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Studies towards the improvement of an anti-cocaine monoclonal antibody for treatment of acute overdose

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ABSTRACT

There is currently no clinically-approved antidote for cocaine overdose. Efforts to develop a therapy via passive immunization have resulted in a human monoclonal antibody, GNCgzk, with a high affinity for cocaine (K_d = 0.18 nM). Efforts to improve the production of antibody manifolds based on this antibody are disclosed. The engineering of an HRV 3C protease cleavage site into the GNCgzk IgG has allowed for increased production of a F(ab′)$_2$ with a 20% superior capacity to reduce mortality for cocaine overdose in mice.

It is estimated that there are about 1.5 million people 12 and older that are current users of cocaine in the United States,[1] and from 2001 to 2014 there has been a 42% increase in the number of deaths attributed to cocaine use.[2] Additionally, studies indicate cocaine remains the most commonly used illicit stimulant in Europe, with an estimated 2.4 million users aged 15–34.[3] Despite its prevalence, there is no currently available clinical antidote to treat acute cocaine toxicity. Methods to treat cocaine overdose only lie in management of the symptoms: arrhythmia, seizure, and hyperthermia.[4] Thus, it is desirable to find a more direct antidote therapy for cocaine intoxication. One approach is to target the cocaine molecules themselves using a monoclonal antibody to sequester the cocaine in the blood stream and lower the relative concentration of drug affecting the central nervous system, and mitigating the psychoactive effects.

Previously, we developed a murine monoclonal antibody (mAb), GNC92H2 IgG, which exhibited therapeutic utility in rodent models with an affinity of approximately 2 nM for cocaine.[5–7] This success was followed by the isolation of a hybridoma from a transgenic xenomouse producing a fully human mAb, GNCgzk.[8–10] Compared to GNC92H2, this clone was found to produce antibodies with a greatly increased affinity for cocaine (K_d = 0.18 nM) as well as higher specificity against cocaine metabolites.[10] Importantly, GNCgzk has a fast association rate ($k_{on} = 4.3 \times 10^7$ M^{-1} s^{-1}), and this key property allowed for GNCgzk to demonstrate complete prophylactic protection from cocaine overdose. Moreover, the administration of the GNCgzk IgG was able to decrease lethality by 40% when administered 3 min after administration of an LD_{50} dose of cocaine. This was improved through the construction of the F(ab′)$_2$ fragment of the GNCgzk antibody, which demonstrated complete protection from lethality at 3 min post-cocaine treatment.[10] However, production of the F(ab′)$_2$ suffered from a low yield following pepsin digestion of the GNCgzk IgG, limiting its clinical viability.

Many advances in antibody technology have allowed for the ex vivo improvement of mAb manifolds.[11] In order to lower the production costs of producing the GNCgzk IgG, a new single-chain variable fragment with an IgG1 crystallizable fragment (scFv-Fc) construct was designed (Fig. 1). The benefits of using a scFv construct lies in the ease of production: engineering flexibility, speed of biosynthesis, and higher yields.[12] However, the lack of a constant region results in rapid blood clearance, which is overcome

Abbreviations: mAb, monoclonal antibody; scFv, single-chain variable-fragment; Fc, crystallizable fragment; PK, pharmacokinetic; SPR, surface plasmon resonance; HRV, human rhinovirus.

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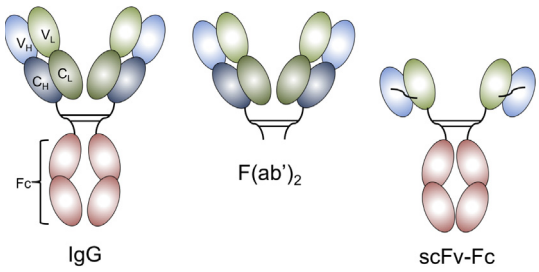

	IgG	F(ab')$_2$	scFv-Fc
MW (kDa)	150	50	105
Reduction in mortality (compared to saline)	30%	50%	30%
K_d (μM)	3.8	33.3	8.8

Figure 1. Cartoon depiction of the different antibody manifolds of GNCgzk with a summary of key characteristics.

by combing the scFv with a Fc portion.[13] Combined, this fusion scFv-Fc construct provides a format with more easily tunable pharmacokinetic (PK) properties.

PK studies were initiated by examining the serum stability of the GNCgzk scFv-Fc. Mice were injected with 15 mg/kg of purified fusion protein, and blood samples were taken at 1, 24, 48, and 72 h after injection. The concentration of scFv-Fc retained in the blood was measured by surface plasmon resonance (SPR) using a Biacore 3000 instrument (GE Healthcare) equipped with a research-grade CM5 sensor chip. A cocaine-BSA conjugate was immobilized on the chip surface and samples from each time point were measured.[14] The GNCgzk scFv-Fc exhibited an adequate serum half-life of 3.9 days (Fig. 2) in mice, an improvement over the GNC92H2 counterpart.[7]

The GNCgzk scFv-Fc was then tested in a cocaine overdose model in mice.[15] Male CD-1 mice (Charles River Laboratories, Wilmington, MA) were used in the overdose studies and catheters were implanted as previously described.[10] Briefly, animals were administered a dose of cocaine hydrochloride (NIDA, Rockville, MD) dissolved in sterile 0.9% saline, intraperitoneally (ip) at 10 mL/kg. The GNCgzk scFv-Fc was found to decrease mortality by 30% at a dose of 66 mg/kg 3 min after administration of 110 mg/kg cocaine (Fig. 3a). Seizures and ataxia are also phenotypic effects of cocaine overdose. However, these data were skewed due to the high mortality rate of the control group, preventing a good measure of seizures and ataxia (Fig. 3b and c).

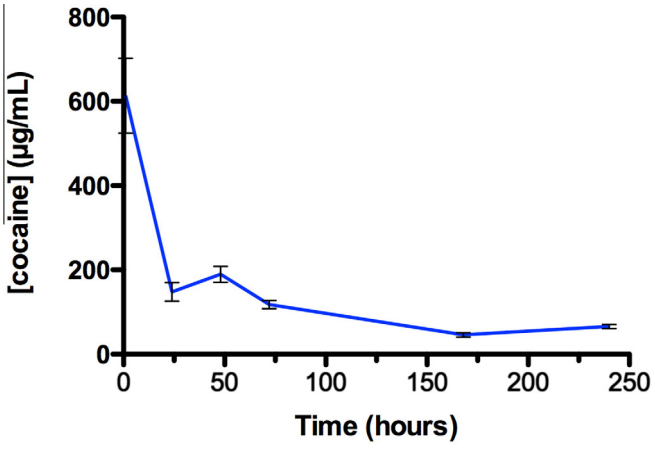

Figure 2. Average time-dependent serum concentrations of GNCgzk scFv-Fc determined by SPR (mean ± SEM; $n = 9$). Half-life of the GNCgzk scFv-Fc was determined to be 3.9 days.

The effects of the GNCgzk scFv-Fc were not as robust as the IgG or F(ab′)$_2$ format in reducing mortality. The GNCgzk F(ab′)$_2$ had previously shown complete survival of all animals at a dose of 66 mg/kg.[10] In order to more readily access the F(ab′)$_2$ construct, a human rhinovirus (HRV) 3C protease cleavage site (LEVLFQGP) was engineered into the hinge region of the GNCgzk IgG plasmid, encoding the modified IgG^{HRV} protein. The use of the HRV 3C protease resulted in quantitative conversion of the IgG^{HRV} to F(ab′)$_2^{HRV}$.

A radioimmunoassay (RIA) was employed[16] in order to determine the affinity of the GNCgzk IgG^{HRV}, F(ab′)$_2^{HRV}$, and scFv-Fc for cocaine. Pooled serum samples from each treatment group were incubated with L-[benzoyl1-3,4-^{3}H(*N*)]-cocaine tracer (spec. activity = 26 Ci/mmol; PerkinElmer, Boston, MA), and through equilibrium dialysis using 5000 Da MWCO plates (Harvard Apparatus, Holliston, MA) with a cold competitor, the affinity of each construct was obtained.[17] The relative affinity (K_d) for cocaine for each construct was determined to be 3.8, 33.3, and 8.8 μM for the IgG^{HRV}, F(ab′)$_2^{HRV}$, and scFv-Fc, respectively. The relatively high K_d for the F(ab′)$_2^{HRV}$ was surprising, since it had been previously demonstrated to be the most effective intervention.[10]

To explore the possibility that the HRV cleavage site affected the capacity of the F(ab′)$_2^{HRV}$ construct in preventing acute cocaine toxicity, it was tested in mice by ip. injection 3 min after a cocaine dose of 110 mg/kg was administered. Although it did not exhibit the complete loss of mortality as previously demonstrated, it outperformed the GNCgzk IgG^{HRV} by reducing the mortality by 50%, as opposed to 30% (Fig. 4a). It also exhibited a strong attenuation of ataxia and modest reduction in the seizure severity score over the 40 min time course (Fig. 4c and d). Thus, despite having the highest K_d, the F(ab′)$_2^{HRV}$ still outperformed its IgG^{HRV} counterpart.

There is currently no antidote for the treatment of acute cocaine overdose in the clinic. Efforts to produce such an antidote through passive immunization have produced a viable mAb, GNCgzk. While the engineering of a scFv-Fc construct of the GNCgzk mAb imparted easier production, it lacks the same efficacy as the full-length IgG and F(ab′)$_2$ counterparts. Thus, in order to make the F(ab′)$_2$ more accessible, an HRV cleavage site was engineered into the hinge region of the IgG plasmid to give quantitative yields of F(ab′)$_2$ from the IgG construct. This F(ab′)$_2$ was able to decrease mortality by 20% over the IgG, as well as attenuate phenotypic behaviors of cocaine overdose. Investigations into the use of the GNCgzk F(ab′)$_2$ are ongoing and will consist of further characterization of the binding mode between the IgG and F(ab′)$_2$, as well as further development as a clinically-viable cocaine antidote.