



**TV-1380 (ALBUMIN-FUSED MUTATED BChE) ATTENUATES THE  
CARDIOVASCULAR AND RESPIRATORY EFFECTS INDUCED BY COCAINE  
IN CYNOMOLGUS MONKEYS**

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## ABSTRACT

Cocaine abuse and dependence are global problems with serious medical and social consequences. There is no available treatment for cocaine overdose, facilitation of cocaine abstinence, or reduction of relapse in addicts who have achieved abstinence. Human plasma butyrylcholinesterase (BChE) contributes to normal cocaine metabolism and has been considered for use in treating cocaine abusers. Enhancing cocaine hydrolysis by BChE via its mutagenesis and binding this enzyme to human serum albumin has resulted in a molecule (TV-1380) with extended catalytic activity, prolonged plasma half-life, and stability. Teva Pharmaceutical Industries Ltd is the owner of TV-1380 and this study was performed on their behalf to evaluate the cardiovascular and respiratory safety of TV-1380 alone on 6 cynomolgus monkeys and to examine the potential cardiotoxic effects of the combination of TV-1380 and cocaine on an additional 6 monkeys. Parameters evaluated included mortality, cageside and clinical observations, body weights, body weight changes, heart rate, blood pressure (systolic, diastolic, and mean arterial blood pressure), electrocardiogram waveform, body temperature, respiration rate, saturated blood oxygen, and end-tidal carbon dioxide. Treatment with TV-1380 alone when administered by intramuscular injection had no adverse effect on body temperature, respiratory parameters, or cardiovascular parameters mentioned above. The intravenous administration of cocaine at 1 mg/kg resulted in expected increases in body temperature and in cardiovascular parameters. However, pre-treatment with TV-1380 three hours prior to administration of cocaine attenuated the maximum effect and maximum change in these endpoints induced by cocaine; the most notable effects were on body temperature, heart rate, and rate-pressure product. In many cases the return to baseline occurred more quickly when animals received TV-1380 prior to administration of cocaine than when cocaine was administered alone.

## INTRODUCTION

Cocaine abuse and dependence in the US are problems with devastating medical and social consequences (Schrank, 1992; Marzuk et al., 1995), but there is no reliable means to treat cocaine overdose or reduce the likelihood of relapse in users who have achieved abstinence. Treatment of cocaine addiction, therefore, is complex and must address a variety of problems such as the psychobiological, social and pharmacological aspects of the patient's drug abuse.

Human plasma butyrylcholinesterase (BChE) is known to contribute to natural cocaine metabolism (Inaba et al., 1978) and has been considered for use in treating cocaine addiction (Gorelick, 1997). Recent advances have enhanced the catalytic efficiency of the enzyme making it a potential method to blunt cocaine toxicity and to reduce or eliminate the central effects associated with cocaine-seeking behavior. Fusion of the mutant BChE enzyme with human serum albumin resulted in a recombinant protein with favorable pharmacokinetics, high catalytic efficiency, and an extended plasma half-life similar to that of other fusion proteins (Duttaroy et al., 2005). In rats, the recombinant human serum albumin-mutant BChE fusion protein prevented seizures when a normally lethal cocaine injection was given, lowered brain cocaine levels even when administered after the drug, and provided rescue after convulsions commenced. It also selectively blocked cocaine-induced reinstatement of drug seeking in rats that had previously self-administered cocaine (Brimijoin et al., 2008).

Teva Pharmaceutical Industries Ltd is the owner of the recombinant human serum albumin-mutant BChE fusion protein, TV-1380, and this study was performed on their behalf to evaluate the cardiovascular and respiratory safety of TV-1380 alone on 3 male and 3 female naïve cynomolgus monkeys and to examine the potential cardiotoxic effects of the combination of TV-1380 and cocaine on an additional 3 male and 3 female naïve monkeys.

## MATERIALS AND METHODS

### *Test Animals and Husbandry*

**Table 1. Test Animal Details**

Sex	Males	Females
<b>Species and Strain</b>	Cynomolgus monkeys of Chinese origin	
<b>Supplier</b>	AVANZA Stock Colony (original supplier: Harlan)	
<b>Method of Identification</b>	Chest tattoo	
<b>Number of Animals Transferred</b>	7	7
<b>Number Used on Study</b>	6	6
<b>Age at First Dose</b>	3-4 years old	
<b>Weight Range at First Dose</b>	3.0 to 3.6 kg	2.3 to 3.2 kg
<b>Disposition of Extra Animals</b>	All animals were transferred back to the AVANZA stock colony.	

**Table 2. Animal Husbandry Details**

<b>Feed</b>	Certified Global Harlan Teklad Laboratory Diet 2055
<b>Water</b>	Automatic watering system
<b>Housing</b>	Individually housed in stainless steel cages suspended on stainless steel racks. Each cage was affixed with a cage tag containing pertinent animal and study information.
<b>Temperature Range</b>	18 to 29°C
<b>Humidity Range</b>	30 to 70%
<b>Light Cycle</b>	12-hour light/12-hour dark, interrupted as necessary for study-related events
<b>Air Changes</b>	Minimum of 10 air changes per hour

### *Implantation of Telemetry Units and Vascular Access Ports*

A sterile telemetry unit and a vascular access port (VAP) were implanted into each animal. A gel-filled pressure transducer (for blood pressure measurement) was inserted into the left femoral artery and secured. Two electrocardiogram (ECG) leads (one positive, one negative) were tunneled through the subcutaneous tissue to

the left lateral thoracic region between the last 2 ribs (positive lead) and on to the right cranial thoracic region (negative lead). The VAP was implanted by making a small slit in the femoral vein, and one end of a flushed cannula was inserted and anchored to the femoral vessel with non-absorbable suture. The other end of the cannula was tunneled through the subcutaneous tissue and externalized through a skin incision between the last rib and the pelvic bone, where it was connected to the VAP. The assembled VAP was placed in a subcutaneous or intramuscular pocket, anchored in place with a non-absorbable suture, flushed, and locked with heparinized saline.

### ***Recovery, Jacket Acclimation, and Signal Verification***

Animals underwent at least a 13-day recovery period prior to initiation of dosing. During this time, antibiotics and analgesics were administered as necessary and animal behavior and surgical incisions were observed daily. ECG and blood pressure signals were verified at the completion of the telemetry implantation and daily during the post-operative recovery period with approximate 2-minute recordings daily. Additionally, during the week prior to dosing, the signal was verified for an extended period of time ( $4 \pm 0.25$  hours) to ensure signal stability.

The VAP was checked and flushed at the completion of each implantation on the day of surgery, daily for the first 3 days post implantation, and at least once per week thereafter until the first dosing day.

Each animal was initially fitted with the infusion jacket system used for cocaine/vehicle infusion and acclimated to the jacket for approximately 30 minutes at a time during the week prior to the first dose.

### ***Neat Test and Control Articles***

**Table 3. Neat Test and Control Articles Details**

<b>Name</b>	<b>Purity</b>
TV-1380	98.7%
Formulation Buffer (PMTT, pH 7.2)	Not Provided / Assumed 100%
Sterile Water for Injection, USP (SWFI)	Not Provided / Assumed 100%
Cocaine HCl	100%
0.9% Sodium Chloride for Injection, USP (SCFI)	Not Provided / Assumed 100%

## Experimental Design

**Table 4. Study Groups and Treatment**

Study Group	Study Day	Treatment	Test/Interaction Article Dose Level (mg/kg)	Test/Interaction Article Dose Concentration (mg/mL)	Type of Data Collection
1	1	Formulation Buffer	0	0	Cardiovascular
	4	Formulation Buffer	0	0	Respiratory
	8	TV-1380	15	30	Cardiovascular
	11	TV-1380	15	30	Respiratory
2	15	Formulation Buffer	0	0	Cardiovascular
		Saline	0	0	
	18	Formulation Buffer	0	0	Cardiovascular
		Cocaine	1	5	
	22	TV-1380	15	30	Cardiovascular
		Cocaine	1	5	

The maximum dose volume of TV-1380 formulation or formulation buffer administered was 0.5 mL/kg. Each dose was administered to a separate site, alternating between the left and right hind limb; the total volume administered to each site was less than or equal to 0.25 mL/kg/site. Each separate dose was administered by intramuscular (IM) injection over  $30 \pm 15$  seconds using a sterile needle and syringe.

The infusion line with attached needle was preloaded with the appropriate volume of cocaine or interaction article vehicle followed by a separator air bubble that filled the rest of the infusion line (total volume 0.85 mL). The line was attached to an extension line preloaded with saline which was attached to an infusion pump with the medication cassette containing saline. After programming the pump start and stop times, the pump was placed in the pocket of the infusion jacket.

The interaction article (cocaine or its vehicle) was administered  $3 \pm 0.25$  hours after test article or formulation buffer administration. The interaction article was administered via intravenous (IV) infusion at a dose volume of 0.2 mL/kg

(using the pump via an implanted vascular access port) for approximately 30 seconds followed by an approximately 90-second flush (infusion) of the separator air bubble and saline. The total duration of interaction article dosing was  $120 \pm 10$  seconds.

**Table 5. Animal Observations and Measurements**

Procedure	Frequency of Testing
Cageside Observations	$\geq 2$ Daily
Physical Examinations	Prior to test article or formulation buffer dosing on each day.
Body Weight	Prior to test article or formulation buffer dosing on each day.

***Cardiovascular Profiling and Body Temperature***

Parameters: Heart rate, blood pressure (systolic, diastolic and mean arterial blood pressure), ECG waveform and body temperature were recorded on Study Days (SD) 1, 8, 15, 18 and 22 with DSI (Data Science International, St. Paul, MN) Telemetry System DataQuest A.R.T. 3.1, and collected continuously for  $1 \text{ hour} \pm 10 \text{ minutes}$  prior to IM dosing, continuously from the time of IM dose completion through  $9 \pm 0.25$  hours postdose and again from  $22-24 \pm 0.5$  hours following the IM dose. The rate-pressure product was calculated (heart rate multiplied by systolic pressure).

Data Collection: The heart rate, blood pressure (systolic, diastolic and mean arterial blood pressures) and body temperature data were sampled as presented in Table 6.

**Table 6. Telemetry Sampling Timepoints**

Animals	Timepoints
<b>Group 1</b>	45 ± 5 minutes and 30 ± 1 minutes prior to IM dosing of TV-1380 or formulation buffer
	15 ± 2 minutes, 30 ± 5 minutes, 1 ± 0.25 hours, 2 ± 0.25 hours, and 24 ± 0.5 hours after IM dosing of TV-1380 or formulation buffer
<b>Group 2</b>	45 ± 5 minutes and 30 ± 1 minutes prior to IM dosing of TV-1380 or formulation buffer
	At 15 ± 2 min intervals after IM dosing and 5 ± 1 minutes prior to IV infusion of cocaine or saline (3 ± 0.25 hours after IM injection)
	At 1-minute intervals for the first 30 minutes after IV infusion of cocaine or saline is complete, at 15-minute intervals for the next 5.5 hours, and at 15-minute intervals from 22-24 ± 0.5 hours after IM injection of TV-1380 or formulation buffer

The maximum hemodynamic change was determined as the greatest absolute value change (the greatest increase or the greatest decrease from the baseline) within the defined timepoint. The time of maximum hemodynamic effect was determined as the 1-minute interval in which the parameters reached their maximum change. The maximum hemodynamic effect is the value for that time interval.

Representative ECG waveforms (approximately 10- and/or 30-second duration) were collected from telemetry recordings as shown in Table 7. The RR-interval was calculated and reported from the measured heart rate. QTc was calculated based on QT interval and measured heart rate (Spence et al. 1998).

**Table 7: ECG Sampling Timepoints**

<b>Animals</b>	<b>Timepoints</b>
<b>Group 1</b>	45 ± 5 minutes and 30 ± 1 minutes prior to IM dosing of TV-1380 or formulation buffer
	15 ± 2 minutes, 30 ± 5 minutes, 1 ± 0.25 hours, 2 ± 0.25 hours, and 24 ± 0.5 hours after IM dosing of TV-1380 or formulation buffer
<b>Group 2</b>	45 ± 5 minutes and 30 ± 1 minutes prior to IM dosing of TV-1380 or formulation buffer
	15 ± 2 minutes, 30 ± 5 minutes, 1 ± 0.25 hours, and 2 ± 0.25 hours after IM dosing of TV-1380 or formulation buffer
	At the peak time of maximal hemodynamic effect during the 5 minutes after IV infusion of cocaine
	30 ± 5 minutes, 2 ± 0.25 hours, 5 ± 0.25 hours and 21 ± 0.5 hours after completion of IV infusion of cocaine or saline

### ***Respiratory Profile***

On SD 4 and 11, respiration rate, saturated blood oxygen (SpO<sub>2</sub>) and end-tidal CO<sub>2</sub> (ETCO<sub>2</sub>) was collected prior to IM dosing of TV-1380 or formulation buffer and at 15 ± 2 and 30 ± 5 minutes, 1 ± 0.25, 2 ± 0.25 and 24 ± 0.5 hours after IM dosing of formulation buffer or TV-1380. Data was collected with Surgivet V9004 Capnograph (Waukesha, WI). Respiratory rates were manually collected.

### ***Termination***

All animals were removed from study one week following the last dose.

### ***Statistical Analyses***

Descriptive statistics (mean, standard deviations, and N) were calculated for all measurement data.

## RESULTS

### *Animal Disposition and Clinical Observations*

Treatment with TV-1380 alone at 15 mg/kg had no effect on mortality, clinical, or cageside observations. Observations noted were of no toxicological importance and were not attributed to TV-1380 administration.

### *Body Weights and Body Weight Changes*

Treatment with TV-1380 alone at 15mg/kg had no effect on body weights or body weight changes. All animals either remained the same or gained weight throughout the study.

### *Respiratory Profile*

Treatment with TV-1380 alone at 15mg/kg had no effect on respiration rate, SpO<sub>2</sub>, or ETCO<sub>2</sub>. These parameters remained within normal physiological ranges for cynomolgus monkeys following administration of either the formulation buffer or TV-1380 at a dose of 15 mg/kg.

### *Cardiovascular Profiling and Body Temperature*

The mean maximum effect and mean maximum change (the greatest increase or the greatest decrease from the baseline) in each parameter at each treatment are presented in Table 8 and 9, respectively.

The administration of cocaine at 1 mg/kg resulted in an increase in body temperature of approximately 1°C, and in all cardiovascular parameters; the heart rate and rate-pressure product increased by 100 bpm and 16,260 bpm\*mmHg, respectively. However, pre-treatment with TV-1380 at 15 mg/kg three hours prior to administration of cocaine attenuated the maximum effect and change in these endpoints with the most notable effects being on body temperature (decrease of approximately 1°C), heart rate (decrease of approximately 55 bpm), and rate-pressure product (decrease of approximately 5200 bpm\*mmHg). An examination of individual figures indicates that the heart rate, systolic pressure, diastolic pressure, mean arterial pressure and the rate-pressure product returned to baseline

by approximately 30, 18, 16, 18, and 32 minutes, respectively after cocaine administration when animals received TV-1380 as compared to administration of cocaine alone after which the respective return to baseline occurred at approximately 120, 90, 80, 80, and 120 minutes (See Figures 1-6).

### *Electrocardiogram Evaluation*

No biologically important ECG findings were noted when 15 mg/kg of TV-1380 or 1 mg/kg of cocaine were administered alone to cynomolgus monkeys. However, the combination of TV-1380 (15 mg/kg) and cocaine (1 mg/kg) caused QTc shortening and QTc prolongation. The normal range for monkeys is 0.16 to 0.28 seconds, and in some animals the QTc interval was as low as 0.12 to 0.14 seconds and as high as 0.30 to 0.46 seconds. The significance of these QTc findings is not known. QT and QTc prolongation are observed in normal telemetered animals. No atrial or ventricular arrhythmias or abnormal T-wave changes were observed in any animal in this study. Therefore, the absence of any cardiac arrhythmias suggests that the combination of TV-1380 and cocaine is not biologically or toxicologically significant.

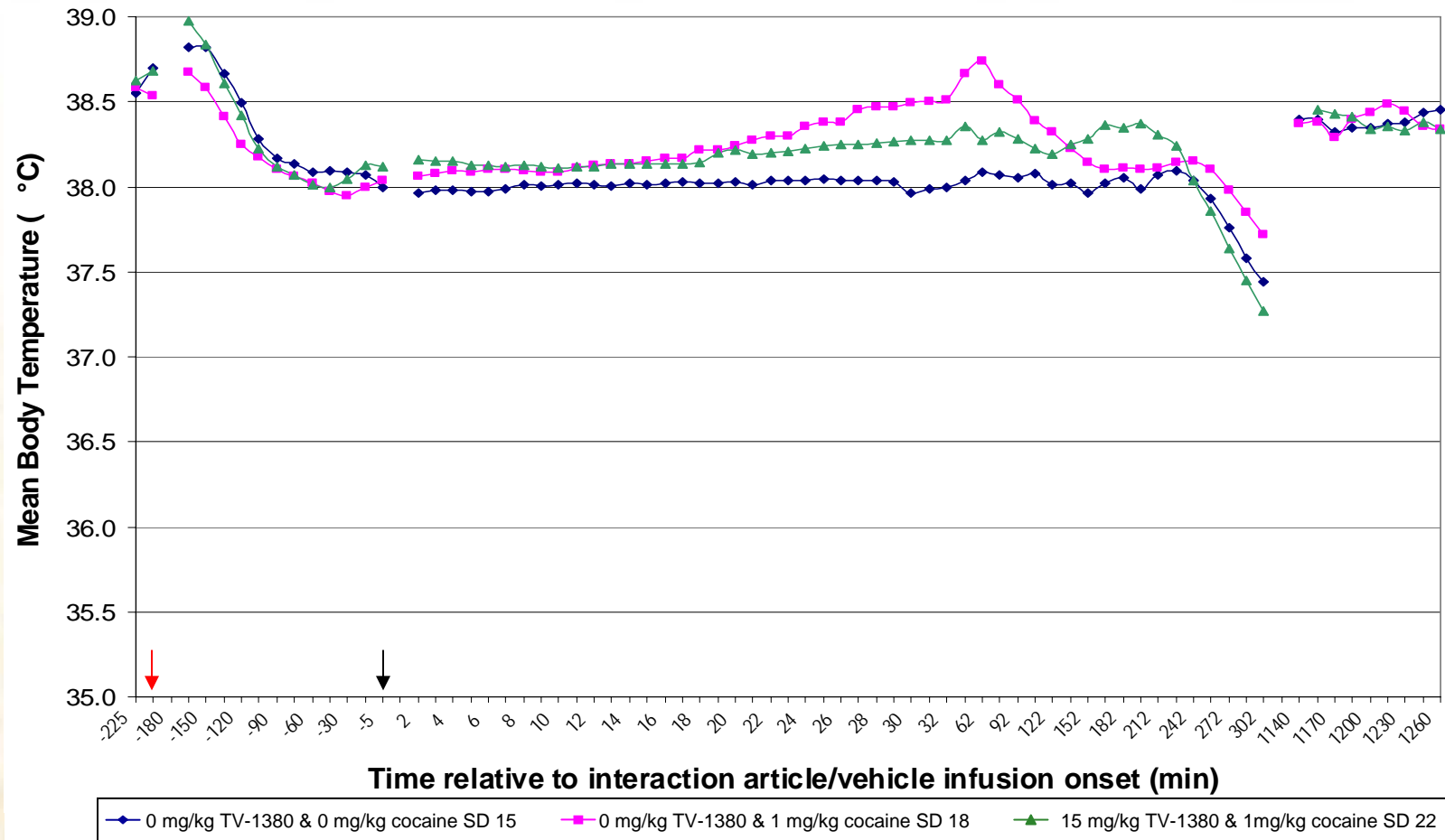
**Table 8: Averaged Individual Maximum Effects  $\pm$  SD on Body Temperature Following Cessation of Interaction Article Infusion and Averaged Individual Cardiovascular Parameter Maximum Effects  $\pm$  SD During the Five Minutes Immediately Following Cessation of Interaction Article Infusion**

Dose Number/ Study Day	Dose Level TV-1380/ Cocaine (mg/kg)	Body Temperature (°C)			Heart Rate (bpm)			Systolic Blood Pressure (mmHg)			Diastolic Blood Pressure (mmHg)			Mean Arterial Pressure (mmHg)			Rate-Pressure Product (bpm*mmHg)		
			$\pm$	SD		$\pm$	SD		$\pm$	SD		$\pm$	SD		$\pm$	SD		$\pm$	SD
5/15	0/0	37.9	$\pm$	0.5	138	$\pm$	31	120	$\pm$	15	82	$\pm$	14	100	$\pm$	15	18190	$\pm$	4184
6/18	0/1	38.8	$\pm$	0.2	237	$\pm$	11	168	$\pm$	19	119	$\pm$	11	143	$\pm$	13	35799	$\pm$	2673
7/22	15/1	37.8	$\pm$	0.7	185	$\pm$	29	156	$\pm$	18	108	$\pm$	10	131	$\pm$	13	27563	$\pm$	5710

**Table 9: Averaged Individual Body Temperature Maximum Changes  $\pm$  SD Following Cessation of Interaction Article Infusion and Averaged Individual Cardiovascular Parameter Maximum Changes  $\pm$  SD During the Five Minutes Immediately Following Cessation of Interaction Article Infusion**

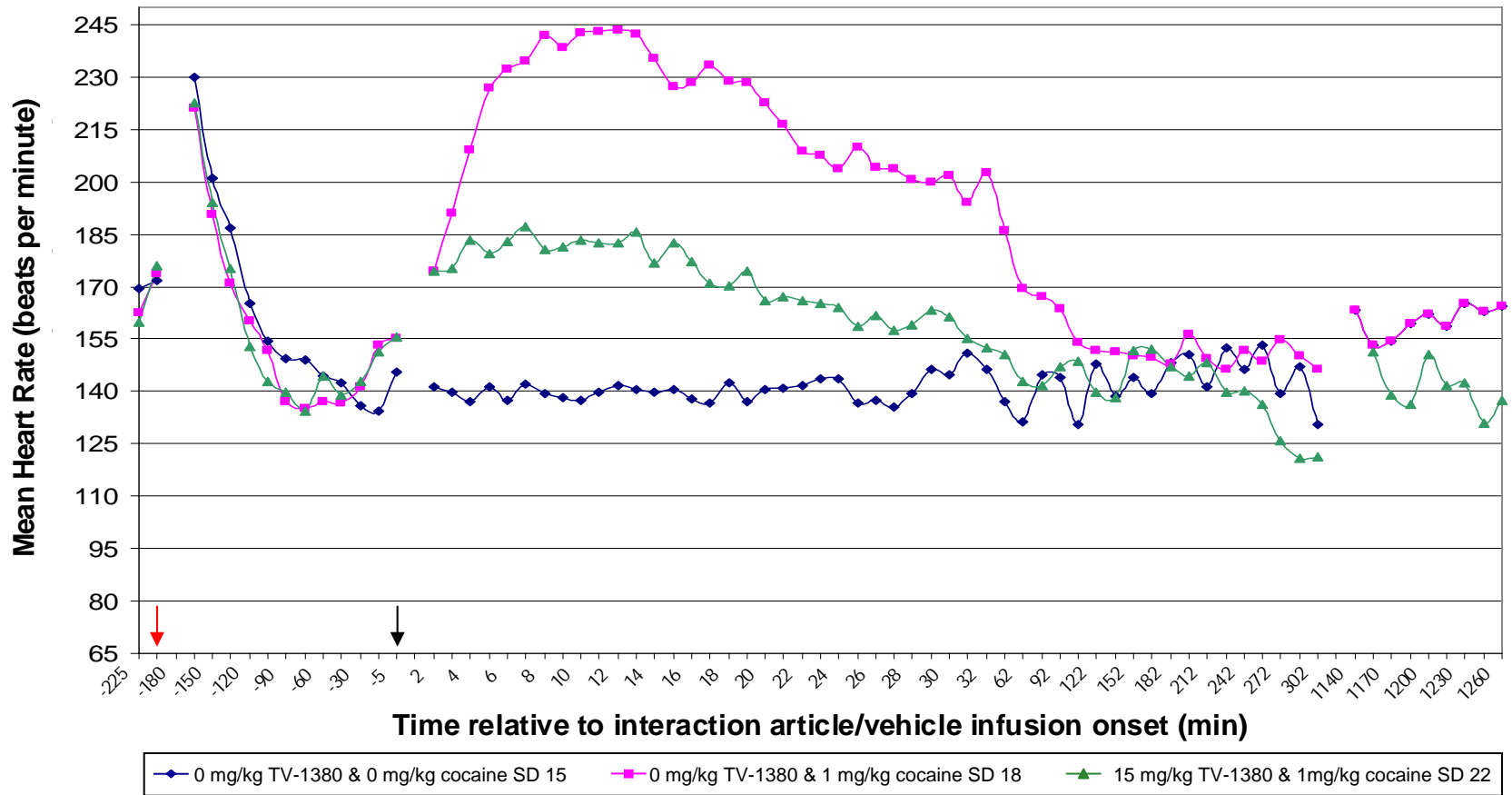
Dose Number/ Study Day	Dose Level TV-1380/ Cocaine (mg/kg)	Body Temperature (°C)			Heart Rate (bpm)			Systolic Blood Pressure (mmHg)			Diastolic Blood Pressure (mmHg)			Mean Arterial Pressure (mmHg)			Rate-Pressure Product (bpm*mmHg)		
			$\pm$	SD		$\pm$	SD		$\pm$	SD		$\pm$	SD		$\pm$	SD		$\pm$	SD
5/15	0/0	-0.2	$\pm$	0.4	-5	$\pm$	23	-1	$\pm$	10	-3	$\pm$	9	-3	$\pm$	10	5718	$\pm$	3057
6/18	0/1	0.8	$\pm$	0.3	80	$\pm$	19	40	$\pm$	14	31	$\pm$	8	35	$\pm$	10	21976	$\pm$	2025
7/22	15/1	-0.2	$\pm$	0.7	35	$\pm$	35	32	$\pm$	12	23	$\pm$	10	27	$\pm$	10	16767	$\pm$	5869

**Figure 1: Study Group 2 Males and Females Pooled Mean Body Temperature (°C)**



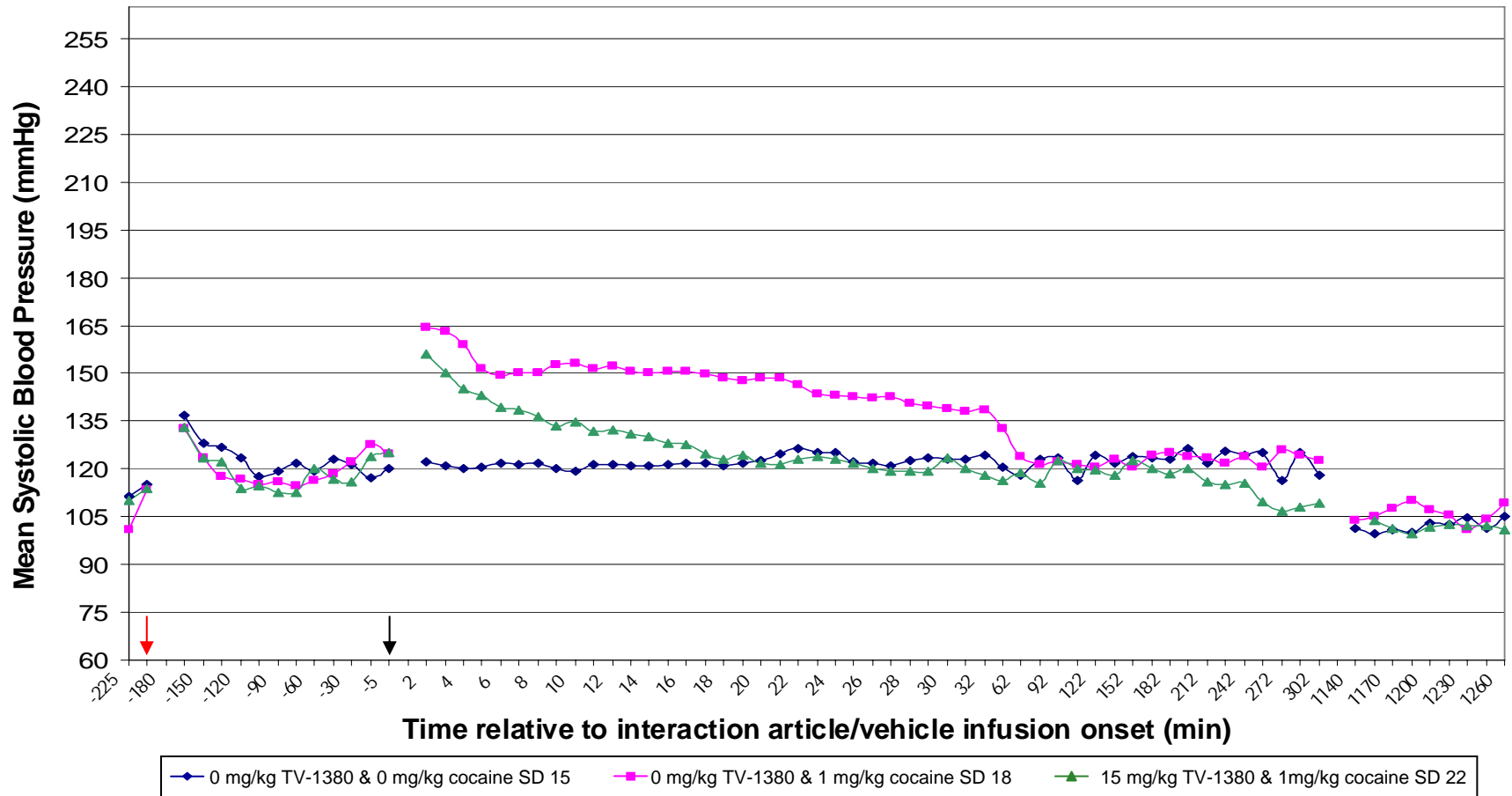
**Note:** The red arrow represents the time of injection of TV-1380 or formulation buffer.  
The black arrow represents the start of saline or cocaine infusion.

**Figure 2: Study Group 2 Males and Females Pooled Mean Heart Rate (beats per minute)**

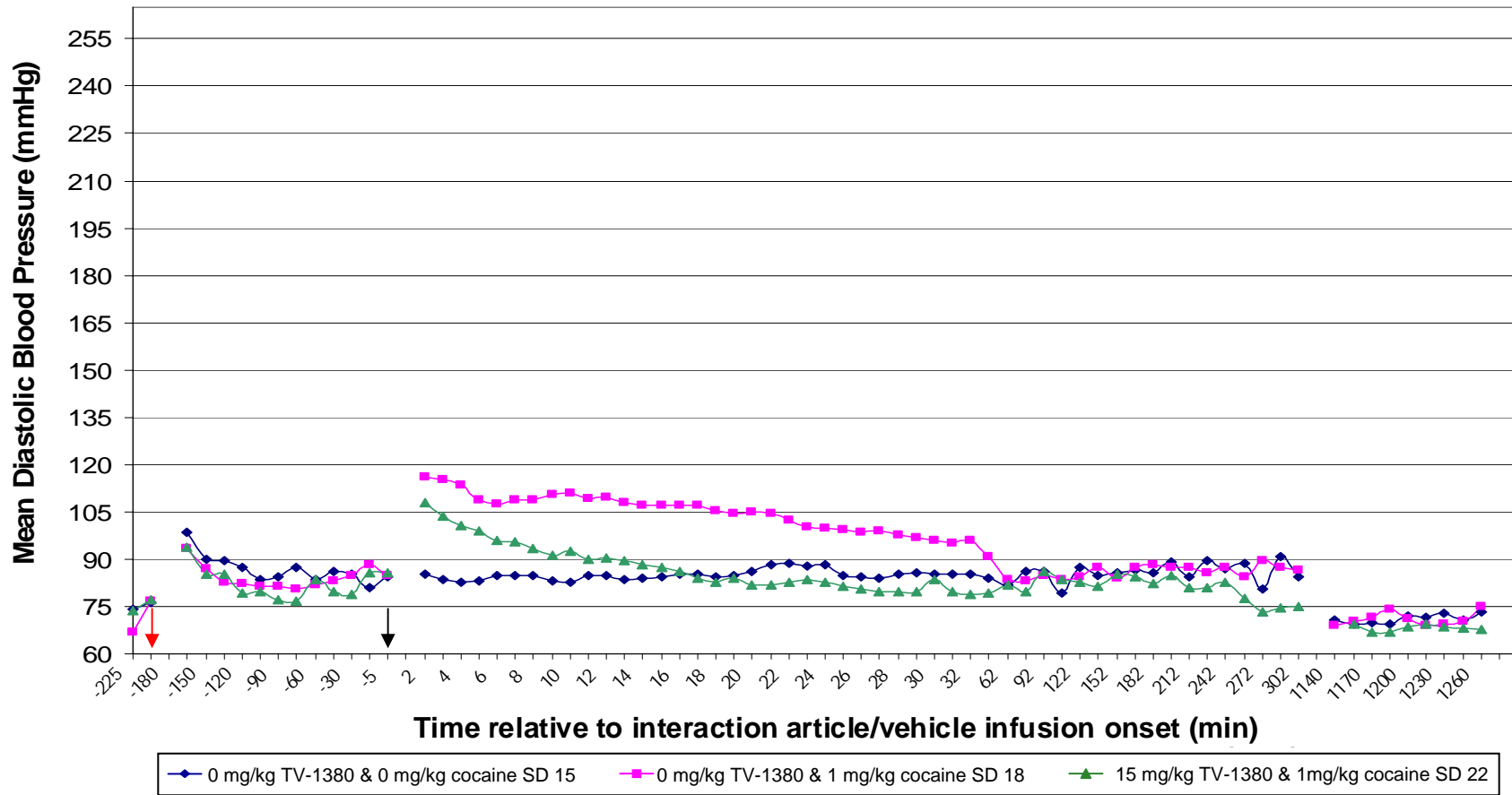


**Note:** The red arrow represents the time of injection of TV-1380 or formulation buffer.  
The black arrow represents the start of saline or cocaine infusion.

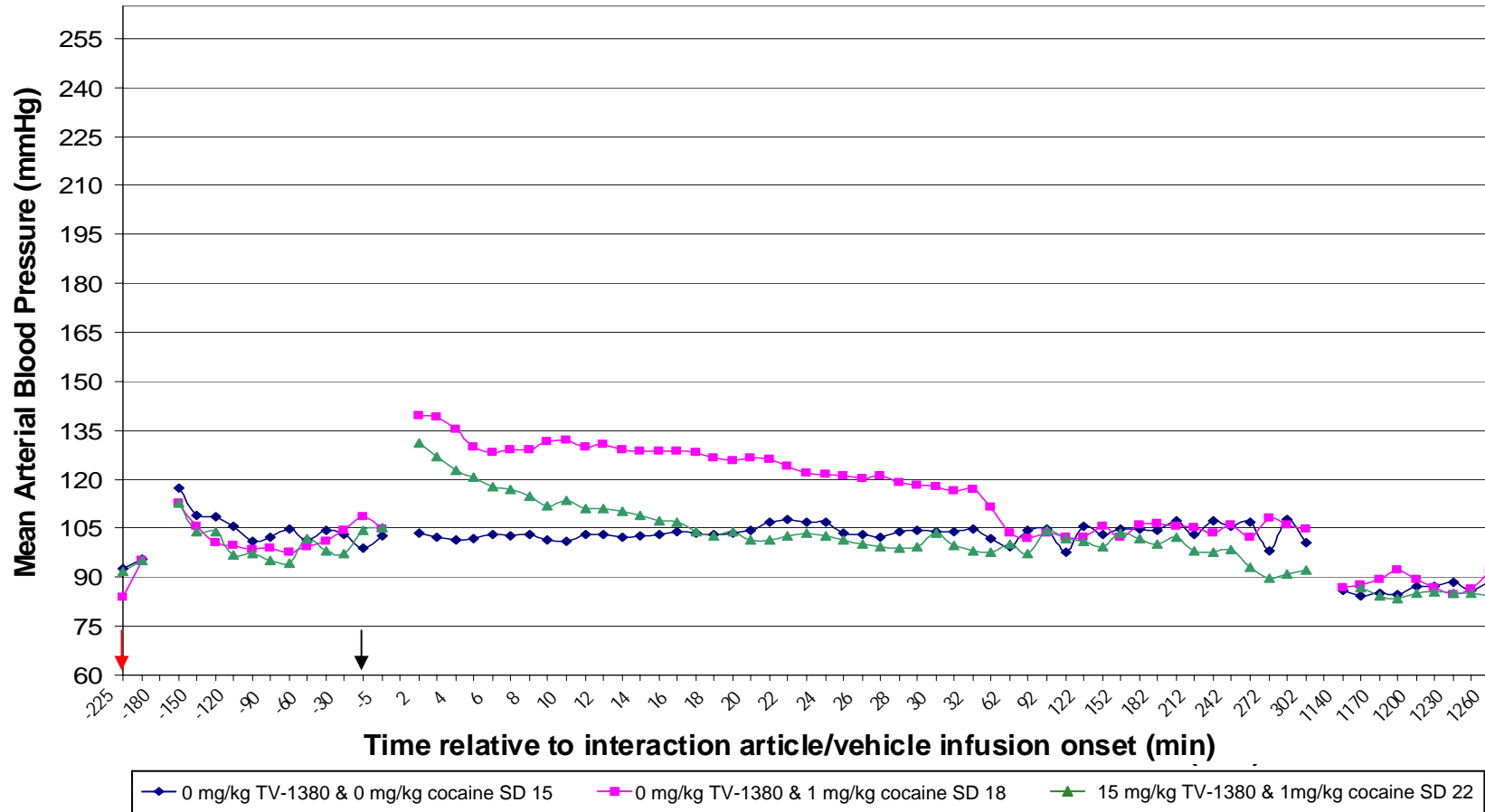
**Figure 3: Study Group 2 Males and Females Pooled Mean Systolic Blood Pressure (mmHg)**



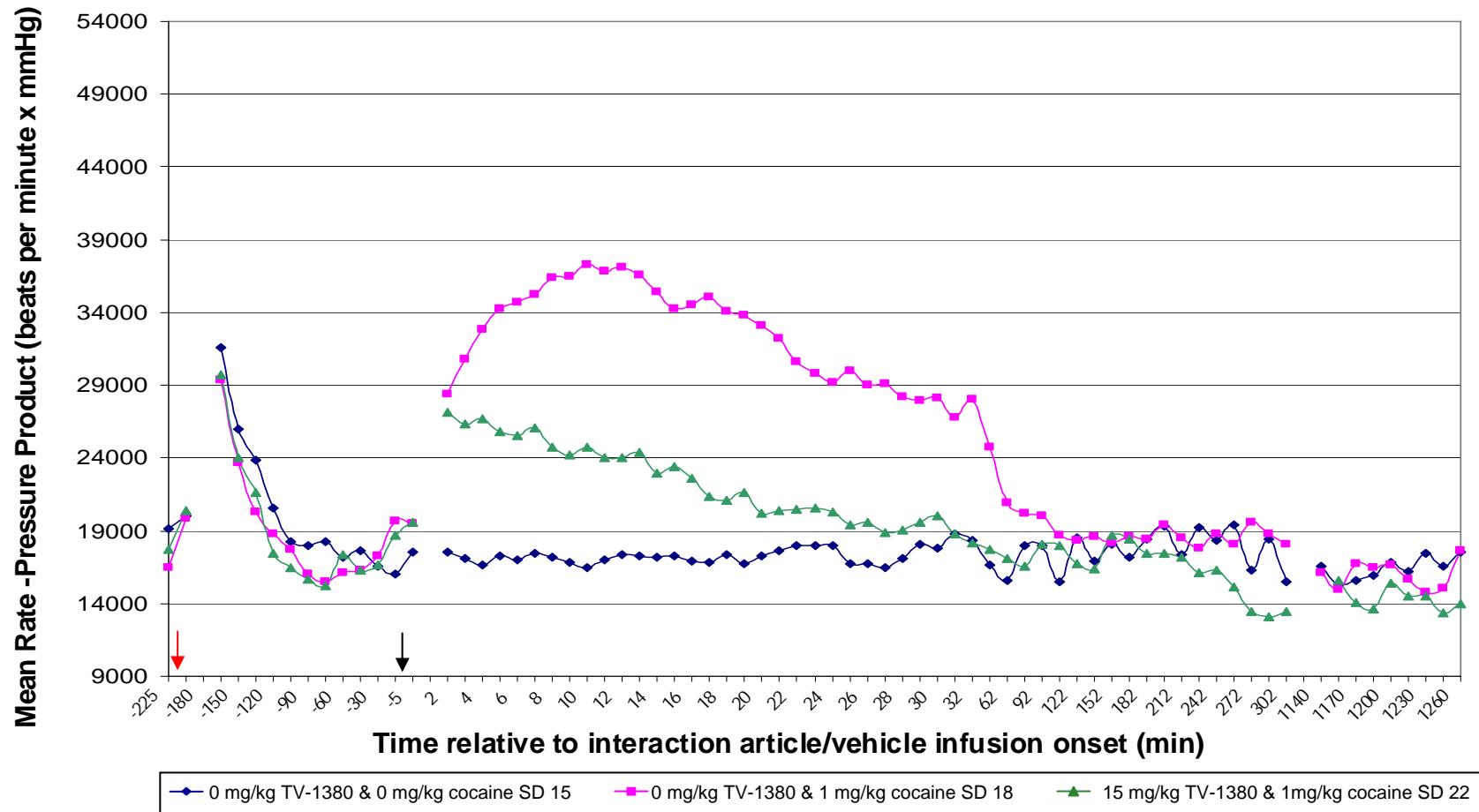
**Figure 4: Study Group 2 Males and Females Pooled Mean Diastolic Blood Pressure (mmHg)**



**Figure 5: Study Group 2 Males and Females Pooled Mean Arterial Blood Pressure (mmHg)**



**Figure 6: Study Group 2 Males and Females Pooled Mean Rate Pressure Product (beats per minute x mmHg)**



**Note:** The red arrow represents the time of injection of TV-1380 or formulation buffer.  
The black arrow represents the start of saline or cocaine infusion.

## CONCLUSIONS

Treatment with TV-1380 alone at 15 mg/kg by the IM route of administration had no adverse effect on mortality, clinical, or cageside observations. Treatment with TV-1380 alone at 15 mg/kg had no effect on the respiratory parameters (respiration rate, saturated blood oxygen, end tidal carbon dioxide), or on the cardiovascular parameters such as heart rate, blood pressure (systolic, diastolic, and mean arterial blood pressure) or on body temperature.

The administration of cocaine alone at 1 mg/kg by the IV route resulted in an increase in body temperature and in cardiovascular parameters after dosing. However, pre-treatment with TV-1380 at 15 mg/kg, three hours before cocaine administration, attenuated the maximum effect and change in these endpoints with the most notable effects being on body temperature, heart rate and rate-pressure product. In many cases the time to reversal and return to baseline parameters of the effect was reduced by at least 4-fold for all cardiovascular parameters when animals received TV-1380 at 15 mg/kg by IM administration three hours prior to cocaine administration at 1mg/kg by IV route as compared to administration of cocaine alone.

## ACKNOWLEDGMENT

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