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The Concept of Pharmacologic Cocaine Interception as a Treatment for Drug Abuse

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Abstract

Cocaine access to brain tissue and associated cocaine-induced behaviors are substantially reduced in rats and mice by significant plasma levels of an enzyme that rapidly metabolizes the drug. Similar results have been obtained in rodents and humans with therapeutic anti-cocaine antibodies, which sequester the drug and prevent its entry into the brain. We show that an efficient cocaine hydrolase can lead to rapid unloading of anti-cocaine antibodies saturated with cocaine, and we provide a theoretical basis for the hypothesis that dual therapy with antibody and hydrolase enzyme may be especially effective.

1. Introduction

Recent research has opened the door to possible treatments of cocaine abuse with agents that intercept cocaine and prevent its crossing the blood brain barrier and reaching drug-reward centers in the brain. Two promising lines of attack are enzymes that rapidly destroy cocaine [1–3], and anti-cocaine antibodies that sequester injected drug in the circulation where it can be gradually metabolized [4–6]. One can make a compelling rationale for combining these two different approaches, and it is worth considering that idea in some detail.

Butyrylcholinesterase (BChE), abundant in plasma, splits cocaine into ecgonine methyl ester and benzoic acid, which, unlike other cocaine metabolites, do not block transmitter transporters, lack rewarding actions, and exhibit little toxicity [7]. Thus BChE has a unique detoxifying role; equally important, it does not directly affect heart rate, blood pressure, or neurological function [8]. Natural human BChE hydrolyzes cocaine inefficiently, but computationally guided mutations have vastly increased its ability to catalyze that reaction. Early work led to a 40-fold gain in a double mutant that was found to accelerate cocaine metabolism in rats and blunt cardiovascular responses to the drug [2,9,10]. Subsequent focus on the transition-state complex led to the efficient quadruple mutants, “AME” [11], “CocH” [1] and ultimately to a quintuple mutant, “CocH2” [3] with 2000-fold increase in catalytic efficiency for cocaine. These newer hydrolases are promising therapeutics on their own, and

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they may well act synergistically with anti-cocaine antibodies or vaccine. We think it plausible that a combination of immunologic and enzyme therapy will be powerful enough to suppress all reward responses for cocaine. The primary purpose of this paper is to explore the preconditions and pharmacokinetic implications of combination therapy. We also provide evidence that anti-cocaine antibodies with sub-micromolar affinity can reduce free cocaine concentrations below levels associated with drug reward while allowing the drug to be efficiently hydrolyzed by a highly active blood-borne cocaine hydrolase.

2. Materials and Methods

Cocaine-antibody, in the form of immune serum raised in mice against succinyl norcocaine conjugated to keyhole limpet hemocyanin, was prepared under standard conditions using EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) and sulfo-NHS (N-hydroxysulfosuccinimide) [12]. Affinity for cocaine and abundance of cocaine binding sites were determined by equilibrium dialysis [13]. Briefly, aliquots of serum diluted in phosphate buffered saline (PBS) mixed with increasing quantities of radiolabeled ^3H -cocaine (Perkin Elmer, Waltham, MA) were placed one side of equilibrium dialysis chambers (Harvard Apparatus, Holliston, MA) and incubated against PBS on the other side. Serum from unimmunized mice in equivalent conditions demonstrated the absence of nonspecific binding and ensured that equilibrium conditions were reached after incubation for 24 h. Aliquots from each chamber were then analyzed to measure drug concentrations. Calculations of effective average affinity were made using Scatchard plots with saturation binding conditions to determine total specific antibody concentration in terms of binding sites (which ranged from 200 to 700 μM). Cocaine binding to antibody was also assessed by allowing samples of immune sera to react for 30 min at room temperature with fixed quantities of ^3H -cocaine (e.g., 1, or 10 μM), followed by separation of bound and free cocaine in 60 μl aliquots by 5 min centrifugation (at $1000 \times g$) through size-exclusion gel chromatography mini-columns (Centricon YM-10, Fisher Scientific) followed by collection of the void volumes ($\approx 50 \mu\text{l}$) for determination of total radioactivity.

To measure rates of enzymatic hydrolysis in the presence or absence of antibodies, ^3H -cocaine labeled on the benzoic acid moiety was incubated at a concentration of 1 μM either in 1 ml of 0.1 M sodium phosphate, pH 7.4, or in the same buffer supplemented with antiserum containing a 5-fold molar excess of anti-cocaine IgG. The mixtures were pre-incubated with constant stirring at room temperature for exactly 15 min. At that time, a 5- μL baseline sample of the incubation solution was removed and transferred to 300 μL of 0.02 N HCl, which stopped any ongoing hydrolysis owing to serum enzymes and provided a baseline point. Then, 6 μg of cocaine hydrolase in 10 μL was introduced in the form of "Albu-CocH", a cocaine hydrolase based on quadruple mutant human BChE and expressed as a C-terminal fusion to human serum [14]. After 15 sec, sampling and transfer to HCl proceeded as above and continued at 15 second intervals for the next 2 min. Finally, all samples were processed for liberated ^3H -benzoic acid by extraction into toluene pre-mixed with scintillation fluor as described earlier [15].

3. Results and Discussion

The effects of enzyme on free and antibody-bound cocaine are shown in Fig 1. Albu-CocH added to a 1- μM cocaine solution in buffer alone converted essentially 100% of the substrate to benzoic acid within 15 seconds. This experiment was repeated with enzyme added to a 1 μM cocaine solution pre-incubated with antibody in excess, a condition under which cocaine was $> 90\%$ bound to IgG at time zero. In this case hydrolysis was only slightly slower, being 73% complete at 15 seconds, 90% complete at 30 seconds, and 98% complete at 90 seconds. This finding implies that the rate of cocaine dissociation from antibody was fast enough for

hydrolysis in solution to shift the binding equilibrium markedly in less than one minute. Thus it can be concluded that an efficient cocaine hydrolase, if sufficiently abundant, should preserve and complement antibody capacity to bind boluses of i.v. cocaine even when they are repeated at short intervals.

Early studies found that normal BChE shortened cocaine half-life [16] and weakly protected rodents from cocaine toxicity [17]. These findings suggested that more powerful mutated enzymes might be dramatically effective against a range of drug effects. Indeed with CocH in rats, we were able to achieve after-the-fact rescue from massive seizures following a normally lethal cocaine overdose and also selectively abolish drug-primed reinstatement in animals that formerly self-administered cocaine [18]. Also, using gene transfer vectors we found it possible to generate therapeutic enzyme levels for several months after a single treatment [19]. Meanwhile other groups taking immunological approaches with haptene-conjugated cocaine vaccines have elicited multi-micromolar plasma concentrations of anti-cocaine IgG with binding affinities in the low nanomolar range [5,20]. Experimentally, such antibodies have been shown capable of reducing cocaine-seeking behavior in rats [21], while a clinical trial of cocaine vaccine in human users has recently demonstrated a significant increase in the frequency of drug-free urines [22,23].

A theoretical advantage of cocaine antibodies is their ability to bind substantial quantities of drug on a very rapid time scale. Antibodies have fast on-rates for antigen-binding, with half-times measured in milliseconds, and an effective vaccine may generate high levels of circulating immunoglobulin with abundant binding sites. The antiserum used in the present study, for example, contained cocaine-binding IgG at a concentration of 630 $\mu\text{g/ml}$, or about 8 μM , well in excess of the 1–5 μM concentrations of plasma cocaine reached in typical reward-dose scenarios [24,25]. Thus, with easily achievable nanomolar affinities for cocaine [26], it is possible to bind a large fraction of the drug in plasma. Even less avid antibodies at somewhat lower concentrations have a potential to blunt the peak of a drug bolus and slow its rate of rise, a factor considered crucial in determining reward potential [27].

Although enzymes are able to destroy unlimited quantities of drug, they might be too slow to block the rapid 10–20 second onset of cocaine reinforcement. Enzymes may require many seconds or even minutes to inactivate cocaine. Like antibodies, enzymes should bind cocaine quickly, but in practice this binding cannot lower free plasma cocaine levels as effectively as antibody binding. There are two reasons. First, efficient cocaine hydrolases exhibit micromolar K_m values [1,3,14] indicative of dissociation constants in the same range as the expected cocaine concentrations; hence, the binding sites will not saturate. Second, it is impractical to deliver enzyme in amounts commensurate with drug. In our recent studies, iv enzyme injection or transduction with viral vectors led to very high plasma cocaine hydrolase activities, near 0.5 U/ml [9,18,19,28]. However, the enzyme protein level corresponding to such activity is only 0.5 μM . Thus, only a small fraction of the free cocaine could bind even if affinity were much higher than estimated. In short, enzyme treatment must rely on catalytic function rather than binding.

When a drug arrives in small doses or by a gradual mode of delivery, either an enzyme or an antibody may by itself serve as an effective barrier, but larger or repeated drug doses, especially when delivered iv, are likely to overwhelm either type of molecule in isolation. Given together, however, antibody and enzyme could act synergistically in the following manner. We hypothesize that antibodies will rapidly bind much of the incoming cocaine while the enzyme clears away free excess drug and then destroys the remainder as it dissociates from the immunoglobulin. The in vitro experiment described in the present work supports our prediction that this off-loading can be quick enough to reset the system before another dose arrives. Figure 2 schematically represents the potential effects of antibody and

enzyme, alone and in combination, on plasma and brain levels of cocaine delivered i.v. or by inhalation.

Two important questions remain to be resolved in developing a combined therapy with anti-cocaine vaccine and cocaine hydrolase (delivered directly or by means such as gene transfer): 1) What cocaine binding affinity is optimal for the IgG? 2) What is the best relative ratio of IgG to enzyme protein? These questions deserve investigation at the mathematical level (i.e., by pharmacokinetic modeling) and experimentally (i.e., by detailed studies of cocaine hydrolysis under controlled conditions in vitro and of cocaine uptake and lifetime in key tissues of live animal subjects). The limited information at hand is sufficient, however, to make basic points clear. One is that antibody affinity must be neither extremely high nor extremely low. IgG affinity for a given protein antigen is driven primarily by dissociation rate. Ultra-high affinities ($\ll 1$ nM) are unsuitable because dissociation will be too slow for enzymatic degradation to occur on a reasonable time scale (less than ≈ 5 min). At the other extreme, low affinity antibodies ($K_d > 1$ μ M) are unsuitable because, with 1–5 μ M plasma cocaine levels expected after a rewarding dose [24,25], only a modest fraction of the drug is bound even when the IgG is comparably abundant. The antibody tested here falls between these extremes, with an apparent K_d for cocaine binding of 33 nM.

It might be questioned whether a combination therapy could ever be helpful, because, if a repeated cocaine injection is delayed until cocaine has already dissociated from the antibody, there is no need for enzyme, but if the new injection occurs earlier, even a dual treatment will fail. This proposition supposes that antibody in the first setting will present enough empty binding sites to make the enzyme superfluous, while in the second setting the antibody will be overwhelmed and the enzyme must act entirely on its own. In response, while admitting that a satisfactory outcome is not guaranteed, we reason that combination therapy should be superior when there is repeated dosing. Let us consider three such cases. In case one (dosing intervals long with respect to spontaneous antibody-cocaine dissociation), antibody is initially effective, and enzyme then prevents the dissociating drug from reaching the brain. In case two (shorter dosing intervals), enzyme hastens the net dissociation by shifting the equilibrium towards zero plasma levels while again preventing drug from reaching the brain. In case three (ultra short dosing intervals), antibody prevents an initial bolus of drug in brain and then remains saturated but the enzyme at least mitigates the effects of the second bolus. All three outcomes would be superior to single treatment with either agent.

Finally we should consider “catalytic antibodies”. Since it has been possible to create antibodies capable of cocaine hydrolysis [4,29,30], it is worth raising the question whether they by themselves could serve the dual function of binding and metabolizing cocaine. Although this idea is attractive in principle, it must be realized that catalytic antibodies are likely to have far lower affinity for antigen than do non-catalytic molecules. For example, K_m values for cocaine hydrolysis ranged from 200 to 1000 μ M in most of the monoclonal antibodies reported by the groups of Landry [30,31] and Janda [32], suggesting dissociation constants in the high micromolar range. Furthermore, a normal polyclonal response to vaccination with cocaine would not yield large quantities of such highly specific molecules. In comparison, combinations of optimally active enzyme with antibody of optimal efficiency will be easier to produce and substantially more effective than even a passively administered monoclonal IgG molecule that inherently has only modest ability to catalyze cocaine hydrolysis. This combination therapy for cocaine addiction has the advantage of offering substantial help for addicts motivated to give up their addiction, without requiring any additional therapeutic agents to be taken.

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Figure 1.

Percent hydrolysis of free and antibody-bound cocaine is shown as a function of time. Drug (1 μ M) was prepared at zero time in antibody-rich serum, "AB" ($\approx 5 \mu$ M IgG) or saline, "SAL". CocH (0.1 mg/ml) was added at 15 min (arrow), when baseline was $< 1\%$ hydrolysis.

Figure 2.

Schematic illustration of cocaine uptake into brain after i.v. administration to subjects under four different conditions: “unprotected” (no pretreatment), “enzyme” (provided with an efficient cocaine hydrolase), “antibody” (given high affinity anti-cocaine IgG), and “enzyme + antibody” (given both pretreatments). Panels read from left to right as a time sequence. As shown, an unprotected subject will accumulate cocaine in brain after each repeated injection. An enzyme treated subject will not, so long as hydrolysis is nearly complete before the second injection. An antibody treated subject may do better than an enzyme treated subject at first, but a second injection can overwhelm the protection. Dual treatment is most effective.