Qijin Chau

Assignment #3 Paper Summary

Introduction/Background

Drug addiction is a brain disorder defined by an irresistible need to seek out and consume drugs. Having drug addiction, there is a large chance of relapse when exposed to drugs or drug-associated cues. Cocaine is a commonly abused drug that is quick to create addiction. Even after long periods of abstention, re-exposure to cocaine can lead to relapse. Currently, modern medication is insufficient in meeting the needs for treating cocaine use, relapse, or even the emergencies that result from cocaine overdose. Even though many different pharmacological targets and behavioral interventions have been explored to counteract cocaine addiction, a successful market-approved medication for treating cocaine addiction or relapse has not been developed. This study is attempting to endeavor upon an unexplored approach to treat drug addiction, specifically cocaine. Butyrylcholinesterase (BChE) is a natural enzyme located in hepatocytes and plasma that is found to be able to hydrolyze cocaine at low catalytic efficiency into benzoic acid and ecgonine methyl ester. These products are low in toxicity and rewarding properties. Through protein engineering, the catalytic strength and substrate specificity of human BChE (hBChE) has been significantly improved. The team is trying to fill the knowledge gap of skin gene therapy by using CRISPR (clustered regularly interspaced short palindromic repeats) to edit skin epidermal stem cells; an approach that has never been explored or attempted before.

Main goal

The main goal of the study is finding a way to stably, safely, and effectively deliver the engineered hBChE into mice, while allowing continuous expression. The eventual hope is for the enzyme to stifle the desire for cocaine and to protect against an overdose in humans.

Results

The first results revealed that engineered hBChE can be expressed for cocaine hydrolysis through CRISPR-edited epidermal stem cells. The CRISPR-mediated genome editing was carried out in mice epidermal stem cells. To make this possible, the team developed DNA vectors to serve as vehicles to carry the hBChE gene into the cells. Using $Cas9^{D10A}$ and two guide RNAs that target the mouse Rosa26 locus, the modified hBChE gene was delivered to the experimental mice. The engineered epidermal cells showed strong expression and secretion of hBChE displayed by analytical techniques like immunoblots in *Figure 1c*. The secreted hBChE protein also proved functional in breaking down cocaine shown in *Figure 1e*. To make sure that the modified epidermal cells are not capable of forming tumors, the ability for the cells to survive and grow in the absence of anchorage to the extracellular matrix and their neighboring cells were examined. The epidermal stem cells with hBChE targeting were deemed unable for anchorage independent growth.

The second results revealed that embedment of engineered epidermal stem cells can protect mice from cocaine-seeking behavior and cocaine overdose. A model was developed by culturing epidermal stem cells on top of mouse dermis. Even with expression of hBChE, the cultured cells were able to stratify into skin-like tissue in vitro (*Figure 2a)*. These tissues were transplanted into host animals with similar genotypes to investigate the potential therapeutic effect of hBChE expression in vivo. Cocaine can block dopamine reuptake and elevate extracellular levels of dopamine, resulting in locomotor stimulation and reward-related behaviors. Dopamine and cocaine levels in both GhBChE mice and control mice grafted with WT epidermal cells (GWT mice) after a small cocaine injection were measured to test if the expression of BChE can remove cocaine quickly and lead to reduced extracellular dopamine

and locomotor activity. Microdialysis was performed in the nucleus and mass spectrometry was used to quantify the results. The data strongly suggested that skin-derived hBChE can effectively hydrolyze cocaine and reduce extracellular levels of dopamine as shown in *Figure 2e*. Then, to determine whether engrafting hBChE-expressing cells can protect mice from the lethal toxicity of cocaine, various doses were given to the grafted and control mice while calculating lethality rates. For the GWT mice, it was observed that 80 $mg \cdot kg^{-1}$ cocaine induced roughly 50% lethality while 120 and 160 $mg \cdot kg^{-1}$ induced 100% lethality (*Figure 2i*). Meanwhile, doses ranging from 40 to 160 $mg \cdot kg^{-1}$ of cocaine had nearly 0 lethality in GhBChE mice, suggesting that the engraftment of hBChE-expressing cells can protect mice from the toxicity of cocaine overdose. Lastly, to determine whether engrafting hBChE-expressing cells can affect cocaine-induced reinstatement of drug seeking, the mice were put on recovery along with extinction training then given a dose of cocaine injection. The results suggested that skin-derived hBChE efficiently disrupted cocaine-induced reinstatement because the previously cocaine-associated environment was solely restored in the GWT mice.

The third results revealed that engineered human epidermal stem cells can deliver hBChE in vivo. Human skin tissue was cultured to test the practicality of gene therapy with human epidermal stem cells. To perform CIRPSR-mediated genome editing in human cells, vectors were developed. The first vector encoded two guide RNAs targeting the human adeno-associated virus integration site 1 gene locus and the second was an AAVS1-targeting vector that held the engineered hBChE. Using immunoblots and ELISA shown in *Figure 4c, d*, engineered human epidermal cells were determined to exhibit strong hBChE production. After stratifying, the engineered cells formed skin tissues in vitro, which were then transplanted to nude hosts (*Figure 4e*). Grafted skin exhibited no negative effects in vivo, proving that editing of human epidermal stem cells does not significantly alter cellular dynamics and continuance (*Figure 4f*). These results suggest the potential clinical relevance of skin gene delivery for the treatment of cocaine abuse and overdose in the future.

Conclusions

The study demonstrates that engraftment of genome-edited skin stem cells can be used to provide a long-term active cocaine hydrolase inside mice and potentially humans. Skin epidermal stem cells can be utilized for successful gene therapy in vitro, then this efficient genetic manipulation can provide benefits in vivo with minimal risk. For cocaine addicts and individuals with a potentially high risk of cocaine abuse who seek help or treatment, the skin gene therapy approach with hBChE expression is a scientific break-through that offers an effective therapeutic option to address several key aspects of drug abuse. The therapy can reduce the development of cocaine-seeking behavior, prevent cocaine-induced reinstatement of drug-seeking behavior, and protect against cocaine overdose after skin transplantation.

Future directions

Further studies are required to determine the protective effect of hBChE in response to extremely high doses of cocaine and cue-induced relapse. In a different study, bacteria cocaine esterase was concluded to prevent cocaine-induced toxicity, however these effects were depreciated when very high doses of cocaine were used. Additionally, on top of the drug effects, environmental cues also play a significant role in cocaine craving, abuse, and relapse. Proved to be a strong cocaine hydrolase, however the effects of hBChE against cue-induced relapse are unknown. While testing hBChE lethality protection from cocaine, the team could have drastically increased the dosage to see if the hBChE-expressing skin grafts were still effective against extremely high dosages. To test cue-induced relapse, they could first provide a certain food, then always follow up with a dosage of cocaine. Then afterwards, bring the food but not provide the dosage of cocaine to test if the effect of hBChE has any effect upon this empty cue.