

Favorable effects of pyridoxine and folic acid supplementation of shr-sp

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EFFECTOS FAVORABLES DE LA PIRIDOXINA Y EL ÁCIDO FÓLICO ADMINISTRADOS A SHR-SP

RESUMEN

La homocisteína se ha asociado a la enfermedad vascular cerebral (EVC), que realza el interés para la investigación sobre el papel de las vitaminas implicadas en su metabolismo. Ratas Spontaneously Hypertensive Stronke-Prone Rats SHRSP fueron tratados con piridoxina y el ácido fólico en las dosis correspondientes a cinco, diez y veinte veces sus necesidades alimenticias. La presión arterial sistólica y los parámetros generales fueron analizados y comparados al grupo de control. Los correspondientes de las dosis a 10 y 20 veces RDA promovieron un efecto hipotensor, pero los correspondientes a las dosis a 5 veces, no. Los animales tratados en 190 días de la edad presentaron una reducción significativa de la presión arterial sistólica cuando está comparado al grupo de control. Los animales bajo tratamiento no presentaron las muestras de enfermedad cerebral detectadas naturalmente en ese acoplamiento. Nuestros resultados sugieren que las vitaminas B6 y B9 podrían ser beneficiosas para la terapia preventiva del EVC.

Palabras clave: enfermedad vascular cerebral, SHRSP, piridoxina, ácido fólico.

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ABSTRACT

Homocysteine has been associated to the stroke, which enhances the interest for the investigation about the role of vitamins involved on its metabolism. Stroke Prone rats were treated by pyridoxine and folic acid at doses correspondent to five, ten and twenty times their nutritional needs. The systolic blood pressure and general parameters were analyzed and compared to control group. The doses correspondents to 10 and 20 times RDA promoted a hypotensive effect, but doses correspondents to 5 times, did not. The treated animals at 190 days of age presented a significant reduction of systolic blood pressure: 204 ± 3 mmHg when compared to control group: 230 ± 5 mmHg. At seven months of age the animals under treatment did not present signs of stroke naturally detected in that linkage. Our results suggest that vitamins B6 and B9 could be beneficial for preventive therapy of stroke.

Key words: SHRSP, stroke, folic acid, pyridoxine

The cerebrovascular disease is any abnormality of the brain resulting from the pathologic process of the blood vessels e.g. occlusion of the lumen by a thrombus or embolus, rupture of the vessel, an altered permeability of the vessel wall and an increased viscosity or other change in quality of blood that can lead to an infarction of the brain or spontaneous hemorrhage into the brain or subarachnoid space^{1,2}. It's a leading cause of mortality and disability in Western countries, particularly in the elderly³.

Increasing data suggest that oxidative stress plays detrimental roles in cerebrovascular disease. Ischemia and reperfusion can induce an excess of free radicals that can lead to oxidative modification of protein, lipid and DNA molecules. The concept of neuro-protection involves inhibition of a cascade of

pathological molecular events occurring under ischemia and leading to calcium influx, activation of free radical reaction and cell death^{4,5,6,7}.

Plasma homocysteine level is a strong independent risk factor for vascular disease, such as stroke,^{8,9} and several studies have linked homocysteine to blood pressure, specially systolic blood pressure¹⁰⁻¹². The mechanism by which homocysteine promotes vascular disease includes increased thromboses, consumption of nitric oxide and endothelial injury, and it can be considered a hydroxy radical producer^{13,14,15}.

Folate and Pyridoxine are important regulators of the metabolism of homocysteine in the body¹⁶, and studies have shown an inverse relationship between levels of these factors and levels of homocysteine in the blood¹⁷. In addition, randomized controlled trials have demonstrated that folate supplementation reduce blood levels of homocysteine, decreasing the risk for stroke. In the same way, in previous studies we showed that folate and pyridoxine combines supplementation reduced systolic blood pressure in SHR rats¹⁸.

Therefore, the main goal of this study was to evaluate the effects of different doses of combined therapy of folic acid and pyridoxine on general biological and neurological parameters including blood pressure in stroke prone models.

MATERIAL AND METHODS

2.1 Animals and diet

Experiments were carried out using 12 male spontaneously hypertensive stroke-prone rats (SHR-SP) at 18 weeks of age. The animals maintained in Metabolic cages at bioterium with controlled: temperature (22° C), humidity (60± 10%), air exhaustion Cycle (15min/h) and light /dark cycle of 12 hours. Received Nuvilab rat chow (Nuvital Co) and water ad libitum. After ten days of basal period the animals were divided in two groups: control (n=6) and treated (n=6). Treated group received vitamin B6 (pyridoxine) plus B9 (folic acid) diluted in water by orogastric gavage at doses correspondent to five, ten and twenty times their nutritional needs. The animals from control group received water by orogastric gavage in order to maintain the same stress as the treated group was submitted. The doses were established according to the nutritional needs determined by the Committee on Nutrition for Laboratory Animal Diet from the National Academy of Sciences of USA. Rats were studied for 12 weeks and each four weeks the dose was changed. All procedures were carried out in accordance with the conventional guidelines for experimentation with

animals (NIH Publication N°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.

2.2 Biological Parameters

The general condition of the animals was examined every day for the 12 weeks. The animals were weighed on the day before starting and thereafter twice a week during this assay. Water intake and food consumption were measured daily and urine output as well.

The systolic blood pressure was measured twice a week by plethysmography. The average of 3 pressure readings was obtained.

Daily, the general neurological parameters were analyzed. Spontaneous mobility was evaluated by allowing the animals to walk freely for 60 s. Under these conditions, the mobility of the animals was scored as follows: normal locomotion = 0, wide or tight circling towards the paretic side =1. Signs of ataxia are most frequently observed in this test.

The sensory test was done by San Diego instruments (USA). The SDI Tail Flick Analgesia Meter measures pain sensitivity in rats as they respond to the application of heat to a small area of their tails. The animal's tail is placed over a window located on the Tail Flick platform. A foot switch activates an intense light beam to heat the tail at a reliable, reproducible rate.

When the rat feels discomfort, it flicks its tail which automatically stops the timer. The reaction time from activation of the light beam to the tail flick is automatically presented on a digital display.

2.3 Data analysis

The differences between group mean values were evaluated by Student's t-test for an unpaired observations. Values were considered to be significantly different when P value was less than 0.05.

RESULTS

Folic acid and pyridoxine at dose 5 times RDA did not have significant hypotensive effect at four weeks of treatment (data not shown). However, from the week 4 to 8, the treated group under supplementation at 10 times RDA (second dose) had a significant ($p<0,05$) reduction of systolic blood pressure ($H_{>19\pm7mmHg}$).

On the other hand, the highest dose (20 times RDA) did not provoke a sharp decrease of systolic blood pressure, but maintained it at $204\pm3mmHg$ when compared to $230\pm5mmHg$ of control group at

the same age.

Signs of toxic effect were not observed in this study, even when high doses were used (date not shown).

During this assay, tests of spontaneous mobility recognized in five rats from control group recurrent signs of transient ischemic attack, characterized by abnormality of balance, coordination and behavior changes. During acute phase, loss of body weight was also observed. One rat of treated group presented sign of ataxia at doses correspondent to 10 times RDA, however, at 20 times RDA there was no attack anymore.

The sensory motor test showed that those rats not treated presented a significant ($p < 0,05$) increase Time of response: 1.40 seconds versus 0.80 seconds (treated group).

In the same way, at 30 weeks of age, the majority of those rats under treatment did not present signs of stroke, however, 83% of rats from control group presented a transitory ischemic attack.

DISCUSSION

Once the blood supply to an area of the brain is blocked, there are a series of events over time that leads to the death of brain tissue. The aim of the treatment is to prevent or slow this cascade of events, and to restore normal brain flow as quickly as possible. In this assay, the maneuvers able to reduce the hypertension, which is one of the major risk factors of stroke and vascular dementia are welcome.

In the same way, an imbalance between production and scavenging of superoxide anion which also results in hypertension even by the inactivation of nitric oxide or an increased oxidative stress from the resultant peroxy nitrite produced promotes an inflammatory process attributed to the stroke event as well. Increasing values of plasma homocysteine has been associated to the oxidative stress and hyperhomocysteinemia is one of the newly recognized independent risk factors of stroke^{15,16,17,18}.

The spontaneously hypertensive rat stroke prone, a recognized model of heritable hypertension-associated cerebrovascular injury^{19,20}, seems to present high plasma and tissue homocysteine levels.

Therefore, it raises some questions whether the supplementation of B6 and B9 vitamins involved on homocysteine metabolism would be a benefit on decreasing systolic blood pressure^{21,22}.

In the present study, the stroke prone model did not respond to the therapy with doses corres-

pondent to five times their nutritional needs, as observed in SHR model¹⁸ which suggests that SHR-SP might be more resistant to the therapy in general. Indeed, Nabika, Cui and Masuda (2003) had already demonstrated a different susceptibility between those linkages²³.

On the other hand, the SHR-SP were responsive to doses correspondent to ten times their RDA, when we were able to observe a significant reduction of systolic blood pressure.

Additionally, the supplementation also prevented the stroke in 83.3% of the rats, since some signs such as ataxia, loss of balance, partial paralysis were not detectable. At the same time, our experiment showed that the neurological tests, here applied, were trustworthy identifying the early ischemic events.

The treatment clearly protected the animals against the stroke since this event naturally expected at 18 weeks of age, according to literature, was postponed to 30 weeks of age and was not observed in those treated animals.

In general, B6 and B9 at supraphysiological doses might provoke toxic effects²⁴, however, in the present assay, the combined therapy even with doses at 20 times RDA, curiously did not provoke signs of toxicity.

Regarding the antihypertensive effect here reported, our findings suggest that combined pyridoxine and folic acid therapy was effective on the control of systolic blood pressure and should be thought of as one of the alternative therapies to prevent stroke.

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