EFFECTIVENESS OF HIGH –DOSE RIBOFLAVIN IN MIGRAINE PROPHYLAXIS: A RANDOMIZED CONTROLLED TRIAL.

J. Schoenen, MD, PhD; J. Jacquy, MD; and M. Lenaerts, MD
Neurology 1998; 50:466 - 470

ABSTRACT Deficit of mitochondrial energy metabolism may play a role in migraine pathogenesis. We found in a previous open study that high-dose riboflavin was effective in migraine prophylaxis. We now compared riboflavin (400 mg) and placebo in ***55*** patients with migraine in a randomized trial of 3 months duration. Using an intention-to-treat analysis, riboflavin was superior to placebo in reducing attack frequency *(p* = 0.005) and headache days ***(p*** = 0.012). Regarding the latter, the proportion of patients who improved by at least 50%, i.e. "responders," was 15% for placebo and 59% for riboflavin *(p* = 0.002) and the number-needed-to-treat for effectiveness was 2.3. Three minor adverse events occurred, two in the riboflavin group (diarrhea and polyuria) and one in the placebo group (abdominal cramps). None was serious.
Because of its high efficacy, excellent tolerability, and low cost, riboflavin is an interesting option for migraine prophylaxis and a candidate for a comparative trial with an established prophylactic drug. NEUROLOGY 1998; 50:466-470 A mitochondrial dysfunction resulting

A mitochondrial dysfunction resulting in impaired oxygen metabolism may play role in migraine. Migraine headache can be a prominent feature in patients affected by the syndrome of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS).7,8 Riboflavin (vitamin B2) is the precursor of flavin mononucleotide and flavin adenine dinucleotide, which are required for the activity of flavoenzymes involved in the electron transport chain. Given to patients with MELAS or mitochondrial myopathies on the assumption that at large doses it might augment activity of mitochondrial complexes I and **11,** riboflavin was able to improve clinical and biochemical abnormalities. Against this background, we performed an open pilot study of high-dose (400 mg/day) riboflavin as a prophylactic treatment for migraine in 25 patients and found on average a 68% improvement in a severity
Index.13 Because of this encouraging result, the effectiveness of riboflavin in migraine prophylaxis was studied in a randomized placebo-controlled trial. **Methods.** *Patients.* The study was conducted in six centers in Be?@urn and the Grand Duchy of Luxemburg under the sponsorship of the Belgian Migraine Society. Patients were recruited between March 1995 and March 1996, and the trial was completed on July 31, 1996. The design was in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Medicine, University of Liege. Patients (ages 18 to 65 years) were eligible for the study if they met the International Headache Society (IHS) diagnostic criteria for migraine with or without aural4 and had a history of migraine of at least 1 year, had between two and eight attacks per month, had no more than ***5*** days of interval headaches per month, had no analgesic or ergotamine overconsumption, and had no serious organic or psychiatric disease. Women were required to have adequate contraceptive protection. Written informed consent was obtained. This was a double-blind, randomized, two-parallel group trial of oral riboflavin 400 mg (Riboflavinum D 2914A, Federa, Brussels) and placebo (Avicel RC 581" 850 mg + p-carotene 0.473 mg) taken in capsules once daily. Avicel is not absorbed, and to the best of our knowledge, low doses of p-carotene, used to blind for color, have no effect on migraine. At the first visit, eligible patients received placebo for a l-month baseline treatment. At the second visit, they were randomized to riboflavin or placebo only if they had presented at least one migraine attack during the preceding month.

The study medications were randomized in 10 blocks of 10 packages, each block comprising ive placebo and five active treatments. The random order of medications was different for each block and known only to the pharmacist of the Citadelle Hospital (Liege), who prepared the study material. Each investigator received one to three treatment blocks containing 10 packages of trial medication.

Each package, numbered serially from 1 to 100, contained three boxes of 35 placebo riboflavin capsules labeled “B,” “C,” or “D” for the successive 3 months of the randomized treatment period. Ten to 30 boxes of 35 placebo capsules labeled “A” were given simultaneously for the 1-month baseline treatment. In each center, patients were allocated in sequence to the randomized phase, starting with the lowest treatment package number. No stratification was performed. Sealed envelopes containing the treatment codes were added to treatment material; no code was broken before complete data analysis. The total blinded and randomized treatment period was thus ***3*** months with monthly control visits. Patients were provided with a diary in which they had to record each migraine attack: headache severity on a four-point scale ***(3,*** severe; 2, moderate; 1, mild; 0, no pain), presence of nausea and vomiting, name and number of acute headache medication, and headache duration in hours. Diaries were cross-checked by the investigator and the patient at each visit. Acute treatments varied between patients and centers: oral or rectal analgesics with antiemetics, oral or subcutaneous sumatriptan, and, in a few cases, ergotamine-containing preparations. Compliance was assessed by counting the returned capsules at each monthly visit. Primary efficacy variable was change of attack frequency in month 4 compared with the baseline month 1 (placebo) in accordance with IHS guidelines. I5 Secondary outcome variables were reduction of migraine headache days, of mean duration per day, of mean severity per day, of a migraine index (headache days + mean severity), of days with nausea, vomiting, and of mean number of tablets, suppositories, or injections taken per day for the acute treatment of the attacks. The proportion of responders (i.e., patients having an improvement equal or superior to 50%) was calculated for attack frequency, headache days, and migraine index. We also measured the change of outcome variables between the first baseline month and the averaged ***3*** subsequent months to evaluate a global effect during the randomized treatment period. Patients were interviewed about possible adverse events at each control visit. For responder rates (and for adverse events), the number of patients needed to treat (NNT) was determined with the formula; *Statistical analyses.* Sample size calculations were based on the following response rates: 30% for placebo based on published trials and *70%)* for riboflavin inferred from an 80%1 responder rate in the open trial. To detect a significant difference between the two treatments *(5%)* two sided significance level) with an 80% power, the minimum size of each treatment group was estimated at 28 patients."

Statistical analysis (STATISTICA, STATSOFT Inc.) was carried out on an intention-to-treat basis. In the patients' diaries, migraine headaches separated by less than 24 hours were counted as one single attack. Values for patients who dropped out were included according to the last value-carried-forward method. The Mann-Whitney U test was used for comparison of primary and secondary outcome variables between the two treatment groups, chisquare for demographic and nosographic data, Fisher's two-tailed exact test for responder rate, and the sign test for changes between baseline and the second, third, or fourth month of treatment within placebo and riboflavin groups. All statistical tests were two-tailed. $p < 0.05$ was considered significant.

Results. *Study population.* Eighty patients were recruited by six centers. Four patients were lost to follow-up, 5 withdrew consent, and 16 (20%) did not report any migraine attack during the first month of baseline placebo treatment. Fifty-five patients (69%) were randomized to receive the study medication, 27 placebo (49%), and 28 riboflavin 400 mg (51%>O).n e patient in the placebo group had to be excluded from the intention-to-treat analysis because of protocol violation (frequent interval headaches and simultaneous enrollment in another clinical trial).

Four patients (7%) dropped out after randomization: three after 2 months in the placebo arm (two for inefficacy, one lost to follow-up) and one two weeks after randomization in the riboflavin arm because of diarrhea. Groups were comparable in demographic and migraine features, as illustrated in table l, which summarizes patient characteristics as taken from history at the first visit before enrollment in the trial.

Treatment efficacy. The change from baseline to month 4 in attack frequency, the primary outcome variable, was significantly different between riboflavin and placebo (table 2). Attack frequency decreased after 1 month of treatment with riboflavin and further during the last month. The difference between riboflavin and placebo became statistically significant at month 4. When month **4** was compared with month **1**, there was also a significant difference between placebo and riboflavin for migraine days and migraine index and with a higher *p* value for severity, duration, and days with nausea and vomiting but not for acute medication use. The 50% responder rate was significantly higher in riboflavin- than in placebo treated patients. There were no obvious differences in treatment results across centers (see figure 1).

No significant change of any outcome variable was detected in the course of treatment within the placebo group. By contrast, in the riboflavin group, attack frequency and headache days were lower in months 2 *(p* = 0.0001 and *p* = 0.0051, ***3*** *(p* = 0.0021, and **4** *(p* = 0.008 and *p* = 0.0001) and days with nausea were lower in month 4 (*p* = 0.024) compared with month **1.** Because statistical differences between treatment groups, however, emerged during the last month of treatment, we also analyzed the data by comparing with month 1 the averaged values of months 2, ***3,*** and 4 combined. The differences between the riboflavin and placebo groups remained significant for attack frequency *(p* = 0.005) and headache days *(p* = 0.012) (figure 2) and for the migraine index *(p* = 0.012) but not for the other efficacy variables. When the 50% responder rate was considered, the number of patients needed to treat for effectiveness of riboflavin 400 mg compared with placebo was 2.3 for headache days, 2.8 for attack frequency, and 3.1 for migraine index.

There were no significant changes of blood pressure or body weight (placebo, **+0.4** kg; riboflavin, +0.2 kg) in either group. As judged from capsule counts, compliance was excellent; the maximum number of capsules returned at a follow-up visit was eight (mean, 3.5) in patients who completed the trial. Only three adverse events were recorded during the trial. One woman in the riboflavin group had diarrhea 2 weeks after starting the drug and withdrew from the study. On follow-up, her symptoms disappeared within 72 hours. Another patient receiving riboflavin complained of polyuria but completed the study. In the placebo group, one patient mentioned recurrent abdominal cramps of moderate intensity that did not interrupt the trial. Comparing riboflavin with placebo, the number of patients needed to treat for adverse effects was 33.3.

Discussion. This randomized controlled trial demonstrates that a daily oral dose of 400 mg riboflavin is significantly superior to placebo for migraine prophylaxis and confirms therefore the results of our previous open study.I3 The effect of riboflavin begins after 1 month but is maximal only after **3** months of treatment. It is most pronounced on attack frequency and the number of days with migraine headache. Riboflavin's superiority over placebo is marginal for headache severity, duration, acute antimigraine drug consumption, or gastrointestinal symptoms associated with the attack. **A** 2-month lag before significant improvement over placebo has been observed with other antimigraine prophylactics (e.g., with flunarizine), and most trials have shown that the predominant effect of prophylactic pharmacotherapy is on migraine attack frequency.'" These features may be related to the pharmokinetics and the mode of action of these drugs. For instance, impaired mitochondrial energy metabolism is likely to be only one functional abnormality that predisposes to migraine. It may not play a crucial role in all patients. It is conceivable that a clinical effect due to pharmacologic intervention on the mitochondrial metabolism builds up more slowly than one brought about by receptor blockade (e.g., the one due to betablockers). Whether efficacy of riboflavin may increase further beyond **3** months of treatment remains to be determined. As expected, the 50% responder rate was lower in the present trial ***(56%*** for attack frequency, 59% for headache days) than in the open study (80%). It

compares favorably, however, with that of other prophylactic drugs used in migraine and is likely to be clinically relevant, because the therapeutic "gain" over placebo is **37%** for attack frequency.
Even though historical comparisons with published trials must be taken with reservation, such an advantage over placebo is close to that calculated from studies of other established antimigraine prophylactics: 42% for beta-blockers and flunarizine, **37%** for valproate. 20,21
Using a placebo during the first baseline month, as we did in accordance with IHS guidelines, lHS revents in theory randomization of a proportion of placebo responders, which in our study was +20% of recruited patients. Nonetheless, the responder rate to placebo during the active treatment period is similar in the present study and in the valproate trial that used a design with a treatment-free baseline.2n In a review of available randomized controlled trials, I6 valproate had a calculated NNT for effectiveness of **1.6,** which is better than the 2.3 calculated for riboflavin 400 mg. However, valproate had also a low number-to-treat (2.4) for adverse effects, whereas riboflavin's NNT is at **33.3,** suggesting that this many subjects need to be treated before an adverse effect occurs. One major advantage of riboflavin over established prophylactic drugs is therefore its excellent efficacy side-effect profile. High-dose oral magnesium 22 and cyclandelate which have also an excellent tolerability were recently investigated in the prophylaxis of migraine. Their effectiveness seems by far inferior to that of riboflavin, because for 50% responder rates, the therapeutic gain over placebo did not exceed **18.4%** for magnesium or 7.4% for cyclandelate. The hypothesis that riboflavin exerts its beneficial effects in migraine by increasing complex I and I1 activity and mitochondrial energy metabolism can be tested by nuclear magnetic resonance spectroscopy studies. The assumption of a mitochondrial dysfunction in migraine is only based on biochemical investigations.
None of the mutations or deletions of mitochondrial DNA known to occur in mitochondriopathies such as MELAS were detected in migraine up to Although unlikely, another mode of action can thus not be excluded. In view of riboflavin's efficacy in migraine prophylaxis, it may be of interest to study the effects of other well-tolerated drugs enhancing mitochondrial activity, such as lipoic acid. It may also be worthwhile for cost reasons to explore the antimigraine effect of lower doses of riboflavin. The dose of 400 mg was chosen in the present trial because comparable high doses were previously used to treat mitochondriopathies 9-12 and because of the lack of toxicity. Despite the fact that intestinal absorption of riboflavin is a saturable process, there is evidence that prolonged retention of the vitamin in the small intestine can increase the total amount absorbed.25

Acknowledgments
We acknowledge the assistance of **V.** De Pasqual for analyzing the data.
Disclosure
J. Schoenen is Research Director at the National Fund for Scientific Research (Belgium).
Appendix
List of investigators: J. Jacquy, Charleroi (n = **20**); P. Laloux, Mont-Godinne (n = ***5);*** M. Lenaerts, Luxembourg (n = 16); P. Louis, Antwerpen (n = 10); J. Schoenen, Liege (n = ***25);*** A. Vakaet, Gent (n = **4**)
References
1.Welch KMA, Levine SR, D'Andrea G, Schultz L, Helpern JA. Preliminary observations on brain energy metabolism in migraine studied by in vivo "phosphorus NMR spectroscopy.
Neurology 1989; 39: 538-541.
2. Montagna P, Sacquegna T, Cortelli P, Lugaresi E. Migraine as a defect of brain oxidative metabolism: a hypothesis. J. Neurol 1989; 236: 124-125.
3. Barbiroli B, Montagna P, Cortelli P, et al. Abnormal brain and muscle energy metabolism shown by 31P magnetic resonance spectroscopy in patients affected by migraine with aura. Neurology
1992; 42: 1209-1214.

4. Montagna P, Cortelli P, Monari L, et al. 31P-Magnetic resonance spectroscopy in migraine without aura. Neurology 1994;
5. Sangiorgi S, Mochi M, Riva R, et al. Abnormal platelet mitochrondrial function in patients affected by migraine with and without aura. Cephalalgia 1994; 14: 21-23.
6. Watanabe H, Kuwabara T, Ohkubo M, Tsuji S, Yuasa T. Elevation of cerebral lactate detected by localized 'H-magnetic resonance spectroscopy in migraine during the interictal period.
Neurology 1996; 47: 1093 - 1095.
7. Montagna P, Gallassi R, Medori R, et al. MELAS syndrome: characteristic migrainous and epileptic features and maternal transmission. Neurology 1988;38:751-754.
8. Goto Y, Horai S, Matsuoka T, Koga Y, Nihei K, Kobayashi M, **February 1998** NEUROLOGY *50* **469** 44:666-668. Nonaka I. Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS): a correlative study of the clinical features and mitochondrial DNA mutation. Neurology 1992; 42: 545 - 550.
9. Arts WFM, Scholte HR, Boggard JM, Kerrebijn KF, Luyt-Houwen IEM. NADH-CoQ reductase deficient myopathy: successful treatment with riboflavin. Lancet 1983;2:581-582.
10. Penn AMW, Lee JWK, Thuillier P, et al. MELAS syndrome with mitochondrial tRNALe" **'UUR)** mutation: correlation of clinical state, nerve conduction, and muscle **31P** magnetic resonance
spectroscopy during treatment with nicotinamide and riboflavin. Neurology 1992;42:2147-2152.
11. Antozzi C, Garavaglia B, Mora M, et al. Late-onset riboflavinresponsive myopathy with combined multiple acyl coenzyme. A dehydrogenase and respiratory chain deficiency. Neurology
12. Scholte HR, Busch HF, Bakker HD, Bogaard JM, Luyt-Houwen IE, Kuyt LP. Riboflavin-responsive complex I deficiency. Biochim Biophys Acta 1995; 1271:75 - 83.
13. Schoenen J, Lenaerts M, Bastings E. High-dose riboflavin as a prophylactic treatment of migraine: results of an open pilot study. Cephalalgia 1994; 14: 328 - 329.
14. Headache Classification Committee of the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. Cephalalgia
1988;8(suppl 7):l-96.
15. International Headache Society Committee on Clinical Trials in Migraine. Guidelines for controlled trials of drugs in migraine. First edition. Cephalalgia 1991;ll:l-12. 1994;44:2153-2158.
16. McQuay H, Carroll D, Jadad AR, Wiffen P, Moore A. Anticonvulsant drugs for management of pain: a systematic review. Br Med J 1995;311:1047-1052.
17. Armitage P, Berry G. Statistical methods in medical research, 3rd ed. Oxford: Blackwell Scientific Publications, 1994.
18. Andersson KE, Vinge E. P-Adrenoceptor blockers and calcium antagonists in the prophylaxis and treatment of migraine. Drugs 1990;39:355-373.
19. Toda N, Tfelt-Hansen P. Calcium Antagonists. In: Olesen J, Tfelt-Hansen P, Welch KMA, eds. The headache. New York: Raven Press, 1993.
20. Jensen R, Brinck T, Olesen J. Sodium valproate has a prophylactic effect in migraine without aura: a triple-blind, placebo controlled crossover study. Neurology 1994; 44: 647 - 651. 21. Mathew NT, Saper JR, Silberstein SD, et al. Migraine prophylaxis with divalproex. Arch Neutol 1995; 52: 2481 - 2486.
22. Peikert A, Wilimzig C, Kohne-Volland R. Prophylaxis of migraine with oral magnesium: results from a prospective, multi-center, placebo-controlled and double-blind randomized
study. Cephalalgia 1996; 16: 257 - 263.
23. Diener HC, Foh M, Iaccarino C, et al. Cyclandelate in the prophylaxis of migraine: a randomized, parallel, double-blind study in comparison with placebo and propranolol. Cephalalgia 1996;16:441-447.
24. Klopstock T, May A, Seibel P, Papagiannuli E, Diener HC, Reichmann H. Mitochondrial DNA in migraine with aura. Neurology 1996; 46: 1735 - 1738.

25. Levy G, Mosovich LL, Allen JE, Yaffe SJ. Biliary excretion of riboflavin in man. J Pharm Sci 1972; 61: 143 - 144.