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Acute anticonvulsant effects of capric acid in seizure tests in mice

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ABSTRACT

Capric acid (CA10) is a 10-carbon medium-chain fatty acid abundant in the medium-chain triglyceride ketogenic diet (MCT KD). The purpose of this study was to characterize acute anticonvulsant effects of CA10 across several seizure tests in mice. Anticonvulsant effects of orally (p.o.) administered CA10 were assessed in the maximal electroshock seizure threshold (MEST), 6-Hz seizure threshold, and intravenous pentylenetetrazole (i.v. PTZ) seizure tests in mice. Acute effects of CA10 on motor coordination were assessed in the grip and chimney tests. Plasma and brain concentrations of CA10 were measured. Co-administration studies with CA10 and another abundant medium-chain fatty acid, caprylic acid (CA8) were performed. CA10 showed significant and dose-dependent anticonvulsant properties by increasing seizure thresholds in the 6-Hz and MEST seizure tests; it was ineffective in the i.v. PTZ seizure test. At higher doses than those effective in the 6-Hz and MEST seizure tests, CA10 impaired motor performance in the grip and chimney tests. An enhanced anticonvulsant response in the 6-Hz seizure test was produced when CA8 and CA10 were co-administered. An acute p.o. administration of CA10 resulted in dose-proportional increases in its plasma and brain concentrations. CA10 exerted acute anticonvulsant effects at doses that produce plasma exposures comparable to those reported in epileptic patients on the MCT KD. An enhanced anticonvulsant effect is observed when CA10 and the other main constituent of the MCT KD, CA8, were co-administered. Thus, acute anticonvulsant properties of CA10 and CA8 may influence the overall clinical efficacy of the MCT KD.

1. Introduction

Clinical efficacy of high fat and low carbohydrate/protein ketogenic diets (KDs) in suppressing seizures in epileptic patients has long been recognized and, in recent years, their use has been on the rise in patients suffering from seizures and epilepsy syndromes uncontrollable by antiepileptic drugs (AEDs) (Caraballo and Vining, 2012; Stafstrom and Rho, 2012). That KDs are effective when AEDs fail suggests that these two treatment modalities affect seizures and related events through different mechanisms. While the pharmacological actions of AEDs are relatively well understood and can be ascribed to their interactions with a specific receptor or multiple receptors in the CNS (Porter et al., 2012), the clinical efficacy of KDs cannot be similarly explained and multiple mechanisms of action have been proposed and continue to emerge (Danial et al., 2013; Masino and Rho, 2012).

There are two types of KDs in current clinical use: the classic KD (Wilder, 1921) and the medium-chain-triglyceride (MCT) KD (Huttenlocher et al., 1971). Both diets have similar proportions of the main dietary constituents; however, they differ in the type of fatty acids contained in the triacylglycerol component of the diet. The classic Wilder's KD contains long-chain fatty acids (i.e. those with aliphatic tail of more than 12 carbons) while the MCT KD contains medium-chain fatty acids of 6–12 carbons in length with the 8-carbon caprylic acid (CA8) being the most abundant (50–75% content), followed by the 10-carbon capric acid (CA10, 23–45% content), and with minimal amounts of the 6-carbon caproic (1–3% content) and 12-carbon lauric (1–5%) acids (Bach and Babayan, 1982; Traul et al., 2000). Although refractory epilepsies have been shown to benefit from both KDs, the classic KD is favored by the

Abbreviations: AED, antiepileptic drug; ANOVA, analysis of variance; CA10, capric acid; CA8, caprylic acid; CL, confidence limit; CNS, central nervous system; CS_{50}, the current intensity required to induce seizure response in 50% of mice; ERK, extracellular signal-regulated kinase; KD, ketogenic diet; MAPK, p38 mitogen-activated kinase; MCT, medium-chain triglyceride; MEST, maximal electroshock seizure threshold; PTZ, pentylenetetrazole; SEM, standard error of the mean; TD_{50}, the dose predicted to produce motor impairment in 50% of mice.

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clinicians due to its apparent better safety profile, particularly in adult patients (Freeman et al., 2007; Hartman and Vining, 2007; Kossoff and Rho, 2009; Liu and Wang, 2013; Miranda et al., 2012; Neal et al., 2009; Than et al., 2005).

In comparison to long-chain fatty acids in the classic KD, medium-chain fatty acids in the MCT KD may offer some metabolic advantages such as faster absorption from the gastrointestinal system into the portal circulation and faster oxidation in the liver that is less influenced by the need of concurrent decrease in carbohydrate supply (Bach and Babayan, 1982; Henderson, 2008; Johnson et al., 1990; McGarry and Foster, 1971). This facilitated biochemical availability of medium-chain fatty acids results in faster metabolic energy expenditure, faster production of ketone bodies and perhaps initiation of changes (metabolic and non-metabolic) relevant to the anticonvulsant effect of the KDs (Bach and Babayan, 1982; Papamandjaris et al., 1998). In contrast to long-chain fatty acids, chemical properties of medium-chain fatty acids (i.e., solubility in aqueous media, presence in an unbound fraction in the blood, affinity to carrier-mediated transporters) allows them to cross the blood–brain barrier and exert direct pharmacological effects in the CNS (Dahl et al., 1956; Ebert et al., 2003; Edmond et al., 1998; Johnson et al., 1990; Mitkov and Toreva, 1979; Rapoport, 2001; Spector, 1988; Walker et al., 1970). Indeed, there is evidence that certain medium-chain fatty acids may have acute anticonvulsant properties in seizure tests in rodents (Chang et al., 2013; Liu and Pollack, 1994; Perlman and Goldstein, 1984; Wlaz et al., 2012). For example, the main constituent of the MCT KD, CA8, was anticonvulsant across several seizure tests that are routinely used in screening for potential AEDs (Giardina and Gasior, 2009; Smith et al., 2007; Wlaz and Löscher, 1998). Increased plasma levels of medium-chain fatty acids have been reported in epileptic patients treated with the MCT KD (Haidukewych et al., 1982; Sills et al., 1986a, 1986b).

Thus, the present study aimed to further characterize potential acute anticonvulsant effects of MCT's fatty acids by testing the second most abundant fatty acid in the MCT KD, CA10. To allow for direct comparisons, CA10 was tested in a comparable fashion to previous CA8 testing in our lab (Wlaz et al., 2012). Additionally anticonvulsant effects of a combined administration of CA10 and CA8 were evaluated. The findings of this study further support that the MCT KD may provide an acute anticonvulsant effect through the direct action of its main constituents, CA8 and CA10, in addition to the anticonvulsant effects produced by a metabolic conversion of MCT to ketone bodies and/or other physiological/biochemical changes.

2. Material and methods

2.1. Animals

Experimentally naïve male albino Swiss mice weighing 25–30 g (Laboratory Animals Breeding, Słaboszów, Poland) were housed up to 10 per cage under controlled laboratory conditions (ambient temperature, 22–23 °C, relative humidity, 45–55%, 12 h light/dark cycle, light on at 6:00 a.m.). Chow pellets (Murigran, Agropol S.J., Motycz, Poland) and tap water were continuously available. The animals were allowed to acclimatize to the vivarium for at least 3 days before they were used for experiments. The experiments were performed during the light phase of the light/dark cycle between 9:00 and 14:00 h and after a minimum 30-min period of acclimation to the experimental room. All experimental procedures were unblinded.

The experimental protocol was approved by the Local Ethics Committee at the Medical University of Lublin (licenses numbers 55/2009 and 25/2010), and all experimental procedures were performed in strict compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drugs

Capric acid (CA10; MW = 172.26 g; also called decanoic acid), caprylic acid (CA8; MW = 144.21 g; also called octanoic acid), and pentylenetetrazole (PTZ) were used in the present study (Sigma-Aldrich, Poznań, Poland). Both CA10 and CA8 were suspended in a 0.5% aqueous solution of methyl cellulose (Sigma-Aldrich) and were administered orally (p.o.) by gastric gavage in a volume of 10 ml/kg. The p.o. route of administration for CA10 and CA8 was selected to model oral administration of the MCT diet in epileptic patients. PTZ was dissolved in saline and administered intravenously (i.v.). The pretreatment time for CA10 (30 min) was selected to correspond to its maximal anticonvulsant efficacy as established in a series of pilot experiments. Brain tissue levels of CA10 at different pretreatment times were also determined (see below). Doses of CA10 and CA8 were expressed in mmol/kg.

CA10 was tested in seizure tests under conditions that allowed detection of anticonvulsant as well as pro-convulsant effects of CA10 (Gasior et al., 2012; Giardina and Gasior, 2009), as described in detail below.

2.3. Intravenous PTZ seizure threshold test

I.v. PTZ seizure threshold assessment was performed using parameters (i.e., PTZ concentration, 10 mg/ml; infusion rate, 0.2 ml/min) as validated before (Gasior et al., 2012; Wlaz et al., 2012). Specifically, three sequentially-occurring behavioral endpoints of seizure activity were used to determine the threshold for seizure induction: (1) first myoclonic twitch (rapid upward flick of rigid tail), (2) clonus (repeated jerking movements of all four limbs lasting at least 5 s) with loss of the righting reflex (clonic convulsions, or clonus), and (3) tonic forelimb extension (tonic convulsions, or tonus). The time between the start of the infusion and the onset of each of these endpoints was recorded and used to calculate seizure thresholds for each endpoint separately. Seizure thresholds were calculated using the following formula: threshold dose of PTZ [mg/kg] = (PTZ concentration [mg/ml] × infusion rate [ml/s] × infusion duration [s] × 1000)/body weight [g] and were expressed as the dose of PTZ (in mg/kg) needed to produce a given endpoint as noted above. The infusion was stopped at the beginning of the tonic convulsions, which were usually lethal for the mice. All surviving animals were euthanized immediately after the end of the infusion.

2.4. Six-hertz (6-Hz) seizures

Psychomotor seizures (6-Hz seizures) were induced by applying a square-wave alternating current (frequency, 6 Hz; duration, 3 s) via corneal electrodes delivered from a Grass S48 stimulator coupled with a constant current unit CCU1 (both from Grass Technologies, West Warwick, RI, U.S.A.) as described elsewhere (Gasior et al., 2012; Wlaz et al., 2012). Convulsions in this test were characterized by a stunned (fixed) posture, which was often followed by rearing, forelimb clonus, and twitching (Barton et al., 2001; Giardina and Gasior, 2009). At the end of the convulsions, animals resumed normal exploratory behavior. Control animals always exhibited more than 10 s of abnormal seizure behavior (ie, stunned posture, often followed by rearing, forelimb clonus, and twitching) whereas treated animals were considered to be protected if the abnormal seizure behavior was less than 10 s in duration. Seizure threshold in this test was assessed by recording convulsions after different current intensities were applied to groups of 19–20 mice treated with different doses of CA10 according to an 'up-and-down' method (Giardina and Gasior, 2009; Kimball et al., 1957). Each mouse was stimulated only once at any given current intensity and the presence or absence of seizure activity was judged as described above. If the mouse responded with convulsions (as described above), the next mouse was stimulated with a current of an intensity 0.06-log step lower than the previous one. If, however, the mouse did not respond with convulsions, the next mouse was stimulated with a current