

The Response of Young and Adult Rats to the Riboflavin Supplementation

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ABSTRACT

The aim of this article was to study the response of young and adult rats on the supplementation of diet with riboflavin. Twenty-four young and adult normotensives (Wistar) male rats, subdivided into two groups: treated (10mg riboflavin/Kg of body weight) and control (receiving vehicle) were daily evaluated for physical and behavioural aspects. Systolic blood pressure was determined twice a week and liver toxicity was investigated at the end of treatment. Data were evaluated using one-way ANOVA and $p < 0.05$ was significant. There were no changes on general health aspects of the treated rats; however, the supplementation provoked a significant ($p < 0.05$) systolic blood pressure reduction.

Key words: Riboflavin, antioxidant, Wistar

INTRODUCTION

In an aerobic systems, it is essential the balance between the oxide-reducing agent and the antioxidant system. The free radicals generated endogenously as a direct consequence of the metabolism of O₂ and also in non-physiological situations, such as exposure to xenobiotics of the cell cause the incomplete reduction of O₂ (Ross et al., 1991).

In general, the cells are well armed with a number of defense mechanisms to prevent the accumulation of oxidative stress inducers (Marmol et al., 2007) which can be accomplished in two ways: eliminating the agents before it causes injury or repairing the damage occurred (Hebbel, 1986). However, these could be loss as a result of ageing process (Barja, 2002; Barja, 2004;

Camougrand and Rigoulet; 2001). This was confirmed in a number of studies such as Mendonza - Núñez et al., (2007), who comparing the healthy young and elderly individuals observed that the elderly group had lower levels of reduced-glutathione (GSH), one of the most important actors of the antioxidant defense system (Benzi et al., 1989). Besides that, food recordatory assays have demonstrated a significant reduction in the consumption of an antioxidant vitamin with increase in the age of healthy individuals (Olivieri et al., 1994). These findings closely associate aging to vascular and brain oxidative stress (Alpin et al., 1999).

On the other hand, Kripke et al., (1998) and Mastaloudis et al., (2001) suggested that the prevention of diseases by supplementation of an antioxidant supplements, such as vitamins A, C

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and B2 in healthy individuals of 45 years or more was not justified, considering the fact that before 60 years of age, there was no significant increase in oxidative stress. For riboflavin, Sanatelli *et al.*, (1988) reported EEG abnormalities by a child with epilepsy during long term of riboflavin supplementation, although there were no much evidences about it is toxicity.

It is also important to mention that riboflavin seems to have an ambiguous role on oxidative stress control. According to Massey (2000), flavins are thought to contribute to oxidative stress through their ability to produce superoxide, but at the same time, flavins are frequently involved in the reduction of hydroperoxides, products of oxygen-derived radical reactions.

In view of such controversy, there is an increasing interest to identify whether adult rats react to vitamins modulation in the diet compare to young rats. Therefore, the aim of this work was to investigate if supraphysiological supplementation of riboflavin was able to modulate the systolic blood pressure and general biological parameters of young and adult normotensives rats.

METHODOLOGY

Animals and supplementation

Twenty-four young (eight weeks) and adults (18 weeks) normotensives male Wistar rats obtained from the colony of bioterium of the Federal University of the State of Rio de Janeiro, were kept in cages in individual metabolic control conditions: temperature (21 ± 2 °C), humidity ($60 \pm 10\%$), air exhaustion cycle (15 min/h) and 12 h–dark/light cycle (artificial lights, 7 a.m.–7 p.m.) and fed a standard diet Nuvilab (Nuvital®) plus water ad libitum. After a baseline period of 10 days, the rats were subdivided into groups with n=6 in each: control and treated. The treated groups received riboflavin (R4500-Sigma®) at 10mg/Kg of body weight by oral gavage during two weeks and control groups received vehicle (water). All the procedures were carried out in accordance with the conventional guidelines for experimentation with animals (NIH Publication No. 85–23, revised 1996). The experimental protocols used in this study were approved by the Ethics Committee for Animal Experimentation at the Federal University of Rio de Janeiro State.

Physiological parameters

The animals maintained in metabolic cages were submitted to a daily evaluation of water and food intake, body weight, diuresis and physical aspects: distribution and coloring of hair; bleeding, stains, cracks, opacification and colouring of mucous. The behavioural aspects and motor-sensory parameters were also investigated following Whishaw and Kolb methodology (Whishaw and Kolb, 2005). Systolic blood pressure was determined through non-invasive method of plethysmography (Vianna *et al.*, 1992).

Statistical analysis

Data were evaluated using the student's t test and analysis of variance one-way ANOVA, $p < 0.05$ was considered significant.

Toxicity

Under deeper anesthesia: eter inhalation plus thiopental sodium (25 mg/kg) via intraperitoneal, the rats were submitted to an incision and removal of the liver which was submersed on physiological saline solution and the liver weight was determined following the method of Sherle. (Scherle, 1970). The results were expressed as weight of organ (g)/100g of body weight of animal.

RESULTS

The supplementation did not alter overall physiological parameters of young and adult rats. There were no presence of ataxia or convulsions under riboflavin supplementation. The sensory-motor response was obtained with values ranging from 1 min, and it was no age dependent. The macroscopic evaluation of liver confirmed the absence of riboflavin's toxicity under supraphysiologic doses for both groups (Table 1). The control young group had blood pressure around 102.08 ± 1.64 mmHg and the treated group from 97.8 ± 1.2 (baseline period) to 88.27 ± 1.12 mmHg (treatment period) (Fig. 1). On the other hand, the adult rats presented a smaller reduction; however, it was also significant ($p < 0.05$). The systolic blood pressure of control group was 105.9 ± 1.55 and the treated group varied from 105.2 ± 0.83 (baseline period) to 99.8 ± 1.65 mmHg (treatment period) (Fig. 2).

Table 1 - Effects of riboflavin on biological parameters of young and adult Wistar rats. The values represent the mean \pm SD of 6 animals (control) and 6 animals (treated) for both groups.

Physiological Parameters	Groups	Body Weight	Diuresis	Food intake	Water intake	Liver Weight
		(g)	(ml)	(g)	(ml)	(g/100g bw)
Younger Rats	Control	320,96 \pm 24,15	6,24 \pm 1,53	19,67 \pm 2,03	28,53 \pm 3,55	4,11 \pm 0,32
	Treated	356,51 \pm 16,53	5,77 \pm 1,82	22,07 \pm 1,07	28,12 \pm 1,54	3,73 \pm 0,18
Adult Rats	Control	272,42 \pm 15,99	4,67 \pm 2,23	16,94 \pm 2,14	26,62 \pm 3,63	3,48 \pm 0,21
	Treated	275,91 \pm 14,92	6,68 \pm 5,41	19,09 \pm 3,04	29,97 \pm 6,45	3,31 \pm 0,18

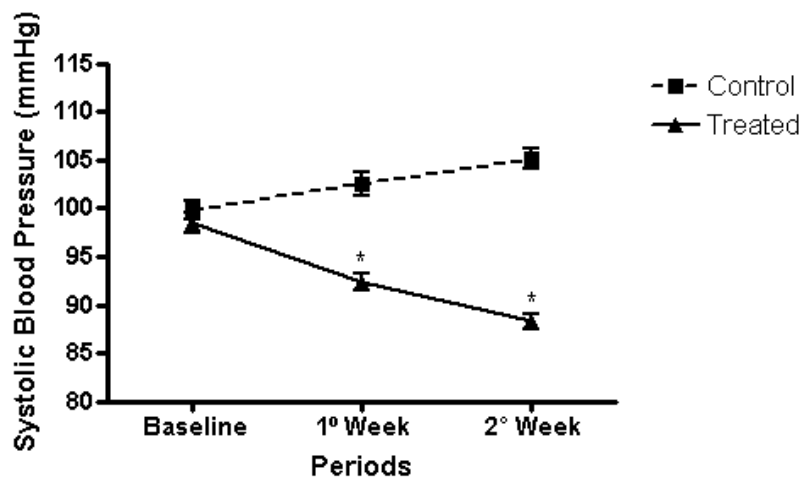


Figure 1 - Effects of supplementation of riboflavin on systolic blood pressure of Wistar young group. The values represent the mean \pm SD. * $p < 0,05$.

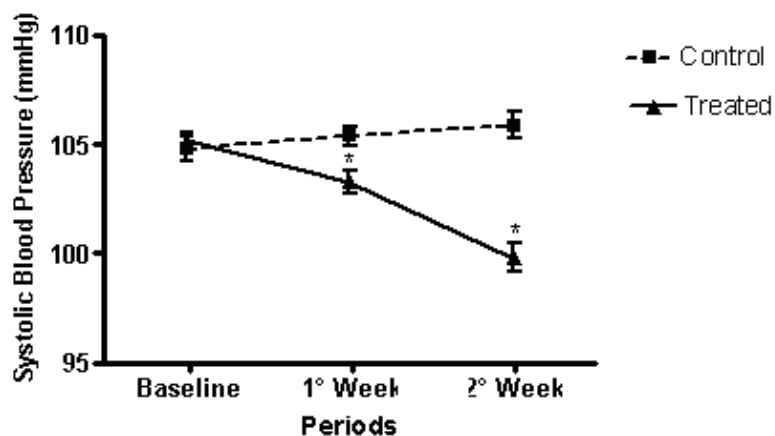


Figure 2 - Effects of supplementation of riboflavin on systolic blood pressure of Wistar adult group. The values represent the mean \pm SD. * $p < 0,05$.

DISCUSSION

During old age, generation of free radicals seems to be more pronounced. Such a biochemical alteration might increase the risk of oxidative damage to macromolecules (DNA, proteins, carbohydrates and lipids), favoring the presence or complications of a great number of acute and chronic diseases: diabetes mellitus, atherosclerosis, hypertension, acute myocardial infarct, stroke and ischemia-reperfusion (Hicks et al., 1996). Human cells suffer from 10,000 hits of free radicals daily. This number increases with aging and added to certain pro-oxidant factors such as smoking, alcohol addict, accentuated physical activity, inadequate nutrition, masculine gender, drug consumption and psychological stress determine the genes of chronic diseases during old age (Ames et al., 1993; D'Almeida et al., 1998).

In this study, the supraphysiological supplementation did not provoke the side effects. Indeed, there were no signs of neurological damage since the response to sensory-motor test was faster around 1 min instead of that usually presented by the neurologically compromised animals: 2-4 min (Carstens et al., 1993). The treatment was clearly able to induce the systolic blood pressure reduction on both groups. However adult rats were more resistant to the supplementation, reaching a systolic blood pressure reduction of 6 mmHg versus 17 mmHg presented by the younger rats. Such lesser response by old rats could be associated to the evidence that efficiency of an antioxidant system decreases during the ageing (Jones et al., 2002).

The physiological role of riboflavin shown here could probably be attributed to its chemical structure: the presence of isoalloxazine ring (Fig. 3) (Colibus and Mattevi, 2006). Such chemical configuration gives to this vitamin high affinity to react with various substrates, mainly molecular oxygen (Massey, 1994; Mattevi, 2006). Even

redox / ionic / electronic state of flavin has different chemical properties that can be adjusted by the means of protein environment (hydrogen bonds, hydrophobic interactions...) (Colibus and Mattevi, 2006). Flavin-dependent oxidases use dioxygen as an electron acceptor to generate hydrogen peroxide, whereas monooxygenases / hydroxylases use dioxygen to insert an oxygen atom to the substrate. The reaction catalyzed by the monooxygenases involves the formation of flavin-peroxide intermediate, which results from the addition of molecular oxygen of the C4a atom of the flavin (Fig. 4).

Flavoenzymes are known to take part in an ever-growing number of cellular processes and it is becoming evident that their function is not restricted to energy metabolism. The structural and functional diversity of flavin-dependent enzymes is astonishing and reflects the chemical versatility of the flavin molecule. Furthermore, advances in enzymological, biophysical and structural methods, with more powerful computational techniques, will provide new tools to address many open questions (Shi et al., 2006). In the future, probably the mechanistic properties of the flavin, especially in relation to the role of tunneling (Liang and Klinman, 2004), the mechanism of electron transfer from and to the flavin (Toogood et al., 2004; Leys et al., 2003), and the modulation of reactivity to oxygen, can be finally understood.

This study was an important point of departure, as it showed a link between riboflavin supplementation and its physiological effect on blood pressure modulation, an important parameter to evaluate the health status. On the other hand, a number of analyses should be done in order to identify if the effect of riboflavin is associated exclusively to its chemical structural characteristic or can be associated to its role on metabolic pathways.

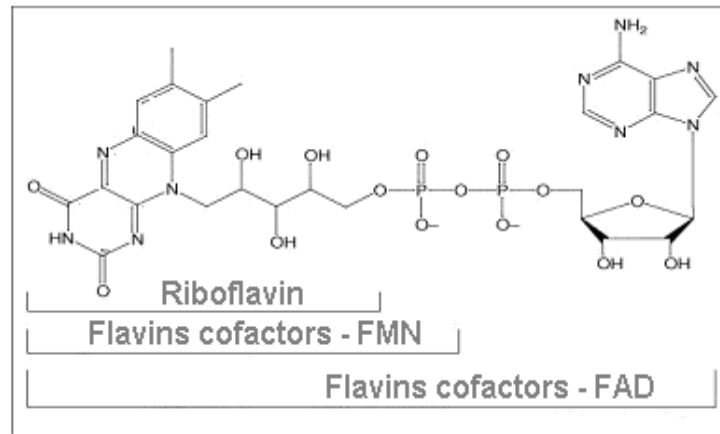


Figure 3 - Isoalloxazine ring of riboflavin and its flavin cofactors.

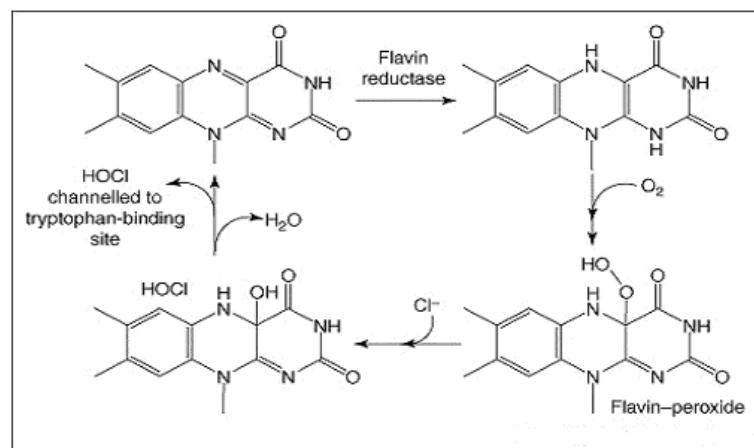


Figure 4 - Reaction mechanism between riboflavin and molecular oxygen.

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RESUMO

O processo do envelhecimento e alguns transtornos, incluindo hipertensão, foram estreitamente associados ao estresse oxidativo. Em relação à riboflavina (vitamina B2), existe uma possibilidade de que suas propriedades antioxidantes podem contribuir para controlar esse evento. Assim, esse estudo utilizou vinte e quatro ratos machos jovens e velhos normotensos (Wistar), sendo subdivididos em dois grupos: tratado (riboflavina 10 mg / kg de peso corporal) e

o controle (recebendo veículo). Foram avaliados diariamente aspectos físicos e comportamentais. A pressão arterial sistólica foi determinada duas vezes por semana e a toxicidade hepática foi investigada no final do tratamento. Os dados foram avaliados usando ANOVA one-way e $p < 0,05$. A suplementação não alterou os aspectos de saúde geral dos ratos tratados, no entanto, a suplementação provocou uma significativa ($p < 0,05$) redução da pressão arterial sistólica.

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