Altered Arginine Metabolism in Complete Hydatidiform Mole

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Abstract

Objective: Nitric oxide is considered a key inhibitor of cellular proliferation and inducer of differentiation *in vitro*, and some hyperproliferative conditions have been shown to involve decreased production of nitric oxide due to overexpression of arginase. This study set out to investigate a possible role for such a process in complete hydatidiform mole.
Materials and Methods: Plasma arginase levels and plasma nitric oxide levels were measured in 29 patients with complete hydatidiform mole and in 27 healthy pregnant women.
Results: Compared with healthy pregnant controls, patients with complete hydatidiform mole had significantly higher mean plasma levels of nitric oxide (36.78±7.97 vs 28.60±7.32 μmol/L; p = 0.001), and -though not significantly- higher plasma levels of arginase activity (16.72±6.03 vs 11.26±5.11 U/g protein; p = 0.085).
Conclusion: The significance of this apparent breakdown in the co-regulation of nitric oxide synthase and arginase in the pathophysiology of complete hydatidiform mole remains to be determined.

Keywords: arginine, arginase, nitric oxide, nitric oxide synthase, complete hydatidiform mole

Özet

Komplet Mol Hidatiformda Değişmiş Arjinin Metabolizması

Amaç: Nitrik oksitin *in vitro* hücresel proliferasyon inhibitörü ve diferansiasyon indükleyicisi olduğu düşünülmektedir. Bazı hiperproliferatif durumlarda, arjinazın artışına bağlı olarak nitrik oksit üretiminde azalma olduğu gösterilmiştir. Bu çalışma, komplet mol hidatiformda bu sürecin olası rolünü araştırmak için düzenlenmiştir.
Materyal ve Metot: Yirmi yedi sağlıklı gebe kadın ve 29 komplet mol hidatiform hastasında plazma arjinaz ve nitrik oksit düzeyleri ölçüldü.
Sonuçlar: Sağlıklı gebe kontrol grubuyla karşılaştırıldığında, komplet mol hidatiform hastalarında ortalama plazma nitrik oksit düzeyleri anlamlı olarak yüksek (36.78±7.97 vs 28.60±7.32 μmol/L; p = 0.001) ve plazma arjinaz düzeyleri, istatistiksel olarak anlamlı olmasa da yüksek (16.72±6.03 vs 11.26±5.11 U/g protein; p = 0.085) bulunmuştur.
Tartışma: Arjinaz ve nitrik oksit sentaz koregülasyonundaki belirgin bozukluğun, komplet mol hidatiform fizyopatolojisindeki önemi araştırılmalıdır.

Anahtar sözcükler: arjinin, arjinaz, nitrik oksit, nitrik oksit sentaz, komplet mol hidatiform

Introduction

Complete hydatidiform mole (CHM) is a gestational trophoblastic disease, one of a heterogeneous group of diseases characterized by abnormally proliferating trophoblastic tissues (1).

L-arginine is an important modulator of immune system activation. It is converted to nitric oxide (NO) and citrulline by the enzyme nitric oxide synthase (NOS) also to ornithine and urea by the enzyme arginase (Figure 1). Both enzymes are active at inflammatory sites. As arginase and NOS share a common substrate, the regulation of arginase is linked with NO production, and it has been suggested that the balance of L-arginine metabolism between these two pathways has important pathophysiological effects. The regulation of L-arginine metabolism in tissues that possess both arginase and NOS activities is poorly understood. Arginine metabolizing pathways reciprocally (i.e. NOS inhibits arginase, Arginase inhibits NOS) inhibit each other *in vitro* at multiple levels, including substrate competition and protein and mRNA stability and expression. There is evidence that arginase activity can be increased by diabetes (2), hypertension (3), and also induced by inflammatory stimuli (4) and certain cytokines (5, 6) in various cell types.

NO is considered a key inhibitor of cellular proliferation and inducer of differentiation *in vitro* and is an important mediator in a number of physiological processes (7, 8). In the body, it is produced by the oxidation of arginine by NOS.

NOS activity has been studied in trophoblast cells (9),

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Table 1. Demographic characteristics of patients with complete hydatidiform mole and healthy pregnant controls

	Mean±SD*		
Variables	Complete hydatidiform mole (n=29)	Controls (n=27)	P
Age (years)	31.1±7.9	28.3±4.4	NS†
Gestational age (weeks)	12.2±3.0	12.6±4.6	NS
Gravidity	5.6±3.2	6.2±2.5	NS
Parity	4.4±3.2	4.4±2.1	NS
Abortion	0.4±0.6	0.7±0.9	NS

*SD, standard deviation.

†NS, not significant.

pregnancy (10), and trophoblastic diseases (11). Ariel et al. have suggested that this enzyme plays a role in implantation and vascular invasion (11). NOS has been observed in solid human tumor tissues, including gynecological cancer (12) and breast cancer (13). Moriyama et al. suggested that increased plasma nitrite/nitrate levels may correlate with tumor volume in patients with hepatocellular carcinoma (14).

The regulation of L-arginine metabolism in CHM is unknown and necessitates further interpretation of the two pathways. We have previously reported that high plasma levels of NO in CHM (15). However, to the authors' knowledge, no study has simultaneously investigated arginase and NO activities in CHM. This study hypothesized that the usual reciprocal balance between activities of arginase and NOS, which appears important in regulation of proliferation and differentiation, might be altered in CHM. Therefore, the study measured arginase activity and NO production in the plasma of patients with CHM.

Materials and Methods

Patients

Fifty-six women patients attending a University Hospital participated in this prospective case-control study. There were two groups of patients. The first group comprised 29 patients suffering from CHM, mean gestational age 12.2 weeks (±3.0) according to last menstrual period. The second group comprised 27 normal pregnant healthy women in the first trimester of pregnancy who served as controls; each had a single viable fetus, mean (±SD) gestational age 12.6 weeks (±4.6) as estimated by ultrasonography. Mean ages of patients and controls were 31.1 years (±7.9) and 28.3 years (±4.4) respectively.

Admission criteria for the patient group were absence of previous smoking, cardiovascular disease, diabetes mellitus, renal disease, primary hypertension, connective tissue disease, infection, hemorrhage, recent transfusion, history of antioxidant intake or medication history. Demographic characteristics are shown in Table 1. Diagnosis of trophoblastic disease was based on histopathologic examination of molar tissue, showing lack of identifiable embryonic or fetal tissues, with chorionic villi showing generalized hydatidiform swelling, and diffuse trophoblastic hyperplasia resulting from abnormal fertilization. Informed, written consent was obtained from all patients.

Blood Sampling

Blood was sampled before evacuation in the patient group. After an overnight fast, blood (4 ml) was withdrawn from the antecubital vein using a Vacutainer tube (BD Vacutainer Systems, Oxford, United Kingdom) and centrifuged at 1000 g for 10 min. The plasma was separated and stored in aliquots at –80°C until needed.

Arginase Assay

Plasma arginase activity was measured according to the method of Geyer and Dabich with some modification for plasma (16). Briefly, plasma was diluted 10 times with a solution of 5 mmol/L Mn^{++} and incubated for 8 min at 55°C. Then 0.2 ml preincubated plasma, 0.4 ml 25 mmol/L L-arginine, and 0.4 ml 40 mmol/L carbonate buffer (pH 9.7) were incubated for 60 min at 37°C. The reaction was stopped and the sample deproteinized by adding 1 ml of 1N HClO4. Supernatants were obtained by centrifugation for 5 min at 5000 rpm, and urea levels were measured spectrophotometrically using the thiosemicarbazide-diacetylmonoxime-urea (TDMU) method. One unit of plasma arginase was defined as the enzyme activity that produces 1 mmol urea per minute. Arginase activity was expressed as U/g protein. Protein was determined using the Biuret method (17).

NO Assay

NO is a very labile molecule (18). In aqueous solution, NO reacts with molecular oxygen and accumulates in the plasma as nitrite and nitrate ions. These stable oxidation end-products can be measured readily in biological fluids and have been used *in vitro* and *in vivo* as indicators of NO production (19). First, nitrate reductase (37°C for 1 hour) was used to convert all the nitrates present in plasma into nitrites. Assay for total nitrites was then carried out according to the method of Tracey et al. using potassium nitrate as standard (20). Griess reagent, an equal mixture of 1% sulfanilamide in 5% orthophosphoric acid and 0.1% N-1-naphtyl ethylenediamine (Sigma), was added for development of color, and readings were taken after 10 minutes in 96-well assay plates at 540 nm (Tekniko-Microwell system; Organon, Netherlands). Results were expressed as

µmol/L NO. The limit of detection of the method was 0.34 µmol/L nitrite. Intra-test variance was 9% and inter-test variance was 17%.

Statistical Analysis

All statistical calculations were performed using SPSS for Windows (version 10.0). Since the spread of results showed a normal distribution ($p > 0.05$), parametric tests were used. After applying the Kolmogorov-Smirrov test to the data, demographic differences between groups and results for arginase and NO levels were analyzed using Student's t test. Data are reported as the mean±SD. Differences were considered significant at $p \leq 0.05$.

Results

There were no significant differences in mean age, gestational age, gravidity, parity or abortion for patients with CHM and controls (Table 1).

Compared with controls, patients with CHM had significantly higher mean plasma levels of NO (36.78±7.97 vs 28.60±7.32 µmol/L; $p = 0.001$), and -though not significantly- higher plasma levels of arginase activity (16.72±6.03 vs 11.26±5.11 U/g protein; $p = 0.085$) (Table 2).

Discussion

To the best of our knowledge this is the first study examining arginase activity together with NO levels in CHM. In this study, we found that plasma arginase activity and NO levels were both elevated in patients with hydatidiform mole.

The regulation of arginase is linked with NO production, and it has been suggested that the balance of L-arginine metabolism between these two pathways has important pathophysiological effects (21, 22). Arginase is a key enzyme in nitrogen metabolism, forming urea and L-ornithine from L-arginine. Ornithine is a precursor of proline and polyamines, both of which are involved in cellular proliferation, wound healing and macrophage activation (8). Arginase is often co-localized with NOS and they maintain a complex relationship, regulating each other and competing for their common substrate. It is a mitochondrial enzyme with various regulatory functions including immunoprotection (23) and its expression has been evaluated in numerous human tumor types.

Table 2. Arginase activity and concentrations of nitric oxide (NO) in normal pregnancy and in complete hydatidiform mole

	Mean±SD*		
Variables	Complete hydatidiform mole (n=29)	Controls (n=27)	P
Arginase (U/g protein)	16.72±6.03	11.26±5.11	0.085
NO (µmol/L)	36.78±7.97	28.60±7.32	0.001

*SD, standard deviation.

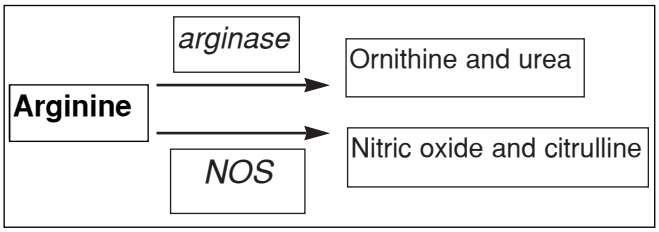

Figure 1. Arginase and nitric oxide synthase (NOS) related metabolic pathways of arginine.

Arginase expression appears crucial in regulating the cellular immune response and the inflammatory process during critical illness (8).

In normal pregnancy, circulating NO levels vary, the highest activity of NO synthase being found in first-trimester villi, with a significant fall in activity in the third trimester (10). There is evidence that invasive trophoblasts can release the vasoactive agent NO, although its physiological function in this setting is not understood (24). Ariel et al. have suggested that NO release by trophoblastic cells may play a role during the process of vascular invasion, in placentation as well as in trophoblastic disease (11). *In vitro*, nitric oxide is also considered a key inhibitor of cellular proliferation and inducer of differentiation.

During late pregnancy, arginase activity has been found to increase significantly in animals (25,26). Given the relationship between arginase and NOS, this could be anticipated. Arginase is also found in the human placenta (27). However, a study by Carpintero et al. found no increase in serum arginase activity in the first, second, or third trimester of pregnancy (28). It is possible that arginase activity in pregnancy is increased significantly in the involved tissues, while not increased in the serum (29). A study in guinea pigs found levels of arginase in myometrium underlying the placental implantation site to be much greater than those in kidney and more than twice those in myometrium opposite the implantation site (30). In rats, inhibiting uterine arginase activity arrests embryonic development (31). This could be secondary to effects on polyamine synthesis rather than to increase in NO.

Arginase has been accorded a key role in regulating the production of NO by depleting supplies of arginine (32). Thus, increase/decrease in the level of arginase results in a decrease/increase in NO production. This study has produced the surprising result that, in complete hydatidiform mole, levels of both arginase and NO are increased. Barring the presence of some as yet unknown mechanism for NO production, this could only result from presence of such an excess of arginine (possibly produced by Krebs cycle) that both mechanisms could be accommodated.

This disturbance of the usual arginine metabolism could, if confirmed, be of some importance in the etiology of complete hydatidiform mole and deserves further investigation.

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