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Evaluation of Arginine-Nitric Oxide Pathway in Patients with Hyperthyroidism

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ABSTRACT

There is a reciprocal regulation of arginase and nitric oxide synthase in L-arginine metabolizing pathways. Nitric oxide has been claimed as an important role player in hyperthyroidism in recent years. The fact that arginase is an important part of regulation of arginine has not been investigated yet in terms of nitric oxide synthase activity in hyperthyroidism. This study aims to investigate arginase, manganese (a cofactor of arginase) and total nitrite levels (a metabolite of nitric oxide) and their relationship to the arginine- nitric oxide pathway in patients with hyperthyroidism. Arginase activities, manganese, and total nitrite levels were measured in plasma samples from 50 patients with hyperthyroidism and 50 healthy age- and gender-matched control subjects. Plasma arginase activities were found to be significantly lower and total nitrite level higher in patients with hyperthyroidism than those of controls. Manganese levels were not significantly different in hyperthyroidism group compared to those of controls. Our results demonstrate that the arginine-nitric oxide pathway is implicated in the cardiovascular manifestations of hyperthyroidism which may be of clinical relevance.

Keywords: hyperthyroidism, arginase, manganese, nitric oxide

INTRODUCTION

Thyroid diseases are common endocrine disorders in humans and accompanied by important changes in hemodynamic and cardiac function [1-3]. Thyroid hormones have multiple effects on the cardiovascular system, exerted through both direct and indirect mechanisms of action. Patients with thyroid disease often have clinical manifestations suggesting changes in cardiovascular hemodynamics [3,4]. Hyperthyroidism manifests a hyperdynamic circulation with increased cardiac output, increased heart rate and decreased peripheral resistance [5].

The mechanisms by which thyroid hormones affect vascular physiology are not fully understood. However, few data are available regarding the effects of thyroid hormones on endothelial function. Endothelium is metabolically active and plays a key role in vascular hemostasis by secreting numerous autocrine and paracrine substances. One of the major endothelium-derived vasoactive mediators is nitric oxide (NO). It is well known that NO is an important factor regulating vascular tone which accounts for the powerful vasodilator effects of endothelium-derived relaxing factor [6,7]. L-arginine is the main source of NO generation via NO synthase (NOS). L-arginine is also a substrate of the enzyme arginase. Arginase and NOS compete with each other for L-arginine because they use the same substrate [8]. There is a reciprocal regulation between arginase and NOS in the pathways of L-arginine metabolism. [8-10]. Arginase requires manganese (Mn) as a cofactor for its catalytic activity and stability [11]. There is a direct correlation between arginase activity and Mn level in the body [12]. Arginase activity related studies are available in various endocrine disorders such as diabetes and metabolic syndrome [13,14].

Our main objective in this study was to investigate the arginine-NO pathway by measuring plasma total nitrite, arginase and Mn in hyperthyroidism. Although there are some studies dealing with NO levels, NOS activity and plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of NOS in the hyperthyroidism [1,15-19], to our knowledge, this is the first report which has investigated arginase activity and Mn levels together with NO levels in hyperthyroidism.

MATERIALS AND METHODS

All subjects were informed about the study and their prior written informed consent was obtained. If cooperation with patients is impossible, consent was obtained from the relatives of the patients and the hospital authority. The investigation conformed to the principles outlined in the Declaration of Helsinki.

Patients

Fifty patients with hyperthyroidism were included in this study. Causes of hyperthyroidism were Graves' disease (n=35), toxic adenoma (n=9) and toxic multinodular goiter (n=6). The

control group consisted of fifty age- and sex-matched healthy subjects. The patients and control subjects were also matched according to smoking. Patients with regular drug ingestion, chronic systemic diseases such as diabetes mellitus, hypertension, etc., patients with liver and kidney disease were excluded from the study. Clinical diagnosis was supported by plasma fT3, fT4, TSH and thyroid scan determinations.

Sample Collection and Preparation

Venous blood samples were collected after the patients had fasted overnight. Samples drawn from an antecubital vein were immediately transferred into heparin-containing tubes and centrifuged at 2500 rpm for 10 minutes. Plasma samples were kept at -70°C for subsequent assays and all the measurements were performed at the same time.

Measurement of Plasma Levels of Total Nitrite

The NO content of the plasma was measured in ELISA (ELx808 Absorbance Microplate Reader, BioTec Instrument Inc. Vermont, USA) by using commercial kits (Nitrate/nitrite colorimetric assay kit, Cayman Chemical Co. Catalog No: 780001, Michigan, USA). This kit employs a nitrate reductase for enzymatic reduction of nitrate to nitrite, prior to measurement of the nitrite levels using the Griess reagent, as described previously [20]. The nitrite level measured using this system represents the total levels of both NO metabolites (nitrate and nitrite). Results were expressed as micromoles of NO per liter (µmol/L).

Measurement of Plasma Arginase Levels

Plasma arginase activity was measured according to the method of Geyer and Dabich [21] with some modification for plasma. The urea level was measured spectrophotometrically through the method of thiosemicarbazide-diacetyl-monoxime urea in the supernatants. One-unit plasma arginase was defined as the enzyme activity that produces 1 µmol of urea per minute. In addition, protein was determined using the Biuret method. As enzyme activity was very low in plasma samples, the specific activity was expressed as units per gram protein by the measurement of the activity in 1ml plasma per hour divided by the amount of protein in 1ml of plasma.

Measurement of Plasma Mn Levels

Determination of plasma Mn was performed by Perkin Elmer AAnalyst 800 Atomic Absorption Spectrometer (Shelton, CT, USA), according to the method of Brodie and Routh [22]. Plasma was diluted (1 : 2) with aqueous 0.1% Triton X-100 solution, and thoroughly mixed by a vortex mixer. The diluted plasma was used directly for the analysis. The total volume inserted in the tube was 20 µL. All determinations were run in duplicate, and individual values were averaged. Absorption readings were measured at peak height. The variation coefficient for replicate measurement was less than 3%.

Statistical Analyzes

SPSS for Windows computing program version 16.0 (IBM Inc, Chicago, IL) was used for the analyses. Data were analyzed using parametric statistical methods. The Student's t tests were used for pair-wise comparisons. Bivariate comparisons were examined using the Pearson rank correlation coefficients (r) and values corrected for ties. Two-tailed significance values were used. $p < 0.05$ was considered significant in all tests.

Table 1. The characteristics and plasma levels of arginase, Mn and total nitrite in patients with hyperthyrodism and controls

Parameter	Control (n=50)	Hyperthyroidism (n=50)	
Age (mean ± SD), years	39.5 ± 6.4	42.7 ± 8.9	p>0.05
Gender	20 M /30 F	18 M / 32 F	p>0.05
Smoking	25 (%50)	23 (%46)	p>0.05
Total nitrite (µmol/L)	7.92 ± 2.69	9.79 ± 4.17	p<0.01
Arginase (U/g protein)	9.77 ± 4.62	7.76 ± 3.71	p<0.05
Manganese (µg/L)	2.64 ± 1.76	2.16 ± 1.71	p>0.05

RESULTS

As to the social and demographic data (e.g. age, or sex), patients and the controls showed homogeneity, and there were no significant differences between the groups (p >0.05).

As shown in **Table 1**, plasma NO level of patients with hyperthyroidism was significantly higher (p<0.05), whereas plasma arginase activities were significantly lower (p<0.05) than that of control subjects. We did not observe any significant correlations between these parameters in patients with hyperthyroidism and controls.

DISCUSSION

There is no study which has investigated arginase activity and Mn levels together with NO levels in hyperthyroidism. In this study plasma arginase activities were found to be significantly lower and total nitrite level higher in patients with hyperthyroidism than those of controls.

A limited number of studies investigating the NO levels in patients with hyperthyroidism are available. Also, there are studies about NOS and ADMA, which an endogenous inhibitor of NOS. However, the findings on NO levels in patients with hypothyroidism are controversial in the literature. Some researchers [15,23] have found higher plasma levels of NO in consistent with our results, and others [16,17] have determined lower levels of NO in contradiction with our results. Quesada et al. [1] have found high NOS activity in all tissues of hyperthyroid rats. The NOS catalyzes nitric oxide synthesis from L-arginine. The high activity of this enzyme is compatible with high levels of NO. On the contrary, Hermenegildo et al. [16] have found high levels of ADMA, an endogenous inhibitor of NOS, in hyperthyroid patients. A great number of studies in the literature suggest that there is a competition between arginase and NOS and that they control each other's levels. In a study, the arginase was found to be the major pathway of L-arginine metabolism in woman with metabolic syndrome [13]. Also according to our previous results, in patients with Alzheimer disease had high NO levels and low arginase activity [24]. Therefore, we measured arginase and NO levels together based on this reciprocal relationship. Our results indicate that NOS is the major pathway that metabolizes L-arginine in patients with hyperthyroidism.

To our knowledge there is no report about arginase activity in patients with hyperthyroidism. This study is the first report on the arginase activity in these patient groups. In our study, plasma arginase activities were significantly lower in patients with hyperthyroidism than those of controls. The decreased arginase activity can be explained by low Mn concentrations, because arginase requires Mn for its catalytic activity and

stability and Mn deficiency reduces the arginase activity. But in our study, there was no statistically significant difference between the Mn levels. The mechanism responsible for the reduction of arginase activity in hyperthyroid patients is not known. One possible reason of low arginase activities may result from high NO levels. However, it is not clear whether low arginase activity due to a reciprocal arrangement leads to a high NO level or whether a high NO level leads to low arginase activity.

Mn levels in patients with hyperthyroidism also tended to be lower, but significance was not reached. If the number of cases is increased, a significant difference can be found between Mn levels of both groups. In our study, the number of cases is a limiting factor that may affect Mn results. In the literature, we found only two studies of the erythrocyte levels of Mn in patients with hyperthyroidism. In 1984, Aihara et al. [25] showed that there was no statistically significant difference between erythrocyte Mn levels of both groups which supports the findings of our study. In the other study, contrary to our results, serum Mn levels were found to be significantly higher in hyperthyroidism [26]. Importance of iodine and selenium in thyroid metabolism is well known, but the roles of other essential trace elements including Mn on thyroid hormone homeostasis remain unclear. Mn levels were higher in other thyroid diseases such as non-toxic diffuse goiter and euthyroid multinodular goiter [27,28].

In conclusion, lower arginase and higher NO levels found in patients with hyperthyroidism may suggest a pathway that favors NO synthesis. This study has a potential to open new perspectives concerning the relationships between arginase activity/NO level and hyperthyroidism. Moreover, the finding of this study demonstrates that arginine-NO pathway may be implicated in the pathogenesis of hyperthyroidism which may be a high clinical relevance. On the other hand, further clinical studies that would have more patients and controls are needed to reach more comprehensive results and to clarify the relationship between NO metabolism and hyperthyroidism.

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