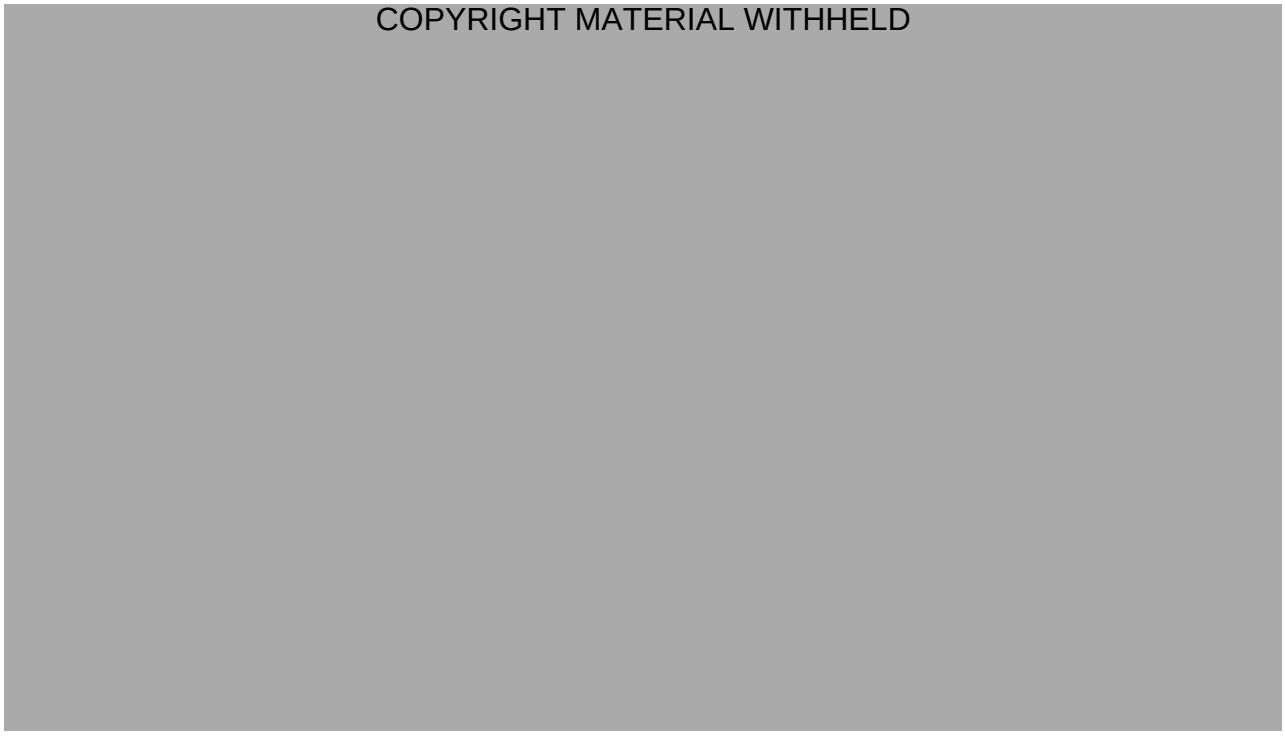
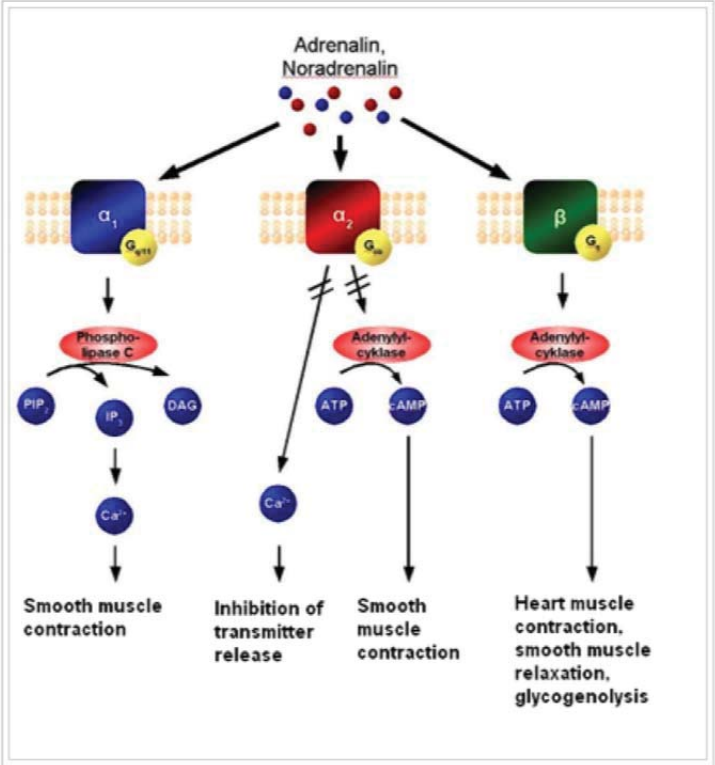


Figure 2: Epinephrine Synthetic pathway (Goodman & Gillman, 2011**)**

Mechanism of Action: Epinephrine (adrenaline), an endogenous sympathomimetic catecholamine targets adrenergic receptors (α- and β-adreno-receptors belonging to a class of G-protein coupled receptors), and causes vasoconstriction through its binding with α-receptors (α1 and α2) at high concentration and vasodilation via β-receptors (β1, β2, and β3) at low concentration.

Epinephrine binds its receptor that associates with heterotrimeric G protein. The G protein associates with adenylate cyclase that converts ATP to cAMP. This initiates a chain of chemical reactions to ultimately signal a cellular response (Fig. 3).



The mechanism of adrenergic receptors. Adrenaline or noradrenaline are receptor ligands to either α_1, α_2 or β-adrenergic receptors. α_1 couples to G_q, which results in increased intracellular Ca^{2+} which results in smooth muscle contraction. α_2, on the other hand, couples to G_i, which causes a decrease of cAMP activity, resulting in smooth muscle contraction. β receptors couple to G_s, and increases intracellular cAMP activity, resulting in e.g. heart muscle contraction, smooth muscle relaxation and glycogenolysis.

Figure 3: The mechanism of Epinephrine action

Effect of epinephrine on vital organ system is presented as below (Table 1) Goodman & Gillman 12e, Section II.

Table 1: Effect of epinephrine on vital organ system

4.2 Primary Pharmacology: Studies from the Literature

Pharmacodynamics

Non-clinical studies (one in vivo study in rats, one in vivo study dogs, and two studies in sheep (one in vitro and one in vivo) submitted by the Sponsor were reviewed and discussed herein to determine the pharmacological effects of epinephrine in animals.

Levy et al. 2003
Effects of epinephrine and norepinephrine on hemodynamics, oxidative metabolism, and organ energetics in endotoxemic rats

Key Findings
The hyperlactemia is not related to cellular hypoxia in sepsis model of Wistar rats following epinephrine treatment.

Purpose
To determine whether an increased lactate concentration in sepsis model of Wistar rats is the result of hypoxia, thermogenic or any other metabolic pathway triggered by epinephrine.

Method
Sepsis was developed in rats following an intravenous infusion of 15 mg/kg Escherichia coli O127:B8 endotoxin.

After 90 minutes, sepsis and time matched saline treated control rats were treated for 90 to 180 min with epinephrine (0.2 and 1μg/kg/min) or norepinephrine (0.2 and 1μg/kg/min).

The mean arterial pressure (MAP), aortic, renal, mesenteric and femoral blood flow, arterial blood gases, lactate, pyruvate, and nitrate were measured at baseline and 90 and 180 min after endotoxin exposure.

Results

MAP decreased (from 115 ± 11 to 76 ± 8 mmHg, $p < 0.01$) while heart rate increased (from 369 ± 9 to 430 ± 14 bpm, $p < 0.05$) in endotoxin treated sepsis rats. The lactate concentration increased with a high lactate/pyruvate (L/P) ratio (Table 2). The mean efficient dose of epinephrine (2.8μg/kg/min) or norepinephrine (3.1μg/kg/min) increased heart rate (from 370 ± 8 and 360 ± 9 to 431 ± 10 and 420 ± 12 bpm, respectively, $p < 0.05$). Epinephrine and norepinephrine treatment in sepsis rats increased MAP over baseline values. Epinephrine increased aortic blood flow while renal blood low decreased with both drugs.

Table 2: Time course of arterial metabolic parameters at baseline and 180 minutes in saline, epinephrine, or norepinephrine control groups, and endotoxemic rats treated with or without epinephrine or norepinephrine from 90 to 180 minutes (Sponsor's table)

	Glucose (mmol/l)		Lactate (mmol/l)		Lactate/pyruvate ratio		Arterial ketone body ratio	
	Baseline	180 min	Baseline	180 min	Baseline	180 min	Baseline	180 min
Saline	2.34±0.30	2.56±0.50	1.6±0.7	1.9±1.0	16±5	19±7	0.92±0.3	1.1±0.5
Epi	2.40±0.32	3.50±0.70*	1.5±0.6	2.1±0.5*	15±5	16±4	1.2±0.4	1.4±0.5
Nor	2.40±0.40	2.60±0.50	1.5±0.5	1.6±0.4	16±4	17±5	1.1±0.4	0.9±0.5
Endo	2.20±0.50	2.45±0.60	1.5±0.7	4.5±2.1*	16±4	31±12*	1.39±0.4	0.60±0.4*
Endo-Epi	2.30±0.40	4.60±1.30*	2.0±0.8	5.1±2.0**	15±3	28±9*	1.1±0.5	0.5±0.2*
Endo-Nor	2.50±0.50	3.70±0.50*	1.3±0.4	4.4±2.1*	16±4	32±12*	0.95±0.5	0.50±0.3*

*$p<0.05$ vs. baseline in the same group, **$p<0.05$ vs. baseline in the same group and compared with other groups

Plasma lactate concentration increased with a stable L/P ratio with epinephrine and did not change with norepinephrine compared to endotoxin values. Epinephrine and norepinephrine did not change tissue L/P ratios concentration in muscle, heart, gut, or liver when compared to base line endotoxin values (Table 3).

Table 3: Course of tissue lactate (nmol/mg wet/weight) and lactate/pyruvate (L/R) ratio at baseline and 180 minutes in saline, epinephrine, or norepinephrine control groups, and endotoxemic rats treated with or without epinephrine or norepinephrine from 90 to 180 minutes (Sponsor's table)

	Muscle	Liver	Gut	Kidney	Heart
Lactate					
Saline	2.2±1.2	1.2±0.7	2.0±0.9	0.75±0.4	1.2±0.3
Epi	4.2±1.3*	1.4±0.6	1.8±0.7	0.80±0.5	1.1±0.2
Nor	2.7±1.0	1.3±0.5	1.9±0.8	0.60±0.2	1.5±0.3
Endo	7.1±2.0*	4.2±1.2*	4.3±0.9*	3.6±1.5*	7.3±2.2*
Endo-Epi	6.1±2.0*	4.8±1.3*	6.6±1.5*	4.2±1.1*	5.0±1.5*
Endo-Nor	6.3±2.0*	4.4±1.2*	5.2±1.1*	4.1±1.2*	6.0±1.2*
L/P ratio					
Saline	22±8	24±6	14±5	19±10	17±8
Epi	19±6	22±5	14±5	17±8	18±9
Nor	20±5	22±7	15±4	18±9	20±9
Endo	82±12*	60±14*	29±9*	42±12*	35±9*
Endo-Epi	76±15*	70±14*	33±7*	80±18*	42±10*
Endo-Nor	80±14*	75±19*	32±9*	85±12*	40±9*

*$p<0.05$ vs. saline

Minneci et al. 2004
Differing effects of epinephrine, norepinephrine, and vasopressin on survival in a canine model of septic shock (in vivo study in dogs)

Key Findings
Epinephrine infusion caused a decrease in the 28-day survival in a dog model of septic shock, and norepinephrine and vasopressin did not show any significant improvement.

Purpose
To study the effects of epinephrine, norepinephrine, and vasopressin on survival in canine septic shock model.

Methods
Beagle dogs (n=78, 12-28 months old weighing 10-12 kg) were induced with sepsis by implantation of a fibrin clot containing live *Escherichia coli* 0111:B4 into the abdominal cavity.

After 6 hrs of implantation, dogs with varying degree of sepsis were randomized and given epinephrine (0.2, 0.8, or 2.0 μg/kg/min), norepinephrine (0.2, 1.0, or 2.0 μg/kg/min), vasopressin (0.01 or 0.04 U/min), or placebo.

Serial hemodynamic and biochemical variables were measured 7 days prior (baseline), and at 6, 24, and 48 hours after implantation, using arterial and pulmonary artery catheters, laboratory tests, and gated radionucleotide cineangiograms of the left ventricle.

To alleviate the pain, animals were given epidural medication (morphine sulfate (0.1 mg/kg) and bupivicaine (1.25 mg/kg), that did not significantly affect the MAP and left ventricular ejection fraction.

Euthanasia was performed on or before 28 days and counted as a non-survivor based on the criteria of a predetermined pain score, respiratory rate under 5 breaths per minute, seizure activity, or uncontrolled hemorrhage for more than 2 minutes, or if in pain or distress that could not be relieved.

Cox Proportional Hazards survival models were used to assess significant differences in survival data.

Results:
A progressive decrease was observed in survival ($p < 0.06$), MAP ($p < 0.05$), cardiac index ($p < 0.02$), and ejection fraction ($p = 0.02$) as the dose of bacterial exposure was increased (Fig. 4).

Source: Minneci 2004

Figure 4: Characteristics of the canine sepsis model with three different bacterial challenges in control animals receiving intravenous fluids and antibiotics without vasopressor therapy (Sponsor's Figure)

The 28-day survival was decreased with epinephrine, while norepinephrine and vasopressin did not show a significant improvement in survival (Fig.5).

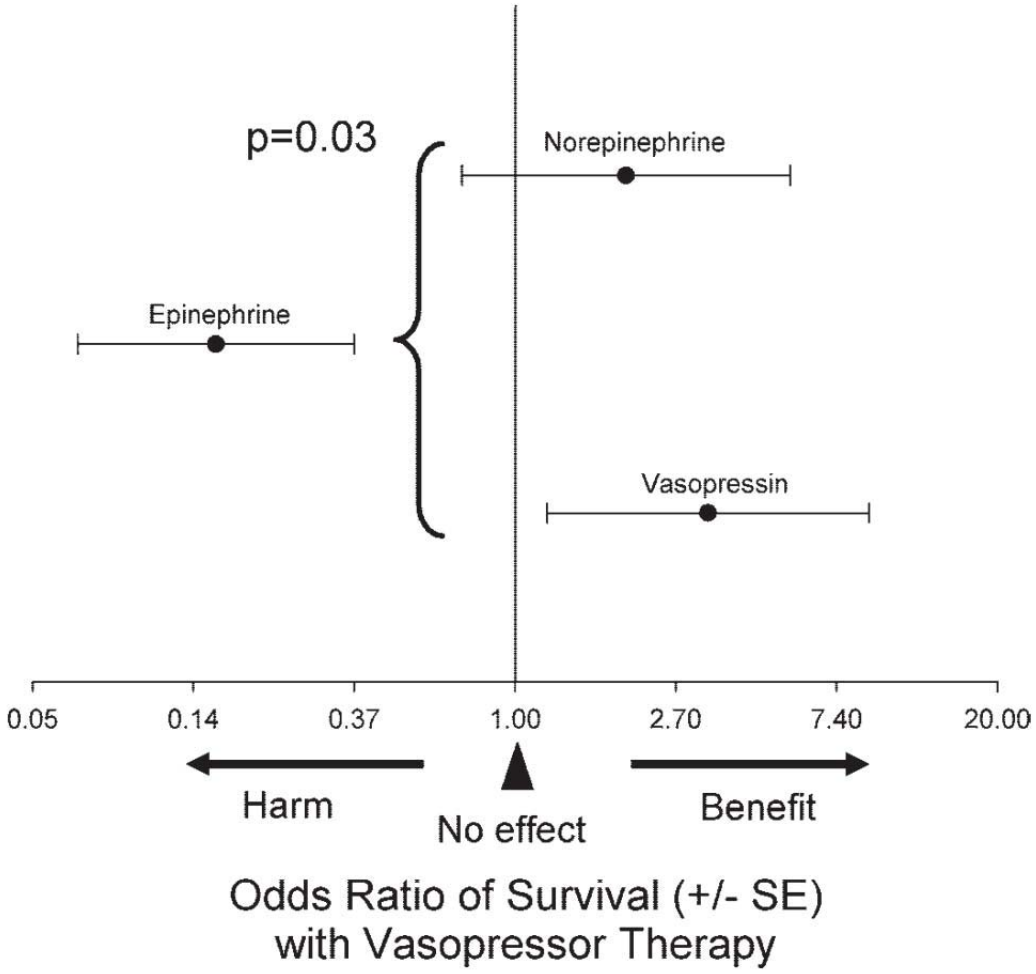


Figure 5: Effects of vasopressors on survival. The odds ratios of survival (means, closed circles; ±, horizontal lines) with vasopressor therapy averaged over all bacterial challenge and drug dose levels are shown. Overall, the effect of epinephrine on outcome was significantly different from the effects of norepinephrine and vasopressin ($P = 0.03$). Compared with controls, epinephrine had a harmful effect and norepinephrine and vasopressin had beneficial effects on survival.

A significant decrease was observed in cardiac index, ejection fraction, pH, systemic vascular resistance and serum creatinine after epinephrine infusion when compared with infected controls, norepinephrine and vasopressin while MAP, pulmonary capillary wedge pressure, central venous pressure, and heart rate, did not differ among epinephrine, norepinephrine, and vasopressin in canine model of septic shock (Fig.6).

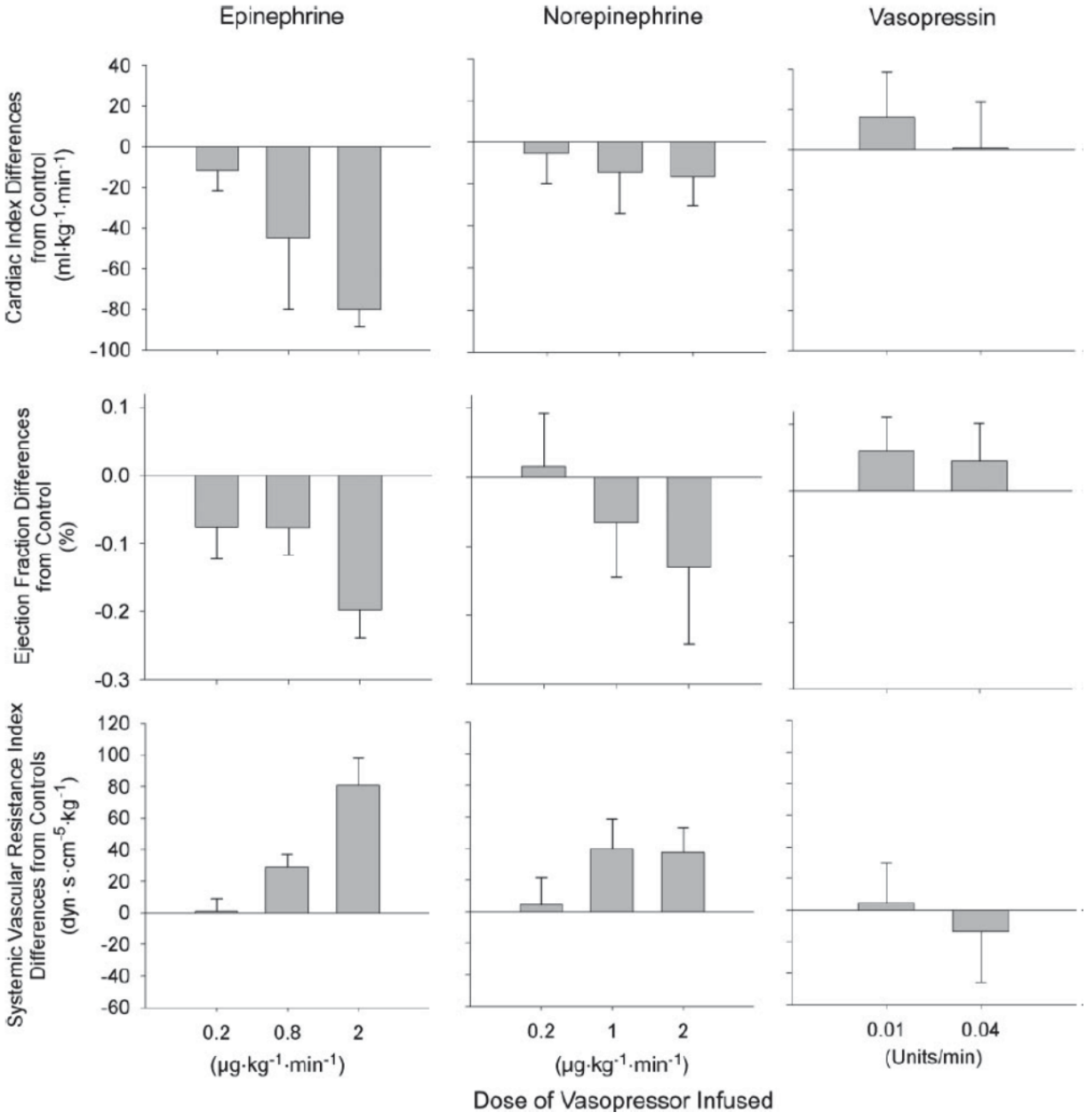


Figure 6: Effects of vasopressors on physiological parameters of cardiac index, ejection fraction, and systemic vascular resistance on canine sepsis models as differences from nonvasopressor treated controls (Sponsor's Figure).

Myburgh et al, 2006
An appraisal of selection and use of catecholamines in septic shock - old becomes new again (in vitro study using a sheep preparation)

Key Findings
Infusion of epinephrine, norepinephrine or dopamine significantly and equivalently increased MAP, cardiac output and right atrial pressure with a little change in systemic vascular resistance or heart rate in physiological sheep preparation.

Purpose
This review article describes the effects of epinephrine (0-60 µg/kg/min), norepinephrine (0-60 µg/kg/min) or dopamine (0-60 µg/kg/min) infusion on systemic hemodynamics in physiological sheep preparation (*in vitro study*).

Results
The data has shown that infusion of epinephrine, norepinephrine or dopamine significantly increased the MAP, cardiac output and right atrial pressure in an equivalent manner with a little change in systemic vascular resistance (SVR) or heart rate (Fig.7). Effects of low dose levels were more predominant on β- receptors while the high dose on α- receptors.

Source: Myburgh 2006

Figure 7: Effects of epinephrine, norepinephrine, and dopamine on cardiovascular parameters in a physiological ovine preparation (Sponsor's figure)

Di Giantomasso 2005
The hemodynamic and metabolic effects of epinephrine in experimental hyperdynamic septic shock (in vivo study in sheep)

Key Findings
The blood flow to the heart, gut, and kidney were increased at the onset of sepsis with an induced hypotension and hyperlactemia in sheep model of septic shock. Epinephrine infusion significantly improved the MAP (69 vs. 86 mmHg) and cardiac output (6.4 vs. 7.1 l/min) and decreased renal blood flow (330 vs. 247 ml/min). Epinephrine was also associated with metabolic effects, decreased mesenteric, coronary and renal conductance.

Purpose
To determine the effect of epinephrine treatment on blood flow of vital organs, global and regional hemodynamics, and metabolic function in sheep model of septic shock.

Methods
Sepsis was induced in merino cross-ewes (n=7) weighing between 35-45 kg by intravenous bolus injection of $3x10^9$ colony forming units (CFU) of *E.coli*. Following the onset of sepsis (5/6 hrs. after infusion), sheep were randomly given epinephrine (0.4 μg/kg/min) or vehicle for 6 hours.

The MAP, cardiac output, heart rate, sagittalsinus, coronary, mesenteric and renal flows were measured throughout the infusion period.Urinary flow was measured and sampled every 2 hours for analysis.

Arterial blood samples for analysis of arterial blood gases, serum urea, creatinine and electrolytes were measured with automated analyzer at 0, 30, 60, 120, 240 and 360 minutes during the observation period. Two-hour creatinine clearance was calculated at 2, 4 and 6 hours during the septic state. No fluid boluses, mechanical ventilation or antibiotics were given.

Results
Tachycardia (heart rate>140 bpm), fever (temperature >41°C), and tachypnea (respiratory rate >30 breaths/min) developed after intravenous infusion of *E. coli*.

Onset of hypotension (85 ± 7 vs. 69 ± 8 mmHg, $p < 0.05$), increase in cardiac output (4.0 ± 0.9 vs. 6.4 ± 1.2 L/min, $p < 0.05$) and increase in total peripheral conductance (cardiac output/MAP; 55.1 ± 6.2 vs. 91.8 ± 13.2 mL/min/mmHg, $p < 0.05$) were the major hemodynamic changes observed as a result of hyperdynamic sepsis (Fig. 7).

Administration of epinephrine improved the hemodynamic state by increasing MAP (69 ± 8 vs. 86 ± 13 mmHg, $p < 0.05$) and cardiac output (6.4 ± 1.2 vs. 7.1 ± 1.6 L/min, $p < 0.05$) compared to vehicle control (Fig. 6). Epinephrine also decreased the heart rate (133.1 ± 9.6 vs. 120.5 ± 25.9 bpm, $p < 0.05$) and total peripheral conductance (91.8 ± 13.2 vs. 85.1 ± 26.0 mL/min/mmHg), Fig. 8.

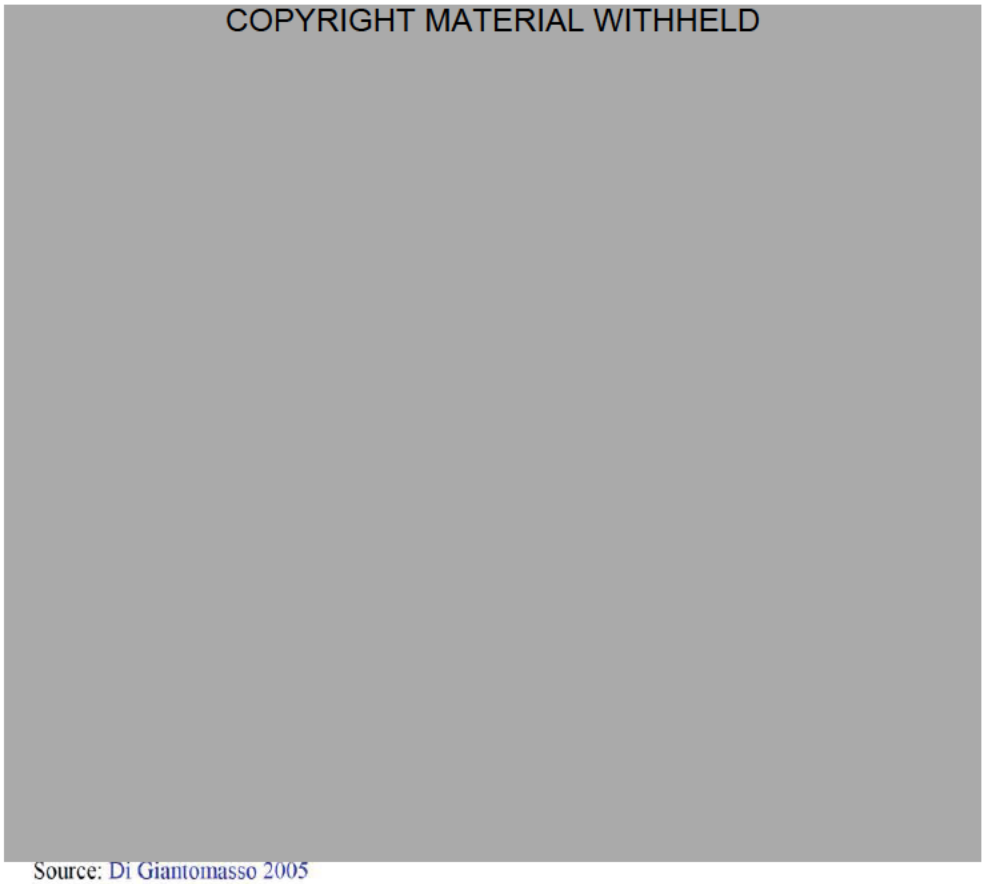
Source: Di Giantomasso 2005

Figure 8: Graphic representation of the effect of hyperdynamic sepsis with vehicle (squares) and epinephrine (triangles) on MAP, heart rate, cardiac output, and total peripheral conductance

Onset of sepsis increased the renal blood flow (330 ± 101 vs. 214 ± 75 mL/min, $p < 0.05$) and renal conductance (from 4.7 ± 0.6 vs. 2.6 ± 0.6 mL/min/mmHg, $p < 0.05$).

The renal blood (330 ± 101 vs. 247 ± 54 mL/min, $p < 0.05$) and renal conductance (4.7 ± 0.6 vs. 3.5 ± 0.8 mL/min/mmHg, $p < 0.05$) were also reduced following epinephrine administration, while an increase in total urine output (293 ± 224 vs. 544 ± 497 mL per 6 hours, $p < 0.05$) was observed.

At the same time no changes (75.6 ± 31.8 vs. 73.8 ± 37.8 mL/min, not significant [NS]) were observed in mean creatinine clearance compared to vehicle control (Fig. 9).



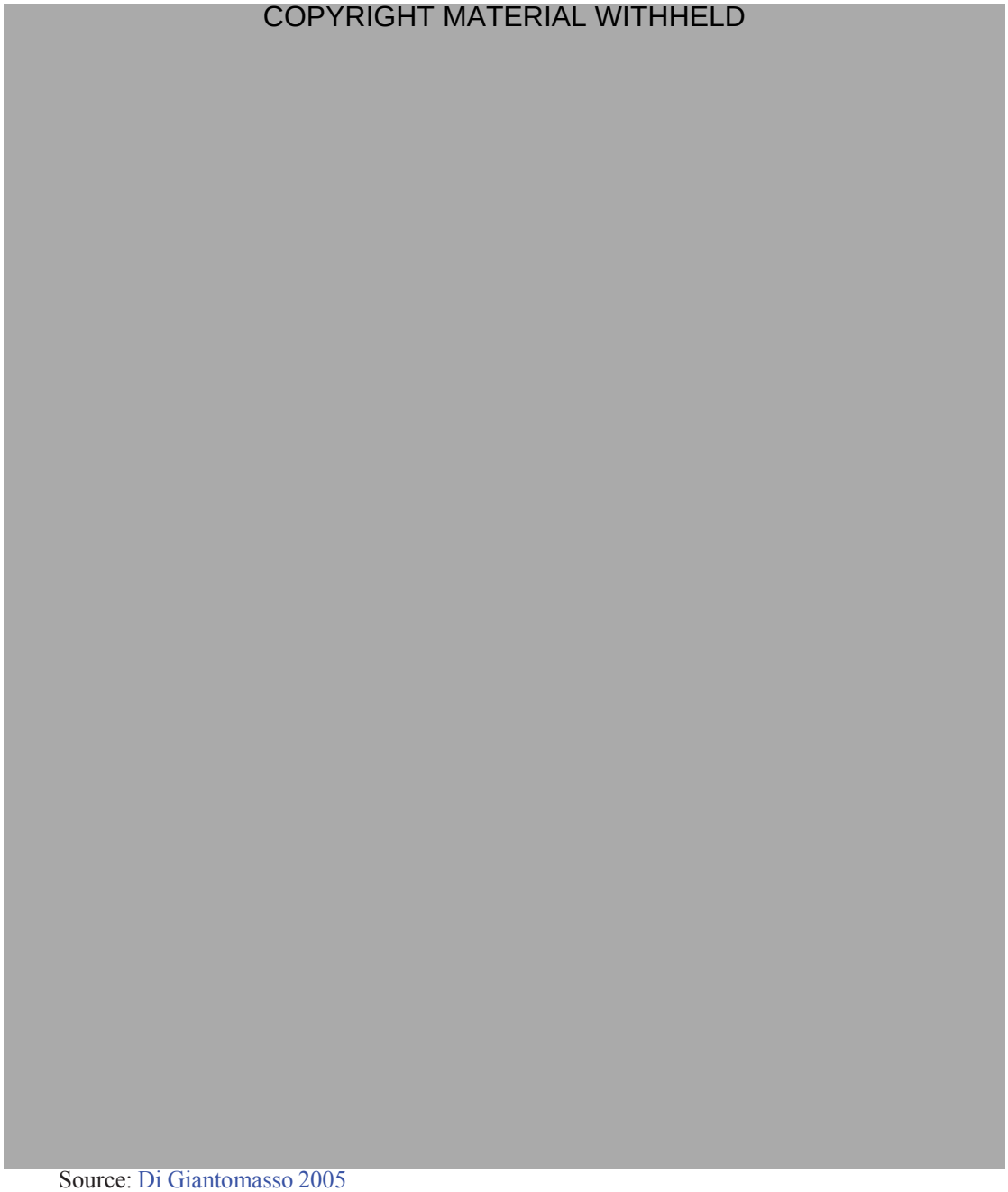
Source: Di Giantomasso 2005

Figure 9: Graphic representation of renal blood flow and renal conductance during hyperdynamic sepsis with vehicle (squares) and with epinephrine (triangles)

Onset of sepsis increased the mesenteric circulation and superior mesenteric artery flow (859 ± 170 vs. 516 ± 221 mL/min, $p < 0.05$) and the administration of epinephrine did not affect the mesenteric flow (859 ± 170 vs. 824 ± 407 mL/min, NS) when compared to vehicle control.

The mesenteric conductance increased by sepsis (6.1 ± 1.8 vs. 12.7 ± 2.9 mL/min/mmHg, $p < 0.05$) was significantly decreased (12.7 ± 2.9 vs. 10.1 ± 5.0 mL/min/mmHg, $p < 0.05$) following administration of epinephrine when compared to vehicle control (Fig. 10).

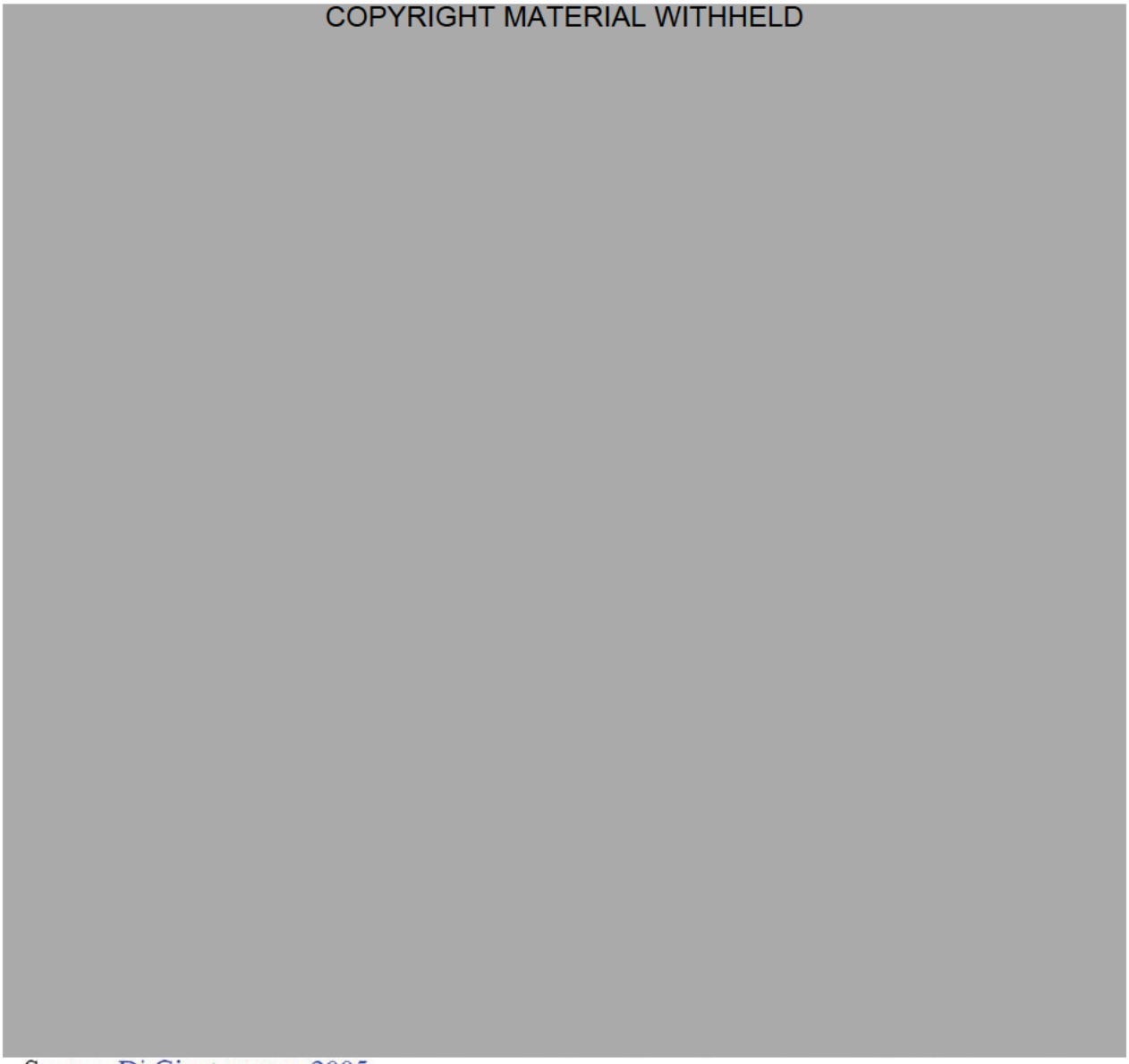
The coronary blood flow (24.5 ± 8 vs. 51.1± 12.1 mL/min, $p < 0.05$) and conductance (0.27 ± 0.10 vs. 0.75 ± 0.18 mL/min/mmHg, $p< 0.05$) were almost doubled at the onset of sepsis.



Source: Di Giantomasso 2005

Figure 10: Graphic representation of mesenteric blood flow and mesenteric conductance during hyperdynamic sepsis with vehicle (squares) and with epinephrine (triangles)

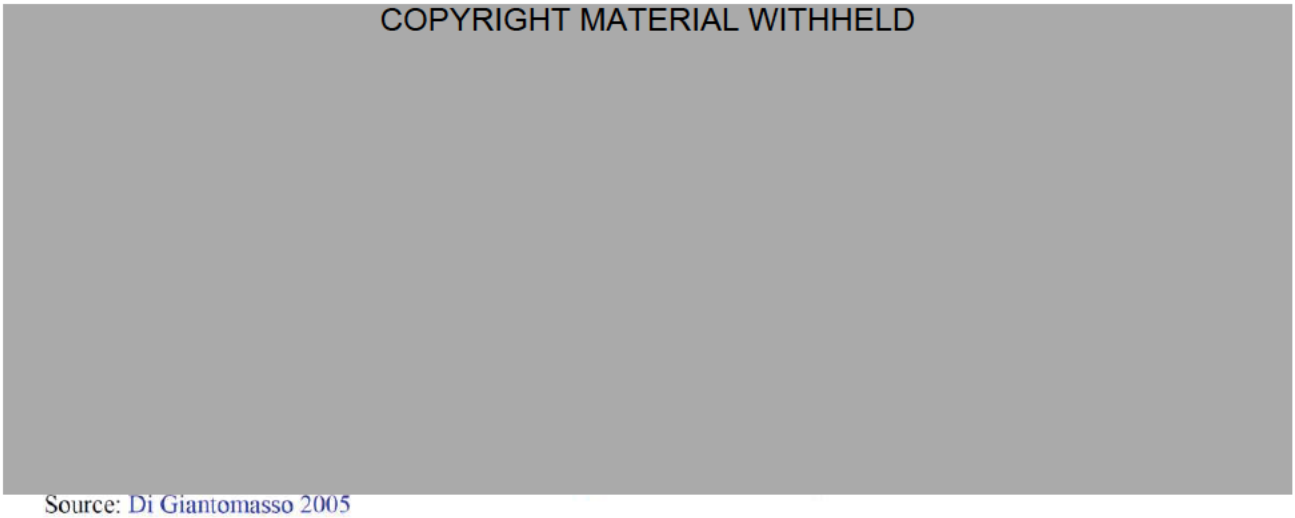
Epinephrine administration did not affect the coronary blood flow compared to vehicle (51.1 ±12.1 vs. 53.1 ± 25.9 mL/min, NS), however, asignificant decrease was observed in conductance (0.75 ± 0.18 vs. 0.64 ± 0.34 mL/min/mmHg, $p < 0.05$), Fig. 11.



Source: Di Giantomasso 2005

Figure 11: Graphic representation of coronary blood flow and coronary conductance during hyperdynamic sepsis with vehicle (squares) and with epinephrine (triangles)

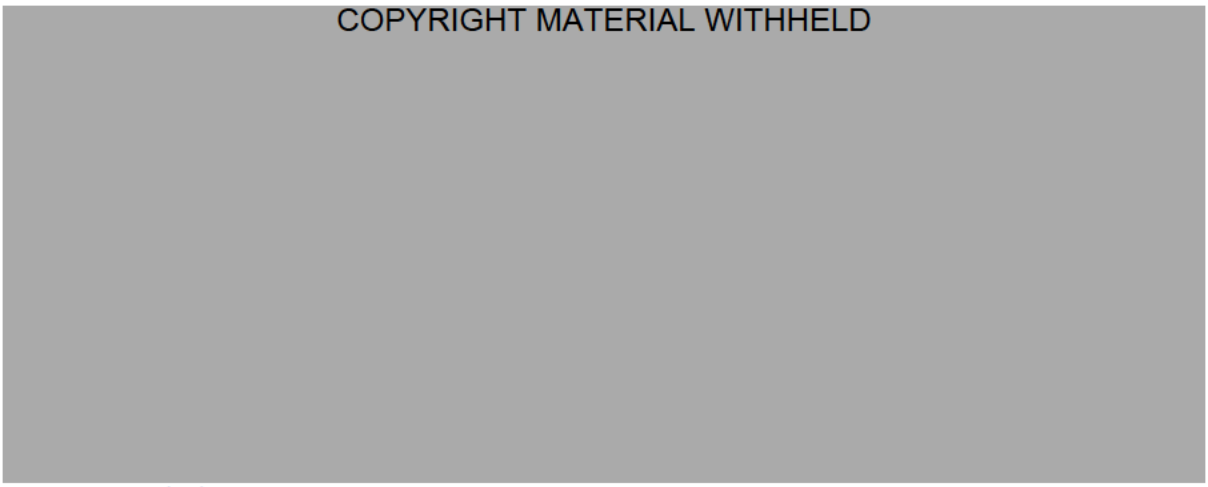
The myocardial performance as evaluated by systole (875 ± 170 vs. 1535 ± 333, $p < 0.05$) and stroke volume (49.2 ± 6.3 mL/beat vs. 60.5 ± 12.9 mL/beat, $p < 0.05$), that were decreased at the onset of sepsis, were significantly increased by epinephrine administration (Fig. 12).



Source: Di Giantomasso 2005

Figure 12: Graphic representation of stroke volume and df/dt during hyperdynamic sepsis with vehicle (squares) and with epinephrine (triangles)

The sagittal sinus flow was stable during sepsis when compared to baseline (13.8 ± 3.2 vs. 14.1 ± 1.6 mL/min, NS) and it was not altered with epinephrine when compared to vehicle (14.1 ± 1.6 vs. 15.1 ± 10.0 mL/min, NS), Fig. 13.



Source: Di Giantomasso 2005

Figure 13: Graphic representation of sagittal sinus blood flow during hyperdynamic sepsis with vehicle (squares) and with epinephrine (triangles)

Onset of sepsis increased the blood lactate concentration (0.7 ± 0.2 vs. 1.8 ± 0.7 mmol/L, $p < 0.05$), and effects were further potentiated by epinephrine treatment (1.8 ± 0.7 vs. 15.7 ± 4.8 mmol/L, $p < 0.05$). Epinephrine-induced hyperlactatemia seems to be associated with a metabolic acidosis (serum bicarbonate: 25.± 3.0 vs. 15.7 ± 3.0 mmol/L, $p < 0.05$) when compared to vehicle. Administration of epinephrine led to hyperglycemia (2.6 ± 0.5 vs. 13.5 ± 5.8 mmol/L, $p < 0.05$) and hypokalemia (4.3 ±0.4 vs. 3.0 ± 0.3 mmol/L, $p < 0.05$) when compared to vehicle control.

4.3.1 Safety Pharmacology

Sponsor has not conducted any safety pharmacology study. The published studies have shown that epinephrine produced a dose-dependent and potent increase in all cardiovascular parameters at 0.1–1.0 mg/kg (Fig. 14/15/16). Mean QT/QTc intervals were not influenced. Counting of extra-systoles showed a dose-dependent increase during intravenous drug administration starting at a threshold dose of 0.1 mg/kg (Hauser et al, 2005).

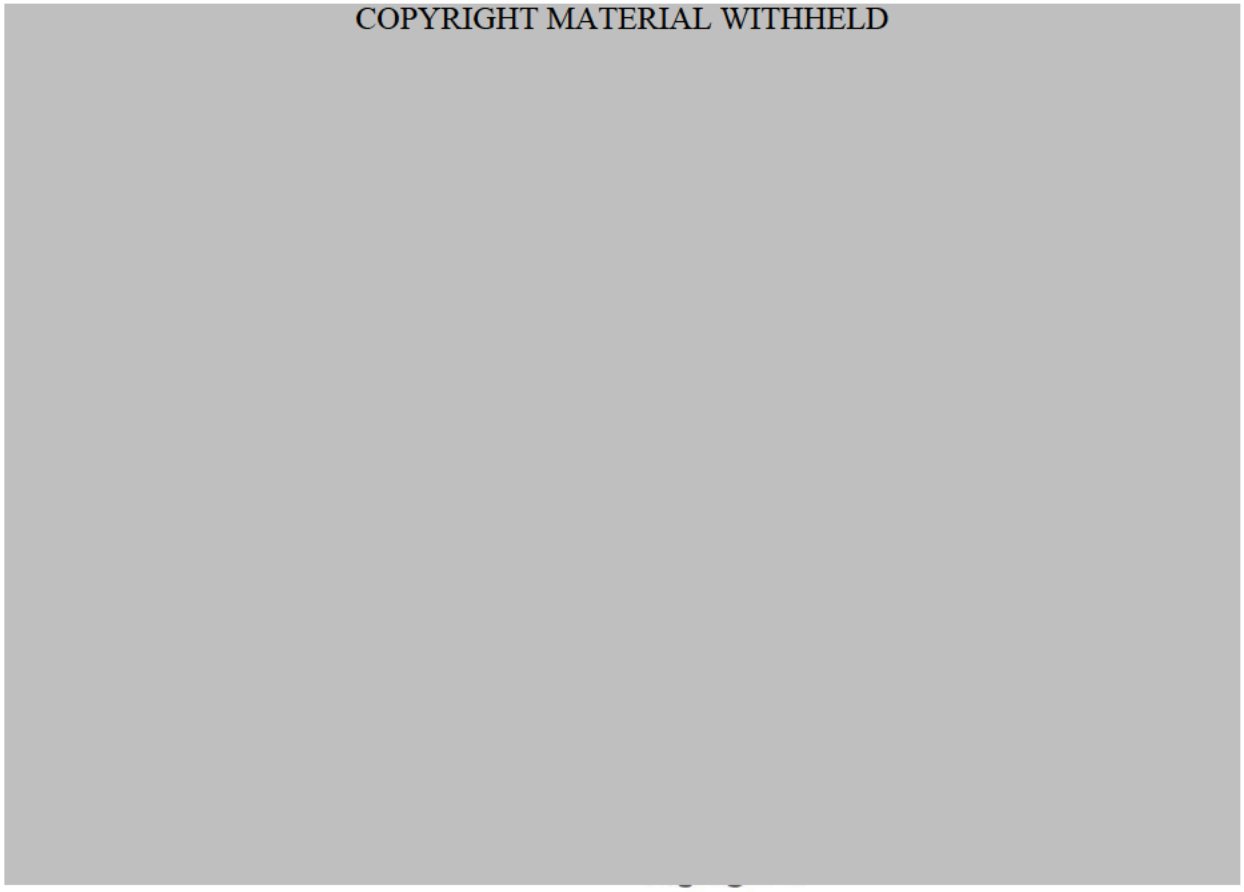


Figure 14: Effects of selected reference compounds on heart rate (HR) in anaesthetized guinea pigs after intravenous administration. Each curve represents the meanTS.E.M. *P <0.05, **P <0.01, ***P <0.001 versus pre-drug values

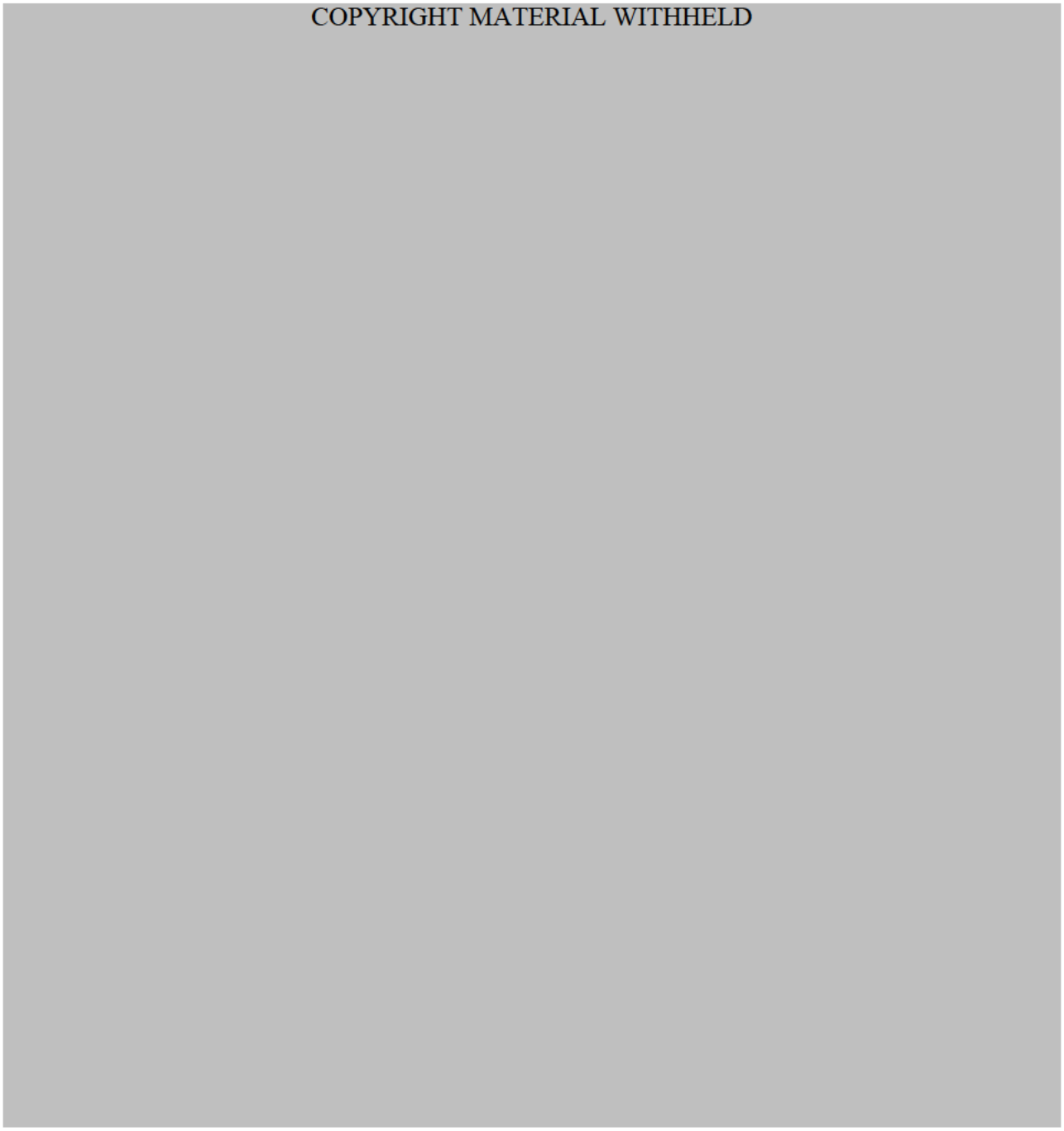


Figure 15: Effects of selected reference compounds on (A) SAP and (B) DAP in anaesthetized guinea pigs after intravenous administration of different doses. Each curve represents the meanTS.E.M. *P <0.05, **P <0.01, ***P <0.001 versus pre-drug values.

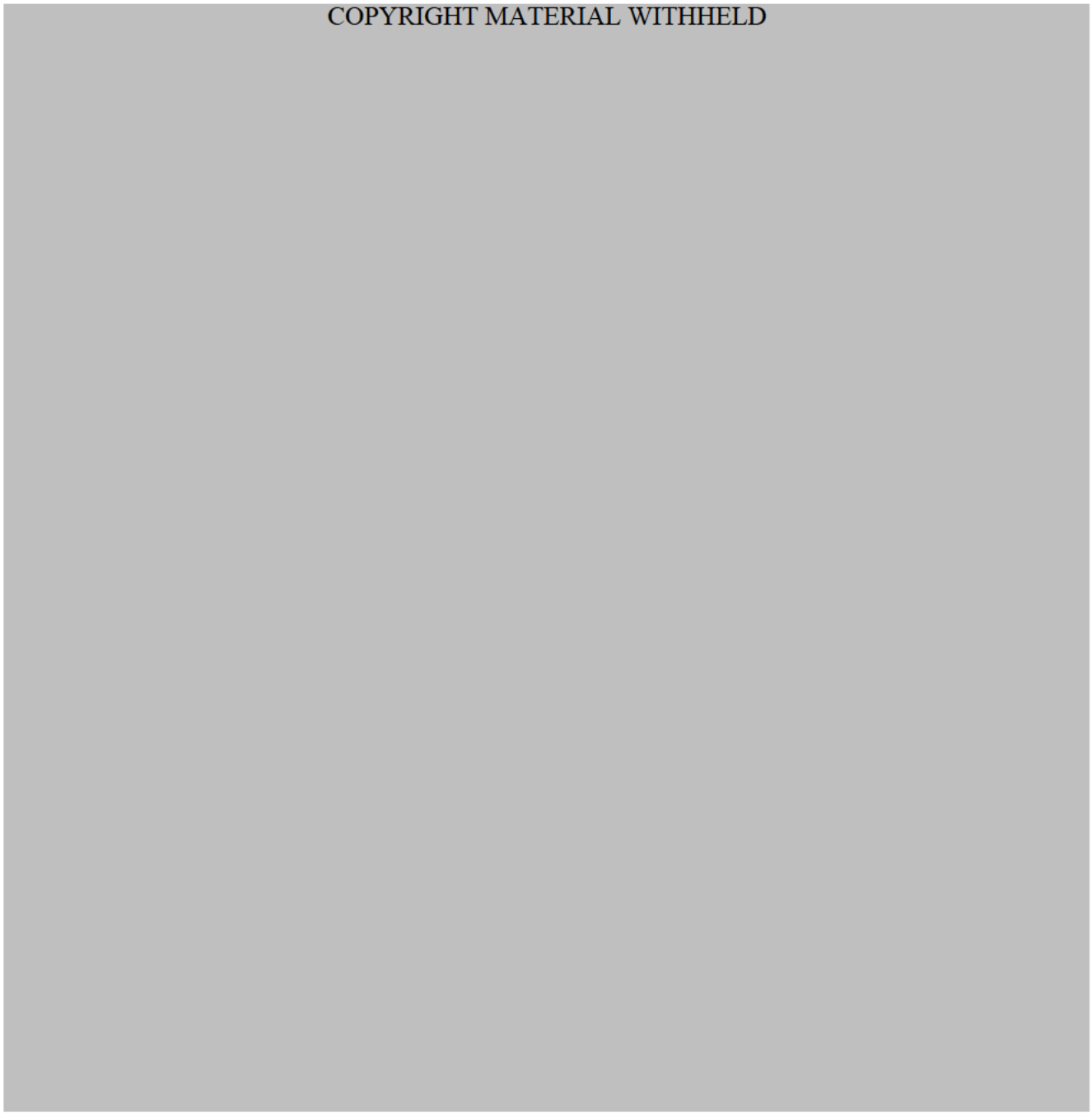


Figure 16: Effects of reference compounds on (A) LVP and (B) dp/dtmax in anaesthetized guinea pigs after intravenous administration of different doses. Each curve represents the meanTS.E.M. *P <0.05, **P <0.01, ***P <0.001 versus pre-drug values

5 Pharmacokinetics/ADME/Toxicokinetics

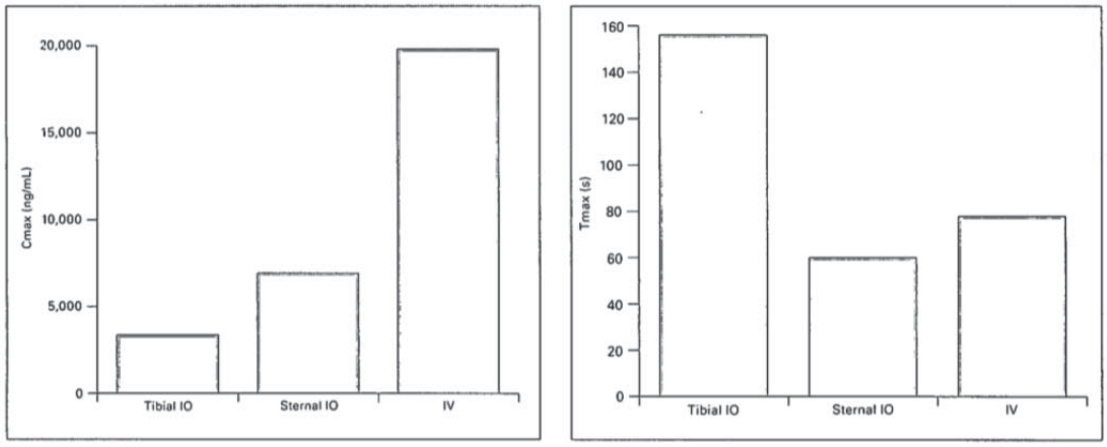
Sponsor did not conduct any PK/ ADME/Toxicokinetics study. Data from published literature have shown that an intravenous administration of 1 mg of epinephrine in a porcine model of cardiac arrest during cardiopulmonary resuscitation resulted in a serum concentration 5.87 and 2.86 times greater when compared with tibial intraosseous (IO), sternal IO routes, respectively.

Burgert et al (2012)
Comparison of tibial intraosseous, sternal intraosseous, and intravenous routes of administration on pharmacokinetics of epinephrine during cardiac arrest: a pilot study.

The maximum concentration (Cmax) of epinephrine for tibial IO route of administration ranged from 1,038 to 5,260 ng/mL, with a mean (±SD) of 3,371 ± 1,561 ng/mL, tbe Cmax for tbe sternal IO route ranged from 3,314 to 18,617 ng/mL (mean, 6,924 ± 6,551), while the Cmax for tbe IV route ranged from 8,579 to 38,768 ng/mL (mean, 19,810 ± 12,323). A significant difference in Cmax between the sternal 10 and IV routes (P = .009) and tibial 10 and IV routes (P = .03) were noticed while no significant difference between the tibial and sternal IO routes (P = .75) were found. The IV administration of 1 mg of epinepbrine resulted in a serum concentration 5.87 and 2.86 times greater than for the tibial and sternal routes of administration, respectively **(A).**

The time to maximum concentration (Tmax) of epinephrine was also compared across the 3 groups (Figure 2). The Tmax for tbe tibial IO route ranged from 150 to 180 seconds (mean, 156 ± 13 seconds); for the sternal IO route from 30 to 120 seconds (mean, 60 ± 42 seconds); and for the IV route, from 30 to 180 seconds (mean, 78 ± 69 seconds). There were significant differences in Tmax between the tibial IOand IV (P = .04) and between the tibial IO and sternal IO (P = .02) groups but no difference were observed between the sternal 10 and IV groups (P =.56) **B.**

A. The maximum concentration (Cmax) **B. Time to maximum concentration (Tmax)**



The Mean Maximum Concentration (Cmax) and Mean Time to Maximum Concentration (Tmax) of epinephrine Administered via 3 Routes in a Porcine Model of Cardiac Arrest During Cardiopulmonary Resuscitation

6 GENERALTOXICOLOGY

6.1 Single-Dose Toxicity

No single dose toxicology studies were conducted by the Sponsor. The LD_{50} data (Table 4) from published literature have demonstrated that epinephrine is toxic at higher dose levels (Lewis, 2004).

Table 4: The Median Lethal Dose(s) of Epinephrine (Sponsor's table)

Source: TOXNET

A hemolytic study carried out by the Sponsor to determine if epinephrine is hemolytic at the desired dose dilution is presented below.

6.2 Repeat-Dose Toxicity Studies

No repeat dose toxicology study conducted by the Sponsor. Data from published reports suggest that multiple IV injections of epinephrine could induce glaucoma in rabbits (Grant WM, 1986).

Results from Toxicology and carcinogenesis studies of L-epinephrine hydrochloride conducted by exposing groups of F344/N rats and B6C3Fl mice of each sex to an aerosol containing epinephrine hydrochloride (0 or 12.5-200 mg/m3 for 14 days, 13 weeks, 15 months, or 2 years) are discussed below (NTP, 1990).

14-Day NTP Study (1990)

Mortality (100%) was observed in male rats exposed to epinephrine hydrochloride at a dose of 50 mg/m3 or females at 100 mg/m3 more (Table 5) and in mice (3/5) at a dose of 50 mg/m3 or more (Table 6). Observed clinical signs at higher dose (100 and 200 mg/m3) were an increased respiratory rate and excessive lacrimation in rats and dyspnea in mice.

Table 5: Survival and Median Body Weights of Rats in the 14-Day In halation Study of Epinephrine Hydrochloride (NTP)

Concentration (mg/m^3)	Survival (a)	Mean Body Weights (grams) Initial (b)	Necropsy	Change (c)	Necropsy Weight Relative to Controls (percent)

Table 6: Survival and Median Body Weights of Mice in the 14-Day In halation Study of Epinephrine Hydrochloride (NTP)

Concentration (mg/m^3)	Survival (a)	Mean Body Weights (grams) Initial (b)	Necropsy	Change (c)	Necropsy Weight Relative to Controls (percent)

13-Week NTP Study (1990)

Increased respiratory rates in mice and rats, organ weight increase in heart and adrenal glands in rats (Table 7), increase in adrenals and liver weight of mice (Table 8) were observed when animals were exposed to 40 mg/m3 epinephrine hydrochloride.

Minimal focal squamous metaplasia occurred in the respiratory epithelium of the nasal mucosa of rats (5/9♂ and 4/10♀) and mice (3/10♂ and 1/9 ♀) exposed to 40 mg/m3. Degenerative lesions of the laryngeal muscle were seen in male and female rats exposed to 20 or 40 mg/m3 when compared with control aerosol treated animals.

Table 7: Organ Weights for rat in the 13-Week Inhalation Study of Epinephrine Hydrochloride (a)

Organ	Control	2.5 mg/m³	5 mg/m³	10 mg/m³	20 mg/m³	40 mg/m³

Inflammation in the glandular stomach was seen in male and female mice exposed to 10, 20, and 40 mg/m3, and uterine atrophy was seen in 7/10 female mice exposed to 40 mg/m3.

Table 8: Change in Organ weights for mice in the 13-Week Inhalation studies of Epinephrine hydrochloride (a)

Organ	Control	2.5 mg/m³	5 mg/m³	10 mg/m³	20 mg/m³	40 mg/m³
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15 month and 2-year Studies

F344/N rats (60) and B6C3F1 mice (60) were exposed to l-epinephrine hydrochloride at a dose concentration of 0, 1.5, or 5 mg/m3 for 6 hours/day for 15 months and for 103 weeks.

Blood samples were collected prior to sacrifice of the animals and analyzed for erythrocyte and leukocyte counts, hemoglobin concentration, hematocrit value, and leukocyte differential count.

Necropsy was performed for brain, liver, and right kidney and histopathologic examinations were conducted on control and high dose animals.

There were no drug related clinical signs observed in rats and mice exposed with L-epinephrine hydrochloride for 15 months or 2-years.

Increased incidences of minimal-to-mild nonneoplastic lesions were observed in the nasal passage of exposed male and female mice (Table 9). Neoplasm of the nasal passage were not seen in rats.

Table 9: Nasal Passage Lesions Observed in Mice in the 2-Year Inhalation Studies of Epinephrine Hydrochloride

Site/Lesion	Male			Female		
	Chamber Control	1.5 mg/m^3	3 mg/m^3	Chamber Control	1.5 mg/m^3	3 mg/m^3
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7 GENETIC TOXICOLOGY

No genetic toxicology studies were conducted/submitted by the Sponsor.

Data from NTP studies (1990) have shown an equivocal response of epinephrine when tested in *Salmonella typhimurium* strain TA100 in the absence of metabolic activation system (S-9) and negative in the presence of metabolic activation (S9).

No mutagenic activity was observed in TA100 in the presence of Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver S9 or in strains TA98, TA1535, or TA1537 with or without S9 (1990).

The responses observed in the CHO cell assay for induction of sister chromatid exchanges were considered to be negative and equivocal in the presence or absence of S9 activation. Epinephrine did not show any chromosomal aberrations in CHO cells with or without S-9 fraction.

8 CARCINOGENICITY

No carcinogenic effects were observed in male or female F344/N rats or B6C3F1 mice exposed to aerosols containing 1.5 or 3 mg/m3 epinephrine hydrochloride for 2 year.

However, these studies were considered to be inadequate because the concentrations used, which were chosen to represent multiples of therapeutic doses given by inhalation, and were too low for the animals to have received an adequate systemic challenge from the drug (NTP, Report 380, 1990).

9 REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

There are no reproductive and developmental toxicology studies conducted by the Sponsor. An excerpt from the reference listed drug (Twinject, NDA 020800) indicating the dose used and its effects is presented here as below:

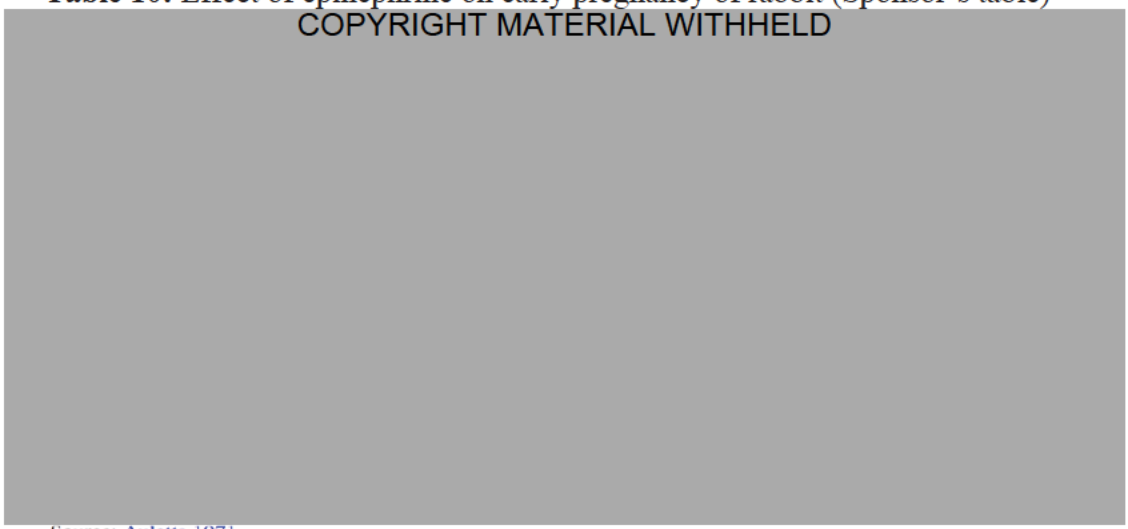
Species	Dose/Route	Multiples of Maximum recommended dose
Rabbit	1.2 mg/kg (SC) (Fetal anorexia & spontaneous abortions)	30 times (SC/IM) mg/m^2 basis
Mice	1 mg/kg (NOAEL=0.5mg/kg)	7 times (SC/IM) mg/m^2 basis
Hamsters	0.5 mg/kg	5 times (SC/IM) mg/m^2 basis

The data from published literature suggest that IV infusion of epinephrine in pregnant rabbits led to increased maternal blood pressure, uterine vasoconstriction, placental cynosis & CV alterations in fetus (Doull & Classen in, Casarett & Doull's Toxicology 3rd edition1986).

Epinephrine has been shown to affect the ovum transport and the motility of the rabbit oviduct (Longley et al 1968) and impair implantation in rats (Crist & Hulka, 1970). As presented in Table 15, decreased number of implantations and fetuses were observed in rabbits treated with epinephrine on Days 6 to 7 and 7 to 9 ($P<0.01$ and $P<0.01$ respectively).

In addition to this, epinephrine has been shown to interfere with ovum implantation and fetus survival in rabbits and having a potential teratogenic activity as gastroschisis in one fetus (Table 10) at Day 6/7 (Auletta, 1971) and abnormally absent aortic arches related to dysrhythmogenesis (Rajala et al, 1988).

Table 10: Effect of epinephrine on early pregnancy of rabbit (Sponsor's table)



Source: Auletta 1971

Hemorrhages, edema and necrosis of distal extremities were observed when 5-50 µg of adrenalin was injected directly into rabbit fetuses at 18 to 22 days of gestational age (Shepard, 1986). In

addition to hemorrhages of head, skin, and extremities more than 50% aortic arch anomalies were seen in the surviving embryos 20-200 µg when epinephrine was dropped on chorio-allantoic membrane of chick once per day on the 10th, 11th or 12th day of incubation (Shepard, 1986).

The reproductive and developmental toxicity studies have shown developmental effects in rabbits at a subcutaneous dose of 1.2 mg/kg (approximately 30 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis).

In mice epinephrine infusion caused developmental effects at a subcutaneous dose of 1 mg/kg (approximately 7 times the maximum recommended daily subcutaneous or intramuscular dose on mg/m2 basis).

In hamsters epinephrine infusion caused developmental effects at a subcutaneous dose of 0.5 mg/kg (approximately 5 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis) as found in the listed approved epinephrine drug product.

10 SPECIAL TOXICOLOGY STUDIES

10.1 ASTM Hemolysis-Direct Contact Method (ASTM F 756-08)

Conducting laboratory and location:	(b) (4) [redacted]
Study number(s):	12J0271G-M01G
Date of study initiation:	Oct. 19, 2012
Drug lot/batch number:	09101
GLP compliance:	Yes
QA statement:	Yes

Key Study Findings

The test article (epinephrine) was found hemolytic at higher concentration (undiluted, 1:10 and 1:100 dilutions in PBS) while it did not show any hemolytic activity at lower concentration (1:1000 dilution in PBS) when evaluated using direct contact method protocol.

Purpose

To evaluate the compatibility of test article (epinephrine) with human blood and to assess its hemolytic potential to cause blood cell lysis *in vitro*.

Methods

A 3 mL pooled human blood, obtained from three different donors [redacted] (b) (4) was centrifuged at 800g for 15 min. The plasma samples were removed, diluted (1:1) in cyanmethemoglobin (CMH) diluent and incubated at room temperature for 3-5 minutes.

The absorbance was used to calculate the plasma free hemoglobin (PFH). As per ASTM guidelines, the blood with PFH value of < 2 mg/mL is acceptable. A 20 µL sample from pooled blood was used to determine total hemoglobin concentration in blood.

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The hemolytic evaluation was performed as per standard protocol [redacted] (b) (4) described in ASTM Hemolysis Test Standard F 756-08 and using the following supplies (Table 11). A standard hemoglobin curve was prepared to determine the hemoglobin concentration at 540 nm (Table 12).

Table 11: Supplies (Sponsor's table)

Reagents/Materials	Lot Number	Manufacturer	Expiration Date
Negative Control: High Density Polyethylene	MKBB6695 Sterilization Batch # 237	[redacted] (b) (4)	N/A 1/31/13
Positive Control: DI Sterile Water	DI650		4/15/13
(Ca Mg Free) 1X Phosphate Buffer Saline (PBS)	30001215		6/2013
Human Blood #1 Human Blood #2 Human Blood #3	40743 F 42827 F 49770 M		11/22/12
Anticoagulant (0.13M Sodium Citrate Solution)	C877381		2/2014
Cyanmethemoglobin Diluent	31332		4/2014
Cyanmethemoglobin Standards (80 mg/dL)	27011		9/2013

Table 12: Standard Hemoglobin Concentration (Sponsor's table)

Hb Concentration (mg/mL)	Absorbance (540nm)
0.01	0.0044
0.02	0.0107
0.05	0.0320
0.10	0.0645
0.30	0.2025
0.50	0.3460
0.70	0.4871

Slope	1.4292
Y-intercept	5.7765×10^{-3}
Correlation	0.99996

Direct Contact Method

Blank, negative and positive controls, diluted and undiluted blood samples (1:10, 1:100, and 1:1000) in triplicates were prepared (at a ratio of 1.0 mL of blood per 7.0 mL of total volume). Samples were incubated at 37 ± 2°C for 3 hours with gentle mixing at every 30 minutes (Table 13).

Table 13: Preparation of test article and controls (Sponsor's table)

Name	Total Surface Area/Weight	Extraction Ratio	Total Volume (mL)	Vehicle	Diluted Blood Ratio (mL)	Diluted Blood (mL)
Test Article (Undiluted, 1:10, 1;100, and 1:1000)			7.0		1:7	1.0
Negative Control	2.0 g	0.2 g/1 mL	10.0	PBS	1:7	1.4
Blank			7.0	PBS	1:7	1.0
Positive Control			7.0	Sterile DI Water	1:7	1.0

A series of dilutions was prepared as follows:

Dilution	Volume of Preceding Dilution (mL)	Volume of Diluent (PBS)(mL)
Undiluted	N/A	N/A
1:10	3.0 mL (from undiluted test article)	27.0
1:100	3.0 mL (from 1:10 dilution)	27.0
1:1000	3.0 mL (from 1:100 dilution)	27.0

The suspensions were decanted and centrifuged at 800g for 15 minutes. The supernatant for each sample was carefully removed and placed into a second set of screw-capped tubes. A 1.0 mL of each supernatant was added to 1.0 mL of CMH diluent and incubated at room temperature for 3-5 minutes. The absorbance of each solution was read at 540 nm and colors obtained were evaluated (Table 14).

Table 14: Color Determinations (Sponsor's table)

Supernatant	Color	Particulates or Precipitants
Test Article (Undiluted)	Transparent Brown	None
Test Article (1:10 dilution)	Transparent Pink	None
Test Article (1:100 dilution)	Clear	None
Test Article (1:1000 dilution)	Clear	None
Negative Control	Clear	None
Positive Control	Transparent Burgundy-Reddish	None
Blank (PBS)	Clear	None

Validity of the Test: The test is considered valid, if the negative control replicates have a blank corrected hemolytic index of< 2% and the positive control replicates have a blank corrected hemolytic index above the blank corrected negative control replicates of at least 5%.

Interpretation: Hemolytical grade is assigned based on Hemolytic Index as recommended by ASTM guideline (Table 15). Toxicological significance is assigned by the client based on the nature of the tissue contact, duration of contact, surface area to body ratios, the nature of the device, and any other pertinent information.

Table 15: Hemolytic Grades

Blank Corrected Hemolytic Index Above the Negative Control (%)	Hemolytic Grade
0 - ≤ 2	Non-hemolytic
> 2 - ≤ 5	Slightly hemolytic
> 5	Hemolytic

Table adopted from ASTM F756-08: Standard Practice for Assessment of Hemolytic Properties of Materials

The percent hemolysis was calculated using the following formula:

$$\text{Blank corrected \% hemolysis} = \frac{(Abs^{Sample*} - Abs_{(mean)}{}^{Blank}) \times 100}{Abs_{(mean)}{}^{DBH} - Abs_{(mean)}{}^{Blank}}$$

Abs = Absorbance

DBH = Diluted Blood Hemoglobin

Sample* = Test Article, Negative Control, Positive Control

Results

Based on ASTM criteria, the hemolysis results for the controls were acceptable. The negative control had an average hemolytic index of 0% contact and the positive control had an average hemolytic index of 92.90%.

The Hemolytic grades for the test article and positive control above the negative control are presented in Table 8. A corrected hemolytic index above the negative control for the test articles was 329.46%, 54.01%, 15.12%, and 1.06% for the undiluted, 1:10, 1:100, and 1:1000 dilutions respectively. A corrected hemolytic index for the positive control was 92.90%.

Results for the blank corrected hemoysis% for the test article (undiluted) should have been theoretically below 100% as it was observed for the positive control (92.90%), but due to unusual high absorbance readings, the average was 329.46%. The high absorbance reading was caused by light scattering.

The supernatant had a transparent brownish color that became darker (more brown and opaque) when it reacted with the cyanmethemoglobin reagent causing light scattering by unknown particulates rather than hemoglobin molecules; therefore causing the blank corrected hemolysis% to appear higher than 100%.

11 INTEGRATED SUMMARY AND SAFETY EVALUATION

11.1 Introduction

Epinephrine is an endogenous sympathomimetic catecholamine and has been used as a vasopressor for over 50 years in treating hypotension associated with septic shock and in improving the systemic arterial blood pressure.

This submission is primarily based on published literature as a 505(b)(2) application and safety information from Twinject (NDA 020800, an approved drug) as the listed reference drug:

Listed Drug	How supplied/ route of administration	Indications	Dosage
Twinject (0.3 mg epinephrine/mL) Shionogi Inc distributed by Greenstone LLC (label date 1/2010 NDA 020800; Approval 5/2003	Single use auto-injector for subcutaneous or intramuscular injection	Epinephrine auto-injector is indicated in the emergency treatment of severe allergic reactions (Type I).	The dosage is 0.3 mg delivered in1 mL and is for use by patients who weigh 30 kg (approx. 65 pounds) or greater.

There are several FDA approved epinephrine drug products (1 mg/mL, 1:1000 dilution) currently being marketed as intramuscular or subcutaneous administration for the treatment of anaphylactic shock (EpiPen and Twinject), and anesthetic combination products that contain epinephrine (such as Septocaine; Octocaine; Xylocaine with epinephrine).

11.2 Pharmacology

Epinephrine causes vasoconstriction through its binding with α-receptors (α1 and α2) at high concentration and vasodilation via β-receptors (β1, β2, and β3) at low concentration. Epinephrine binds its receptors that associate with heterotrimeric G protein and initiates a chain of chemical reactions to ultimately signal a cellular response.

Administration of epinephrine USP, 1:1000 (1 mg/mL) Injection (0.05 to 2.0 µg/kg/min) is proposed by Belcher to provide hemodynamic support for increasing systemic arterial blood pressure (to achieve a desired M AP ≥ 70 mmHg) and in acute hypotensive states associated with septic shock.

11.3 Toxicology

Data from nonclinical studies (Giantomasso et al, 2005; Goodman & Gillman, 2011; Myburgh, 2006) have shown that epinephrine provides a significant improvement in restoring MAP and myocardial performance, by increasing contractility, stroke volume, and cardiac output in a dose dependent manner. However, in a canine model of septic shock the 28-day survival was found to be decreased with epinephrine infusion (Minneci et al, 2004).

Epinephrine caused significant decrease in cardiac index, ejection fraction and pH and a concomitant increase in systemic vascular resistance and serum creatinine when compared with norepinephrine and vasopressin.

The blood flow to the heart, gut, and kidney were increased at the onset of sepsis with an induced hypotension and hyperlactemia. Epinephrine infusion significantly improved the MAP (69 vs. 86 mmHg) and cardiac output (6.4 vs. 7.1 l/min) and decreased renal blood flow (330 vs. 247 ml/ min).

Epinephrine was also associated with metabolic effects, decreased mesenteric, coronary and renal conductance in a sheep model of septic shock (Levy et al 2003).

Data from hemolysis study conducted by the Sponsor have shown that epinephrine at 1:1000 dilution is non-hemolytic.

11.4 Genotoxicity

An equivocal response of epinephrine was found (NTP, 1990) when tested in *Salmonella typhimurium* strain TA100 in the absence of metabolic activation system (S-9) and negative in the presence of metabolic activation (S9) or in strains TA98, TA1535 or TA1537 with or without S9 (NTP1990).

11.5 Carcinogenicity

Carcinogenic studies conducted by the inhalation route were found negative. However, they were considered inadequate and non-GLP (NTP, 1990).

11.6 Developmental Toxicology

The reproductive and developmental toxicity studies have shown developmental effects in rabbits at a subcutaneous dose of 1.2 mg/kg (approximately 30 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis).

In mice epinephrine infusion caused developmental effects at a subcutaneous dose of 1 mg/kg (approximately 7 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis).

In hamsters, epinephrine administration caused developmental effects at a subcutaneous dose of 0.5 mg/kg (approximately 5 times the maximum recommended daily subcutaneous or intramuscular dose on a mg/m2 basis) as found in the listed approved epinephrine drug product.

Data obtained from published literature have shown that epinephrine was toxic in rats, mice and dogs at high dose levels (Table 10 & 11) and interferes with ovum implantation and fetal survival in the rabbit (Auletta, 1971).

Epinephrine is shown to have potential teratogenic activity as observed by the fetuses with gastroschisis (Auletta FJ., 1971), and abnormally absent aortic arches related to dysrhythmogenesis (Rajala GM et al, 1988) were also reported.

11.7 Conclusion

Data from nonclinical studies have shown that epinephrine affects various vascular beds (e.g. heart, kidney, skin) by increasing contractility, stroke volume, and cardiac output, and significantly improves the mean arterial pressure (MAP) and myocardial performance in a dose dependent manner.

In addition to this epinephrine has been shown to have an implantation loss, incidence of arrested fetuses and gastroschisis (teratogenic potential) in rabbits.

The proposed dosing regimen of epinephrine (0.05 to 2.0 µg/kg/min/iv) is based on previous human experience and published single and multiple dose animal studies (NTP, 1990).

The submitted NDA-205029 is approvable from a Pharmacology and Toxicology perspective.

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/s/

RAMA S DWIVEDI
07/10/2013

THOMAS PAPOIAN
07/11/2013
Concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 205029 **Applicant:** BELCHER PHARMACEUTICALS LLC **Stamp Date: 12/04/2013**

Drug Name: (b) (4) **(epinephrine)** **NDA/BLA Type:**

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?			Yes. Submission is primarily based on published literature.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?			Yes.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?			See comment # 4
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			N/A

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			None yet identified.
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? _____Yes___

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

None

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None

Rama Dwivedi — January 30, 2013
Reviewing Pharmacologist — Date

Thomas Papoian — January 30, 2013
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

THOMAS PAPOIAN
01/31/2013