CWR tripeptide is a promising activator of SIRT1 in the fight against Alzheimer's disease

Review Article

Alzheimer's disease (AD) affected approximately 48 million people worldwide in 2015 and is one of the most financially costly diseases in developed countries, costing the US healthcare system, for example, $100 billion annually. The cause of AD remains unclear and treatment has therefore concentrated on managing symptoms.

A group of enzymes that may be involved in protection from age-related diseases such as AD is the Sirtuin family of NAD^+-dependent deacetylases. One Sirtuin, SIRT1, appears to control production of the amyloid beta peptides (A**β**) that make up amyloid plaque involved in AD. From studies using an AD mouse model, SIRT1 appears to have therapeutic potential that has led to the development of SIRT1 modulators such as resveratrol (Res). In this study by Rahul Kumar and his colleagues, a novel peptide SIRT1 activator was designed using *in silico* docking studies and synthesized. The tripeptide, CWR, was tested for its effect on the activity of recombinant SIRT1 protein and also determining its cytotoxicity. CWR was found to be a potent allosteric activator of SIRT1 both by molecular docking and *in vitro* analysis, increased cell viability and no toxicity to human erythrocytes were also observed.

SIRT1 is a target for the treatment of Alzheimer's disease

Sirtuin 1 (SIRT1), a member of the Sirtuin family of NAD^+-dependent deacetylases, has been recently shown to reduce amyloidogenic processing of the β-amyloid precursor protein involved in AD both *in vitro* and *in vivo*. For example, mice engineered to develop amyloid plaques and AD symptoms were improved when SIRT1 was overexpressed in the brain and exacerbated when SIRT1 was deleted. Based on this and other discoveries, Rahul Kumar and his colleagues decided to investigate the possibility of developing a peptide drug candidate directed to activate SIRT1. They chose to focus on peptides since these are known to be potent, specific and have a safe mode of action.

Design through molecular docking

A library of 8000 tripeptides was built to include all possible combinations of amino acids and the tripeptides were tested *in silico* against the crystal structure of SIRT1 in the open state. This screening involved docking experiments between a grid surrounding the active site, which was kept rigid, and the flexible tripeptides. The top ten tripeptides were selected based on their docking scores, number of hydrogen bonds formed, and hydrophobic interactions.

The ten peptide candidates were then synthesized using a PS3 Automated Solid Phase Peptide Synthesizer using Fmoc chemistry. The CWR peptide was synthesized on pre-loaded Wang resin by deprotecting the Fmoc-Arg-Wang resin with 20% piperidine in N,N-dimethylformamide (DMF) and activating Fmoc-Trp-OH with uronium salt 2-(1Hbenzotrizole-1yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HBTU) in the presence of base N-methylmorpholine (NMM) followed by coupling for 1 h. This procedure was repeated with Fmoc-Cys-OH to yield the final sequence Fmoc-Cys-Trp-Arg-Wang resin. Finally, the N-terminal Fmoc was removed and the resin was cleaved from the peptide with trifluoroacetic acid. The peptide was purified by reverse phase chromatography.

The tripeptide CWR shows potential as an allosteric SIRT1 activator

The peptides were screened for their ability to activate SIRT1 using a fluorescence-based deacetylase assay based on recombinant SIRT1. This screening showed that the peptide CWR was clearly the most effective in activating SIRT1, doubling the rate of deacetylation at about 105 µM. CWR had the highest docking score and highest number of hydrogen bonds that, together with a large number of hydrophobic interactions, suggested that it had the highest affinity for the allosteric site of SIRT1.

Kumar and colleagues then investigated the mechanism of activation of SIRT1 by CWR by studying the kinetics using the activity assay run at different concentrations of CWR. The tripeptide had no effect on V_{max}, but at a concentration of 120 µM it reduced the K_m from 507 to 72 µM, leading the researchers to conclude that CWR is an allosteric activator of SIRT1.

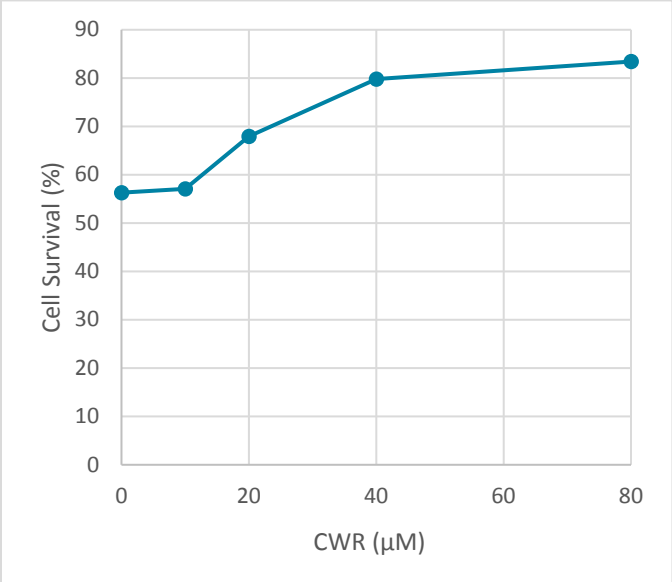
CWR raises SIRT1 activity in AD serum and IMR-32 cell line

The researchers then tested the effect of different concentrations of CWR on SIRT1 activity in the serum from AD patients. Their fluorescence assay showed that CWR increased SIRT1 activity by over 20% compared to untreated serum.

Treating cells of the IMR-32 neuroblastoma cell line tested the effect of CWR peptide on *in vivo* SIRT1 activity. The activity of SIRT1 was measured using an antibody directed against the acetylated form of p53-K382, a known target residue of SIRT1. Treatment of IMR-32 cells with CWR showed a dose-dependent decrease in the acetylation level of p53-K382, and adding the SIRT1 inhibitor nicotinamide prevented CWR from activating SIRT1. Together, these results confirmed that CWR is indeed a specific activator for the SIRT1 allosteric site.

CWR protects IMR-32 from cytotoxicity and shows minimal hemolytic effect

Treating the IMR-32 cell line with $A\beta_{25-35}$ peptide induces cytotoxicity associated with the amyloid plaque involved in AD. This effect could be greatly reduced by co-incubating with CWR (see Figure). Incubating red blood cells with CWR at as high as 1 mM showed minimal toxicity to RBCs.



CWR peptide protects CMR-32 cell line from the toxicity of $A\beta_{25-35}$ peptide

The promise of CWR

There is increasing interest in allosteric activation of SIRT1 since this may open new avenues of therapeutic intervention in a range of age-related diseases, including Alzheimer's disease. With its efficacy *in vitro* and *in vivo* together with low toxicity, the tripeptide CWR developed in this study is a promising starting point for the development of small molecule activators of SIRT1 that could be used in the fight against this debilitating disease.

Reference

Design, synthesis of allosteric peptide activator for human SIRT1 and its biological evaluation in cellular model of Alzheimer's disease. Kumar R, et al. Eur J Med Chem. 2016 Nov 3. pii: S0223-5234(16)30939-4. doi: 10.1016/j.ejmech.2016.11.001. [Epub ahead of print] https://www.ncbi.nlm.nih.gov/pubmed/27836195