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Research Report

Anticonvulsant properties of an oral ketone ester in a pentylenetetrazole-model of seizure

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ABSTRACT

The ketogenic diet is known to have an anti-epileptic effect; in fact it is currently used to treat drug resistant epilepsies. The efficacy of this diet is thought to be correlated to the elevation of blood ketone bodies. Because of problems with compliance to this diet, there is an interest in evaluating alternative pharmacological treatments that can have anti-seizure effects by elevating ketone bodies. In the present experiment, an orally administered synthetic ketone ester (R,S – 1,3-butanediol acetoacetate diester, or BD-AcAc2) was evaluated for its anti-seizure efficacy in a rat model. The threshold for seizure induction with progressive intravenous infusion of pentylenetrazole (PTZ) was evaluated in anesthetized Wistar rats two hours after a single 1 ml intragastric administration of BD-AcAc2 (i.e. 4 g/kg b.w., treated group) or water (control group). After correction for the dose of anesthetic, the results showed that the administration of BD-AcAc2 induced an elevation of the PTZ threshold (140±11 mg/kg for the treated group, 122±6 mg/kg for the control group), along with an increased level of blood β-hydroxybutyrate (2.7±0.3 mM for the treated group, 1.4±0.1 mM for the control group). This result suggests that ketone esters may pave the road towards the establishment of a "ketogenic diet in a pill".

Abbreviations: ANCOVA, analysis of covariance; KD, ketogenic diet; BD-AcAc2, 1,3-butanediol acetoacetate diester; CNS-OT, central nervous system oxygen toxicity; ATA, atmospheres absolute; PTZ, pentylenetetrazole; βHB, β-hydroxybutyrate; cEEG, cortical-electroencephalogram; AcAc, acetoacetate; ROS, reactive oxygen species; RNS, reactive nitrogen species

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1. Introduction

The ketogenic diet (KD) is a high fat (about 90% of total dietary kcal ingested), adequate protein, and low-carbohydrate diet that is rigidly calculated and tailored to the needs of each patient. It is primarily used for the treatment of drug-resistant epilepsies in childhood and is often only implemented as a last resort. Currently, the diet is constrained to some specific foods; this leads to problems with compliance, as a consequence of unpleasant palatability.

Recently, a number of authors have proposed new formulations of KD in "tablets", using synthetic compounds to induce the same pharmacological effects of a KD, without following its dietary restrictions (Rho and Sankar, 2008). It is widely believed that the anti-seizure effects associated with the KD are principally produced as a consequence of the metabolism of ketone bodies (notably β-hydroxybutyrate, acetoacetate and acetone). A direct anticonvulsant action has been recognized in particular for the administration of acetoacetate and acetone, as reported in some experimental models (Bough and Rho, 2007; Masino and Rho, 2012).

Recently, D'Agostino et al. (2013) have employed a synthetic precursor of ketone bodies in the form of a ketone diester, specifically 1,3-butanediol acetoacetate diester (BD-AcAc2), to prevent the grand mal-like seizures induced by central nervous system oxygen toxicity (CNS-OT). These authors demonstrated a significant increase in the seizurogenic threshold of 574±116% in animals treated with BD-AcAc2 compared to water-treated controls.

In order to further evaluate the anti-seizure properties of orally administered ketone bodies, in the present study we tested BD-AcAc2 in an adult Wistar rat model of seizures induced by progressive e.v. injection of pentylenetetrazole (PTZ).

2. Results

2.1. General experimental variables

Table 1 shows the mean values of several variables recorded for each experimental group. Body weight, β-ketonemia, blood glucose, urethane dose and colonic temperature can all have an effect on the seizure threshold, but all these variables did not differ significantly between the two experimental groups, allowing the comparison of the results between the two groups and supporting the conclusion about the effect of the treatment. As expected, the BD-AcAc2-treated group had significantly higher blood levels of β-hydroxybutyrate (βHB) compared to the control group at the time of PTZ infusion start ($P<0.05$). It can be noted that βHB was already high in both groups before the gavage treatment (normal values are expected to be lower than 1.0 mM); this finding was likely due to over-night fasting, which was necessary in the present model to assure an empty stomach at the time of the gavage and to have a reproducible time course of the effect. In addition, in the water-gavaged group, βHB at the time of starting the PTZ infusion was lower than before the gavage; this is a broadly discussed effect of urethane anesthesia, which also explains the increase in blood glucose levels observed at this time in both groups (Sánchez-Pozo et al., 1988).

2.2. Seizure PTZ-threshold.

A positive and significant correlation was observed between the dose of urethane injected (g/kg b.w.) and the PTZ-threshold (mg/kg b.w.); the slope of the linear regression between these two variables did not differ between the two experimental groups. These findings made it mandatory and allowed to consider the dose of urethane as a covariate and to perform an analysis of covariance (ANCOVA) to correct the PTZ-threshold for the dose of urethane. No other significant correlations were observed between the PTZ-threshold and the other experimental variables of Table 1. The mean values of the observed PTZ-thresholds were higher in the treated group compared to the control group (Table 1); the ANCOVA demonstrated a significant effect for the treatment taking the urethane dose as covariate ($F_{1,13}=5.62$, $P<0.05$).

3. Discussion

The main results from the present study showed that the oral administration of BD-AcAc2 in Wistar rats leads to a significant increase in blood βHB and an increased latency to

Table 1 – Main general variables. BD-AcAc$_2$: group treated by gavage with oral ketone ester before seizure induction. Water: group treated by gavage with water before seizure induction. The asterisk indicates statistical significant difference between the two groups (*$P<0.05$). The PTZ threshold was corrected for the dose of urethane considered as a covariate; the statistical comparison between the two groups for this variable was done by ANCOVA.

Treatment by gavage	BD-AcAc$_2$	Water
Weight	248±6	236±4
β-ketonemia before gavage (mM)	2.1±0.2	2.3±0.2
Blood glucose before gavage (mg/dL)	95±12	95±17
Urethane dose (g/kg)	1.9±0.2	1.7±0.2
Colonic temperature 2 h after gavage (°C)	36.4±0.1	36.3±0.3
β-ketonemia 2 h after gavage (mM)	2.7±0.3	1.4±0.1*
Blood glucose 2 h after gavage (mg/dL)	132±19	166±40
PTZ threshold (mg/kg)	147±18	121±15
PTZ threshold corrected by urethane dose as covariate (mg/kg)	140±11	122±6*