Blocking the Lipid Metabolism as a New Treatment Strategy for Multiple Sclerosis

Anne Skøttrup Mørkholt [§], Agnete Larsen*, Jette G. K. Nieland[#], Shohreh Issazadeh[$], Søren Nielsen [§] and John Dirk Nieland [§]

[§] Laboratory of Metabolism Modifying Medicine, Department of Health Science and Technology, Aalborg University, Denmark
[*] Department of Biomedicine, Aarhus University, Denmark
[#] Meta-IQ ApS, Aarhus, Denmark
[$] Biotech Research & Innovation Centre, Copenhagen University, Denmark
Correspondence: jdn@hst.aau.dk

Abstract

Multiple Sclerosis is a complex disease, which has been regarded as both a neurodegenerative and inflammatory disease characterized by damage to myelin sheaths of neurons. Normally, lipids of the CNS are important for a lot of functions e.g. hiding the myelin sheath proteins for the immune system and structure of myelin sheaths. Disturbances in metabolic pathways, as seen in Multiple Sclerosis, result in an upregulated lipid metabolism, thus changing the composition and level of lipids compared to healthy individuals. By blocking Carnitine Palmitoyl Transferase-1A (CPT-1A), a key molecule involved in lipid catabolism, the metabolism is reversed to glucose metabolism. This block of lipid metabolism causes reparation of myelin sheaths, re-lipidation of myelin sheath proteins thereby shielding these for the immune system and finally, restoration of signaling capacity of receptors using lipids.

People carrying CPT-1A mutations are protected against developing Multiple Sclerosis and other CNS diseases. These mutations reduce or delete the activity of CPT-1A. The Hutterite and Inuit populations are carrying CPT-1A mutations and the prevalence of developing Multiple Sclerosis is 1/1100 and 1/50000, respectively. Normally, the prevalence in these regions is 1/350.

Experimental Autoimmune Encephalomyelitis (EAE) models in rats and mice were established in order to test the effect of Etomoxir, a CPT-1A antagonist. The mice EAE model shows > 50 % healthy mice after two weeks of treatment with Etomoxir. In addition, the inflammatory response is downregulated and the myelin sheaths are repaired. The rat EAE model shows 25 % disease free animals after treatment with Etomoxir started at day 7.

Blocking the lipid catabolism through inhibition of CPT-1 opens new avenues for the treatment of Multiple Sclerosis and stands for a paradigm shift in the understanding of the development and treatment of Multiple Sclerosis and other CNS diseases.

Introduction

Hypothesis

- Multiple Sclerosis is characterized by dysfunction of lipid metabolism and inflammation.

Metabolic Pathways

- Normally, the brain utilizes both glucose and fatty acids for cellular energy production (glycolysis and beta-oxidation), whereas in conditions with stress the metabolism shifts to lipid utilization (Figure 1).
- CPT-1 is a key molecule involved in lipid metabolism.
- Conversion of acyl-CoA to acyl-carnitine by CPT-1 serves as an central regulator of the metabolism of the cell.

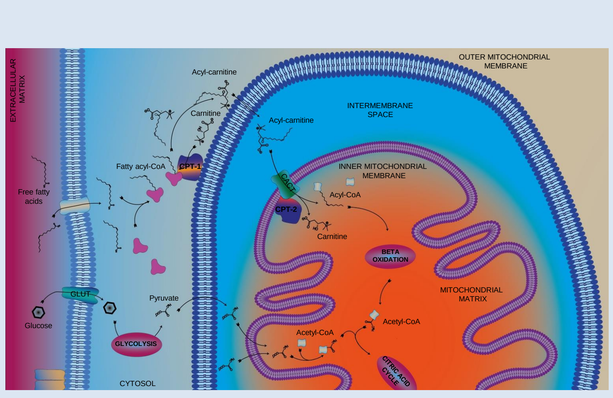

Figure 1 – Metabolic Pathways.

Multiple Sclerosis and Lipid Metabolism

- In Multiple Sclerosis a link between glucose and lipid metabolism has been found (Figure 2).
- **Healthy condition:** the brain utilizes glucose metabolism to generate the energy needed to exert its function. When challenged due to stress (pathological, psychological or physical) the metabolism shifts to lipid metabolism.
- **Pre-diseased condition:** if this shift in metabolism continues for a long period lipids can not be replenished to myelin sheath proteins, e.g. myelin basic protein. Moreover, Prostaglandin-E2 (PGE2) production is initiated and the immune system is attracted.
- **Diseased condition:** T- and B-cells are stimulated by exposed citrullinated un-lipidated proteins, which induces an inflammatory response resulting in further stress, upregulated lipid metabolism, exposure to citrullinated un-lipidated proteins and production of PGE2.
- **Treatment:** By inhibition of CPT-1 the lipid metabolism and the inflammatory response are blocked, thereby reversing the development of Multiple Sclerosis.

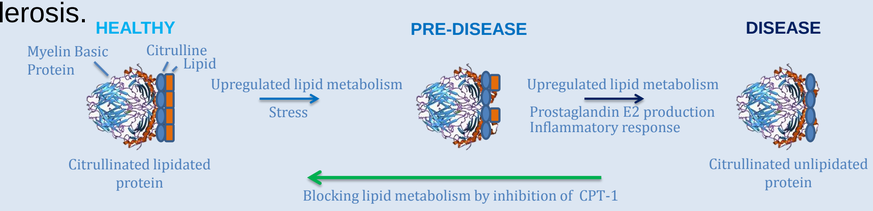

Figure 2 – Mode of Action Model of upregulated Lipid Metabolism in Multiple Sclerosis.

Methods

Experimental Autoimmune Encephalomyelitis Mice Model

12 weeks old female C57BL/6 mice with an average weight of 23.5 g were injected intradermal with Myelin Oligodendrocyte Glycoprotein (MOG_{35-55}) conjugated with Freund's adjuvant. The mice were weighted and scored daily. At day 10 after disease induction, the mice were treated with Etomoxir (MIQ-001) and compared to a placebo group (Figure 3).

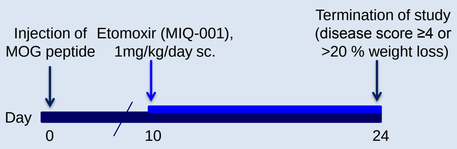

Figure 3 – Timeline of the Experimental Autoimmune Encephalomyelitis model in mice.

Experimental Autoimmune Encephalomyelitis Rat Model

2 months old female Lewis rats with an average weight of 200 g were injected intradermal with MOG_{35-55} conjugated with Freund's adjuvant. The rats were weighted and scored daily. In the first study, rats were treated with Etomoxir at day 7 and compared to a placebo group (not illustrated). In the second study, rats were treated at day 1 or day 5 with either Etomoxir or Interferon-β (Figure 4).

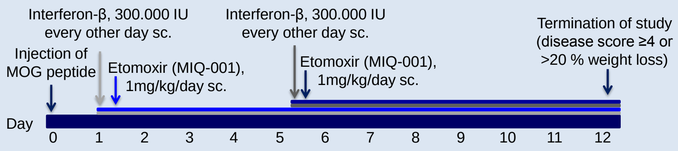

Figure 4 – Timeline of the Experimental Autoimmune Encephalomyelitis model in rats.

Conclusions

- Treatment with Etomoxir ameliorates characteristic symptoms of Multiple Sclerosis investigated by the well-established EAE model of Multiple Sclerosis.
- Treatment with Etomoxir restores neuronal function in 50 % of mice (day 24) and 25 % of rats (day 7).
- CPT-1 is a key molecule involved in lipid metabolism and people with a CPT-1a mutation are protected from developing all types of Multiple Sclerosis.

Perspectives

- Generation of Inuit mice with a CPT-1a mutation.
- Immunohistochemistry for CPT-1a and myelin basic protein from sections of Multiple Sclerosis patients.
- Etomoxir is ready for clinical phase II trials in secondary progressive Multiple Sclerosis and acute optic neuritis.

Results

Etomoxir Blocks Lipid Metabolism in a mice EAE model of Multiple Sclerosis

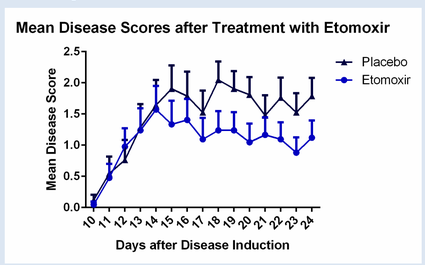

Figure 5 - Mean disease score of day 10 to 24 after disease induction in mice receiving either Etomoxir or placebo. The statistical analysis applied is a two-way repeated measure ANOVA test with a Sidak's multiple comparisons post hoc test. Results are presented as mean ± SEM. There is no significant difference in mean disease score between treatment with Etomoxir (n=21) and placebo (n=21).

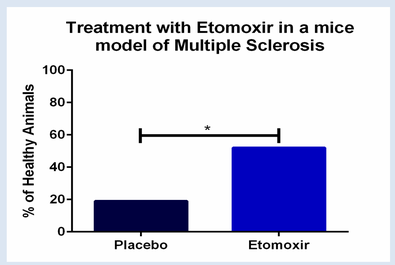

Figure 6 – Percentage of healthy animals (corresponding to a score 0 at day 24 or/and a sum disease score of max 7) after two weeks of treatment with either Etomoxir or placebo. The statistical analysis applied is a chi-square test (5.081, df 1). There is significant difference between treatment with Etomoxir (n=21) and placebo (n=21), *p=0.0242.

Etomoxir decreases Diseases Scores in a rat EAE model of Multiple Sclerosis

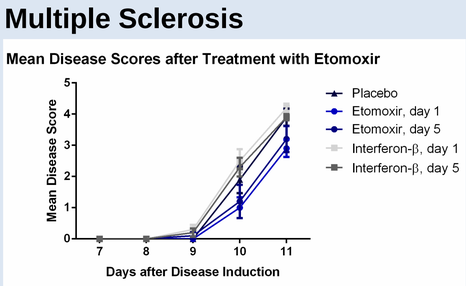

Figure 10 - Mean disease score of day 7 to 11 after disease induction in rats treated with either Etomoxir, interferon-β or placebo. The statistical analysis applied is a two-way repeated measure ANOVA test with a Tukey's multiple comparisons post hoc test. Results are presented as mean ± SEM. At day 10 and 11, statistical significant differences between treatment with Etomoxir (day 1 and 5) and Interferon-β (day 1 and 5) are found.

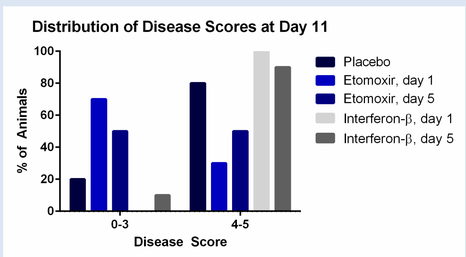

Figure 11 - Percentage of rats in the five treatment groups distributed over disease score 0 to 3 and disease score 4 to 5 at day 11 after disease induction (n=10 in each group).

Etomoxir Blocks Lipid Metabolism in a rat EAE model of Multiple Sclerosis

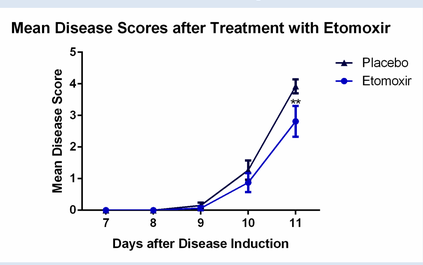

Figure 7 - Mean disease score of day 7 to 11 after disease induction in rats receiving either Etomoxir or placebo. The statistical analysis applied is a two-way repeated measure ANOVA with a Sidak's multiple comparisons post hoc test. Results are presented as mean ± SEM. There is significant difference in mean disease score between treatment with Etomoxir (n=16) and placebo (n=26) at day 11, **p=0.0013.

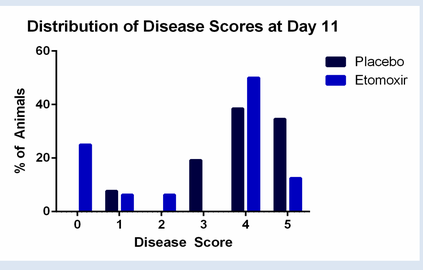

Figure 8 - Percentage of rats in the two groups distributed over the different disease scores at day 11 after disease induction (n=16 in Etomoxir and n=26 in placebo group).

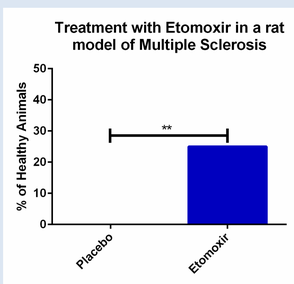

Figure 9 - Percentage of healthy animals corresponding to a score 0 at day 11 after treatment with Etomoxir or placebo. The statistical analysis applied is a chi-square test (7.184, df 1). There is significant difference between treatment with Etomoxir (n=16) and placebo (n=26), **p=0.0074.

Humane Data

Hutterites

- Mutation in CPT-1a makes the molecule non-functional.
- 30-60 % have mutation in at least one allele of CPT-1a.
- Low incidence of Multiple Sclerosis (1/1100).

Inuits

- Mutation in CPT-1a reduces the function of the molecule to 25 %.
- 98 % have mutation in one allele of CPT-1a.
- Low incidence of Multiple Sclerosis (1/50.000).

Normal prevalence of Multiple Sclerosis in these regions is 1/350.

	Mutation in CPT-1a	CPT-1a Activity of Mutated Type compared to Wild Type	Percentage of People with CPT-1a Mutation (at least in one allele)	Frequency of Multiple Sclerosis
Canadian Population	Wild type	100 %	~ 0 %	1/350
Hutterites	2129 G to A → AA710 Gly to Glu	~ 0 %	60 %	1/1100
Inuits	1436 C to T → AA479 Pro to Leu	25 %	98 %	1/50.000

Table 1 – Overview of CPT-1a mutations in the Canadian people, Hutterites, and Inuits.