Is chronic rapeseed oil diet more neuroprotective than chronic corn/sunflower diet?

Evaluation by audiogenic seizure test in magnesium-deficient mice (MDDAS)

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Abstract: *Polyunsaturated fatty acids (PUFA) and specifically omega3 have been shown to exert a potent protecting effect on both cardiac and neuronal functions. Rapeseed oil contains 9% of alphalinolenic acid (18-3n-3, ALA), whereas corn and sunflower oils (18:2n-6, linoleic acid rich) do not. The aim of the present study was to compare in mice the putative protective effects of ALA, by testing two chronic diets containing either rapeseed oil (ALA rich) or a corn/sunflower blend (devoided of ALA) using an epilepsy model, allowing the detection of neurotoxic or neuroprotective activities: the MDDAS test (Magnesium Deficiency-Dependent Audiogenic Seizure test). After a 30 day-Mg-deprivation period, neuronal hyperexcitability appeared only in the corn/sunflower fed group, suggesting a protecting effect of the rapeseed oil. The number of convulsive mice was twice reduced in the rapeseed group and all of them recovered whereas in the corn/sunflower group all the mice had seizures and 43% died. The pattern of seizures with the rapeseed diet showed an increase in the first two step durations (latency and wild running), and a non significant slight decrease in the third (convulsions) and the fourth (recovery) ones. These results suggest a GABAergic-like effect. The increases in the first 2 phases were also indicative of a likely effect on Na^+ channels, which was also observed using the maximum electroshock seizure test. These preliminary results indicate that adapted chronic dietary intake of rapeseed oil, an ALA rich monounsaturated oil, could help to control neuronal disorders as here shown in our model of magnesium-deficient mice.*

Key words: *epilepsy, magnesium deficiency omega-3, alphalinolenic acid, rapeseed oil*

Introduction

In the last decade, different *in vivo* and *in vitro* studies have demonstrated the beneficial effect of polyunsaturated fatty acids (PUFA) and specifically omega3 on cardiac and neuronal excitability. This protective effect may be of clinical relevance in the prevention of both cardiovascular and brain dysfunctions including epileptic seizures [1, 2].

Among omega3, alphalinolenic acid (18:3n-3, ALA) was shown to be protective against both arrythmia and ischemia [3-6]. ALA is found in vegetable oils like rapeseed, soya, nuts, or linseed and represents 9% of the highly monounsaturated (60%) rapeseed oil whereas it is absent in polyunsaturated/omega6 rich sunflower or corn oils.

The aim of the present paper was to study whether dietary rapeseed oil could be of interest against audiogenic seizures in magnesium-deficient mice (MDDAS test: Magnesium Deficiency-Dependent Audiogenic Seizure test). This test is a pluripotent model of epilepsy also allowing the detection of neurotoxic or neuroprotective activities. The MDDAS test has been validated previously [7] in adult magnesium-deficient mice individually exposed to a calibrated audiogenic stimulus (100 dBA, 10 kHz, 15 sec). It is characterized by 4 successive steps: Latency, Wild Running, Convulsions (Tonic Seizure) and Recovery, the duration of which is recorded as a mechanistic approach. Neurotoxic treatments decrease the 1st and 2nd phases and increase the 3rd and 4th phases. Neuroprotective treatments using various anti epileptic drugs showed different patterns according to their main mechanism of action, either phenitoinergic or GABAergic, antioxidative or anti-inflammatory [7].

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). Female Swiss OF1 mice, were purchased from Janvier (Le Genest-St-Isle, France) and divided into two groups (n = 14). Each group was fed for 30 days, Mg-deficient diets (50 ± 5 mg/kg) prepared as previously described [8], containing 5% vegetable oils, either rich in ALA (rapeseed) or poor in ALA (corn/sunflower 3:1). They were placed five per cage and maintained on a 12h light-dark cycle at 21 ± 1° C. They had free access to food and, in order to avoid an additional input, distilled water.

At the end of the deprivation period, the body weight gain was measured. The mice were afterwards transferred individually in a plexiglass cage in an Apelex type 01-1668B actimeter (Bagneux, France) and allowed to explore for a 3 minute period. Their locomotor activity was measured by the crossing of the photocell activity meter and automatically recorded. The experiment was carried out in a sound proof room between 9: 00 and 13: 00 to reduce the confounding influence of diurnal variation in motility.

Maximun ElecroShock test (MES) was induced via a pair of auricular clip electrodes by means of an electroshock stimulator (Karl Kolbe, Scientific Technical Supplies, Frankfurt, Germany). MES test measured the capacity of a test compound to provide complete protection against threshold seizures (tonic hindlimb extension in 100% of mice followed by clonic seizures) induced by 4 mA, 0.2 sec duration, 50 Hz, sinewave form. It allows the detection of drugs acting on Na^+ voltage-dependent channels. For the 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 stimula-

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tion. Audiogenic seizures were videotaped. The test measured the capacity of a test compound to provide complete protection against threshold seizures induced by 100 dBA. The duration of 4 successive phases [Latency, Wild Running, Convulsions, Recovery] was recorded in seconds.

Statistical analysis. Data were expressed as mean ± SEM and analysed by Student's t-test.

Table 1. *Comparison of the two magnesium-deficient diets on body weight gain, locomotor activity at the end of the deprivation period (n = 14 per group).*

Parameters Diets	Body Weight (g)	Locomotor activity
Corn/Sunflower (ALA deficient diet)	26.6 ± 0.88	152.7 ± 37.9
Rapeseed (ALA rich diet)	26.1 ± 1.18	97.0 ± 22.5*

* Significant at $p < 0.05$ between the two groups.

Results

The body weight was similar in both groups after 30 days of the two Mg-deficient diets: about 26 g *(table 1)*. The individual spontaneous locomotor activity, measured for 3 min (Apelex actimeter), showed that magnesium deficiency induced central nervous hyperexcitability (NHE) in the corn/sunflower group as compared to the rapeseed group (152.7 ± 37.9 *vs.* 97.0 ± 22.5). In the MES test, the mean intensity responsible for a tonic seizure in 50% of the mice was slightly higher in the rapeseed group (4.5 mA) than in the corn/sunflower group (4 mA, NS). In addition, the rapeseed fed mice recovered more rapidly (data not shown). In the MDDAS test, the mice did not respond in the same way, depending on their diet: (i) the number of convulsive mice was lower in the rapeseed group (50%) as compared to the corn/sunflower group (100%). In addition, all the mice convulsing in the rapeseed group recovered whereas 43% died in the corn/sunflower group. (ii) The pattern of seizures was also different. The first two phases of the audiogenic seizure test 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 corn/sunflower group, Convulsions and Recovery durations showed a tendency to decrease slightly *(table 2)*.

Table 2. *Comparison of the two magnesium-deficient diets on the pattern of MDDAS test (n = 14 per group).*

Diets	% of convulsing mice	Latency (sec)	Wild running (sec)	Convulsions (sec)	Recovery (sec)
Corn/sunflower (ALA deficient diet)	100	4.0 ± 1.4	2.3 ± 0.4	1.7 ± 0.4	46.5 ± 6.7
Rapeseed (ALA rich diet)	50	6.7 ± 5.5*	3.7 ± 0.5*	1.6 ± 0.5	43.3 ± 4.1

* Significant at $p < 0.05$ between the two groups.

Discussion

Results reported in this work suggest a potential therapeutic value of ALA for convulsive pathologies as previously proposed by others in different animal seizure models [9, 2]. Firstly, magnesium deficiency induces in Mg-deficient/ALA poor diet group a central nervous hyperexcitability which did not appear in the Mg-deficient/ALA rich diet group. Secondly, in the MES test, the rapeseed fed group resisted slightly better to voltage stimulation (4.5 mA) than corn/sunflower oil fed group (4mA), indicating that sodium channel inhibition might be at least partly implicated in the rapeseed protective effect. Interestingly, phenitoinergic drugs (phenitoin, carbamazepine), which are the most used drugs in preventing epilepsia, act on the same channels. Finally, in the MDDAS test, the number of convulsive mice was significantly lower in the rapeseed group (50% instead of 100% in the corn/sunflower group) and the crisis was less severe since no mice died whereas 43% died in the other group. The global pattern showed an important increase in the first step duration, suggesting as previously voltage-dependent Na^+ channel inhibition. But, the global pattern would be rather GABAergic.

In any case, a rapeseed diet must not be considered as an antiepileptic drug; but it may help to reduce the susceptibility to epilepsia.

Interestingly, ALA has been shown to be neuroprotective both *in vivo* (in kainate induced seizure test) and *in vitro* (on seizure-like activity using glutamate neurons) and was associated with blockade of glutamatergic transmission (6).

To conclude, these preliminary results suggest that chronic dietary intake of rapeseed oil, an ALA rich monounsaturated oil, could help to control neuronal disorders as here shown in our model of magnesium-deficient mice.

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