Effect of a dietary combined vitamin C and E supplementation on the liver, kidneys and brain arginase activity in non-pregnant and pregnant rats with streptozotocin-induced diabetes

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ABSTRACT

As nitrogen metabolism is exacerbated during diabetes mellitus or/and pregnancy, and because arginase catalyses the arginine hydrolysis into ornithine and urea, this enzyme activity as well as the ornithine content were investigated in tissues (liver, kidney and brain) from pregnant or non pregnant streptozotocin (STZ) diabetic rats, dietary supplemented or not with antioxidant vitamins (C and E). Sixty 12 week old female Wistar rats were randomly divided into 6 equal groups according to their gestational status and the dietary treatments. The groups 1 (non pregnant) and 2 (pregnant) received only intraperitoneal injection of citrate buffer and served as controls, whereas the animals of the groups 3 (non pregnant) and 4 (pregnant) were treated with STZ (40 mg/kg) and those of the groups 5 (non pregnant) and 6 (pregnant) were submitted to STZ administration and to a dietary combined ascorbate and -tocopherol acetate (1g and 600 mg/kg food respectively) supplementation for 20 days. Except for the kidneys from not supplemented diabetic rats in which the arginase activity and the ornithine content were significantly increased in pregnant rats, no significant difference was evidenced between pregnant and non pregnant females. The arginase activity and the ornithine content were dramatically enhanced in the liver of diabetic animals (pregnant or not) (p < 0.001), whereas these 2 parameters were reduced in kidney and in brain, particularly in the non pregnant females. By contrast, in supplemented animals, tissue enzyme activities and ornithine quantities were similar to those observed in healthy rats, showing that vitamin C and E oral treatment significantly attenuate nitrogen perturbations linked to diabetes. These results suggest that the oxidative stress would participate to the development of metabolic disorders and organ dysfunctions observed in diabetes mellitus.

Key words: Diabetes, Arginase, Ornithine, Vitamin C and E combination, Pregnancy.

RÉSUMÉ:

Effets de l’addition simultanée de vitamines E et C dans l’alimentation sur l’activité de l’arginase dans le foie, le rein et le cerveau de rats gestantes ou non rendues diabétiques par la streptozotocine.

Comme le métabolisme azoté est exacerbé durant le diabète sucré et/ou la gestation, et puisque l’arginase catalyse l’hydrolyse de l’arginine en ornithine et en urée, cette activité enzymatique et la teneur en ornithine ont été mesurées dans les tissus (foie, rein et cerveau) de rats rendus diabétiques par la streptozotocine (STZ) et supplémentées ou non par des vitamines anti-oxydantes (vitamines C et E). Soixante rats Wistar âgées de 12 semaines ont été réparties en 6 groupes égaux selon leur état de gestation et les traitements reçus. Les groupes 1 (femelles non gestantes) et 2 (femelles gestantes) n’ont reçu qu’une injection intra péritonéale de tampon citrate et ont servi de contrôles, tandis que les animaux des groupes 3 (non gestants) et 4 (gestants) ont été traités par la STZ (40 mg/kg) et ceux des groupes 5 (non gestants) et 6 (gestants) ont reçu une administration de STZ et une alimentation enrichie en ascorbate et en acétate d’-tocophérol (1g et 600 mg/kg d’aliment respectivement) pendant 20 jours. A l’exception des reins prélevés sur les animaux diabétiques non supplémentés dans lesquels l’activité arginase et la quantité d’ornithine ont été significativement augmentées lors de la gestation, aucune différence significative n’a été constatée entre les femelles gestantes ou non. En revanche, l’activité arginase et la teneur en ornithine ont été considérablement augmentées dans le foie des animaux diabétiques gestants ou non (p < 0.001) alors qu’une diminution de ces 2 paramètres a été observée dans le rein et le cerveau, particulièrement chez les femelles non gestantes. Chez les rats supplémentés, les activités enzymatiques et les concentrations en ornithine dans les 3 tissus ont été semblables à celles mesurées chez les contrôles, ce qui montre que le traitement oral en vitamines anti-oxydantes atténuait significativement les perturbations du métabolisme azéotiles liées au diabète sucré. Ces résultats suggèrent qu’un stress oxydatif pourrait participer au développement des altérations métaboliques et des dysfonctionnements organiques inhérents.

Mots-clés: diabète sucré, arginase, ornithine, vitamines C et E, gestation.

Introduction

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) catalyses the hydrolysis of L-arginine to L-ornithine and urea in the final step of the urea cycle and is therefore present mainly in the liver [33]. Much lower arginase activity is found in other organs such as kidney in which the urea cycle is not complete [1]. During pregnancy, the hepatic formation of urea is depressed [25] leading to a reduced urinary excretion [17]. This metabolic particularity would result from a down-
regulation of arginase or from a preferential α-amino acids utilisation for foetus growth in pregnant females.

The most frequently studied antioxidant vitamins are vitamins C and E. Vitamins C and E have been given to normal and pregnant diabetic rats, leading to a decrease in the oxidative stress [20, 26, 31, 36, 37]. Combination of vitamins C and E can also be safely used in high doses in prevention of oxidative stress in diseases such diabetes (non-pregnant and pregnant rats) and cardiovascular disease [10, 27, 28, 30, 38]. Several studies have evidenced some relationships between vitamin E and arginase. PARK and TAPPEL [32] reported that rats fed with a vitamin E supplemented diet had a lower liver arginase activity than those fed with a vitamin E deficient diet. Moreover, dietary vitamin E administration significantly reduced the liver arginase activity increase induced by high doses of prednisolone in rats [12]. In addition, it has been established that vitamin C stabilizes the arginase activity [3, 4].

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. Insulin dependent diabetes mellitus (IDDM) is characterized by a series of complications that affect many organs. Human diabetic patients also suffer from a wide variety of complications such as atherosclerosis, retinopathy, liver and kidney damage [9]. Increased activity of liver arginase was demonstrated in diabetic animals [2, 6, 13, 14, 22, 40, 43]. However, whether vitamin C and E supplementation could modify arginase and the ornithine values in diabetic rats is currently unknown. Therefore, we decided to investigate effects of vitamin C and E supplementation on arginase activity and ornithine content in non-pregnant and pregnant diabetic rats with streptozotocin - induced diabetes.

Material and methods

CHEMICALS

All chemicals were obtained from Sigma Chemical Inc. (St. Louis, MO, USA) and all organic solvents from Merck Chemical Inc. (Darmstadt, Germany) except vitamin C and E. The oral form of vitamin C (ascorbic acid) and vitamin E (d-l-α-tocopheryl acetate) was obtained from F. Hoffman La Roche (Istanbul, Turkey). Streptozotocin (STZ) was purchased from SERVA GmbH (Heidelberg, Germany). All reagents were in analytical grade.

ANIMALS AND DIETS

The Medical Faculty Experimentation Ethics Committee of our university approved the experimental procedure of the study. At the beginning of the study, thirty non pregnant and thirty pregnant female Wistar rats (12 week old, weighing 150-165 g) bred in our laboratory were used. They were housing individually in stainless-steel cages in a pathogen-free University Laboratory Animal Research facility at 22-24°C with light from 08.00 to 20.00 with free access to water. All animals were fed with a commercial diet (Elazig Feed Factory, Elazig, Turkey) including the ingredients shown in Table 1 during the experiment.

Table 1: Diet composition of 12 week old Wistar rats.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Proportion, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>25.1</td>
</tr>
<tr>
<td>Barley</td>
<td>20.2</td>
</tr>
<tr>
<td>Soybean</td>
<td>36.0</td>
</tr>
<tr>
<td>Wheat</td>
<td>9.7</td>
</tr>
<tr>
<td>Fish flour</td>
<td>3.2</td>
</tr>
<tr>
<td>Meat-bone flour</td>
<td>2.4</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>1.7</td>
</tr>
<tr>
<td>Salt</td>
<td>1.1</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.2</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin/mineral mix*</td>
<td>0.2</td>
</tr>
<tr>
<td>Vitamin A 12 000 000 IU; vitamin C 50 mg; vitamin D₃ 400 000 IU; vitamin E 30 mg; vitamin K₃ 2.5 mg and vitamins B₆ 3 mg; B₂ 7mg; B₄ 4 mg and B₃ 15 mg; nicotinamide 4 mg; calcium-D pantothene 8 mg; Folic acid 1 mg; biotin 45 mg; folic acid 1 mg; Mn 80 mg; Fe 40 mg; Zn 60 mg; Cu 5 mg; I 0.4 mg; Co 0.1 mg; Se 0.15 mg and antioxidant (butyhydroxytoluol) 30 mg. The Vitamin C and E supplemented diet contained extra an ascorbic acid (1 g) and dl-α-tocopheryl acetate (600 mg) combination per kg food.</td>
<td>6 In per kg mixture: vitamin A 12 000 000 IU; vitamin C 50 mg; vitamin D₃ 400 000 IU; vitamin E 30 mg; vitamin K₃ 2.5 mg and vitamins B₆ 3 mg; B₂ 7mg; B₄ 4 mg and B₃ 15 mg; nicotinamide 4 mg; calcium-D pantothene 8 mg; Folic acid 1 mg; biotin 45 mg; folic acid 1 mg; Mn 80 mg; Fe 40 mg; Zn 60 mg; Cu 5 mg; I 0.4 mg; Co 0.1 mg; Se 0.15 mg and antioxidant (butyhydroxytoluol) 30 mg. The Vitamin C and E supplemented diet contained extra an ascorbic acid (1 g) and dl-α-tocopheryl acetate (600 mg) combination per kg food.</td>
</tr>
</tbody>
</table>

INDUCTION OF DIABETES AND PREGNANCY

After 2 week acclimatization, diabetes was induced (day 0) in female rats with streptozotocin (STZ) using a previously described protocol [26]. STZ was intraperitoneally (i.p.) administered at a dose of 40 mg/kg body weight dissolved in citrate buffer (0.1 M, pH 4.5). Control rats received i.p. citrate buffer. Blood glucose was measured 10 days after induction of diabetes. Diabetes was defined as a blood glucose concentration >20 mmol/L. Ten days after STZ or citrate buffer injection the female rats were mated overnight with non-diabetic male rats. The morning on which sperm was found in the vaginal smear was designated as the gestational day 0.

This experimental study was composed of six groups of ten animals: the first and second groups (receiving citrate buffer injection) were used as non-pregnant and pregnant controls. The third and fourth groups (receiving STZ injection) were the non-pregnant and pregnant diabetic groups. The animals of the fifth (non-pregnant) and sixth (pregnant) groups received STZ injection and antioxidant vitamin C and E (1 g ascorbic acid and 600 mg dl-α-tocopheryl acetate/kg food) supplementation for 20 days. Both pregnant and non pregnant animals were anaesthetized with ether for 5 minutes then slaughtered on day 20 and then the liver, kidneys and brain were collected from all animals.

BIOCHEMICAL ANALYSIS

Organs were excised and rinsed in cold saline (0.9 % NaCl). The tissues were weighed and homogenized with 10 volumes...
EFFECTS OF VITAMIN C AND E COMBINATION ON ARGINASE ACTIVITY IN DIABETIC RATS

Results

The arginase activity was maximal in the liver and minimal in the brain in all groups. The highest ornithine contents were found in kidneys whereas values measured in the liver and in the brain were roughly similar (Table 2). Dramatic increases of the arginase activity and ornithine content were observed in the liver from not supplemented diabetic animals compared to the controls (p<0.001). By contrast, the arginase activities in kidney (p<0.001) and in brain (p<0.05) were significantly depressed in non pregnant diabetic females compared to the non pregnant controls. In pregnant diabetic females, the kidney arginase activity was also significantly lowered (p<0.001) while the enzyme activity in the brain was quite similar to that measured in pregnant healthy rats. The ornithine concentrations in kidney and in brain seemed to be lowered in not supplemented diabetic animals (groups 3 and 4) compared to the controls although only significant differences were evidenced in kidney between non pregnant females (p<0.05) and in brain between pregnant animals (p<0.01) (Table 2).

On the other hand, tissue arginase activities and ornithine quantities were closely related to the corresponding control values (groups 1 and 2) in pregnant or not diabetic animals which received vitamin C and E supplementation (groups 5 and 6) (Table 2).

Pregnancy did not modify the tissue arginase activities and the ornithine concentrations in control rats. But, the arginase activity and the ornithine content were markedly increased in kidney from pregnant not supplemented diabetic animals (group 4) compared to non pregnant diabetic females (group 3) (p<0.001) whereas these 2 parameters measured in the liver and in the brain were identical in the both groups. No significant difference was evidenced between pregnant and non pregnant diabetic females supplemented with vitamin C and E (groups 5 and 6).

Discussion

Glucose uptake by hepatic cells is assured by a specific protein carrier, which the expression onto cell membranes is dependent of insulin [11]. Consequently, during diabetes mellitus, glucose transport into hepatic cells is defective, but compensatory mechanisms such gluconeogenesis from glycerol and α-amino acids increase in diabetic animals. The deamination of amino acids is catalyzed by many enzymes (aminotransferase and glutamate dehydrogenase), and produces carbon skeletons that are subsequently used as an energy source in diabetes, whereas the residual ammonium ions are excreted through urea formation. Increased food consumption, amino acid metabolism, and increased ratio blood glucagon/insulin occur during IDDM and would increase the urea cycle activity. The liver arginase activity is

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>Not supplemented diabetic rats</th>
<th>Vitamin C and E supplemented diabetic rats</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginase</td>
<td>256.03±23.74a</td>
<td>276.40±18.83a</td>
<td>608.86±107.74b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ornithine</td>
<td>35.46±4.17a</td>
<td>32.49±2.11a</td>
<td>104.33±21.21b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginase</td>
<td>15.22±1.08b</td>
<td>18.71±1.81b</td>
<td>11.84±1.24b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ornithine</td>
<td>82.64±4.23b</td>
<td>92.49±3.54b</td>
<td>75.19±9.21b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginase</td>
<td>1.17±0.24b</td>
<td>0.76±0.20a</td>
<td>1.17±0.29b</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ornithine</td>
<td>26.43±0.94bc</td>
<td>27.54±2.75a</td>
<td>19.33±2.44abc</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values with different superscripts within the same row, were statistically significant.

Table 2: Effect of vitamins C and E combination on arginase activity (units/mg of protein) and ornithine content (nmol/mg of protein) in liver, kidneys and brain in non-pregnant and pregnant diabetic rats treated by streptozotocin (40mg/kg i.p.) and in the respective controls.
increased in diabetic animals [2, 6, 13, 14, 22, 40, 43]. In our study, this enzyme activity is markedly enhanced (by around 200%) in non-pregnant and pregnant diabetic rats, probably in response to the high rate of protein catabolism [39, 41].

However, the activation of hepatic arginase was related to an increase of free Mn$^{2+}$ concentration in the liver [6, 7, 14]. This oligo-element is a cofactor for arginase and modifies the enzyme conformation [18, 35] and its susceptibility to proteolysis [5, 35]. BOND et al [6] have demonstrated that liver arginase protein amounts were similar in control and in diabetic rats, whereas the enzyme activity was dramatically increased in diabetic rats. But, UPADHYAYA et al. [43] have reported that liver arginase mRNA and protein contents were greater in diabetic rats than in controls. Paradoxically, the arginase activity diminished in the pancreas [23] and in the kidneys [13] from diabetic rats. In the present study, significant decreases of the enzyme activity were also evidenced in the kidney and in the brain of non pregnant STZ treated rats. On the contrary, UPADHYAYA et al. [41] have noticed an enhancement of arginase activity in diabetic kidney. The differential tissue expression of arginase could be partially explained by the existence of two distinct structural gene loci. One of these 2 loci is responsible for the arginase expression in the liver, while the second expresses arginase in insulin-nondependent tissues such as brain and kidney [16]. Consequently, the lack of insulin would probably cause directly or indirectly an adverse regulation of these 2 loci: up regulation of the first, and down regulation of the second. In extra hepatic tissues, arginase is also involved in the polyamine biosynthesis [29], by providing ornithine for subsequent formation of putrescine [45] and for other metabolic purposes (glutamate/proline pathway) [19]. The results of this study indicate that ornithine concentrations in the tissues are also affected by streptozotocin-induced diabetes in rats and ornithine concentrations are coincident with the activities of arginase in the tissues.

Arginase activities are reported to be increased in liver, kidney, small intestine, myometrium and stomach with advancing pregnancy [34, 44]. However, we found in the present study that pregnancy had no marked effect on tissue arginase activities in healthy or in the diabetic rats except in the kidney of diseased animals. Nevertheless, albeit these divergent results, the previously reported decreases of uraemia and of urinary urea excretion during pregnancy are probably not directly linked to tissue arginase activity variations. The preferential use of $\alpha$-amino acids for foetal protein synthesis would probably induce the reduction of protein catabolism and nitrogen excretion in the maternal organism.

Vanadate and insulin treatments were found to restore the increased activity of liver arginase nearly to the control values [42]. In the same way, serum arginase activity was increased in alloxan treated rats, and L-arginine treatment could normalize enzyme activity [24]. MENDEZ and ARREOLA [23] reported that the pancreatic arginase activity and the putrescine concentration diminished under alloxan treatment but they were markedly elevated by L-arginine in the diabetic group.

PARK and TAPPEL [32] reported that rats fed with a vitamin E supplemented diet for 40 days had lower liver arginase activity than those fed with a vitamin E deficient diet. High doses of prednisolone induced increases of liver arginase activity in rats but did not affect renal activity. A dietary vitamin E administration significantly reduced the hepatic activity and paradoxically increased renal arginase activity in prednisolone-treated rats [12]. Similarly, the combined administration of vitamins C and E to streptozotocin-induced diabetic rats restores the tissue arginase activities and ornithine contents nearly to control values. As we have already demonstrated that a dietary supply with vitamins E and C significantly decreased glycaemia in STZ-treated rats [27], it would be relevant to look for a correlation between arginase activity in liver or in serum and glycaemia in order to verify if this enzyme activity would be considered as a marker of the disease.

As a conclusion, pregnancy did not directly affect arginase activities and ornithine contents in healthy and diabetic rats. But, significant variations of these 2 parameters were evidenced mainly in the liver during experimental STZ-induced diabetes, and they were completely abrogated by a combined vitamin C and E dietary supplementation, suggesting that the improvement of antioxidant systems would attenuate the metabolic disorders directly due to diabetes mellitus or indirectly to the occurrence of an oxidative stress. Nevertheless, the molecular mechanisms are unknown and require further investigations.

References

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