Neuroprotective potential of chronic rapeseed oil diet evaluated by audiogenic seizures test in magnesium-deficient mice.

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Abstract

The magnesium deficiency-dependent audiogenic seizures (MDDAS) test has been validated in adult mice fed for 25-32 days a synthetic 50 ppm Mg-containing diet, rich in omega6 fatty acids, brought by sunflower:corn (1:3) oils. In the present paper, we compared two groups of mice fed either the reference magnesium-deficient diet or a similar diet containing as exclusive lipid source the highly monounsaturated rapeseed oil rich in alphalinolenic acid. This omega 3 rich oil did not change the body weight gain or the magnesium concentration as compared to the omega 6 diet. In contrast, it did not induce the motor hyperactivity observed in the group fed sunflower:corn diet and significantly protected the mice both from audiogenic seizures (50% instead of 100%) and from death since all the convulsive mice survived in the omega 3 group as compared to 50% in the omega 6 group. The MDDAS pattern revealed an increase in the durations of the two first phases (latency and wild running) in the omega 3 group, suggesting that the neuroprotective effect would be mediated mainly through the Na^+ voltage-gated channels. The seizure durations were unchanged whereas the recovery duration tended to decrease suggesting a possible slight antioxidant/anti-inflammatory potential of the rapeseed diet.

Key words: Rapeseed Oil, sunflower:corn oil, omega 6, omega 3, alphalinolenic acids, audiogenic seizure test, magnesium deficit

Introduction

Long-chain polyunsaturated fatty acids (PUFA) are essential components of the central nervous system and are brought in the form of their short chain precursors. In the last decade, many *in vivo* and *in vitro* studies have emphasized the beneficial effect of PUFA, notably omega 3, on cardiac and neuronal excitability. These data suggest that omega 3 supplementation may be of clinical relevance in the prevention of both cardiovascular and brain dysfunctions including epileptic seizures (Lauritzen et al., 2000).

Among omega 3, alphalinolenic acid [18:3n-3 or ALA] which is known to protect against both arythmia and ischemia, represents 9% of the highly monounsaturated (60%) rapeseed oil whereas ALA is absent in omega 6 rich sunflower or corn oils.

The audiogenic seizure test (MDDAS) test has been validated previously (Bac et al., 1998; Maurois et al., 2001) in adult magnesium-deficient mice individually exposed to a calibrated audiogenic stimulus (100dBA, 10kHz, 15 sec). It is characterized by 4 successive phases: wild running latency, wild running, tonic seizure and recovery (or death), the comparative duration of which may be indicative of underlying mechanisms exhibited by the tested compounds. This seizure test is discriminatory, distinguishing between phenytoinergic, GABAergic and ethosuccimide compounds. It is also suitable for evaluation of neuroprotective compounds, namely those presenting antioxidant and/or anti-inflammatory properties (Bac et al., 1998; Maurois et al., 2001).

The aim of the present study was to compare the neuroprotective potential of two magnesium-deficient diets containing either rapeseed or sunflower:corn oils at the same concentration in the MDDAS test.

Materials and Methods

The investigation conforms to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH, No 85-23, revised 1996).

Animals: Female Swiss OF1 mice (n= 16), 6-weeks old, weighting 20-22g, were purchased from Janvier (Le Genest-St-Isle, France) and were assigned at random to two magnesium-deficient diets (50 ± 5 mg/kg) prepared as previously described (Maurois et al., 1989) containing 5% vegetable oils, either omega 6 (sunflower:corn 1:3) or omega 3 (rapeseed), for 25-32 days. They were placed eight per cage and maintained on a 12:12h light-dark schedule at 21 ± 1°C. They had free access to food and to distilled water which avoids additional magnesium input. In current practice, in order to avoid food oxidation, fresh food was lyophylised and frozen at -20°C. It was given to mice every day in sufficient amount. At the end of the deprivation period, the body weight, serum magnesium concentration, locomotor activity were measured in mice and the MDDAS test was performed.

Plasma magnesium measurement: Magnesium concentrations were determined by atomic spectrophotometry in plasma

and expressed in mg/mL according to Rousselet & Durlach (1971).

Locomotor activity: Mice were transferred individually in an Apelex type 01-1668B actimeter (Bagneux, France). They were allowed to explore for a 2 min period. Their spontaneous activity was measured for another 3 minutes by the crossing of the photocell activity meter and automatically recorded. The experiment was carried out in a sound proof room between 9:00 a.m. and 13:00 p.m. to reduce the confounding influence of diurnal variation in motility.

Magnesium-deficiency dependent audiogenic seizures (MDDAS) test: Individual animals were placed in a 9 dm^3-volume test chamber (30, 20 and 15 cm for length, width and height, respectively) and exposed for 15 sec to an acoustic signal of 10 ± 0.1 kHz frequency and 100 ± 1 dBA intensity. This acoustic stimulus signal was produced by a signal generator and projected via a high frequency speaker mounted on the roof of the chamber. The noise level was measured close to the animal's ear by an external decibel-meter probe. Each animal was subjected to a single audiogenic stimulation. The test measured the capacity of a test compound to provide complete protection against threshold seizures induced by 100 dBA and the audiogenic pattern may indicate the underlying mechanisms exhibited by tested compounds. Audiogenic seizures were videotaped and the duration in seconds of each phase was recorded.

Statistical analysis: Results were expressed as mean ± S.E.M. and analysed by Student's t-test.

Results

At the end of the deprivation period, the plasma level in magnesium-deficient mice was 75% decreased as compared with the initial magnesium levels in both groups (5.70 ± 0.51 and 5.55 ± 0.43 in the omega 6 and omega 3 fed groups respectively *vs* 21.52 ± 1.26 mg/L). The body weight gain was similar in both groups. In contrast, the individual spontaneous locomotor activity, measured for 3 min (Apelex actimeter), showed that magnesium deficiency induced hyperexcitability in the omega 6 group but not in the omega 3 rapeseed group (152.7 ± 37.9 *vs* 97.0 ± 22.5).

In the MDDAS test, the number of convulsive mice was significantly lower in the omega 3 rapeseed group (50%) as compared to the omega 6 sunflower:corn group (100%). In addition, all the mice convulsing in the rapeseed group recovered whereas 50% died in the omega 6 group. The pattern of seizures was also different. The time periods of the first two phases increased significantly ($p<0.05$) in the rapeseed group: latency and wild running durations were 6.7 ± 5.5 and 3.7 ± 0.5 sec instead of 4.0 ± 1.4 and 2.3 ± 0.4 sec respectively in the omega 6 group, while tonic seizures and recovery durations remained similar in the two groups. However the recovery phase showed a non significant tendency to decrease.

Table I : Comparison of the two magnesium-deficient diets on the pattern of MDDAS test

Diets	n	Convulsing mice (%)	Latency (sec)	Wild running (sec)	Tonic seizures (sec)	Recovery (sec)
Rapeseed	8	50	6.7 ± 5.5*	3.7 ± 0.5*	1.7 ± 0.4	46.5 ± 6.7
Sunflower:corn	8	100	4.0 ± 1.4	2.3 ± 0.4	1.8 ± 0.5	43.3 ± 4.1

*significant at $p<0.05$

Discussion

The serum magnesium decrease and the body weight gain were similar in both groups of mice indicating that the two parameters were not dependent on the lipid composition of the diet, in our experimental conditions. In contrast, locomotor activity measurements showed that the omega 6 diet induced hyperexcitability whereas the omega 3 diet did not. Hyperexcitability in magnesium-deficient mice fed the sunflower:corn diet has already been reported (Durlach et al., 2000) whereas the results obtained in magnesium deficient mice fed rapeseed oil have never been described until now. Consequently, it seems that the rapeseed oil exerts a significant neuroprotective effect in mice, which has been also observed in the MDDAS test. Indeed, the rapeseed diet decreased the percentage of convulsive mice and, among them, the number of fatal issues. This result could be ascribed to the high percentage of alphalinolenic acid present in the rapeseed oil, which was shown to prevent neuronal death and brain dysfunctions including epileptic seizures *in vitro* and *in vivo*, in animals treated with kainate or after hippocampal lesions (Vreugdenhil et al., 1996; Xiao & Li, 1999; Lauritzen et al., 2000). In a model of focal brain ischemia in mice, alphalinolenic acid was also shown to confer in addition to neuronal protection, and in agreement with our findings, a long term survival (Heurteaux et al., 2006).

The audiogenic pattern brings additional informations. It appeared clearly that the rapeseed diet increased the first two phase durations as compared to mice fed the sunflower:corn diet. This means that the rapeseed diet increased the seizure threshold in magnesium-deficient mice, as do the phenitoinergic compounds commonly used in the treatment of epilepsy (Bac et al., 1998; Maurois et al., 2001). This effect indicates that the neuroprotective effect of the rapeseed diet would be mainly mediated by Na^+ voltage dependant channels. It can be ascribed to alphalinolenic acid itself but it may be also assumed that the chronic dietary supply of the short-chain essential precursor, alphalinolenic acid, present in the rapeseed oil could lead to a high level of docosohexaenoic acid (DHA), a major compound of the membrane phospholipids of neural tissues (Marszalek JR, et al., 2005). The potent hyperexcitability inhibition observed also by Lauritzen et al. (2000) after injection of ALA has been at least partly ascribed to a decrease in synaptic glutamate transmission, involving the partial inhibition of voltage-sensitive Na^+ and Ca^{2+} channels (Linden & Routtenberg, 1989) and the opening of background K^+ channels which are activated by polyunsaturated fatty acids such as docosahexaenoic and alphalinolenic acid (TREK-1 and TRAAK) (Fink et al., 1998). These channels are abundant in the brain where they are located both pre- and post-synaptically, and are insensitive to saturated fatty acids, which offer no neuroprotection (Lauritzen et al., 2000). K^+ channels activated by polyunsaturated fatty acids might be important in the blockade of glutamatergic transmission by polyunsaturated fatty acids, resulting in a potent

neuroprotective effect in the MDDAS test, since hyperfunction of the glutamatergic system is involved in seizure induction (Bac et al., 1998; Lauritzen et al., 2000).

Finally we paid attention to the slight decrease in the recovery phase duration during with the rapeseed oil diet. This tendency must be confirmed on a greater number of mice, since it may be associated to an anti-inflammatory/antioxidant properties of the rapeseed oil. Indeed, whereas the omega 6 sunflower/corn diet provide linoleic acid which is the precursor of arachidonic acid implicated in pro-inflammatory processes, the omega 3 rapeseed diet provide alphalinolenic acid precursor of DHA fatty acid which are involved in the anti-inflammatory processes (Calder, 2006).

Conclusion

Chronic consumption of rapeseed oil, an alphalinolenic acid rich monounsaturated oil, could help to prevent or reduce neuronal disorders presenting signs or mechanisms observed in magnesium deficient mice, an animal model of audiogenic seizures. The study of this interesting neuroprotective effect of the rapeseed oil, acting as most of the antiepileptic drugs on Na^+ channels, is still in progress and may be of clinical relevance.

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