Addition of a Nitric Oxide Inhibitor to a More Biocompatible Peritoneal Dialysis Solution in a Rat Model of Chronic Renal Failure

Marijke de Graaff,1 Anniek Vlijm,1 Machteld M. Zweers,1 Annemieke M. Coester,1 Frédéric Vandemeule,2 Dirk G. Struij,1,3 Raymond T. Krediet1

Biocompatible dialysis solutions have been developed to preserve peritoneal membrane morphology and function. Compared with a conventional solution, a combination of glycerol, amino acids, and dextrose in a bicarbonate/lactate buffer (GLAD) led to less peritoneal fibrosis and fewer vessels in a chronic peritoneal exposure model in the rat. However, no concomitant reduction in small-solute transport was observed. We hypothesized that this result could be attributable to peritoneal vasodilation induced by vasoactive substances such as nitric oxide.

The aim of the present study was to investigate whether fast transport of small solutes and proteins induced by exposure to GLAD could be influenced by L-Nγ-methyl-L-arginine acetate (L-NMMA), an inhibitor of NO. These investigations used our rat model of long-term peritoneal exposure with chronic renal failure.

All rats underwent peritoneal catheter implantation and a 70% nephrectomy. Thereafter, the rats were allocated to 3 groups: 16 weeks of peritoneal exposure to GLAD and L-NMMA, to GLAD only, or to buffer (bicarbonate/lactate without any osmotic agent). Afterward, a standard peritoneal permeability analysis adjusted for the rat was performed. Subsequently, the rats were euthanized, and tissue samples were obtained for morphometric determinations. No effect of L-NMMA on the transport of small solutes and proteins was found. Also, no effect on morphology was found. Our findings make it unlikely that NO is directly involved, being more in favor of a direct effect of amino acids on peritoneal transport.

Keywords
Amino acids, glycerol, peritoneal exposure model, nitric oxide, NO

Introduction
Long-term peritoneal dialysis (PD) can lead to morphologic and functional alterations of the peritoneum (1). These alterations are probably caused by chronic exposure to bioincompatible dialysis solutions. Extremely high concentrations of glucose and the presence of glucose degradation products are among the most important culprits (2). We developed a long-term peritoneal exposure model in Wistar rats, with and without chronic renal failure, in which such alterations could be induced and alternative solutions tested (3,4).

A recent study showed that, compared with a conventional dialysis solution, a mixture of osmotic agents and bicarbonate/lactate buffer [glycerol 1.4%, amino acids 0.5%, dextrose 1.1% (GLAD)] used in a peritoneal exposure model in rats with renal failure resulted in a reduced number of peritoneal vessels (5). However, transport of small solutes was not reduced. We hypothesized that these unexpected findings might be attributable to vasodilation.

Dilation of peritoneal vessels can be caused by exposure to amino acids or by upregulation of endothelial NO synthase (eNOS). A study in stable PD patients exposed to an amino acid–based solution (as compared with a glucose-based solution) showed

From: 1Division of Nephrology, Department of Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands; 2Baxter R&D, Nivelles, Belgium; and 3Dianet Foundation Amsterdam–Utrecht, Netherlands.
higher peritoneal blood flow and higher mass transfer area coefficients (MTACs) of small solutes. This effect was independent of vasodilating prostaglandins, and it was also unlikely to be mediated by NO (6). On the other hand, uremic rats have showed increased expression of eNOS and a beneficial effect of NOS inhibition by \( \text{N}^\gamma \text{-nitro-L-arginine methyl ester (L-NAME)} \) on permeability changes during peritonitis (7,8). Production of NO can be inhibited by \( \text{l-arginine analogs (9)} \) such as L-NAME and \( \text{N}^\gamma \text{-monomethyl-L-arginine (L-NMMA)} \). The latter is able to inhibit both constitutive and inducible NO synthase (10).

The aim of the present study was to investigate whether fast transport of small solutes and proteins induced by exposure to GLAD could be influenced by L-NMMA in our rat model of chronic peritoneal exposure with renal failure.

**Materials and methods**

Male Wistar rats (Harlan CBP, Zeist, Netherlands) with a body weight of 280 – 300 g were divided into 3 groups:

- Experimental group: exposure to GLAD with L-NMMA
- Positive control group: exposure to GLAD only
- Negative control group: exposure to buffer only (bicarbonate/lactate solution without any osmotic agent)

All dialysis solutions were provided by Baxter, Nivelles, Belgium. The rats were housed one to a cage under standardized conditions. All rats underwent catheter implantation and a one-step 70% nephrectomy as previously described (4,5). After the 70% nephrectomy, the animals received a nephroprotective diet to avoid premature mortality from uremia during the period of investigation.

For 16 weeks, all rats received daily infusions of the designated study solution. Before instillation, all PD fluids were preheated to 37°C and heparinized (5 IU/mL). The rats received 6 mL/100 g body weight dialysis fluid intraperitoneally until their body weight exceeded 420 g. Thereafter, the infusion volume was set at 20 mL dialysis fluid daily. To monitor renal function, blood samples were drawn under isoflurane anesthesia every 2 weeks by tail vein puncture. In the GLAD L-NMMA group, L-MNNA (1 mg/kg body weight) was added to the GLAD solution and administered intraperitoneally.

After the experimental period of 16 weeks, a standard peritoneal permeability analysis adjusted for the rat (SPARa) was performed (11). The rats were kept in metabolic cages for a 24-hour urine collection the day before the SPARa to allow residual renal function to be calculated. The SPARa is a modification of the human standard peritoneal permeability analysis (12) that uses dextran 70 (Hyskon: Medisan Pharmaceuticals AB, Uppsala, Sweden) as a volume marker. Every SPARa was performed in a 4-hour dwell with 3.86% Physioneal solution (Baxter Healthcare, Dublin, Ireland). After the SPARa, omental tissue was obtained for morphometric analysis. To judge the extent and distribution pattern of fibrosis, the omentum was stained with pico sirius red (Edward Gurr, BDH brand—VWR International, West Chester, PA, U.S.A.). The scoring was classified as follows: 0 = normal presence of fibrous tissue (compared with tissues from untreated rats from other studies), 1 = mild fibrosis, 2 = moderate excess, 3 = severe deposition of fibrosis. Platelet endothelial cell adhesion molecule 1 antibody (goat anti-rat CD31: Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) was used to count the number of vessels per microscopic field in the rat omental tissue. The vessel density was scored using computer-aided morphometrics.

Peritoneal transport (urea and creatinine) was expressed as dialysate-to-plasma (D/P) ratios and MTACs. Glucose absorption was calculated as the difference between the glucose in the dialysate at the start of the SPARa and the glucose in the dialysate at the end of the SPARa relative to the instilled quantity of glucose. Peritoneal function parameters and fluid kinetics such as net ultrafiltration rate, lymphatic absorption rate, transcapillary ultrafiltration rate, and free water transport were calculated as previously described (12–14). Albumin, immunoglobulin G, and fibrinogen concentrations in plasma and dialysate were measured by ELISA.

Statistical analyses were performed using the SPSS software package (version 16.0: SPSS, Chicago, IL, U.S.A.). An analysis of variance with a Bonferroni correction was used to compare the study groups.

**Results**

Not all animals completed the study. Nephrectomy complications and catheter problems were the reasons for dropout in 32%. After the SPARa, 3 animals were excluded because they had signs of peritonitis on
morphology examination of peritoneal tissues. At the end of the experimental period, 24 rats could be analyzed. Severity of kidney failure after 16 weeks was comparable in the 3 groups. Creatinine clearances were 2.5 ± 0.6 mL/min (mean ± standard deviation) for the GLAD L-NMMA group, 2.3 ± 0.5 mL/min for the GLAD group, and 2.3 ± 1.1 mL/min for the buffer group.

Table I summarizes the parameters of peritoneal solute and fluid transport. Solute transport was higher in both GLAD groups than in the buffer group. The difference reached significance for the GLAD group for D/P urea and D/P albumin. Both GLAD groups showed significantly higher glucose absorption. We observed no differences between the GLAD L-NMMA and GLAD groups, meaning that no effect on peritoneal solute transport parameters of L-NMMA was observed. The GLAD L-NMMA and GLAD groups both showed reduced sodium sieving, but this reduction was not reflected in the amount of free water transport.

Table II shows the semiquantitative scoring of fibrosis and vessels. All groups showed similar fibrotic changes in the omental tissue and a similar number of vessels per microscopic field, without any effect of L-NMMA.

**Discussion**

The addition of L-NMMA to a GLAD dialysis solution had no effect on peritoneal function and morphology. The present study therefore failed to solve the discrepancy between the reduction in the number of peritoneal vessels (5) and the unaltered solute transport rates with exposure to GLAD as compared with exposure to a conventional dialysis solution.

Three possible explanations for the absence of an effect of L-NMMA should be considered:

- First, the dose of L-NMMA used in the study could have been too low. However, the same dose of L-NMMA administered intravenously to patients with septic shock caused an increase in blood pressure (15). It could therefore be expected that L-NMMA applied locally might inhibit NO synthesis in the peritoneal cavity.
- Second, it is possible that L-NMMA has an effect only immediately after administration. In our study design, L-NMMA was administered daily for 16 weeks. After those 16 weeks, the rats did not receive L-NMMA on the specific day of the peritoneal function test.
- Third, it may be that NO is uninvolved in stable, noninfected PD. Observations in a rabbit model of PD support this idea. The addition of L-arginine, the substrate of NO synthesis, had no more effect on peritoneal transport parameters than did the inhibitor L-NMMA (16). Addition of the NO donor nitroprusside in the same rabbit model increased peritoneal albumin clearance 86%. An exchange with a 1.1% amino-acid solution and one with

<table>
<thead>
<tr>
<th>Variable</th>
<th>GLAD L-NMMA (n=9)</th>
<th>GLAD (n=7)</th>
<th>Buffer (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/P urea</td>
<td>0.78±0.07</td>
<td>0.81±0.09b</td>
<td>0.69±0.06</td>
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<tr>
<td>D/P creatinine</td>
<td>0.58±0.07</td>
<td>0.62±0.11</td>
<td>0.51±0.10</td>
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<td>MTAC urea</td>
<td>0.20±0.04</td>
<td>0.22±0.08</td>
<td>0.15±0.02</td>
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<tr>
<td>MTAC creatinine</td>
<td>0.12±0.03</td>
<td>0.13±0.04</td>
<td>0.09±0.03</td>
</tr>
<tr>
<td>Glucose absorption (%)</td>
<td>72±3b</td>
<td>69±4b</td>
<td>62±6</td>
</tr>
<tr>
<td>Net ultrafiltration rate (μL/min)</td>
<td>46±15</td>
<td>51±15</td>
<td>44±12</td>
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<tr>
<td>Lymphatic absorption rate (μL/min)</td>
<td>31±8</td>
<td>31±17</td>
<td>28±12</td>
</tr>
<tr>
<td>Transcapillary ultrafiltration rate (μL/min)</td>
<td>79±12</td>
<td>83±14</td>
<td>73±10</td>
</tr>
<tr>
<td>Maximal dip D/P sodium</td>
<td>0.10±0.02b</td>
<td>0.10±0.02b</td>
<td>0.14±0.04</td>
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<tr>
<td>Free water transport (mL at 60 min.)</td>
<td>3.1±0.3</td>
<td>3.4±1.0</td>
<td>3.6±0.6</td>
</tr>
<tr>
<td>D/P albumin (×10³)</td>
<td>12.4±4.8</td>
<td>14.8±5.9b</td>
<td>8.6±1.7</td>
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<tr>
<td>D/P immunoglobulin G (×10³)</td>
<td>9.2±7.3</td>
<td>7.0±2.0</td>
<td>4.4±1.2</td>
</tr>
<tr>
<td>D/P fibrinogen (×10³)</td>
<td>4.6±3.8</td>
<td>1.5±0.6</td>
<td>2.6±2.5</td>
</tr>
</tbody>
</table>

a Data are expressed as mean ± standard deviation.
b p < 0.05 versus buffer.

GLAD = glycerol, amino acids, and dextrose in a bicarbonate/lactate buffer; L-NMMA = Nγ-methyl-L-arginine acetate; D/P = dialysate-to-plasma ratio; MTAC = mass transfer area coefficient.
Effects of NO on the Peritoneal Membrane

nitroprusside increased small-solute transport in PD patients to some extent, but only nitroprusside doubled peritoneal protein clearances (17). Kinetic modeling in the same study showed that the major effect of nitroprusside is on the large-pore radius, a parameter that amino acids hardly influence.

Conclusions

It can be concluded that the absence of a decrease in small-solute transport is most likely attributable to the vasodilating effects of the amino acids present in GLAD. No indication is present that transport would be induced by inflammation leading to induction of NO synthase. These results support further investigations in humans.

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References

16 Douma CE, Zweers MM, de Waart DR, van der Wardt AB, Krediet RT, Struijk DG. Substrate and

<table>
<thead>
<tr>
<th>Variable</th>
<th>GLAD (n=9)</th>
<th>L-NMMA (n=7)</th>
<th>Buffer (n=8)</th>
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</thead>
<tbody>
<tr>
<td>Submesothelial area</td>
<td>1.7±0.5</td>
<td>1.3±0.5</td>
<td>1.0±0.8</td>
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<tr>
<td>Intersegmental area</td>
<td>1.2±0.4</td>
<td>1.1±0.4</td>
<td>1.3±0.5</td>
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<tr>
<td>Perivascular area</td>
<td>1.3±0.5</td>
<td>1.6±0.5</td>
<td>1.3±0.5</td>
</tr>
<tr>
<td>Overall fibrotic score</td>
<td>4.2±0.8</td>
<td>4.0±0.6</td>
<td>3.5±1.3</td>
</tr>
<tr>
<td>Vessels per microscopic field (n)</td>
<td>22±3</td>
<td>21±8</td>
<td>16±3</td>
</tr>
</tbody>
</table>

a Data are expressed as mean ± standard deviation. No significant differences were observed.

GLAD = glycerol, amino acids, and dextrose in a bicarbonate/lactate buffer; L-NMMA = Nγ-methyl-L-arginine acetate.


Corresponding author:
Marijke de Graaff, Academic Medical Center, Division of Nephrology, Department of Medicine, Room A01-132, PO Box 22660, Amsterdam 1100 DD Netherlands.
E-mail: marijke.degraaff@amc.uva.nl