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(54) Title: BCHE ALBUMIN FUSIONS FOR THE TREATMENT OF COCAINE ABUSE

(57) Abstract: A method of attenuating a biological effect of cocaine exposure in a primate. Such method includes administering to the primate an amount of a BChE-albumin fusion protein comprising the amino acid substitutions A227S, S315G, A356W, and Y360G, wherein the amount of the fusion protein is effective to cause attenuation of the biological effect of cocaine exposure in the primate.



WO 2011/071926 A1

- 1 -

5 **BChe ALBUMIN FUSIONS FOR THE TREATMENT OF COCAINE ABUSE**

This application claims priority of U.S. Provisional Applications Nos. 61/412,205, filed November 10, 2010 and 61/283,791, filed December 8, 2009, the contents of which are
10 hereby incorporated by reference in their entirety.

Throughout this application various publications, published patent applications, and patents are referenced. The disclosures of these documents in their entireties are hereby
15 incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

Background

20 Cocaine abuse and dependence have disastrous medical and social consequences which have made the development of an effective treatment a high priority (Pan, Y., Gao, D., Yang, W., Cho, H., Yahg, G., Tai, H., Zhan, C., "Computational redesign of human butyrylcholinesterase for anticocaine medication," *PNAS*,
25 102(46):16656-61, 2005). However, as stated by Brimijoin et al.: "there is no reliable means to treat cocaine overdose or reduce the likelihood of relapse in users who have achieved abstinence. Human plasma butyrylcholinesterase (BChE) contributes to normal cocaine metabolism and has been
30 considered for use in treating cocaine toxicity" (Brimijoin, S., Gao, Y., Anker, J., Gliddon, L., LaFleur, D., Shah, R., Zhao, Q., Singh, M., Carroll, M., "A Cocaine Hydrolase Engineered from Human Butyrylcholinesterase Selectively Blocks Cocaine Toxicity and Reinstatement of Drug Seeking in Rats,"
35 *Neuropsychopharmacology*, 33:2715-25, 2008).

- 2 -

Wild-type BChE, while important for cocaine metabolism in the body, has low catalytic efficiency with cocaine. The low cocaine hydrolase activity of wild-type BChE would require the use of prohibitively large quantities of purified enzyme for treatment of cocaine abuse or overdose. Mutagenesis performed on human BChE with the goal of enhancing the cocaine hydrolase activity resulted in the development of the double mutant A328W/Y332A (residues 356 and 360 relative to full length BChE) that has a k_{cat} that is 40-fold higher than wild-type BChE, with only a slightly increased K_M (Sun H., Shen M., Pang Y., Lockridge O., BrimiJoin S., "Cocaine Metabolism Accelerated by a Re-Engineered Human Butyrylcholinesterase" *Journal of Pharmacology and Experimental Therapeutics*, 302(2):710-716, 2002).

Further experimentation utilizing molecular dynamics to simulate the transition state for the first chemical reaction step of BChE catalyzed hydrolysis of cocaine resulted in the BChE mutant A227S/S315G/A356W/Y360G that has a catalytic efficiency which is 500-fold greater than wild-type BChE and is greater than other previously designed BChE mutants (Pan, Y., Gao, D., Yang, W., Cho, H., Yang, G., Tai, H., Zhan, C., "Computational redesign of human butyrylcholinesterase for anticocaine medication," *PNAS*, 102(46):16656-61, 2005).

To obtain a form of the A227S/S315G/A356W/Y360G BChE mutant that may be suitable for therapeutic use, the BChE mutant designed by Pan et al. was fused at its C terminus to human serum albumin (HSA) because it has been observed that similar fusions exhibit favorable pharmacokinetic properties with high stability and extended plasma half lives. It was observed that the BChE-albumin fusion comprising the above mutations retains high catalytic efficiency with cocaine and exhibits a plasma half-life of 8 hours after i.v. injection to rats. (BrimiJoin

- 3 -

S., Gao, Y., Anker J., Gliddon L., LaFleur D., Shah R., Zhao, Q., "A Cocaine Hydrolase Engineered from Human Butyrylcholinesterase Selectively Blocks Cocaine Toxicity and Reinstatement of Drug Seeking in Rats" *Neuropsychopharmacology*,
5 33:2715-25, 2008).

To date, there has been no effective method for treating cocaine abuse or overdose in primates developed that utilizes a BChE-albumin fusion comprising the mutations A227S, S315G,
10 A356W, and Y360G.

- 4 -

Brief Summary of the Invention

The subject invention provides a method of attenuating a biological effect of a cocaine exposure in a primate comprising administering to the primate an amount of a fusion protein comprising

(a) a mutant butyrylcholinesterase (BChE) polypeptide comprising the sequence

EDDIIATKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQSLTKWSDIWNA
TKYANSCCQNIQSFPGFHGSEMWNPTDLSCLYLNWVWPAPKPKNATVLIWIYGG
10 GFQTGTSSLHVYDGKFLARVERVIVVSMNYRVGALGFLALPGNPEAPGNMGLFDQQLA
LQWVQKNIAAFGGNPKSVTLFGESSGAASVSLHLLSPGSHSLFTRAILQSGSFNAPWA
VTSLYEARNRTLNLAKLTGCSRENETEITIKCLRKNKDPQEILNLNFAFVVPYGTPLGVNF
GPTVDGDFLTDPDILLELGQFKKTQILVGVNKDEGTWFLVGGAPGFSKDNNSIITRK
EFQEGKLIFFPGVSEFGKESILFHYTDWVDDQRPENYREALGDVVGDYNFICPALEFT
15 KKFSEWGNNAFFYYFEHRSSKLPWPEWGMVHGVEIEFVFGPLPLERRDNYTKAEIILS
RSIVKRWANFAKYGNPNETQNNSTSWPVFKSTEQKYLTLNTESTRIMTKLRAQQCRFW
TSFFPKV (SEQ ID NO:1),

(b) a human serum albumin (HSA) polypeptide comprising the sequence

20 DAHKSEVAHRFKDLGEENFKALVLIIFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES
AENCCKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFQHKDDNPPLRLV
RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELFFAKRYKAAFTCECQAADK
AACLLPKLDELREDEGKASSAKQRLKCASLQKFGERAFAKAWAVARLSQRFPAEFAEVS
KLVTDLTKVHTECCHGDLLECADRADLAKYICENQDSISSKLKECCEKPLEKSHCI
25 AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLRLR
LAKTYETTLEKCCAAADPHECYAKVFDEFKPLVEEPQNLKQNCLEFQLGGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTFFVSDRVTKCCTESLVNRRPCFSALEVDETYVPKEFNAETTFHADICTLSEKERQ
IKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKKADDKETCFAEEGKKLVAASQ
30 AALGL (SEQ ID NO:2), and

(c) a signal peptide comprising the sequence
MRPTWAWWLFLVLLLALWAPARG (SEQ ID NO:3),

- 5 -

wherein the amount of the fusion protein is effective to cause attenuation of the biological effect of the cocaine exposure in the primate.

- 6 -

Description of the Figures

Figure 1 (SEQ ID NO: 4) shows the amino acid sequence of AlbuBChE, a BChE-albumin fusion protein comprising the mutations A227S, S315G, A356W, and Y360G.

5

Figure 2 shows AlbuBChE serum concentration-time profile in individual cynomolgus monkeys following a single IM administration of 0.2, 1 or 5 mg/kg AlbuBChE dose (top panel linear scale, lower panel semi-logarithmic scale).

10

Figure 3 shows mean AlbuBChE serum concentration-time profile in cynomolgus monkeys following a single IM administration of 0.2, 1 or 5 mg/kg AlbuBChE dose (top panel linear scale, lower panel semi-logarithmic scale).

15

Figure 4 shows mean cocaine concentration vs. time in control animals (n=2) and as a function of time post-AlbuBChE dose (n=3).

20 Figure 5 shows mean benzoylecgonine concentration vs. time in control animals (n=2) and as a function of time post-AlbuBChE dose (n=3).

25 Figure 6 shows mean ecgonine methyl ester concentration vs. time in control animals (n=2) and as a function of time post-AlbuBChE dose (n=3).

30 Figure 7 shows a comparison of mean cocaine concentration-time profile for Day 1 cocaine control group with Day 11 (240 hr) post-AlbuBChE administration at 0.2, 1 or 5 mg/kg.

Figure 8 shows AlbuBChE PK/PD analysis in cynomolgus monkeys following a single IM administration of 0.2, 1 or 5 mg/kg AlbuBChE dose. Cocaine was administered IV at a dose of 1 mg/kg

- 7 -

in control animals or at 2, 48, 96, 120 and 240 hr post-AlbuBChE dose.

Figure 9 shows cocaine metabolic pathways.

5

Figure 10 shows mean cocaine $AUC_{(0-t)}$ following 1 mg/kg IV cocaine dose in control animals (n=2) and as a function of time post-AlbuBChE dose (n=3).

10 Figure 11 shows mean benzoylecgonine $AUC_{(0-t)}$ following 1 mg/kg IV cocaine dose in control animals (n=2) and as a function of time post-AlbuBChE dose (n=3).

15 Figure 12 shows AlbuBChE serum concentration-time profile in individual squirrel monkeys following a single IM administration of 5 mg/kg AlbuBChE dose (semi-logarithmic scale).

20 Figure 13 shows mean cocaine concentration vs. time in control animals (n=2) and as a function of time post-AlbuBChE dose (n=3).

25 Figure 14 shows mean ecgonine methyl ester concentration at 5 min following 1 mg/kg IV cocaine dose in control animals (n=2) and as a function of time post-AlbuBChE dose (n=3).

Figure 15 shows mean benzoylecgonine concentration at 5 min following 1 mg/kg IV cocaine dose in control animals (n=2) and as a function of time post-AlbuBChE dose (n=3).

30

Figure 16 shows a summary of squirrel monkey blood levels of cocaine and the cocaine metabolites ecgonine methyl ester (EME) and benzoylecgonine (BZ) at 5 minutes (top panel) and 30 minutes (bottom panel) following cocaine injection.

- 8 -

Figure 17 shows AlbuBChE PK/PD analysis in Squirrel Monkeys following a single IM administration of 5 mg/kg AlbuBChE dose. Cocaine was administered IV at a dose of 1 mg/kg in control animals (n=2) or at 2, 72 and 96 hr post-AlbuBChE dose (n=3).

Figure 18 shows mean heart rate vs. time in cynomolgus monkeys prior to and following a single IM administration of 15 mg/kg AlbuBChE dose or formulation buffer.

10

Figure 19 shows mean heart rate pressure product vs. time in cynomolgus monkeys prior to and following a single IM administration of 15 mg/kg AlbuBChE dose or formulation buffer.

15 Figure 20 shows mean arterial blood pressure vs. time in cynomolgus monkeys prior to and following a single IM administration of 15 mg/kg AlbuBChE dose or formulation buffer.

20 Figure 21 shows mean diastolic blood pressure vs. time in cynomolgus monkeys prior to and following a single IM administration of 15 mg/kg AlbuBChE dose or formulation buffer.

25 Figure 22 shows mean systolic blood pressure vs. time in cynomolgus monkeys prior to and following a single IM administration of 15 mg/kg AlbuBChE dose or formulation buffer.

Figure 23 shows mean body temperature vs. time in cynomolgus monkeys prior to and following a single IM administration of 15 mg/kg AlbuBChE dose or formulation buffer.

30

Figure 24A shows mean respiration rate vs. time in cynomolgus monkeys prior to and following a single IM administration of 15 mg/kg AlbuBChE dose or formulation buffer. Data for male cynomolgus monkeys is shown in Figure 24B, top panel, and data

- 9 -

for female cynomolgus monkeys is shown in Figure 24B, bottom panel.

Figure 25A shows mean SPO₂ levels vs. time in cynomolgus monkeys prior to and following a single IM administration of 15 mg/kg AlbuBChE dose or formulation buffer. Data for male cynomolgus monkeys is shown in Figure 25B, top panel, and data for female cynomolgus monkeys is shown in Figure 25B, bottom panel.

Figure 26 shows mean ETCO₂ levels vs. time in cynomolgus monkeys prior to and following a single IM administration of 15 mg/kg AlbuBChE dose or formulation buffer. Data for male cynomolgus monkeys is shown in Figure 26B, top panel, and data for female cynomolgus monkeys is shown in Figure 26B, bottom panel.

Figure 27 shows mean arterial blood pressure vs. time in cynomolgus monkeys following a cocaine dose of 1 mg/kg administered IV three hours post 15 mg/kg AlbuBChE dose. Cocaine dose was administered at t=0.

Figure 28 shows mean diastolic blood pressure vs. time in cynomolgus monkeys following a cocaine dose of 1 mg/kg administered IV three hours post 15 mg/kg AlbuBChE dose. Cocaine dose was administered at t=0.

Figure 29 shows mean systolic blood pressure vs. time in cynomolgus monkeys following a cocaine dose of 1 mg/kg administered IV three hours post 15 mg/kg AlbuBChE dose. Cocaine dose was administered at t=0.

Figure 30 shows mean heart rate vs. time in cynomolgus monkeys following a cocaine dose of 1 mg/kg administered IV three hours

- 10 -

post 15 mg/kg AlbuBChE dose. Cocaine dose was administered at t=0.

5 Figure 31 shows mean rate pressure product vs. time in cynomolgus monkeys following a cocaine dose of 1 mg/kg administered IV three hours post 15 mg/kg AlbuBChE dose. Cocaine dose was administered at t=0.

10 Figure 32 shows mean body temperature vs. time in cynomolgus monkeys following a cocaine dose of 1 mg/kg administered IV three hours post 15 mg/kg AlbuBChE dose. Cocaine dose was administered at t=0.

15 Figure 33 shows squirrel monkey response rate (top panels) and number of injections (bottom panels) during self administration sessions over consecutive days where either cocaine or saline was available for self-administration. Responding was tracked for five days following vehicle or AlbuBChE administration (5 mg/kg).

20 Figure 34 shows levels of reinstatement of cocaine self-administration following administration of AlbuBChE or AlbuBChE vehicle. The AlbuBChE or vehicle was given i.m. Two, 48 and 96 hrs later 0.3 mg/kg cocaine i.v. (left panels) or 0.1 mg/kg cocaine i.v. (right panels) was given 5 min before a saline substitution session.

25 Figure 35 shows the modulation of cocaine's discriminative-stimulus effects in methamphetamine trained subjects by AlbuBChE.

30 Figure 36 shows methamphetamine's discriminative-stimulus effects in methamphetamine trained subjects after AlbuBChE administration.

- 11 -

Figure 37 shows the relative serum concentration of an albumin fusion compared to a non-fusion peptide where the albumin fusion is dosed once-weekly and the non-fusion peptide is dosed
5 daily.

Figure 38 shows a SDS-PAGE of AlbuBChE purified from CHO cells. Lane 1 is a molecular weight marker, and lanes 2 and 4 show purified AlbuBChE under reducing and non-reducing conditions,
10 respectively.

Figure 39 shows the number of beam crossings vs. time in rats treated with wild-type BChE or Albu-Coch prior to a cocaine dose, rats given a cocaine dose alone, and rats given neither
15 cocaine nor BChE. Locomotor activity was assessed by detecting infrared beam breaks as described in Brimijoin et al.

Figure 40 shows the concentration of AlbuBChE vs. time in mice following a dose of AlbuBChE and also shows cocaine hydrolysis
20 (represented as the percentage of cocaine converted to benzoic acid (%BA) within 60 minutes of cocaine administration) vs. time post AlbuBChE dose.

Figure 41 shows the cocaine content in brain and heart collected from rats (n=6) given an AlbuBChE (3mg/kg) dose or saline, through the tail vein, followed ten minutes later by 30
25 μ Ci 3H-cocaine (3.5 mg/kg), also through the tail vein. Brains and hearts were collected 10 minutes post 3H-cocaine dose. Left to right, control data is bars 1 and 3, while AlbuBChE data is
30 bars 2 and 4.

Figure 42 shows cocaine (left plot) and benzoate levels (right plot) in brain, heart and plasma collected from rats given a 3 mg/kg AlbuBChE dose or saline 10 minutes prior to a 30 μ Ci 3H-

- 12 -

cocaine dose (3.5 mg/kg). Brain, heart and plasma were collected 10 minutes post cocaine dose.

Figure 43 shows the experimental design of a study examining the ability of AlbuBChE to protect from lethal overdose.

Figure 44 shows the percentage of rats exhibiting hyperactivity, seizures, and death in response to a dose of 100mg/kg cocaine given ten minutes after a dose of 0, 1, 3, or 10 mg/kg AlbuBChE.

Figure 45 shows the concentration of cocaine vs. time in Sprague Dawley rats given an IV dose of AlbuBChE at 0, 2, and 10 mg/kg followed five minutes later by an IP dose of cocaine at 60 or 100 mg/kg.

Figure 46 shows a behavioral model of addiction and relapse. "S" and "D" represent saline and the drug cocaine, respectively. Animals are trained to emit a lever press for a cocaine infusion. After stabilization (maintenance), saline is substituted for cocaine and the behavior is allowed to extinguish. In the subsequent reinstatement phase, priming injections of cocaine are given, alternating with saline.

Figure 47 shows the selective blockade of cocaine-primed reinstatement of drug-seeking behavior resulting from AlbuBChE treatment. Rats that had previously self-administered cocaine and extinguished when cocaine was replaced with saline were primed with an IV injection of saline (S), cocaine (C, 10 mg/kg), or amphetamine (A, 2 mg/kg). AlbuBChE was administered IV (E, 2 mg/kg) two hours before the behavioral session.

Figure 48 shows the number of lever presses per session in rats following saline injection, cocaine injection (10 mg/kg, IV),

- 13 -

or AlbuBChE (2 mg/kg, IV) followed by cocaine (10 mg/kg, IV) after an addiction phase and forced abstinence.

Figure 49 shows AlbuBChE bioavailability, half-life, and time
5 for maximum concentration, T_{max}, in cynomolgus monkeys following a single intravenous (IV), subcutaneous (SC), or intramuscular (IM) injection. AlbuBChE absolute bioavailability for IM and SC routes of administration was calculated relative to IV at the 3 mg/kg dose level.

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- 14 -

Detailed Description of the Invention

The subject invention provides a method of attenuating a biological effect of a cocaine exposure in a primate comprising administering to the primate an amount of a fusion protein comprising

(a) a mutant butyrylcholinesterase (BChE) polypeptide comprising the sequence

EDDIIITATNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQSLTKWSDIWNA
TKYANSCCQNIQSFPGFHGSEMWNPTDLSEDCLYLVNWIIPAPKPKNATVLIWIYGG
10 GFQTGTSSLHVYDGKFLARVERVIVVSMNYRVGALGFLALPGNPEAPGNMGLFDQQIA
LQWVQKNIAAFGGNPKSVTLFGESSGAASVSLHLLSPGSHSLFTRAILQSGSFNAPWA
VTSLYEARNRTILNLAKLTGCSRENETEIIKCLRNDPQEIILLNEAFVVPYGTPLGVNF
GPTVDGDFLTDMPDILLELGQFKKTQILVGVNKDEGTWFLVGGAPGFSKDNNSIITRK
EFQEGLIKIFPGVSEFGKESILPHYTDWVDDQRPENYREALGDVVGDNFYFICPALEFT
15 KKFSEWGNNAFFYYFEHRSSKLPWPEWMGMVHGYETEFVGLPLERRDNYTKAEIILS
RSIVKRWANFAKYGNPNETQNNSTSWPVFKSTEQKYLTLNTESTRIMTKLRAQQCRFW
TSFFPKV (SEQ ID NO:1),

(b) a human serum albumin (HSA) polypeptide comprising the sequence

20 DAHKSEVAHRFKDLGEENFKALVLIIFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES
AENCDSLSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPPLRLV
RPEVDVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELFFAKRYKAAFTTECCQAADK
AACLLPKLDELDEGKASSAKQRLKASLQKFGERAFKAWAVARLSQRFPAEFAEVS
KLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
25 AEVENDEMPADLPSLAADFVESKDVCKNYAEAKDVFLGMFLYFYARRHPDYSVVLRLR
LAKTYETTLKCCAAADPHECYAKVFDEFKPLVEEPQNLKQNCLEFQELGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTVPVSDRVTKCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQ
IKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKKADDKETCFAEEGKKLVAASQ
30 AALGL (SEQ ID NO:2), and

(c) a signal peptide comprising the sequence
MRPTWAWWLFVLVLLALWAPARG (SEQ ID NO:3),

- 15 -

wherein the amount of the fusion protein is effective to cause attenuation of the biological effect of the cocaine exposure in the primate.

- 5 In an embodiment of the method, fusion protein comprises the sequence

MRPTWAWWLFLVLLLLALWAPARGEDDIIATKNGKVRGMNLTVFGGTVTAFLGIPYAQ
PPLGRRLRFKKPQSLTKWSDIWNATKYANSCQNIDQSFPGFHGSEMWNPNLTLSEDCL
YLNWVWPAPKPKNATVLIWIYGGGFQGTGSSSLHVYDGKFLARVERVIVVSMNYRVGAL
10 GFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESSGAASVSLHLL
SPGSHSLFTRAILQSGSFNAPWAVTSLYEARNRTLNLAKLTGCSRENETEIIKCLRNK
DPQEILLNEAFVVPYGTPLGVNFGPTVDGDFLTDMPDILLELGQFKKTQILVGVNKDE
GTWFLVGGAPGFSKDNNSIITRKEFQEGLKIFFPGVSEFGKESILFHYTDWVDDQRPE
NYREALGDVVGDYNFICPALEFTKKFSEWGNNAFYYFEHRSSKLPWPEWMGMVHGIE
15 IEFVFGLEPLERRDNYTKAEELLSRSIVKRWANFAKYGNPNETQNNSTSWPVFKSTEQK
YLTNLNTESTRIMTKLRAQQCRFWTSFFPKVDAHKSEVAHRFKDLGEENFKALVLIIFA
QYLQQCPFEDHVKLNEVTEFAKTCVADESAENCDSLHTLFGDKLCTVATLRETYGE
MADCCAKQEPEPERNECFLOHKDDNPNLRLVRPEVDVMCTAFHDNEETFLKKYLYEIA
RHPYFYAPELLFFAKRYKAAFTCCQAADKAACLLPKLDELDEGKASSAKQRLKCAS
20 LQKFGERAFAKAWAVARLSQRFPKAEFAEVSKLVTDLTQVHTECCHGDLLECADDRADL
AKYTCENQDSISSKLEKCEKPLEKSHCIAEVENDEMPADLPSLAADFVESKDVCKN
YAEAKDVFLGMFLYEYARRHPDYSVLLLRRLAKTYETTLKCCAAADPHECYAKVFDE
FKPLVEEPQNLKQNCLEFQELGEYKFQNALLVRYTKKVPQVSTPTLVEVSRNLGKVG
SKCKKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCTESLVNRRPCFSALE
25 VDETYVPKEFNAETFTFHADICTLSEKERQIKQTALVELVKHKPKATKEQLKAVMDD
FAAFVEKCKKADDKETCFAEEGKKLVAASQAALGL (SEQ ID NO:4).

In another embodiment of the method, the fusion protein is administered prior to the cocaine exposure.

30

In another embodiment, the fusion protein is administered up to one day prior to the cocaine exposure.

- 16 -

In another embodiment, the fusion protein is administered up to 216 hours prior to the cocaine exposure. The fusion protein may be administered 1, 2, 3, 4, 5, 6, 7, 8, or 9 days prior to the cocaine exposure.

5

In yet another embodiment of the method, the fusion protein is administered after the cocaine exposure. In a further embodiment, the fusion protein is administered up to six hours following the cocaine exposure. In another embodiment, the fusion protein is administered up to one hour after the cocaine exposure.

10

In yet another embodiment of the method, the cocaine exposure is a single cocaine exposure.

15

In yet another embodiment of the method, the cocaine exposure is a recurring cocaine exposure. In a further embodiment of the method, the recurring cocaine exposure is cocaine abuse or cocaine dependence.

20

In yet another embodiment of the method, the recurring cocaine exposure comprises at least six single cocaine exposures in a twelve month period.

25

In yet another embodiment of the method, the recurring cocaine exposure comprises at least twenty single cocaine exposures during the primate's lifetime.

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In yet another embodiment of the method, the biological effect is caused by cocaine overdose in the primate and the attenuating is treating or preventing the biological effect.

In yet another embodiment of the method, the biological effect is an increase in blood pressure.

- 17 -

In a further embodiment of the method, the duration of the increase in blood pressure is reduced by 60-90%.

- 5 In a further embodiment of the method, the duration of the increase in blood pressure is reduced by about 78%.

In yet another embodiment of the method, the biological effect is an increase in heart rate or body temperature. In a further
10 embodiment of the method, the degree of attenuation is 45%-70%.
In a further embodiment of the method, the degree of attenuation is 57%.

- 15 In yet another embodiment of the method, the biological effect is cocaine seeking behavior in the primate.

In yet another embodiment of the method, the cocaine seeking behavior occurs during a period of cocaine abstinence following the cocaine exposure.

- 20 In yet another embodiment of the method, the cocaine seeking behavior follows a relapse.

- 25 In yet another embodiment of the method, administration of the fusion protein two hours before the relapse attenuates cocaine seeking behavior by the primate immediately following the relapse.

- 30 In yet another embodiment of the method, administration of the fusion protein two hours before the cocaine exposure results in a 50% to 100% reduction in the cocaine seeking behavior by the primate.

- 18 -

In yet another embodiment of the method, attenuation of cocaine seeking behavior is observed up to four days following administration of the fusion protein.

- 5 In yet another embodiment of the method, the administration of the fusion protein results in a lowering of total cocaine exposure in the primate than without the administration.

- 10 In yet another embodiment of the method, attenuating cocaine seeking behavior results in a period of cocaine abstinence in the primate. In a further embodiment of the method, the period of abstinence is 2 weeks to 3 weeks.

- 15 In yet another embodiment of the method, attenuating cocaine seeking behavior results in a larger proportion of days in which the primate is not exposed to cocaine than without the administration.

- 20 In yet another embodiment of the method, attenuating cocaine seeking behavior results in a larger number of consecutive days in which the primate is not exposed to cocaine than without the administration.

- 25 In yet another embodiment of the method, attenuating cocaine seeking behavior results in a lessening of severity of cocaine dependence or abuse as evaluated by the cocaine selective severity assessment (CSSA) or Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV).

- 30 In yet another embodiment of the method, the effective amount of the fusion protein is an amount which reduces the primate's serum cocaine level to about 0 ng/ml within about 30 minutes of a 1 mg/kg intravenous cocaine dose.

- 19 -

In yet another embodiment of the method, the administration of the fusion protein reduces the primate's serum cocaine level to less than 12% of the serum cocaine level without the administration within about 5 minutes of a 1 mg/kg intravenous cocaine dose.

In yet another embodiment of the method, the administration of the fusion protein reduces the primate's serum cocaine level to 7% of the serum cocaine level without the administration within about 5 minutes of a 1 mg/kg intravenous cocaine dose.

In yet another embodiment of the method, the fusion protein is administered only once, daily, semi-weekly, weekly, bi-weekly, or monthly.

In another embodiment, the fusion protein is administered weekly or twice weekly.

In another embodiment, the fusion protein is administered as a single dose following the cocaine exposure.

In another embodiment, the fusion protein is administered as a single dose following a cocaine overdose.

In yet another embodiment of the method, the fusion protein is administered by intramuscular injection or subcutaneous injection.

In yet another embodiment of the method, the fusion protein is in a formulation buffer comprising 10mM sodium phosphate, 200mM mannitol, 60mM trehalose, and 0.01% (w/v) polysorbate 80, pH 7.2.

- 20 -

In yet another embodiment of the method, the fusion protein is present in the formulation at a concentration of at least 30 mg/ml.

- 5 In yet another embodiment of the method, attenuation of the biological effect is observed up to 72 hours after administration of the fusion protein.

10 In yet another embodiment of the method, the primate is a human.

In a further embodiment of the method, the cocaine exposure is a single cocaine exposure of 10 mg to 60 mg or a recurring cocaine exposure wherein each single cocaine exposure of the
15 recurring cocaine exposure is 10 mg to 60 mg.

In yet another embodiment of the method, the effective amount of the fusion protein is an amount which reduces the human's serum cocaine level to about 0 ng/ml within about 30 minutes of
20 a 40 mg intravenous cocaine dose.

In yet another embodiment of the method, the effective amount of the fusion protein is 0.06 mg/kg to 5 mg/kg. In another embodiment of the method, the effective amount of the fusion
25 protein is 0.06 mg/kg, 0.3 mg/kg, 1.6 mg/kg, or 4.8 mg/kg.

In yet another embodiment of the method, the effective amount of the fusion protein is 50 mg to 300 mg. In another embodiment of the method, the effective amount of the fusion protein is 50
30 mg, 100 mg, 150 mg, or 300 mg.

An embodiment of the BChE-albumin fusion protein comprising the amino acid substitutions A227S, S315G, A356W, and Y360G is shown in Figure 1 (SEQ ID NO: 4). The amino acid sequence

- 21 -

shown in Figure 1 comprises a heterologous signal peptide, shown by underlining, a BChE domain comprising amino acids E29 to V529 of human BChE, and human serum albumin (HSA), shown in italics. The amino acid substitutions A227S, S315G, A356W, and Y360G are shown in bold and are underlined in Figure 1. The numbering of the substitutions is relative to that of full length BChE. The protein encoded by the amino acid sequence of Figure 1 is referred to as "AlbuBChE" throughout this application.

10

It is understood that all combinations of the above described embodiments of the invention are within the scope of the invention.

15 As used herein, "primate" refers to any of an order of mammals that are characterized especially by advanced development of binocular vision, specialization of the appendages for grasping, and enlargement of the cerebral hemispheres and that include humans, apes, monkeys, and related forms.

20

As used herein, "degree of attenuation" refers to the decrease in a biological effect of cocaine exposure that is observed following administration of a BChE-albumin fusion protein as compared to the biological effect of cocaine exposure observed in the absence of the BChE-albumin fusion protein. The degree of attenuation is calculated by the following formula:

$$\text{degree of attenuation} = \frac{\Delta_{\text{BChE-albumin absent}} - \Delta_{\text{BChE-albumin present}}}{\Delta_{\text{BChE-albumin absent}}}$$

30

For example, if cocaine exposure raises a baseline temperature of 38°C to 38.7°C in the absence of a BChE-albumin fusion protein, and cocaine exposure raises a baseline temperature of

35

- 22 -

38°C to 38.3°C in the presence of a BChE-fusion protein, the degree of attenuation is 57.1% $((0.7^{\circ}\text{C} - 0.3^{\circ}\text{C})/0.7^{\circ}\text{C})$.

As used herein, "a single cocaine exposure" refers to one exposure of cocaine isolated from any other exposure of cocaine. "A recurring cocaine exposure" refers to more than one single cocaine exposure. The recurring cocaine exposure may be a regular or an irregular pattern of single cocaine exposures beginning with the second or subsequent single cocaine exposure in the subject. An individual experiencing recurring cocaine exposure may meet the criteria for cocaine dependence or cocaine abuse of the Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV).

As used herein, the term "total cocaine exposure" refers to the aggregate cocaine exposure during a given time interval. Total cocaine exposure may be measured during or after a period of a treatment designed to attenuate cocaine seeking behavior or other biological effect of cocaine exposure.

As used herein, the term "a period of cocaine abstinence" refers to a period of time following cocaine exposure where the primate does not experience a new cocaine exposure.

As used herein, the term "relapse" refers to a cocaine exposure following a period of cocaine abstinence.

It is understood that where a parameter range is provided, all tenths of integers within that range are provided by the invention. For example, "0.2 mg/kg to 15 mg/kg" includes 0.2 mg/kg, 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg etc. up to and including 15.0 mg/kg.

- 23 -

An animal dose may be converted to a human equivalent dose (HED) by using the conversion table found in the publication "Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers," U.S. Department of Health and Human Services, Food and Drug Administration Center for Drug Evaluation and Research (CDER), July 2005. Doses for cynomolgus monkeys in mg/kg may be converted to an HED in mg/kg by dividing the cynomolgus monkey dose by 3.1. Doses for squirrel monkeys in mg/kg may be converted to an HED in mg/kg by dividing the squirrel monkey dose by 5.3.

List of Abbreviations

15	AUC _(0-t)	Area under the plasma concentration-time curve from time zero up to time of last detectable concentration (tz)
	AUC _(0-∞)	Area under the plasma concentration-time curve from time zero to infinity
20	%AUC _{ex}	Percentage of AUC that is due to extrapolation from tz to infinity
	BChE	butyrylcholinesterase
	BQL	Below Quantitation Limit
	CL	clearance
	C _{max}	maximum concentration
25	CocH	cocaine hydrolase
	ELISA	enzyme linked immunosorbent assay
	GFR	glomerular filtration rate
	hr	hour(s)
	HRP	horseradish peroxidase
30	IM	intramuscular
	IV	intravenous
	LLOQ	lower limit of quantitation
	min	minute(s)
	NCA	noncompartmental analysis
35	NIDA	National Institute on Drug Abuse
	PBS	phosphate buffered saline
	PD	pharmacodynamics
	PK	pharmacokinetics
	RT	room temperature
40	Rsq	Coefficient of determination
	%RSD	Percent Relative Standard Deviation
	SD	Standard Deviation
	SC	subcutaneous
	SOP	standard operating protocol

- 24 -

$t_{1/2}$	half-life of the terminal phase
t_{max}	time of maximum concentration
V_z	volume of distribution from the terminal phase

- 5 The BChE-albumin fusion protein shown in Figure 1 (AlbuBChE) was applied to trials as described in the examples below.

- 25 -

**Example 1 - A Pharmacokinetic and Pharmacodynamic Study
Following a Single Intramuscular Administration of AlbuBChE and
Multiple Intravenous Doses of Cocaine to Cynomolgus Monkeys**

5 Objectives

The objective of this trial was to determine the pharmacokinetic (PK) profile of AlbuBChE in male cynomolgus monkeys following a single intramuscular administration at 0.2, 1 or 5 mg/kg AlbuBChE dose levels and to determine AlbuBChE
10 activity as a function of time by intravenous administration of 1 mg/kg dose of cocaine at 2, 48, 96, 120, and 240 hours after AlbuBChE dose administration.

Rationale

15 AlbuBChE is a fusion protein of human serum albumin (HSA) and a genetically modified form of human butyrylcholinesterase (BChE) that exhibits high catalytic efficiency for the hydrolysis of cocaine to benzoic acid. AlbuBChE is under development as a potential intervention in preventing relapse to drug-seeking
20 behavior. Fusion of a protein to albumin has been shown to improve the pharmacokinetic properties of the protein by reducing the clearance and extending the half-life. A longer half-life is expected to translate into a longer dosing interval and better compliance with a drug regimen.

25

Rationale for Species Selection

Cynomolgus monkeys (*macaca mulatta*) were selected for this study based on anatomical, physiological, and biochemical similarities to humans, which may facilitate extrapolation of
30 observed pharmacokinetic and pharmacodynamic properties to humans. Monkeys are known to express butyrylcholinesterase, which is a component of this drug. Monkeys are also physiologically responsive to cocaine.

- 26 -

Rationale for Dose Level and Route

In a previous study, AlbuBChE pharmacokinetic profile was evaluated in cynomolgus monkeys. In that study, the test article was administered SC (7.8, 2.4 and 0.78 mg/kg), IM (2.4 mg/kg) and IV (2.4 mg/kg). The pharmacokinetic properties of AlbuBChE were linear throughout the SC doses measured. The choice of IM for this study was based on the greater bioavailability in IM as compared to SC (79% and 35-39% respectively) observed in the previous study. In addition, AlbuBChE IM dose levels of 0.2, 1 and 5 mg/kg were selected to define AlbuBChE pharmacodynamic range in cynomolgus monkeys.

The dose of cocaine at 1 mg/kg IV was selected as a dose sufficiently high to yield a physiologically relevant response and achieve measureable concentration of cocaine in blood, while not excessively stimulating the animals.

The number of animals in each group is the minimum number of animals per group necessary for assessment of inter animal variability. As this is a pilot study, only one sex (males) was evaluated.

Test Solutions

The test article, AlbuBChE, was stored in a stock solution as a frozen liquid formulation containing 29.9 mg/mL at $-70 \pm 15^{\circ}\text{C}$. Prior to dosing, the test article formulation was thawed at room temperature. When the test article formulation was completely thawed, the container was mixed by gentle inversion and diluted with the appropriate volume of test article diluent to achieve a concentration of 20 mg/mL. This formulation was serially diluted with the test article diluent to achieve concentrations of 4 and 0.8 mg/mL.

- 27 -

Cocaine hydrochloride, the pharmacodynamic test article, was purchased from Sigma. Dose levels were expressed as the hydrochloride salt.

5 **Experimental Design**

The study included a total of 11 naive, adult male cynomolgus monkeys divided into four dose groups:

10 a control group of two cynomolgus monkeys that only received a single IV dose of cocaine;

15 three dose groups that were treated with a single IM dose of AlbuBChE at 0.2, 1 or 5 mg/kg. Three naive, adult male cynomolgus monkeys were used in each dose group. Following AlbuBChE treatment, a 1 mg/kg IV dose of cocaine was administered at 2, 48, 96, 120, and 240 hours after AlbuBChE dose administration.

20 A summary of the dose designation and dose levels can be found in Table 1. Individual doses were calculated based on body weights recorded on the day of dose administration. Animals were not fasted prior to dose administration.

25

30

Table 1: Group Designations and Dose Levels

Group	Treatment	AlbuBChE Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals
1	Single IV dose of Cocaine	1	1.0	1	2
2	Single IM dose of AlbuBChE	0.2	0.8	0.25	3
	Single IV dose of Cocaine at 2, 48, 96, 120, and 240 hours after AlbuBChE dose administration ¹	1	1.0	1	
3	Single IM dose of AlbuBChE	1	4	0.25	3
	Single IV dose of Cocaine at 2, 48, 96, 120, and 240 hours after AlbuBChE dose administration ¹	1	1.0	1	
4	Single IM dose of AlbuBChE	5	20	0.25	3
	Single IV dose of Cocaine at 2, 48, 96, 120, and 240 hours after AlbuBChE dose administration ¹	1	1.0	1	

¹All times will be within ± 5 minutes of the listed times**5 Samples Collection for AlbuBChE**

A single intramuscular (IM) administration of AlbuBChE was administered on study day 1 and blood was collected at the time points outlined in Table 2. Approximately 0.5 mL of whole blood was collected from the femoral vein into serum separate

10 tubes (SST).

Table 2: Pharmacokinetics for AlbuBChE

Time Point	Sample Volume	Collection Device
Pre-dose, 1, 3, 6, 12, 24, 36, 48, 72, 96, 128, 144, 168, 216, and 264 hours post dose	~0.5 mL	SST

15 Samples Collection for Cocaine

Approximately 0.5 mL of whole blood was collected from the femoral vein at 5, 10, 15, 20, 30, 40 and 60 minutes following

- 29 -

each dose of cocaine (Study Day 1 of the control group and Study Day 1 (2hr), 3 (48 hr), 5 (96 hr), 6 (120 hr), and 11 (240 hr) post-AlbuBChE dose administration). Blood was placed into K2EDTA blood collection tubes with esterase inhibitor
5 diisopropylfluorophosphate (DFP). Tubes were inverted several times and placed on wet ice upon collection. Samples were centrifuged at 2-8°C within 45 minutes of collection. The resultant plasma was recovered and a single 200 µL aliquot of plasma was placed into polypropylene tubes. Plasma samples were
10 frozen over dry ice and stored at -75 ± 15°C.

Assay Methods

The following outline describes the ELISA-based assay employed in the measurement of AlbuBChE concentrations in monkey serum
15 samples obtained pre-administration and at different times after administration.

Immulon 4 HBX plates are coated with 100 µL of anti-BChE mAb 002-01 (Abcam ab17246) at 1 µg/mL in PBS, overnight at 4 °C.
20 Blocking is done with 2% Casein in 1x PBS, 200 µL/well, 2 hours at room temperature. After washing, 100 µL of diluted serum samples are added to the plates along with standards. Standards are generated through 3.64 fold, serial dilution of AlbuBChE from 2000 to 3.1 ng/mL. Serum samples and standards are
25 maintained at 10% serum by dilution with buffer containing pooled cynomolgus monkey serum. A wash step precedes detection with 100 µL of anti-HSA mAb-6502-HRP at 0.04 µg/mL for 1 hour. Wells are washed again, prior to developing with 100 µL of TMB substrate. After 15 minutes, the reaction is terminated with
30 100 µL/well of 1N H2SO4 and read on SpectraMax plate reader at 450/570 nm. Values for unknown serum samples are calculated by interpolation of standard curve generated by 4-parameter fit of AlbuBChE standards. The limit of quantitation in cynomolgus serum was 21.1 ng/mL.

- 30 -

LC-MS Quantitation of Cocaine in Serum

The following outline describes the LC-MS-based assay employed for the measurement of cocaine in plasma samples. Prior to MS analysis, plasma samples, calibration standards and controls were extracted using supported liquid extraction (SLE-ISOLUTE, Biotage). Twenty-five microliters of samples (25 μ L) received 150 μ L of 5% Ammonium hydroxide and 25 μ L internal standard solution (Cocaine-d3). After mixing and transfer to SLE plate, samples were eluted with methylene chloride and evaporated to dryness. After resuspension in 50 μ L of reconstitution solution - 5% ACN in 10 mM ammonium acetate in water, 10 μ L was injected onto Thermo Hypercarb column at 0.5 ml/min. Mobile phase was biphasic. Mass spectrophotometric detection was performed using Api 4000, APCI positive interface and Multiple Reaction Monitoring (MRM). Plasma concentrations of cocaine, benzoylecgonine and ecgonine methyl ester were determined using this assay.

PK Analysis of AlbuBChE

AlbuBChE pharmacokinetic parameter values were determined using serum concentration-time profiles for individual animals. The computer software WinNonlin Professional (Version 4.0.1 Pharsight Corporation, USA) was used. Specifically the model for non-compartmental analysis with extravascular input was applied. If there were fewer than 3 data points in the terminal phase of the serum concentration curve, a terminal phase half-life and PK parameters derived from the half-life were not calculated for that profile. Parameters analyzed include t_{max} , C_{max} , $t_{1/2}$, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ were calculated.

PK Analysis of Cocaine

When the data permits, pharmacokinetic parameter values for cocaine and its metabolites were determined using plasma

- 31 -

concentration-time profiles for individual animals at all the study days of cocaine administration. The computer software WinNonlin Professional (Version 4.0.1 Pharsight Corporation, USA) was used. Specifically the model for non-compartmental analysis with IV bolus input for cocaine and extravascular input for the metabolites. If there were fewer than 3 data points in the terminal phase of the plasma concentration curve, a terminal phase half-life and PK parameters derived from the half-life were not calculated for that profile.

10

PK/PD Analysis

AlbuBChE PK/PD relationship was defined using the computer software WinNonlin Professional (Version 4.0.1 Pharsight Corporation, USA). Specifically the direct sigmoidal inhibitory Effect E_{max} model was used in which maximum effect (E_{max}) as measured by cocaine AUC was assumed at AlbuBChE concentration of zero. The model equation can be described as follows:

Inhibitory Effect Sigmoid E_{max} , $C = 0$ at E_{max} , $C = \text{infinity}$ at E_0 .

20

$$E = E_{max} - (E_{max} - E_0) * (C^{**Gamma} / (C^{**Gamma} + EC50^{**Gamma}))$$

The sigmoidal model was selected due to the higher activity observed at the 48 hr timepoint compared to the 2 hr timepoint.

25

The PD parameter used in the analysis was cocaine AUC. PK parameters used in the model were AlbuBChE plasma concentrations at 2, 48, 96, 120, and 240 hr post-AlbuBChE dose. Due to the fact that AlbuBChE concentration was not measured at 2 hr post-AlbuBChE dose, the concentration at 3 hr postdose was used in the analysis with the assumptions that AlbuBChE concentration at 3 hr post AlbuBChE dose may reflect AlbuBChE conc. at 2 hr post AlbuBChE dose.

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- 32 -

Statistical Methods

Summary statistics of the concentration profiles and PK parameter values by experimental group were calculated using the descriptive statistics function in WinNonlin. Statistical parameters reported are N, mean, SD and percent coefficient of variation (%CV).

Results**AlbuBChE Concentration Profile**

AlbuBChE concentration-time data and summary statistics are listed in Tables 3 and 4. Individual animal AlbuBChE serum concentration-time profiles following a single AlbuBChE IM injection of 0.2, 1 or 5 mg/kg are shown in Figure 2. Mean serum concentration-time profiles for the three AlbuBChE dose groups are shown in Figure 3.

Following IM injection, all animals had measurable AlbuBChE concentrations. Inter-animals variability per dose group appears to be reasonable as indicated by %CV that ranged from 8.2 to 78.4 for all time points. AlbuBChE serum concentration increased with increasing dose.

- 33 -

Table 3: Individual and Mean AlbuBChE Serum Concentration (ng/mL) vs. time (hr) Profile in Cynomolgus Monkeys following a single IM administration of 0.2, 1 or 5 mg/kg/animal AlbuBChE dose.

5

Time (hr)	AlbuBChE Dose: 0.2mg/kg IM			Mean (ng/mL)	SD	%CV
	17691	17692	17693			
0	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--
1	37.1	235.4	111.0	128	100	78.4
3	176.1	278.5	280.7	245	59.8	24.4
6	144.0	212.1	272.4	210	64.2	30.7
12	88.4	143.1	197.9	143	54.8	38.3
24	52.3	95.2	135.7	94.4	41.7	44.2
36	29.8	68.3	87.4	61.8	29.3	47.4
48	<LLOQ	66.4	80.7	73.6	n=2	--
72	<LLOQ	44.5	39.5	42.0	n=2	--
96	<LLOQ	30.1	<LLOQ	<LLOQ	--	--
128	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--
144	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--
168	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--
216	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--
264	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--

Time (hr)	AlbuBChE Dose: 1.0 mg/kg IM			Mean (ng/mL)	SD	%CV
	17694	17695	17696			
0	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--
1	1058.9	462.4	1598.6	1040	568	54.6
3	1665.5	1374.0	1956.0	1665	291.0	17.5
6	1269.3	1266.3	1596.1	1377	189.6	13.8
12	884.3	968.1	1049.5	967	82.6	8.5
24	630.1	673.8	572.1	625	51.0	8.2
36	373.4	471.7	348.2	398	65.3	16.4
48	289.4	362.7	264.4	306	51.1	16.7
72	122.1	235.0	133.0	163	62.3	38.1
96	87.0	131.7	75.3	98.0	29.8	30.4
128	36.2	72.0	38.1	48.8	20.1	41.3
144	27.4	47.7	31.5	35.5	10.7	30.2
168	<LLOQ	39.5	<LLOQ	<LLOQ	--	--
216	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--
264	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--

10

- 34 -

Time (hr)	5.0 mg/kg IM			Mean (ng/mL)	SD	%CV
	17697	17698	17699			
0	<LLOQ	<LLOQ	<LLOQ	<LLOQ	--	--
1	2580.0	6570.9	3821.0	4324	2042	47.2
3	4899.0	12683.9	6024.0	7869	4207.6	53.5
6	6016.0	17686.8	6530.3	10078	6594.7	65.4
12	4178.0	11105.8	5091.0	6792	3764.0	55.4
24	2836.0	5034.0	3325.0	3732	1154.1	30.9
36	1572.0	2929.0	1901.0	2134	707.9	33.2
48	1329.7	2644.4	1604.9	1860	693.4	37.3
72	637.7	1190.8	723.6	851	297.7	35.0
96	415.1	732.6	454.7	534.1	173.0	32.4
128	217.2	325.9	224.3	255.8	60.8	23.8
144	158.4	254.7	163.3	192.1	54.2	28.2
168	118.9	165.5	130.4	138.3	24.3	17.6
216	73.7	91.6	79.4	81.6	9.14	11.2
264	41.9	50.9	47.2	46.7	4.52	9.69

-- Not applicable
LLOQ 27.2 ng/ml

5 **Table 4: Summary of Mean AlbuBChE Serum Concentration (ng/ml) vs. time (hr) in Cynomolgus Monkeys following a single IM administration of 0.2, 1, or 5 mg/kg/animal AlbuBChE dose.**

Time (hr)	0.2 mg/kg	1 mg/kg	5 mg/kg
1	128	1040	4324
3	245	1665	7869
6	210	1377	10078
12	143	967	6792
24	94.4	625	3732
36	61.8	398	2134
48	73.6	306	1860
72	42.0	163	851
96	--	98.0	534
128	--	48.8	256
144	--	35.5	192
168	--	--	138
216	--	--	81.6
264	--	--	46.7

-- Not applicable
LLOQ 27.2 ng/ml

- 35 -

Concentration Profile of Cocaine, Benzoylecgonine and Ecgonine methyl ester

LC/MS analysis was performed on all samples to analyze for cocaine, benzoylecgonine and ecgonine methyl ester levels in monkey plasma. Mean plasma concentration-time profiles in control animals and following 0.2, 1, or 5 mg/kg can be found in Tables 5, 6, and 7 and Figures 4, 5 and 6 for cocaine, benzoylecgonine and ecgonine methyl ester, respectively.

In general, cocaine plasma concentrations appear to decrease as a function of AlbuBChE dose and increase as a function of time post-AlbuBChE administration. Cocaine concentrations at 240 hr post-AlbuBChE administration appears to be in the same range as Day 1 cocaine control group (Figure 7). This suggests that AlbuBChE was not active at 240 hr post-AlbuBChE administration of 0.2, 1 or 5 mg/kg. This also indicates that cocaine kinetics do not show time dependent kinetics in cynomolgus monkeys making the comparison of Day 1 single IV dose cocaine control to the multiple dose cocaine profile following AlbuBChE administration feasible.

As one might expect based on cocaine metabolic pathway (Figure 9), benzoylecgonine plasma concentrations appear to decrease as a function of AlbuBChE dose and increase as a function of time post-AlbuBChE administration.

Theoretically, plasma concentration of ecgonine methyl ester should increase following AlbuBChE administration (Figure 9). This increase was only evident at the cocaine dose administered at 2 hr post-AlbuBChE administration for all three AlbuBChE dose levels. Ecgonine methyl ester plasma concentration could not be easily distinguished from control following cocaine administration at 48, 96, 120 or 240 hr post-AlbuBChE administration.

- 36 -

Table 5: Mean Cocaine Plasma Concentration (ng/mL) vs. time (min) in Cynomolgus monkeys following cocaine IV dose of 1 mg/kg in control animals (n=2) or at 2, 48, 72, 96, 120 and 240 hr post-AlbuBChE IM dose of 0.2, 1, or 5 mg/kg (n=3 per dose group).

Control and 0.2 mg/kg AlbuBChE Dose

Time (min)	Control Day 1	2 hr	48 hr	96 hr	120 hr	240 hr
5	371	481	235	360	349	453
10	393	370	203	301	312	379
15	305	273	187	255	267	297
20	251	227	146	189	218	254
30	226	67.0	121	165	160	189
40	188	115	77.8	118	141	133
60	138	58.6	43.2	60.5	64.7	89.5

Control and 1 mg/kg AlbuBChE Dose

Time (min)	Control	2 hr	48 hr	96 hr	120 hr	240 hr
5	371	308	176	265	356	397
10	393	235	140	245	263	340
15	305	179	152	218	233	300
20	251	73.7	128	208	202	259
30	226	102	87.0	162	161	218
40	188	96.9	62.3	123	141	181
60	138	19.7	29.1	64.8	79.7	117

Control and 5 mg/kg AlbuBChE Dose

Time (min)	Control	2hr	48 hr	96 hr	120 hr	240 hr
5	371	145	126	176	298	346
10	393	77.9	89.9	166	217	283
15	305	43.5	82.7	174	216	283
20	251	12.8	49.5	136	161	226
30	226	110	36.7	99.0	139	211
40	188	4.96	18.9	80.6	113	161
60	138	2.26	5.74	35.2	49.5	111

- 37 -

Table 6: Mean Benzoylecgonine Plasma Concentration (ng/mL) vs. time (min) in Cynomolgus monkeys following cocaine IV dose of 1 mg/kg in control animals (n=2) or at 2, 48, 72, 96, 120 and 240 hr post-AlbuBChE IM dose of 0.2, 1, or 5 mg/kg (n=3 per dose group).

Control and 0.2 mg/kg AlbuBChE Dose

Time (min)	Day 1 Control	2 hr	48 hr	96 hr	120 hr	240 hr
5	47.6	50.6	27.3	49.7	55.1	53.5
10	65.4	49.7	29.4	47.7	46.9	53.3
15	63.0	50.4	29.0	52.6	53.1	62.9
20	72.0	65.1	28.2	55.1	55.9	63.7
30	82.4	28.6	37.1	56.5	64.2	69.8
40	87.2	58.0	36.9	55.2	61.4	74.5
60	96.9	60.5	35.6	57.8	66.2	80.9

Control and 1 mg/kg AlbuBChE Dose

Time (min)	Day 1 Control	2 hr	48 hr	96 hr	120 hr	240 hr
5	47.6	30.1	24.0	43.9	37.2	40.0
10	65.4	33.2	23.7	41.2	38.9	47.2
15	63.0	32.5	24.9	40.0	42.2	55.0
20	72.0	32.5	26.7	43.7	40.2	56.7
30	82.4	28.9	29.8	47.4	51.3	64.6
40	87.2	38.4	29.6	46.7	50.9	68.3
60	96.9	29.6	29.8	49.6	54.4	75.2

Control and 5 mg/kg AlbuBChE Dose

Time (min)	Day 1 Control	2 hr	48 hr	96 hr	120 hr	240 hr
5	47.6	22.3	20.0	44.2	44.6	44.9
10	65.4	23.7	21.0	43.5	47.5	56.4
15	63.0	22.0	24.4	49.7	50.3	71.9
20	72.0	22.9	23.7	51.1	57.3	83.4
30	82.4	31.1	25.9	56.1	63.6	88.4
40	87.2	21.3	25.5	57.7	69.5	101
60	96.9	20.1	24.4	60.5	69.2	108

- 38 -

Table 7: Ecgonine Methyl Ester Plasma Concentration (ng/mL) vs. time (min) in Cynomolgus monkeys following cocaine[®] IV dose of 1 mg/kg in control animals (n=2) or at 2, 48, 72, 96, 120 and 240 hr post-AlbuBChE IM dose of 0.2, 1, or 5 mg/kg (n=3 per dose group).

Control and 0.2 mg/kg AlbuBChE Dose

Time (min)	Day 1 Control	2 hr	48 hr	96 hr	120 hr	240 hr
5	24.4	51.6	21.1	26.4	18.9	20.1
10	34.5	81.9	19.5	39.4	28.9	23.1
15	57.4	83.2	23.9	24.5	27.6	25.1
20	39.0	154	25.8	29.8	34.5	28.3
30	77.6	261	33.9	49.8	136	33.9
40	60.8	120	50.7	50.2	98	38.8
60	80.9	120	37.0	43.4	82.9	41.4

10

Control and 1 mg/kg AlbuBChE Dose

Time (min)	Day 1 Control	2 hr	48 hr	96 hr	120 hr	240 hr
5	24.4	235	201	138	42.7	92.7
10	34.5	227	32.2	39.8	27.4	21.9
15	57.4	276	48.2	42.9	37.8	25.9
20	39.0	301	56.2	47.3	31.8	29.5
30	77.6	291	63.8	57.3	104	41.3
40	60.8	202	64.9	64.4	68.6	51.8
60	80.9	189	57.3	59.9	76.8	54.3

Control and 5 mg/kg AlbuBChE Dose

Time (min)	Day 1 Control	2 hr	48 hr	96 hr	120 hr	240 hr
5	24.4	337	119	100	45.5	127
10	34.5	321	68.9	60.9	46.0	26.1
15	57.4	363	102	82.2	96.5	30.7
20	39.0	331	107	104	60.1	35.1
30	77.6	266	108	111	79.9	49.6
40	60.8	308	106	126	122	53.1
60	80.9	248	108	127	187	58.0

15 Pharmacokinetic Analysis of AlbuBChE

AlbuBChE pharmacokinetic parameter values following a single IM dose of 0.2, 1 or 5 mg/kg AlbuBChE dose in individual animals

- 39 -

and descriptive statistics per group can be found in Table 8. Mean PK parameter values for AlbuBChE following IM dosing at 0.2, 1 or 5 mg/kg are summarized in Table 9.

- 5 In general absorption from IM site of administration was rapid with measureable concentrations observed at the first sample collected (1 hr postdose). Maximum concentration was observed at 3 hr for the 0.2 and 1 mg/kg dose level. T_{max} values was slightly longer (6 hr) for the 5 mg/kg dose.
- 10 AlbuBChE exposure increased with increasing dose. Dose normalized C_{max} and AUC values appear to increase as a function of dose suggesting a more than proportional increase in exposure as a function of dose. This was also accompanied with
- 15 a increase in terminal elimination $t_{1/2}$ as a function of dose particularly between 1 and 5 mg/kg dose levels where $t_{1/2}$ value almost doubled.

20 **Table 8: Individual and Mean AlbuBChE Pharmacokinetic Parameter Values in Cynomolgus Monkeys following a single IM administration of 0.2, 1 or 5 mg/kg/animal AlbuBChE dose.**

0.2 mg/kg AlbuBChE Dose								
Animal #	R_{sq}	No. Points Lambda-z	$t_{1/2}$ (hr)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(0-4)}$ (hr*ng/mL)	$AUC_{(0-\infty)}$ (hr*ng/mL)	AUC %Extrap
17691	1.00	3	15.3	3	176	2746	3404	19.3
17692	1.00	3	42.1	3	279	7878	9704	18.8
17693	0.980	7	24.4	3	281	8479	9867	14.1
Mean			27.2		245	6368	7658	17.4
SD			13.6		59.8	3151	3686	2.90
%CV			50.0		24.4	49.5	48.1	16.7

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- 40 -

1 mg/kg AlbuBChE Dose

Animal #	R _{sq}	No. Points Lambda-z	t _{1/2} (hr)	T _{max} (hr)	C _{max} (ng/mL)	AUC _(0-t) (hr*ng/mL)	AUC _(0-∞) (hr*ng/mL)	AUC %Extrap
17694	0.990	6	28.4	3	1666	43128	44251	2.54
17695	0.992	8	34.3	3	1374	51298	53255	3.67
17696	0.992	6	30.3	3	1956	46185	47564	2.90
Mean			31.0		1665	46871	48357	3.04
SD			3.03		291	4128	4554	0.581
%CV			9.76		17.5	8.81	9.42	19.1

5 mg/kg AlbuBChE Dose

Animal #	R _{sq}	No. Points Lambda-z	T _{1/2} (hr)	T _{max} (hr)	C _{max} (ng/mL)	AUC _(0-t) (hr*ng/mL)	AUC _(0-∞) (hr*ng/mL)	AUC %Extrap
17697	0.999	4	63.4	6	6016	201754	205584	1.86
17698	1.00	3	56.4	6	17687	437848	441992	0.938
17699	1.00	3	65.5	6	6530	235983	240442	1.85
Mean			61.8		10078	291862	296006	1.55
SD			4.73		6595	127581	127623	0.532
%CV			7.66		65.4	43.7	43.1	34.3

5

Table 9: Mean Pharmacokinetic parameter values for AlbuBChE following a single IM injection in cynomolgus monkeys at 0.2, 1 or 5 mg/kg.

AlbuBChE Dose	0.2 mg/kg IM	1.0 mg/kg IM	5.0 mg/kg IM
t _{1/2} (hr)	27.2	31.0	61.8
t _{max} (hr)	3	3	6
C _{max} (ng/mL)	245	1665	10078
AUC _(0-t) (hr*ng/mL)	6368	46871	291862
AUC _(0-∞) (hr*ng/mL)	7658	48357	296006
AUC %Extrap	17.4	3.04	1.55
Dose Normalized C _{max}	1226	1665	2016
Dose Normalized AUC _(0-∞)	38292	48357	59201

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- 41 -

Pharmacokinetic Analysis of Cocaine and metabolites

Summary table of mean cocaine pharmacokinetic values per dose group are presented in Table 10.

5 Cocaine pharmacokinetic profile was well characterized for all dose groups and time points with one exception: plasma concentration-time profile for the cocaine dose administered at 2 hr post-AlbuBChE dose was variable for all dose groups. As such, the terminal elimination $t_{1/2}$ could not be accurately
10 characterized in most of the animals on this cocaine dosing occasion.

Cocaine $AUC_{(0-t)}$ appears to decrease as a function of AlbuBChE dose and increase as a function of time post-AlbuBChE
15 administration. Similarly, cocaine systemic plasma clearance appears to increase as a function of AlbuBChE dose and return to control values as a function of time post-AlbuBChE dose. At 240 hr post-AlbuBChE administration cocaine AUC and clearance appear to be in the same range as Day 1 cocaine control group.

20 For all three AlbuBChE dose levels, maximum AlbuBChE effect on cocaine clearance or AUC was observed at 48 hr postdose. The duration of effect was related to AlbuBChE dose levels. Following the 5 mg/kg dose, AlbuBChE effect on cocaine AUC and
25 clearance was evident up to 120 hr postdose. At the 1 mg/kg dose, AlbuBChE effect on cocaine AUC or clearance may still be evident at 96 to 120 hr post-AlbuBChE dose relative to control or the 240 hr values. As AlbuBChE dose decreased to 0.2 mg/kg, so did the duration of effect with 48 hr being the last time
30 point with elevated cocaine clearance. Based on this data, a once or twice weekly dosing regimen of AlbuBChE is likely.

As can be observed in Figure 5, the concentration-time profile of benzoylecgonine did not include a terminal

- 42 -

elimination phase. As such, pharmacokinetic characterization of benzoylecgonine was limited to characterization of $AUC_{(0-t)}$. Summary table of mean AUC values as a function of AlbuBChE dose and time post-AlbuBChE dose in comparison with cocaine control
5 group can be found in Table 11. Consistent with cocaine metabolic pathway (Figure 9), benzoylecgonine AUC decreased as a function of AlbuBChE dose and increase as a function of time post-AlbuBChE administration as shown in Figure 11.

10 As can be observed in Figure 6, the concentration-time profile of ecgonine methyl ester was flat and did not include a terminal elimination phase. As such, no pharmacokinetic characterization of ecgonine methyl ester was conducted. Theoretically, ecgonine methyl ester AUC should increase
15 following AlbuBChE administration (Figure 9). This increase was only evident at the cocaine dose administered at 2 hr post-AlbuBChE administration for all three AlbuBChE dose levels. AlbuBChE effect on ecgonine methyl ester could not be easily distinguished from control following cocaine administration at
20 48, 96, 120 or 240 hr post-AlbuBChE administration.

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Table 10: Mean cocaine pharmacokinetic parameter values in Cynomolgus Monkeys following 1 mg/kg cocaine IV dose in control animals (n=2) or at 2, 48, 72, 96, 120 and 240 hr post-AlbuBChE IM dose of 0.2, 1, or 5 mg/kg (n=3 per dose group).

5

Study Time (hr)	AlbuBChE Dose (mg/kg)	t _{1/2} (min)	C ₀ (ng/mL)	AUC _(0-t) (min*ng/mL)	AUC _(0-∞) (min*ng/mL)	AUC % Extrap	Cl (mL/min/kg)	V _{ss} (mL/kg)
Day 1	Control	41.8	371	14604	23407	35.4	48.5	2679
2	0.2	NA	628	11878	NA	NA	NA	NA
48	0.2	21.2	274	7713	9094	15.1	110	3503
96	0.2	21.1	451	11145	13008	14.9	78.1	2506
120	0.2	23.0	391	11611	14059	16.1	72.5	2338
240	0.2	29.2	549	13693	17650	21.6	57.4	2181
2	1	NA	422	7880	NA	NA	NA	NA
48	1	19.0	226	5965	6764	11.9	150	4304
96	1	23.9	288	10044	12313	18.4	81.6	2940
120	1	28.6	491	11526	14838	22.4	67.4	2687
240	1	31.0	467	14364	19623	26.7	51.1	2288
2	5	NA	271	3289	NA	NA	NA	NA
48	5	11.4	177	3015	3110	3.0	325	5729
96	5	21.3	190	6624	7729	14.2	129	4111
120	5	22.3	411	9378	11046	14.5	93.0	2857
240	5	33.8	424	12952	18578	29.2	55.6	2637

NA Not applicable or not determined because data did not permit.

Table 11: Summary Table of Mean Benzoylcegonine AUC_(0-t) (min*ng/mL) in control animals and as a function of time post-AlbuBChE dose.

10

	2 hr	48 hr	96 hr	120 hr	240 hr
Control	4641	--	--	--	--
0.2 mg/kg	3136	1989	3270	3583	4089
1 mg/kg	1960	1668	2741	2809	3660
5 mg/kg	1405	1441	3222	3624	5088

- 44 -

PK/PD Analysis

Based on the mechanism of action of AlbuBChE it was anticipated that a direct effect inhibitory Emax model would be able to characterize the PK/PD relationship of AlbuBChE and cocaine.

5

PK and PD data used in the analysis are shown in Table 12. The fit resulting from the direct Sigmoidal inhibitory Effect Emax model are shown in Figure 8.

10 The data clearly shows the inverse relationship between AlbuBChE serum concentration and cocaine exposure. AlbuBChE serum concentration that may result in 50% decrease in cocaine concentration (EC50) was estimated by the model to be ~600 ng/mL. E_{max} , E_0 , and Gamma values were 12909 (min*ng/mL), 1096
15 (min*ng/mL), and 0.708, respectively.

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Table 12: AlbuBChE PK/PD analysis Cynomolgus Monkeys following a single IM administration of 0.2, 1 or 5 mg/kg AlbuBChE dose. Cocaine was administered IV at a dose of 1 mg/kg in control animals or at 2, 48, 96, 120 and 240 hr post-AlbuBChE dose.

5

PK parameters: AlbuBChE plasma concentrations per animals at 3[#], 48, 96, 120 and 240 hr post-AlbuBChE dose.

PD parameter: cocaine AUC₍₀₋₄₎ per animal at 2, 48, 96, 120 and 240 hr post-AlbuBChE dose.

Animal ID	AlbuBChE IM Dose (mg/kg)	Time (hr)	AlbuBChE Conc. (ng/mL)	Cocaine AUC ₍₀₋₄₎ (min*ng/mL)	Animal ID	AlbuBChE IM Dose (mg/kg)	Time (hr)	AlbuBChE Conc. (ng/mL)	Cocaine AUC ₍₀₋₄₎ (min*ng/mL)
17689	0	2	0	18335	17694	1	96	87	9166
17690	0	2	0	10872	17695	1	96	132	9925
17691	0.2	2	107	13317	17696	1	96	75	11042
17692	0.2	2	257	10812	17697	5	96	415	6428
17693	0.2	2	196	11504	17698	5	96	733	6887
17694	1	2	1362	6597	17699	5	96	455	6556
17695	1	2	918	9746	17691	0.2	120	0	11293
17696	1	2	1777	7297	17692	0.2	120	0	11428
17697	5	2	3740	4594	17693	0.2	120	0	12111
17698	5	2	9627	3039	17694	1	120	36	10451
17699	5	2	4923	2234	17695	1	120	72	11561
17691	0.2	48	0	8550	17696	1	120	38	12568
17692	0.2	48	66.4	7315	17697	5	120	217	7954
17693	0.2	48	80.7	7276	17698	5	120	326	9620
17694	1	48	389	6025	17699	5	120	224	10560
17695	1	48	363	5033	17691	0.2	240	0	13669
17696	1	48	264	6839	17692	0.2	240	0	12762
17697	5	48	1330	3323	17693	0.2	240	0	14649
17698	5	48	2644	2628	17694	1	240	0	14533
17699	5	48	1605	3095	17695	1	240	0	13998
17691	0.2	96	0	13936	17696	1	240	0	14560
17692	0.2	96	30	8978	17697	5	240	58	11173
17693	0.2	96	0	10522	17698	5	240	71	13745
					17699	5	240	63	13937

10 #

AlbuBChE concentration was not measured at 2 hr post-AlbuBChE dose, the 3 hr post-AlbuBChE dose was used in the analysis.

- 46 -

Animal Observations

The animals were observed throughout the study. There were no deaths during the study, and there were no clinical or cageside observations associated with administration of AlbuBChE at either dose. Administration of cocaine at a dose of 1 mg/kg without pre-treatment with the test article resulted in hyperactivity and increased respiratory rate with abdominal breathing. Cocaine-related observations were not observed for five days following pre-treatment with the test article at AlbuBChE doses ranging from 0.2 to 5 mg/kg but returned on study days 6 and 11.

Conclusions

AlbuBChE was well tolerated in cynomolgus monkeys following a single IM dose of 0.2, 1 or 5 mg/kg.

AlbuBChE absorption from IM site of administration was rapid. T_{max} was observed at 3 hr for the 0.2 and 1 mg/kg and 6 hr for the 5 mg/kg dose. AlbuBChE exposure appears to increase in a more than proportional manner as a function of dose. Terminal elimination $t_{1/2}$ increased from 31 to 62 hr between 1 and 5 mg/kg dose levels.

Cocaine was administered to control animals on Day 1 and at 2, 48, 96, 120 and 240 hr post-AlbuBChE dose. After each cocaine administration, multiple samples were drawn with the objective of determining the pharmacokinetic profile in response to decreasing serum levels of AlbuBChE. Cocaine $AUC_{(0-t)}$ decreased as a function of AlbuBChE dose and increase as a function of time post-AlbuBChE administration.

The duration of effect was related to AlbuBChE dose levels. AlbuBChE effect on cocaine AUC and clearance was evident up to 120 hr for 5 mg/kg dose, 96-120 hr for 1 mg/kg dose and 48 hr

- 47 -

for the 0.2 mg/kg dose. Based on this data, a once or twice weekly dosing regimen of AlbuBChE is likely.

PK/PD relationship in cynomolgus monkeys appears to indicate an
5 inverse relationship between AlbuBChE serum concentration and cocaine exposure. AlbuBChE serum concentration that may result in 50% decrease in cocaine concentration (EC_{50}) was estimated by the direct Sigmoidal Inhibitory Effect E_{max} model model to be
~600 ng/mL.

10

Example 2 - AlbuBChE Pharmacokinetic Pharmacodynamic (PK/PD) Study in Squirrel Monkeys

Objective

15 To evaluate AlbuBChE pharmacokinetics and pharmacodynamics following a single 5 mg/kg intramuscular injection of AlbuBChE to Squirrel monkeys. AlbuBChE pharmacodynamic effect was measured following cocaine IV administration at a dose of 1 mg/kg in control animals or at 2, 72 and 96 hr post-AlbuBChE
20 dose.

Study Design

Dose Level: AlbuBChE dose level of 5 mg/kg was selected based upon prior studies in Cynomolgous monkeys in which this dose
25 level was found to be effective in decreasing cocaine exposure. Cocaine IV dose of 1 mg/kg was based on literature reports that such dose is sufficient to cause an effect in monkeys without causing excessive hyperactivity. The IM route of administration for AlbuBChE dosing was selected because this is an intended
30 route of administration to humans.

Table 13: Test System and Neat Materials

Species	Primate –Squirrel – non-naïve ~ 0.8-1.2 kg
Wash out Period	~1 week
In Life Period	1 week, not including wash out
Number of Animals / Sex / Group	3 males for AlbuBChE dose group 2 Control with vehicle
Number of Groups (including Control)	1 dose groups + Control
Total number of animals on study	5
Test Article	AlbuBChE stock formulation was stored in a freezer (-75 ± 15°C) and protected from light.
Pharmacodynamic Test Article	Cocaine hydrochloride was stored under ambient conditions protected from light.
Formulation Procedures	The AlbuBChE stock formulation was thawed at room temperature and once thawed was inverted gently. The stock was diluted with Formulation Buffer to achieve a concentration of 20 mg/mL. The cocaine hydrochloride formulation was prepared by adding the required amount of cocaine hydrochloride to the formulation container, adding the required amount of saline to the container, and stirring until a clear formulation was observed. The final concentration was expressed as the hydrochloride salt. The formulation was filtered through a 0.22-µm PVDF syringe filter. The formulation was stored at -75 ± 15°C until needed.

Table 14: Group Designation and Dosage Levels

5

Group	Treatment	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals
1	Single IV dose of Cocaine	1	1.0	1	2
2	Single IM dose of AlbuBChE	5	20	0.25	3
	Single IV dose of Cocaine at 2, 72 and 96 hours after AlbuBChE dose administration	1	1.0	1	

AlbuBChE Pharmacokinetic Samples

Blood samples (0.4 mL) were collected from the femoral vein and placed into serum separator tubes (SST) at pre-dose, 24, 72, 96

- 49 -

& 336 hrs post-dose (samples collected at 336 hr (14 days) post-dose were intended to evaluate immunogenicity. The samples were also analyzed for AlbuBChE concentration). Tubes were maintained at room temperature for at least 1 hour, but not to exceed 4 hours prior to centrifugation. Samples were centrifuged and at least 200 μ L of serum was harvested and maintained on dry ice prior to storage at approximately -70°C.

Cocaine Pharmacokinetic Samples

Approximately 0.4 mL of whole blood was collected from the femoral vein after every cocaine dose at 5 and 30 minutes postdose. Blood was placed into K₂EDTA blood collection tubes with esterase inhibitor (diisopropylfluorophosphate (DFP)). Tubes were inverted several times and placed on wet ice upon collection. Samples were centrifuged at 2-8°C within 45 minutes of collection. The resultant plasma will be recovered and a single 200 μ L aliquot of plasma was placed into polypropylene tubes. Plasma samples was frozen over dry ice and stored at approximately -70°C.

AlbuBChE Sample Analysis

The following paragraph briefly describes the ELISA based assay employed in the measurement of AlbuBChE concentrations in serum samples.

Immulon 4 HBX plates are coated with 100 μ L of anti-human BChE mAb 002-01 (Abcam ab17246) at 1 μ g/mL in PBS, overnight at 4°C. Blocking is done with 2% casein in phosphate buffered saline (PBS), 200 μ L/well, 2 hours at room temperature. After washing, 100 μ L of diluted serum samples are added to the plates along with standards. Standards are generated through 2.6 fold, serial dilution of AlbuBChE from 420 to 5.2ng/mL. Serum samples and standards are maintained at 10% serum by dilution with buffer containing pooled cynomolgus monkey serum. A wash step

- 50 -

precedes detection with 100µl of anti-HSA mAb-6502-HRP at 0.04 µg/mL for 1 hour. Wells are washed again, prior to developing with 100µL of tetramethylbenzidine substrate. After 15 minutes the reaction is terminated with 100 µL/well of 1N H₂SO₄ and read on SpectraMax plate reader at 450/570 nm. Values for unknown serum samples are calculated by interpolation of standard curve generated by 5-parameter fit of AlbuBChE standards. Serum samples collected at predose and on Day 14 postdose were also analyzed for immunogenicity.

10

AlbuBChE Pharmacokinetic and PK/PD Analysis

AlbuBChE pharmacokinetic parameter values were determined using serum concentration-time profiles for individual animals. The computer software WinNonlin Professional (Version 4.0.1 Pharsight Corporation, USA) was used. Specifically the model for non-compartmental analysis with extravascular input was applied.

In spite of the limited amount of data, an attempt was made to characterize PK/PD relationship. The computer software WinNonlin Professional (Version 4.0.1 Pharsight Corporation, USA) was used. Specifically the direct Inhibitory Effect E_{max} model was use in which E_{max} was assumed at AlbuBChE concentration of zero. The model equation can be described as follows:

25

$$E = E_{max} * (1 - (C / (C + EC50)))$$

The PD parameter used in the analysis was cocaine plasma concentration at 5 min post-cocaine dose. PK parameters used in the model were AlbuBChE plasma concentrations at 2, 72 and 96 hr post-AlbuBChE dose. Due to the fact that AlbuBChE concentration was not measured at 2 hr post-AlbuBChE dose, the 1st time sample collected was used in the analysis (24 hr post-

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- 51 -

AlbuBChE dose) with the assumptions that AlbuBChE concentration at 24 hr post AlbuBChE dose may reflect AlbuBChE concentration at 2 hr post AlbuBChE dose. Due to the limited data and the assumptions used in the analysis, parameter values from this PK/PD analysis need to be viewed as approximate estimates.

Results

AlbuBChE

Table 15 and Figure 12 provide AlbuBChE serum concentration-time profiles in three male Squirrel monkeys following a single intramuscular administration of 5 mg/kg AlbuBChE dose. Table 16 summarizes AlbuBChE Squirrel monkeys pharmacokinetic parameter values.

AlbuBChE terminal elimination slope was well characterized as indicated by Rsq values being higher than 0.9. Area under the curve was also well characterized as indicated by %AUC extrapolated being less than 20%. The initial absorption phase of AlbuBChE concentration-time profile was not characterized since the first sample was collected at 24 hr postdose. As such, T_{max} and C_{max} reported are apparent since actual T_{max} can be expected to occur between 3 and 6 hr. AlbuBChE exposure was consistent for the three animals, with differences in levels only clearly evident after 2 weeks. For example, AUC values for the three animals were in a tight range with %CV of ~7%. AlbuBChE terminal elimination $t_{1/2}$ was estimated to range from 45.5 to 65.5 hr (average half-life of 56.6 hours).

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- 52 -

Table 15: AlbuBChE Serum Concentration (ng/mL) vs. time (hr) Profile in individual Squirrel Monkeys following a single IM administration of 5 mg/kg/animal AlbuBChE dose.

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Time (hr)	MKY 547	MKY 3434	MKY 53B
0	< LLOD	< LLOD	< LLOD
24	2340.2	2296.7	2036.7
72	436.9	517.7	433.7
96	247.5	247.5	233.5
336	23.4	19.0	7.0

Table 16: AlbuBChE Pharmacokinetic Parameter Values in individual Squirrel Monkeys following a single IM administration of 5 mg/kg/animal AlbuBChE dose.

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MKY ID	Rs _q	No. Points Lambda-z	t _{1/2} (hr)	T _{max} (hr)	C _{max} (ng/mL)	AUC(0-t) (hr*ng/mL)	AUC(0-∞) (hr*ng/mL)	AUC %Extrap
547	0.990	3	65.5	24	2340	135454	137666	1.61
3434	0.983	3	58.8	24	2297	136268	137881	1.17
53B	0.997	3	45.5	24	2037	120596	121056	0.380
Mean						130773	132201	
SD						8822	9652	
%CV						6.75	7.30	

Cocaine and metabolites

Cocaine was administered IV at a dose of 1 mg/kg in control animals (n=2) or at 2, 72 and 96 hr post-AlbuBChE dose (n=3). The bioanalytical method measured the plasma concentration of cocaine and two of its metabolites: ecgonine methyl ester and benzoylecgonine. Cocaine metabolic pathway is shown in Figure 9, ecgonine methyl ester metabolite is formed directly from cocaine through the butyrylcholine esterase enzyme. As such,

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- 53 -

this metabolite can be predicted to increase following AlbuBChE administration.

Individual and mean cocaine plasma concentration (ng/mL) vs.
5 time (hr) data in control and AlbuBChE treated Squirrel
monkeys are shown in Table 17. A summary of the mean cocaine
concentrations vs. time are shown in Figure 13. Highest cocaine
concentrations were observed in the control animals. The lowest
cocaine levels were observed at 2 hr following AlbuBChE
10 administration reaching values that are approximately 7% of
control. Cocaine concentrations increased as a function of time
post-AlbuBChE administration yet even at 96 hr post-AlbuBChE
administration, cocaine concentration were still 60% of control
animals.

15 Individual and mean ecgonine methyl ester plasma concentration
(ng/mL) vs. time (hr) data in control and AlbuBChE treated
Squirrel monkeys are shown in Table 18. A summary of the mean
ecgonine methyl ester concentrations are shown in Figure 14. As
20 expected, ecgonine methyl ester concentration at 5 min after
cocaine administration were low in the control animals. The
values were ~40 fold higher in the AlbuBChE treated animals
with the highest concentrations observed at 2 hr post-AlbuBChE
dose. Ecgonine methyl ester concentrations decreased as a
25 function of time post-AlbuBChE administration yet even at 96 hr
post-AlbuBChE administration, ecgonine methyl ester
concentration were still higher than in the control animals.

Individual and mean benzoylecgonine plasma concentration
30 (ng/mL) vs. time (hr) data in control and AlbuBChE treated
Squirrel monkeys are shown in Table 19. A summary of the mean
benzoylecgonine concentrations are shown in Figure 15. As
illustrated, AlbuBChE effect on the cocaine metabolite
benzoylecgonine was less pronounced.

- 54 -

Figure 16 shows a summary of the blood levels of cocaine and the cocaine metabolites ecgonine methyl ester (EME) and benzoylecgonine (BZ) at 5 and 30 min following the cocaine injection. Compared to a control cocaine injection following administration of vehicle, 2 hours following administration of AlbuBChE cocaine blood levels were significantly reduced at both the 5- and 30-min time points ($F_{3,7} = 7.34$, $p < 0.05$ and $F_{3,7} = 14.4$, $p < 0.005$ respectively). Seventy-two hours following AlbuBChE cocaine levels were still significantly below control levels 30-min post cocaine. The effects of AlbuBChE were not significant at either 5 or 30 min time point 96 hours following administration. The effects of AlbuBChE on EME levels were similar, but in the opposite direction to those of cocaine. Blood levels of EME were elevated at both the 5- and 30 min time point 2 hr following AlbuBChE administration ($F_{3,7} = 5.6$, $p < 0.05$ and $F_{3,7} = 33.3$, $p < 0.001$). Blood levels of EME remained significantly elevated at 72 hours following AlbuBChE for the 30 min time point. While blood levels of BZ appeared to be slightly reduced 2 hr following AlbuBChE, these effects were not significant.

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- 55 -

Table 17: Cocaine Plasma Concentration (ng/mL) vs. time (hr) in individual Squirrel Monkeys following a single IM administration of 5 mg/kg AlbuBChE dose. Cocaine was administered IV at a dose of 1 mg/kg in control animals (n=2) or at 2, 72 and 96 hr post-AlbuBChE dose (n=3).

Cocaine Concentration at 5 min following Cocaine dose at 2, 72 and 96 hr post-AlbuBChE Dose

Time Post-AlbuBChE Dose	MKY 547	MKY 3434	MKY 53B	Mean	SD	%CV
2	0.575	20.0	22.4	14.3	12.0	83.5
72	41.3	176	104	107	67.4	62.9
96	97.9	191	112	134	50.2	37.5

<LLOQ (1 ng/mL)

10

Cocaine Concentration at 30 min following Cocaine dose at 2, 72 and 96 hr post-AlbuBChE Dose

Time Post-AlbuBChE Dose	MKY 547	MKY 3434	MKY 53B	Mean	SD	%CV
2	0.581	0.115	0.261	0.319	0.238	74.7
72	65.0	57.9	44.9	55.9	10.2	18.2
96	62.9	75.6	67.1	68.5	6.47	9.44

<LLOQ (1 ng/mL)

15

Cocaine Concentration at 5 and 30 min following cocaine dose in control animals

Time post cocaine dose	MKY 548	MKY 27B	Mean
5 min	185	239	212
30 min	82.6	154	118

<LLOQ (1 ng/mL)

Summary Table of Mean Cocaine Concentration vs. time in control animals and as a function of time post-AlbuBChE dose

20

Time Post Cocaine (min)	Control	2 hr Post-AlbuBChE	72 hr Post-AlbuBChE	96 hr Post-AlbuBChE
5	212	14.3	107	134
30	118	0.319	55.9	68.5

- 56 -

Table 18: Ecgonine Methyl Ester Plasma Concentration (ng/mL) vs. time (hr) in individual Squirrel Monkeys following a single IM administration of 5 mg/kg AlbuBChE dose. Cocaine was administered IV at a dose of 1 mg/kg in control animals (n=2) or at 2, 72 and 96 hr post-AlbuBChE dose (n=3).

Ecgonine Methyl Ester Concentration at 5 min following Cocaine dose at 2, 72 and 96 hr post-AlbuBChE Dose

Time Post-AlbuBChE Dose	MKY 547	MKY 3434	MKY 53B	Mean	SD	%CV
2	109	417	333	286	159	55.6
72	29.3	101	73.2	67.8	36.2	53.3
96	40.5	72.3	35.8	49.5	19.9	40.1

<LLOQ (1 ng/mL)

Ecgonine Methyl Ester Concentration at 30 min following Cocaine dose at 2, 72 and 96 hr post-AlbuBChE Dose

Time Post-AlbuBChE Dose	MKY 547	MKY 3434	MKY 53B	Mean	SD	%CV
2	252	314	218	261	48.7	18.6
72	61.3	112	81.7	85.0	25.5	30.0
96	25.7	76.8	45.2	49.2	25.8	52.4

<LLOQ (1 ng/mL)

Ecgonine Methyl Ester Concentration at 5 and 30 min following cocaine dose in control animals

Time post cocaine dose	MKY 548	MKY 27B	Mean
5 min	1.61	12.8	7.21
30 min	1.27	1.34	1.31

<LLOQ (1 ng/mL)

- 57 -

Summary Table of Mean Ecgonine Methyl Ester Concentration vs. time in control animals and as a function of time post-AlbuBChE dose

Time Post Cocaine (min)	Control	2 hr Post-AlbuBChE	72 hr Post-AlbuBChE	96 hr Post-AlbuBChE
5	7.21	286	67.8	49.5
30	1.31	261	85.0	49.2

5 **Table 19: Benzoyllecgonine Plasma Concentration (ng/mL) vs. time (hr) in individual Squirrel Monkeys following a single IM administration of 5 mg/kg AlbuBChE dose. Cocaine was administered IV at a dose of 1 mg/kg in control animals (n=2) or at 2, 72 and 96 hr post-AlbuBChE dose (n=3).**

Benzoyllecgonine Concentration at 5 min following Cocaine dose at 2, 72 and 96 hr post-AlbuBChE Dose

Time Post-AlbuBChE Dose	MKY 547	MKY 3434	MKY 53B	Mean	SD	%CV
2	4.83	23.3	20.1	16.1	9.87	61.4
72	9.93	41.7	28.8	26.8	16.0	59.6
96	15.5	36.2	26.3	26.0	10.4	39.8

<LLOQ (1 ng/mL)

15 **Benzoyllecgonine Concentration at 30 min following Cocaine dose at 2, 72 and 96 hr post-AlbuBChE Dose**

Time Post-AlbuBChE Dose	MKY 547	MKY 3434	MKY 53B	Mean	SD	%CV
2	11.1	20.8	10.9	14.3	5.66	39.7
72	20.7	33.4	21	25.0	7.25	29.0
96	18.9	37.6	21.5	26.0	10.1	39.0

<LLOQ (1 ng/mL)

20 **Benzoyllecgonine Concentration at 5 and 30 min following cocaine dose in control animals**

Time post cocaine dose	MKY 548	MKY 27B	Mean
5 min	34.4	31.6	33.0
30 min	25.1	29.9	27.5

<LLOQ (1 ng/mL)

- 58 -

Summary Table of Mean Benzoylecgonine Concentration vs. time in control animals and as a function of time post-AlbuBChE dose

Time Post Cocaine (min)	Control	2 hr Post-AlbuBChE	72 hr Post-AlbuBChE	96 hr Post-AlbuBChE
5	33.0	16.1	26.8	26.0
30	27.5	14.3	25.0	26.0

5

AlbuBChE PK/PD Relationship

In spite of the limited number of time points and animals, an attempt was made to characterize AlbuBChE PK/PD relationship in male Squirrel monkeys. PK and PD data used in the analysis are shown in Table 20. The individual data and the fit resulting from the direct Inhibitory Effect E_{\max} model are shown in Figure 17. The data clearly shows the inverse relationship between AlbuBChE serum concentration and cocaine levels. AlbuBChE serum concentration that may result in 50% decrease in cocaine concentration (EC₅₀) was estimated by the model to be ~400 ng/mL. E_{\max} was 213 ng/mL. Due to the limited data and the assumptions used in the analysis, parameter values from this PK/PD analysis need to be viewed as approximate estimates.

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- 59 -

Table 20: AlbuBChE PK/PD analysis Squirrel Monkeys following a single IM administration of 5 mg/kg AlbuBChE dose. Cocaine was administered IV at a dose of 1 mg/kg in control animals (n=2) or at 2, 72 and 96 hr post-AlbuBChE dose (n=3).

5

PK parameters: AlbuBChE plasma concentrations at 24[#], 72 and 96 hr post-AlbuBChE dose

PD parameter: cocaine plasma concentration at 5 min post-cocaine dose.

MKY ID	Time Post-AlbuBChE Dose (hr)	PK AlbuBChE (ng/mL)	PD Cocaine concentration at 5 min post-cocaine dose (ng/mL)
548 (Control)	2	0	185
27B (Control)	2	0	239
547	2	2340.2	0.575
	72	436.9	41.3
	96	247.5	97.9
3434	2	2296.7	20.0
	72	517.7	176
	96	247.5	191
53B	2	2036.7	22.4
	72	433.7	104
	96	233.5	112

10

Due to the fact that AlbuBChE concentration was not measured at 2 hr post-AlbuBChE dose, the 1st time sample collected was used in the analysis (24 hr post-AlbuBChE dose) with the assumptions that AlbuBChE concentration at 24 hr post AlbuBChE dose may reflect AlbuBChE conc. at 2 hr post AlbuBChE dose.

15

Conclusions

AlbuBChE pharmacokinetic profile was characterized in three male Squirrel monkeys following a single IM 5 mg/kg AlbuBChE dose. Extent of variability in exposure was minimal (~7%). AlbuBChE terminal elimination t_{1/2} was estimated to range from 45.5 to 65.5 hr.

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- 60 -

Cocaine was administered IV at a dose of 1 mg/kg in control animals (n=2) or at 2, 72 and 96 hr post-AlbuBChE dose (n=3). AlbuBChE caused a decrease in cocaine exposure. The effect was most pronounced at 2 hr post-AlbuBChE dose (<7% of control).
5 Cocaine exposure was ~60% of control at 96 hr post-AlbuBChE dose.

Consistent with AlbuBChE mechanism of action, exposure to the cocaine metabolite ecgonine methyl ester increased ~40 fold at
10 2 hr post-AlbuBChE administration and decreased as a function of time post-AlbuBChE dose.

AlbuBChE effect was less pronounced on the cocaine metabolite, benzoylecgonine.
15

PK/PD relationship in Squirrel monkeys appears to indicate an inverse relationship between AlbuBChE serum concentration and cocaine levels. AlbuBChE serum concentration that may result in 50% decrease in cocaine concentration (EC_{50}) was estimated by
20 the direct Inhibitory Effect E_{max} model model to be ~400 ng/mL. Due to the limited data and the assumptions used in the analysis, parameter values from this PK/PD analysis need to be viewed as approximate estimates.

25 **Example 3 - AlbuBChE: A Cardiovascular Safety Pharmacology Study in Cynomolgus Monkeys with or without Administration of Cocaine**

Objective

30 To evaluate the cardiovascular and respiratory safety of AlbuBChE administered by the intramuscular (IM) route in 3 male and 3 female cynomolgus monkeys. In addition, the effect of cocaine (administered by the intravenous (IV) route), AlbuBChE

- 61 -

and their combination on cardiovascular safety was evaluated in another group of 3 male and 3 female cynomolgus monkeys.

Study Design

5 Prior to the start of the study, the monkeys were surgically instrumented with telemetry transmitters and vascular access ports. The following CV parameters were monitored: systolic pressure, diastolic pressure, mean arterial blood pressure, mean heart rate and mean rate-pressure product. Body
10 temperatures changes were also monitored.

Study Group 1 (3 males and 3 females), served as a control group for testing the effect of AlbuBChE alone compared to its vehicle. Monkeys were administered (IM) with the control
15 article AlbuBChE formulation buffer on Study Days (SDs) 1 and 4. Three hours post formulation buffer administration respiratory and cardiovascular (CV) parameters were recorded, respectively.

20 A single IM dose of the test article (AlbuBChE at 15mg/kg) was administered on SD 8 and 11. Three hours after AlbuBChE dosing (corresponding to T_{max} of AlbuBChE) CV and respiratory parameters were monitored, respectively.

25 The effect of pretreatment with AlbuBChE prior to cocaine dose on CV parameters was tested on the second group (Group 2) of animals (3 males and 3 females). First baseline CV parameters were recorded on SD 15 after an IM administration of AlbuBChE formulation buffer followed by a single IV administration of
30 saline (cocaine vehicle), 3 hours later. The effect of cocaine on CV parameters was measured on SD 18, on which the animals received a single IM injection of AlbuBChE formulation buffer followed by a single IV dose of cocaine (1mg/kg, IV), 3 hours later.

- 62 -

The effect of pretreatment with AlbuBChE on cocaine - induced changes in CV parameters was monitored on SD 22. CV parameters were recorded subsequent to a single IM dose of AlbuBChE (15mg/kg) followed by a single IV dose of cocaine (1mg/kg), 3 hours later (see Table 21).

All animals were observed at least twice a day for morbidity, mortality, injury, and availability of food and water. The physiological responses to test article administration, including blood pressure, heart rate, body temperature, and the electrocardiograph (ECG), were monitored.

Table 21: Group Designation and Dosage Levels

Study Group	Study Day	Treatment	Test Article or Interaction Article Dose Level (mg/kg)	Test Article or Interaction Article Dose Conc. (mg/ml)	Number of Animals	
					Male	Female
1	1	Formulation Buffer-CV	0	0	3	3
	4	Formulation Buffer-Respiration	0	0		
	8	AlbuBChE-CV	15	30		
	11	AlbuBChE-Respiration	15	30		
2	15	Formulation Buffer-CV	0	0	3	3
		Saline-CV	0	0		
	18	Formulation Buffer-CV	0	0		
		Cocaine-CV	1	5		
	22	AlbuBChE-CV	15	30		
		Cocaine-CV	1	5		

- 63 -

Results

All animals survived to study termination.

5 Study Group 1

AlbuBChE at 15mg/kg (IM) did not produce any significant changes in the recorded CV parameters, nor in body temperature compared to baseline values determined with AlbuBChE formulation buffer, as shown in Figures 18-26.

10

Study Group 2

Within two minutes post cocaine (1.0 mg/kg, IV) administration mean arterial blood pressure (MAP) was increased by about 35 mmHg above baseline value of 105 mmHg. Pre-treatment with 15mg/kg AlbuBChE prior to cocaine dose showed that not only was the maximum blood pressure was decreased by AlbuBChE but the time to reversal of the effect was also truncated by 4.5 fold (17 minutes upon pretreatment with AlbuBChE prior to cocaine dose compared to 77 minutes when cocaine was given alone), as shown in Figure 27.

20

Administration of cocaine (1.0 mg/kg, IV) elicited a rapid increase in heart rate from a baseline value of 140 beats/min reaching a peak value of 240 beats/min (70% increase) within about 3 minutes. This cocaine - induced elevation in heart rate was sustained for about 15 min dissipating gradually and returning to baseline value 2 hours after cocaine administration. Pre-treatment with AlbuBChE blunted cocaine - induced rise in heart rate showing a rapid but moderate increase of 30% to a peak value of 182 beats/min within about 3 minutes. This mild increase in heart rate was reversed completely returning to baseline value already 30 minutes after cocaine dose, as shown in Figure 30.

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- 64 -

Administration of cocaine (1.0 mg/kg , IV) resulted in a mild and step wise increase in body temperature with a peak value of 38.7°C at 45 minutes post-dose compared to base line temperature of 38°C. Pre-treatment with AlbuBChE (15 mg/kg, IM) prior to cocaine dose caused only a subtle increase in body temperature to a value of 38.3°C, as shown in Figure 32.

Example 4 - AlbuBChE: A Cocaine Self-Administration and Reinstatement of Cocaine Self-Administration Study in Squirrel Monkeys

Methods

Subjects

Adult male squirrel monkeys (*Saimiri sciureus*) weighing 0.8 to 1.2 kg were used as subjects. All monkeys were individually housed in a humidity and temperature controlled room and had free access to water. The monkeys were fed following any experimental procedures an amount of food (Lab Diet 5045, PMI Nutrition International, Richmond, IN; Banana Softies, Bio-Serv, Frenchtown, NJ) determined to maintain a stable weight. Fresh fruit, vegetables and environmental enrichment were also provided daily. The animal care facilities were fully accredited by AAALAC and all experiments were approved by the NIDA Intramural Research Program Animal Care and Use Committee.

Cocaine Self-Administration

Three monkeys were trained to self-administer 30 µg/kg/inj i.v. cocaine. Details of the self-administration training procedure can be found elsewhere (Justinova et al., 2003). In brief, the monkeys were placed in a seated position in a Plexiglas restraint chair. The chair was enclosed in a larger acoustical chamber. On the front wall of the restraint chair there was a response lever and stimulus lights. A green light signaled the

- 65 -

beginning of the session. The monkeys were trained to make 10 responses (fixed-ratio, FR, 10) to receive an i.v. injection of cocaine. The injection of cocaine was accompanied by the green light turning off and a yellow stimulus light being presented
5 for 2 sec. Following each cocaine injection there was also a 60-sec timeout. At the end of the timeout, the green light was turned on. Sessions lasted for 1 hour. Occasionally, saline was substituted for cocaine. Following the establishment of stable cocaine self-administration and reliable extinction of
10 responding following saline substitution, AlbuBChE (5 mg/kg, i.m.) or its vehicle (i.m.) were given 2 hours prior to a self-administration session. Self-administration responding was then measured for 5 consecutive days. Following each drug test or saline substitution, responding for 30 µg/kg/inj cocaine was
15 reestablished for at least 5 days. Following reinstatement testing (see below), the dose of cocaine available for self-administration was lowered to 10 µg/kg/inj and the effect of AlbuBChE (5 mg/kg, i.m.) was again determined.

20 *Reinstatement of Cocaine Self-Administration*

Saline was substituted for cocaine in monkeys that were reliably self-administering 30 µg/kg/inj i.v. cocaine. Responding rapidly decreased and remained at that low level over a number of days. The vehicle of AlbuBChE was then given
25 2 hrs before cocaine (0.3 mg/kg, i.v.), which was given 5 min prior to a session where saline was available for self-administration to determine whether cocaine would reinstate self-administration responding. The same dose of cocaine was also given prior to saline self-administration sessions 48 and
30 96 hours later. Subsequently, AlbuBChE (5 mg/kg, i.m.) was given 2 hours prior to a second sequence of 3 reinstatement tests with 0.3 mg/kg i.v. cocaine. Following these tests, the monkeys were returned to baseline where 30 µg/kg/inj cocaine was available for self-administration. Subsequently, saline

- 66 -

was again substituted for cocaine and a second series of reinstatement tests were conducted in a similar manner except that 0.1 mg/kg i.v. cocaine was given prior to reinstatement sessions.

5

Drugs

AlbuBChE was supplied by TEVA Pharmaceutical (Netanya, Israel) in a frozen solution at a concentration of 30 mg/ml. Once thawed the solution was diluted to 15 mg/ml with vehicle and then given to the monkeys in a volume of 0.33 ml/kg. Cocaine (NIDA, Baltimore, MD) was prepared in sterile saline and given in a volume of 0.2 ml for self-administration. When given as a pretreatment for the reinstatement studies, cocaine was given in a volume of 0.3 ml/kg.

15

Data Analysis

The blood level data were analyzed separately for each metabolite and time point (5 or 30 min). A one-way analysis-of-variance (ANOVA) was performed with follow-up Dunnett tests using the results of monkeys treated with vehicle as the control. The self-administration data were analyzed separately for response rate or injections following either vehicle treatment or AlbuBChE treatment. Control data were included in the analysis by taking the average of the last 3 days for the immediately preceding baseline condition. A within subjects ANOVA was then performed with follow-up tests contrasting days following vehicle or AlbuBChE with the control. For the reinstatement tests, a two way ANOVA was performed for both response rate and injections, with time post vehicle or AlbuBChE as the within-subjects factor and pretreatment (vehicle or AlbuBChE) as the between subjects factor. Follow-up contrast compared vehicle and AlbuBChE at each time point.

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- 67 -

Results

In monkeys self-administering 30 µg/kg/inj cocaine, AlbuBChE reduced self-administration. The panels of Figure 33 show response rate (top panel) and injections (bottom panel) over consecutive days. The first five days show baseline responding. Saline was then substituted for cocaine and both rate ($F_{5,10} = 37.0$, $p < 0.001$) and injections ($F_{5,10} = 188.7$, $p < 0.001$) were significantly reduced for the 5 days of substitution when compared to the average of the last 3 days of the preceding baseline period. Following a 5 day return to baseline, monkeys were treated with vehicle and given 5 days of self-administration training. When compared to the average of the last 3 days of the preceding baseline period, vehicle treatment had no effect on injections ($F_{5,10} = 2.6$, $p = 0.10$). An overall effect of days was observed for response rate ($F_{5,10} = 4.6$, $p < 0.05$), however follow-up contrasts failed to reveal any significant change in responding from control on any of the 5 days following vehicle treatment. Following vehicle treatment, monkeys were given 5 mg/kg AlbuBChE and cocaine self-administration was tracked for 5 days. Significant changes from the average of the last 3 days of the previous baseline were observed for both response rate ($F_{5,10} = 4.6$, $p < 0.05$) and injections ($F_{5,10} = 14.1$, $p < 0.001$). Follow-up contrast revealed that response rate was different from control on days 1-4 following treatment and injections were significantly different from control on day 1 and 4 following treatment.

Following reinstatement testing (see below), monkeys were returned to self-administration at a lower cocaine dose (10 µg/kg/inj). Decreasing the maintenance dose decreased rate of responding and number of injections (Figure 33 right panels). Following baseline stability, monkeys were given a vehicle injection and self-administration continued for 5 days.

- 68 -

Neither response rate nor injections significantly changed following vehicle injection compared to the average of the last 3 days on baseline. AlbuBChE (5 mg/kg) was then given and cocaine self-administration continued for an additional 5 days.

5 Both response rate and injections were decreased 2 hrs following the AlbuBChE injection, but these effects failed to reach significance.

In monkeys that were responding for 30 µg/kg/inj cocaine, substituting saline for cocaine lead to a rapid decrease in responding. The AlbuBChE vehicle was then given i.m. Two, 48 and 96 hrs later 0.3 mg/kg cocaine i.v. was given 5 min before a saline substitution session. The administration of cocaine led to a reinstatement of cocaine self-administration responding as shown in the black bars in the left panels of Figure 34. When 5 mg/kg AlbuBChE was given i.m., and 2 hrs, 48 hrs and 96 hrs later 0.3 mg/kg cocaine i.v. was given immediately before a saline substitution session, reinstatement of responding was significantly blocked at the 2 hr time point for both response rate ($F_{1,4} = 16.9$, $p < 0.05$) and injections ($F_{1,4} = 34.9$, $p < 0.001$). Monkeys were subsequently tested in an identical manner with 0.1 mg/kg cocaine as the reinstatement dose. While reinstatement to the lower cocaine dose was more variable, a similar pattern of results was observed with the effect of 5 mg/kg AlbuBChE again significant at 2 hrs for both responses ($F_{1,4} = 15.2$, $p < 0.05$) and injections ($F_{1,4} = 129.7$, $p < 0.001$).

In 4 monkeys that were trained to discriminate 0.6 mg/kg methamphetamine from saline, cocaine fully substituted for methamphetamine at a dose of 0.3 mg/kg (Figure 35, top panel). When 5 mg/kg AlbuBChE was given 2 hrs prior to the determination of the cocaine dose-effect function, 0.3 mg/kg cocaine failed to generalize to the methamphetamine cue.

- 69 -

Partial substitution (approximately 70%) was observed at a higher cocaine dose (1.0 mg/kg). Twenty-four hrs following AlbuBChE, the 0.3 mg/kg dose of cocaine still failed to completely generalize to the methamphetamine cue (approximately 70%), but 1.0 mg/kg now fully generalized to methamphetamine. By 48 hrs following AlbuBChE, the 0.3 mg/kg dose fully generalized to the methamphetamine cue. Pretreatment with AlbuBChE 2 hrs prior to the determination of the methamphetamine dose-effect function did not affect generalization of methamphetamine to the methamphetamine cue (Figure 36, top panel). Pretreatment with AlbuBChE did not affect the rate of responding under either treatment condition (Figures 35 and 36, bottom panels).

The monkeys trained to self-administer cocaine and also tested on the reinstatement procedure received a total of 4 injections of AlbuBChE. Blood was taken for determination of AlbuBChE antibodies after the first, second and fourth AlbuBChE injections. Following the first 2 injections, antibody levels were below the level of detection (titer level of 20). Following the fourth injection, however, antibody levels appeared elevated. In 2 monkeys the antibody titer was slightly elevated over the limit of detection (21 and 43). For the third monkey, antibody levels were clearly elevated (643). The concentration of AlbuBChE in the blood for this monkey one week following the AlbuBChE injection was also reduced (27 ng/ml vs 55 and 53 ng/ml for the other 2 monkeys).

Discussion

The results of these studies clearly show that pretreatment with a modified form of BChE is effective in antagonizing the behavioral effects of cocaine in a non-human primate. This work extends previous work with this compound in rodents, where it was shown that AlbuBChE could antagonize the toxic effects

- 70 -

of cocaine in rats and also block cocaine-induced reinstatement of cocaine self-administration (Brimijoin et al., 2008). Here, pretreatment with 5 mg/kg AlbuBChE reduced both response rate and injections in monkeys self-administering 30 µg/kg/inj cocaine. In these same monkeys tested for reinstatement of cocaine self-administration following self-administration extinction, 5 mg/kg AlbuBChE was capable of blocking reinstatement of responding by either 0.3 mg/kg or 0.1 mg/kg cocaine. When the dose available for self-administration was reduced to 10 µg/kg/inj, AlbuBChE again appeared to reduce the response rate and number of injections received, although these effects with 10 µg/kg/inj cocaine were more variable and failed to reach significance. Finally, the 5 mg/kg dose of AlbuBChE was also able to antagonize the generalization of cocaine to a methamphetamine discriminative stimulus, but this effect was specific to cocaine as AlbuBChE was not able to antagonize the discriminative stimulus effects of methamphetamine.

When tested against 30 µg/kg/inj cocaine self-administration, the effects of AlbuBChE were still evident 3 days later, although at a reduced effectiveness. On reinstatement and discrimination, however, the effects of AlbuBChE were not evident 48 hours following treatment. The half-life of AlbuBChE was shown to be around 56 hours and effects on cocaine metabolism were still evident 72 hours after AlbuBChE administration, so it might have been expected that some effect on these procedures would have been evident at 72 hours. A number of factors could contribute to this failure to see a reduction in reinstatement or cocaine generalization at 72 hrs. During self-administration smaller doses of cocaine are given over an extended period of time. In the other procedures a relatively larger dose of cocaine was administered as a bolus injection. It may be easier for AlbuBChE to metabolize the cocaine before it reaches the brain under the self-

- 71 -

administration conditions than under the other conditions. When AlbuBChE metabolizes cocaine under self-administration, its effect is to institute extinction conditions. Behavioral recovery from extinction may also prolong the effects of AlbuBChE treatment on self-administration. In the reinstatement study, responding produced saline and the stimulus previous associated with reinforcement. When the monkeys reinstated responding following a cocaine injection, the monkeys still received saline for responding, but they also received the stimulus previously associated with cocaine. The presentation of this stimulus may also have facilitated continued responding under the reinstatement conditions (Spealman et al., 1999), masking some of the effect of AlbuBChE.

The effects of AlbuBChE were almost certainly related to its effects on the metabolism of cocaine. The administration of AlbuBChE decreased the amount of cocaine in the blood for at least 72 hours. The fact that EME levels were also increased for at least 72 hours following AlbuBChE suggests that AlbuBChE was metabolizing cocaine similarly to native BChE (Jones, 1984). This time course for cocaine metabolism is similar to that seen for the self-administration experiment where the monkeys were reinforced with 30 µg/kg/inj cocaine. Further, AlbuBChE had no effect on response rate in the discrimination study, suggested that it does not produce a non-specific effect on operant responding independent of the presence of cocaine in the blood.

The observation of relatively high levels of AlbuBChE antibodies in one monkey following its fourth injection indicates that AlbuBChE might lose some of its effectiveness over multiple injections. While the results for that one particular monkey did not appear to be different from the other

- 72 -

2 monkeys tested, the observation of antibodies suggests that some of the AlbuBChE would be bound by antibodies which might decrease its ability to metabolize cocaine. Further work will be needed to determine how prevalent the antibody production is
5 in primates and whether the levels of antibody observed are sufficient to decrease the effectiveness of AlbuBChE in metabolizing cocaine.

Cocaine continues to show up in both emergency room mentions
10 and medical examiner reports in the DAWN surveys. As such, a drug that could counteract the toxic effects of cocaine may be useful adjunct to emergency room treatment for patients abusing cocaine. An advantage of using a drug that metabolizes cocaine is that it will be specific for cocaine and should have minimal
15 side effects. However, it would be necessary to confirm that a patient is using cocaine as AlbuBChE would not be effective against toxicity produced by other drugs of abuse, such as the amphetamines, that might produce a similar spectrum of toxic effects.

20 In addition to treating the toxic effects of cocaine, AlbuBChE might also be useful as an adjunct to other treatments for cocaine abuse. A person with a sufficient blood level of AlbuBChE who takes cocaine would be expected to experience a
25 reduced subjective effect of cocaine. In the context of the current study, this translates to less cocaine self-administration, reduced reinstatement of cocaine self-administration and the failure of cocaine to generalize to the methamphetamine discriminative stimulus. As relapse to cocaine
30 abuse remains a problem in treatment, the reduced subjective effects of cocaine would be expected to reduce relapse. For someone undergoing treatment that does relapse to use, the presence of AlbuBChE in the blood would metabolize much of the cocaine and potentially reduce the likelihood of continued

- 73 -

cocaine use. This would require an individual to continue taking AlbuBChE throughout the period of time that relapse would be expected. Therefore, it will be critical to determine how prevalent the development of antibodies to AlbuBChE is and
5 how that might effect its ability to metabolize cocaine.

In conclusion, pretreatment of squirrel monkeys with AlbuBChE was able to reduce the amount of cocaine in the blood following a cocaine bolus treatment. Pretreatment with AlbuBChE was also
10 able to reduce cocaine self-administration in monkeys that had been trained to self-administer 10 and 30 µg/kg/inj cocaine. AlbuBChE was able to block cocaine-induced reinstatement of cocaine self-administration for 2 different doses of cocaine. Finally, AlbuBChE was also able to antagonize the
15 discriminative effects of cocaine in squirrel monkeys. The finding that AlbuBChE was able to antagonize the behavioral effects of cocaine in 3 different models of cocaine's behavioral effects suggests that it should be effective as a potential treatment of cocaine abuse.

20

**Example 5 - A double-blind, placebo-controlled, single ascending dose of AlbuBChE followed by multiple doses of cocaine to evaluate the pharmacokinetic and pharmacodynamic parameters of cocaine after AlbuBChE administration in cocaine recreational
25 volunteers**

Objective

To determine cocaine blood levels following multiple doses subsequent to AlbuBChE administration, and to determine the
30 behavioral, psychological and safety effects of cocaine following multiple doses subsequent to AlbuBChE administration. To evaluate the pharmacokinetics, safety, and tolerability of AlbuBChE.

- 74 -

Study Design

This is a 5-arm, parallel-group, active and placebo-controlled, double blind, randomized study, to compare treatment with
5 AlbuBChE with placebo as a negative control.

Forty (40) non-treatment seeking healthy adults who have used cocaine are randomly assigned to five (5) treatment groups. Each group will contain eight subjects.

10

Group A (low dose): Subjects receive a single, intramuscular injection of 50 mg of AlbuBChE.

Group B (intermediate dose): Subjects receive a single, intramuscular injection of 100 mg of AlbuBChE.

15 Group C (high-intermediate dose): Subjects receive a single, intramuscular injection of 150 mg of AlbuBChE.

Group D (high dose): Subjects receive a single, intramuscular injection of 300 mg of AlbuBChE.

20 Group E (negative control): Subjects receive a single, intramuscular injection of placebo.

The study is divided to 5 cohorts of 8 subjects per cohort with 2 to 3 days apart.

25 *Inpatient Hospital Phase*

Subjects remain resident in the clinic from the evening before Day 1 (check in; Day -1) until the morning of Day 11. Subjects return to the clinic for follow-up visit on Day 18±2.

30 Day -1: On the morning of day -1, a 40 mg intravenous dose of cocaine is administered.

Day 1: A single, intramuscular dose of AlbuBChE or placebo is administered.

- 75 -

Day 1, 4, 8 and 10: On Day 1, at AlbuBChE C_{max} (3 hours post dose) and at the morning of days 4 (72 hours), 8 (168 hours) and 10 (216 hours) at the corresponding time of AlbuBChE/
5 placebo administration, a 40 mg intravenous challenge dose of cocaine is administered.

Saline infusions are included (randomized with cocaine infusions, administered 2 hours apart) on each day of cocaine
10 administration, and have the infusion type order single blinded.

Study Drug Administration

Planned AlbuBChE doses are as described above for groups A-E.
15 Each subject receives a single dose of AlbuBChE or placebo by intramuscular (IM) injection. 40 mg of cocaine hydrochloride, prepared as a 1 mg/ml saline solution is injected into a forearm vein at the rate of 1 mg/second using a constant rate infusion pump.

20

Study Duration

The duration of the study (from screening until the End of Study (EOS) visit) for each subject is approximately 11 to 12 weeks.

25

Study Population

A total of Forty (40) male subjects aged 18-55 who are recreational cocaine users. Subjects who discontinue the study for any reason after dosing are not replaced.

30

Main Inclusion/Exclusion Criteria

Inclusion Criteria

1. Male volunteers aged 18-55 years, inclusive;

- 76 -

2. Body mass index (BMI) 18-33 kg/m² and weight of at least 50 kg;
3. Healthy, as determined by no clinically significant medical history, physical examination, electrocardiogram (ECG),
5 vital signs and laboratory results at screening;
4. Currently use cocaine by smoking, intranasal or i.v. route; defined as at least once in a month prior to screening, 6 times in the past year and 20 times over lifetime. Current use is confirmed through a positive Urine Drug Test (UDS)
10 prior to in-patient period (i.e. at screening or between screening and Day -1);
5. Able to understand the nature of the trial and any hazards of participating in it;
6. Able to communicate satisfactorily with the Investigator and
15 to participate in, and comply with the requirements of the entire trial; and
7. Willingness to give written consent to participate, after reading the information and consent form and after having the opportunity to discuss the trial with the Investigator
20 or his delegate and sign the Informed Consent.

Exclusion Criteria

1. Current or history of alcohol or drug dependence according to DSM-IV-TR (Diagnostic and Statistical Manual of Mental
25 Disorders - last version) criteria (excluding nicotine and caffeine), or participation in rehabilitation program (except smoking cessation);
2. History of severe allergic or anaphylactic reactions;
3. Known allergy or hypersensitivity to natural or recombinant
30 butyryl cholinesterase (BChE), human serum albumin (HSA) or any other component of the formulation;
4. History of any clinically significant (as determined by the Principal Investigator) cardiac, endocrine, haematological, hepatic, immunological, metabolic, urological, pulmonary,

- 77 -

- neurological, dermatological, psychiatric, renal, or other major disease;
5. History of any malignant disease;
6. Major trauma or surgery in the 2 months before screening or at any time between screening and check-in;
7. Acute infection within 2 weeks before screening or at any time between screening and check-in;
8. Clinically significant abnormal ECG findings at screening or check-in visits, as determined by the Principal Investigator;
9. Blood pressure outside the ranges 90-140 mmHg systolic or 45-90 mmHg diastolic (measured after a rest of at least 5 min) at screening or check-in. Blood pressure may be re-tested in the supine position at intervals of 5-10 min. The pressure elevation is considered sustained if either the systolic or the diastolic pressure exceeds the stated limits after three assessments, and thus the subject may not be randomized;
10. Heart rate <40 beats/min (measured after a rest of at least 5 min) at screening or check-in;
11. Known history of or a positive test result for human immunodeficiency virus (HIV) types 1 or 2 at screening;
12. Known history of, or a positive test result for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) at screening;
13. Use of prescription or over-the-counter (OTC) medication (other than paracetamol (acetaminophen), $\leq 2\text{g/day}$ or ibuprofen, $\leq 650\text{ mg/day}$), investigational drugs, vitamins, or herbal remedies, within 2 weeks before dosing or within 5 half-lives before dosing, whichever is longer;
14. Participation in another clinical trial of a new chemical entity or a prescription medicine within the 3 months before dosing or within 5 half-lives before dosing, whichever is longer;

- 78 -

15. Loss of >400 mL blood (e.g. as a blood donor) within
2 months before dosing, or received any blood, plasma, or
platelet transfusions within 3 months before check-in, or
who have planned donations during the study or within
5 3 months after the study;
16. Subject consumes greater than 20 cigarettes per day on
average, in the month prior to screening, or be unable to
abstain from smoking (or use of any nicotine-containing
substance) for at least 6 hours (so that all pre-dose and
10 post-dose/infusion-related assessments can be completed
uninterrupted);
17. Positive UDS for amphetamine, cocaine (only on at Day -1),
opioids, cannabinoids, and benzodiazepines. Positive result
for benzodiazepines and cannabinoids acceptable as long as
15 stable or decreasing due to long half-lives. Positive UDS
for other drugs will result in rescheduling at discretion
of investigator/designee; Positive breath alcohol test will
also result in rescheduling at discretion of
investigator/designee;
- 20 18. Exposure to pesticides or any other organophosphates (e.g.
agricultural workers);
19. Inability to participate or successfully complete the
study, in the opinion of their general practitioner or the
Investigator, because the volunteer is:
- 25 a. mentally or legally incapacitated, or unable to give
consent for any reason;
- b. in custody due to an administrative or a legal decision,
or under supervised parole, or being admitted to a
sanitarium or social institution;
- 30 c. unable to be contacted in case of emergency; or
- d. unlikely to cooperate or comply with the clinical study
protocol or is unsuitable for any other reason.

- 79 -

Safety, Tolerability, and Immunogenicity Analysis*Safety*

Vital signs, including blood pressure, heart rate (part of the PD parameters) and body temperature are monitored. Physical examinations, clinical laboratory tests, and 12-lead ECGs are performed, with 12-lead ECGs after each cocaine infusion, at pre-infusion, 30 minutes and 2 hours post-infusion, and 8 hour post-first infusion. Telemetry is performed for 4 hours immediately after each cocaine injection.

10

Samples for BChE and acetyl cholinesterase (AChE) activity are collected but are not all necessarily assayed. The activity of BChE and AChE is determined using a colorimetric method based on the Ellman assay, which serves as a neutralization assay for the endogenous enzymes. Sampling timepoints are at screening, pre-dose and 168 hours (Day 8), 240 hours (Day 11) and follow-up (Day 18±2) post-dose. The blood tests for Ellman assay take place before administration of cocaine (where relevant).

20 Intravenous infusions of cocaine are immediately terminated if any of the following occur:

1. Systolic BP of 180 mm Hg or greater;
2. Diastolic BP of 100 mm Hg or greater;
3. HR of 130 bpm or greater; or
- 25 4. Behavioral manifestations of psychostimulant toxicity (agitation, psychosis, or inability to cooperate with study procedures).

Subjects are considered to have not tolerated IV cocaine infusions if any of the following occur:

- 30 1. Acute chest pain or shortness of breath;
2. Systolic BP of 180 mm Hg or greater;
3. Diastolic BP of 120 mm Hg or greater;

- 80 -

4. HR of $[220 - \text{age} \times 0.85]$ bpm or greater (i.e., > than 170 bpm for a 20 year old subject);
5. Neurologic or psychiatric events (e.g., panic or psychosis);
- 5 6. Clinically significant ECG abnormalities (including heart block, three or more sustained ectopic ventricular beats, or tachyarrhythmias);
7. Stopping criteria for further cocaine infusion do not return to acceptable limits within appropriate time frames
- 10 (e.g., 30 min);
8. Stopping criteria for further cocaine infusion are met for a second time within the protocol; or
9. Any condition that in the clinical judgment of the PI that is of sufficient magnitude to present a danger to the
- 15 subject.

Tolerability of AlbuBChE IM injection

Local tolerability and pain (evaluated by the visual analogue scale (VAS)) at the injection site are evaluated during the

20 first 24 h after dosing. Timepoints are: 20 min, 1, 2, 4, 8 and 24 hours post AlbuBChE dose.

Adverse events (AEs) are monitored throughout the study.

25 *Immunogenicity (IG)*

Samples for immunogenic levels are collected but are not all necessarily assayed. Titers for antibodies against HSA, human butyryl cholinesterase (hBChE) and AlbuBChE are evaluated. Sampling timepoints are: pre-dose and 168 hours (Day 8), 240

30 hours (Day 11) and follow-up (Day 18±2) post-dose. The blood tests for IG assays take place before administration of cocaine (where relevant).

- 81 -

Pharmacokinetic Variables and Sampling

To determine serum concentration of AlbuBChE, blood samples are collected before dosing and at several time points after dosing. Where the data permits, the following pharmacokinetic

5 (PK) parameters are calculated:

	C_{max}	maximum observed serum concentration
	t_{max}	time to achieve C_{max}
10	$AUC_{(0-t)}$	area under the serum concentration-time curve from 0 h to the time of the last quantifiable concentration
	$AUC_{(0-\infty)}$	area under the serum concentration-time curve extrapolated to infinity
15	AUC_{ext}	percentage of $AUC_{(0-\infty)}$ due to extrapolation from t_{last} to infinity
	V_d/f	apparent volume of distribution during terminal phase
	CL/f	apparent total body clearance
20	λ_z	terminal elimination rate constant, estimated by linear regression on the terminal phase of the semi-logarithmic concentration versus time curve
	$t_{1/2}$	apparent terminal elimination half-life
	MRT	mean residence time

25 Additional parameters may be calculated if deemed necessary. Sampling timepoints for PK of AlbuBChE in serum are as follows: pre-dose and 20, 40 min, 1 hour, 1.5 hours, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, 12 hours, 18 hours, 24 hours (Day 2), 36 hours (Day 2), 48 hours (Day 3), 72 hours (Day 4), 96 hours
30 (Day 5), 120 hours (Day 6), 144 hours (Day 7), 168 hours (Day 8), 192 hours (Day 9), and 216 hours (Day 10) post-dose. Where applicable, sampling timepoints for PK of AlbuBChE are taken just before cocaine infusion.

- 82 -

Pharmacodynamics Variables and Sampling

Blood samples are collected before dosing and at several time-points after each dosing of cocaine. *In vivo* cocaine levels (and its metabolites benzoylecgonine and ecgonine methyl ester) are determined by validated LC/MS-MS method and are correlated to AlbuBChE blood levels. Cocaine exposure (C_{max} , t_{max} , $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and V_d) and clearance (CL , λ_z , $t_{1/2}$) are determined. PD sampling timepoints of cocaine levels in serum after each cocaine dose are as follows: pre-dose, 2, 5, 10, 15, 30, 45, 60, 90, and 120 minutes post dose.

Blood pressure and heart rate are measured pre-infusion and 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 300 minutes post infusion.

Behavioral and psychological effects (Subjective effects)

Subjective effects are measured using a computerized (21 CFR 11 validated) visual analogue scale (VAS). The time points for collection of subjective effects match with those for cocaine blood samples. The following set of VAS are: Drug Liking, Take Drug Again, Overall Drug Liking, High, Good Effects, Bad Effects, Feeling Rush, Desire for Cocaine, Feeling Anxious, Over-Stimulated, and Any Drug Effects.

PD sampling timepoints of VAS parameters after each cocaine dose are as follows: pre-dose, 2, 5, 10, 15, 30, 45, 60, 90, and 120 minutes post dose.

Urine Collection

Urine is collected for metabolic investigations over a 52 hour interval after cocaine administration on Day -1, Day 4 and Day 8. The collection ranges are: (i) immediately after cocaine

- 83 -

dose, followed by collection intervals of (ii) two intervals of three hours, and (iii) nine intervals of six hours.

Statistical Analysis

5 Pharmacokinetics

PK parameters are derived from serum concentrations for each dose level (number of subjects, mean, SD, geometric mean (for AUCs and C_{max}), coefficient of variation, minimum, median, and maximum). Dose proportionality (for AlbuBChE only) using the
10 power model is evaluated for C_{max} , AUC_{0-t} , and $AUC_{0-\infty}$.

Pharmacodynamics

Time course of PD effects (subjective and objective) is determined. PD parameters include: E_{max} (maximum effect), TE_{max}
15 (time to E_{max}), time averaged area under the effect curve (TA_AUE), partial AUE for early time points, and cumulative AUE.

Safety

20 Safety data is determined using descriptive statistics and change from Baseline, where appropriate, for each treatment group. AEs are coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by system organ class and preferred term.

25

Bioanalytical

Frozen serum samples for determining AlbuBChE concentrations and antibodies are shipped on dry ice for analysis. Analyses of cocaine and metabolite levels is performed using validated
30 methods.

- 84 -

Results*Safety, Tolerability and Immunogenicity*

AlbuBChE is safe to administer to humans. No significant safety
5 issues are observed with intramuscular (IM) AlbuBChE doses of
50 mg to 300 mg. AlbuBChE is well tolerated, with no
unacceptable side effects. Local tolerability and pain at the
IM injection site are acceptable, with no significant adverse
events. The administration of 50 mg to 300 mg of AlbuBChE as a
10 single dose does not provoke a significant immune response.
Titers for antibodies against HSA, human butyryl cholinesterase
(hBChE) and AlbuBChE are all within acceptable limits.

Pharmacokinetic Variables

15 Maximum observed serum concentration of AlbuBChE, C_{max} ,
increases with increasing dose of AlbuBChE. AlbuBChE absorption
from the IM site of administration is rapid, with a short time
to achieve C_{max} . $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ indicate that AlbuBChE
levels persist for a significant and clinically useful amount
20 of time. V_d/f values indicate that the majority of AlbuBChE is
present in the circulation during the terminal phase. CL/f
values indicate that AlbuBChE is cleared from the body at an
acceptable rate. Apparent terminal elimination half-life values
indicate a weekly or twice weekly dosing of AlbuBChE to
25 maintain therapeutic levels. The mean residence time of
AlbuBChE is within clinically acceptable limits.

Pharmacodynamic Variables

30 Maximum observed serum concentration of cocaine, C_{max} , decreases
with increasing dose of AlbuBChE. C_{max} levels are achieved very
rapidly following a 40 mg intravenous dose of cocaine, with a
short t_{max} . $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ of cocaine and its metabolites
benzoyllecgonine and ecgonine methyl ester indicate that cocaine

- 85 -

is more rapidly metabolized in subjects receiving a dose of AlbuBChE prior to cocaine exposure than subjects receiving placebo. Cocaine $AUC_{(0-t)}$ decreases as a function of AlbuBChE dose and increases as a function of time post-AlbuBChE administration. V_d values indicate that the majority of cocaine is present in the circulation during the terminal phase. CL values indicate more rapid cocaine clearance following administration of AlbuBChE compared to placebo, with clearance rate decreasing as time post AlbuBChE administration increases. Similarly, apparent terminal elimination half-life values indicate more rapid cocaine clearance following administration of AlbuBChE compared to placebo, with clearance rate decreasing as time post AlbuBChE administration increases.

15 *Behavioral and psychological effects*

Subjects administered AlbuBChE report significantly different results on the visual analogue scale (VAS) compared to subjects administered placebo. AlbuBChE administration decreases cocaine liking. AlbuBChE administration decreases the desire to take cocaine again. AlbuBChE administration decreases overall drug liking. AlbuBChE administration decreases the "high" associated with cocaine exposure. AlbuBChE administration decreases the subjective feeling of "good effects" after cocaine exposure. AlbuBChE administration decreases subjective feeling of "rush" associated with cocaine exposure. AlbuBChE administration decreases the desire for cocaine. AlbuBChE administration reduces feelings of anxiousness following cocaine exposure. AlbuBChE administration reduces feelings of being over-stimulated following cocaine exposure. AlbuBChE administration reduces the reporting of "any drug effects."

Subjects administered AlbuBChE prior to cocaine exposure do not report significantly different "bad effects" following AlbuBChE administration compared to subjects administered placebo.

- 86 -

AlbuBChE reduces the feeling of "bad effects" following cocaine exposure.

Discussion

5 The present Example determines cocaine blood levels following multiple doses of cocaine subsequent to AlbuBChE administration, and determines the behavioral, psychological and safety effects of cocaine following multiple doses subsequent to AlbuBChE administration in an inpatient setting.

10 The primary measure of a successful treatment of cocaine abuse and dependence in an outpatient setting is a period of abstinence during and following treatment. The facilitation of a period of abstinence, such as a two or three week period of
15 abstinence, indicates a successful treatment. Secondary outcome measures include reduction in cocaine levels or amount of cocaine intake, overall proportion of cocaine non-use days, proportion of successful subjects, the largest number of consecutive cocaine non-use days, and severity of cocaine
20 dependence as evaluated, for example, by the cocaine selective severity assessment (CCSA).

Previous studies have found that AlbuBChE has a half life of approximately 8 hours in rats and have speculated that the
25 potential half-life of AlbuBChE would range from 1 to several days in humans. (Brimijoin et al., 2008; Gao et al. 2009). Gao et al. noted that the observed half-life of monomeric AlbuBChE in rats is shorter than that of native tetrameric BChE, and speculated that the half-life of AlbuBChE could be increased by
30 post-translational modifications such as polyethylene glycosylation.

The present Example determines that the half-life of AlbuBChE when administered by intramuscular injection is dose dependent,

- 87 -

with half-life values increasing with increasing dose. The half-life values at the specified dosages allow for a weekly or twice weekly dosing schedule without the need for post-translational modifications such as polyethylene glycosylation.

5 Previous studies have also reported that intravenous administration of AlbuBChE to rats can cause a modest increase in blood pressure and mild lethargy. (Brimijoin et al. 2008). In contrast, a significant increase in blood pressure is not
10 observed when AlbuBChE is administered to humans by intramuscular injection, nor is lethargy reported in a significant number of subjects.

Human subjects administered AlbuBChE do not report a
15 significant increase in cocaine cravings. In contrast, desire to use cocaine is significantly decreased following AlbuBChE dosing and remains significantly depressed for up to one week following a single administration of AlbuBChE.

20 The findings of the present Example indicate that intramuscular administration of AlbuBChE to humans at the specified dosages does not result in any unacceptable side effects and that the specified dosages will allow for the successful treatment of biological effects of cocaine exposure.

25

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- 89 -

What is claimed is:

1. A method of attenuating a biological effect of a cocaine exposure in a primate comprising administering to the primate an amount of a fusion protein comprising:

(a) a mutant butyrylcholinesterase (BChE) polypeptide comprising the sequence

EDDIIATKNGKVRGMNLTVFGGTVTAFLGIPYAQPPLGRLRFKKPQSLTKWSDIWNNA
TKYANSCCQNIQSFPGFHGSEMWNPNNTDLSEDCLYLVNWI PAKPKKNATVLIWIYGG
GFQTGTSSLHVYDGKFLARVERVIVVSMNYRVGALGFLALPGNPEAPGNMGLFDQQLA
LQWVQKNIAAFGKNPKSVTLFGESSGAASVSLHLLSPGSHSLFTRAILQSGSFNAPWA
VTSLYEARNRTLNLAKLTGCCSRENETEIIKCLRNKDPQEILLNEAFVVPYGTPLGVNF
GPTVDGDFLTDMPDILLELGQFKKTQILVGVNKDEGTWFLVGGAPGFSKDNNSIITRK
EFQEGLKIFFPGVSEFGKESILFHYTDWVDDQRPENYREALGDVVGVDYNFICPALEFT
KKFSEWGNNAFFYYFEHRSSKLPWPEWMGMHGYEIEFVFGLPLERRDNYTKAEIILS
RSIVKRWANFAKYGNPNETQNNSTSWPVFKSTEQKYLTLNTESTRIMTKLRAQQCRFW
TSFFPKV (SEQ ID NO:1),

(b) a human serum albumin (HSA) polypeptide comprising the sequence

DAHKSEVAHRFKDLGEENFKALVLI AFAQYLQQCPFEDHVKLVNEVTEFAKTCVADES
AENCCKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFQHKDDNPNLRLV
RPEVDVMCTAFHDNEETFLKKYLYEIAARRHPYFYAPELLFFAKRYKAAFTTECCQAADK
AACLLPKLDELDEGKASSAQRLKASLQKFGERAFAKAWAVARLSQRFPKAEFAEVS
KLVTDLTKVHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCI
AEVENDEMPADLP SLAADFVESKDVCKNYAEAKDVFLGMFLY EYARRHPDYSVVL LRL
LAKTYETTL EKCCAAADPHECYAKVFDEFKPLVEEPQNLIKQNCLEFQ LGEYKFQNA
LLVRYTKKVPQVSTPTLVEVSRNLGKVGSKCKHPEAKRMPCAEDYLSVVLNQLCVLH
EKTVPVSDRVTKCTESLVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQ
IKKQTALVELVKHKPKATKEQLKAVMDDFAAFVEKCKADDKETCF AEKGKLV AASQ
AALGL (SEQ ID NO:2), and

(c) a signal peptide comprising the sequence
MRPTWAWWLFLVLLALWAPARG (SEQ ID NO:3),

- 90 -

wherein the amount of the fusion protein is effective to cause attenuation of the biological effect of the cocaine exposure in the primate.

2. The method of claim 1, wherein the fusion protein comprises the sequence

MRPTWAWWLFVLVLLALWAPARGEDDIIATKNGKVRGMNLTVPGGTVTAFLGIPYAQ
PPLGRLRFKKPQSLTKWSDIWNATKYANSCCNIDQSFPGFHGSEMNPNTDLSDECL
YLNWVIPAPKPKNATVLIWIYGGGFQTGTSSLHVYDGKFLARVERVIVSMNYRVGAL
GFLALPGNPEAPGNMGLFDQQLALQWVQKNIAAFGGNPKSVTLFGESSGAASVSLHLL
SPGSHSLFTRAILQSGSFNAPWAVTSLYEARNRTLNLAKLTGCSRENETEIIKCLRNK
DPQEILLNEAFVVPYGTPLGVNFGPTVDGDFLTDMPDILLELGQFKKTQILVGVNKDE
GTWFLVGGAPGFSKDNNSIITRKEFQEGLEKIFFPGVSEFGKESILFHYTDWVDDQRPE
NYREALGDVVGDNFICPALEFTKKFSEWGNNAFFYYFEHRSSKLPWPEWMGMHGYE
IEFVFGLEPLERRDNYTKAEILSRSIVKRWANFAKYGNPNETQNNSTSWPVFKSTEQK
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FAAFVEKCKKADDKETCFAEEGKKLVAASQAALGL (SEQ ID NO:4).

3. The method of claim 1 or claim 2, wherein the fusion protein is administered prior to the cocaine exposure.

4. The method of claim 3, wherein the fusion protein is administered up to one day prior to the cocaine exposure.

- 91 -

5. The method of claim 3, wherein the fusion protein is administered up to 216 hours prior to the cocaine exposure.

6. The method of claim 1 or claim 2, wherein the fusion protein is administered after the cocaine exposure.

7. The method of claim 6, wherein the fusion protein is administered up to one hour after the cocaine exposure.

8. The method of any one of claims 1-7, wherein the cocaine exposure is a single cocaine exposure.

9. The method of any one of claims 1-7, wherein the cocaine exposure is a recurring cocaine exposure.

10. The method of claim 9, wherein the recurring cocaine exposure is cocaine abuse or cocaine dependence.

11. The method of any one of claims 9-10, wherein the recurring cocaine exposure comprises at least six single cocaine exposures in a twelve month period.

12. The method of any one of claims 9-10, wherein the recurring cocaine exposure comprises at least twenty single cocaine exposures during the primate's lifetime.

13. The method of any one of claims 1-12, wherein the biological effect is caused by cocaine overdose in the primate and the attenuating is treating or preventing the biological effect.

14. The method of any one of claims 1-13, wherein the biological effect is an increase in blood pressure.

- 92 -

15. The method of claim 14, wherein the duration of the increase in blood pressure is reduced by 60-90%.

16. The method of claim 15, wherein the duration of the increase in blood pressure is reduced by about 78%.

17. The method of any one of claims 1-13, wherein the biological effect is an increase in heart rate or body temperature.

18. The method of claim 17, wherein the degree of attenuation is 45%-70%.

19. The method of claim 18, wherein the degree of attenuation is 57%.

20. The method of any one of claims 1-13, wherein the biological effect is cocaine seeking behavior in the primate.

21. The method of claim 20, wherein the cocaine seeking behavior occurs during a period of cocaine abstinence following the cocaine exposure.

22. The method of claim 20, wherein the cocaine seeking behavior follows a relapse.

23. The method of claim 22, wherein administration of the fusion protein two hours before the relapse attenuates cocaine seeking behavior by the primate immediately following the relapse.

24. The method of any one of claims 20-23, wherein administration of the fusion protein two hours before the

- 93 -

cocaine exposure results in a 50% to 100% reduction in the cocaine seeking behavior by the primate.

25. The method of any one of claims 20-24, wherein attenuation of cocaine seeking behavior is observed up to four days following administration of the fusion protein.

26. The method of any one of claims 20-25, wherein the administration of the fusion protein results in a lowering of total cocaine exposure in the primate than without the administration.

27. The method of any one of claims 20-26, wherein attenuating cocaine seeking behavior results in a period of cocaine abstinence of 2 weeks to 3 weeks.

28. The method of any one of claims 20-27, wherein attenuating cocaine seeking behavior results in a larger proportion of days in which the primate is not exposed to cocaine than without the administration.

29. The method of any one of claims 20-28, wherein attenuating cocaine seeking behavior results in a larger number of consecutive days in which the primate is not exposed to cocaine than without the administration.

30. The method of any one of claims 20-29, wherein attenuating cocaine seeking behavior results in a lessening of severity of cocaine dependence or abuse as evaluated by the cocaine selective severity assessment (CSSA) or Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV).

31. The method of any one of claims 1-30, wherein the effective amount of the fusion protein is an amount which

- 94 -

reduces the primate's serum cocaine level to about 0 ng/ml within about 30 minutes of a 1 mg/kg intravenous cocaine dose.

32. The method of any one of claims 1-31, wherein the administration of the fusion protein reduces the primate's serum cocaine level to less than 12% of the serum cocaine level without the administration within about 5 minutes of a 1 mg/kg intravenous cocaine dose.

33. The method of claim 32, wherein the administration of the fusion protein reduces the primate's serum cocaine level to 7% of the serum cocaine level without the administration within about 5 minutes of a 1 mg/kg intravenous cocaine dose.

34. The method of any one of claims 1-33, wherein the fusion protein is administered weekly or twice weekly.

35. The method of any one of claims 1-34, wherein the fusion protein is administered by intramuscular injection or subcutaneous injection.

36. The method of any one of claims 1-35, wherein the fusion protein is in a formulation buffer comprising 10mM sodium phosphate, 200mM mannitol, 60mM trehalose, and 0.01% (w/v) polysorbate 80, pH 7.2.

37. The method of claim 36, wherein the fusion protein is present in the formulation at a concentration of at least 30 mg/ml.

38. The method of any one of claims 1-37, wherein attenuation of the biological effect is observed up to 72 hours after administration of the fusion protein.

- 95 -

39. The method of any one of claims 1-38, wherein the primate is a human.

40. The method of claim 39, wherein the cocaine exposure is a single cocaine exposure of 10 mg to 60 mg or a recurring cocaine exposure wherein each single cocaine exposure of the recurring cocaine exposure is 10 mg to 60 mg.

41. The method of claim 40, wherein the effective amount of the fusion protein is an amount which reduces the human's serum cocaine level to about 0 ng/ml within about 30 minutes of a 40 mg intravenous cocaine dose.

42. The method of any one of claims 39-41, wherein the effective amount of the fusion protein is 0.06 mg/kg to 5 mg/kg.

43. The method of claim 42, wherein the effective amount of the fusion protein is 0.06 mg/kg, 0.3 mg/kg, 1.6 mg/kg, or 4.8 mg/kg.

44. The method of any one of claims 39-41, wherein the effective amount of the fusion protein is 50 mg to 300 mg.

45. The method of claim 44, wherein the effective amount of the fusion protein is 50 mg, 100 mg, 150 mg, or 300 mg.

MRPTWAWWFLVLALLALWAPARG

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Figure 1

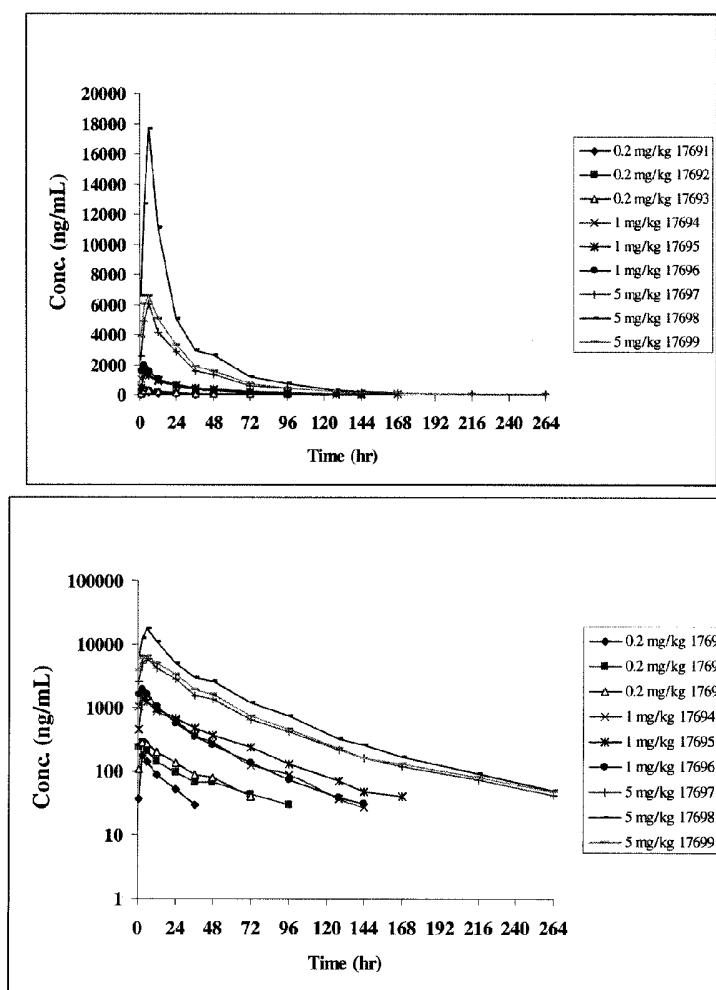


Figure 2

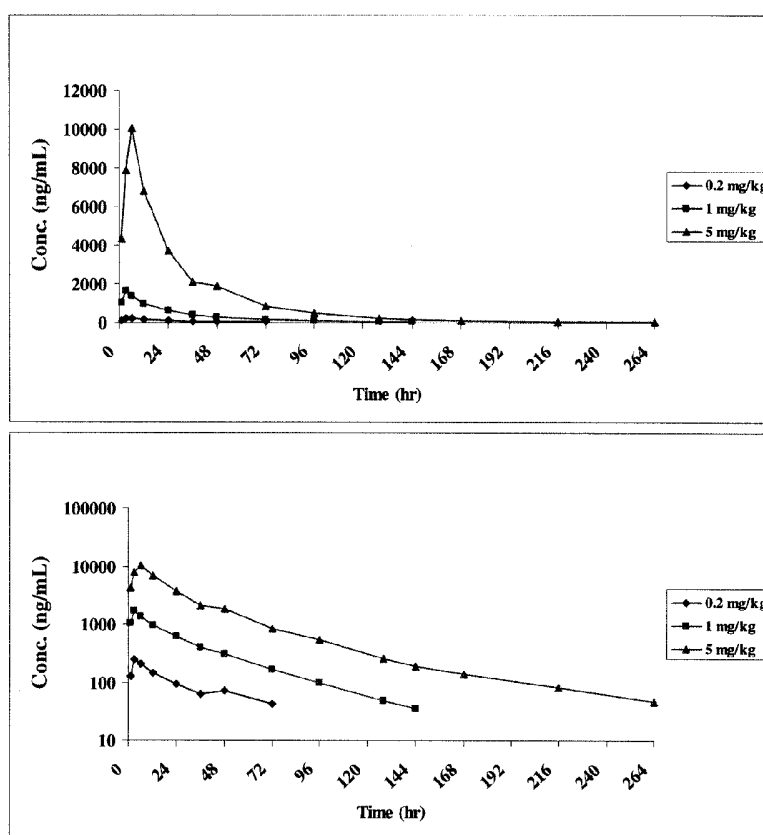


Figure 3

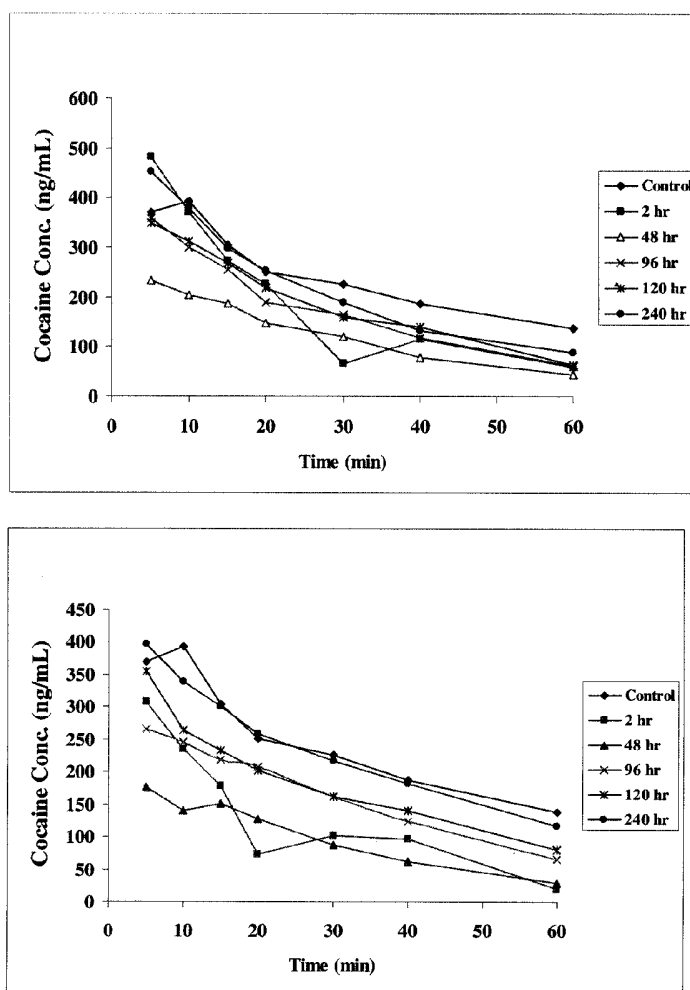


Figure 4A

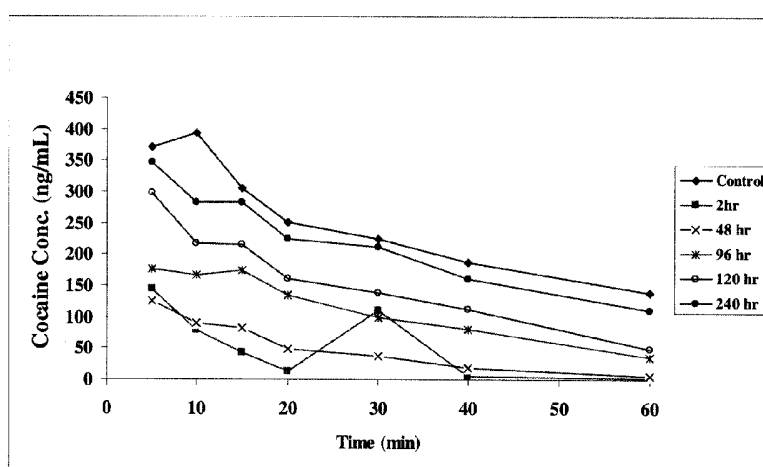


Figure 4B

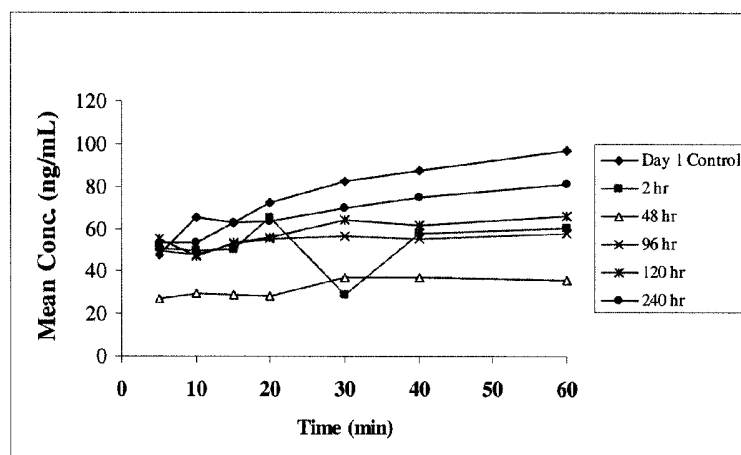


Figure 5A

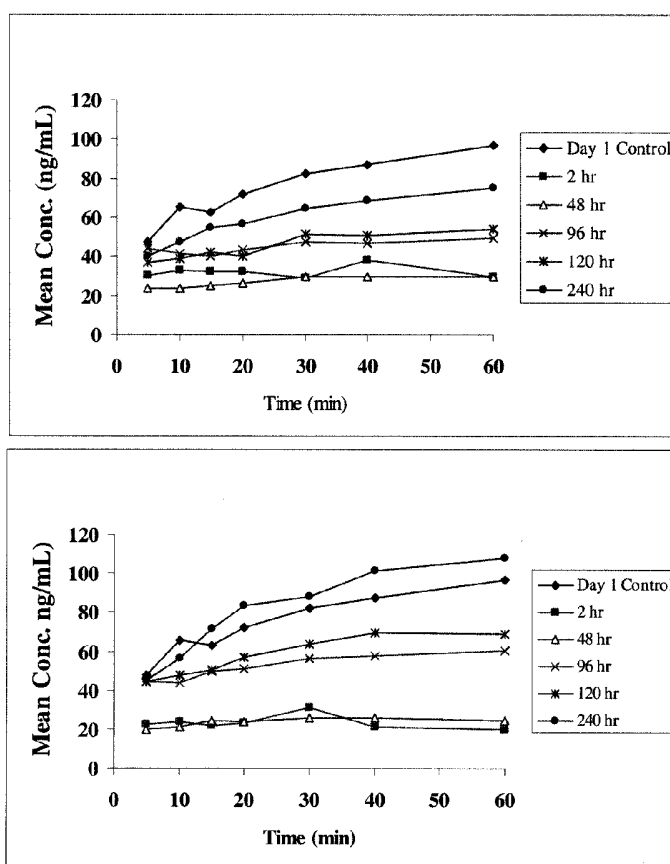


Figure 5B

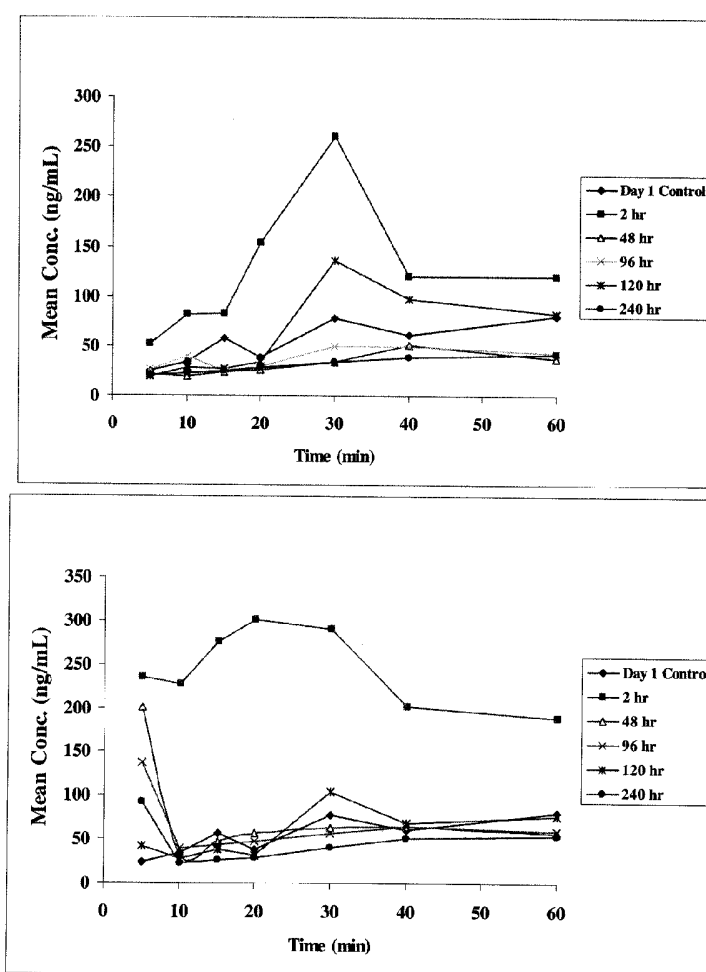


Figure 6A

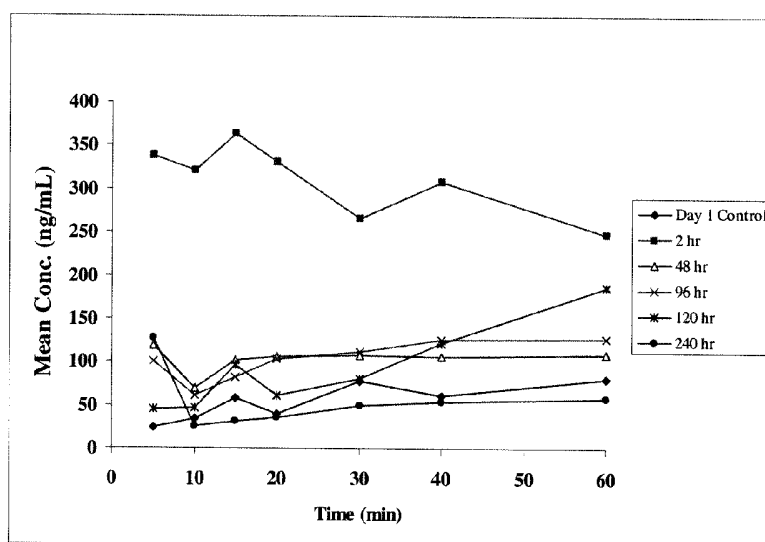


Figure 6B

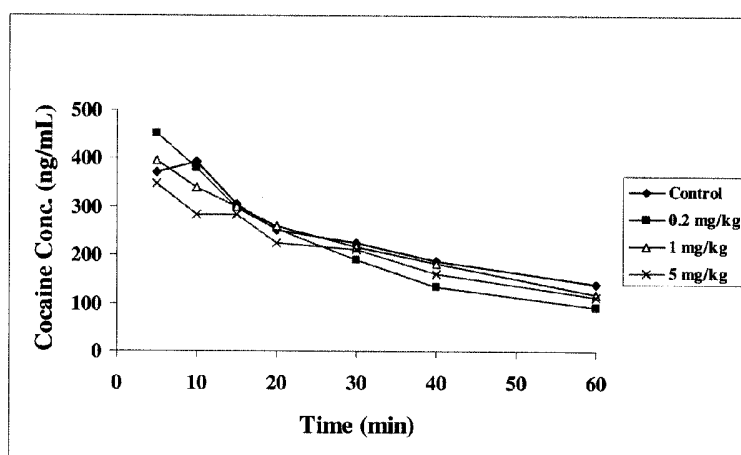


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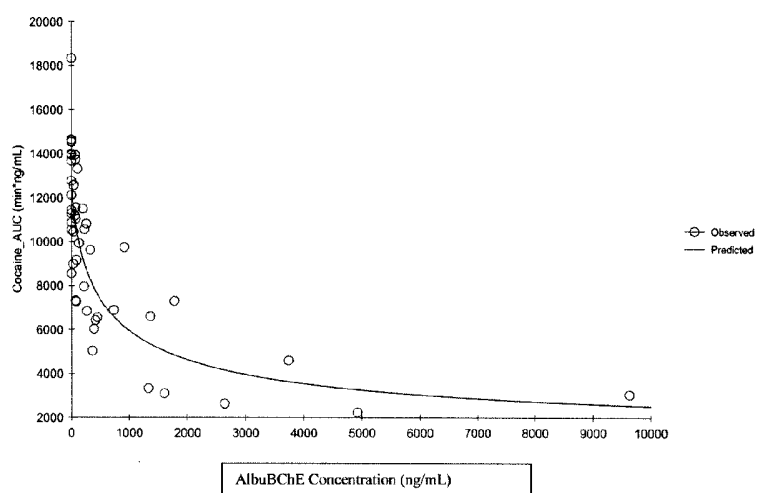


Figure 8

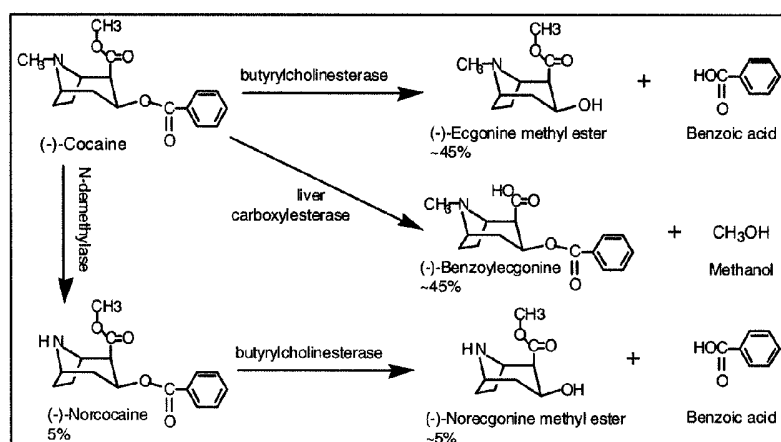


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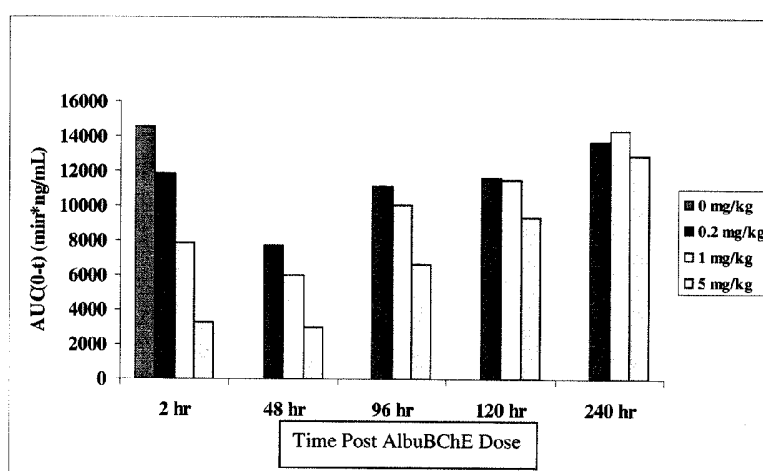


Figure 10

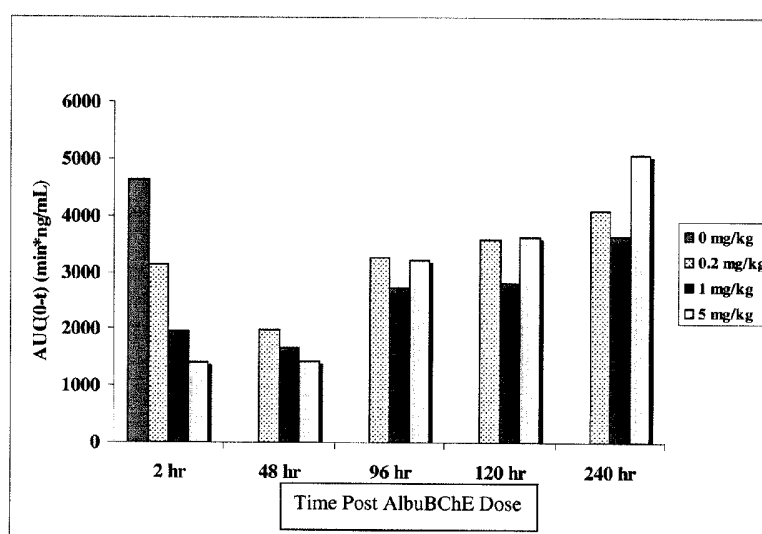


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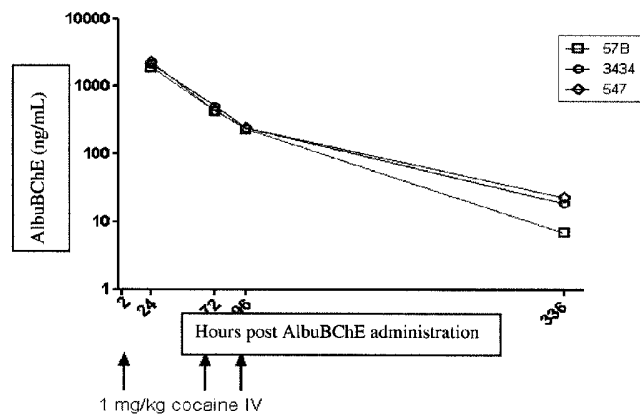


Figure 12

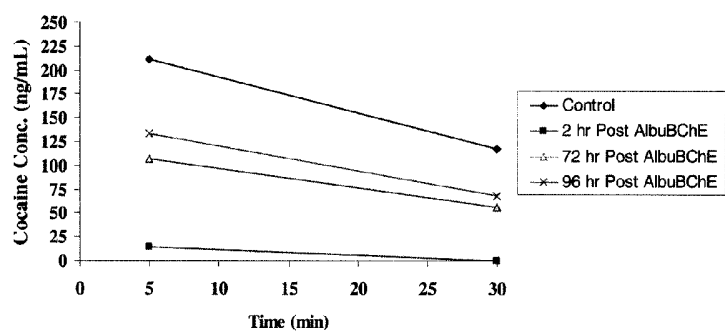


Figure 13

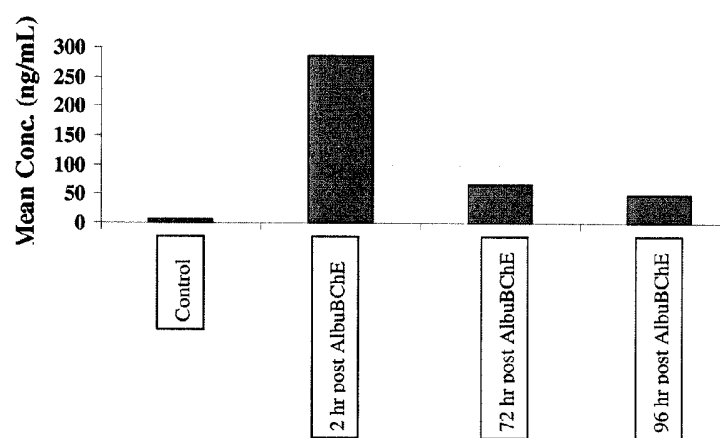


Figure 14

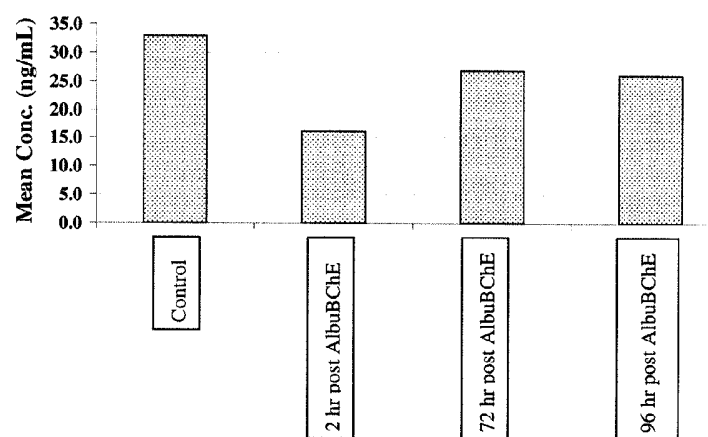


Figure 15

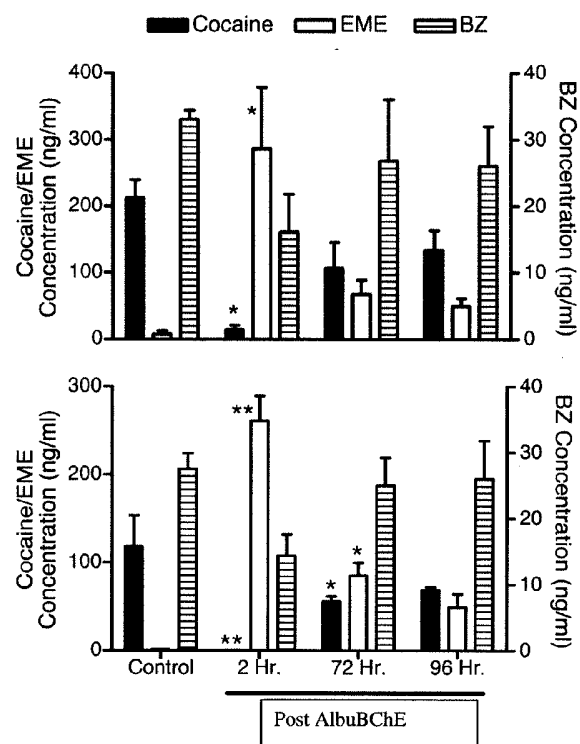


Figure 16

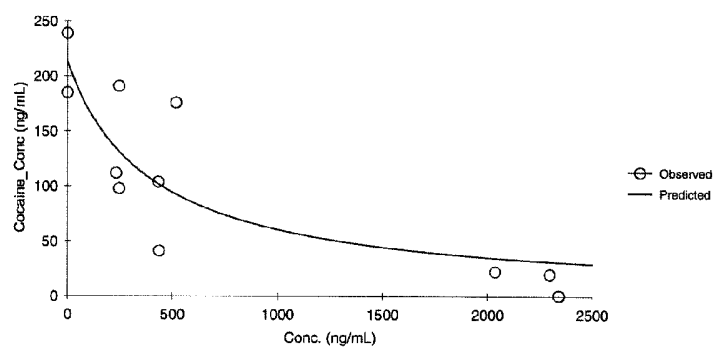


Figure 17

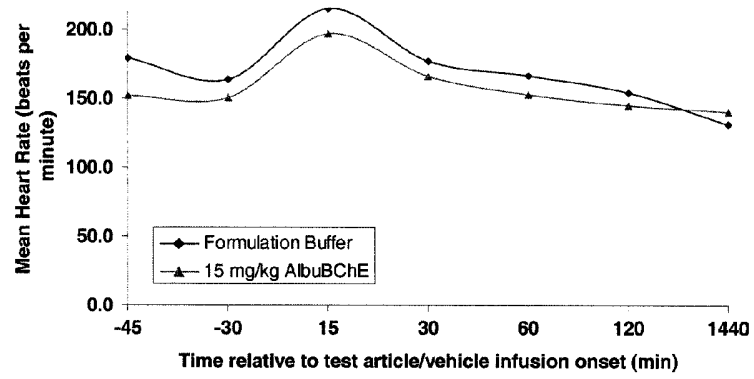


Figure 18

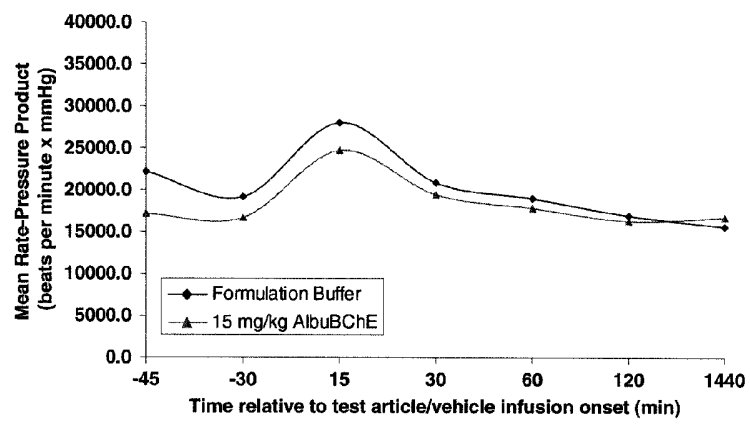


Figure 19

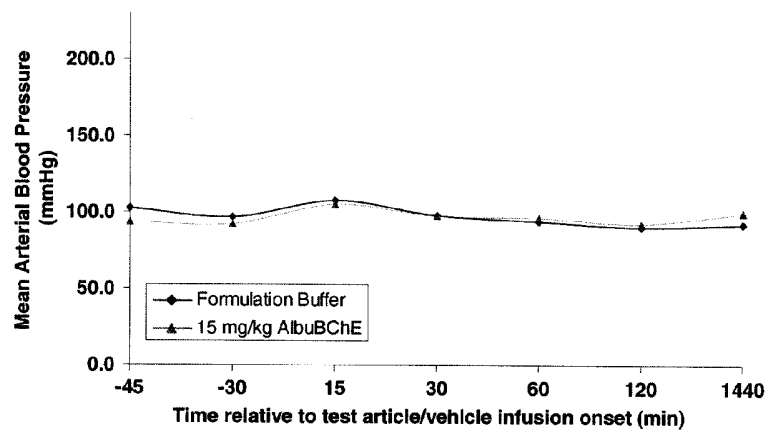


Figure 20

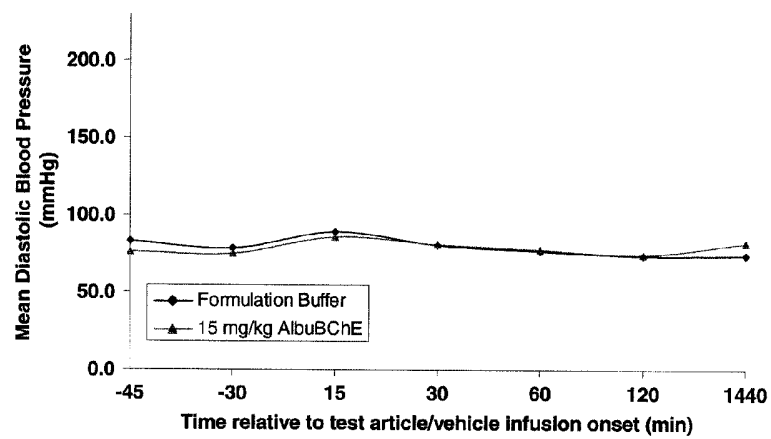


Figure 21

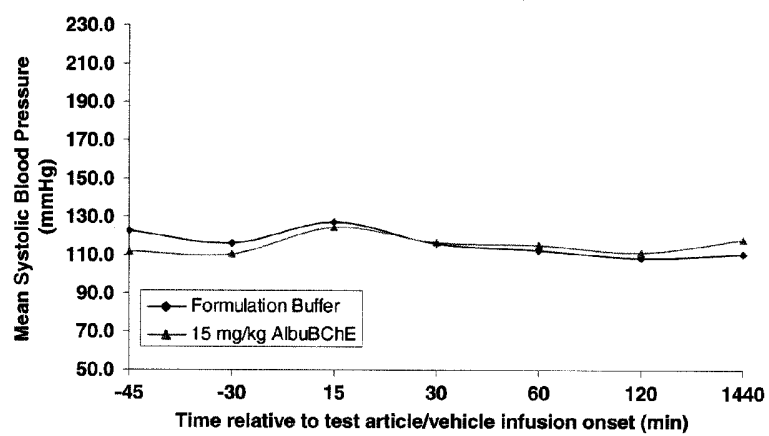


Figure 22

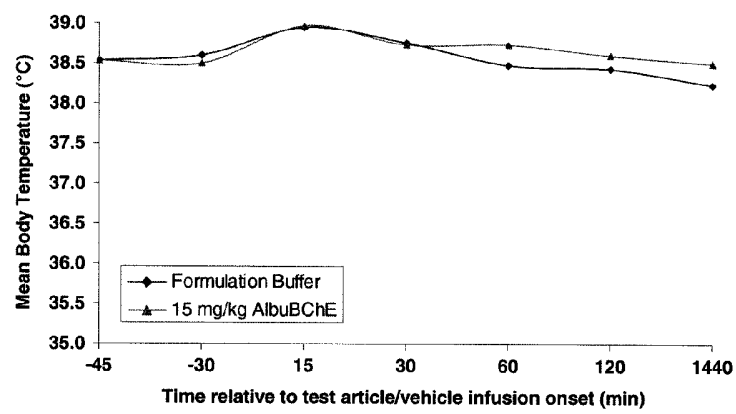


Figure 23

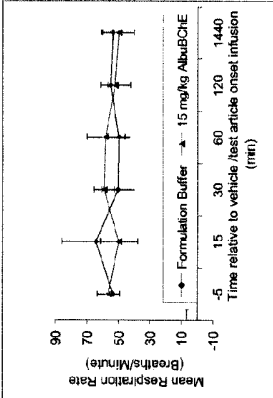
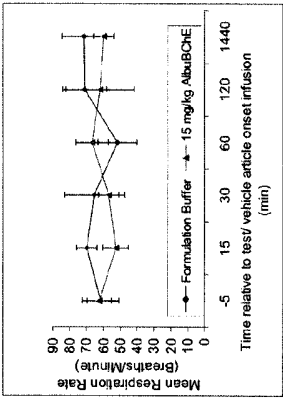


Figure 24B

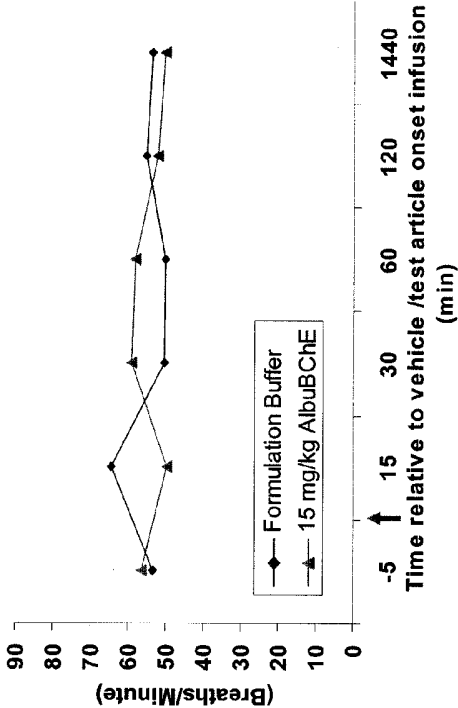


Figure 24A

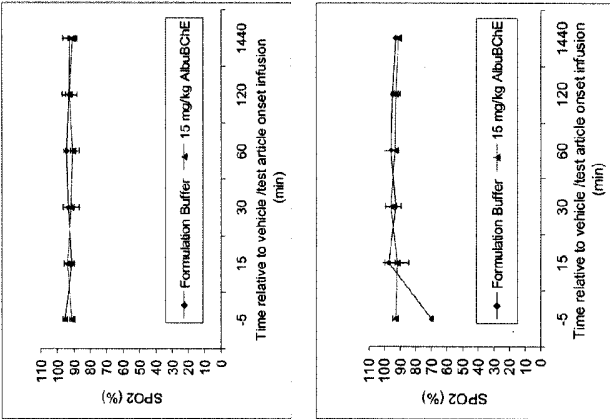


Figure 25B

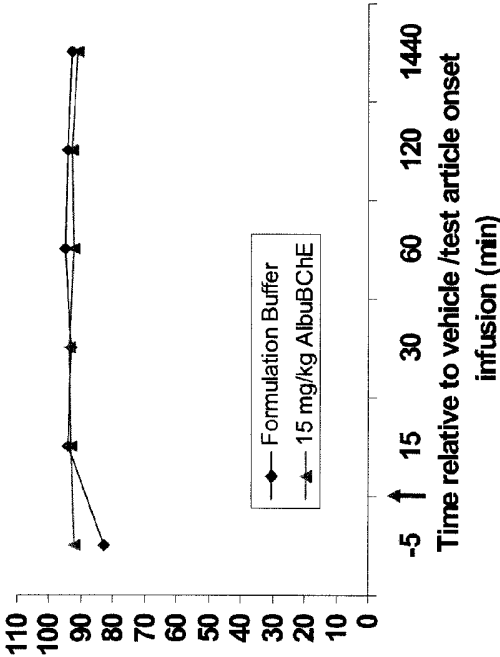


Figure 25A

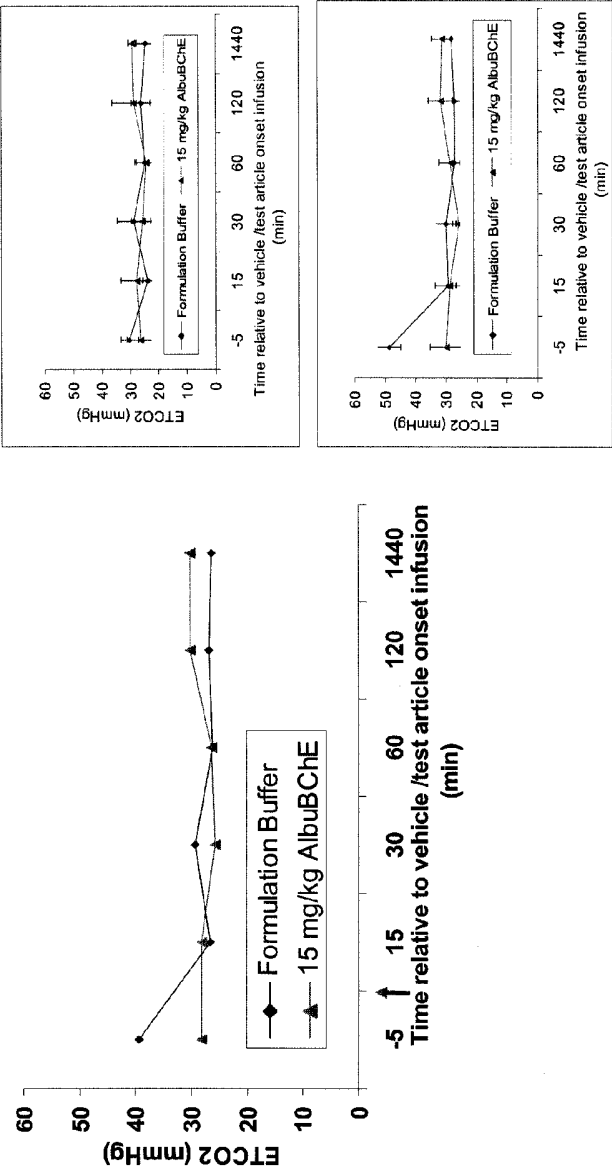


Figure 26B

Figure 26A

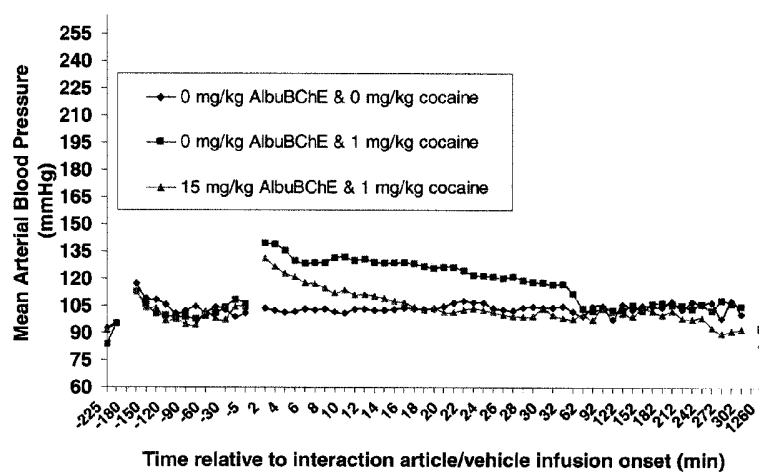


Figure 27

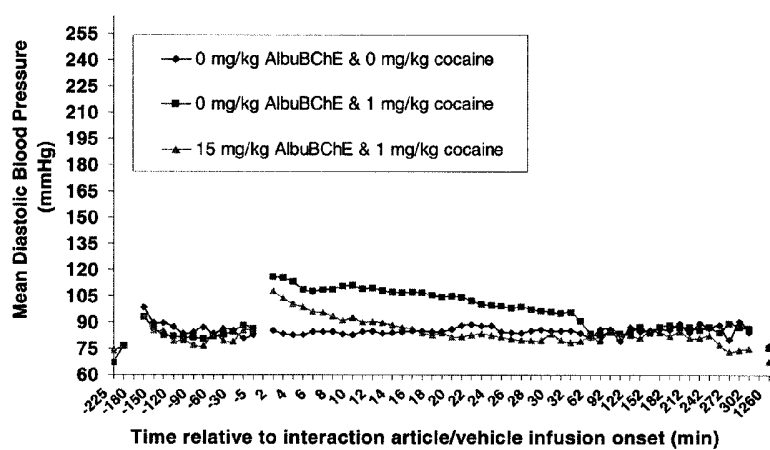


Figure 28

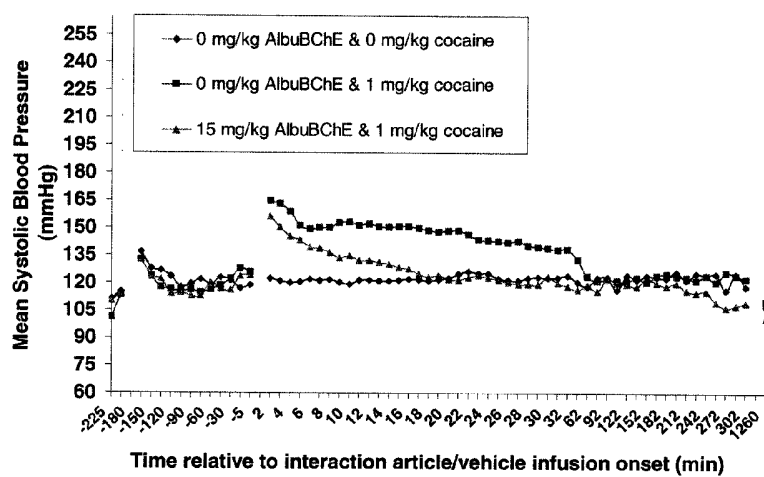


Figure 29

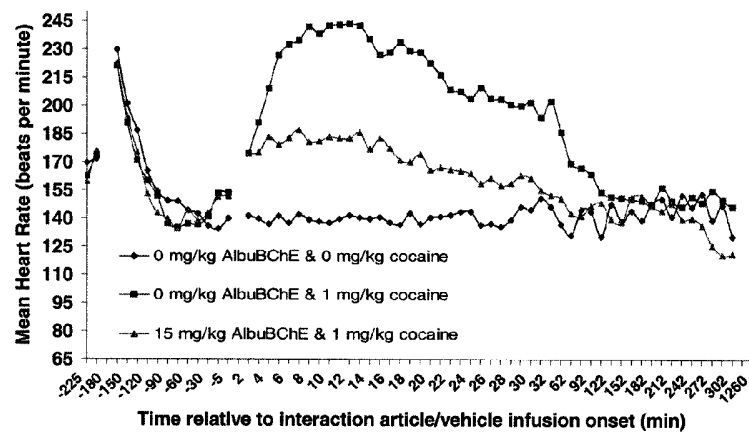


Figure 30

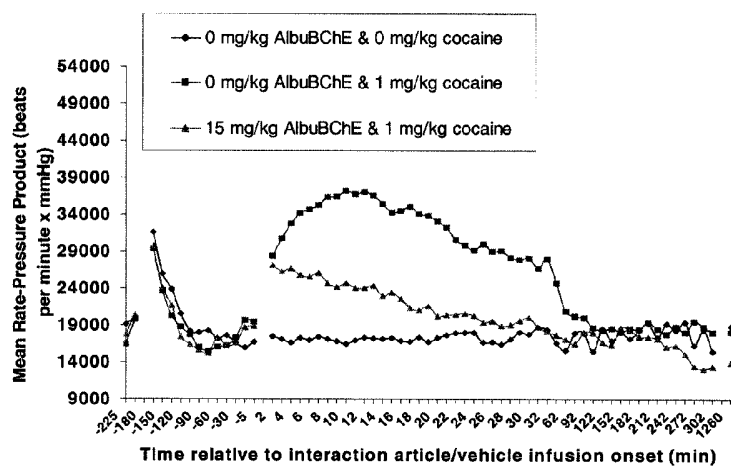


Figure 31

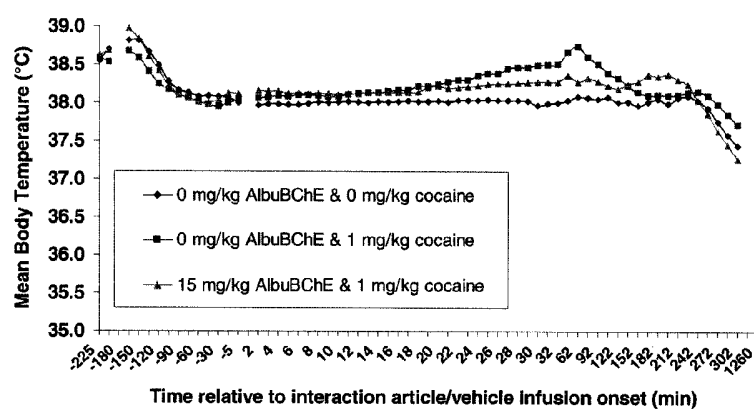


Figure 32

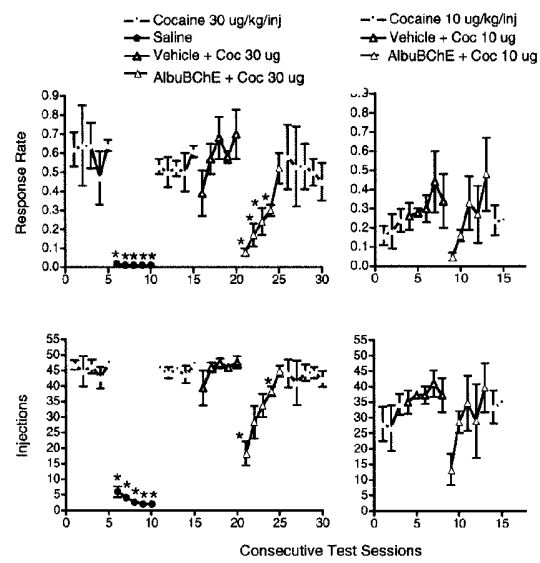


Figure 33

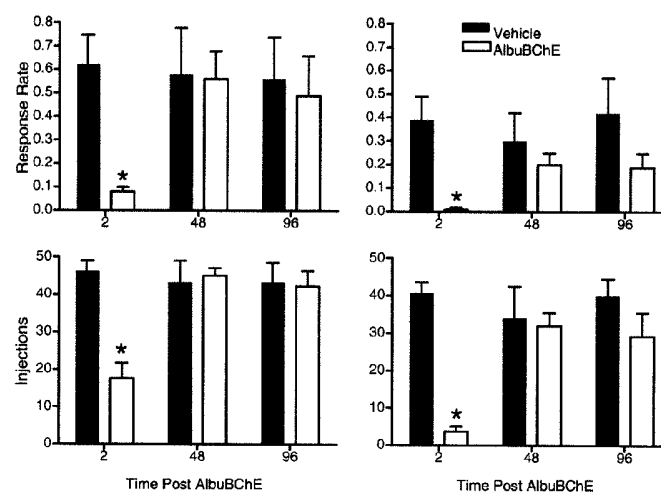


Figure 34

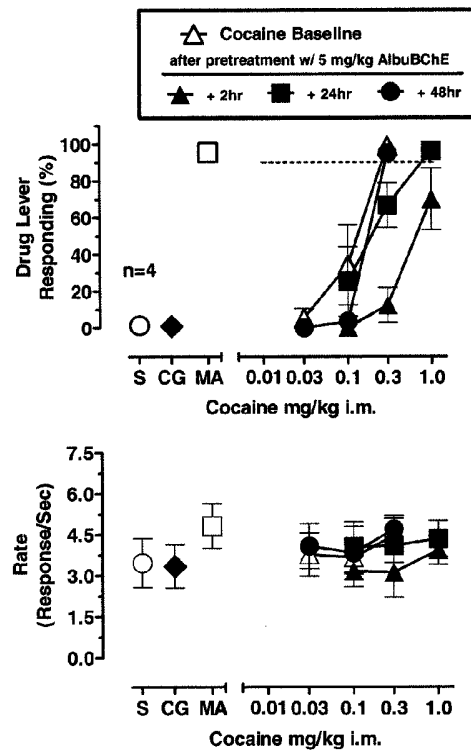


Figure 35

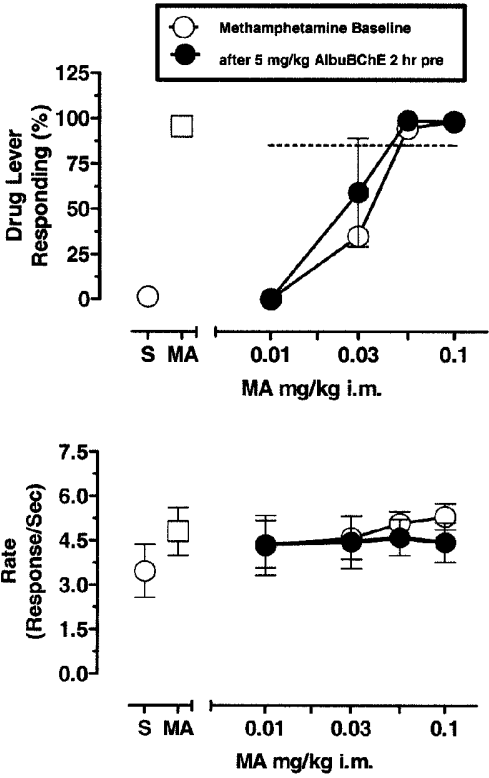


Figure 36

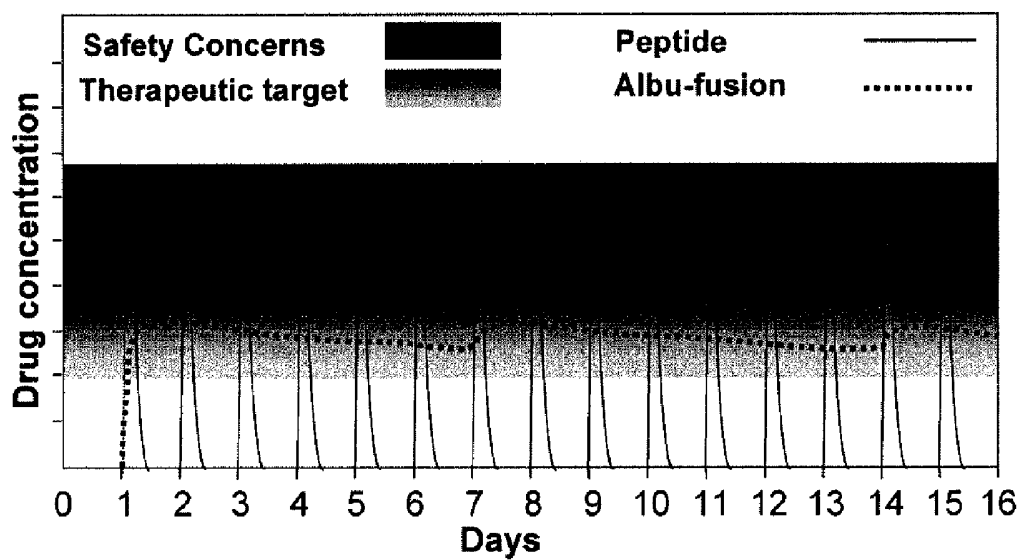


Figure 37

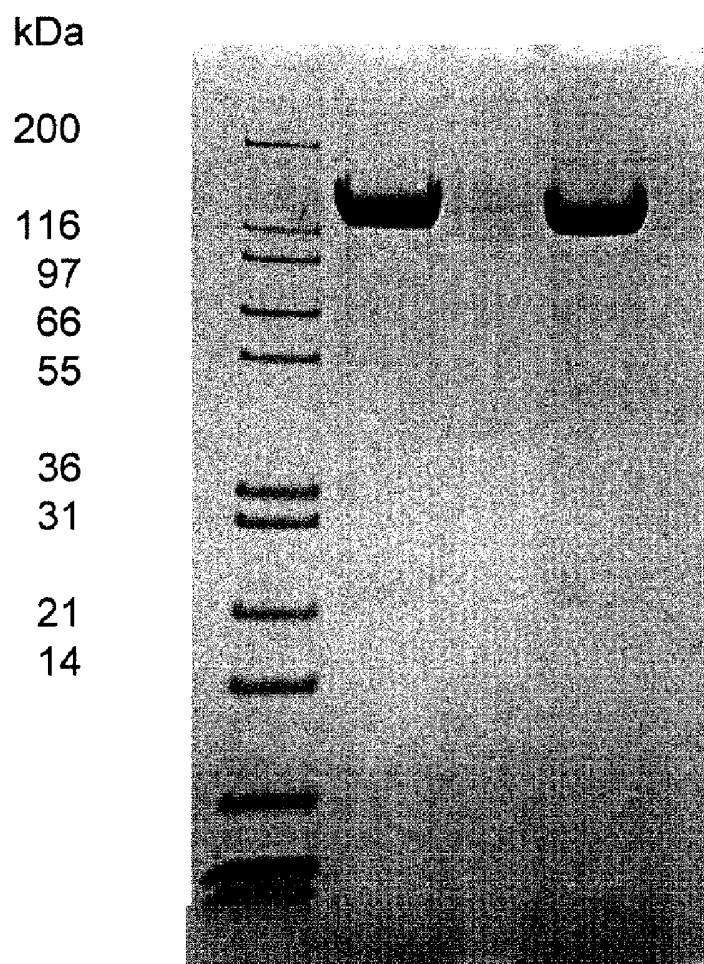


Figure 38

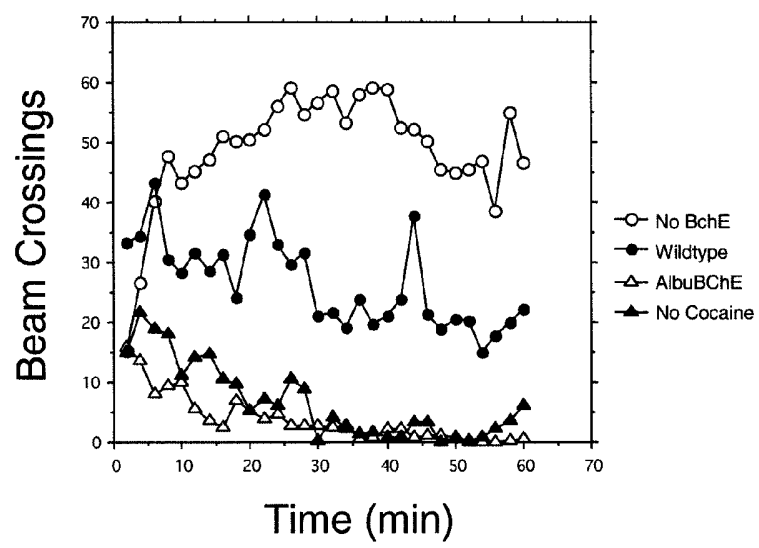


Figure 39

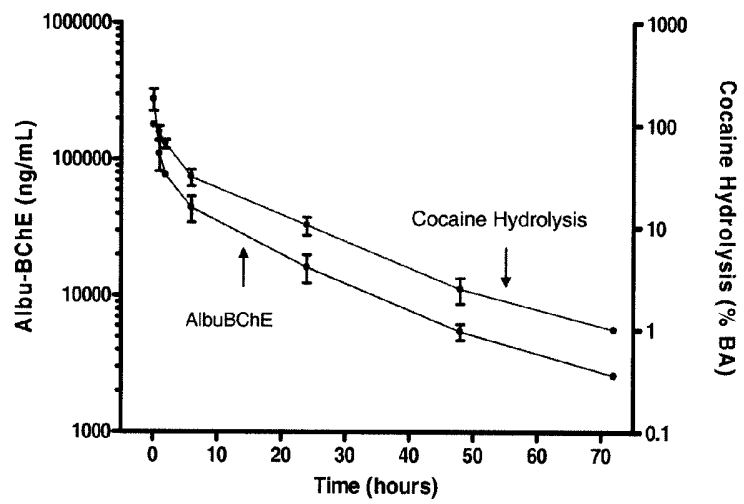


Figure 40

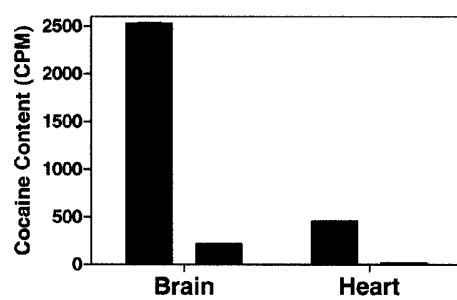


Figure 41

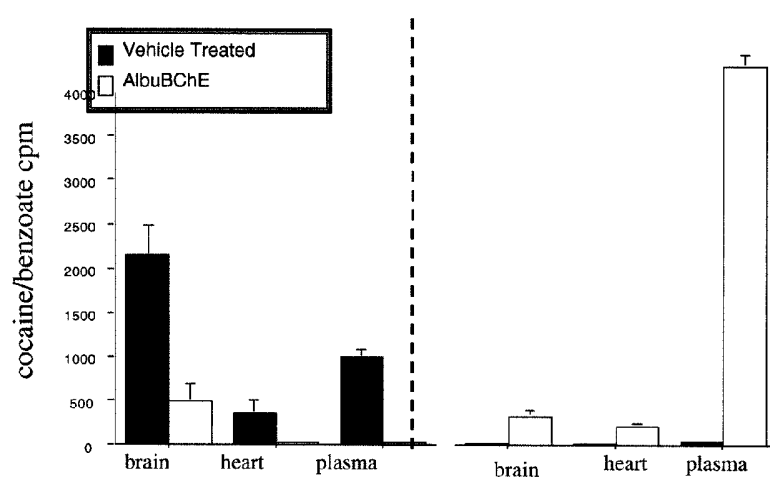


Figure 42

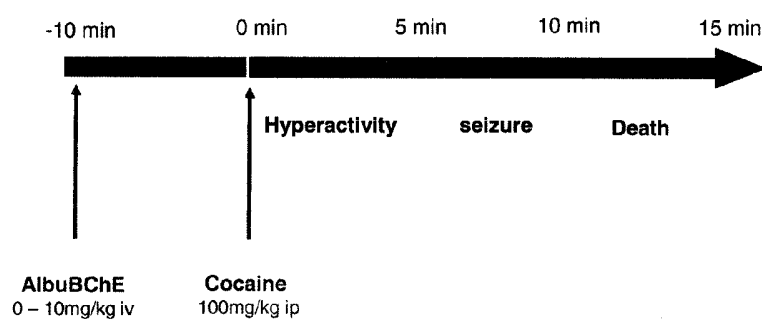


Figure 43

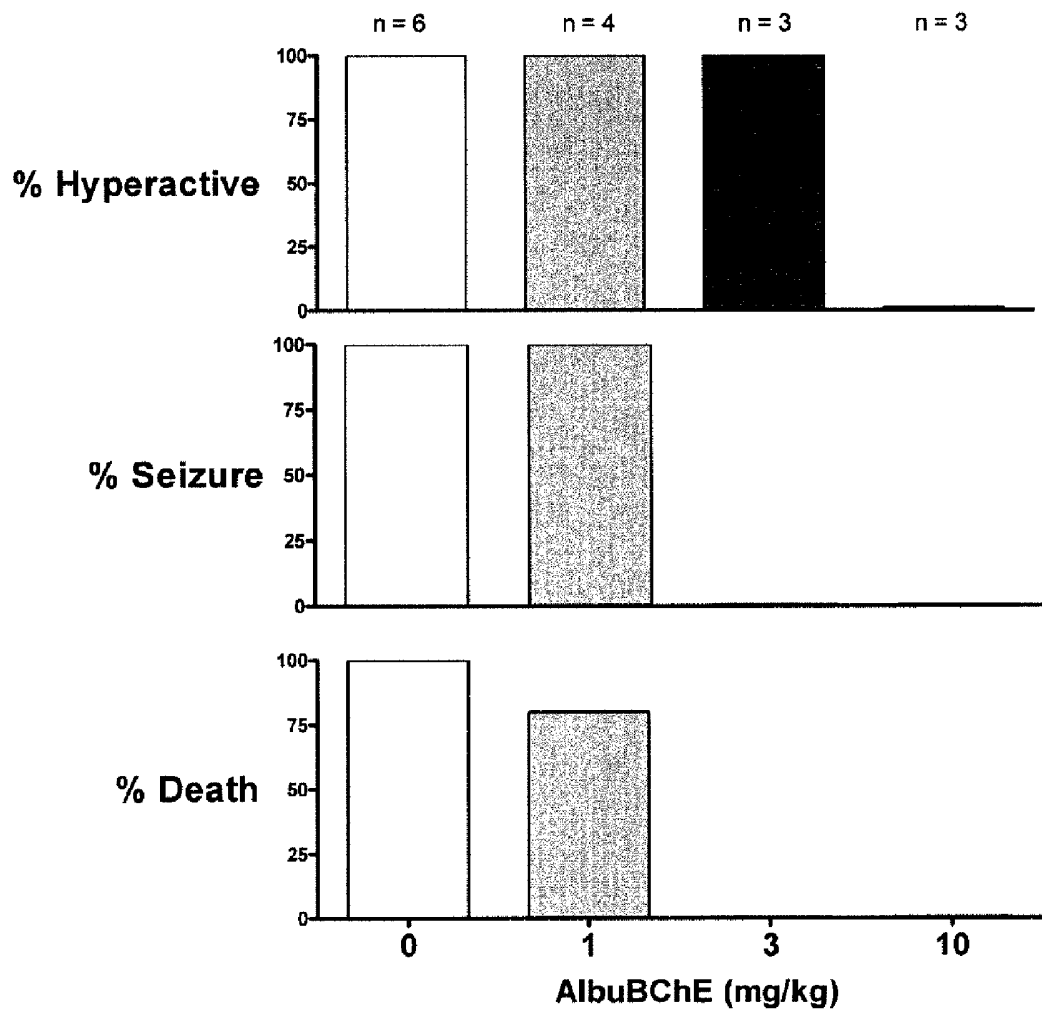


Figure 44

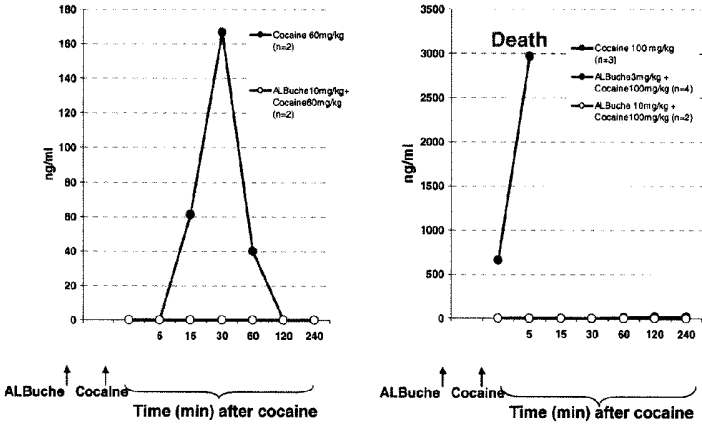


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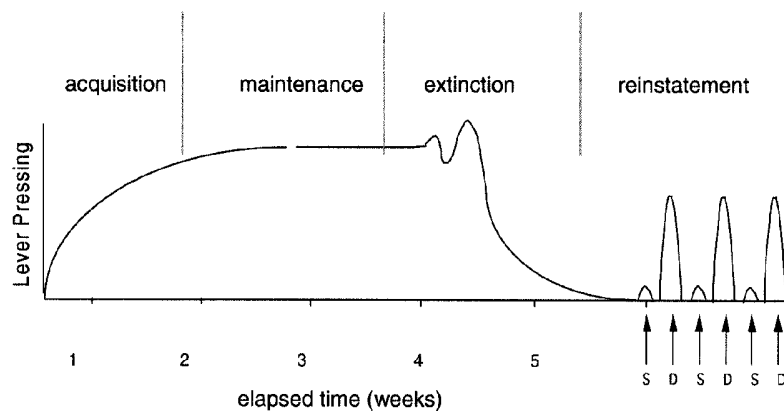


Figure 46

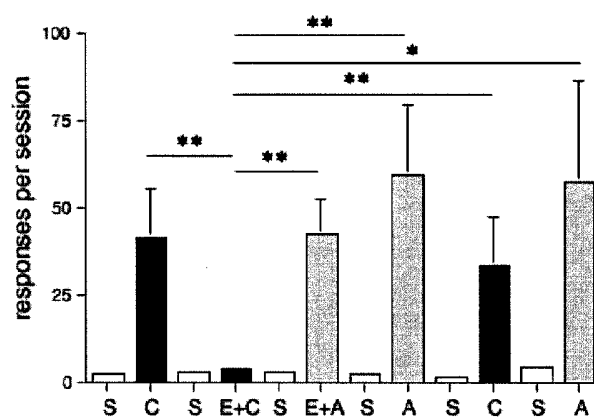


Figure 47

Treatment following addiction phase and forced abstinence	Lever Press (per session)
Saline injection	0
Cocaine injection	21
Albu-BChE followed by Cocaine	0

Figure 48

	<u>AlbuBChE</u>	<u>AlbuBChE</u>	<u>AlbuBChE</u>
Route	SC	IM	IV
Dose (mg/kg)	3	3	3
Bioavailability (%)	39%	79%	100%
Half-life (t _{1/2}) (hr)	36	35	28
T _{max} (hr)	24	3	0.083

Figure 49