

Erythrocyte L-Arginine Uptake in Peritoneal Dialysis Patients Changes over Time

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During long-term exposure to continuous ambulatory peritoneal dialysis (PD), the characteristics of the peritoneal membrane may be altered. The substrate for nitric oxide synthesis is L-arginine, which may enter cells via the y^+ and y^+L transport systems. Peritoneal membrane characteristics may depend on vascular function and the L-arginine-NO pathway. Maximal capacity for L-arginine transport is higher in patients with a lower dialysis adequacy index. Our aim was to evaluate erythrocyte L-arginine uptake in PD patients at the start and end of a 3-year interval.

Our longitudinal study evaluated 8 stable patients on PD who were not using NO donors and who had been free of peritonitis for at least 1 month. Uptake of L-arginine was measured in 2003 and again in 2006. Maximal transport capacity (V_{max} in micromoles per liter-cells per hour) and half-saturation constant (k_m , in micromoles per liter) were measured in erythrocytes using ^{14}C as a marker and N-ethylmaleimide as inhibitor of the y^+ system.

For the years 2003 and 2006 respectively, mean \pm standard deviation for total L-arginine uptake V_{max} was $749 \pm 182 \mu\text{mol/L-cells/h}$ and $1146 \pm 365 \mu\text{mol/L-cells/h}$ ($p = 0.016$, paired t-test), for y^+L V_{max} was $180 \pm 58 \mu\text{mol/L-cells/h}$ and $515 \pm 142 \mu\text{mol/L-cells/h}$ ($p = 0.002$), and for y^+ V_{max} was $556 \pm 177 \mu\text{mol/L-cells/h}$ and $662 \pm 267 \mu\text{mol/L-cells/h}$ (nonsignificant). The total y^+L and y^+ k_m were not significantly different.

The L-arginine maximal uptake capacity in erythrocytes increased after 3 years of PD treatment. These findings agree with the suggestion of an association between y^+L activity and dialysis adequacy or uremia toxicity. Peritoneal membrane character-

istics may depend on vascular function and the L-arginine-NO pathway.

Key words

Membranes, cationic amino acid transporter, amino acids, nitric oxide

Introduction

The substrate for synthesis of the vasodilator nitric oxide is L-arginine, which is involved in blood flow regulation and vascular tone and permeability (1). Production of NO depends on the intracellular presence of its precursor, which enters the erythrocyte via cationic amino-acid transporter systems such as y^+ and y^+L (2). Because ultrafiltration and permeability for peritoneal solutes depend on the interaction between membrane and capillary surface areas, it is likely that the L-arginine-NO pathway may be involved in peritoneal permeability modulation during peritoneal dialysis (PD) treatment (3).

Changes in membrane transport systems have been described in erythrocytes from uremic patients, and it has been postulated that the abnormalities are important contributors to the pathophysiology of the uremic syndrome (4-6). Erythrocyte uptake of L-arginine does not change across the various peritoneal membrane functional categories as measured by a peritoneal equilibration test, although maximal transport capacity is higher in patients with a lower dialysis adequacy (Kt/V) index (7). Because the characteristics of peritoneal membrane exposed to long-term continuous ambulatory PD may be altered (8,9), the aim of the present study was to evaluate erythrocyte L-arginine uptake in PD patients at the start and end of a 3-year interval.

Patients and methods

Our cross-sectional survey at the Renal Unit of Hospital São Lucas enrolled 8 adult PD patients, free of

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peritonitis for at least 1 month before blood sampling. All were clinically stable, without inflammatory or infectious complications, and they were not using NO donors. Informed consent was obtained from all participants, and the local scientific and ethics committees approved the study protocol.

Erythrocyte L-arginine uptake assays were performed. Heparinized blood was centrifuged and washed three times with ice-cold saline solution (platelets and white cell layer discarded) for separation of erythrocytes. All samples were kept on ice until uptake assays were performed. Erythrocytes were separated into two aliquots, and one aliquot was incubated with *N*-ethylmaleimide (NEM) before uptake measurement.

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 concentrations of L-arginine (8.8, 15.5, 24.5, 51.0, 100, and 300 µmol/L) and ¹⁴C as a marker. Uptake was interrupted by transferring the sample tubes onto ice. Erythrocytes were then washed free of extracellular radioactivity, lysed (triton 0.1% volume:volume), and protein-precipitated (trichloroacetic acid 5% weight:volume) to recover their intracellular content. Radioactivity was then determined in a liquid-scintillation counter. Uptake was corrected to micromoles per liter – cells per hour. Maximal transport capacity (V_{\max} in micromoles per liter – cells per hour) and half-saturation (k_m in micromoles per liter) were derived from Michaelis–Menten kinetics, using a computer application (Enzfitter for MS-DOS: Biosoft, Cambridge, U.K.). All L-arginine uptake assays were performed in duplicate, and NEM (0.8 mmol/L) was used to measure the NEM-insensitive fluxes corresponding to activity of the y^+L transport system. The NEM-sensitive uptake was considered to be the uptake via the y^+ transport system.

Results are expressed as mean ± standard deviation. The paired Student *t*-test or Mann–Whitney test was used for comparisons. The SPSS software, version 11 for Windows (SPSS, Chicago, IL, U.S.A.), was used for all statistical analyses.

Results

Mean age of the 8 patients (6 women, 2 men) was 49 ± 16 years in 2003. Mean time on dialysis was 25.6 months (range: 9 – 50 months). The causes of end-stage renal disease were hypertensive nephropathy ($n = 3$), polycystic kidney disease ($n = 1$), chronic

glomerulonephritis ($n = 1$), systemic lupus erythematosus ($n = 1$), and others ($n = 2$).

Table I shows the initial and final Michaelis–Menten kinetics of L-arginine uptake via the y^+ and y^+L transport systems. For the years 2003 and 2006 respectively, total L-arginine uptake V_{\max} was 749 ± 182 µmol/L – cells/h and 1146 ± 365 µmol/L – cells/h ($p = 0.016$, paired *t*-test), mean y^+L V_{\max} was 180 ± 58 µmol/L – cells/h and 515 ± 142 µmol/L – cells/h ($p = 0.002$), and mean y^+ V_{\max} was 556 ± 177 µmol/L – cells/h and 662 ± 267 µmol/L – cells/h (nonsignificant). The total y^+L and y^+ k_m were not significantly different.

Discussion

An increment in mean total erythrocyte L-arginine uptake V_{\max} was detected after a 3-year follow-up period in stable PD patients. Transport changes were attributable to an increase in the y^+L system V_{\max} component, with no change observed in the y^+L half-saturation constant or in y^+ kinetics. In a previous study, no significant difference was observed in the maximal transport capacity or the half-saturation constant for both L-arginine transporters, y^+ and y^+L , between the various peritoneal membrane functional categories (7). Moreover, a negative correlation between the maximal transport capacity of the y^+ system and Kt/V was observed (7).

Long-term PD is associated with a reduction in residual renal function (10) and a reduction in total Kt/V (8), resulting in reduced clearance of uremic toxins. Transport of L-arginine has been shown to be altered in undialyzed uremic patients and in hemodialysis and PD patients (11–13), and L-arginine

TABLE I Michaelis–Menten kinetics of L-arginine uptake via the y^+ and y^+L transport systems, mean ± standard deviation

	2003	2006	<i>p</i> Value
V_{\max} (µmol/L–cell/ h)			
Total	749±182	1146±365	0.016
y^+	556±177	662±267	0.260
y^+L	180±58	515±142	0.002
k_m (µmol/L)			
Total	75±15	94±37	0.151
y^+	76±24	108±67	0.202
y^+L	63±39	107±58	0.151

^a $p < 0.05$, paired *t*-test.

V_{\max} = maximal transport capacity; k_m = half-saturation constant.

supplementation may possibly be beneficial in uremic patients (14). Increased y^+ system V_{\max} in the erythrocytes of uremic patients was reported to increase lysine transport (4). Increased V_{\max} may be the result of trans-stimulation, the acceleration of transporter activity by L-arginine or other compatible substrates for the L-arginine transporters (2). Endogenous analogs of L-arginine, such as asymmetric dimethylarginine, are increased in uremia (15,16), and they also have affinity for L-arginine transporters (2). These analogs are inhibitors of the NO synthases and would induce a reduction in NO production (17) despite increased L-arginine uptake. We did not measure plasma and intracellular L-arginine concentration and the levels of its analogs.

Conclusions

The findings of the present study agree with the suggestion of an association between L-arginine transport activity and dialysis adequacy or uremic toxicity.

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