Inflammatory status is observed in patients with chronic renal failure (CRF). The relationship between oxygen free radical production and dialysis could play an important role in protein oxidation. Carbonyl protein plasma level is an important tool in the study of protein stress, and it is related to the arterial intima thickness in the atherosclerosis process.

We studied protein oxidative stress in 21 peri-toneal dialysis (PD) patients and 42 hemodialysis (HD) patients as compared with 32 undialyzed patients with CRF. Carbonyl protein plasma levels were measured in nanomoles per milligram protein by the ELISA method (Winterbourn et al).

Dialysis patients had a higher protein carbonyl content than did CRF patients (0.1265 ± 0.04 nmol/mg vs. 0.1594 ± 0.03 nmol/mg, p < 0.0002). Patients on PD had a lower level than patients on HD (0.1452 ± 0.03 nmol/mg vs. 0.1665 ± 0.04, p < 0.004).

Glucose administration in PD is known to be able to increase glucose degradation products (GDPs) and advanced glycosylation end-products (AGEs) with high carboxylic and oxidative stress. In our study, the carbonyl protein level was higher in HD patients than in PD patients, perhaps because more protein oxidative stress is associated with hemodialysis technique or because the PD patients had greater residual renal function.

Key words
Chronic renal failure, hemodialysis, oxidative stress, carbonyl protein

Introduction
Inflammatory status is observed in dialyzed and undialyzed chronic renal failure (CRF) patients. The relationship between oxygen free radical production and dialysis could play an important role in protein oxidation. Oxidative reactions most frequently involve free-radical intermediates that have direct or indirect participation in the inflammatory response (1). That response is amplified by the hemobioincompatibility of dialysis systems and solutions, which worsen the pro-oxidant status of uremic patients or which, by activating signalling cascades, mediate proliferation, differentiation, and cell death (2).

The accumulation of oxidized proteins depends upon the balance between pro-oxidant, antioxidant, and proteolytic activity. That oxidatively modified forms of proteins have been demonstrated to accumulate during oxidative stress and in some pathologic conditions has focused attention on physiologic and non physiologic mechanisms for the generation of reactive oxygen species (3,4) (Figure 1).

Hemodialysis is associated with increased oxidant stress. That observation appears to be due to an increased production of free radicals during hemodialysis, a net reduction of many antioxidants, and factors relative to the uremic state. Several studies show the amplified inflammatory response during hemodialysis being produced by various factors and mechanisms (Figure 2). The phenomenon relates to the interaction of uremic toxins, endotoxins, dialysate, and membrane (5,6).

Glucose in peritoneal dialysis solutions increases the auto-oxidation and protein glycation, increasing oxidative factors and reducing the antioxidant response by the polyol pathway. Oxidative stress is associated with an accelerated atherosclerosis process and is accompanied by protein changes (7—9) (Figure 3).
The carbonyl content of plasma proteins is an important tool in the study of protein oxidative stress, and it is related to the arterial intima thickness in the atherosclerosis process. Many ways exist to produce protein carbonyls; they result from the interaction between free radicals and proteins or carbohydrates, and from auto-oxidation of lipids (10).

The aim of our study was to evaluate protein oxidative stress in peritoneal dialysis (PD) patients, hemodialysis (HD) patients, and undialyzed CRF patients.
Protein Oxidative Stress in Dialysis Patients

Patients and methods

Patients

The study involved 21 PD patients [10 men, 11 women; mean age: 59.9 years (range: 16—84 years); mean time on PD: 38±8 months], using the Fresenius Medical Care StayÆSafe system with glucose as the osmotic agent.

42 HD patients [22 men, 20 women; mean age: 60.5 years (range: 38—86 years); time on HD: 51±12 months], using Fresenius Medical Care polysulfone low-flux (F8) membranes.

32 undialyzed patients with CRF (22 men, 10 women; mean age: 57.6 years (range: 20—86 years).

No patients were diabetic.

Analysis of protein carbonyl content

In all of the patients, the carbonyl content of plasma proteins was measured in nanomoles per milligram by the ELISA method [Winterbourn et al (5)].

Results

Table I shows the results of the ELISA measurements.

Discussion

Glucose administration in PD is known to increase glucose degradation products (GDPs) and advanced glycation end-products (AGEs) with high carbonyl and oxidative stress. In our study, the carbonyl protein level was higher in HD patients than in PD patients, perhaps because more protein oxidative stress is associated with hemodialysis technique or because the PD patients had greater residual renal function.

Conclusions

1. Dialysis patients had higher carbonyl protein plasma levels than did undialyzed CRF patients.
2. Patients on PD had lower carbonyl protein plasma levels than did patients on HD.

References


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<table>
<thead>
<tr>
<th>Patient group</th>
<th>Results of ELISA (nmol/mg)</th>
<th>p Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undialyzed CRF</td>
<td>0.1265±0.04</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>All dialysis</td>
<td>0.1594±0.03</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>0.1452±0.03</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>0.1665±0.04</td>
<td>&lt;0.004</td>
</tr>
</tbody>
</table>

a By Student t-test.

CRF = chronic renal failure.
Endothelial Nitric Oxide Synthase Gene Polymorphism in Dialysis Patients

Ana de Prado, Teresa Doate, Ester Martinez, Alba Herreros, Antonio Cabezás, Enric Andrés, Angeles Ortiz, José M. Cubero, José M. Pou

Nitric oxide is an important factor in the regulation of vasodilator tone. In vascular cells, NO is synthesized by endothelial nitric oxide synthase, a key enzyme of the endogenous vasodilator system. Some studies have described the interaction between NO and the other factors that promote vasodilatation in vascular smooth muscular cells. Some of those factors are angiotensin-converting enzyme (ACE), transforming growth factor β (TGFβ), and endothelial oxide nitric synthase (eNOS).

Polymorphism that can alter the expression or the function of the eNOS protein has been identified in the eNOS gene in the promoter and codification zones. One of the recently identified variants of the eNOS gene is a G-to-T conversion at nucleotide position 894 within exon 7 of the eNOS cDNA, resulting in a replacement of glutamic acid by aspartic acid at codon 298 (Glu298Asp). That mutation could be related to hypertension and worse prognosis in nephropathy prevalence and evolution.

The mutation of the eNOS gene has been studied to determine if it could be related to cardiovascular problems. The aim of the present study was to investigate whether the Glu298Asp variant in the eNOS gene varied among patients on dialysis.

Patients and methods
The Glu298Asp genotype of eNOS gene was determined in 62 patients on hemodialysis (HD) and 29 on continuous ambulatory peritoneal dialysis (PD). The two dialysis groups were also compared to 109 healthy controls. Identification of the Glu298Asp variant in exon 7 was performed by enzymatic amplification and restriction fragment length polymorphism (RFLP) analysis.

No significant differences were seen between the control group and the dialysis patients, or between the HD and the PD patients.

Key words
Nitric oxide, endothelial nitric oxide synthase gene, hemodialysis

Introduction
Nitric oxide is an important factor for the regulation of vasodilator tone. In response to stimuli such as hypoxia and shear stress, vascular endothelial cells synthesize NO by endothelial nitric oxide synthase (eNOS) (1). The NO produced enzymatically from L-arginine by eNOS in the endothelium diffuses to the vascular smooth cells (VSMCs) and increases cGMP levels, which mediate muscular relaxation.

Some studies have described the interaction between NO and other factors such as angiotensin-converting enzyme (ACE), transforming growth factor β (TGFβ), and eNOS, which all promote vasodilatation in VSMCs.

Polymorphism that can alter the expression or the function of the eNOS protein has been identified in the eNOS gene in the promoter and codification zones. One of the recently identified variants of the eNOS gene is a G-to-T conversion at nucleotide position 894 within exon 7 of the eNOS cDNA, resulting in a replacement of glutamic acid by aspartic acid at codon 298 (Glu298Asp). That mutation could be related to hypertension and worse prognosis in nephropathy prevalence and evolution.

The mutation of the eNOS gene has been studied to determine if it could be related to cardiovascular problems. The aim of the present study was to investigate whether the Glu298Asp variant in the eNOS gene varied among patients on dialysis.

Patients and methods
The Glu298Asp genotype of eNOS gene was determined in 62 patients on hemodialysis (HD) and 29 on continuous ambulatory peritoneal dialysis (PD). The dialysis groups included 40 men and 34 women (mean age: 55.9 ± 14.4; time on dialysis: 44.5 ± 10 months). The two dialysis groups were also compared to 109 healthy control subjects (mean age: 56 ± 7 years).

Identification of the Glu298Asp variant in exon 7 was performed by enzymatic amplification and restriction fragment length polymorphism (RFLP) analysis (5). The genotyping of the polymorphism was performed by polymerase chain reaction (PCR) amplification with primers 5′-CATGAGGCTCAG-
CCCAGAAC-3’ (sense) and 5’-AGTCAATCCCTTTGGTGCTCAC-3’ (antisense), followed by *Mbo*I restriction endonuclease. The 206 bp PCR product was cleaved into 119 bp and 87 bp fragments in the presence of a T at nucleotide 894. The presence of a T corresponds to the amino acid aspartic acid (Asp).

**Results**

In the control group, the observed frequencies of eNOS genotype were GG, 39.8%; GT, 43%; and TT, 17.2%. In patients on dialysis, the frequencies were (HD subgroup) GG, 40.3%; GT, 38.7%; and TT, 21.7%; and (PD subgroup) GG, 41.6%; GT, 50%; and TT, 8.6%.

We found no difference in the distribution of the studied polymorphism between the 93 controls subjects and the 74 dialysis patients (Table I). No significant difference was seen between the two groups of dialysis patients.

**Discussion**

Various studies have reported that the Glu298Asp variant can be associated with hypertension, acute myocardial infarction, vascular problems, and progression of diabetic nephropathy (4). The etiology for most of the renal diseases is not clear, although vascular changes can be a possible origin. For this reason, eNOS may play a role in renal diseases.

The Glu298Asp variant is not correlated with different levels of NO. Differences are seen between Japanese and Caucasian populations. Although eNOS may be implicated in renal alterations, it may not play a main role; or perhaps the defective eNOS DNA variants manifest in kidney disease only as local pathophysiologic disturbances. Further studies in groups of patients with varying degrees of renal and cardiovascular disease must be performed before conclusions can be drawn.
References


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Glucose Suppresses Peritoneal Inflammatory Reactions and Mesothelial Hyperplasia Caused by Intraperitoneal Saline Infusion

In the past, we had observed that infusion of normal saline into the peritoneal cavity stimulates an inflammatory response. In the present study, we examined what effect the addition of glucose to normal saline would have on the peritoneal inflammatory response and change in peritoneal morphology.

After catheter implantation, rats were infused intraperitoneally (IP) for 3 days with Dianeal 1.36% (Baxter Healthcare Corporation, Deerfield, IL, U.S.A.). Dialysate samples were collected on day 3 after a 4-hour dwell. Next, rats were exposed to either NaCl (n = 7) or NaCl with glucose 250 mmol/L (Glu, n = 7) twice daily for 4 weeks. After 2 weeks and 4 weeks of the study, dialysate samples were collected after a 4-hour dwell to analyze the activity of inflammatory reaction. At the end of the experiment, imprints of peritoneal mesothelium were taken. Control animals (C, n = 6) did not undergo catheter implantation or the dialysis procedure.

The inflammatory reaction cell count, cell differentiation, nitric oxide production, protein loss, and monocyte chemoattractant protein-1 (MCP-1) concentration in dialysate expressed as a percentage of the initial value did not change during the study in rats exposed to NaCl. On the other hand, in Glu-treated animals, the protein concentration was decreased after 4 weeks of the study (74% ± 23%, p < 0.05), as was MCP-1 (24% ± 12%, p < 0.05). The nitrites concentration was decreased after 2 weeks (72% ± 19%; p < 0.05). Intrapерitoneal adhesions were found in 6 rats of the NaCl group (86%) and in only 4 rats (57%) of Glu group. In the NaCl rats, a higher density of mesothelial cells was observed (2792 ± 510 cells/mm²) as compared with Glu rats (2028 ± 561 cells/mm²; p < 0.05) and with control rats (1629 ± 422 cells/mm², p < 0.05). The NaCl group also showed a higher nucleus:cytoplasm surface ratio (0.25 ± 0.03) as compared with the Glu group (0.18 ± 0.02, p < 0.01) and with the control group (0.14 ± 0.01, p < 0.01).

Addition of glucose to normal saline suppresses the peritoneal inflammatory response and mesothelial hyperplasia occurring with intraperitoneal infusion of NaCl solution alone.

Key words Glucose, intraperitoneal inflammation, mesothelial hyperplasia

Introduction Long-term exposure of the peritoneum to dialysis fluid in dialyzed patients results in progressive changes in peritoneal membrane permeability to solutes and water (1). Simultaneously, alterations in morphology occur, including both mesothelial cell changes and disorganization of the peritoneal interstitium (2). In the absence of bacterial peritonitis, such histologic and physiologic alterations are believed to result from bioincompatibility of peritoneal dialysis fluids.

Among the potential bioincompatible components of the dialysis solutions, glucose is one of the most important. Glucose is used as an osmotic agent in most commercially available peritoneal dialysis solutions. Toxic effects of glucose have at least two possible mechanisms: direct metabolic alterations owing to the high glucose concentration itself and alterations secondary to the hyperosmolality of peritoneal dialysis fluids (3, 4).

On the other hand, peritoneal changes also appear to be related to chronic intraperitoneal inflammation induced by the process of peritoneal dialysis (5). Intrapерitoneal infusion of any fluid was shown to result in mechanical irritation of the peritoneum, followed by activation of intraperitoneal inflammatory cells (6). In our previous studies, we showed that phosphate-buffered saline (PBS) which is an isotonic solution with a physiologic pH (and therefore may be
considered a biocompatible dialysis fluid) caused a severe inflammatory response in rats after intraperitoneal administration (7). An intriguing conclusion of our study was that glucose suppressed the peritoneal inflammation caused by PBS, as measured by cell count and neutrophil:macrophage ratio in dialysate, and by nitrites in dialysate (an index of nitrous oxide synthesis). However, the effects on the morphology of mesothelial cells of the inflammatory reaction caused by PBS alone and PBS supplemented with glucose have not been studied.

In the present study, we tried to estimate the effect of the addition of glucose to normal saline on the peritoneal inflammatory response and mesothelial cell morphology in chronically dialyzed rats.

Materials and methods
The study was performed on 20 male Wistar rats weighting between 250 g and 350 g. At the beginning of the study, peritoneal catheters were implanted into 14 rats according to a previously described method (8). (The 6 rats that did not undergo catheter implantation and dialysis made up the control group.)

For the first 2 days, all rats with implanted catheters were injected intraperitoneally with Dianeal 1.36% (Baxter Healthcare Corporation, Deerfield, IL, U.S.A.) supplemented with antibiotics [gentamicin 5 mg/L (Polfa, Tarchomin, Poland) and cefazolin 50 mg/L (Eli Lilly, Florence, Italy)]. The fluid was allowed to absorb. On the third day, after a 4-hour dwell, dialysate samples (5 mL from each rat) were collected to characterize the activity of the inflammatory reaction at baseline. The rats were then randomly divided into two groups and were infused twice daily for a further 4 weeks with 20 mL of various solutions also supplemented with antibiotics:

¥ Group NaCl (n = 7): 0.9% NaCl (Polfa, Kutno, Poland)
¥ Group Glu (n = 7): 0.9% NaCl supplemented with glucose, 250 mmol/L (Sigma, St. Louis, MO, U.S.A.)

To analyze the activity of the inflammatory reaction for the given dialysate after 2 weeks and 4 weeks of the study, samples of dialysate (5 mL from each rat) were collected 4 hours after injection of the given solution in the respective group.

At the end of the study, the rats were humanely killed by bleeding, and the peritoneal cavity was opened to estimate the presence of adhesions in the peritoneal cavity. Also, imprints of mesothelial cells from the visceral peritoneum of the liver were taken to characterize their morphology.

Analysis of the inflammatory reaction
Immediately after dialysate drainage, a cell count (in a hemocytometer) and cell differentiation measurements were performed manually. The neutrophil:macrophage ratio (Ne:Ma, expressed as percentage) was then calculated for every rat.

In dialysate samples, total protein was measured using the colorimetric method described by Lowry (9). Additionally, the concentration of dialysate nitrites (an index of nitrous oxide synthesis) was determined after reduction of nitrates to nitrites with nitrate reductase (Roche, Mannheim, Germany). The nitrite concentration in each dialysate sample was measured using Griess reagent (10), and the monocyte chemoattractant protein-1 (MCP-1) concentration was measured using an ELISA kit (Biosource, Camarillo, CA, U.S.A.).

Mesothelial cell imprints
Imprints of the mesothelial monolayer from the visceral peritoneum were taken after the peritoneal cavity was opened. Glass slides coated with 1% agar (Sigma, St. Louis, MO, U.S.A.) were stuck to the liver surface for 30 seconds, allowing mesothelial cells to be peeled off that surface. Next, fixation was done using 96% ethanol. Slides were stained with hematoxylin (Quimica Clinica Aplicada, Amposta, Spain). The density of mesothelial cells and the nucleus:cytoplasm surface ratio was determined by light microscopy (observed at 200× magnification) supported with digital image analyzing equipment (Screen Measurement 4.0, Prague, Czech Republic).

Statistical analysis
The results obtained in the dialysate from each group after 2 and 4 weeks of exposure to dialysis fluids were compared with the initial values and between the groups in both periods.

Results are expressed as mean ± standard deviation. The statistical analysis was performed using repeated-measures ANOVA and post-hoc analysis with the nonparametric Dunn test or the Mann—Whitney test, as appropriate. A p value of less than 0.05 was considered significant.
Results

Inflammatory reaction

In the NaCl group, the activity of the intraperitoneal inflammatory parameters did not change during the 4-week experiment. It was the same at the end of 4 weeks as it was immediately after catheter insertion (Figure 1). In contrast, the intensity of the intraperitoneal inflammation decreased significantly in Glu group during the study. The total protein concentration was significantly reduced after 4 weeks exposure as compared with the initial results (p < 0.05, Figure 1(C)). However, the nitrite concentration was already diminished after 2 weeks (p < 0.05), and the decrease was even more strongly expressed after 4 weeks [p < 0.01; Figure 1(D)].

Additionally, after 4 weeks exposure, the dialysate cell count was significantly lower in the Glu group than in NaCl group (1443 ± 516 cells/mm³ vs. 2771 ± 1600 cells/mm³, p < 0.05), as was the dialysate total protein concentration (328 ± 101 mg/dL vs. 418 ± 81 mg/dL, p < 0.05) and MCP-1 level (134 ± 57 pg/mL vs. 363 ± 292 pg/mL, p < 0.05). The Ne:Ma ratio was also lower (49% ± 34% vs. 66% ± 29%); however, the difference was not statistically significant.

* p < 0.05, **p < 0.01 (both with respect to initial value). Ne:Ma = neutrophil to macrophage ratio.

FIGURE 1 Peritoneal inflammation markers after 2 and 4 weeks of exposure to 0.9% NaCl alone (white bars) and to 0.9% NaCl supplemented with glucose 250 mmol/L (striped bars).
Alterations in morphology

Intraperitoneal adhesions were found in 6 rats of the NaCl group (86%) and in only 4 rats (57%) of the Glu group.

Rats exposed to NaCl revealed higher mesothelial cell density than did rats exposed to Glu (2792 ± 510 cells/mm² vs. 2028 ± 561 cells/mm², \( p < 0.05 \)) and the non dialyzed control rats (1629 ± 422 cells/mm², \( p < 0.05 \)). The nucleus:cytoplasm surface ratio was also higher in NaCl rats than in Glu rats (0.25 ± 0.03 vs. 0.18 ± 0.02, \( p < 0.01 \)) and in non dialyzed control rats (0.14 ± 0.01, \( p < 0.01 \), Figure 2).

Discussion

In the present study, we showed that physiologic saline solution causes severe inflammatory reaction when administered intraperitoneally. In rats that were injected with physiologic saline, the dialysate cell count, peritoneal permeability to proteins, and nitric oxide concentration remained high for 4 weeks of the experiment. On the other hand, supplementation of the same solution with glucose resulted in lower concentrations of the mentioned factors (Figure 1).

Because we did not drain the entire quantity of intraperitoneal fluid after a 4-hour exposure, we do not know the cumulative values of inflammatory markers. Their concentrations depend on the volume of dialysate, which may be larger in the Glu group owing to the hypertonicity of the injected solution. Therefore, the effect of dilution of the inflammatory markers must be considered in that group.

Still, the alterations in the morphology of the peritoneal mesothelium and the prevalence of intraperitoneal adhesions confirm our hypothesis that the concentration of inflammatory markers, rather than their cumulative value, is a predictive factor for peritoneal injury (Figure 2). Our observations lead us to conclude that glucose suppresses the intraperitoneal inflammation and mesothelial hyperplasia that occur during chronic intraperitoneal administration of 0.9% saline.

Previously, we observed that chronic exposure of the peritoneum to PBS resulted in a severe inflammatory reaction and that that reaction was milder when PBS was supplemented with glucose or mannitol (7). Earlier Wang et al also reported that physiologic saline cannot be considered a biocompatible dialysis solution owing to its influence on lymphatic flow and probable damaging effect on peritoneal tissues (11).

In contrast to our findings of mesothelial cell abnormalities, Hekking et al observed, in a study in rats, a higher density of mesothelial cells after chronic ex-

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**Figure 2** Imprints of mesothelial monolayer obtained from a rat dialyzed with 0.9% saline alone (upper photograph), a rat dialyzed with saline supplemented with glucose (middle photograph) and from a non dialyzed control rat (lower photograph). Slides were stained with hematoxylin and were observed at 200× magnification.
posure to dialysis fluid containing high glucose concentrations as compared with saline fluid (12). Yet, in that study, the volume of dialysis fluid was small (only 10\(^\text{mL}\), once daily) and the duration of the experiment was shorter than that in our experiment.

Is the observed suppressive effect of glucose beneficial or is it not?

Inhibition of mesothelial hyperplasia may be a reflection of a lower degree of peritoneal injury, but it may also indicate ineffective mechanisms of regeneration. Shostak et al. noticed a higher prevalence of apoptotic mesothelial cells and a depletion of growth capabilities after chronic in vivo exposure to glucose (13). Lower concentrations of nitric oxide and MCP-1 are probably related to low activity of peritoneal macrophages and a lower degree of peritoneal damage. But the peritoneal cavity may be more susceptible to peritonitis when phagocyte function is depleted (14).

Further studies have to be performed (based on the same experimental system) to evaluate the function of peritoneal phagocytes and the microscopic changes of the peritoneum in connection with inflammatory reaction. The influence of other osmotic solutes, such as mannitol, on peritoneal inflammation and mesothelial hyperplasia caused by saline also need to be examined to distinguish the hypertonic effect of glucose from its metabolic action.

References


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Small-Solute and Middle-Molecule Clearances During Continuous Flow Peritoneal Dialysis

John K. Leypoldt, John M. Burkart

Previous theoretic and clinical studies have shown that continuous flow peritoneal dialysis (CFPD) provides a high dose of small-solute removal; however, the dose of middle-molecule removal with CFPD therapy has not been evaluated. We used a variable-volume, two-compartment model to calculate theoretical steady-state solute kinetic profiles during CFPD, continuous ambulatory peritoneal dialysis (CAPD), and hemodialysis using a high-flux dialyzer (HFHD), for an anuric 70-kg patient and two measures of dose: equivalent renal clearance (EKR) and standard Kt/V (stdKt/V).

Dose measures during each therapy were calculated for five solutes: urea, creatinine, vitamin B₁₂, inulin, and β₂-microglobulin. Fluid (1 L daily) was assumed to accumulate in and to be removed from the extracellular space, and non renal clearance was assumed to be zero for all solutes except β₂-microglobulin.

Calculated doses for CFPD were higher than for CAPD or HFHD when assessed by either EKR or stdKt/V. Dose enhancements for CFPD were highest for small solutes, but were still considerable for middle molecules. We conclude that CFPD achieves higher doses than CAPD or HFHD for both small-solute and middle-molecule removal.

Key words
β₂-Microglobulin, continuous flow peritoneal dialysis, dose, middle molecules, urea

Introduction
Small-solute clearances during current forms of peritoneal dialysis (continuous ambulatory peritoneal dialysis and automated peritoneal dialysis) are limited predominantly by low dialysate flow rates. Early experience with peritoneal dialysis using a high and continuous flow of dialysis solution [most frequently called continuous flow peritoneal dialysis (CFPD)] showed impressive increases in clearances of small solutes, such as urea and creatinine (1—3). Those early clinical experiences have been confirmed in recent years (4—7), identifying a potential strategy for increasing the dose of small-solute removal during peritoneal dialysis. The impact of a high and continuous flow of dialysate on peritoneal clearances of middle molecules during CFPD has not previously been studied.

The present study describes theoretical predictions for peritoneal clearances of small solutes and middle molecules during CFPD. The predictions were made using a variable-volume, two-compartment model of solute kinetics, similar to that described previously (8). Using two measures of dose [the equivalent renal clearance (EKR) described by Casino and Lopez (9) and the standard Kt/V (stdKt/V) described by Gotch (10,11)], solute clearances for CFPD were compared with those for continuous ambulatory peritoneal dialysis (CAPD) and for hemodialysis using a high-flux hemodialyzer (HFHD). Solute clearances and dose measures were computed for urea as well as for other marker molecules of various molecular weights.

Methods
Solute kinetics were simulated theoretically using a variable-volume, two-compartment model (8) for three different therapies: CFPD, CAPD, and HFHD. Steady-state solute concentration profiles were simulated for five surrogate uremic solutes spanning a broad range of molecular weights (Table I). No binding to plasma proteins was assumed to occur for any solute.

The total volume of distribution for each solute was partitioned into a perfused compartment from
which solute was directly removed and a non-perfused compartment from which solute transport occurred only into the perfused compartment. Solute volumes of distribution were allowed to vary with time owing to interdialytic fluid intake and intradialytic fluid removal. The total volume of distribution for urea, creatinine, and vitamin B\(_\text{12}\) was assumed to be the volume of total body water. Of that total, one third was assumed to be within the perfused (extracellular) compartment, and two thirds, within the non-perfused (intracellular) compartment. The total volume of distribution for inulin and \(\beta\text{-}2\)-microglobulin was assumed to be the extracellular compartment, with intravascular (one quarter of extracellular volume) and interstitial (three quarters of extracellular volume) spaces as the perfused and non-perfused compartments respectively.

All computer simulations were performed for an anephric patient with a post-dialysis weight of 70 kg; total body water was assumed to be 50% of the post-dialysis weight. That latter value was a compromise between the value estimated from anthropometric equations (12) and the lower value estimated from urea kinetics during chronic hemodialysis therapy (13,14). All fluid gained by and removed from the patient was assumed to move into and out of the extracellular compartment; the relative amounts removed from the intravascular and interstitial fluids were assumed to be proportional to the compartment volumes (one quarter and three quarters, respectively).

Solute generation was assumed to occur only within the perfused compartment. Endogenous solute generation rates were assumed as constant for urea, creatinine, and \(\beta\text{-}2\)-microglobulin [(8) Table I]. Vitamin B\(_\text{12}\) and inulin are not naturally generated within the body; nevertheless, to predict steady-state solute concentration profiles for calculating dose measures, it was assumed that those solutes were generated at constant rates.

Solute transfer between the compartments was assumed as proportional to the solute concentration difference; the proportionality constant was defined as the intercompartmental transfer constant, or clearance \(K_{IC}\). The values of \(K_{IC}\) for each solute were assumed to be identical to those reported previously [(8) Table I].

Excess fluid removal during HFHD was assumed as constant throughout the treatment. During a CFPD dwell, the rate of fluid removal was assumed as constant. During a CAPD dwell, the rate of fluid removal depends on the glucose concentration in the dialysate; it was assumed as equal to the time-dependent ultrafiltration rate predicted by the model of Pyle [(15) see Appendix]. Weekly fluid removal was matched to weekly fluid accumulation so that overall patient volume was at steady state. The rate of fluid intake or accumulation was assumed as constant and equal to 1 L daily.

Solute removal from the body was evaluated differently for each therapy; the mathematical details of the relationships are described in the Appendix. Daily CFPD therapy consisted of an 8-hour, 2-L CFPD treatment and two 8-hour, 2-L CAPD dwells using 1.5% glucose—containing dialysis solution. Daily CAPD therapy consisted of four 4-hour, 2-L CAPD dwells using 1.5% glucose—containing dialysis solution and one 8-hour, 2-L CAPD dwell using 4.25% glucose—containing dialysis solution. Solute transport across the peritoneal membrane was calculated accounting for both diffusive and convective transport (see Appendix for details). Solute transport rates by diffusion and convection were assumed to be related to the permeability-area product or mass transfer-area coefficient (PA), and 1 minus the solute reflection coefficient (\(\sigma\)), respectively, for the peritoneal membrane. Table II lists the assumed values of those parameters for CAPD. Previous work has shown that PA values for urea and creatinine during CFPD are substantially higher than those during CAPD (18). We therefore assumed that PA values during CFPD are twice those during CAPD for all solutes.

For HFHD, three 3-hour treatments per week were simulated on a traditional Monday/Wednesday/Friday schedule. Solute clearances during HFHD were calculated assuming the use of a high-flux dialyzer, a blood flow rate of 400 mL/min and a dialysate flow

### Table I: Solute characteristics

<table>
<thead>
<tr>
<th>Solute</th>
<th>Molecular weight (mg/min)</th>
<th>(G^a) (mg/min)</th>
<th>(K_{IC}^b) (mL/min)</th>
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<td>Urea</td>
<td>60</td>
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<tr>
<td>Creatinine</td>
<td>113</td>
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<tr>
<td>Vitamin B(_\text{12})</td>
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<td>125</td>
</tr>
<tr>
<td>Inulin</td>
<td>5,200</td>
<td>0.3</td>
<td>90</td>
</tr>
<tr>
<td>(\beta\text{-}2)-Microglobulin</td>
<td>11,800</td>
<td>0.17</td>
<td>40</td>
</tr>
</tbody>
</table>

\(a\) Solute generation rate.  
\(b\) Intercompartmental transfer constant or clearance.
rate of 800 mL/min to achieve a single-pool urea Kt/V of 1.4 per treatment. The dialyzer mass transfer-area coefficients for each solute in the present study were similar to those assumed by Clark et al. (8) and are shown in Table II. Dialyzer solute clearances during HFHD were also adjusted for ultrafiltration (see Appendix). Non renal clearance (Kr) was assumed to be zero except for β2-microglobulin, where it was assumed to be 3 mL/min (19).

For each solute, a variable time-step, fourth-fifth—order Runge—Kutta algorithm (20) was used to compute steady-state solute concentration profiles by iterative solution of the mass balance equations (see Appendix) over a 10-week interval. From the steady-state concentration profiles, several indices were calculated. The time-averaged concentration (TAC) was obtained for each solute and therapy combination as a time-weighted and distribution volume—weighted average of the intradialytic and interdialytic concentrations, assuming that those concentrations were linear during the intradialytic, post-dialysis rebound, and interdialytic periods (8). The immediate post-dialysis rebound period was assumed to be 1 hour for urea, creatinine, and vitamin B₁₂, and 4 hours for inulin and β2-microglobulin. With the assumed solute generation rate (G) and calculated whole-body TAC values known, the EKR was then calculated (9) as

\[ \text{EKR} = \frac{G}{V} \]  

The mean pretreatment concentration (MPC) was calculated as the highest daily concentration in the perfused compartment (during CFPD and CAPD) or by averaging the three pretreatment concentrations in the perfused compartment (during HFHD). The standard Kt/V (stdKt/V) for each solute was then calculated by the equation

\[ \text{stdKt/V} = \frac{G \times t}{\text{MPC}/V} \]  

where \( t \) and \( V \) denote the total weekly time and volume of distribution for the solute of interest.

**Results**

Solute concentrations during CAPD were relatively time-independent; the concentrations in the perfused and non perfused compartments were very similar in magnitude, but dependent on the solute. Concentrations in the perfused compartment decreased during an 8-hour CFPD treatment by 24% for urea, 13% for creatinine, 7% for vitamin B₁₂, 5% for inulin, and —6% for β2-microglobulin. However, the differences in solute concentration between the perfused and non perfused compartments were uniformly small (<1 mg/dL), indicating little solute disequilibrium during CFPD. Post-dialysis concentration rebounds of urea and inulin in the perfused compartment after HFHD were approximately 25% and 73% respectively. Those values are larger than the values reported previously by Clark et al (8), reflecting the higher efficiency of hemodialysis treatments simulated in the present study.

Table III shows calculated values of EKR and stdKt/V. Values of EKR were higher during CFPD than during CAPD or HFHD for all solutes. Values of stdKt/V were also higher during CFPD than during CAPD or HFHD for all solutes, except for β2-microglobulin. Figures 1 and 2 show the magnitude of the increases in EKR and stdKt/V for each solute during CFPD over CAPD and HFHD.

**Discussion**

Because of rapid removal rates, two-compartment models are required for simulations of solute concentration profiles during intermittent artificial kidney therapies. The concentration profiles simulated in the present study for HFHD confirm previous work indicating that significant post-dialysis rebound occurs in the perfused compartment for all solutes (8), but especially for middle molecules (21). The present study also shows that the magnitude of solute disequilibrium...
during CFPD and CAPD is quite small, suggesting that future kinetics studies of those therapies may be performed using simpler single-compartment models.

The results of the present study show that doses for small-solute and middle-molecule removal are both higher during CFPD than during either CAPD or HFHD. The magnitude of the increase in dose depends on whether EKR or stdKt/V is used for evaluation; nevertheless, the primary conclusion of the present study is relatively independent of the dose measure employed.

The mechanisms for the higher dose during CFPD as compared with CAPD depend on the solute. For example, the increase in urea and creatinine removal during CFPD is attributable both to the increase in the concentration difference across the peritoneal membrane and to the increase in PA. In contrast, the increased dose for middle-molecule removal was attributable primarily to the increase in PA.

It should be emphasized that our clearance and dose estimates are likely conservative. We have assumed that PA values during CFPD are twice those observed during CAPD; however, limited experimental studies have shown even larger increases in clearances and PA values for small solutes such as urea and creatinine (1,3,18). Because the magnitude of the increase in PA for middle molecules has not been evaluated experimentally, future studies regarding peritoneal transport during CFPD should include investigations of middle-molecule removal in addition to evaluations of small-solute removal.

Two limitations of the present study should be mentioned. First, we have assumed that no protein binding of solutes occurs. That assumption is likely good for some small solutes, such as urea and creatinine, but it may not be realistic for other molecules of interest, especially vitamin B₁₂ (22). Second, our theoretical predictions are limited by the accuracy of the
assumed transport parameters. For example, differences in peritoneal membrane transport characteristics among patients may yield clinical results different from those predicted here. Future studies should evaluate differences in solute transport during CFPD and CAPD with respect to patient transport status.

**Conclusion**

We conclude that CFPD achieves higher doses for both small-solute and middle-molecule removal than does CAPD or HFHD.

**Appendix**

The equations governing solute concentrations in the perfused (p) and non perfused (np) compartments are based on mass balances within each compartment as described previously (8):

\[
\frac{d(C_p V_p)}{dt} = G - K_{IC}(C_p - C_{np}) - J_s - K_{NR} \infty C_p \quad [A1]
\]

\[
\frac{d(C_{np} V_{np})}{dt} = K_{IC}(C_p - C_{np}) \quad [A2]
\]

\[
\frac{dV_p}{dt} = -J_v, \quad \text{and} \quad [A3]
\]

\[
\frac{dV_{np}}{dt} = 0, \quad [A4]
\]

where \( J_s \) and \( J_v \) denote solute and volume removal rates, respectively; and \( C_p \) and \( C_{np} \) denote solute concentrations in plasma water. For HFHD, \( J_s \) was set equal to 0 during inter-treatment intervals.

The two preceding equations describe changes in intercompartmental volumes only for urea, creatinine, and vitamin B\(_12\). When considering inulin and \( \beta_2 \)-microglobulin, changes in volume for the perfused and non perfused compartments were assumed as follows:

\[
\frac{dV_p}{dt} = -J_v/4 \quad [A5]
\]

\[
\frac{dV_{np}}{dt} = -3\delta J_v/4. \quad [A6]
\]

Solute and volume removal rates depended on the treatment modality. Solute removal rates during CFPD and CAPD were calculated from this equation (15):

\[
J_s = PA(C_p - C_d) + (1 - \sigma) Q_f \infty C_p, \quad [A7]
\]

where PA and \( 1 - \sigma \) during CAPD dwells are as reported in Table II, and \( Q_f \) denotes the transperitoneal ultrafiltration rate. During CFPD treatment, values of PA were assumed to be twice the values reported in Table II.

The change in the dialysate solute concentration with time during CAPD dwells is governed by this equation:

\[
\frac{d(C_d V_d)}{dt} = J_s - Q_d \infty C_d, \quad [A8]
\]

where \( V_d \) denotes the volume of dialysis solution within the peritoneal cavity and \( Q_d \) denotes the rate of fluid absorption from the peritoneal cavity.

During CFPD, changes in the dialysate solute concentration are governed alternatively by this equation:

\[
\frac{d(C_d V_d)}{dt} = J_s - (Q_d + Q_{pd} + Q_f) \infty C_d, \quad [A9]
\]

where \( Q_{pd} \) denotes the continuous flow of dialysis solution into the peritoneal cavity during that therapy (assumed as 200 mL/min).

Dialysate solute concentration entering the peritoneal cavity during CFPD was assumed to be 0, and the initial concentration during CAPD was assumed to be 0. During CFPD, the transperitoneal ultrafiltration rate was assumed as constant during the treatment. The constant value was assigned to maintain overall weekly fluid balance.

During CAPD dwells, the transperitoneal ultrafiltration rate was assumed to be time-dependent as described previously (15,23). The coefficients of the exponential equation describing transperitoneal ultrafiltration depended on the assumed glucose concentration of the freshly infused dialysis solution for each exchange. The fluid absorption rate from the peritoneal cavity was assumed to be the rate defining the decrease in peritoneal volume after osmotic equilibration between plasma and the dialysis solution (15), and residual volume between exchanges was assumed to be 0.

During HFHD, the solute removal rate was described by this equation:

\[
J_s = (K_d + 0.4\infty Q_f) \infty C_p, \quad [A10]
\]

where \( K_d \) denotes solute clearance in the absence of ultrafiltration, which was calculated using standard formulas (24) from the blood water and dialysate flow rates and the \textit{in vivo} \( K_d A \) values reported in Table II. The second term in the parentheses of equation [A10]
denotes an approximate correction factor for increased solute removal owing to ultrafiltration. That correction factor has been shown to be approximately valid for both urea and β₂-microglobulin (25).

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References

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