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TV-1380 attenuates cocaine-induced changes in cardiodynamic parameters in monkeys and reduces the formation of cocaethylene

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ABSTRACT

Background: TV-1380 is a rationally mutated, human BChE fused to human serum albumin that has high hydrolytic enzymatic activity against cocaine and as well as an extended elimination half-life.

Objective: The present studies examined the safety of TV-1380 and its protective effect when given to monkeys alone or concomitantly with cocaine and ethanol.

Methods: A set of studies was conducted in monkeys with TV-1380. The parameters tested included telemetric assessment of cardiovascular parameters, clinical pathology, plasma analysis of cardiac troponin I, ex-vivo analyses of cocaethylene and PK analysis of serum concentrations of TV-1380, cocaine and its metabolites, and histopathological examinations.

Results: TV-1380 treatment in monkeys was well tolerated. TV-1380 pretreatment prior to cocaine significantly attenuated the cardiac effects of cocaine and reduced cocaine-induced elevations in serum cardiac troponin I. TV-1380 changed the metabolic fate of cocaine resulting in decreased exposure to benzoylecgonine, while increasing the exposure to ecgonine methyl ester in plasma. TV-1380 reduced the plasma levels of the toxic metabolite cocaethylene formed after co-administration of ethanol and cocaine.

Conclusion: The results of this study demonstrate that TV-1380 not only accelerates the elimination of cocaine, but also protects the treated animal from the cardiac effects of cocaine, and inhibits the formation of the toxic cocaethylene metabolite when cocaine is given together with ethanol, supporting further clinical development of modified BChE products as possible treatments for cocaine abuse.

1. Introduction

Cocaine use impacted the lives of 18 million people in 2014, as reported by the United Nations Office on Drugs and Crime in 2016 (UNODC, 2016). In addition to the known hazards of cocaine use and abuse, cocaine can cause cardiovascular pathologies, including myocardial infarction, heart failure, arrhythmias, aortic dissection, and endocarditis (Rezkalla and Kloner, 2007; Stankowski et al., 2015).

Presently, there are no approved pharmaceuticals to treat cocaine addiction (Shorter et al., 2015). A potential approach to treatment of cocaine addiction is the administration of rational, mutagenic, hydrolytic enzymes that can accelerate cocaine metabolism (Sun et al., 2002). Butyrylcholinesterase (BChE) is the main endogenous enzyme that hydrolyses cocaine to its inactive metabolites, ecgonine methyl ester and benzoic acid in plasma (Xie et al., 1999; Inaba et al., 1978). Carboxylesterase-1 (CE1) is active in the liver and hydrolyzes cocaine to produce benzoylecgonine and methanol. Oxidative enzymes in the liver can also metabolize cocaine to produce norcocaine, which can be

further metabolized by BChE hydrolysis to norecgonine methyl ester (Fig. 1). Cocaine elimination can be enhanced by using exogenously added BChE. This treatment is well tolerated (Morishima et al., 1999). However, because its half-life is short and it has low catalytic activity on cocaine, endogenous BChE was not developed as a treatment for cocaine addiction. TV-1380 (AlbuBChE/Albu-Coch), was developed as a quadruple mutant form of human BChE (A199S/S287G/A328W/Y332G) with approximately 1000x fold higher hydrolytic activity (Pan et al., 2005) against cocaine than BChE, and a longer half-life due to its fusion at its carboxy-terminus with recombinant human serum albumin (HAS; Schlindler et al., 2013; Gao et al., 2008).

Clinical trials for TV-1380 as a treatment for cocaine dependence and overdose were initiated when pretreatment with TV-1380 was found to accelerate cocaine metabolism in monkeys (Schlindler et al., 2013), attenuate the toxic effects of cocaine, prevent convulsions, and prevent priming-induced relapse to cocaine use in rats (Brimjoin et al., 2008). In 2016, the clinical development of TV-1380 was halted after a phase II clinical trial did not result in an enhanced rate of abstinence

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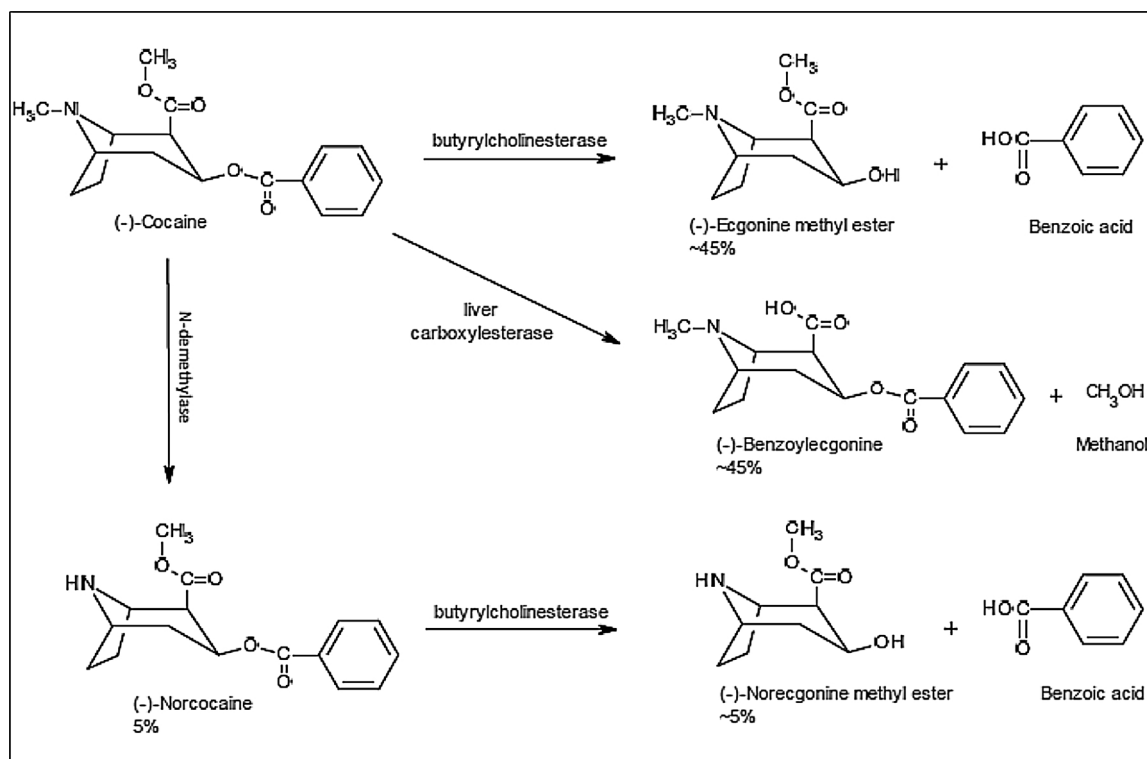


Fig. 1. Metabolic pathways for cocaine elimination in humans. With permission from Xie et al. (1999). *Molecular Pharmacology* 55, 83–91.

among the treated users (Gilgun-Sherki et al., 2016).

Part of the development program of TV-1380 involved assessing its safety in monkeys, and in the course of these studies, it found that TV-1380 may have benefits as a treatment to reduce the cardiotoxic effects that can occur with cocaine abuse, in particular when it is consumed in combination with alcohol.

Thus, the studies described herein are investigations of the potential of TV-1380 to reduce cocaine-induced changes of cardiodynamic parameters. First, we measured cardiac safety parameters in treated cynomolgus monkeys and the blood concentrations of cocaine and its metabolites in the presence or absence of TV-1380. Secondly, we investigated the interaction with ethanol, since a vast majority of cocaine users consume cocaine in combination with alcohol (European Monitoring Centre for Drugs and Drug Addiction, 2009) because of a more intense feeling of 'high' beyond that perceived with either drug alone (Pennings et al., 2002). Alcohol is known to react with cocaine to produce cocaethylene, an active cytotoxic metabolite (Julien et al., 2011) that is implicated in the cardiotoxicity of cocaine (Wilson et al., 2001; McCance-Katz et al., 1998). Cocaethylene has a similar psychoactive effect as cocaine, but is associated with liver damage, seizures and the risk of immediate death is known to be 18–25 times higher than with cocaine alone (Andrews, 1997). To address this complication, we have tested the effect of TV-1380 on the generation of cocaethylene in monkeys treated with cocaine and ethanol.

2. Materials and methods

2.1. Animal husbandry

The animal studies complied with Good Laboratory Practice (GLP) and were carried out at AVANZA (Gaithersburg, MD, USA) and MPI Research, Inc. (Mattawan, MI, USA) laboratories in cynomolgus monkeys (*Macaca fascicularis*), originally supplied by Harlan or Alpha Genesis at the age range of 3–8 years old. The animals had unrestricted access to water and were fed a daily amount of diet (Lab Diet) supplemented with fresh fruits, vegetables, and other enrichment foods. All

animals were housed in a humidity- and temperature-controlled room. The animal care facilities were fully accredited by AAALAC International and all experiments were approved by the Institutional Animal Care and Use Committee (IACUC). Male monkeys were housed individually, and female monkeys were pair-housed in stainless steel double-sized cages.

2.2. Study designs

2.2.1. Subchronic toxicology study in monkeys

Twenty male and twenty female sexually mature cynomolgus monkeys were divided into four groups, a control group ($n = 6/\text{gender}$) that received the vehicle (formulation buffer) and three groups that received TV-1380; 10 mg/kg ($n = 4/\text{gender}$), 20 mg/kg ($n = 4/\text{gender}$) and 50 mg/kg ($n = 6/\text{gender}$). Animals were administered the formulations twice weekly for 13 weeks (26 doses) via two bolus intramuscular (IM) injections. In the high dose and control groups, 2 animals per sex were maintained for an off treatment/recovery period of 4 weeks. All animals were observed for morbidity, mortality, clinical signs, clinical pathology evaluation, body weights, food and water consumption. At termination, necropsy examinations were performed.

2.2.2. Cardiovascular and respiratory safety study

Six male and six female animals with implanted telemeterized units were assigned to 4 treatment groups. Group 1 ($n = 3/\text{gender}$) animals were injected twice with the vehicle (formulation buffer), once on Day –8 to measure baseline cardiovascular and ECG parameters, and once on Day –4 to measure baseline respiration parameters. On day 1 of the study, TV-1380 was administered IM at a dose level of 15 mg/kg and cardiovascular and ECG parameters were assessed. Treatment with TV-1380 was repeated on Day 4 and respiration endpoints were followed (Table 1). The second phase of the study started after Group 1 was completed. Group 2 ($n = 3/\text{gender}$) monkeys were used to test the interaction of TV-1380 and cocaine. Baseline of the cardiovascular parameters was established at Day –4. On Day 1, animals were treated with vehicle and with cocaine alone at 1 mg/kg by IV injection. On Day

Table 1
Cardiovascular and respiratory safety study design.

Group Number	Number Animals	Study day	Treatment	Dose Level (mg/kg)	Type of Data Collection
1	3 per gender	–8	Vehicle	0	Cardiovascular
		–4	Vehicle	0	Respiratory
		1	TV-1380	15	Cardiovascular
		4	TV-1380	15	Respiratory
2	3 per gender	–4	Vehicle	0	Cardiovascular
			Saline	0	
		1	Vehicle	0	Cardiovascular
			Cocaine	1	
		4	TV-1380	15	Cardiovascular
			Cocaine	1	

4, the monkeys were first dosed with TV-1380 IM 15 mg/kg, followed three hours later by IV cocaine 1 mg/kg administration. Cardiovascular endpoints were measured after each treatment.

2.2.3. Cocaine metabolism study

A total of 11 naïve, adult male cynomolgus monkeys were divided into four dose groups: A control group (n = 2) that only received a single IV dose of cocaine (1 mg/kg) and three dose groups (n = 3) that were pre-treated with a single administration of TV-1380 (0.2, 1 or 5 mg/kg) by the IM route followed by administration of 1 mg/kg cocaine IV dose repeated five consecutive times, at 2, 48, 96, 120, and 240 h after TV-1380 administration.

2.2.4. Study on the effect of TV-1380 on cocaine metabolism in the presence of ethanol

Four animals were used in this study in several phases (Table 2). Between each phase of treatment, a seven-day washout period was introduced. In the first phase, the four animals were treated with vehicle (formulation buffer) and three hours later were administered ethanol (1.5 g/kg) orally, followed by cocaine administration 30 min later. In Phase 2 of the study, which was performed one week later, TV-1380 (5 mg/kg) was given first to the four monkeys, followed three hours later by an oral administration of ethanol (1.5 g/kg) and then cocaine (IV, 1 mg/kg) which was given 30 min post ethanol treatment. In Phase 3, a similar sequence of TV-1380 pretreatment followed by ethanol and cocaine administration as in Phase 2 was given to the animals, but with a lower dose of ethanol (0.5 mg/kg rather than 1.5 mg/kg). Blood samples for determination of the plasma concentrations of cocaine and its metabolites were collected at pre-dose, 5, 10, 15, 20, 30, 40, 60, and 120 min following completion of the IV cocaine dose.

2.3. Procedures

2.3.1. Cardiovascular and respiratory examinations

Monkeys were implanted with a sterile telemetry unit (Data Science

Table 2
Study design of cocaine metabolism in combination with ethanol in monkeys.

Phase	Treatment combination	Route of Administration	Study time
Phase 1	Vehicle (0 mg/kg)	IM	Week 1
	+ Ethanol (1.5 g/kg)	PO	
	+ Cocaine (1 mg/kg)	IV	
Phase 2	TV-1380 (5 mg/kg)	IM	Week 2
	+ Ethanol (1.5 g/kg)	PO	
	+ Cocaine (1 mg/kg)	IV	
Phase 3	TV-1380 (5 mg/kg)	IM	Week 3
	+ Ethanol (0.5 g/kg)	PO	
	+ Cocaine (1 mg/kg)	IV	

IM – intramuscular PO – oral gavage IV – intravenous.

International (DSI) Telemetry System DataQuest A.R.T. 3.1. St. Paul, MN) and a vascular access port (VAP). Telemetry units were implanted in the abdomen of each animal. Animals underwent at least a 14-day-post-operative recovery period prior to initiation of dosing. The telemetry unit signals were verified for an extended period of time to ensure stability. Heart rate, QT, PR, RR, and QRS intervals were measured from the representative electrocardiogram (ECG) waveform. The RR interval was calculated and reported from the measured heart rate. QTc was calculated based on QT interval and heart rate (Spence's correction). The rate pressure product was calculated as the heart rate multiplied by the systolic pressure.

2.3.2. Measurement of respiration

Respiration rate, saturated blood oxygen (SpO₂) and end-tidal CO₂ (ETCO₂) were measured on specific days (noted as Respiratory). Data was collected with a Surgivet V9004 Capnograph (Waukesha, WI). Respiratory rates were manually recorded.

2.3.3. Histopathology

Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on sections of tissues from all animals at the end of the dosing period in the sub-chronic 13 weeks toxicity study.

2.3.4. Measurement of cardiac troponin I in plasma

Serum cardiac troponin I (cTnI) evaluations were conducted on animals in the cocaine metabolism in combination with ethanol study. Blood samples were collected from the femoral vein in alert animals and transferred into standard tubes. Serum analysis of cTnI was conducted at Antech Labs (USA). Serum was collected and analyzed using a solid phase chemiluminescent immunoassay method (Siemens Diagnostics Imulite System).

2.3.5. Ex-vivo catalytic activity of TV-1380 on cocaethylene compared to cocaine

Human serum from healthy donors was spiked with different amounts of TV-1380 and then diluted 1/100 in PBS (Table 3). The spiked material was incubated with cocaine or cocaethylene for various time intervals. A liquid chromatography tandem mass spectrometry (LC/MS/MS) method was used to determine substrate concentrations at each time point. Km and kcat were calculated and the ratio kcat/Km was used to compare the efficacy of TV-1380 activity on both substrates. The study was conducted at the Tandem Laboratories (Salt Lake City, UT, USA).

2.3.6. Analysis of cocaine, cocaine metabolites or TV-1380 levels in plasma and pharmacokinetic evaluation

Analysis of serum cocaine and its metabolites: benzoylecgonine,

Table 3

Kinetic parameters determined in vitro for (–)-cocaine and cocaethylene hydrolyses catalyzed by TV-1380 in comparison with published data on wildtype BChE.

		TV-1380	Wild type -BChE	Reference for Wild type
Cocaine	Km (μM)	4.75	4.5	Sun et al. (2002)
	kcat (min ⁻¹)	1499	4.1	
	kcat/Km (M ⁻¹ min ⁻¹)	3.26 × 10⁸	9.11 × 10⁵	
Cocaethylene	Km (μM)	11.1	7.5	Hou et al. (2014)
	kcat (min ⁻¹)	7.97	3.3	
	kcat/Km (M ⁻¹ min ⁻¹)	7.3 × 10⁷	4.40 × 10⁵	

Mean of two experiments.

cocaethylene, ecgonine methyl ester, norcocaine, and norecgonine methyl ester was performed using validated LC/MS/MS methods (Tandem Labs USA, West Trenton, NJ). For the cocaine, benzoylecgonine and ecgonine methyl ester methods, the lower limits of quantification were 1.0 ng/mL and upper limits of quantification were 1000–10000 ng/mL. For cocaethylene, the lower limit of quantification was 0.050 ng/mL and upper limit of quantification was 50 ng/mL. Serum sample analysis for TV-1380 concentration was conducted using a validated ELISA method. The WinNonlin Professional software (version 4.0.1) was used to calculate pharmacokinetic parameters by a non-compartmental modeling of IV bolus for cocaine and extravascular input modeling was applied for the metabolites. If there were fewer than three data points in the terminal phase of the plasma concentration curve, the terminal phase half-life and PK parameters derived from the half-life were not calculated for that profile.

2.4. Drugs

TV-1380 (AlbuBChE/Albu-Coch) was manufactured and supplied as a lyophilized material by Teva Pharmaceutical (USA) and was reconstituted with sterile water for injection to final concentrations of 30 or 100 mg/mL. The reconstituted solution was then diluted with buffer (10 mM sodium phosphate, 200 mM mannitol, 60 mM trehalose dihydrate, 0.01% polysorbate 80 or 50 mM sodium phosphate, 115 mM mannitol, 35 mM trehalose, and 0.03% (w/v) polysorbate 80, pH 7.2) and given to the monkeys by intramuscular injection in a volume of 0.167 mL/kg or 0.5 mL/kg. Cocaine (Mallinckrodt, St. Louis, MO, USA) was dissolved in saline. Cocaine was injected by an intravenous infusion in volume of 0.2 mL/kg or 1 mL/kg. Ethanol (Spectrum Chemical Labs, New Brunswick, NJ, USA) was diluted from a stock of 95% in distilled water and given to the animals by gavage at a dose volume of 3.75 mL/kg at dose levels concentrations of 0.5 and 1.5 g/kg.

2.5. Statistical analysis

Repeated measures mixed models with treatment groups, time and sex as fixed effects and animals ID as random effect were employed to see the effect of cocaine administration on cardiovascular events, and separately, the effect of TV-1380 administration on cocaine's effect on cardiovascular events. The effects of TV-1380 on the pharmacokinetic exposure parameter ($AUC_{0-\infty}$) of cocaine and its metabolites (benzyl-ecognine and ecognine methyl-ester) and the respiratory parameters (sPO₂, ETCO₂ and respiration rate) were analyzed using a two-way ANOVA with a nested Dunnett test.

Inferential analyses were conducted using [®]JMP Statistical Discovery software version 13.1.0 from [®]SAS Institute Inc. Cary, NC. Additional descriptive statistics such as mean, standard error (SE), and % coefficient of variation (%CV) were calculated using Excel (Microsoft).

3. Results

Studies described herein are part of the preclinical safety package that was carried out to support clinical trials of TV-1380 in healthy volunteers (Cohen-Barak et al., 2015) and in cocaine users (Shram et al., 2015; Gilgun-Sherki et al., 2016).

3.1. Sub-chronic 13-week toxicity study of TV-1380 in cynomolgus monkeys

Treatment of monkeys with TV-1380 at doses up to 50 mg/kg twice a week for 13 weeks was well tolerated and all animals survived the study without showing treatment-related adverse clinical signs, changes in food consumption, or effects on body weight and body weight gain. Hematological, blood and urine chemistry investigations conducted at the end of dosing period demonstrated no treatment related effects in

either sex at any dose level.

Histopathological examination revealed findings of minimal severity at the injection sites and the adjacent sciatic nerve without dose dependency. The treatment-related findings at the injection sites consisted of perivascular infiltrates of either lymphocytes or mixed leukocytes and a low degree of lymphoid infiltration. There was no associated vascular degeneration or necrosis. The findings in the sciatic nerve consisted of perivascular lymphoid infiltrates in the loose connective tissue surrounding the sciatic nerve in males at all doses and females at 10 mg/kg and 20 mg/kg. There was no cellular damage at the sciatic nerve itself. This is an expected finding when proteins are injected and is common to many other injected proteins (Engelhardt, 2008). These findings showed a trend for recovery following a 28 day of washout and were considered non-adverse.

3.2. Cardiovascular and respiratory investigations in cynomolgus monkeys

The effect of TV-1380 on the cardiovascular and respiratory systems in monkeys was tested in the presence and absence of cocaine in six male and six female monkeys. The animals were divided into two groups, which were administered TV-1380 consecutively according to the sequence described in Table 1. Treatment with TV-1380 alone at 15 mg/kg in both Group 1 and 2 had no effect on respiration rate, oxygen saturation (SpO₂), or End Tidal CO₂ (ETCO₂).

As shown in Fig. 2, the administration of cocaine at 1 mg/kg by IV injection resulted in an increase in body temperature ($p < 0.01$), which peaked at about 60 min after dosing (maximum change of approximately 0.5 °C) and in an immediate increase in heart rate (maximum change of 80 bpm), blood pressure (maximum change of 40 mmHg in systolic pressure and 31 mmHg in diastolic pressure) and mean arterial pressure (maximum change of 35 mmHg) (all $p < 0.01$). The (heart) rate pressure product was similarly affected (maximum change of 21,976 bpm*mmHg), all of which peaked within 5–10 min (Fig. 2). These effects were greater than the changes in heart rate and pressure that occurred after vehicle and are attributed to the normal response to manipulation.

Pretreatment of monkeys with TV-1380 at 15 mg/kg, 3 h before cocaine administration attenuated the effects induced by cocaine alone (Fig. 2; all $p < 0.01$). The most notable effects of TV-1380 were reduction of body temperature (maximum change of -0.5 °C), heart rate (maximum change of 35 bpm) and heart rate-pressure product (maximum change of 14,438 bpm*mmHg). The heart rate, systolic and diastolic pressure, mean arterial pressure, and the rate-pressure product in TV-1380 pretreated animals also returned to baseline faster (16–32 min) than in the animals that received cocaine alone (80–120 min).

No cardiac arrhythmias, no abnormal T wave changes and no atrial or ventricular arrhythmias were observed in any animal either with cocaine alone, TV-1380 alone or in combination. All QT intervals in the treated animals were within normal limits.

3.3. Cocaine metabolism of TV-1380 treated cynomolgus monkeys

Analysis of plasma in cocaine treated monkeys pretreated with different doses of TV-1380 indicated that TV-1380 accelerates the elimination of cocaine as a function of TV-1380 dose (Fig. 3A). This effect was most pronounced and statistically significant in the first few hours after TV-1380 pretreatment and diminished as time progressed, consistent with the elimination of TV-1380 from the plasma (Fig. 4), and by 240 h post-TV-1380 administration the hydrolytic effect of TV-1380 was no longer apparent. The elimination of cocaine under the influence of TV-1380 was also associated with reduced plasma levels of benzoylecognine, which is the metabolite formed by liver carboxypeptidase (Fig. 3B), suggesting a shift towards the off-liver BChE pathway. Reciprocally, the plasma levels of the BChE dependent cocaine metabolite, ecgonine methyl ester, were increased following TV-

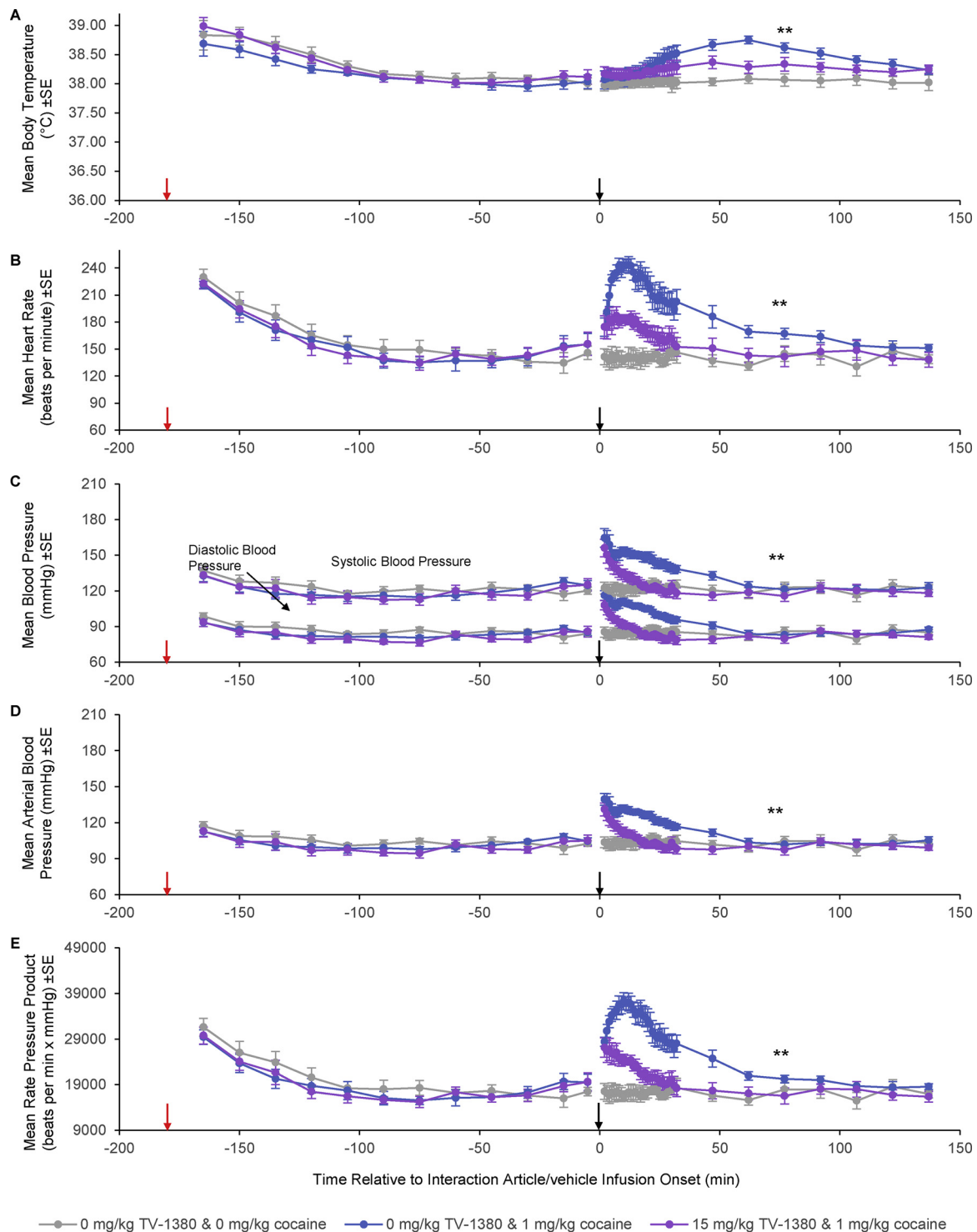


Fig. 2. Cardiovascular response to cocaine, TV-1380 or combination in treated cynomolgus monkeys. Mean (SE) responses from males and females monkeys (n = 3/ gender) are shown: mean body temperature (°C) (A), mean heart rate (beats per minute) (B), mean systolic and diastolic blood pressure (mmHg) (C), mean arterial blood pressure (mmHg) (D), rate pressure product (beats per minute x mmHg) (E). The red arrow represents the time of injection of TV-1380 or formulation buffer. The black arrow represents the start of saline or cocaine injection. Asterisks ** indicate statistically significant (p < 0.01) differences in cardiovascular events due to the administration of TV-1380 to animals treated with cocaine (purple line) versus animals treated with cocaine and no treatment with TV-1380 (blue line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

1380 (Fig. 3C), reflecting the enhanced metabolism by this pathway. TV-1380 pharmacokinetic profile was characterized at all dose levels (Fig. 4). All animals had measurable TV-1380 concentrations. TV-1380 exposure increased with increasing dose. TV-1380 T_{max} ranged from 3 h (for 0.2 and 1 mg/kg dose) to 6 h (for the 5 mg/kg dose). Terminal elimination ($t_{1/2}$) of TV-1380 increased from 31 to 62 h with

increasing TV-1380 dose. The effect of TV-1380 on cocaine metabolism was both time and dose dependent.

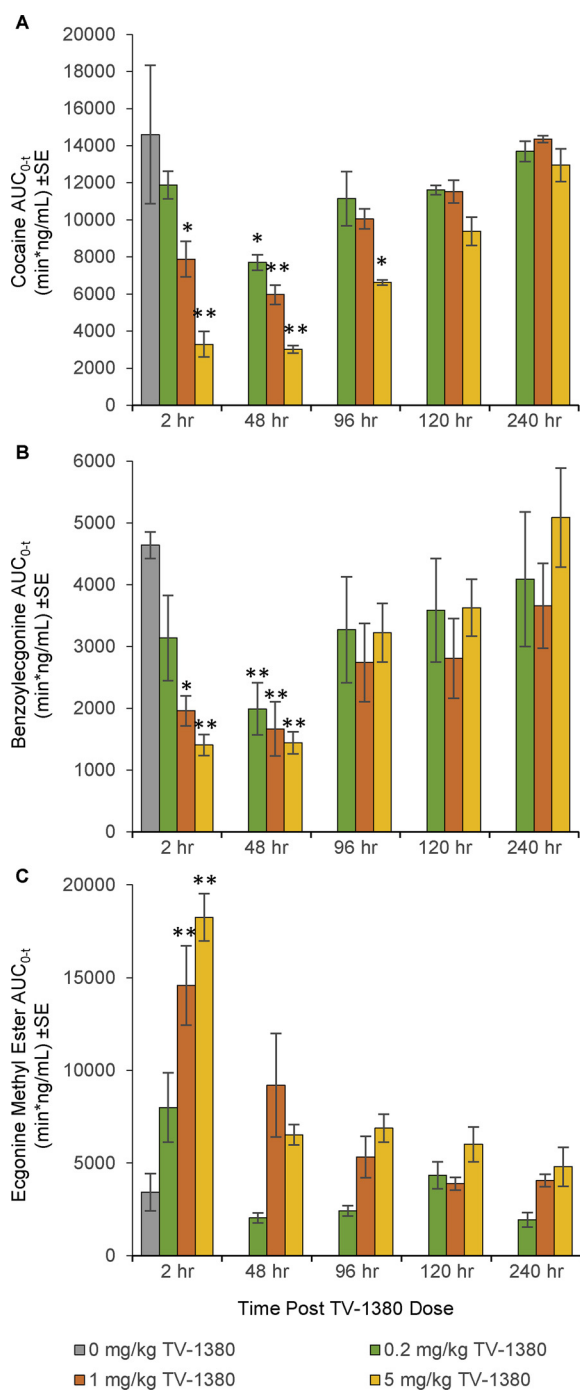


Fig. 3. Plasma exposure of cocaine and its metabolites following TV-1380 treatment in male cynomolgus monkeys. Mean (SE) exposure of cocaine AUC(0-t) (A) benzoylcegonine AUC(0-t) (B) ecgonine methyl ester AUC(0-t) (C) following 1 mg/kg IV cocaine dose following pretreatment with control (n = 2) or TV-1380 various doses (n = 3). Pretreatment with TV-1380 occurred at 2, 48, 96, 120, and 240 h prior to cocaine administration. Significant differences in the AUC_{0-t} of cocaine and metabolites benzoylcegonine and ecgonine methyl ester between no TV-1380 and different doses of TV-1380 (0.2, 1, and 5 mg/kg) are indicated with asterisks; * p < 0.05, ** p < 0.01.

3.4. Ex vivo catalytic activity of TV-1380 on cocaethylene compared to cocaine

To investigate the effect of TV1380 on cocaethylene metabolism, human serum was spiked with different amounts of TV-1380 and incubated with either cocaine or cocaethylene as substrates. The kinetic

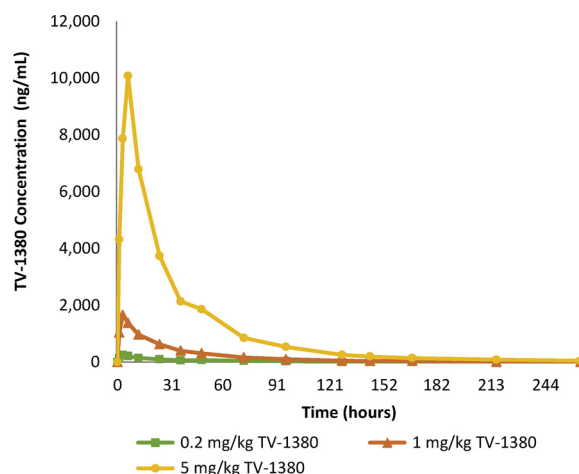


Fig. 4. Serum concentration TV-1380 (ng/mL) vs. time (hour) profile in male cynomolgus monkeys. Average exposure following a single intramuscular administration of TV-1380 dose 0.2, 1 or 5 mg/kg/animal.

parameters of the enzymatic reactions (Table 3) indicate that TV1380 is considerably more potent in the hydrolysis of cocaine than in the hydrolysis of cocaethylene, with kcat average values of 1499 (min^{-1}) and 7.97 (min^{-1}) respectively. Km values were similar, but slightly higher for the cocaethylene substrate. The kcat/Km values for these two reactions were calculated as 3.26×10^8 and 7.3×10^7 ($\text{M}^{-1} \text{min}^{-1}$), respectively. These catalytic values are two and three orders of magnitude higher for both substrates than the values published for the wild type enzyme (Hou et al., 2014; Sun et al., 2002) and indicate that TV-1380 is effective as a hydrolytic enzyme for cocaethylene.

3.5. Cocaine metabolism in combination with ethanol in TV-1380 treated cynomolgus monkeys

In light of the noted catalytic activity of TV-1380 on cocaethylene as seen in our in-vitro study, we have examined if TV-1380 can effectively metabolize cocaethylene in vivo in monkeys. To this end, we characterized the pharmacokinetic profiles of cocaine and its metabolites when cocaine and ethanol are given with and without TV-1380 pretreatment. Four adult male cynomolgus monkeys were treated consecutively in 3 study phases, separated by a washout period according to the schedule described in Table 2.

The results of this experiment are illustrated in Fig. 5, panels A-D. The plasma concentrations of cocaine (Panel A) demonstrate that the IM administration of 5 mg/kg TV-1380 to the monkey completely eliminated the exposure to cocaine regardless of the levels of co-administered ethanol (either 1.5 g/kg or 0.5 g/kg). TV-1380 also completely eliminated the exposure to the two metabolites; cocaethylene (Panel B) and benzoylcegonine (Panel C). In contrast, the plasma levels of ecgonine methyl ester (Panel D) were markedly increased in monkeys which received TV-1380 prior to cocaine and alcohol regardless of the level of the alcohol dose (0.5 or 1.5 g/kg). The markedly reduced exposure to both benzoylcegonine and cocaethylene following TV-1380 pretreatment indicates that in the presence of TV-1380, the hydrolysis of cocaine to benzoylcegonine by hCE1 (Brzezinski et al., 1997) carboxylesterase is shifted towards the formation of ecgonine methyl ester. Since the formation of cocaethylene is critically linked to the hydrolysis by hCE1 carboxylesterases (Farre et al., 1997; Parker and Laziure, 2010), the TV-1380 shift in cocaine metabolism diminished the production of cocaethylene under the study conditions. The data also show that the presence of alcohol does not modify the catalytic efficiency of TV-1380.

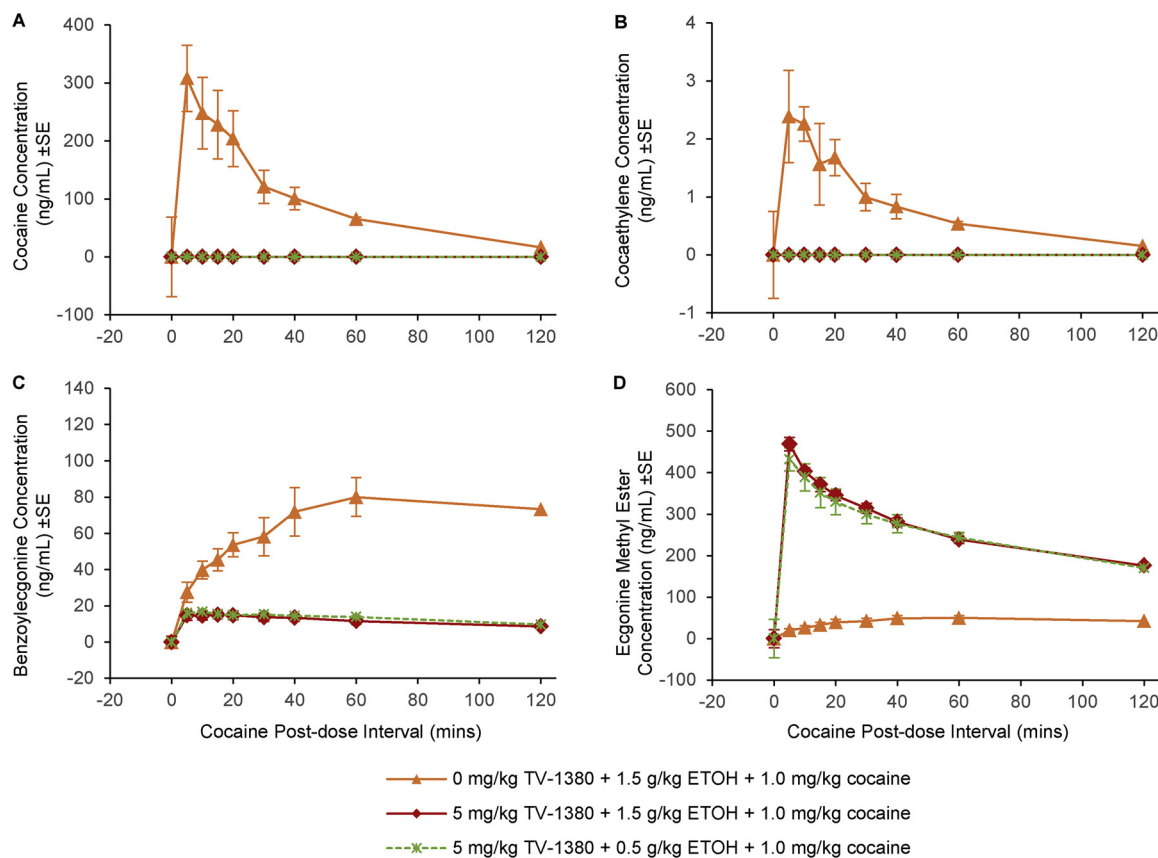


Fig. 5. Plasma concentrations in male monkeys after IM administration of TV-1380, oral ethanol and IV cocaine. Time-dependent mean (SE) concentrations of cocaine (A) cocaethylene. (B) benzoylecgonine (C) and ecgonine methyl ester (D).

3.6. Measurement of plasma levels of cardiac troponin I

Cardiac troponin I (cTnI) concentration in peripheral blood is a known biomarker for cardiotoxicity (Wallace et al., 2004). In our investigation of the metabolic fate of cocaine in the presence of alcohol and TV-1380, serum samples were also analyzed for cTnI (Fig. 6). The combination of ethanol and cocaine produced a marked increase in cTnI levels, which peaked at about 10 h after cocaine administration and lasted for several hours; a small elevation above background was still

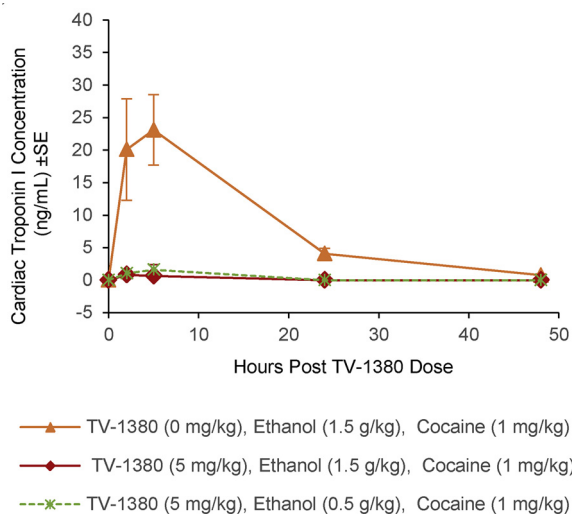


Fig. 6. Plasma concentrations of cardiac Troponin I (cTnI) concentration in male monkeys. Mean (SE) cardiac troponin I (cTnI) concentration for monkeys given IM administration of TV-1380, oral ethanol and IV cocaine.

seen at 24 h. This response was abrogated in animals pretreated with TV-1380, possibly as the result of the noted shift in the metabolic pathway away from the production of cocaethylene.

4. Discussion

TV-1380 is a rationally mutated, human butyrylcholinesterase enzyme (BChE) fused to human serum albumin that was designed as a long-acting, potent cocaine hydrolytic enzyme. Considering its potential use as a chronic medication for cocaine dependence, it was essential to investigate the safety of TV-1380 in monkeys in a repeat dose study and examine its effect on cocaine metabolism. The results of the 13-week repeat dose toxicity study, presented here, demonstrate the favorable safety profile of TV-1380 (up to 50 mg/kg/twice a week) showing tolerability and lack of any treatment related effect. All tested safety parameters were unaffected by TV-1380 treatment including cardiovascular and respiratory endpoints.

The protective effect of TV-1380 from cocaine induced toxicity was previously tested in rats and was reported (Brimijoin et al., 2008). Here we examined the protective effect of TV1380 in monkeys in the presence of the cocaine, and focused on cocaine-induced changes in cardiodynamic parameters. The dose of cocaine selected for this study was 1 mg/kg, a dose that is not associated with severe CNS signs. While the administration of intravenous injection of cocaine alone (1 mg/kg) resulted in an increase in body temperature, and a small transient rise in blood pressure and heart rate (beyond the stress response to the injection itself), pre-treatment with TV-1380 (15 mg/kg) 3 h prior to cocaine administration attenuated these effects. The effects noted in this study for cocaine at 1 mg/kg are mild, but consistent with the known pressor response of the molecule (Gao and Brimijoin, 2004). It is worth noting that overt cocaine cardiotoxicity in animals is mainly seen at doses higher than those employed in our study (Hearn et al., 1991).

Investigations of the cocaine clearance in the monkeys have confirmed our previous observations in rats; TV-1380 accelerates the clearance of cocaine in a dose and time dependent manner. Pretreatment with TV-1380 was most effective when given a few hours before cocaine administration, but a clear effect could still be seen at 48 h. Effect was also dependent on the dose of TV-1380; 5 mg/kg inhibited the exposure to cocaine by more than 75% (when given 2 h before cocaine), a dose of 1 mg/kg produced about 50% inhibition and the dose of 0.2 mg/kg did not cause a statistically significant change.

hBChE primarily eliminates cocaine by hydrolysis to the two known cocaine metabolites: ecgonine methyl ester and benzoic acid, which are considered non-toxic (Xie et al., 1999). TV1380 was shown in our studies to dramatically change the metabolic profile of cocaine in monkeys by shifting the metabolic clearance away from liver carboxypeptidase towards the BChE-dependent pathway, thereby decreasing exposure to benzoylecgonine while increasing the exposure to the BChE metabolite, ecgonine methyl ester. These results are consistent across species (Schlindler et al., 2013) and seen also in treated humans (Shram et al., 2015).

The concurrent use of alcohol and cocaine is a common practice among drug abusers, as it enhances the euphoric effect and reduces some of the unpleasant late effects of cocaine. However, the combination of cocaine and alcohol also results in the formation of the active and toxic metabolite cocaethylene, the ethyl ester of cocaine (Andrews, 1997; Parker and Laizure, 2010). Cocaethylene is more potent than cocaine in mediating lethality in mice (Hearn et al., 1991) and the combined use of cocaine and ethanol may present a greater health risk than cocaine alone (Andrews, 1997).

Our studies indicate that formation of cocaethylene as a metabolite of cocaine in the presence of ethanol in monkeys is markedly inhibited when the monkeys are pretreated with TV1380. This effect occurred concomitantly with the induced decrease in the levels of benzoylecgonine and with the increase in the levels of ecgonine methyl ester, all indicative of the shift in the metabolic pathway away from that dependent on liver carboxypeptidase. The data also indicate that the generation of cocaethylene is dependent on the carboxypeptidase mediated hydrolysis. This observation is consistent with earlier publication on cocaethylene metabolism, showing its dependence on carboxypeptidase activity (Brzezinski et al., 1997).

Our results have also demonstrated a marked increase in the levels of plasma cardiac troponin I (cTnI) in monkeys treated concomitantly with ethanol and cocaine. cTnI is one of the newest and most specific markers of cardiac injury and its enhanced levels in these monkeys may be related to the generation of cocaethylene. Since this metabolite is associated with general toxicity and particularly cardiotoxicity, the elevation of cTnI levels in the treated animals and the inhibition of its formation by TV-1380 may have important beneficial therapeutic activities.

Our in-vitro enzymatic studies with isolated enzymes in human plasma suggest that TV1380 has a much greater hydrolytic activity than wild type BChE towards cocaethylene as a substrate (supported by Hou et al., 2014). Such high catalytic activity can potentially lead to the rapid removal of cocaethylene from the circulation, as was demonstrated in vivo in monkeys.

This effect of TV-1380 on the accelerated catabolism of cocaethylene is in addition to the effect of TV-1380 on the formation of cocaethylene, through the shift in the metabolic pathway away of the endogenous carboxypeptidase (hCE1) mediated hydrolysis. The two mechanisms are not mutually exclusive and act in concert to reduce the levels of cocaethylene in plasma. These data suggest that TV-1380 can be effective in the treatment of cocaine overdose, not only because it accelerates the catabolism of cocaine, but also as it reduces the formation of cocaethylene, an active toxic metabolite, and possibly also accelerates the degradation of this toxic metabolite.

The results presented in this article reveal a promising safety profile of TV-1380 in treated monkeys, in addition to a potential advantage for

TV-1380 in the reduction of the toxic metabolite, cocaethylene, and the favorable effects on markers of cardiac damage. These effects should encourage renewed efforts of pharmaceutical companies to develop new modified BChE enzyme products that will have the needed catabolic activity for cocaine and cocaethylene and eventually help in the treatment of cocaine addiction.

Conflict of interest

LSD, DS, HH, AG, and MR are employees of Teva Pharmaceuticals Ltd., the sponsor of the development of TV-1380.

Role of funding source

[Teva Pharmaceuticals, who funded several of the studies of this report, was involved in the study design, in the collection, analysis and interpretation of the data, in the writing of the report and in the decision to submit the article for publication.] This work was supported by Global Research and Development Teva Pharmaceutical Industries, Netanya Israel.

Contributors

All authors were responsible for the studies concept and designs. AG performed the ex vivo catalytic data analysis. All authors assisted with data analysis and interpretation of findings. LSD drafted the manuscript. DS, MR, and HH provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

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