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Triheptanoin - a medium chain triglyceride with odd chain fatty acids: a new anaplerotic anticonvulsant treatment?

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Abstract

The triglyceride of heptanoate (C7 fatty acid), triheptanoin, is a tasteless oil used to treat rare metabolic disorders in USA and France. Heptanoate is metabolized by β-oxidation to provide propionyl-CoA, which after carboxylation can produce succinyl-CoA, resulting in anaplerosis – the refilling of the tricarboxylic acid cycle. Heptanoate is also metabolized by the liver to the "C5 ketones", β-ketopentanoate and/or β-hydroxypentanoate, which are released into the blood and thought to enter the brain via monocarboxylate transporters. Oral triheptanoin has recently been discovered to be reproducibly anticonvulsant in acute and chronic mouse seizures models. However, current knowledge on alterations of brain metabolism after triheptanoin administration and anaplerosis via propionyl-CoA carboxylation in the brain is limited. This review outlines triheptanoin's unique anticonvulsant profile and its clinical potential for the treatment of medically refractory epilepsy. Anaplerosis as a therapeutic approach for the treatment of epilepsy is discussed. More research is needed to elucidate the anticonvulsant mechanism of triheptanoin and to reveal its clinical potential for the treatment of epilepsy and other disorders of the brain.

1. Introduction

Dysfunction of metabolic processes appears to play a major role in conditions that include seizures as well as certain forms of epilepsy. This notion is corroborated by two main types of observations. 1) Mutations in genes that are involved in energy and/or ATP metabolism are associated with epileptic seizures, e.g. glucose transporter 1 (GLUT1) deficiency, but also mutations of mitochondrial constituents. 2) Several manipulations of metabolic pathways are efficacious in rodent seizure models and/or epilepsy patients. This includes the ketogenic diet (as discussed in this supplement), fructose-1,6-bisphosphate in rat epilepsy models (Lian et al., 2007; Lian et al., 2008) and 2-deoxy-D-glucose in certain rat and mouse models (Garriga-Canut et al., 2006; Stafstrom et al., 2009). Triheptanoin is a medium chain triglyceride containing three odd chain fatty acid heptanoate molecules. It is a clear tasteless oil which can easily be added to any diet. Roe, Brunengraber and colleagues discovered triheptanoin as an oral anaplerotic treatment for metabolic disorders (Roe et al., 2002;

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Brunengraber and Roe, 2006; Roe and Mochel, 2006). This sparked interest in its potential for the treatment of epilepsy, resulting in the recent finding that triheptanoin feeding is anticonvulsant in three mouse epilepsy models. In this review we discuss the current knowledge of triheptanoin in terms of its anticonvulsant and metabolic effects and its clinical potential in comparison to the ketogenic diet.

2. Triheptanoin's anticonvulsant profile

At the time of writing this article, triheptanoin feeding as an anticonvulsant treatment in rodents has only been evaluated by two laboratories. In 2008 it was described that short term feeding of triheptanoin within a context of a ketogenic diet inhibited cortical spreading depression in young rats (de Almeida Rabello Oliveira et al., 2008). The Borges' laboratory investigated the effect of oral triheptanoin in the context of a more regular "low fat" diet, a composition largely based on the clinical studies by Roe. Given that in clinical studies up to 35% of the daily caloric intake is provided in the form of triheptanoin, standard rodent chow was modified accordingly to include 170 ml of triheptanoin per kg rodent diet (Willis et al., 2010). Other components of regular rodent chows, such as 150 g sucrose and some of the complex carbohydrates and fats were omitted to accommodate the amount triheptanoin added. Fed to mice, the dietary intake of protein, antioxidants, vitamins and minerals was similar between standard versus triheptanoin diet. In metabolic cages, a 30 g mouse consumed on average, 5 g of triheptanoin-containing diet per day, corresponding to a dose of 0.85 ± 0.2 g triheptanoin per day (average and standard error of the mean for 4 experiments). In our initial experiments up to two weeks of triheptanoin feeding did not induce reproducible anticonvulsant activity in acute mouse seizure models, such as the fluorothyl, 6 Hz and pentylenetetrazole (PTZ) (i.v.) tests (Willis et al., unpublished). In contrast, we found reproducible anticonvulsant effects after ≥ three weeks of feeding in one acute and two chronic mouse seizure models in CD1 and CF1 mice, respectively. In the maximal electroshock threshold test in CD1 mice, we recently found a small but reproducible increase of the critical current at which 50% of mice seize (Willis et al., unpublished). We are currently investigating the minimum triheptanoin feeding amount and time required for this effect. In the corneal kindling model we found a reproducible delay in the kindling process in CF1 mice. This effect is similar to results found with low doses of levetiracetam in the same model (Matagne et al., 2008) and valproate, phenobarbital and lacosamide in the rat amygdala kindling model (Brandt et al., 2006; Silver et al., 1991). Lastly, we used a second hit pentylenetetrazole (PTZ, i.v.) test in CF1 mice that were subjected to pilocarpine-induced status epilepticus (PILO-SE). Mice and rats that experience PILO-SE develop spontaneous seizures (Turski et al., 1984; Turski et al., 1983) and increased sensitivity to PTZ. In our hands, there was no evidence of spontaneous seizures or increased seizure threshold in mice that did not develop SE (no SE mice, Willis et al., 2010). In two experiments, triheptanoin reproducibly increased the PTZ seizure threshold in CF1 mice that had experienced PILO-SE. The fact that there was no effect of triheptanoin in the PTZ test in no SE mice suggests that triheptanoin feeding is particularly effective in mice with spontaneous recurrent seizures.

Table 1 summarizes and compares the anticonvulsant profiles of triheptanoin and some of the most commonly used antiepileptic drugs and the ketogenic diet. The table needs to be interpreted with caution, because not all the specific conditions of animal epilepsy models used could be taken into account. For example, PTZ models vary between different laboratories and anticonvulsant efficacy of certain drugs is dependent on the PTZ administration route and the rodent used (Löscher et al., 1991). Also, data on the anticonvulsant effects of ketogenic diets in animal models can vary across laboratories (Susan Masino, Adam Hartman personal communication (Hartman et al., 2007; Hartman et al., 2008; Samala et al., 2008; Borges, 2008). To our knowledge, antiepileptic drug efficacy

has not yet been described in the combination of the chronic pilocarpine model with a second hit seizure susceptibility test in the mouse, but the same model has been characterized in the rat (Blanco et al., 2009). The data by Blanco and colleagues suggest that this model is a useful tool to identify treatments with efficacy in pharmacoresistant epilepsy, because the model is resistant to the most commonly used antiepileptic drugs, valproate, phenobarbital and phenytoin. In summary, the anticonvulsant profile of triheptanoin in the context of a regular diet is different from that found with other established treatments for epilepsy. This raises the hope that triheptanoin therapy may benefit patients with medically refractory epilepsy. The next paragraphs discuss anaplerosis as triheptanoin's possible mechanism of anticonvulsant action and triheptanoin's clinical potential.

3. Anaplerosis and triheptanoin

In aerobic metabolism, ATP production is largely dependent on the tricarboxylic acid (TCA) cycle. A reduction in the levels of TCA cycle intermediates and subsequently acetyl-CoA oxidation and energy production may be a contributor to seizures in "epileptic" brains. Anaplerosis is the refilling of deficient metabolites of the TCA cycle (Kornberg, 1966), involving carboxylation of pyruvate and propionyl-CoA (Fig. 1). In the brain, pyruvate carboxylase is the main anaplerotic enzyme (Patel, 1974, Sonnewald and Rae, 2010). Two other enzymes, phosphoenol-pyruvate carboxykinase and malic enzyme have the potential to increase the levels of TCA intermediates oxaloacetate and malate, respectively. However, both enzymes appear to only work in the decarboxylation direction in the brain (Patel, 1974). The anaplerotic pathway from propionyl-CoA via methylmalonyl-CoA to succinyl-CoA has been well studied in peripheral tissues, such as skeletal muscle, liver and heart (Fig. 1; e.g. Nuutinen et al., 1981; Martini et al., 2003; Reszko et al., 2003; Owen et al., 2002). The enzymes involved are propionyl-CoA carboxylase (EC 6.4.1.3), methylmalonyl-CoA epimerase (EC 5.1.99.1) and methylmalonyl-CoA mutase (EC 5.4.99.2). Propionyl-CoA carboxylase is a biotin dependent enzyme consisting of a heteropolymer of α and β subunits, encoded by two different genes *PCCA* and *PCCB* (Lamhonwah et al., 1986). Functional mutations in either gene can result in propionic acidemia (MIM ID # 606054), which in many patients appears to result in epileptiform activity with a high manifestation rate of clinical seizures (Haberlandt et al., 2009). Anaplerotic molecules metabolized to propionyl-CoA and propionyl-CoA carboxylase pathway include the branched chain amino acids, isoleucine and valine, propionate, and molecules containing uneven fatty acids, such as triheptanoin (Fig. 1).

Triheptanoin supplies the body with heptanoate which can either be oxidized to propionyl-CoA directly or is metabolized by the liver to the "C5 ketones", β-ketopentanoate and/or β-hydroxypentanoate, which are released into the blood (Roe et al., 2002; Kinman et al., 2006; Brunengraber and Roe, 2006; Roe and Mochel, 2006; Deng et al., 2009; Gu et al., 2010). Heptanoate is likely to enter the brain via diffusion, while C5 ketones may cross the blood brain barrier and enter cells of the brain via monocarboxylate transporters. Increasing anaplerosis through the propionyl-CoA pathway has the potential to be a powerful new approach to optimize TCA activity in the diseased brain, resulting in increased production of reducing equivalents, oxidative phosphorylation and ATP and also amino acid neurotransmitters. This concept is intriguing especially in the light of the hypothesis that increased ATP and/or energy production may underlie the anticonvulsant mechanism of action of the KD (DeVivo et al., 1978; Bough et al., 2006; Masino and Geiger, 2008; Masino et al., 2009).

5. Anaplerosis as a therapeutic approach for the treatment of epilepsy

There are several reasons why a therapeutic approach to increase anaplerosis in the brain appears to be viable. It is plausible that TCA cycle intermediates are reduced in chronic epilepsy, because α-ketoglutarate is the precursor for the neurotransmitters glutamate and GABA and oxaloacetate for aspartate. Increased neurotransmission, that is, release of these substances, such as during seizures, can reduce the levels of TCA cycle intermediates. The following studies in three different epilepsy models during the chronic phase corroborate this notion. In the hippocampal formation of lithium PILO-SE rats, levels of glutamate, aspartate (indicative of the TCA intermediates α-ketoglutarate and oxaloacetate), N-acetyl aspartate, adenosine triphosphate plus adenosine diphosphate and glutathione were decreased (Melo et al., 2005). Glutamate concentrations were also decreased in the hippocampi of rats after kainate-induced SE (Alvestad et al., 2008). In PILO-SE mice, forebrain levels of malate and also propionyl-CoA were decreased (Willis et al., 2010). These findings are consistent with our hypothesis that increased anaplerosis may protect “epileptic” brains against seizures.

6. Anaplerosis via triheptanoin in the brain?

To obtain a measure of brain anaplerosis via triheptanoin, Willis et al., (2010) compared steady state forebrain metabolite levels from mice with and without PILO-SE after three weeks of feeding triheptanoin vs standard diet. In the chronic phase in PILO-SE mice, triheptanoin feeding nearly restored brain levels of propionyl-CoA and increased methyl-malonyl-CoA, suggesting that triheptanoin could be anaplerotic through this pathway in mice with spontaneous seizures. However, there is still little evidence of substantial anaplerosis through succinyl-CoA in the “normal” or “diseased” brain. Triheptanoin feeding did not increase the forebrain steady state levels of TCA intermediates, aspartate, glutamate or GABA in either PILO-SE or no SE mice, except that malate levels were increased by 25% in mice without PILO-SE. These steady state measurements of metabolites do not give any indication of TCA cycle activity and anaplerotic flux. However, these findings are consistent with low amounts of anaplerosis found when injecting [3-^{14}C]-propionate into mouse striatum and neocortex (Nguyen et al., 2007) and [U-^{13}C]- isoleucine in rat brain (Bak et al., 2009).

There is little known about the cell types in the brain that are involved in the anaplerotic propionyl-CoA carboxylation pathway. The study by Nguyen and colleagues (2007) found five times higher ^{14}C specific activity in glutamine vs. glutamate pools after [3-^{14}C]-propionate injection. This indicates astrocytic metabolism of propionate, because glutamine synthase occurs mainly in astrocytes. On the other hand, in the same study cultured rat cerebellar granule cells exhibited more than three-fold higher activity of propionyl-CoA carboxylase than cerebellar astrocytes. Using in situ hybridisation and immunohistochemistry, expression of propionyl-CoA carboxylase alpha-subunit and methylmalonyl-CoA mutase were found in in neurons in developing and adult rat brain (Ballhausen et al., 2009). Expression was found in cerebellar granule cells, but could not be detected in astroglia *in vivo*. This is a very interesting observation, in light of the fact that pyruvate carboxylase is only found in astrocytes (Yu et al., 1983; Shank et al., 1985; Cesar and Hamprecht, 1995), raising the possibility that anaplerosis via propionyl-CoA can directly occur in neurons.

Many aspects of the anaplerotic propionyl-CoA carboxylation pathway in the brain remain unknown and require further study. The characteristics of enzymatic reactions, such as the conversion of propionyl-CoA via (S)-methylmalonyl-CoA and (R)-methylmalonyl-CoA to succinyl-CoA have been largely investigated in other organs in the past. Little is known

about the expression and regulation of the enzymes responsible, including specifically the β-subunit of propionyl-CoA carboxylase and methylmalonyl-CoA epimerase, but also methylmalonyl-CoA mutase (see Fig. 1). Of particular need is more knowledge about the activity of this pathway in “normal” and “diseased” brains of rodent disease models and human patients. Also, it is still unclear to what extent triheptanoin increases anaplerosis in “normal” or “diseased” brains and if anaplerosis contributes to triheptanoin’s anticonvulsant properties in the mouse. At this time, it is crucial for epilepsy researchers to gain a better understanding of triheptanoin’s anticonvulsant mechanism in the quest to find new improved therapeutic approaches for the treatment of epilepsy.

7. Triheptanoin as a new treatment for epilepsy

Despite the unknown mechanism of triheptanoin’s anticonvulsant action, the fact that triheptanoin has been used safely in several animals and for various metabolic diseases in children and adults should expedite the ethical and regulatory approval processes for a clinical trial in medically refractory patients with epilepsy. In short-term experiments in rats, triheptanoin has been administered intravenously and intraduodenally up to 40% of the caloric requirement (Kinman et al., 2006; Gu et al., 2010). In metabolic studies, there were no signs of metabolic perturbation of liver metabolism, ketoacidosis by C4 or C5 ketones, or propionic acidemia. Similarly, short term intravenous infusion of β-hydroxypentanoate and β-ketopentanoate in dogs did not lead to signs of propionic acidemia as determined by urine analysis (Leclerc et al., 1995).

Roe and his colleagues have shown that triheptanoin can benefit patients of all ages with different metabolic problems, including cardiomyopathy and rhabdomyolysis in very-long-chain acyl-CoA dehydrogenase deficiency (Roe et al., 2002), pyruvate carboxylase deficiency (Mochel et al., 2005), carnitine-palmitoyltransferase II deficiency (Roe et al., 2008), and adult polyglucosan body disease (Roe et al., 2010). The vast experience obtained with the ketogenic diet by researchers, clinicians, dieticians, patients and their families will help to facilitate the investigation and development of triheptanoin as a new anticonvulsant treatment. Chemically, triheptanoin resembles medium chain triglycerides, which have been shown to be an effective treatment for certain children with refractory epilepsy in many studies, including a randomised controlled clinical trial (Neal et al., 2008). The largest concern for epilepsy patients is that triheptanoin may lead to propionic acidemia, which will need to be carefully monitored employing organic acid urine analysis currently used to detect inborn errors of metabolism in newborn infants. Another important issue is that triheptanoin is contraindicated in patients with inborn errors of fatty acid oxidation, such as medium-chain acyl-CoA dehydrogenase deficiency - MCAD, short-chain acyl-CoA dehydrogenase deficiency - SCAD, short-chain-3 hydroxyacyl-CoA dehydrogenase deficiency –SCHAD and HMG CoA (3-hydroxy-3-methyl-glutaryl-CoA) synthase deficiency. Therefore, patients will need to be carefully screened for these disorders, taking into account medical histories, blood acyl-carnitine profiles and urine organic acid analyses. Future studies must address the anticonvulsant mechanism of action of triheptanoin and its potential for clinical use.

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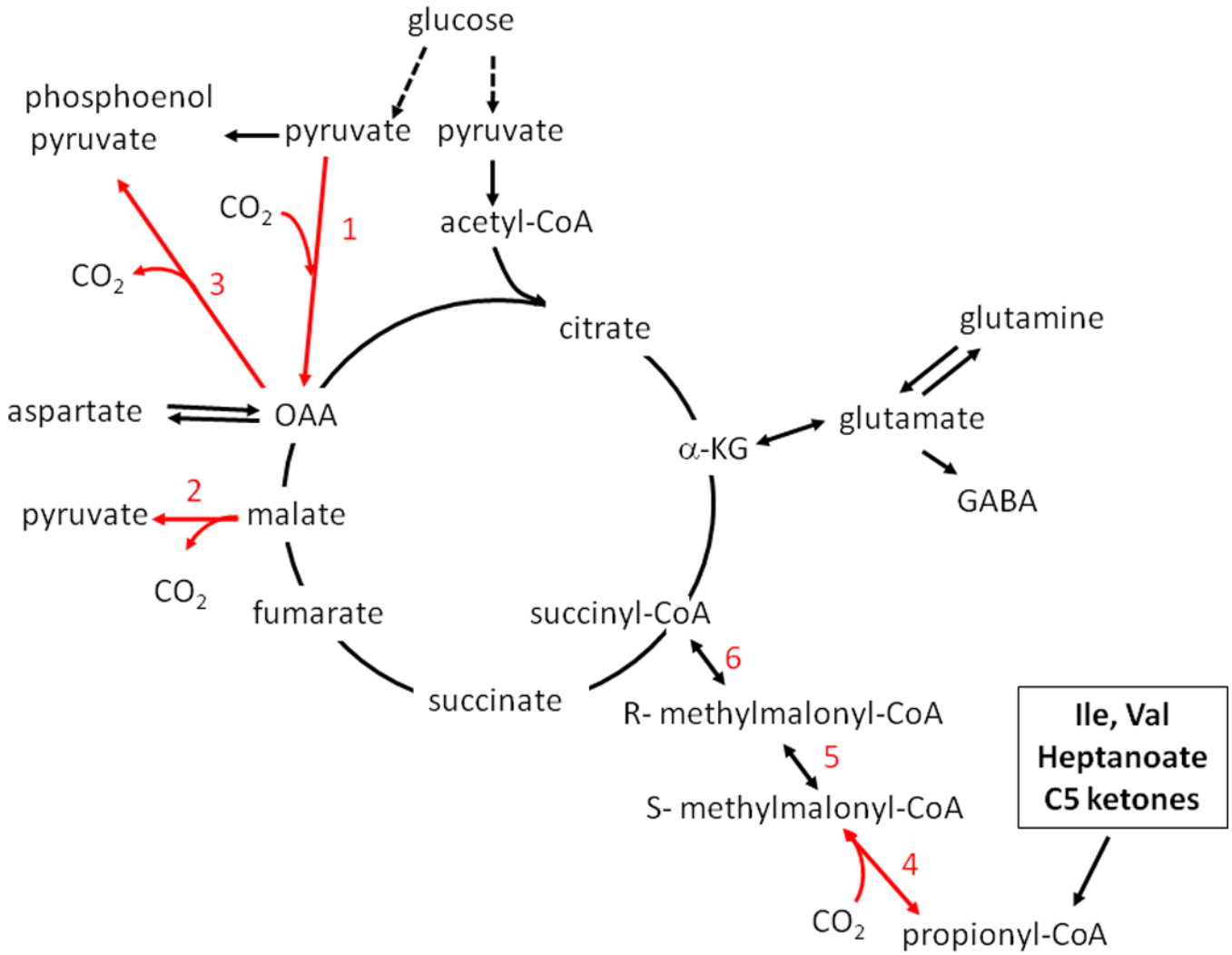

Fig. 1.

Anaplerotic pathways. Enzymes are marked by numbers. In the brain, the main anaplerotic pathway is dependent on pyruvate carboxylase (1). The “reverse” reaction from malate is catalysed by malic enzyme, 2). Phosphoenol-pyruvate carboxykinase (3) is considered to work in the decarboyxlation direction. Anaplerosis via the propionyl-CoA carboxylation pathway using branched chain amino acids (BCAA), heptanoate or the “C5 ketones“β-ketopentanoate and β-hydroxypentanoate involves propionyl-CoA carboxylase (4), methylmalonyl-CoA epimerase (5) and methylmalonyl-CoA mutase (6). Abbreviations: BCAA – branched chain amino acids, α-KG – α-ketoglutarate, OAA- oxaloacetate, Co-A-coenzyme A

Table 1

Comparison of the anticonvulsant properties of triheptanoin in mice compared to other treatments for epilepsy

	Triheptanoin	Ketogenic diet	PHT	VPA	CBZ	LEV
MEST	+ *1*	– *3*	+ *6*	+ *6*	+ *6*	– *11*
PTZ	– *2*	– *3,4*	– *6*	+ *6*	– *6*	– *11*
6Hz	– *1*	+ *3, 4, 5*	+ *7*	+ *7*	+/– *7*	+ *7*
PTZ in PILO-SE rodents	+ *2*	nd	– *8*	– *8*	nd	nd
Corneal kindling:						
A. Delay in kindling progression	+ *2*	nd	nd	nd	nd	+ *12*
B. Protection against fully kindled seizures	(−) *2**	nd	+ *9,10*	+ *9*	+ *9*	+ *9*

Efficacy in mouse seizure models is noted as minus (−) or plus (+) or some (+/−). Exceptions are the PTZ model which was performed in rats for the testing of PHT and VPA.

Abbreviations: KD - ketogenic diet, PHT- phenytoin, VPA-valproate, CBZ- carbamazepine, LEV – levetiracetam.

* note that only one experiment was performed.

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