

Self-assembling Peptide-based Vaccines for Cocaine Addiction

Rajagopal Appavu, Ye Ding, Chunyong Ding, Sonja Stutz, Kathryn Cunningham, Jia Zhou, and Jai S. Rudra
Department of Pharmacology and Toxicology, Center for Addiction Research, Sealy Center for Vaccine Development,
University of Texas Medical Branch, Galveston, TX

Statement of Purpose: Cocaine abuse and dependence is a significant public health challenge in the USA with ~1.4 million current users aged 12 or older¹. Cocaine also accounts for nearly half of the illicit drug-related medical emergencies and currently there are no FDA-approved therapies for acute overdose or treatment of cocaine addiction^{1,2}. Early clinical studies suggest a role for active vaccination to prevent relapse to drug use in abstinent users who voluntarily enter treatment³. In recent years, vaccines that elicit effective anti-cocaine antibodies, which prevent cocaine penetration across the blood-brain barrier and interrupt its rewarding effects, have been successful in animal models¹. However, a primary limiting factor to their success appears to be the degree of immunity evoked by the cocaine antigen and lack of efficient delivery vehicles as small molecules like cocaine will not elicit an immune response unless administered with a carrier protein or an adjuvant. Recently, self-assembling peptides that assemble into β -sheet rich nanofibers have been shown to be effective for eliciting strong antibody responses against conjugated antigens without the need for any exogenous adjuvants⁴. Here, we investigated the ability of self-assembling peptides to elicit antibody responses against small molecule drugs by linking cocaine analogs modified at the P3 site to the self-assembling peptide KFE8. Preliminary studies showed that vaccination with cocaine-bearing KFE8 peptide nanofibers protected against cocaine-induced hyperactivity in mice.

Methods: Cocaine was acquired from the NIH and modified with an appropriate linker and functionality to yield cocaine-based small molecule haptens. The resulting cocaine analog was coupled to the *N*-terminus of the self-assembling peptide KFE8 (FKFEFKFE) using Fmoc chemistry (Fig. 1A) and purified by HPLC (> 90%). The final product was confirmed using mass spectrometry and formation of nanofibers (Coc-KFE8) was determined using transmission electron microscopy (TEM). Male B6 mice were immunized and boosted with Coc-KFE8 nanofibers (N=15) intraperitoneally. Two additional groups of mice received saline (N=9) or cocaine conjugated to BSA (Coc-BSA) (N=12) as a positive control. Antibody titers were evaluated using ELISA and cocaine-induced hyperactivity (20 mg/kg, i.p.) was measured using an open field photobeam activity system.

Results: The newly designed and synthesized cocaine haptens were characterized using ¹H, ¹³C NMR and conjugation of the modified cocaine analog to KFE8 peptide was confirmed by mass spectrometry (data not shown). TEM data indicated that Coc-KFE8 assembled into nanofibers similar to KFE8 (Figs 1B). The designed cocaine analog was found to be non-psychoactive in mice compared to cocaine (Fig. 1C). Mice immunized with Coc-KFE8 nanofibers raised anti-cocaine antibodies

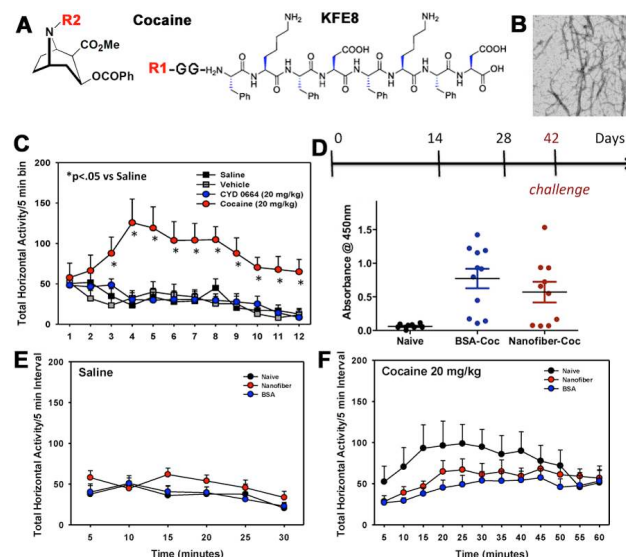


Figure 1. Schematic of Coc-KFE8 synthesis showing orthogonal chemical groups R1 and R2 used to link cocaine to KFE8 (A). TEM images Coc-KFE8 nanofibers (B). The modified cocaine analog generated did not elicit hyperactive behavior in mice (C). Anti-cocaine antibody levels in mice immunized with Coc-KFE8 nanofibers or Coc-BSA (D). No behavioral differences were observed in mice vaccinated and naïve mice in response to saline injections (E) Reduced cocaine-induced hyperactivity in mice vaccinated with Coc-KFE8 nanofibers as measured by total horizontal activity (F).

indicating that self-assembling peptides can adjuvant immune responses against small molecule drugs (Fig. 1D). Similar levels of antibodies were observed in mice vaccinated with Coc-KFE8 nanofibers or Coc-BSA (Fig. 1D). In preliminary behavioral studies, vaccinated mice and naïve mice responded similarly to a saline injection (Fig. 1E), however when challenged with cocaine, mice vaccinated with Coc-KFE8 nanofibers and Coc-BSA displayed suppressed hyperactivity compared to naïve mice (Fig. 1F), suggesting that peptide nanofibers are effective carriers for the development of cocaine vaccines and potentially other small molecule drugs of addiction.

Conclusions: In conclusion, self-assembling peptide nanofibers bearing a novel cocaine analog raised anti-cocaine antibody responses in mice, which protected mice from the hyperactive effects of cocaine. Self-assembling peptides might be attractive carriers for developing vaccines against small molecule addictive drugs.

References: 1. Kosten T. et al. (2014) *Br J Clin Pharmacol* 77:368-74, 2. Kinsey BM et al. (2010) *Exp Rev Vacc*, 9:1109-14, 3. Martell BA et al. (2009) *Arch Gen Psychiatry* 66:1116-23, 4. Rudra et al. (2010) *Proc Natl Acad Sci U S A*, 107:622-27.