Small Molecule Therapeutic Approach for Creatine Transporter Deficiency: Creatine Prodrug Delivery Targeting Fatty Acid Amide Hydrolase

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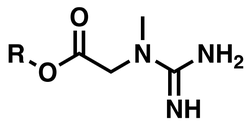
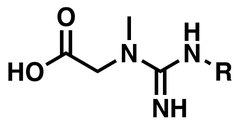
Introduction

Effective **delivery of creatine** or a creatine mimetic has been the goal of numerous therapeutic approaches for the treatment of **Creatine Transporter Deficiency**. The challenge for drug developers has been to find a way to transport molecules across the blood brain barrier (BBB). Recent attempts have utilized **prodrug strategies** that require bioactivation to release creatine from an aliphatic chain. Ideally, these strategies would allow for passive diffusion across the BBB and neuronal membranes, where the aliphatic chain would be cleaved to release creatine.

One such prodrug design uses the carboxylic acid of creatine to form an **ester**[1,2]. However, this is **ineffective** when dosed orally due to the high concentration of plasma **carboxylesterases**. Other prodrug designs utilize the guanidine of creatine to form an **amide**[3]. However, these prodrugs have experienced many challenges **involving rapid inactivation via cyclization, insolubility, instability, and toxicity**.

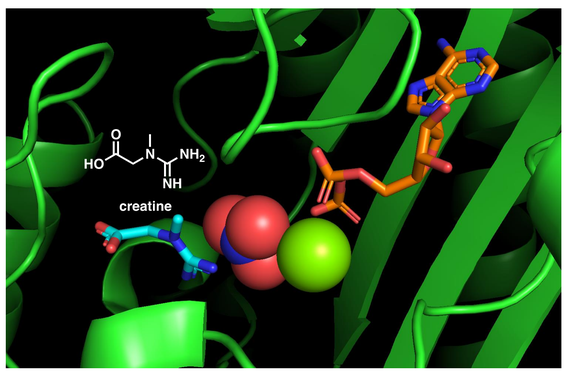


creatine ester prodrug



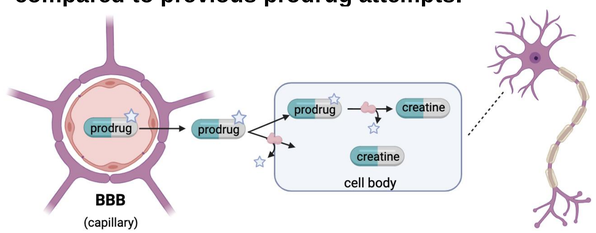
creatine amide prodrug

Even **dual-linkage prodrugs**, with ester and amide moieties, have not proven to avoid the problems each prodrug suffers alone[4]. Therefore, **new prodrugs must be designed** to increase effective oral delivery of creatine.



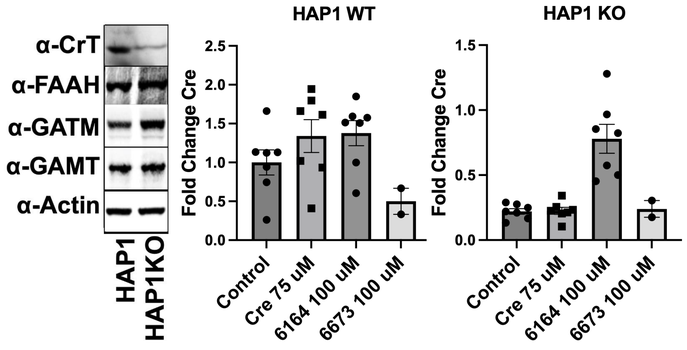
Creatine Delivery Strategies

Creatine amide prodrug strategies identified that the **prodrug** was successfully **hydrolyzed** by **Fatty Acid Amide Hydrolase** (FAAH)[4]. FAAH is a transmembrane serine hydrolase that endogenously hydrolyzes fatty acid amides such as anandamide and oleamide. Recent studies have demonstrated that targeting FAAH for prodrug bioconversion increases CNS bioavailability[5]. **Therefore, a primary goal of our research seeks to develop creatine amide prodrugs that have increased substrate specificity for FAAH, improved solubility, and improved stability compared to previous prodrug attempts.**



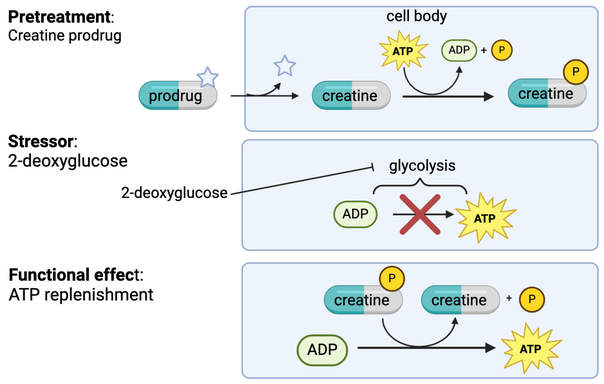
HAP1 Creatine Transporter KO Screen

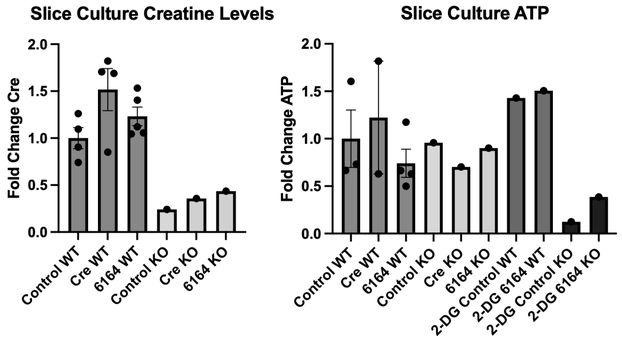
Our group has identified **HAP1 cell cultures** as a **novel screen for creatine prodrug delivery**[6]. Using both WT and KO creatine transporter (CrT) cell lines, prodrug compounds are administered at 100 μM for 24 hours. Cells are then collected, and total creatine levels are measured using enzyme-coupled detection. Prodrug toxicity is screened by measuring total protein concentration of cells post-treatment. Intracellular creatine concentrations are normalized to protein levels.



Hippocampal Slice Culture Screen

Currently, we are **piloting** a **functional screen** in **hippocampal slice cultures** to test compounds successful in HAP1 cells. Hippocampal slices are cultured from WT and CrT KO mice. Slices are pretreated with either 75 μM creatine or 100 μM of prodrug. After 4 hours, slices are treated with 5 mM 2-deoxy-glucose to induce energetic stress. Slices are harvested 24 hours later, and creatine and ATP levels are measured and normalized to protein concentration.



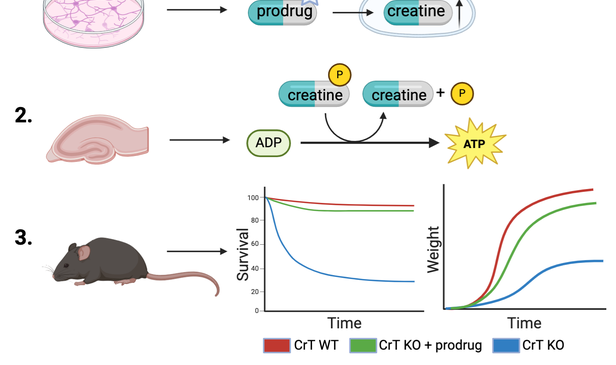


Results

During the development of our screens, we have identified a creatine prodrug**, STF-6164**, which restores intracellular creatine in CrT KO HAP1 cells. However, this prodrug is poorly soluble and displays signs of toxicity at higher concentrations.

Future Directions

We are actively working to develop **more effective alternatives to STF-6164**. During this process, we have been optimizing our hippocampal slice culture functional assay. Upon identification of an improved compound, we will conduct in vivo longitudinal studies that will determine creatine prodrug delivery in CrT KO mice by measuring body weight, survival, and brain creatine levels.



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