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Short communication

The effects of a humanized recombinant anti-cocaine monoclonal antibody on the disposition of cocaethylene in mice

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ABSTRACT

The chimeric human/mouse anti-cocaine monoclonal antibody (mAb) 2E2 and its further humanized variant h2E2 have been reported to sequester a significant portion of cocaine in plasma and decrease cocaine concentrations in the brain in mice and rats. However, many cocaine users co-abuse alcohol, leading to the formation of the centrally active metabolite cocaethylene. This potentially compromises the efficacy of a cocaine-specific immunotherapy. Because h2E2 has high affinity for cocaethylene as well as cocaine, the ability of h2E2 to prevent cocaethylene entry into the brain was investigated. Mice were infused with h2E2 (1.6 μmol/kg i.v.) or vehicle and after one hour were injected with cocaethylene fumarate (1.2 μmol/kg i.v.). At times from 45 s to 60 min, brain and plasma were collected and cocaethylene concentrations were measured using GC/MS. In control mice, a two-compartment pharmacokinetic model generated values for cocaethylene distribution and terminal elimination half-lives of 0.5 and 8.1 min respectively. Initial plasma cocaethylene concentrations increased 13-fold from controls in the presence of h2E2. In brain, h2E2 produced a 92% decrease in the area under the time-concentration curve for cocaethylene. The pharmacokinetics of h2E2 was also characterized in detail. A three-compartment model resolved an initial distribution half-life of 4.4 min and a second distribution half-life of 4.2 h, and a terminal elimination half-life of 7.8 days. The ability of h2E2 to protect the brain from both cocaine and cocaethylene predicts that the clinical efficacy of h2E2 will be retained in cocaine users who co-abuse alcohol.

1. Introduction

Active immunization using cocaine vaccines in clinical trials was able to induce the in vivo production of anti-cocaine antibodies that decreased the use of cocaine in some individuals in which the vaccine elicited a strong immune response [1,2]. This provides proof of concept for the efficacy of immunotherapy for the treatment of cocaine addiction. However, there is considerable variation in the efficacy of the vaccine, as the time it takes to develop an immune response, the amount of antibody elicited, and the affinity and specificity of the antibodies raised varies between individuals [1,2]. The polyclonal antibodies elicited by cocaine vaccines decrease the concentration of cocaine in the brain of immunized mice and rats, which likely accounts for their clinical efficacy. A chimeric/mixed chain IgG1 anti-cocaine monoclonal antibody (mAb) that has a fully human heavy chain and a murine light chain also prevents cocaine entry into the brain in mice [3], and a recombinant version of this mAb with a humanized light chain inhibits cocaine entry into the brain in rats [4], which predicts efficacy as a passive immunotherapy for cocaine abuse. This reengineered mAb, with the preclinical designation h2E2, has 95% sequence homology with human IgG1 and the recombinant protein is produced in gram quantities in a stably transfected mammalian cell line [4]. This passive immunotherapy could provide a more predictable therapeutic response and could also be used as an adjunct to the active immunization approach using cocaine vaccines.

Many cocaine users concurrently use ethanol [5]. When both ethanol and cocaine are present in the liver, the enzyme carboxylesterase-2 catalyzes a transesterification of the methyl ester moiety to an ethyl ester to produce the psychoactive metabolite cocaethylene [6]. In humans who ingest alcohol, between 18% and 34% of cocaine is metabolized to cocaethylene [7]. Like cocaine, cocaethylene has been shown to rapidly enter the brain where it blocks the dopamine transporter and prevents dopamine reuptake. This leads to elevated post-synaptic dopamine levels, causing a centrally mediated pharmacodynamic response analogous to cocaine [8]. For this reason, a decrease in cocaethylene brain concentrations may predict that efficacy of this passive immunotherapy will be maintained in cocaine users who co-abuse alcohol. The mAb h2E2 also has high affinity for cocaethylene [4] but the effects of h2E2, or any other immunotherapy, on the disposition of cocaethylene in vivo have not been investigated. Therefore, the goal of this study was to determine whether h2E2 antagonizes the in vivo distribution of cocaethylene into the brain.

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2. Methods

2.1. Animals

Jugular vein catheterized adult male Swiss Webster mice (25–30 g) were purchased from Taconic Farms (Hudson, NY). Free access to food and water was provided. All studies were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, 1996) and under a protocol approved by the Institutional Animal Care and Use Committee at the University of Cincinnati.

2.2. h2E2 pharmacokinetics

Mice were infused with h2E2 (120 mg/kg i.v over 2 min) and 10 μl blood samples were collected from a small incision at the tip of the tail for up to 5 weeks after injection. The h2E2 concentrations in blood samples were analyzed using an enzyme-linked immunosorbent assay (ELISA) described previously [9]. The amount of h2E2 in varying dilutions of blood samples was compared with a standard curve generated using known concentrations of purified recombinant h2E2.

2.3. The effect of h2E2 on cocaethylene pharmacokinetics

A separate set of mice was infused i.v. with either vehicle (phosphate buffered saline) or h2E2 (120 mg/kg, 1.6 μmol/kg ligand binding sites over 2 min). One hour later cocaethylene fumarate (1.2 μmol/kg i.v.) was rapidly injected. Sodium pentobarbital (50 mg/kg i.p.) was injected to anesthetize the mice 3 min prior to decapitation. At 0.75, 1.5, 3, 5, 10, 20, 40, and 60 min after cocaethylene injection, mice were decapitated and trunk blood was collected in sodium fluoride (16 mg/0.8 mL of blood) to inhibit enzymatic degradation of cocaethylene and heparin (11 units/0.8 mL blood) to prevent blood coagulation. Blood was centrifuged at 5000 *g* for 3 min to separate plasma from red blood cells, and the plasma was removed. A separate sample of whole blood (at least 5 μL) was also preserved to measure hemoglobin content. Whole brain was removed and all samples were placed immediately on dry ice then stored at −20 °C.

Cocaethylene was extracted from brain and plasma samples, then derivatized, and measured by gas chromatography/mass spectrometry (GC/MS) using procedures modified from Norman et al (2007) [3]. Deuterated cocaethylene served as an internal standard and unlabeled cocaethylene with a certified standard concentration were used to generate a standard curve for quantification.

As reported previously for cocaine [3], to correct for the cocaethylene present in blood in the brain, hemoglobin concentrations were determined in plasma and brain samples using spectrophotometric analysis according to the method reported by Choudhri et al. [10] and from a protocol provided by Pointe Scientific, Inc. (Canton, MI).

2.4. Data analysis

All pharmacokinetic data, including cocaethylene and h2E2 pharmacokinetics, was analyzed using Phoenix® WinNonlin® (by Pharsight, a Certara™ company, St. Louis, MO). All plasma data were initially fit using a single compartment model with a bolus injection and a first order elimination. When the latter method provided a poor fit, a two-compartment model was applied with first order distribution between the first and second compartment with clearance only from the first compartment. When this failed to provide a good fit, a three-compartment model with first-order distribution between all compartments was applied. The primary calculated parameters were the distribution half-lives, the terminal elimination half-life, and the volume of distribution at steady state (V_{dss}).

Cocaethylene concentrations in the brain over time were also analyzed using WinNonlin. A single-compartment model with first-order input with lag time and first-order elimination was applied. If this failed to provide a good fit, a two-compartment model with first-order input with lag time and first-order distribution between the compartments and first-order elimination was applied. The primary calculated parameter from the concentration values as a function of time was area under the time-concentration curve (AUC).

All graphs were generated in SigmaPlot (Systat Software Inc., San Jose, CA).

3. Materials

Cocaethylene fumarate was provided by the Research Triangle Institute (Chapel Hill, NC) under the National Institute on Drug Abuse Drug Supply Program. Deuterated cocaethylene-D3 (0.1 mg/ml in acetonitrile), used as an internal standard, the certified unlabeled cocaethylene (1 mg/ml in acetonitrile), used to generate a standard curve, and the derivatizing reagent N,O-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane were purchased from Cerilliant (Round Rock, TX). Recombinant h2E2 was produced from stably transfected CHO-cell lines by Catalent Pharmasolutions (Madison WI) using their proprietary GPEx technology [11]. The human hemoglobin standards and control standards were obtained from Pointe Scientific, Inc. All of the other chemicals and immunoreagents were purchased from Sigma-Aldrich (St. Louis, MO) or Pierce Chemicals (Rockford IL).

4. Results

4.1. mAb h2E2 pharmacokinetics

The detailed study of the time course of decline in h2E2 concentration in mouse plasma resolved three distinct phases. There was an initial rapid first-order decline in concentration with a half-life of 4.4 min (Fig. 1A). This was followed by a longer distribution phase with a half-life of 4.2 h (Fig. 1A/B). The terminal elimination phase had a half-life of 7.8 days (Fig. 1B) and the model generated a value for the V_{dss} of h2E2 of 0.3 L/kg (Fig. 1A/B). Graphs generated by SigmaPlot curve-fitting analysis, also using an exponential decay model with three phases, provided a visually better fit for the data over the first 24 h than did WinNonlin. This model generated a rapid distribution phase of 12.0 min, a longer distribution half-life of 6.0 h, and a terminal elimination half-life of 7.8 days. Between 60 and 120 min after the infusion of h2E2, when the cocaethylene pharmacokinetic studies were completed, the concentration of h2E2 declined by less than 8%.

4.2. Plasma pharmacokinetics of cocaethylene

In control animals, cocaethylene concentrations over time were fit using a two-compartment model. This model generated a distribution half-life of 0.5 min, a terminal elimination half-life of 8.1 min and a V_{dss} of 2.5 L/kg (Fig. 2A). In the presence of h2E2, the initial peak concentration of cocaethylene was 13-fold higher (2940 ng/mL) than in the vehicle-treated group (235 ng/mL). No initial distribution phase was observed so a one-compartment model was applied. This model generated values for the elimination half-life of 11.1 min, and for the V_{dss} 0.1 L/kg (Fig. 2A). Over 60 min, plasma concentrations in control animals declined by approximately 99%, and by 94% in the presence of h2E2.

4.3. Brain pharmacokinetics of cocaethylene

The increase in plasma concentrations observed in the presence of h2E2 (Fig. 2A) was accompanied by a concomitant decrease in brain concentrations (Fig. 2B). Brain cocaethylene concentrations in control animals were fit by a two-compartment model, while a one-compartment model provided an adequate fit in the presence of h2E2 (Fig. 2B). In the presence of h2E2, the mean peak brain concentration