Identifying the Role of Lipid Metabolism in an Experimental Autoimmune Encephalomyelitis Mice Model

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Abstract

Background: Multiple Sclerosis (MS) is a disease characterized by demyelination and inflammation. Dysregulated fatty acid metabolism and mitochondrial function are mechanisms also hypothesized to be involved in the pathogenesis. Carnitine Palmitoyl Transferase 1 (CPT1) is a rate-limiting enzyme, which plays a pivotal role in mitochondrial fatty acid oxidation and blockage of CPT1 results is reversing metabolism from fatty acids to glucose. Moreover, mutations in CPT1a are frequently found among Hutterite and Inuit populations with residual CPT1a activities of 0 % and 22 %, respectively. The prevalence for developing MS in these populations is significantly reduced compared to the Canadian population. Therefore, we hypothesize that a CPT1a mutation protects against MS.

Methods: CRISPR-Cas9 technology was used to develop two mice strains lacking the metabolic enzyme CPT1 required for beta-oxidation. The first mice strain has a base pair shift mutation at position 1436 C to T resulting in an amino acid mutation at position 479 Pro to Leu (c.1436C>T) mimicking the mutation present among Inuits. The second strain has a 13 base pair deletion at position 1436-1448 causing a frame shift and therefore functional knock out of CPT1a, which is comparable to CPT1a activity in the Hutterite population.

An MOG35-55-induced EAE model with wild type and CPT1a (c.1436C>T) mutated mice was established to test the effect of the mutation and treatment with etomoxir. Afterwards, molecular biological tests as well as immunological tests will be performed.

Results: We have generated two different mice strains with mutations in CPT1a similar to the mutations present in Hutterite and Inuit populations. Both mice strains are in the process of being bred until CPT1a homozygosity. Preliminary data concerning CPT1a heterozygous mice showed decreased immune response by lowering the pro-inflammatory cytokine level compared to wild type mice. Experiments are ongoing and results will be presented at the CMSC meeting 2018.

Conclusions: This research indicates that CPT1, a key molecule involved in lipid metabolism, is involved in the immune response as well as disease induction and progression. Inhibition of CPT1 opens up for novel strategies for treating MS by reversing the metabolism and decreasing inflammation.

Background

Multiple Sclerosis is a systemic disease caused by lipid metabolism dysfunction and consequently induction of an inflammatory and autoimmune response.

Normally, the brain utilizes both glucose and fatty acids for cellular energy production (glycolysis and beta-oxidation), whereas under metabolic stress the energy source shifts to fatty acid utilization. This shift induces mitochondrial dysfunction, oxidative stress and inflammation, which are thought to be mediators involved in Multiple Sclerosis (1, 2, 3). Moreover, demyelination leads to exposure of proteins to immune cells, thereby producing autoantibodies to self-antigens leading to autoimmunity (2).

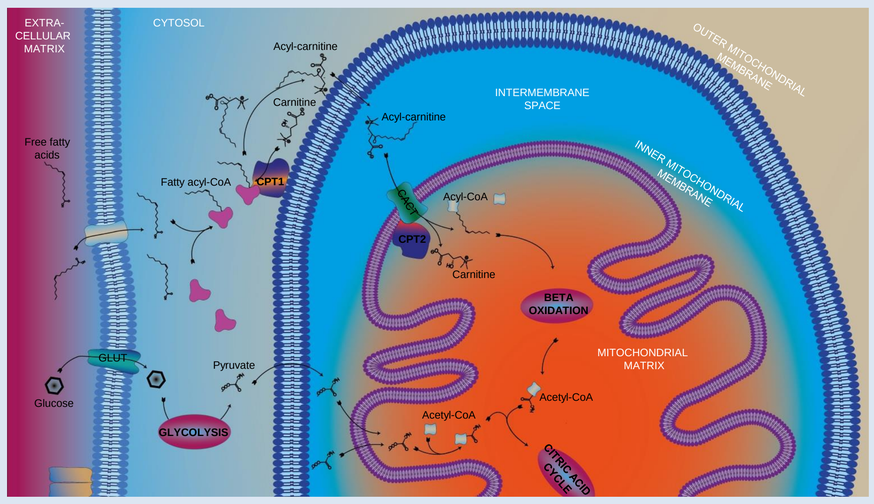

Figure 1. Metabolic pathways in the mitochondria.

Carnitine palmitoyl transferase 1 (CPT1) is a key molecule involved in lipid metabolism. Conversion of acyl-CoA to acyl-carnitine by CPT1 serves as an central regulator of the metabolism of the cell (1, 2).

Studies have shown that treatment with etomoxir, an irreversible blocker of CPT1, resulted in decreased disease activity, inflammation and increased remyelination in an EAE mouse model suggesting that inhibition of CPT1 is a novel potential treatment for Multiple Sclerosis (1, 2).

Results

	Mutation in CPT1a	CPT1a activity	Percentage of people with CPT1a mutation	Frequency of Multiple Sclerosis
Canadian Population	Wild type	100 %	~ 0 %	1/350
Hutterite	2129 G to A → AA710 Gly to Glu	~ 0 %	60 %	1/1100
Inuit	1436 C to T → AA479 Pro to Leu	22 %	98 %	1/50.000

Table 1. Low development of Multiple Sclerosis in human having reduced (Inuit) or loss-of-function (Hutterite) mutations in CPT1a gene.

Acknowledgements

The project is funded by Lundbeck Foundation. We thank Meta-IQ ApS for providing etomoxir and Biomedical Research Laboratory at Aalborg University Hospital for technical assistance.

Objective

To clarify the role of CPT1a mutation and pharmacological inhibition of CPT1 by etomoxir in an auto-immune experimental encephalomyelitis (EAE) model of Multiple Sclerosis.

Methods

We have generated two CPT1a mutant mice strains with CRISPR-Cas9 technology; 1) a CPT1a mutation mimicking the Inuit CPT1a mutation; 1436 C to G → AA479 Pro to Leu, and 2) a CPT1a 13 bp deletion resulting in a functional knock-out comparable to the Hutterite CPT1a mutation.

An experimental autoimmune encephalomyelitis (EAE) mice model was executed and included CPT1a mutated mice (n=2) and C57BL/6J wild type mice (n=3).The mice were immunized with 200 µg Myelin Oligodendrocyte Glycoprotein (MOG_{35-55}) suspended in complete Freund's adjuvant supplemented with *Mycobacterium Tuberculosis H37 Ra* (s.c.). The mice received 500 ng Pertussis toxin on day of immunization and two days later (i.p.). All animals were weighted and neurologically scored (0-5) every day and grip strength were measured at day 2 and day 24. Lymphocytes from spleen were isolated and stimulated with Staphylococcal enterotoxin B (SEB) or MOG_{35-55} for 24 h, whereafter the expression of interleukin-17α, interferon-γ and actin was investigated by RT-qPCR.

Results

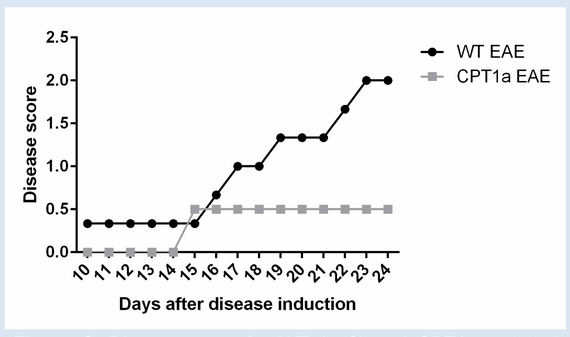

Figure 2. Disease score for WT (n=3) and CPT1a mutated (n=2) mice after EAE induction.

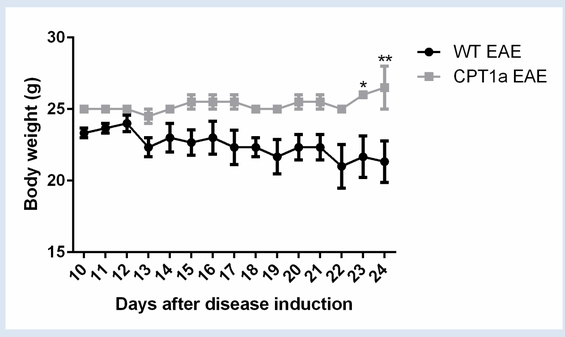

Figure 3. Body weight in grams (g) for WT (n=3) and CPT1a mutated (n=2) mice after EAE induction. The statistical analysis applied is a RM two-way ANOVA with Tukey multiple comparisons test. WT mice show statistical significant lower body weight compared to CPT1a mutated mice at day 23 (p=0.0420) and 24 (p=0.0068). Results are presented as mean ± SEM.

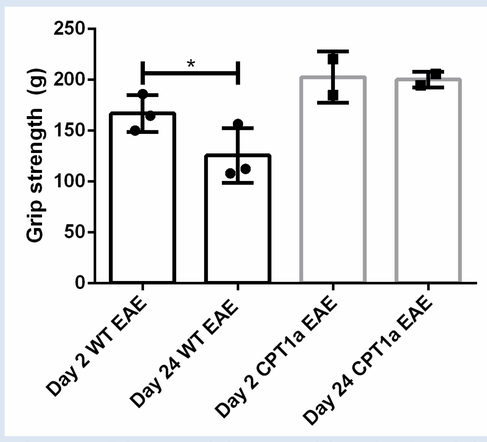

Figure 4. Grip strength in grams (g) at day 2 and day 24 for WT (n=3) and CPT1a mutated (n=2) mice. The statistical analysis applied is a paired t-test. Grip strength decreased significantly over time for WT mice (p=0.0361). Results are presented as mean ± SEM.

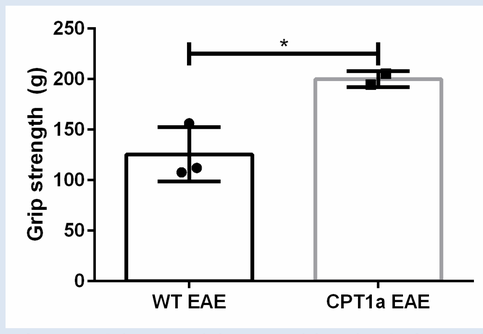

Figure 5. Grip strength in grams (g) at day 24 for WT (n=3) and CPT1a mutated (n=2) mice. The statistical analysis applied is an unpaired t-test. WT mice show significant decreased grip strength compared to CPT1a mutated mice (p=0.0356). Results are presented as mean ± SEM.

Results

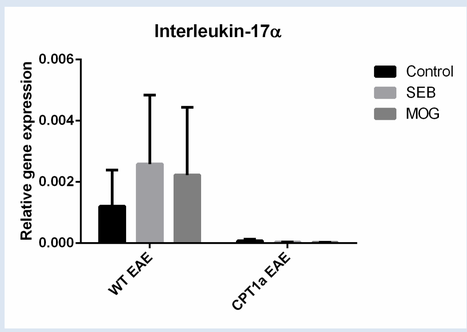

Figure 6. Relative gene expression of interleukin-17α in lymphocytes isolated from WT mice (n=3) and CPT1a mutated mice (n=2). Lymphocytes were stimulated with SEB or MOG. Results are presented as mean ± SEM.

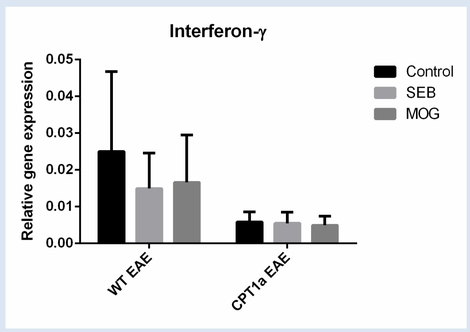

Figure 7. Relative gene expression of interferon-γ in lymphocytes isolated from WT mice (n=3) and CPT1a mutated mice (n=2). Lymphocytes were stimulated with SEB or MOG. Results are presented as mean ± SEM.

Conclusion

Preliminary data regarding biological inhibition of CPT1a by mutations potentially indicate protection against developing Multiple Sclerosis and thereby that lipid metabolism and CPT1a is involved in the pathogenesis of Multiple Sclerosis. These data support the observations of reduced Multiple Sclerosis incidence found in the Hutterite and Inuit population.

References

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