Combination treatment of epilepsy with ketogenic diet and concurrent pharmacological inhibition of cytochrome P450 2E1

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Abstract

While most epileptic patients respond to treatment with existing antiepileptic drugs, there remains a considerable number of patients in whom these drugs do not suffice. Such patients, particularly children, are often treated using the ketogenic diet. This diet imposes a strict limit on carbohydrates; while providing for adequate protein, most of the calories are supplied as triacylglycerol, much of which is metabolized to ketone bodies.

Animal experiments have provided evidence that the anticonvulsant effect of the ketogenic diet is mediated by acetone and correlates with acetone levels. Acetone can be converted in vivo to glucose via acetol and pyruvate; the initial conversion to acetol is catalyzed by cytochrome P450 2E1 (CYP2E1). When CYP2E1 knockout mice are subjected to starvation to induce ketogenesis, they develop blood acetone levels much higher than those observed in wild-type mice. Similarly, pharmacological inhibition of CYP2E1 significantly increases blood acetone levels in rat and man.

Taken together, these observations suggest that pharmacological inhibition of CYP2E1 has the potential to significantly increase the antiepileptic effect of the ketogenic diet. With patients that respond insufficiently to the diet alone, increased acetone levels may improve response. With patients who respond sufficiently to the diet, CYP2E1 inhibitors might allow relaxation of the fairly severe diet regimen and so improve compliance and quality of life.

An existing inhibitor of CYP2E1 is the drug disulfiram. This drug also inhibits the enzyme aldehyde dehydrogenase, which functions in alcohol degradation, and in this capacity has long been used in the treatment of alcohol addiction. Disulfiram inhibits CYP2E1 at conventional therapeutic dosages and increases blood acetone levels in humans and animals. It should therefore be a viable candidate for the proposed drug/diet combination treatment.

Background

The blanket term “epilepsy” covers a fairly diverse range of conditions. This applies to their clinical manifestations; examples are the all-out grand mal seizures, focal seizures, which may affect only one limb or another, and absences, which do not involve any motor manifestations at all. It also applies to pathogenesis, which ranges from biochemical defects in transmitter synthesis or degradation over channel and receptor mutations to anatomical lesions. Lastly, and accordingly, it also applies to therapy. While drugs such as valproate and carbamazepine are useful in more than one form, no single drug is applicable in all forms and situations. Also, while some forms of epilepsy are more readily controlled by drug therapy than others, it seems that refractory cases occur across most forms.

A common motif in all epilepsies is an abnormally high level of neuronal excitability. In keeping with this observation, many antiepileptic drugs broadly inhibit neuronal activity, and accordingly are also used to lower CNS activity in situations ranging from psychoses to narcosis. Several such drugs, in particular the benzodiazepines and the barbiturates, are agonists of the $GABA_A$ receptor, one of the two major inhibitory ionotropic receptors in the brain. Valproate, apart from inhibiting sodium channels, also inhibits the degradation of GABA [1], and therefore indirectly increases $GABA_A$ receptor activity.

The ketogenic diet in the treatment of refractory epilepsy

The therapeutic effect of the ketogenic diet was discovered empirically, and its use actually predates the introduction of modern antiepileptic drugs [2]. It has now largely been superseded by phenytoin and other anticonvulsant drugs. However, it remains in use in a significant number of patients, particularly in children, that fail to respond to anticonvulsant drug therapy. It is completely or partially effective in some, but not all of those patients; this applies across a range of different forms of epilepsy [3].

In the ketogenic diet, the greater fraction (according to [2], between 75% and 83%) of all calories is supplied as triacylglycerol, while carbohydrates and protein are restricted. Carbohydrates yield glucose via degradation alone or via short adapter pathways, while most of the amino acids produced by protein breakdown can be used for gluconeogenesis. Restricting both carbohydrates and protein causes a shortage of glucose. This is compensated by the breakdown of triacylglycerol to fatty acids, which are then converted to ketone bodies. The term comprises the two organic acids β-hydroxybutyrate and acetoacetate as well as acetone, which forms from acetoacetate through decarboxylation. Ketone bodies can partially replace glucose as a source of energy in most tissues, including the brain.

Role of acetone in the anticonvulsant effect of the ketogenic diet

Several alternative explanations have been proposed for the therapeutic effect of the ketogenic diet. In brain tissue slices, acetoacetate and β-hydroxybutyrate promote opening of K_{ATP} channels [4] and promote the presynaptic accumulation of GABA [5]; both of these effects might reduce neuronal excitability. However, recent experiments with animal models of epilepsy point to acetone as the anticonvulsant mediator [6]. This conclusion is based on the following observations:

1. Injection of acetone alone, in the absence of metabolic ketogenesis, is sufficient to inhibit seizure activity [7]; the same is true of isopropanol, which is converted to acetone in vivo [8]. The range of acetone concentrations required for anticonvulsant activity in animal models overlaps with that observed in patients on the ketogenic diet [9].
2. Like several anticonvulsant drugs, acetone has sedative and narcotic properties at high concentrations [10, 11].
3. Acetoacetate, which is the precursor of acetone, has lower anticonvulsant activity than acetone; β-hydroxybutyrate, which is not converted to acetone, has none [12].
4. Metabolites of acetone (see Figure 1) have either weak anticonvulsant activity or none at all [13]. During ketosis, these metabolites do not occur in high concentrations in the blood [14, 15].
5. Using proton NMR, acetone has been detected as the major ketone body in the brain of epileptic children treated with the ketogenic diet [16].

The current proposal is based on the assumption that acetone mediates the anticonvulsant effect of the ketogenic diet. Animal studies that will be necessary to validate the proposed combination therapy will also help to further support the role of acetone, or refute it. Indeed, the use of CYP2E1 inhibitors has been suggested before as a touchstone to distinguish between acetone itself and its metabolites as the anticonvulsant mediators of the ketogenic diet [17].

Acetone metabolism

While textbooks sometimes describe it as a metabolic dead end of ketogenesis that is destined solely for exhalation and excretion, acetone actually undergoes significant metabolism in vivo. The details of this pathway have been elucidated in rats [18]. The initial hydroxylation of acetone to acetol is carried out by cytochrome P450 type 2E1 (CYP2E1; [19]), which is itself induced by ethanol and acetone [20,

21]. Acetol can then be converted to pyruvate via two alternative pathways (Figure 1). In the liver, pyruvate produced from acetone may contribute significantly to the formation of glucose via gluconeogenesis [14, 22]; pyruvate formed in the brain would likely undergo further degradation by pyruvate dehydrogenase.

The importance of CYP2E1 in acetone metabolism has been confirmed in knockout mice, which, upon fasting-induced ketogenesis, develop plasma acetone levels that are several times higher than those observed in the parental strains [23]. Similar results were observed when CYP2E1 was inhibited with diallyldisulfide [24] or disulfiram [25, 26].

CYP2E1

Like many other cytochrome P450 enzymes, CYP2E1 is strongly expressed in the liver. It is also expressed in some areas of the brain, including the hippocampus. CYP2E1 is the key enzyme in the so-called microsomal ethanol oxidation system, which contributes to the degradation of ethanol via acetaldehyde. CYP2E1 also degrades acetone, and in both the liver and the brain it is transcriptionally induced by both ethanol and acetone [27-31]. In the brain, CYP2E1 participates in dopamine degradation [32], but no major disturbance of dopaminergic function has been reported in CYP2E1 knockout mice.

CYP2E1 is also involved in the metabolism of xenobiotics such as acetaminophen, isoniazid, and aniline, as well as halothane and enflurane [30, 33]. Many of the resulting metabolites induce or promote cancer, and pharmacological inhibition of CYP2E1 has been suggested as a means to obstruct this route to carcinogenesis [34, 35]. Induction of CYP2E1, with consecutively increased production of reactive oxygen species, has also been implicated in alcohol-induced [36] and fat-induced [37] forms of hepatitis.

The structure of CYP2E1, with two different inhibitors bound to it, has been solved [38], which opens the way to the development of specific inhibitors. An inhibitor of CYP2E1 that already is in clinical use is disulfiram. Inhibition of CYP2E1 occurs at dosages that are also used in the treatment of alcoholics; this is evident both from drug interactions and from disulfiram-induced acetonemia [25].

The rationale for supplementing the ketogenic diet with CYP2E1 inhibitors

While the ketogenic diet is effective in many epileptic patients, it still falls short in others; a significant

number of patients respond with only partial reduction of seizures or not at all [3, 39, 40]. Results from animal experiments [7, 12] suggest that consistent anticonvulsant action of acetone may require plasma levels that are not consistently obtained by the diet alone [9]. It seems plausible that insufficiently treated patients should benefit from a further increase of diet-induced plasma acetone levels. Genetic and pharmacological studies [23, 25, 26] suggest that such higher, therapeutically more effective levels should be attainable by the proposed diet-drug combination.

The ketogenic diet, while often effective, is hampered by limited patient compliance, which is due to its impact on the quality of life, as well as medical issues such as growth failure, kidney stones, and osteoporosis [41]. Accordingly, less rigid dietary regimens are being explored [42]. It seems possible that combination with pharmacological inhibition of CYP2E1 would permit a relaxation of dietary regimens, while still maintaining acetone levels in the therapeutic range. Such a regimen could then improve compliance in patients who in principle respond satisfactorily to the diet alone, and potentially be a viable alternative for patients who respond to conventional anticonvulsant drugs but experience serious side effects from such treatment.

A candidate CYP2E1 inhibitor: disulfiram

Among the known inhibitors of CYP2E1, the only one in current clinical use is disulfiram, which is therefore the most obvious candidate for animal and clinical studies. Disulfiram was first characterized as an inhibitor of aldehyde dehydrogenase, and as such has been used for more than fifty years to prevent relapse in alcohol addicts. Subsequently, disulfiram was found to also inhibit dopamine-β-hydroxylase [43], CYP2E1 [44], and proteasomes [45]; novel applications based on these effects have been proposed [46]. The drug is metabolized rapidly, and it is the metabolites that are responsible for the various inhibitory effects. Due to its widespread and longstanding use in the treatment of alcoholics, the pharmacokinetics, pharmacodynamics, side effects, and drug interactions of disulfiram are well-tilled ground [30, 47-49].

Pharmacokinetics, metabolism

After oral uptake, disulfiram undergoes rapid and quantitative uptake and metabolism [48]. After initial reduction of disulfiram to diethyldithiocarbamate (DDTC), the latter undergoes methyl conjugation and repeated oxidation (Figure 2). Oxidation can be carried out by several different cytochrome P450 enzymes; CYP2E1 itself is one of them, but not as efficient as several others [50]. Some of the

metabolites are conjugated with glucuronic acid and excreted; a fraction of the sulfur contained in disulfiram is recovered in the urine as sulfate.

Most studies on disulfiram and its active metabolites concern the liver, because alcohol degradation occurs there. Since the brain lacks the liver's abundance of P450 enzymes, it is not clear how efficient formation of the active metabolites in the brain may be.

Inhibition of aldehyde dehydrogenase (ADH)

Inhibition of ADH by disulfiram is the pharmacological rationale of its use in the treatment of alcohol addicts. Alcohol dehydrogenase converts ethanol to acetaldehyde, which in patients that have taken disulfiram accumulates and causes intense hangover-like symptoms (headaches, nausea, and vomiting). The inhibition of aldehyde dehydrogenase is caused by the disulfiram metabolite S-methyl-N,N-diethylthiolcarbamate sulfoxide (DETC-MeSO; Figure 2). Inhibition is irreversible and involves the formation of an N,N-diethylcarbamoyl adduct of the active site cysteine [51].

Aldehyde dehydrogenase also occurs in one of the two alternative degradation pathways for acetol (Figure 1). The possibility that this would influence the proposed application of disulfiram seems remote but cannot be entirely dismissed (see below).

Inhibition of CYP2E1

The inhibition of cytochrome P450 metabolism by disulfiram was initially noticed through drug interactions [52, 53] and later assigned to CYP2E1 [44]. A recent study identified diethyldithiocarbamate as the inhibitory metabolite; mass spectrometry indicated formation of a mixed disulfide between this metabolite and the apo-enzyme [54]. However, in this study, enzyme activity could not be restored by reductive cleavage of the disulfide, suggesting that the enzyme had incurred additional changes, for example the binding of another metabolite to its prosthetic heme group. Spectral effects suggestive of the latter have been reported [53], although that early study did not differentiate between CYP2E1 and other P450 enzymes. In any event, inhibition of CYP2E1 is irreversible and virtually complete at dosages similar to those used in the conventional treatment of alcoholics [55, 56].

The inhibition of CYP2E1 by disulfiram has been exploited in experimental studies [57], but it appears not to have been used with therapeutic intention. In both humans and animal models, CYP2E1 inhibition by disulfiram amplifies blood acetone levels induced by fasting [26, 58].

Dual effect of disulfiram in acetone degradation

Both CYP2E1 and ADH participate in the degradation of acetone. In this pathway, CYP2E1 precedes ADH (Figure 1), and its inhibition should therefore exercise the dominant effect, raising the concentration of acetone and reducing that of downstream metabolites. As discussed above, inhibition of aldehyde dehydrogenase would only concern one of two alternative pathways downstream of acetol, suggesting that its effect will likely be minor. However, a significant increase of the levels of downstream metabolites, relative to levels attained with the diet alone, cannot be entirely ruled out. It is not clear at present whether this would add to or detract from the intended clinical effect of disulfiram. If ADH inhibition should turn out to produce untoward consequences, it may be necessary to consider other inhibitors of CYP2E1 that do not interfere with ADH.

Toxicity

In keeping with its long-standing use in alcohol addiction, there is a considerable variety of known possible side effects of disulfiram. A survey of all reactions reported in a 20 year period in Denmark [47] found an overall incidence of adverse reactions between 1:200 and 1:2000 per treatment year, and a fatality rate of 1:25,000 per treatment year. The most frequent complication was liver toxicity; this may to some extent have been facilitated by preexisting alcohol-mediated liver damage. Neurological and psychiatric symptoms were the next most common.

Intriguingly, several cases of disulfiram-induced seizures have been reported [59, 60], and apparently in at least some of these, alcohol consumption or withdrawal (which also may trigger seizures) were not involved. A biochemical explanation has not been provided. As stated earlier, disulfiram also inhibits dopamine-β-hydroxylase, the enzyme that converts dopamine to norepinephrine. In animal experiments, disulfiram, as well as genetic knockout of the enzyme, promotes the occurrence of seizures after concurrent application of cocaine [61], a powerful inhibitor of presynaptic reuptake of dopamine and norepinephrine. Whether or not the sporadic cases of disulfiram-induced seizures are in any way related to this experimental observation is unknown.

If the inhibition of dopamine-β-hydroxylase by disulfiram should indeed turn out to counteract its intended therapeutic effect, it may be necessary to develop more specific CYP2E1 inhibitors that do not interfere with dopamine-β-hydroxylase. If interference with dopamine degradation by CYP2E1 itself should present a problem, it may be alleviated by the use of inhibitors that are excluded by the blood

brain barrier.

Interaction of disulfiram with hyperbaric oxygen

In rats exposed to hyperbaric oxygen, disulfiram amplified oxygen-induced lung toxicity, which was ascribed to inhibition of superoxide dismutase [62]. The disulfiram dosages used in these experiments were far higher than those used therapeutically in humans.

Hyperbaric oxygen can also induce seizures, which has been ascribed to inhibited transport and presynaptic depletion of GABA [63]. In keeping with this explanation, such seizures can be inhibited with $GABA_A$ receptor agonists [64]. It is interesting to note that the first metabolite of disulfiram, diethyldithiocarbamate (Figure 2), can also inhibit hyperbaric oxygen-induced seizures [65]. Whether or not this observation is related to the antiepileptic action mechanism of disulfiram proposed here has not been established.

Experimental studies

The proposed therapeutic approach needs to be tested in animal experiments and, if these are successful, in clinical studies.

Animal experiments

These should be performed with established pharmacological mouse or rat models of epilepsy. The following experiments should be performed:

1. Wild type and CYP2E1 knockout mice should be subjected to diet regimens varying in stringency, with or without added disulfiram. Blood levels of acetone and its metabolites should be monitored. Behavioral and neurological performance scores should be recorded, and tolerable diet/disulfiram regimes should be established.
2. The effect of different diet/disulfiram combinations on experimentally induced seizure activity should be determined and compared against diet alone and disulfiram alone. CYP2E1 knockout mice should again be included as controls in order to understand possible contributions from the effects of disulfiram on targets other than CYP2E1.
3. Toxicity: Since disulfiram is approved and widely used for the treatment of alcohol addiction, toxicity studies should not be necessary at this stage.

Clinical studies

Given the longstanding use of disulfiram in its established application, it should be possible to limit toxicity studies and focus them on potential novel complications arising from its combination with ketogenic diet or, potentially, with other antiepileptic drugs.

The effectiveness of the proposed combination treatment needs to be studied in various forms of epilepsy. Initial studies might be conducted with a group of patients who show a partial response to ketogenic diet alone, in whom it could be determined whether or not addition of disulfiram improves clinical response. If results are favorable, the studies could be extended to patients who fail to respond to the diet alone. In addition, a combination of disulfiram with a relaxed ketogenic diet could be tried in patients whose seizures are controlled well by a strict diet alone, but who do not tolerate the diet well due to psychological reasons or medical complications.

In current practice, some patients are treated with both antiepileptic drugs and ketogenic diet at the same time. Interactions between acetone and several antiepileptic drugs have been studied in a mouse model [66]. Acetone increased the antiepileptic activities of some anticonvulsants; the degradation of acetone was accelerated by several drugs as well. Overall, no unmanageable interactions were observed. It should be noted, however, that the concentrations of acetone were not very high; ketogenic diet in conjunction with CYP2E1 inhibition would likely result in higher concentrations, which may lead to more significant interactions. This question would also have to be addressed in clinical studies.

Conclusion

Clinical and experimental data from the literature support the notion that the effectiveness of the ketogenic diet in the control of epileptic seizures could be augmented by inhibitors of CYP2E1. An established and well-known drug with such activity is disulfiram. Animal experiments and, if these are successful, clinical studies to explore the validity of this concept seem justified.

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Conflicts of interest

None.

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Legends for Figures

Figure 1: Metabolism of acetone, according to reference [18]. Step 1 may occur spontaneously, but inducible acetoacetate decarboxylase activity has been reported, though no enzyme has been purified [67, 68]. Steps 2 and 7 are catalyzed by alcohol- and acetone-inducible cytochrome P450 activity, mostly CYP2E1. Step 5 is catalyzed by aldehyde dehydrogenase. The drug disulfiram inhibits both CYP2E1 and aldehyde dehydrogenase.

Figure 2: Structures of disulfiram and two of its metabolites. Diethyldithiocarbamate (DDTC) is formed by glutathione-dependent reduction and inhibits CYP2E1 [54]. The second metabolite, S-ethyl-N,N-diethylthiolcarbamate sulfoxide (DETC-MeSO), inhibits aldehyde dehydrogenase; several cytochrome P450 enzymes participate in its formation from DDTC [50].

Figure 1

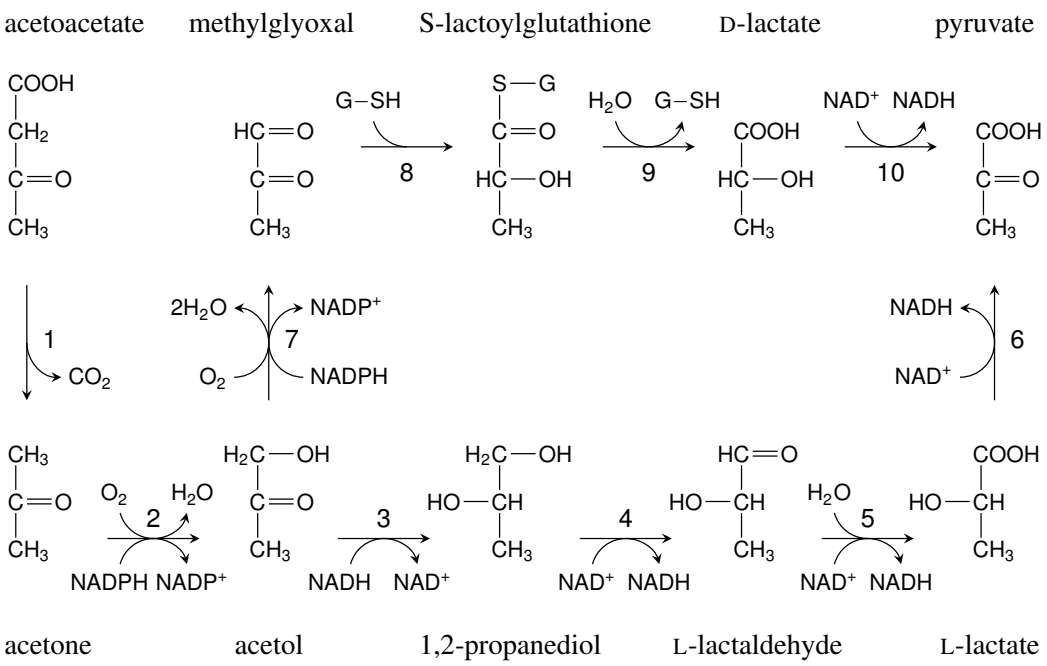
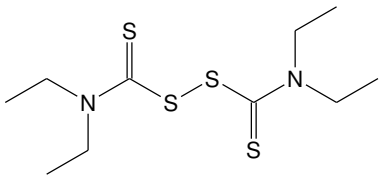
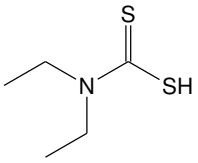

acetoacetate
methylglyoxal
S-lactoylglutathione
D-lactate
pyruvate
G–SH
8
H2O G–SH
9
NAD+ NADH
10
1
CO2
2H2O
NADP+
7
O2
NADPH
NADH
NAD+
6
acetone
acetol
1,2-propanediol
L-lactaldehyde
L-lactate
O2 H2O
2
NADPH NADP+
3
NADH NAD+
4
NAD+ NADH
H2O
5
NAD+ NADH

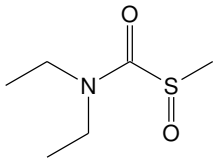
Figure 2



disulfiram



diethyldithiocarbamate



S-methyl-N,N-diethylthiol-carbamate sulfoxide