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Experimental paper

Comparison of a short irradiation (50 sec) by different wavelengths on audiogenic seizures in magnesium-deficient mice: Evidence for a preventive neuroprotective effect of yellow

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Summary: Audiogenic seizures triggered by an acoustic stimulus of determined frequency and amplitude have been described in many laboratory animals in many circumstances including magnesium deficiency. This model, recently validated, was used, in DBA/2 mice, to study the preventive neuroprotective effect of 6 wavelengths of the visible spectrum used in Chromatotherapia* (λ_{max} 440, 484, 528, 572, 616 and 660 nm) at low irradiance. Each short illumination lasted 50 seconds and was followed by 20 minutes of darkness. It appeared that yellow fully protected 16 out of 17 mice from seizure occurrence. Green allowed the survival of 69% of mice but did not protect them from seizure occurrence. On the contrary, the other four colors (orange, red, purple and blue) failed to protect the mice and showed a tendency to accelerate their death. White color was not protective but allowed the difficult survival of 30% of mice. Darkness had no protective effect. These results even though surprising open a great field of investigation.

Key words: magnesium deficiency, seizures, chromatotherapia, neuroprotection, neurotoxicity, phototherapia

Introduction

Various animal models are used to carry out comprehensive research on central neural hyperexcitability. Magnesium deficiency offers an interesting animal model, since in adult DBA/2 mice strain, fed 21 days a 50 ppm magnesium-deficient diet, a generalized seizure episode may be induced by audiogenic stimulus (70 dBA, 10 kHz, 15 sec.) [1]. Magnesium deficiency-dependent audiogenic seizures (MDDAS) in adult mice present three successive phases [1, 2]: (1) wild running latency (2) intensive motor excitation expressed by wild running; (3) clonic then tonic seizures. During the convulsive phase, the animal may die from respiratory failure. We recently documented general characteristics of MDDAS [3] by measuring the three phase durations and if the mouse survived, the recovery duration.

Increased excitatory aminoacid action and decreased efficiency of GABA have been attributed a key role in initiating audiogenic seizures [4-8]. Other or overlapping metabolisms and neurotransmitter changes have also been described in

audiogenic seizures as causative or favouring factors (for a review, see 3).

The MDDAS model represents a seizure test responsive to low doses of antiepileptic drugs and provides an original assessment of the neuroprotective properties of test compounds. So, after hearing about Dr Agrapart's method, we decided to test the Chromatotherapia* efficiency on this model.

Chromatotherapia uses the "vibrations" produced by different wavelengths of the visible spectrum to repair the biological lesions in microorganisms, plants or animals and humans as well [8, 9]. According to the chromatotherapia concept, the properties of magnesium ions are linked to their vibration. When the vibrating effect of magnesium ions is replaced by a "similar vibrating effect of light", the same clinical effect must be expected.

We showed recently [10], that a short irradiation (50 s) of mice by yellow wavelength (550-600 nm), fully protected 9 mice among 10 ($p < 0.001$) from audiogenic seizure occurrence whereas the last one developed fatal audiogenic seizures. In contrast, the color purple aggravated the consequences of magnesium deficiency, by shortening all three phases of the convulsive seizures leading to the precipitated death of all the 9 studied mice ($p < 0.001$).

In the present paper, we studied the effect of a short exposure (50 s) to the six wavelengths used in Chromatotherapy*, on the MDDAS test, in larger groups of adult male DBA/2 mice.

Materials and methods

Diet: Throughout the experiment, mice were given either magnesium-deficient or standard magnesium containing food (50 or 1700 ppm respectively).

Animals: Male DBA/2 mice, 7 weeks old, were purchased from Charles Rivers, France. They were randomly divided into groups of 20 mice per cage. They were kept under a 12L: 12 D schedule (light from 6:00 a.m. - 6:00 p.m.) at 23 ± 1°C and had free access to control food and tap water until they weighed 20 ± 2 g. At the end of the acclimatization period, they were fed for 21 days, *ad libitum*, the magnesium standard (controls) or deficient food (magnesium-deficient groups). They received distilled water to avoid subsequent mineral input. About one third of the magnesium-deficient mice died during this period.

Treatments:

1 – **Chromatotherapia**: Surviving mice were randomly divided in groups of 5 animals per plexiglass cage, set on a mirror. In a dark chamber, groups of 5 animals were subjected to 50 sec of different wavelengths corresponding either to red, purple, blue, green, yellow or orange wavelengths. The light source was placed 50 cm above the cage and the light was reflected by the underlying mirror thus lightening the whole cage. The light source was a standard lamp (KL1500, Schott, France) equipped with an anti-IR filter (Richelet, France). In each experiment, an appropriate colored-filter (Richelet, France) was added to a soft light source to expose the mice to one of the 6 wavelengths of the visible spectrum used in Chromatotherapia* (λ_{max} 440, 484, 528, 572, 616 and 660 nm) corresponding to purple, blue, green, yellow, orange and red respectively. Then, the cages were kept in the dark for an additional 20 min period to avoid subsequent light interference.

2 – **MDDAS**: The audiogenic seizure was initiated -after the darkness resting period- by the emission of a sinusoidal signal (10 Hz ± 0.1 kHz). The sound intensity (70 dBA) was delivered for 15 sec. During the audiogenic assay, only one mouse was present in the experimental field. Care and treatment of mice were according to the guidelines for animal care.

Indices studied: In all the experiments, the number of animals completing audiogenic seizures and/or dying as a result of the MDDAS was recorded. The different phase duration of the audiogenic seizure (wild course latency, wild course and convulsive seizure durations) were recorded. When present, the recovery period- – corresponding to the mouse heaving itself on its legs – was also measured.

Statistical analysis: Results were expressed as mean values ± S.EM The comparison between the different experimental groups was made by ANOVA. Significant differences ($p < 0.05$) were estimated on the basis of Fischer-Scheffe and Dunett's tests.

Results

To calibrate the present assay, standard MDDAS conditions (10 kHz, 70 dBA, 15 sec) were applied to 5 deficient and 5 non-deficient magnesium mice. The deficient mice showed a generalized

seizure episode, whereas the 5 non-deficient did not exhibit signs of neuroexcitability.

A 50 sec yellow lightening of magnesium-deficient mice resulted in a relaxing effect immediately observable during lighting and after the darkness period: mice were notably quiet and their fur was normal. More interestingly, 16 mice of 17 ($p < 0.001$) were fully protected from audiogenic seizure occurrence (one of them presented a wild running which was not followed by convulsive seizures). One developed fatal audiogenic seizures (figure 1). Even when using a stronger noise (80-130 dBA), 2 yellow-treated mice over the 6 tested managed to survive to their seizure episode (data not shown). The other color of interest, even though to a much lesser extent than yellow, was the green one which did not protect more than 6% of the mice from audiogenic seizures, but modified the convulsive pattern of the others, with a series of clonic seizures and a decrease in the tonic seizure duration, both indicating a decrease in the epileptic fit severity. Finally, it allowed two third of them to recover (figure 1). The recovery period was rather rapid (67.5 ± 16.3 s) (table I) and the mice presented thereafter both normal aspect and behaviour. Blue protected 20% of mice from seizure episode and allowed about one third of the convulsive mice to recover but the recovery period was longer than with the green (93.0 ± 23.0 s). In addition the surviving mice presented a lethargic behaviour and a piloerection. The three other colors, purple, red and orange were without significant effect as compared to controls. The use of a white color resulted in 100% convulsant mice of which about one third recovered very hardly (recovery period: 125 ± 56.7 s). The survivors presented a hyperexcitability. Finally, darkness appeared without protective effect and even showed a tendency to accelerate the mouse death.

Table I. Measurement of the different phase durations (expressed as seconds) of the audiogenic seizures in DBA/2 magnesium-deficient mice exposed to various wavelengths for 50 seconds. Yellow treatment was not reported since it protected the mice from seizure occurrence. In the other groups, the convulsive patterns were not similar from one group to another showing for instance a lower severity of the convulsive seizures in the green-treated group.

	Latency	Wild running	Seizures	Recovery
Controls	2.5 ± 0.9	3.3 ± 1.3	12.5 ± 1.4	
Green	6.6 ± 4.9	2.6 ± 0.5	15.2 ± 3.5	67.5 ± 16.3*
Blue	2.5 ± 1.2	2.4 ± 1.5	10.0 ± 2.7	93.0 ± 23.0*
Purple	2.4 ± 2.0	2.9 ± 1.8	13.3 ± 1.6	
Red	1.8 ± 1.0	2.0 ± 0.8	11.4 ± 2.6	
Orange	1.4 ± 0.8	2.1 ± 0.7	13.4 ± 1.3	
White	1.3 ± 1.3	2.8 ± 1.3	17.2 ± 3.2	125 ± 56.72*
Darkness alone	1.8 ± 0.5	2.3 ± 1.6	9.5 ± 1.7	

* $p < 0.01$, *vs* control magnesium-deficient rats

Discussion

Audiogenic seizures in magnesium-deficient mice are an interesting nutritional model of neurological disturbances [3, 11]. Magnesium ions appear to possess many properties that are potentially neuroprotective [12, 13]. According to the chromatotherapia theory [8, 9], a physical energy brought by one wavelength could act as the energy brought by the "corresponding oligoelement". A short (50 sec) yellow exposure (or a longer exposure (4-8 min) to the complementary color, purple) would act "as magnesium ions do" and reciprocally. In the present paper, the six wavelengths used in chromatotherapy were applied for a short period of time (50 sec) with low irradiance. It is noteworthy that the neuroprotective effect of a short yellow exposure previously described [10] was again unequivocally present ($p < 0.001$) using a higher number of mice (16 of the 17 mice studied were protected from audiogenic seizures). However, one mouse died and this may be attributable to a methodologic problem linked both to the simultaneous irradiance of 5 mice and to the mice grouping habits. This may have resulted in an insufficient lightning of the only victim.

Less efficient but still interesting was the green color which did not manage to protect the mice from seizures but reduced the crisis severity and allowed a non negligible number of mice to fully recover.

Blue lightened mice were also partly protected, 20% resisting the sound signal but two thirds of the convulsant mice died and the survivors were severely exhausted.

Orange and red were inefficient.

More surprising was the lack of result with the color purple, in view of the chromatotherapy theory and of our previous results [10] showing an increased neurotoxicity with purple.

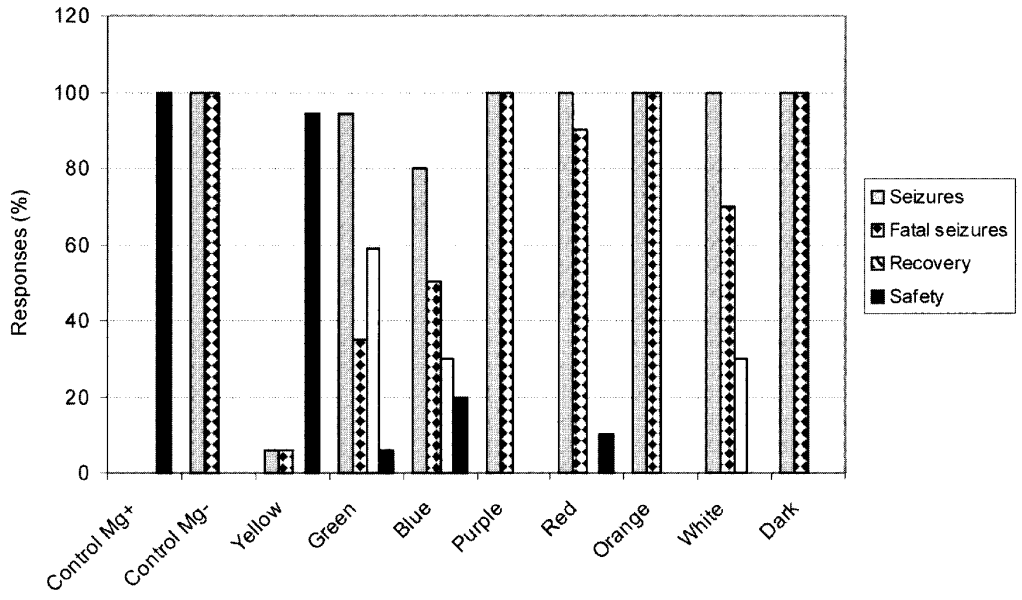

Fig. 1. Effect of 50 second irradiation by different wavelengths in DBA/2 magnesium-deficient mice. Some mice did not exhibit seizures at all (safety). Seizures include both clonic and tonic seizure manifestations; among those mice, some may die (fatal seizure); other may recover (the recovery being completed when the mouse is able to stay on its four legs).

However, it must be noticed that the convulsive pattern of the magnesium-deficient controls used in the present experiment was very different from that we usually observed [10, 11]. It may be assumed that the magnesium deprivation was more drastic than usual, resulting in a very severe magnesium-deficiency induced neurotoxicity and death. Consequently the parameters of the three phases of the audiogenic seizures were so short that it became difficult for us to distinguish between the aggravating and non-efficient wavelengths. In contrast, the high protective effect of yellow observed in the present experiment appeared particularly noticeable in such conditions.

Clinical use of visible light (or some wavelengths) for 2-3 hours has already been reported (phototherapy). It was used mainly as a classic adjuvant anti-depressant treatment [12, 14, 15]. In the present model, a 50 s white light exposure gave less interesting results than some precise wavelengths. It allowed 33% of the mice to recover; the recovery period being important (125.1 ± 56.7 s) and the resulting mice presenting an important hyperexcitability.

Finally, since darkness therapy is also used [12], we assayed the possible neuroprotective role of the 20 minutes of darkness, unsuccessfully since all the mice developped very rapid fatal seizures.

The overall physiological effects of these interesting effects raise question of their impact on neurotransmitters and second messengers. We showed that MDDAS is a nutritional model of neurological disturbances including abnormal behavior (wild running), seizures, and cerebral injury. The wild running responds well to voltage-dependant sodium channel blockers and is not reduced by GABAergic compounds. Conversely, the convulsive phase responds well to GABAergic activity. After the seizure episode, the recovery phase takes place which is largely shortened by free radical scavengers or PAF antagonists [3]. PAF is known to stimulate excitatory aminoacid neurotransmission [16]. This might suggest that all the phases of the MDDAS model, would require distinct cellular pathways. NMDA antagonists are

also involved in neuroprotection but are either ineffective or harmful in patients. Finally, magnesium ions may block Glu-receptors (NMDA and not NMDA receptors) and appears as a potent non toxic neuroprotective agent [17]. In a previous study, we showed that a pharmacological magnesium supply of the various magnesium salts currently used in therapy caused a transient suppression of audiogenic seizures. But, after the end of the treatment, the convulsive seizures rapidly reappeared, except following treatment with the promising long term-efficient magnesium acetyl taurinate [11-13].

Our preliminary results, we called "the Agrapart effect", obtained on audiogenic seizures in magnesium-deficient mice with a short (50 sec) exposure mainly to yellow were sufficiently interesting to be reported. However this experiment raises multiple questions and among them, the long term effect of such a treatment must be questioned and is presently investigated. Secondly the preventive neuroprotective mechanisms of such a complex model cannot be explained in the light of the present results.

It is noteworthy that the use of different wavelengths in animal experimental exposure has already been reported and resulted, for instance, in altered melatonin levels, locomotor activity [18] or ovulation rhythms [19]. These effects, which also could not be explained, were attributed to the presence of photoreceptors, identified in the retina [20-22] where some opsin could play a primary rôle [21, 22], but also in the pineal gland and the deep brain [19] which is involved mainly in the nycthemeral rhythmic activities. Recently, they were also described in the skin [23]. Their physiological significance is at present still unknown.

Conclusion

Chromatography* opens new fascinating perspectives in the field of experimental and clinical research. Further investigations are currently in progress to evaluate the effect of yellow (50 s) when applied simultaneously with the sound stimulation. The short and long-term efficiency of the complementary color (purple) applied for a longer period (4-8 min) must be also evaluated. Finally, the protective effect of Chromatotherapia* on other animal seizure models using GABA antagonists is currently being studied.

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