Prevention of Preeclampsia by High Dose Riboflavin Supplementation

Elsen C[1,2,6], Rivas-Echeverría C[1,3], Sahland K[4], Bogucki P[4], Rivas-Echeverría F[3], Molina L[1], Rivas V[1], Sánchez R[1,3], Pahl L[4], Wallinger R[4], Volz J[5], Wacker J[2], Fruehauf JH[7]

***Abstract*—** Preeclampsia remains a serious obstetrical threat worldwide. Earlier, we had described riboflavin (Vitamin B_2) deficiency as a possible risk factor for preeclampsia in developing countries. Here, we report the outcome of a pilot study examining the impact of high dose riboflavin supplementation on frequency and severity of preeclampsia in Venezuela.

414 women were enrolled since around 20 weeks of gestation and treated with 15 mg/d riboflavin supplementation or placebo until delivery. Results from 255 women were available for evaluation. High dose riboflavin supplementation lead to an approximate 75 percent decrease in the number of cases of severe preeclampsia. Women in the riboflavin group who developed any hypertensive disorder of pregnancy had significantly lower maximum diastolic blood pressures than corresponding women in the placebo group. The study suffered from a significant loss in follow-up, due to political and social problems in Venezuela during the enrolment and follow up time frame of the study; however, we consider the results encouraging in support of further studies into the use of riboflavin to prevent preeclampsia.

***Keywords*—** antioxidants, preeclampsia, pregnancy induced hypertension, prevention of preeclampsia, riboflavin

.

I. Introduction

PREECLAMPSIA remains one of the major risks for pregnant women worldwide and contributes to a considerable number of maternal deaths in the industrialized world and increases four or fivefold the risk of preterm birth[1]. While in Western countries, neonatal intensive care units improve the chances for preterm babies and close monitoring of preeclamptic mothers allows for an expectant management of preeclampsia, such options do not exist for the majority of mothers in the developing world. Recent advances in the molecular understanding of preeclampsia[2] have allowed us a better insight into its pathophysiology, but therapeutic options are still very limited. Prevention of preeclampsia clearly remains a highly desirable aim to improve public health, especially in the developing world.

We have reported our findings from pregnant women in Zimbabwe, where women with riboflavin deficiency had a significantly increased risk of developing preeclampsia[3]. The reason for this increased risk is not entirely clear, but it may be related to the coenzyme function of riboflavin metabolites, flavin adenine dinucleotide (FAD) and flavin adenine mononucleotide (FMN) which are important cofactors for key enzymes; such as glutathione reductase, necessary for the glutathione antioxidant system, and nitric oxidase synthase, which produces the potent vasodilator nitric oxide[4].

Nutritional deficiencies have frequently been hypothesized to contribute to the risk of preeclampsia, and a particular focus has been on the role of antioxidant vitamins[5]. Nutritional deficiencies could help to explain a striking pattern of seasonal changes in the incidence of preeclampsia which has been observed in several developing countries[6] and has been historically reported for countries in Western Europe. Recently, Fawzi et al. [7] reported a 38% reduction in the risk of developing pregnancy-induced hypertension among Tanzanian women who received a multivitamin supplementation which included 20 mg of riboflavin.

We therefore decided to conduct a randomised pilot study with a double-blind, placebo-controlled trial design, to test whether riboflavin supplementation can help to prevent or ameliorate cases of pregnancy-induced hypertension or preeclampsia among Venezuelan women assisted by the Program for the Prevention of Preeclampsia[8].

II. Material and Methods

The study was conducted after ethical approval (Ethical committee of the Health Ministry Mérida State) and in close collaboration with the Venezuelan semi-governmental organization PPP ("Programa de Prevención de Preeclampsia")[8] in the city of Mérida, a provincial capital in

Manuscript received July 26, 2011. This work was supported in part by the Faculty for Clinical Medicine Mannheim of the University of Heidelberg, Germany, by the University of Los Andes, Venezuela by CDCHT-ULA, through grant FA-320-04-07-B; and BASF Aktiengesellschaft, Ludwigshafen, Germany.

1. Programa de Prevención de Preeclampsia (PPP), Mérida, Venezuela
2. Department of Obstetrics&Gynaecology, Fürst-Stirum-Hospital Bruchsal, Germany
3. Universidad de los Andes, Mérida, Venezuela
4. University Hospital Mannheim, Mannheim, Germany
5. Department of Obstetrics&Gynaecology, Klinikum Bielefeld, Germany
6. Department of Obstetrics&Gynaecology, St. Josefs-Hospital Wiesbaden, Germany. email: icchris2001@hotmail.com
7. Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA, and ViThera Laboratories, Cambridge, MA email: johannes@vitheralabs.com

.

Venezuela, during 2002 to 2005. Women were enrolled before or at 20 weeks of gestation, if they met either of the inclusion criteria: (1) primigravidity or (2) multigravidity with previous history of hypertensive disorders of pregnancy. Informed consent was obtained from each subject. The purpose and requirements of the research were explained to them in Spanish by a nurse or physician fluent in the language or a native speaker.

A woman was considered to develop hypertension if her diastolic blood pressure rose to more than 90 mmHg or her systolic blood pressure to more than 140 mmHg, after the 20th week of gestation. Preeclampsia was diagnosed if hypertension in pregnancy was recorded in the presence of a proteinuria ≥300 mg/l according to a dipstick test. Blood pressures were taken by a nurse with the patient sitting on a chair. Cases were categorized as severe preeclampsia if in addition to hypertension and proteinuria, neurologic symptoms were recorded, such as visual disturbances, increased reflexes and irritability, or when the patient suffered eclamptic episodes. Also, preeclamptic cases in which the diastolic blood pressure rose to more than 110 mmHg were classified as severe.

Consecutive numbers had been attributed randomly to allocate women either to the riboflavin or placebo group according to a system of block randomisation which ensured that in every 10 women enrolled there would be five who received each of the two regimens. Patients were attributed study numbers consecutively as they were enrolled in the study. Sealed containers containing either riboflavin or placebo had those study numbers printed on them and were distributed to the women at each of the visits which ensured double blind administration of the study medication. Women were examined every four weeks until 36 weeks and weekly thereafter until delivery.

Plasma samples were obtained during each clinic visit and at delivery. They were centrifugated, frozen and stored at -20C. Determination of flavin adenine dinucleotide (FAD) was performed by HPLC on a C18 Sep-Pak cartridge (Waters) and internal FAD standard (Sigma) according to standard procedures[9].

A total number of 414 women were enrolled and were planned to be followed until delivery. Clinical examination was conducted and serum samples were drawn at four week intervals. Through a double blinded block randomisation procedure performed by a neutral study coordinator; and inclusion of identical looking placebo tablets, neither the participants nor the treating physicians were aware of the treatment which the patient received until the end of the trial. The study medication was produced exclusively for this trial. Women were randomized to receive either 15 mg riboflavin per day, or an identical looking placebo tablet, from 20 weeks gestation onwards. Both were distributed in pre-labelled and sealed containers (bottles). This starting date for the supplementation was chosen for practical reasons (many women do not register with the obstetric services prior to 20 weeks of gestation), and to coincide with the second wave of trophoblast invasion where riboflavin deficiency might play a pathogenic role[3]. No toxicity is known for riboflavin and no upper limit is defined and 15mg/day were chosen to represent a feasible high dose supplementation (10 times the minimum intake). Main outcome parameters included the incidence of hypertensive disorders of pregnancy as well as plasma concentration of FAD (flavin adenine dinucleotide). Statistics were obtained using the Statistical Package for the Social Sciences (SPSS, version 10.0) software. P-values ≤0.05 (using t-tests to compare means and chi-squared tests for over-all numbers) were considered significant. The preliminary calculations implied a prevalence of 10% of preeclampsia in the study cohort. Original power calculations suggested that with a statistical power of 90% and an assumed loss to follow-up of 20% a minimum of 1500 subjects needed to be enrolled to achieve an authoritative result. During the study period severe political and social problems had place in Venezuela, thus the study failed to meet these enrolment goals and therefore is considered as a pilot study only; and in need of further confirmation.

III. Results

Of the 414 enrolled women; 209 were randomised to receive riboflavin and 205 placebo. Characteristics of the patient groups are shown in Table I. Only 255 could be considered for statistical analysis of the main outcome data shown in Table II due to lost in follow up. The loss to follow-up in the entire cohort was 38.4%. One major reason for the loss to follow-up was a general strike in Venezuela, which took place during the study period, and which led to a virtual shutdown of public life from December of 2002 to February of 2003 and slowed down the public health system for several months.

There was a tendency to a reduced incidence of all hypertensive disorders of pregnancy (pregnancy-induced hypertension and preeclampsia) among women receiving riboflavin supplementation (9.5% vs. 14.1% in the placebo group; p=0.34) and the number of preeclampsia cases tended to be lower in women receiving riboflavin compared with women receiving placebo (Table II). We observed two women with severe preeclampsia (1.6%) in the study group vs. eight (6.3%) in the placebo group (including 1 case of eclampsia; p=0.053). The number needed to treat was 22.

	Riboflavin (n=209)	Placebo (205)
Loss to follow-up (%)	39.2	37.6
n=414	Mean (SD)	Mean (SD)
Age [years]	24.2 (5.9)	24.9(6.8)
Gravidity: median (range)	1 (0-11)	1 (0-10)
Parity: median (range)	0 (0-7)	0 (0-8)

ISBN: 978-1-61804-036-7

Gestational age [weeks]	18.3 (5.7)			17.7 (6.5)		
BMI [kg/m^2]	24.4 (4.5)			24.0 (4.6)		
BP systolic [mmHg]	105 (15)			105 (12)		
BP diastolic [mmHg]	64 (10)			64 (9)		
Marital status (%)	married	with partner	single	Married	with partner	Single
	23.4	47.8	27.3	26.8	45.4	25.4
Level of education (%)	no school	primary school	secondary school and academic	no school	primary school	secondary school and academic
	1.0	12.4	86.5	0.5	12.6	86.8

TABLE 1. Summary of patient characteristics at time of enrolment BP=blood pressure, BMI=body mass index, n=number of cases

Women who developed a hypertensive disorder of pregnancy (preeclampsia or pregnancy-induced hypertension, Table II) while receiving riboflavin supplementation had significantly slightly lower maximum diastolic blood pressures, compared with the placebo group (93 mmHg vs. 100 mmHg; p = 0.027).

	Riboflavin (n=127)		Placebo (n=128)		
n=255	mean	standard deviation	Mean	standard deviation	Significance
Duration of pregnancy [weeks]	38.4	3.5	38.9	2.8	p=0.194
Birthweight [g]	3130	602	3158	416	p=0.673
Preeclampsia/ Eclampsia	7 (5.5%)		11 (8.6%)		p=0.237
Patients with any hypertensive disorder: BP sys max [mmHg]	142	19	149	23	p=0.206
Patients with any hypertensive disorder:: BP dia max [mmHg]	93	9	100	9	p=0.027
Pregnancy induced hypertension	5 (3.9%)		7 (5.5%)		p=0.829
Severe Preeclampsia/ Eclampsia	2 (1.6%)		8 (6.3%)		p=0.053

Main outcome parameters

BP=blood pressure, max=maximum, n=number of cases, p=level of significance

Serum levels of FAD were higher in women who received riboflavin supplementation when compared to patients of the placebo group (310 vs. 255ug/l, respectively, at 36 weeks gestation). This may be considered a compliance control, though results failed to reach statistical significance. Plasmatic levels of FAD in newborns were not influenced by the mother's intake of riboflavin or placebo (395 vs. 385 ug/l; n.s.).

IV. Discussion

Prevention of preeclampsia remains a desirable goal for obstetric practice, particularly for countries with limited resources. Nutritional intervention or supplementation with non-toxic compounds such as water soluble vitamins would constitute a highly attractive modality for such a significant medical problem. We studied the impact of high dose (15mg/d) riboflavin supplementation on the frequency and severity of preeclampsia in a high risk population. Our findings are in line with the results of a recent study[10], in which there was also trend towards a reduction in the overall number of preeclampsia cases in women recruited from a rural area in Burkina Faso (West Africa) who had been randomly selected to receive either riboflavin supplementation or placebo. In the present study, however, a reduction in the number of women with severe preeclampsia was demonstrated. It is possible that Neugebauer et al.[10] missed such an effect in their study based on a more limited follow-up. This could explain the relatively low preeclampsia rate in their study (3.6%) compared to reported frequencies of 12-16 % for similar patients populations[11] and could imply that many diagnoses may have been missed. Furthermore, it is likely that the nutritional situation in rural Burkina Faso is worse than in urban Venezuela. Actually plasmatic levels of riboflavin in our cohort (both groups) were high. Riboflavin supplementation given in a situation of empty body storages may not have been sufficient to show an effect on the reduction of the disease.

The mechanism by which riboflavin supplementation could contribute to the amelioration of a clinical course of preeclampsia or pregnancy-induced hypertension is still unclear, although several lines of thoughts exist. One mechanism may be the lowering of homocysteine levels, because higher homocysteine levels have frequently been observed in cases of preeclampsia, although a causal relation has not been proven[12]. In women with heterozygous or homozygous methylenetetrahydrofolate reductase (MTHFR) polymorphisms in which homocysteine accumulates due to an enzymatic defect, riboflavin supplementation as well as folate supplementation has been reported to help lower homocysteine levels by providing coenzyme functions. The coenzyme functions of riboflavin metabolites, FAD and FMN are also at the heart of a second hypothesis. Nitric oxide (NO), an important vasodilatator in the small vessel vasculature, is produced by nitric oxide synthase (NOS). All three isoforms of this enzyme (eNOS (endothelial), nNOS (neural) and iNOS (inducible form) depend on FAD or FMN as coenzymes and riboflavin deficiency could result in hypertension via a reduction of NOS activity and relatively lower NO levels in the vasculature[13].

Chappell et al. [5] initially found hints of a possibly successful prevention of preeclampsia in high risk cases through

ISBN: 978-1-61804-036-7

administration of high doses of antioxidative vitamins E and C; similar results were presented by Rivas-Echeverría et al. [14]. These reports sparked new hopes for the feasibility of preeclampsia prevention by nutritional supplementation. Unfortunately, this finding could not be verified in a larger prospective trial conducted by the same group of researchers[15]. A recent meta-analysis by the Cochrane Library[16] also failed to demonstrate preventative benefit in 566 women supplemented with vitamin E.

V. Conclusion

This randomized, placebo controlled, prospective pilot study of riboflavin supplementation showed a trend towards fewer cases of severe preeclampsia in the riboflavin group and lower blood pressures in women who did develop preeclampsia during riboflavin supplementation. Despite the limited sample size caused by a high drop-out rate, we feel that Vitamin B_2 supplementation may carry a promise for the prevention of preeclampsia. Larger studies, including other populations are needed to investigate this possibility.

Acknowledgment

The Authors would like to thank E. Hannak, A. Osuna and J. Uzcategui for technical and logistic support.

References

[1] Goldenberg RL, Iams JD, Mercer BM, Meis PJ, Moawad AH, Cooper RL, et al. The preterm prediction study: the value of new standard risk factors in predicting early and all spontaneous preterm births. Am J Pub Health. 1998; 88: 233-8

[2] Levine RJ, Thadhani R, Qian C, Lam C, Lim KH, Yu KF, et al. Urinary placental growth ractor and risk of preeclampsia. JAMA. 2005; 293:77-85

[3] Wacker J, Frühauf J, Schulz M, Chiwora F, Volz J, Becker K. Riboflavin deficiency and preeclampsia. Obstet Gynecol. 2000; 96: 38-44

[4] Andrew PJ, Mayer B. Enzymatic function of nitric oxide synthases. Cardiovasc Res. 1999; 43: 522-31

[5] Chappell LC, Seed PT, Briley AL, Kelly FJ, Hunt BJ, et al. Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. Lancet. 1999; 345: 810–6

[6] Wacker J, Schulz M, Frühauf J, Chiwora F, Solomayer E, Bastert G. Seasonal Change in the incidence of preeclampsia in Zimbabwe. Acta Obstet Gynecol Scand. 1998; 77: 712-6

[7] Fawzi WW, Msamanga GI, Urassa W, Hertzmark E, Petraro P, Willett WC, et al. Vitamins and perinatal outcomes among HIV-negative women in Tanzania. N Engl J Med. 2007; 356:1423-31

[8] Rivas-Echeverría C, Echeverría Y, Molina L. Program for the prevention of preeclampsia. Hypertension in Pregnancy. 2000; 19:157.

[9] Capo-Chichi CD, Feillet F, Gueant JL, Amouzou K, Zonon N, Sanni A, et al. Concentrations of riboflavin and related organic acids in children with protein-energy malnutrition. Am J Clin Nutr. 2000; 71: 978-86

[10] Neugebauer J, Zanré Y, Wacker J. Riboflavin supplementation and preeclampsia. Int J Gynecol Obstet. 2006; 93: 136-7

[11] Lydakis C, Beevers M, Beevers DG, Lip GY. The prevalence of preeclampsia and obstetric outcome in pregnancies of normotensive and hypertensive women attending a hospital specialist clinic. Int J Clin Pract. 2001; 55: 361-7

[12] Mignini LE, Latthe PM, Villar J, Kilby MD, Carroli G, Khan KS. Mapping the theories of preeclampsia: the role of homocysteine. Obstet Gynecol. 2005; 105: 411-25

[13] Myatt L, Brockman D, Langdon G, Pollock J. Constitutive calcium-dependent isoforms of nitric oxide synthase in the human placental villous vascular tree. Placenta 1993;14:373-83

[14] Rivas-Echeverría C, Echeverría Y, Molina L. Synergistic use of aspirin, fish oil and vitamins C and E for the prevention of preeclampsia. Hypertension in Pregnancy. 2000; 19:60.

[15] Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AH. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. Lancet. 2006; 367: 1145-54

[16] Rumbold AR, Maats FH, Crowther CA. Dietary intake of vitamin C and vitamin E and the development of hypertensive disorders of pregnancy. Eur J Obstet Gynecol Reprod Biol. 2005; 119: 67-71.