Transperitoneal Transport of Uric Acid: Impact of $p$-Cresol, Sodium Hyaluronan, and Sodium Deoxycholate In Vitro

Our study investigated uric acid transport across isolated parietal peritoneum taken from the anterior abdominal wall of white New Zealand rabbits and placed inside a modified Ussing-type chamber. Values for transfer from the mesothelial to the interstitial side of membrane ($M \rightarrow I$) were calculated using the mathematical model of mass transport and are expressed as a coefficient of diffusive permeability [P (in centimeters per second)]. Four separate series of experiments were done. In the first series, we examined uric acid transfer in control conditions (for 120 minutes). In the second and third series, P was calculated before (15 – 60 minutes) and after introduction of $p$-cresol (0.005 g/dL) or sodium hyaluronan (0.04 g/dL) on the M side of the membrane. In the fourth series, transfer parameters were measured before (15 – 75 minutes) and after (90 – 150 minutes) application of sodium deoxycholate (0.104 g/dL).

The dynamics of transperitoneal transport of uric acid were stable. The values of $P \pm$ standard error of the mean (±0.0001) were 1.936 ± 0.324 cm/s and 2.078 ± 0.186 cm/s. Application of $p$-cresol on the M side of membrane lowered uric acid transport by 10%. Application of sodium hyaluronan produced no change, but application of sodium deoxycholate increased the transfer of uric acid by 155%. These observations may have clinical importance.

**Key words**
Peritoneal transport, uric acid, $p$-cresol, hyaluronan

**Introduction**
Elevated serum levels of uric acid independently increase the risk for kidney disease (1). Hyperuricemia is common in peritoneal dialysis (PD) patients and is significantly associated with the rate of decline of residual renal function (2), but its effect in end-stage renal disease has not yet been thoroughly elucidated. Uric acid is well-known to possibly be a marker of oxidative stress, and it may have a potential therapeutic role as an antioxidant. On the other hand, a strong reducing substance can also act as a pro-oxidant, particularly at elevated levels (3).

Plasma concentrations of $p$-cresol are correlated with uremic syndrome. Lower total and free 4-methylphenol levels are found in patients on PD than in those on hemodialysis, and they are connected *inter alia* with albuminemia status (4,5). $p$-Cresol is produced by intestinal bacteria as a fermentation metabolite, mainly from amino acids such tyrosine and phenylalanine (6,7). This toxin inhibits several biochemical, biologic, and physiologic functions in vivo and in vitro (7–9).

The level of hyaluronan (HA) in PD effluent can predict patient survival and is often used as a surrogate marker for peritoneal inflammation. Moreover, high molecular weight fractions of HA have found applications in fluid for PD (10). The HA molecule is an essential component of peritoneal extracellular matrix. It shows anti-adhesive properties and participates in restoration of peritoneal integrity and remodeling of peritoneum (11–13).

Peritoneal membrane that has been chemically modified by deoxycholate sodium is used as a model for changes of the peritoneum induced by prolonged PD and peritonitis (14–17). This detergent can induce damage to mesothelial cells and generation of free radicals (15).

**Materials and methods**
In our experimental model, fragments of parietal peritoneum from New Zealand male rabbits were placed...
into a modified Ussing-type chamber system (Local Ethics Committee for Animal Research approval 47/2009, Poznań, Poland). The active surface area of the membrane was 1.1 cm². The tissue was connected by peristaltic pump to a fluid reservoir containing Hanks solution (NaCl, 136.88 mmol/L; KCl, 5.36 mmol/L; NaHCO₃, 4.16 mmol/L; CaCl₂, 1.26 mmol/L; KH₂PO₄, 0.44 mmol/L; Na₂HPO₄ × 12 H₂O, 0.34 mmol/L; MgCl₂ × 7 H₂O, 0.41 mmol/L) circulated at a rate of 11 mL/min. Adequate oxygen content and a constant pH of 7.4 in the medium were both maintained by continuous bubbling with a gas mixture consisting of 5% CO₂ and 95% O₂. The whole system was placed in a thermostatic box at 37°C (14).

Four separate series of experiments were done:

- In the first series, uric acid transfer [initial concentration gradient: 20 mg/dL; molecular weight: 168 Da (Serva Electrophoresis, Feinbiochemia, Heidelberg, Germany)] was examined in control conditions for 120 minutes.
- In the second and third series, the coefficient of diffusive permeability ($P$) was determined before (15 – 60 minutes) and after (75 – 120 minutes) application of $p$-cresol [0.005 g/dL, 108 Da (Sigma Chemical, St. Louis, MO, U.S.A.)] or sodium HA [0.04 g/dL, 2000 kDa (Serva Electrophoresis)] on the mesothelial (M) side of the membrane.
- In the fourth series, transfer parameters were measured before (15 – 75 minutes) and after (90 – 150 minutes) application of sodium deoxycholate [0.104 g/dL, 414 Da (POCH, Gliwice, Poland)] on the mesothelial (M) side of the membrane.

Transfer rates of uric acid from the interstitial (I) to the mesothelial side (I→M) of the peritoneal membrane and in the opposite direction (M→I) were studied. Sampling of the medium was carried out at regular intervals (15 minutes). Uric acid concentrations were measured by an enzymatic colorimetric method with uricase and peroxidase (Cormay, Lublin, Poland). The procedures were validated for accuracy, specificity, linearity, and sample stability.

We used a mathematical model of mass transport to estimate $P$ (scaled to the surface area of the in vitro study membrane) for the examined specimen. The changes of $P$ attributable to experimental modifications were determined individually for each experiment as a percentage of the control value before the change and are presented as mean ± standard error of the mean for the whole series. In this way, for each piece of peritoneal membrane, the initial portion of the experiment served as a control for the second portion (14). The statistical analysis used the Wilcoxon test and the Student t-test for paired data (Statistica 8: StatSoft, Tulsa, OK, U.S.A.). The Shapiro–Wilk test was used to evaluate the data distribution. Values of $p$ less than 0.05 were considered statistically significant.

**Results**

During the control experiments, the rate of uric acid passage remained constant over 120 minutes both for transfer directed from the I to M side of membrane and for transfer in the opposite direction. The values of $P$ ± standard error of the mean for I→M and M→I were $1.936 \pm 0.324$ cm/s and $2.078 \pm 0.186$ cm/s respectively (both $\times 0.0001$). Application of sodium HA did not change the bi-directional transfer of uric acid. By contrast, introduction of $p$-cresol into the experimental system lowered I→M and M→I uric acid transport by a mean of 10% ($p < 0.03$). Application of sodium deoxycholate to the experimental system at 75 minutes caused an increase of bi-directional transport of the toxin by a mean of 155% ($p < 0.01$, Figure 1).

**Discussion**

The influence of $p$-cresol, sodium HA, and deoxycholate on transperitoneal transport of low molecular weight solutes such uric acid clearly varies. Clinical and experimental analyses show that uric acid is rapidly transported from the blood to the peritoneal cavity, mainly across small pores (18). Transfer through the peritoneum occurs bi-directionally, depending on the concentration gradient. Assuming that transperitoneal transfer of small solutes is mainly diffusive (by intercellular and transcellular pathways), it is nevertheless impossible to exclude active transport (18,19). In the case of certain small solutes (for example, glucose), transperitoneal transfer may reflect processes other than simple passive passage (19). Earlier research uncovered the active component of urate passage in rabbit renal brush-border membranes (20).

Clinical study in continuous ambulatory PD patients suggests that peritoneal transfer of uric acid can possibly be modified with the use of such modulators of urate transport as pyrazinamide and probenecid, found at the proximal tubules (21). The work presented here analyzed the passage of uric acid, a small negative solute (molecular radius: 3.1 Å;
molecular weight: 168 Da). Comparing the first and second hours of the control experiments, the present study observed stability of transfer. This stability was present in both transfer directions (I → M, M → I), suggesting that it was attributable to the diffusive component of transperitoneal uric acid transport.

Studies of the influence of p-cresol on transfer across the peritoneal membrane are limited, and they relate mainly to the endothelium or the cellular membrane of bacteria (6,22). This toxin has been observed to affect cellular oxygen uptake, cell growth, and the membrane permeability of Escherichia coli (6,23). Moreover, p-cresol has been shown to lower the cytokine-induced expression of adhesion molecules and monocyte adhesion to cytokine-stimulated endothelial cells. Previous studies showed that p-cresol mediates change in endothelial permeability in a Rho kinase–dependent way (22). Considering the foregoing evidence, the modification of transperitoneal uric acid transport that we observed in vitro (lowered by 10%) does not exclude the possibility that the same mechanism is operating here.

In vivo studies of the effect of HA on peritoneal transport of small molecules have produced varying results. As with the present in vitro study, some of those HA studies indicate no change in peritoneal permeability for small and large solutes after HA use; others show a decrease in transfer parameters in the case of macromolecules and an increase for low molecular weight solutes (11,12,24). The ability of HA to inhibit peritoneal permeability for large but not for small compounds is inter alia connected with a reduction of hydroconductivity in the peritoneum. The three-dimensional structure of high weight molecular fractions of HA (>1.6×10^6 Da) make it possible for the molecule to bind to water, with a concomitant change in ultrafiltration (11,13). In earlier in vitro studies, we observed diminished icodextrin (14.6 kDa) transperitoneal transfer after application of HA, without a change in creatinine (113 Da) passage (16). Notably, the lack of such an effect was also observed in the present analysis of uric acid transport in vitro.

In vivo studies show that sodium deoxycholate can induce apoptosis and necrosis of mesothelial cells and can accelerate free-radical reactions. Furthermore, Gotloib et al. observed a considerable increase in the concentration of malondialdehyde in plasma (a result of intensive peroxidation of membrane lipids) 5 minutes after application of deoxycholate (15). Exposure to sodium deoxycholate for 10 minutes caused a 20% increase in glucose concentration and a 65% increase in albumin concentration in the abdominal cavity of animals in a PD model (15). In the present study, sodium deoxycholate also intensified peritoneal uric acid transfer by a mean of 155%. In previous research, this detergent increased the transport of other small molecules (glucose, urea) through the isolated peritoneum (model with both mesothelium and interstitium) and, to an even greater extent, passage of large solutes (albumin, icodextrin, insulin). Comparisons of the in vitro results indicate that, together, the mesothelium and interstitium restrict transfer of low molecular weight compounds to a lesser extent than they restrict macromolecules (14,16,17).

The changes in the coefficient of uric acid transport are probably the result of exfoliation of the mesothelium and looseness of the interstitium because of oxidative stress. Sodium deoxycholate can denude the peritoneal mesothelium (15,17). In our previous studies of isolated parietal peritoneum, we observed a decrease in mesothelium thickness by 20% and an increase in parietal peritoneum thickness by about 40% (connected with a loosening of the tissue) after introduction of sodium deoxycholate (17).
Conclusions
Given the present data, we can conclude that, in vitro, sodium HA does not modify peritoneal transport of uric acid, p-cresol lowers such transport, and sodium deoxycholate intensifies it. From the clinical viewpoint, these observations may be important for the efficiency of the peritoneal membrane.

References

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