The rat as a model for CER-0001 formulation development

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ABSTRACT

Background: CER-0001 is in development as a ketogenic drug for mild-to-moderate Alzheimer's disease (AD). CER-0001 is given at high doses in an attempt to compensate for regional cerebral glucose hypometabolism characteristic of AD. Upon ingestion, CER-0001 leads to the induction of ketosis. Previous studies have shown the formulation of the CER-0001 can influence digestion and absorption of the drug, and hence changes in formulation may influence clinical outcomes. In order to rapidly screen formulations of CER-0001, we have investigated the rat as pharmacokinetic (PK) model of human formulations. PK studies are performed to evaluate the absorption, distribution, metabolism and excretion (ADME) in animals. PK results allow us to define dose, dosing frequency, route of dosing and onset of action.

Methods: We have previously tested several formulations of CER-0001 in human PK studies and found several differences in Cmax, Tmax and AUC related to the release from the emulsions. In the present study we evaluated the PK profiles of these same formulations in rat to determine if rats qualitatively replicated the human results. Healthy young adult male Sprague Dawley rats were used as a test system for this PK study. Five animals per group were used, animals were between 9 to 12 weeks old and the weight variation of animals did not exceed ± 20% of the mean weight. Animals were dosed by oral gavage at Biological Resource Centre (BRC), Agency for Science, Technology and Research (A*STAR), Singapore. Sample analysis was performed by Agilex Biolabs Pty Ltd (Thebarton SA, Australia). Concentrations of acetoacetate and β-hydroxybutyrate in rat serum were determined by LC/MS. Ketone body concentrations (µM) were calculated as a sum of acetoacetate (µM) and β-hydroxybutyrate (µM) concentrations. Ketone body data was analyzed using WinNonlin.

Results: Rats qualitatively mirrored results from human studies. Formulations that exhibited slow release in humans similarly were found to be slow releasing in SD rats. Formulations that exhibited fast release in humans were found to be fast releasing in SD rats.

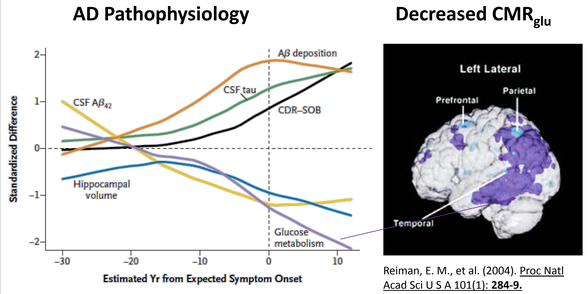
Conclusion: In preliminary studies rats represent a model for CER-0001 formulation development.

BACKGROUND

Rationale for CER-0001 development in AD

AD is characterized by accumulation of aggregated amyloid-beta, neurofibrillary tangles (NFTs) in the cerebral cortex and hippocampus, and region specific declines in the cerebral metabolic rate of glucose (CMR_{glu}).

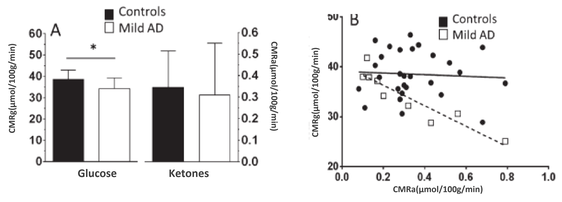
Prior studies have suggested that addressing the declines in glucose metabolism by ketogenic diets or ketogenic treatments may provide a target for AD drug development.



Reiman, E. M., et al. (2004). Proc Natl Acad Sci U S A 101(1): **284-9.**

Ketosis as means to address cerebral glucose hypometabolism in AD

- Under normal conditions, the brain primarily uses glucose as fuel
- Under conditions of low glucose availability ketone bodies are produced from fat stores and can provided up to 60% of brain metabolism
- Arterio-venous sampling studies in AD patients have demonstrated that total CMR_{glu} is decreased relative to healthy aged controls, while oxygen consumption is relatively intact, suggesting a switch in the AD brain from glucose to ketone body metabolism.



Castellano, et al (2014). Lower Brain 18F-Fluorodeoxyglucose Uptake But Normal 11C-Acetoacetate Metabolism in Mild Alzheimer's Disease Dementia. J Alzheimer's Dis.

Prior efficacy studies

A series of studies have been conducted suggesting that induced ketosis could improve cognitive performance in AD.

Reger et al. 2004 studied 20 AD subjects administered a single dose of CER-0001 resulting in a rapid improvement in cognition.

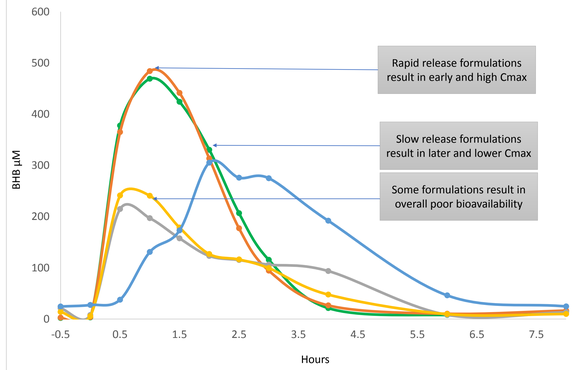
Henderson et al. 2009 studied 152 AD subjects administered CER-0001 for 90 days resulting in significant improvement in ADAS-cog in *APOE4*(-) patients at all dosing visits.

Torosyan, et al. 2018 studied cerebral blood flow with O^{15} PET imaging after CER-0001 administration. Increases in rCBF acutely and after 45 days of CER-0001 administration were noted.

Henderson et al. 2020 studied 413 AD subjects administered CER-0001 for 6 months. No improvements in cognitive performance were noted. Lack of efficacy is suspected to be due to low bioavailability of the formulation used in that study.

Human PK Studies

A series of clinical PK studies were conducted to determine ketogenic profile of different formulations of CER-0001. Some formulations exhibited fast release kinetics with elevated Cmax, while others resulted in delayed release.



Rat PK Studies

METHODS

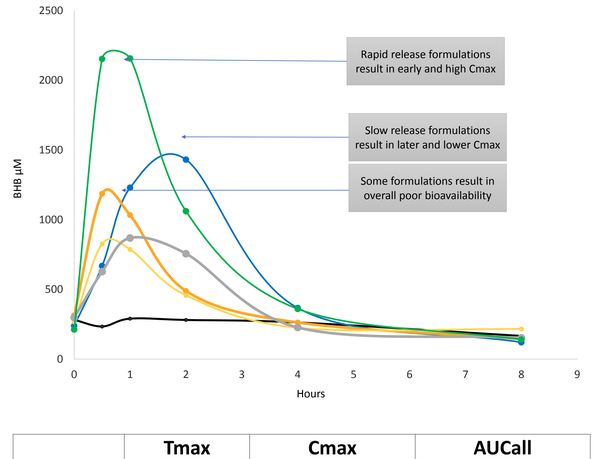
A series of rat PK studies were conducted to determine if rats could provide a tool for rapid screening and assessment of CER-0001 formulations.

Vehicle:	Water
Species:	Sprague Dawley rats
Number and Sex:	30 male animals
Number of groups:	6 groups, 5 animals/group= 30
Formulations:	Vehicle, A, B, C, D, E
Administration:	Oral Gavage
Time points:	0(predose), 0.5, 1, 2, 4, 8 Hours
Animal handling:	ASTAR, Singapore
Analytical:	Agilex, Australia (LC MS)

This study, protocol number BRC IACUC #181383, was approved by the Institutional Animal Care and Use Committee (IACUC) of BRC and a specific amendment for the rat tail vein catheterization for serial blood sampling was also approved. Animals had free access to food and water. Blood was collected by inserting tail vein catheter into lateral tail vein using BD Incyte 22 gauge needle. Concentrations of acetoacetate and β-hydroxybutyrate in rat serum were determined by LC/MS/MS.

RESULTS

Rats qualitatively mirrored results from human studies. Formulations that exhibited slow release in humans similarly were found to be slow releasing in SD rats. Formulations that exhibited fast release in humans were found to be fast releasing in SD rats.



Formula	Tmax		Cmax		AUCall	
	Mean	SD	Mean	SD	Mean	SD
A	0.70	0.27	2361.00	539.26	5698.01	1140.76
B	1.10	0.55	935.60	261.29	3158.83	564.57
C	0.90	0.65	902.60	557.51	2860.42	432.80
D	0.70	0.27	1302.20	413.49	3255.25	762.79
E	1.60	0.55	1717.00	780.38	4800.10	1250.55
Control	1.40	1.67	445.91	224.53	1945.54	1002.00

CONCLUSIONS

We took advantage of series of clinical PK studies to examine how rats compared to humans in the relative ability of different formulations to induce ketosis. PK profiles of rats were qualitatively similar to those of humans. Using rats as model to test formulations allows for faster more cost effective formulation development, and agree well with our dissolution tests. Dissolution tests will add further knowledge to the development of proprietary formulations of CER-0001.

DISCLOSURES

Cerecin is a developing ketogenic treatments for AD and other conditions. Authors are employees of Cerecin.

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