

Hyaluronan Influence on Diffusive Permeability of the Peritoneum *In Vitro*

Teresa Grzelak, Beata Szary, Krystyna Czyzewska

Hyaluronan (HA), an essential component of peritoneal extracellular matrix, participates in restoring peritoneal integrity and remodeling the peritoneum changed by prolonged peritoneal dialysis and repeated peritonitis episodes. The aim of the present study was to compare urea, creatinine, and uric acid transport across the peritoneal membrane in control conditions and after HA application. Experiments were undertaken using rabbit parietal peritoneum and a modified Ussing-type chamber. Values of the transfer directed from the interstitial to the mesothelial side of the membrane (I → M) and in the opposite direction (M → I) were expressed as coefficients of diffusive permeability P. Transperitoneal transport in control conditions (for 120 minutes) and transfer parameters before (15 – 60 minutes) and after HA application (2000 kDa, 0.04 g/dL, 75–120 minutes) were examined. In the control series, stability of bi-directional transport for urea (0.02 g/dL), creatinine (0.1 g/dL), and uric acid (0.02 g/dL) was observed. The values of P ± standard error of the mean for I → M and M → I transfers were respectively 2.293 ± 0.211 and 2.621 ± 0.457 for urea, 1.522 ± 0.102 and 1.865 ± 0.244 for creatinine, and 1.936 ± 0.324 and 2.078 ± 0.186 for uric acid [all $\times 10^{-4}$ cm/s]. Application of HA reduced bi-directional urea transport by a mean of 12%, but did not change the P for creatinine and uric acid. These results show that in vitro HA modifies the dynamics of transport for certain small solutes.

Key words

Peritoneal transport, hyaluronan, urea, creatinine, uric acid

Introduction

The removal of water and uremic toxins from the body of patients with renal disease is one of the priorities of

peritoneal dialysis. Unfortunately, long-term therapy and repeated peritonitis episodes gradually cause morphology and functional changes of the peritoneum acting as a permselective dialysis membrane (1–3). Cytoprotective factors such high molecular weight fractions of hyaluronan [HA (>1000 kDa)] may modify the structure and transport properties of peritoneum (1,4–6). Non sulfated polysaccharide (containing equal amounts of uronic acid and hexamine), the essential component of extracellular matrix, has a modulatory role in water homeostasis, flow resistance, and sieve effect. Hyaluronan in solution forms a three-dimensional chain network with reactive groups (acetamido, carboxyl, and hydroxyl). High molecular weight HA is multi-anionic inhibitor of endothelial cell proliferation and migration with osmotic, anti-adhesive, and anti-oxidant properties as a free-radical scavenger. In contrast, its low molecular fractions (created, for example, during free-radical degradation) stimulate proliferation, sprout formation, and angiogenesis (1,7,8).

Peritoneal transfer characteristics after application of HA have hitherto been investigated in single studies in humans and animals, but the results of those studies varied widely (3,6,7). The aim of the present analysis was to examine the short-term influence of sodium HA of high molecular weight on the dynamics of bi-directional transfer of urea, creatinine, and uric acid across the peritoneal membrane *in vitro*.

Materials and methods

Tissues (fragments of parietal peritoneum isolated from the abdominal wall of New Zealand male rabbits) were immediately placed into a modified Ussing chamber connected by peristaltic pump with a circulation rate of 11 mL/min to a reservoir containing Hanks solution of the following composition: 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, 0.49 mmol/L; MgCl₂ × 6 H₂O, 0.49 mmol/L; MgSO₄ × 7 H₂O, 0.41 mmol/L. A constant pH of 7.4 and adequate oxygen content were both maintained

From: Department of Chemistry and Clinical Biochemistry, Poznań University of Medical Sciences, Poznań, Poland.

in the medium 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 (9,10). The experimental procedure was approved by the Local Ethics Committee for Animal Researches in Poznań, Poland (Nr. 22/2006).

In a control series (15–120 minutes), transport directed from the interstitial to the mesothelial side (I → M) of the peritoneal membrane and in the opposite direction (M → I) was studied for three small-molecule solutes: urea (60 Da; initial concentration gradient: 0.02 g/dL; POCH, Gliwice, Poland), creatinine (113 Da; 0.1 g/dL; Sigma Chemical, St. Louis, MO, U.S.A.), and uric acid (168 Da; 0.02 g/dL; Serva Electrophoresis, Feinbiochemia, Heidelberg, Germany). In separate series, the same parameters were examined before (15–60 minutes) and after (75–120 minutes) application of sodium HA (2000 kDa; 0.04 g/dL; Serva Electrophoresis, Feinbiochemia) on the mesothelial side of membrane. Sampling of the medium was carried out at regular 15-minute intervals. Concentrations of molecules were measured by a kinetic enzymatic method with urease and glutamate dehydrogenase for urea, a modified Jaffe method for creatinine, and an enzymatic colorimetric method with uricase and peroxidase for uric acid (Cormay, Lublin, Poland).

We used a mathematical model of mass transport to estimate the diffusive permeability coefficient P for the examined solutes. The changes of P attributable to experimental modifications were determined individually in each experiment as a percentage of the control value before the change and are presented as mean \pm standard error of the mean (SEM) 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 (9).

The statistical analysis used the Wilcoxon and Student t -tests for paired and unpaired data respectively. A value of $p < 0.05$ was considered statistically significant. The distribution of the data was evaluated using the Shapiro–Wilk test (Statistica, version 7.1; StatSoft, Tulsa, OK, U.S.A.).

Results

In the control series (over 120 minutes), we observed stability of the bi-directional transport for urea, creatinine, and uric acid (Figure 1). The values of P for each molecule were similar in the I → M and M → I transfer directions. The mean values of $P \pm$ SEM for I → M and

M → I transport were, respectively, 2.293 ± 0.211 and 2.621 ± 0.457 for urea, 1.522 ± 0.102 and 1.865 ± 0.244 for creatinine, and 1.936 ± 0.324 and 2.078 ± 0.186 for uric acid (all $\times 10^{-4}$ cm/s). Introduction of HA at 60 minutes into the experiment reduced I → M for urea by 11% ($p < 0.05$) and by 12% ($p < 0.04$) in the opposite direction. The values of P in the case of bi-directional creatinine and uric acid transfer did not change (Figure 2).

Discussion

The results of *in vivo* and *in vitro* studies show that transperitoneal transport of small molecules depends on many different factors. The process is inversely proportional to the molecular mass of the solutes. Furthermore, passage across the peritoneum depends on charge, shape, size, lipid solubility, and degree of binding to proteins. Assuming that transperitoneal transfer of small solutes is mainly diffusive (by intercellular and transcellular pathways), neutral molecules should be transported through the peritoneum more rapidly than are electrically charged molecules (11,12). In the *in vitro* study described here, such relationships have not been always found. In the control series, without

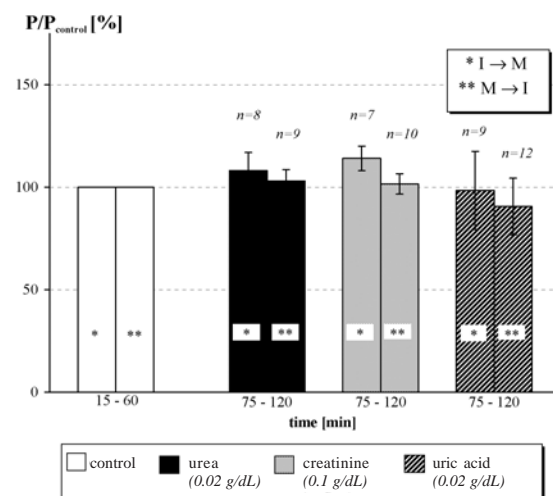


FIGURE 1 Diffusive permeability coefficients (P) of rabbit peritoneum for urea (0.02 g/dL), creatinine (0.1 g/dL), and uric acid (0.02 g/dL) in the control series, expressed as a percentage of the value and standard error of the mean obtained in the first hour of the experiments (open bars). The number of experiments for each subsequent bar is shown. I = interstitial side of the peritoneum; M = mesothelial side of the peritoneum.

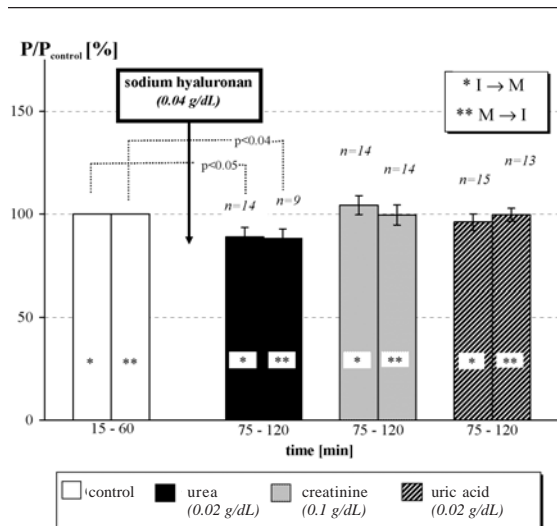


FIGURE 2 Changes in the diffusive permeability coefficients (P) of rabbit peritoneum for urea (0.02 g/dL), creatinine (0.1 g/dL), and uric acid (0.02 g/dL) caused by application of sodium hyaluronan (0.04 g/dL) on the mesothelial side of membrane, expressed as a percentage of the value and standard error of the mean obtained in the first hour of the experiments (open bars). The number of experiments for each subsequent bar is shown. I = interstitial side of the peritoneum; M = mesothelial side of the peritoneum.

HA, the mean values of P for the bi-directional transfer of uric acid (168 Da; $2.007 \pm 0.225 \times 10^{-4}$ cm/s), which has a negative charge at pH 7.4, were similar to those for another analyzed small, neutral solute (for example, urea: 60 Da; $2.457 \pm 0.334 \times 10^{-4}$ cm/s). Moreover, in the case of molecules smaller than uric acid—for example, creatinine (113 Da)—the mean P value was relative low and amounted $1.693 \pm 0.173 \times 10^{-4}$ cm/s. These observations suggest that diffusion is not the sole transfer mechanism through the peritoneum for uric acid.

Previous research uncovered the active component of urate transport in rabbit renal brush-border membranes (13). Clinical study in continuous ambulatory peritoneal dialysis patients suggests the possibility of modifying uric acid peritoneal transfer using modulators of urate transport such pyrazinamide and probenecid, found at the proximal tubules (14). It is worth adding that, in the case of certain small, neutral solutes (for example, glucose), their transperitoneal transfer may reflect processes other than simple passive passage (15).

The possibility of directly applying high molecular weight HA, which is known as a cytoprotective molecule with osmotic, antioxidative, antiangiogenic, and wound healing capability, to the peritoneal cavity has been considered (1,6,7). Animal studies observed that, after long-term HA intraperitoneal application, HA accumulated in interstitial tissue and afterwards was readily metabolized even in uremia. Moreover, increase of endogenous HA in peritoneum was reported in experimental peritoneal dialysis using *N*-acetylglucosamine as the osmotic factor (1,4,5).

The previous clinical and animal studies (in both *in vivo* and *in vitro* conditions) concerning the effects of HA on transperitoneal transport differ in their conclusions. These differences are probably connected to different HA molecular weights and concentrations, exposure times, and peritoneal transport parameters examined (6,7,16–18). Intraperitoneal application of low molecular weight hyaluronan fractions (85 kDa, 280 kDa, and 500 kDa, at 0.01 g/dL) did not change the transcapillary ultrafiltration rate; however, a decrease in this parameter was shown in the case of the high molecular weight compound (4000 kDa at 0.01 g/dL) during 4-hour dwells in animals. Increases in the dialysate concentration of HA (500 kDa) in the range 0.01–0.5 g/dL increases net ultrafiltration. The highest transcapillary ultrafiltration rate has been observed for 0.05 g/dL HA, and the lowest for 0.5 g/dL concentration of glycosaminoglycan (19). Previous research has reported that intraperitoneal application of 500 kDa fractions of hyaluronan (0.01 g/dL) reduce peritoneal fluid absorption and increase the clearance of urea in a 240-minute dwell (16). The osmotic effect of high-concentration HA may counterbalance the decrease in transcapillary ultrafiltration and reduce the hydraulic conductivity of tissue without changing the transcapillary ultrafiltration rate (1).

Clinical studies of peritoneal dialysis dwells of 3 or 6 hours with intraperitoneal application of HA (2000 kDa at 0.01 g/dL and 0.05 g/dL) did not show significant changes in net ultrafiltration and transport parameters for urea, creatinine, and albumin [clearances, dialysate-to-plasma (D/P) ratios, and mass transfer area coefficients], despite a trend toward to decreased fluid reabsorption (6). Long-term HA use (7 days, 1600 kDa, 0.025 g/dL) with standard peritoneal solution diminished K_{BD} coefficients for urea, creatinine, and albumin as compared with those observed in animals undergoing classical dialysis without HA, but had no

effect as compared with a control non dialyzed group (17). Moreover, in another *in vivo* study, authors observed a reduction of transperitoneal protein equilibration, an increase of drained dialysate volume, and a trend toward both toward an increase in clearance and a decrease in the D/P for urea after 4 weeks of HA application during peritoneal dialysis in animals (4).

The present study analyzed the direct, short-term (60 minutes) influence of high molecular weight hyaluronan (2000 kDa, 0.04 g/dL) on small-molecule transport of solutes *in vitro*. The results show a decrease in the *P* for urea and no change in the *P* for creatinine or uric acid. In previous *in vitro* studies, we observed an absence of modifications of transperitoneal transfer in the case of insulin and albumin after HA application (18). The above-mentioned change is probably connected with the creation of a filter-cake on the border surface of the peritoneum and a concomitant resistance to water and transport of certain dissolved small molecules across the membrane *in vitro*. The authors of the previous study suggested that highly polymerized HA (cross-linking possibilities exist, both with itself and with other solutes) builds a sieve structure and thus creates an additional barrier to rapid change in fluid content and transperitoneal passive diffusion of water and molecules (20). In the case of urea, we also cannot exclude a specific physicochemical impact of HA connected with some combination of osmotic pressure, viscosity, sieve effect, degradation on subunits, and interaction with the HA domain. A specific effect of HA in the case of certain small solutes (specifically glucose) are reported in both *in vivo* and *in vitro* studies. The interaction between glucose and the HA domain probably facilitates small-molecule movement and enhancement of absorption of this hexose during dwells in animals after application of HA to the peritoneal cavity and increases diffusion of glucose in a matrix gel containing HA (16).

Conclusions

Peritoneal transfer of urea, creatinine, and uric acid *in vitro* shows stability over 120 minutes of the experiment. Short-term application of high molecular weight sodium HA slightly diminishes bi-directional urea transfer, but does not change the dynamics of transperitoneal transport for creatinine and uric acid. The physicochemical properties of HA suggest the possibility of specific impact on transperitoneal urea transport.

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Corresponding author:

Teresa Grzelak, PhD, Department of Chemistry and Clinical Biochemistry, Poznań University of Medical Sciences, Swiecickiego 6 Str., Poznań 60-781 Poland.

E-mail:

tgrzelak@ump.edu.pl