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L-Arginine erythrocyte transport in normal pregnant and preeclamptic women

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Objective: Uptake of L-arginine by the cell via amino acid transporter systems is the first step for nitric oxide (NO) production. The current study aimed to assess the total L-arginine uptake in erythrocytes of normal pregnant and preeclamptic women.
Study design: Twenty-one normal pregnant and 21 preeclamptic women were studied. To measure total L-arginine uptake in erythrocytes, carbon 14 was used as a marker and Michaelis-Menten kinetic parameters (V_{max} and K_m) were evaluated.
Results: In preeclamptic women, there was a significant increase ($P < .004$) in the mean maximal capacity of transport in erythrocytes (V_{max} = 982.69 μmol/L cells/h ± 433.51) in comparison with normal pregnant women (V_{max} = 584.73 μmol/L cells/h ± 422.33). No significant difference was detected in the half-saturation constant ($P = 0.978$).
Conclusion: The transport kinetics of the NO precursor, L-arginine, is altered in erythrocytes of preeclamptic women. It is possible that abnormal L-arginine uptake may contribute to the pathophysiologic mechanisms of preeclampsia syndrome.

Preeclampsia syndrome (PE) is still a major cause of neonatal and fetal morbidity and mortality. This syndrome, whose etiology remains unknown, is characterized by hypertension and proteinuria, with edema starting after the 20th week of pregnancy. In preeclampsia, increased vasopressor sensitivity and peripheral vascular resistance lead to maternal hypertension and decreased uteroplacental blood flow.[1,2]

Nitric oxide (NO), which is synthesized from L-arginine, is a vascular smooth muscle relaxant that also inhibits platelet aggregation.[3] The L-arginine–NO pathway may be involved in the hemodynamic changes and vasoconstriction of PE,[4] although no cause-effect relationship has been established so far. The first step for NO production is cellular uptake of L-arginine

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via amino acid transporter systems,[5] and abnormalities in the structure or function of these systems in PE could result in altered NO production with changes in vascular relaxation.

The cationic amino acid transporter systems found in endothelial cells mediating the transport of L-arginine are also present in erythrocytes. In addition, erythrocytes are a useful cell model to study membrane transport.[6]

The aim of the current study was to verify the total erythrocyte L-arginine uptake in normal pregnant and preeclamptic women.

Material and methods

Participants

The protocol was approved by the Scientific and Ethics Committee of Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Brazil, and informed consent was obtained from each subject. Twenty-one normal and 21 preeclamptic pregnant women from the obstetric wards and from the obstetric outpatient clinic were included in the study (gestational age >28 weeks). Subjects with diseases known to alter vascular response, such as diabetes mellitus, renal disease, infection, and altered fundi (hypertensive retinopathy) were excluded.

PE syndrome was defined, in accordance with National High Blood Pressure Education Program Working Group[1,2] criteria, as blood pressure higher than 140/90 mm Hg after 20 weeks of gestation concomitant with proteinuria (>300 mg protein excretion/24 hours).

Measurements

Blood pressure was measured with the patient in the left lateral recumbent position and repeated after 20 minutes of rest.

To define the presence of proteinuria, urinary protein was measured by acid precipitation with turbidimetry, estimated as the ratio of protein/creatinine in a urine sample.

Additional routine tests included platelet counting (Coulter counter STKS), creatinine measurements (Jaffé reaction without deproteinization), uric acid (colorimetric readings), and alanine transferase (ALT) and aspartate transferase (AST) (enzymatic testing).

L-arginine uptake into erythrocytes

Blood collected in heparinized tubes was centrifuged and washed three times with ice-cold saline solution (platelets/white cell layer discarded) for separation of erythrocytes. All samples were processed within 6 hours and kept in ice until uptake assays were performed. Total erythrocyte L-arginine uptake was determined by incubating cells for 3 minutes at 37°C (pH 7.4) in a water bath with progressive L-arginine concentrations (20, 40, 60, 80, 100, 120, 140, 180, 200, 300, and 500 μmol/L) and carbon 14 as a marker. Uptake was interrupted by transferring the sample tubes into ice. Erythrocytes were then washed free of extracellular radioactivity, lysed (Triton 0.1% vol/vol), and protein-precipitated (trichloroacetic acid 5% wt/vol) to recover their intracellular content, followed by radioactivity counting in a liquid-scintillation counter. Uptake was corrected to micromoles per liter of cells per hour. Maximal transport capacity (V_{max} in micromoles per liter of cells per hour) and half-saturation (k_m in micromoles liter) was derived from Michaelis-Menten kinetics, using the computer software Enzfitter for MS-DOS (Microsoft Corp, Redmond, Wash). All L-arginine uptake assays were performed in duplicate.

Table I Demographic information of normal and preeclamptic patients

Variable	Pre-eclamptic (mean ± SD [median])	Normal pregnancies (mean ± SD [median])	Level of significance
Age (y)	22.10 ± 5.75 (21)	23.24 ± 4.10 (23)	$P = .463$ (t)
Gestational age (wk)	33.12 ± 3.99 (35)	34.33 ± 3.68 (35)	$P = .550$ (t)
No. of pregnancies	1.29 ± 0.78 (1)	1.95 ± 1.40 (1)	$P = .069$ (MW)
No. of deliveries	0.24 ± 0.70 (0)	0.67 ± 0.97 (0)	$P = .808$ (MW)
Black race	23.8%	19.0%	$P = .706$ (χ^2)

MW, Mann-Whitney.

Statistics

Mean, SD, and median were calculated for all variables. Student t test was used for independent samples. The Mann-Whitney test was used to analyze non-Gaussian data. The correlation between V_{max} and K_m with clinical variables was analyzed with use of the Pearson coefficient of correlation and Spearman and Kendall nonparametric coefficients. $P < .05$ was considered to be statistically significant. The Statistical Package for the Social Sciences (SPSS, Chicago, Ill) was used for all analyses.

Results

Twenty-one patients with PE syndrome and 21 normal pregnancies were studied. Demographic data for all patients appear in Table I. The only significant difference observed between normal and PE women in terms of demographic data concerned number of pregnancies. All clinical variables that characterize PE were significantly different when the two groups were compared (Table II).

The Figure illustrates the mean L-arginine uptake curve of each group. The mean L-arginine V_{max} was

Table II Clinical variables in preeclamptic and normotensive women

Variable	Preeclamptic (average ± SD [median])	Normotensive (average ± SD [median])	Level of significance
Systolic blood pressure (mm Hg)	149.29 ± 17.77 (150)	110.00 ± 12.65 (110)	$P < .001$ (*t*)
Diastolic blood pressure (mm Hg)	97.86 ± 12.31 (100)	74.29 ± 9.26 (70)	$P < .001$ (*t*)
Proteinuria (24-h urine sample, mg/24 h dL)	4020.33 ± 4303.21 (2111)	136.80 ± 122.22 (76)	$P < .001$ (MW)
Platelets (n/dL)	193,476 ± 49,385 (196,000)	232,295 ± 58,523 (224,000)	$P = .021$ (*t*)
Creatinine (mg/dL)	0.70 ± 0.12 (0.70)	0.64 ± 0.11 (0.60)	$P = .034$ (*t*)
Uric acid (mg/dL)	6.61 ± 5.32 (5.60)	4.34 ± 1.33 (4.70)	$P = .016$ (MW)
AST (UI/L [25°C])	15.71 ± 14.40 (11.00)	7.33 ± 2.01 (7.00)	$P = .024$ (MW)
ALT (UI/L [25°C])	21.00 ± 22.78 (12.00)	10.48 ± 2.58 (10.00)	$P = .046$ (MW)
Edema score*	2.38 ± 1.02 (2.00)	0.24 ± 0.62 (0.00)	$P < .001$ (MW)

MW, Mann-Whitney.
*Edema score: from 0 to 4 (0 = no edema; 4 = anasarca).

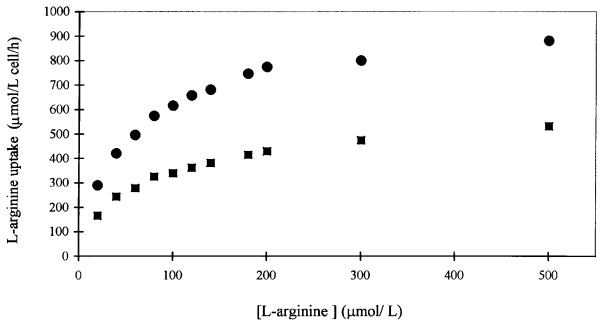

Figure Average L-arginine uptake into erythrocytes in preeclamptic *(circles)* and normotensive women *(squares)*.

significantly increased in PE in relation to normal pregnancies (PE = 982.69 µmol/L cells/h ± 433.51; NP = 584.73 µmol/L cells/h ± 422.33, $P < .004$, Mann-Whitney). Median V_{max} values were 1065 µmol/L cells/h for PE women and 461 µmol/L cells/h for normal pregnant women. The half-saturation constant (K_m) was not significantly different between the groups (PE = 59.31 µmol/L ± 15.67; NP = 59.46 µmol/L ± 20.09, $P = .978$). Median K_m values were 61 µmol/L for PE women and 64 µmol/L for normal pregnant women.

V_{max} was significantly correlated with systolic and diastolic blood pressure, 24-hour proteinuria measurement, presence of edema, and ALT and AST. There were no correlations between any of the clinical variables and K_m (data not shown).

Comment

According to the current results, the maximal capacity of transport (V_{max}) of the NO precursor L-arginine is increased in preeclampsia. L-arginine transport across the cell membrane has been shown to be altered in other disorders, such as chronic renal failure and heart failure.[7,8] This alteration may have an impact on the synthesis of NO; because NO has a regulatory effect on vascular reactivity and blood pressure, we hypothesize that the initial steps of the L-arginine–NO pathway could play a role in preeclampsia, a disorder characterized by hemodynamic changes, increased vascular resistance caused by vasoconstriction, and altered vascular response to pressor agents.[4]

The uptake of a substrate through the cell membrane is often dependent on a concentration gradient; that is, a lower concentration of a specific substrate inside the cell would prompt increased uptake of that substrate. Although L-arginine levels (and the levels of other potential substrates) were not measured in the current study, the observed increase in L-arginine V_{max} indicates an enhanced uptake of the NO precursor. Asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, has been shown to be increased in preeclampsia, whereas plasma L-arginine did not differ from control, resulting in a lower L-arginine/ADMA ratio in the third trimester of pregnancy.[9] On the one hand, the enhanced L-arginine erythrocyte uptake could be merely reflecting a difference in concentration gradient between PE and normal pregnant women; on the other hand, it could be reflecting the presence of other, less obvious differences. For example, Haller et al,[10] using endothelial cells, have suggested that serum from preeclamptic patients may contain factors that increase cell permeability mediated by protein kinase C. In addition, protein kinase C activation stimulates arginine transport in umbilical vein endothelial cells.[11]

The presence of specific intracellular substrates could also cause an acceleration in transmembrane transport, a phenomenon known as transstimulation.[5] If transstimulation was caused by an L-arginine analog (or analogs), this could explain the increased L-arginine uptake even in the presence of decreased NO production, as might be the case in PE. Although L-arginine analogs have an affinity for the same transporter systems involved in L-arginine uptake (y^+ and y^+L),[5] they act as NO inhibitors rather than substrates for the synthesis of

NO.[12] The presence of endogenous nitric oxide synthase (NOS) inhibitors has been described in PE.[9,13]

In PE, both increased and decreased NOS expression has been reported.[14-20] In the case of increased NOS activity,[16,17] the accelerated transport of L-arginine into the cell could be an attempt to compensate for the increased demand for this substrate. However, if transstimulation by NOS inhibitors is present, as described above, NO production would be reduced even in the presence of increased L-arginine V_{max}. Alternatively, if NOS activity is either similar[18,19] or decreased[14,15,21] in normal and PE pregnant women, women with PE would theoretically require a larger supply of L-arginine because of the endothelial vasoconstriction they experience. Another possibility to explain the acceleration of L-arginine transport is the presence of a deficient response of endothelium-derived NO, as previously reported[22,23]; a defective endothelial response would result in the need for more NO to improve vascular relaxation, and therefore a larger supply of L-arginine would be required. It is interesting to note that the administration of exogenous L-arginine has an apparently beneficial effect on blood pressure in PE women.[24]

Finally, the observed correlation between V_{max} and the clinical variables present in PE support the hypothesis that alteration in the transmembrane transport of L-arginine is present in patients with PE syndrome. Further investigation will be required to clarify the clinical and physiologic relevance of this observation.

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