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L-arginine erythrocyte transport increases during pregnancy and immediately postpartum

Adriani O. Galão, MD, Bartira E. Pinheiro da Costa, PhD, Domingos O.L. d'Avila, MD, PhD, Carlos E. Poli de Figueiredo, MD, PhD

Programa de Pós-graduação em Medicina e Ciências da Saúde (Nefrologia), Instituto de Pesquisas Biomédicas/ Faculdade de Medicina/Hospital São Lucas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil

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Objective: This study was undertaken to evaluate erythrocyte membrane transport of L-arginine in pregnancy and immediately postpartum.

Study design: The study comprised 103 women with normal pregnancy, initially evaluated at the second trimester (II), followed into the third trimester (III), and immediately postpartum (PP). Total erythrocyte L-arginine uptake was measured with ^{14}C-L-arginine, at 37°C, for 3 minutes. The maximal transport capacity (V_{max}) and half-saturation constant (K_m) were obtained with the use of Michaelis-Menten kinetics. Results are expressed as mean ± SD. Analysis of variance, followed by Tukey test, was used in statistical analysis ($\alpha \leq .05$).

Results: V_{max} (μmol/L cells per hour) progressively increased at each consecutive time period: 779 ± 283, 946 ± 289, and 1349 ± 390, at II, III, and PP, respectively ($P < .001$). Similarly, K_m (μmol/L) values increased from 56 ± 20 at time II, to 62 ± 18 at time III, and 69 ± 24 at PP ($P < .001$).

Conclusion: Total erythrocyte L-arginine uptake (V_{max} and K_m) increases progressively along normal pregnancy, with a further increase immediately postpartum.

Human pregnancy is associated with multiple physiologic and cardiovascular changes, such as increased plasma volume, cardiac output, and glomerular filtration rate, accompanied by reduction in peripheral vascular resistance and systemic blood pressure.[1]

The role of endothelial-derived nitric oxide in vasodilatation and blood pressure regulation is well established.[2] Nitric oxide (NO) is generated from the oxidative deamination of a guanidino nitrogen of L-arginine. It diffuses out of endothelial cells into adjacent vascular smooth muscle cells, signaling guanylate-cyclase to produce cyclic guanosyne monophosphate, and vascular relaxation. Besides its vascular effects, NO also inhibits platelet aggregation and neutrophyl adhesion.[2]

The L-arginine-NO pathway may be involved in the hemodynamic changes of normal pregnancy, and in pregnancy-induced hypertension.[1] We have previously

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shown that erythrocyte membrane transport of L-arginine is altered in preeclamptic patients in comparison with women undergoing normal pregnancy.[3] L-arginine is carried through the membrane by 2 cationic amino acid transporters: y^+ and y^+L, present in many cell types, such as erythrocytes, endothelial and placental tissue cells.[4] Examining the membrane function during pregnancy may be relevant to understand its physiologic changes and related hypertensive disorders.

The erythrocyte cell membrane has been extensively used in the study of membrane transport processes.[5] Its structure is well known and has a similar composition to that of other cells. A number of transport systems can be adequately evaluated by using this cellular model, which provides a homogeneous cell population without intracellular membranes.

This article reports L-arginine membrane transport kinetics in erythrocytes, during pregnancy, and immediately postpartum of normal pregnant women.

Material and methods

The study was reviewed and approved by the Scientific and Ethics Committee of Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Brazil.

A cohort of healthy pregnant women was prospectively studied during the second and third trimester of pregnancy, and in the immediately postpartum period. Initial blood samples were obtained during their first visit to the Obstetrics Outpatient Clinic. Additional samples were collected during routine pregnancy visits and the immediately postpartum period (<48 hours after delivery). Clinical data of the newborn infants were also collected. Seated blood pressure was measured in the right arm with a mercury sphygmomanometer, after a minimum resting period of 20 minutes in accordance with the National High Blood Pressure Education Program Working Group[6] and American College of Obstetricians and Gynecologists practice bulletin recommendations.[7]

Gestational age was established by the accurate dating of the menstrual period or by an early pregnancy ultrasound before the 12th gestational week. All patients had their gestational age confirmed by an ultrasound during pregnancy. Women with a history of hypertension or diabetes, and those with abnormal urinary sediment and altered fundi were excluded. No patient was seen less than 5 times during the pregnancy, having additional clinical evaluations at 1 week and 1 month postpartum.

Pregnancy was divided as follows: (a) second (II) trimester, from 12th to 28th gestational weeks; (b) third (III) trimester, from 29th gestational week to term; and (c) immediately postpartum (PP), up to 72 hours after delivery.

Flux measurements were performed as previously described.[3,8] Briefly, blood drawn into heparinized tubes was immediately centrifuged (3,000*g*) at room temperature for 10 minutes. The platelets and white cells layer was discarded and red cells were washed 3 times in ice cold saline solution (140 mmo./L NaCl, 5 mmol/L KCl, 10 mmol/L MOPS, 5 mmol/L glucose, pH 7.4). Total erythrocyte L-arginine uptake was measured by incubating the red cells at approximately 10% hematocrit for 3 minutes at 37°C in a water bath, in the presence of increasing L-arginine concentrations (20-500 μmol/L) in saline solution, with the use of ^{14}C-L-arginine as tracer. Fluxes were interrupted by transferring the cell suspension to ice for 3 minutes. Erythrocytes were washed free of the extracellular radioactivity by ressuspension in ice cold saline solution and centrifugation 3 times (14,000 *g*). Cells were then lysed by addition of 0.1% Triton X-100, followed by TCA protein precipitation. The cell suspension was pelleted down by centrifugation at 14,000*g* for 5 minutes, and the supernatant was transferred by pipette and added to vials containing scintillation fluid. The intracellular radioactivity was counted for 2 minutes with a Beckman LS6500 scintillation counter (Beckman Instruments, Inc, Fullerton, Calif). Maximal transport capacity, V_{max} (μmol/L cells per hour), and half-saturation constant, K_m (μmol/L), were derived by using Michaelis-Menten kinetics, with the help of computer software (Enzfitter for MS-DOS; Biosoft, Stapleford, Cambridge, UK). Experimental results were statistically analyzed with analysis of variance (ANOVA), followed by Tukey test; $\alpha \leq .05$ was considered statistically significant.

Results

The study population comprised 103 women (76 white and 27 nonwhite), with mean age of 25.2 ± 6.6 years. Mean number of pregnancies was 2.4 ± 1.9, yet 38 women were undergoing their first pregnancy. Mean gestational age was 21.7 ± 4.0, 32.3 ± 2.3, and 39.6 ± 1.9 weeks for the II, III trimesters, and at delivery, respectively. Clinical characteristics of the cohort are shown in Table I.

Results of the kinetics parameters of transport for each time period are shown in Table II (mean ± SD). As can be seen, V_{max} and K_m increased progressively throughout pregnancy. When the kinetics parameters (V_{max} and K_m) from nulliparous and multiparous women were analyzed separately, no significant difference between them was observed (data not shown).

Perinatal data from 103 neonatal infants were (mean ± SD) as follows: birth weight 3312 ± 489 g, placental weight 638 ± 133 g; 1-minute and 5-minutes Apgar scores 8.3 ± 1.4 and 9.3 ± 1.1, respectively.

Comment

Alterations in erythrocytes membrane transport may be related to endothelial and placental cell membranes

Table I Demographic and clinical characteristics of the pregnant women (n = 103)

Gestational period	II	III	PP	*P**
Height (cm)	1.61 ± 0.06			
Weight (kg)	65.8 ± 12.6[a]	70.5 ± 11.8[b]	72.9 ± 11.9[c]	<.001
SBP (mm Hg)	106 ± 15.3[a]	106 ± 14.0[a]	112 ± 12.3[b]	<.001
DBP (mm Hg)	67 ± 8.0[a]	67 ± 9.8[a]	71 ± 9.8[b]	<.001
Hematocrit (%)	35.7 ± 3.2[a]	34.7 ± 2.8[b]	33.2 ± 4.6[c]	<.001
Hemoglobin (g/dL)	12.0 ± 1.0[a]	11.6 ± 1.0[b]	10.8 ± 1.5[c]	<.001
Creatinine (mg/dL)	0.53 ± 0.09[a]	0.56 ± 0.1[b]	0.66 ± 0.1[c]	<.001
Uric acid (mg/dL)	3.40 ± 0.7[a]	3.48 ± 0.8[a]	4.99 ± 1.3[b]	<.001

Values are mean ± SD.

SBP, Systolic blood pressure; *DBP*, diastolic blood pressure.

* ANOVA, followed by Bonferroni to identify differences (lower case letters represent statistically significant differences among periods).

Table II Membrane transport kinetic constants for erythrocyte L-arginine uptake during pregnancy and postpartum period (n = 103)

Gestational period	II	III	PP	*P**
V_{max} (μmol/L cells per h)	779 ± 283[a]	946 ± 289[b]	1349 ± 390[c]	<.001
K_m (μmol/L)	56 ± 20[a]	62 ± 18[b]	69 ± 24[c]	<.001

Values are mean ± SD.

* ANOVA, followed by Bonferroni to identify differences (lower case letters represent statistically significant differences among periods).

function and to pregnancy adaptive response, although the metabolic capacity of mature red cell is limited compared with typical nucleated cells. As these cells maintain y^+ and y^+L transport system[4] in its mature phase, and hemoglobin has high affinity for NO,[2] it may be useful to evaluate the relationship of erythrocytes with the L-arginine-NO pathway.

In the current study, erythrocyte L-arginine membrane transport was examined in a cohort of healthy women during pregnancy and shortly after delivery, aimed at detecting possible kinetic changes associated with normal pregnancy. The L-arginine maximal transport capacity, V_{max}, and half-saturation constant, K_m, were progressively increased during normal pregnancy. It is conceivable that such adjustments account for physiologic adaptations associated with changes in NO generation. NO synthesized by the endothelial NO synthase (eNOS) is a fundamental determinant of vascular homeostasis and blood pressure regulation.[2] If alterations in erythrocyte membrane transport reflects endothelial cells membrane transport function, the enhanced L-arginine influx could increase intracellular sources to generate NO, leading to the vascular relaxation required by pregnancy. However, the exact NO plasma levels and its role in blood pressure regulation in pregnant women remain unclear. Seligman et al[9] demonstrated that NO synthesis is higher in pregnant than in nonpregnant women. Nobunaga et al[10] suggested that increased NO generation may contribute to maternal vasodilatation and uterine relaxation.

In addition, we have previously shown higher erythrocyte L-arginine maximal transport capacity in preeclampsia compared with normal pregnancy.[3] L-arginine analogues also enter the cells via the cationic amino acid transport system,[4] inhibit NOS, and competitively block cell L-arginine uptake.[11] In preeclamptic women, the endogenous levels of asymmetric dimethilarginine (ADMA) are elevated.[12] ADMA has been considered an explanation to the L-arginine paradox, which shows that the rate of NO synthesis is not affected so much by the absolute concentrations of L-arginine as much as the relative ratio between these amino acids.[13] Furthermore, the existence of a caveolar complex between y^+ transport system and eNOS requires a mechanism for direct delivery.[14] A close link between extracellular L-arginine and membrane-bound eNOS may require the erythrocyte as an L-arginine deliverer to optimize NO production by endothelium.

On the other hand, it has been suggested that the paradigm of NO intercellular communication occurring toward vascular smooth muscle cell to regulate blood pressure homeostasis should be revised.[15] NO transported by red cells participates in dilation of blood vessels. S-nitrosothiol derived from human erythrocytes hemoglobin, and generated from imported NO, is associated with red blood cell membrane and cysteine residues in the hemoglobin-binding cytoplasmic domain of the anion exchanger. Interaction with the anion exchanger promotes the deoxygenated structure in S-nitrosothiol-hemoglobin, which subserves NO group transfer to the membrane. The vasodilatory activity is released from this membrane precinct by deoxygenation.[15] Increased maximal capacity of transport along pregnancy may also favor erythrocyte capture and accumulation of L-arginine, and not only NO, suggesting an exchange mechanism of precursor and product with the endothelium. Erythrocytes can further transport substrates to the placental tissue and to fetal red cells that have increased NO scavenger ability.[16] There are also

findings suggesting that L-arginine transport via y^+L is specifically modulated by oxygen tension, with a strong inverse relationship between system y^+L activity and uterine venous o_2 content differences.[17]

Fetal growth and development are dependent on the adequate provision of substrates from the maternal circulation. Red blood cells act as carriers of amino acids from 1 site in the body to another. It has also been suggested that the role of blood cells in interorgan amino acid transport, in association with high organ blood flow, might ensure fast and efficient delivery of amino acids. In addition, blood cells and plasma may play an independent, and sometimes opposing, role in interorgan amino acid exchange.[18] Also, maternal erythrocyte filterability is suggested to be an indicator of flow state to the placental microcirculation, which is associated with infant birth weight, affecting fetal development.[19]

L-arginine transport has been previously studied in the microvillous plasma membrane (MVM) of the syncytiotrophoblast from human placenta.[20] Initial rate of transport was examined, and not V_{max} and K_m, as in this article. Total L-arginine initial rate of uptake showed a significant negative correlation with gestational age under their in vitro conditions, but the authors concluded that under physiologic conditions MVM cationic amino acid transport would increase between 12 and 41 weeks' gestation.[20] More recently, the same group has shown an increased initial rate of L-arginine uptake in MVM from preeclamptic women[21] compared with those from control placentas. Their data agree with our previous work that shows altered L-arginine uptake kinetics in preeclamptic women.

A sustained increase in L-arginine utilization by other enzyme systems could also further augment substrate requirement. Arginase hydrolysis leads to urea and L-ornithine formation. Arginase is often colocalized with NOS, competing for their common substrate.[22] Enzyme activity is increased during pregnancy,[23] making an increased need of L-arginine appear obvious.

Our results demonstrate modified L-arginine membrane kinetics during pregnancy and immediately postpartum. The change could be required by increased intracellular L-arginine in response to augmented metabolic demands induced by the fetal development[24] and be a necessary adaptation to pregnancy.[1]

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