Hyaluronan Decreases Peritoneal Fluid Absorption in Peritoneal Dialysis

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Abstract. Hyaluronan, exhibiting a high resistance against water flow, acts in the tissue as a barrier against rapid changes in water content. To test whether hyaluronan has any effect on the peritoneal fluid and solute transport, and, in particular, on the peritoneal fluid absorption, a 4-h dwell study with an intraperitoneal volume marker (radiolabeled human serum albumin [RISA]) was conducted in 21 male Sprague Dawley rats (three groups, seven rats in each group). Each rat was injected intraperitoneally with 25 mL of 1.36% glucose solution alone (control group), with 0.005% hyaluronan (HA1 group), or with 0.01% hyaluronan (HA2 group). Dialysate and blood samples were taken frequently for analyses of fluid and solute (urea, glucose, and protein) transport. The intraperitoneal volume was calculated from the dilution of RISA with a correction for RISA disappearance from the peritoneal cavity. This study shows that adding hyaluronan to peritoneal dialysis solution significantly ($P < 0.01$) increased the net peritoneal fluid removal, mainly due to a significant decrease in the peritoneal fluid absorption rate ($P < 0.01$). The diffusive mass transfer coefficients for glucose, urea, and protein did not differ between the three groups. The peritoneal clearance of urea increased significantly in the two hyaluronan groups compared with the control group, due to the increased net fluid removal in the hyaluronan groups. These results suggest that intraperitoneal administration of hyaluronan during a single peritoneal dialysis exchange may significantly increase the peritoneal fluid and solute removal by decreasing peritoneal fluid absorption. (J Am Soc Nephrol 8: 1915–1920, 1997)

Several pharmacological substances have been used to improve the dialysis efficiency of the peritoneum. However, none of them has been clinically used to date (1). Most of these studies tried to increase the peritoneal solute transport rate, whereas only a few aimed to increase the peritoneal ultrafiltration (2,3). Recently, we found that increased peritoneal solute transport rate was associated with increased peritoneal fluid absorption, which resulted in decreased peritoneal fluid removal (4). It has also been reported that peritoneal fluid absorption may greatly decrease the efficiency of peritoneal dialysis (5). Therefore, decreasing the peritoneal fluid absorption may be an efficient way to improve the adequacy of peritoneal dialysis, especially for those patients who have high peritoneal transport rate.

Hyaluronan is a long polysaccharide chain made up of repeating disaccharide units of $N$-acetylglucosamine and glucuronic acid. Hyaluronan is found in most tissues in the body (6,7), and measurable amounts of hyaluronan are found in the drainage dialysis fluid during peritoneal dialysis (8,9). Hyaluronan plays an important role in tissue hydraulic conductivity. It has been proved that hyaluronan exhibits a high resistance against water flow and can thus act in tissue as a barrier against rapid changes in water content (10). Hyaluronan in the interstitium decreases the water permeability of the membrane. Lai-Fook and Brown measured hydraulic conductivity (HC) of the pulmonary interstitium and found that HC was increased in the presence of positively charged molecules. However, the increase in HC was not observed after treatment with hyaluronidase (11). The authors concluded that HC was controlled mainly by hyaluronan. Similar results were reported by Zawieja et al. (12).

If similar effects on peritoneal fluid absorption could be demonstrated by addition of hyaluronan to peritoneal dialysis fluid, this could conceivably improve efficiency of fluid, and therefore solute, removal in peritoneal dialysis. Studies done to investigate the potential use of hyaluronan in peritoneal dialysis have thus far been limited to its potential radical scavenger effect for prevention of peritoneal damage and promotion of tissue healing (13). Wieczorowska et al. found that addition of hyaluronan (0.01%) to saline prevented the peritoneal damage induced by daily intraperitoneal injection of saline. However, whether hyaluronan will affect the peritoneal membrane transport characteristics is still unknown. In this study, we therefore made a detailed analysis of the effect of hyaluronan on the peritoneal fluid and solute transport characteristics in rats.

Materials and Methods

Twenty-one male Sprague Dawley rats with an average body weight of 300 g were randomly divided into three groups. Each rat
was anesthetized initially with a single intraperitoneal injection of 50 mg/kg pentobarbital sodium. This anesthetia was reported not to alter the peritoneal fluid transport in rats (e.g., peritoneal fluid absorption rate and peritoneal lymphatic flow) (14). After 2 h, the rat was given 25 mg/kg pure pentobarbital sodium subcutaneously every hour to maintain the intensity of anesthesia during the experiment. The fur over the abdominal wall was closely shaved. The animal was laid in a supine position and was kept at 37°C with a heating pad (CMA/ Microdialysis, Stockholm, Sweden). Isotonic saline (1 ml/h) was injected subcutaneously to prevent hypovolemia. A multiboloed SILASTIC catheter (0.8 mm internal diameter, Cole Palmer, Chicago, IL) was inserted percutaneously below the xiphoid process, placed in the left lower quadrant of the abdomen, and secured with a purse string stitch. The catheter was used for dialysis fluid infusion and sampling. Each animal was infused intraperitoneally with 25 ml of Dianal® 1.36% glucose dialysis fluid with 0.005% hyaluronan (Sigma, human umbilical cord, protein content <5%, kind gift of Dr. A. Breborowicz, Poznan Medical School, Poznan, Poland) (n = 7, HA1) or 0.01% hyaluronan (n = 7, HA2) or without hyaluronan (n = 7, control group). The dialysis fluids (with or without hyaluronan) contained 18.5 kBq 131I-human serum albumin (RISA) (Isopharma AS, Kjeller, Norway). A priming dose of 0.4 g/L human serum albumin was added in the dialysis solutions to minimize the adhesion of tagged albumin to the surface of the catheter. Solutions were prewarmed to 37°C before instilled intraperitoneally via a three-way valve (Vigo, Connecta, Helsingborg, Sweden) connected to the end of a 0.8-mm catheter over a period of approximately 1 min and allowed to remain in the peritoneal cavity for 4 h. Dialysate samples (0.35 ml) were taken at 0, 3, 15, 30, 60, 90, 120, 180, and 240 min after infusion. Before each sampling, 1 ml of the dialysate was flushed back and forth five times through the catheter. Blood samples were drawn at 0, 120, and 240 min from the tail artery. After 240 min, the peritoneal cavity was opened and the dialysate was collected using syringe and preweighted gauze tissues, and the volume was recorded. The experimental study was approved by the Animal Ethical Committee of the Karolinska Institute at Huddinge Hospital.

Dialysate samples (0.1 ml) and blood samples (0.1 ml of plasma) were analyzed for RISA activity on a gamma counter (Packard Instruments, Meriden, CT) for 10 min each. Dialysate and plasma concentrations of urea (urease-glutamate dehydrogenase method), protein (Coomassie Brilliant Blue dye binding method), and glucose concentrations (hexokinase method) were analyzed using a Multistat Auto Analyser (Instrumentation Laboratory, Spokane, WA). In separate in vitro experiments, no interferences were found between 0.01% hyaluronan and the measurement of these solutes. Hyaluronan in the dialysate effluents was measured using a radiometric assay kit (Pharmacia, Uppsala, Sweden).

Intrapерitoneal dialysate volume was estimated from the dilution of RISA, with corrections made for the elimination of RISA from the peritoneal cavity and the sample volumes (15). In a preliminary study, we performed direct measurements of intraperitoneal volume (with hyaluronan) at 3 and 120 min; we did not find any significant difference in the intraperitoneal volume between the direct measurements and the values estimated from RISA determination, suggesting that the negative hyaluronan charge would not affect the determination of intraperitoneal volume by the RISA dilution method. Note that the intraperitoneal volume at the end of the dwell was directly measured. The peritoneal fluid absorption rate was estimated as the coefficient of RISA elimination from the peritoneal cavity, K_E (ml/min), and the transcapillary ultrafiltration rate was calculated as net volume change plus K_E.

The dialysate over plasma (D/P) concentration ratios for all of the investigated solutes were calculated by dividing the dialysate concentrations of the investigated solutes at a certain time by the aqueous concentrations of the investigated solutes in plasma (16). If no blood sample was taken at the same time as a dialysate sample then the blood concentration of the solute was linearly interpolated from the blood sample taken before and after this moment (17). The D/D_0 for glucose was calculated as the dialysate glucose concentration (D) divided by the glucose concentration in the fresh dialysis solution (D_0). However, the D/D_0 concentration ratio is more sensitive to other parameters than the peritoneal basic transport process; in particular, it is sensitive to intraperitoneal volume. In this study, therefore, we also estimated the effective mass transport coefficients (K_{BD}, ml/min) during a period of dialysate isovolemia (18). The clearance of each investigated solute was calculated as the total amount of the solute in the drained dialysate at 240 min minus the infused amount divided by the mean blood concentration of the solute and the dwell time.

The direct lymphatic absorption of fluid from the peritoneal cavity was assessed as the clearance of RISA from the dialysate to the blood, K_{EB} (ml/min). K_{EB} was calculated from the rate of increase of RISA amount in plasma divided by the average intraperitoneal RISA concentration (19). The plasma volume was set at 3.6 ml/100 g body wt (19,20). The remaining part of fluid absorption to the peritoneal tissue interstitium and capillaries, K_{ET} (ml/min), was calculated as K_E minus K_{EB}.

Two-way ANOVA with repeated measurements and one-way ANOVA were applied to compare intraperitoneal volume, K_E, K_{EB}, K_{ET}, D/P ratios, and K_{BD}. When ANOVA showed a significant difference among the three groups, Scheffe’s F test was used to test the difference between different groups. The results are expressed as mean ± SD. A P value of <0.05 was considered significant.

Results

Fluid Transport

The intraperitoneal volume was significantly higher in the two hyaluronan groups compared with the control group (two-way ANOVA with repeated measurements, P < 0.01), but there was no significant difference between the two hyaluronan groups (Figure 1). The drain volume was 22.7 ± 2.2 ml, 24.6 ± 1.8 ml, and 20.8 ± 1.4 ml for the HA1, HA2, and control groups, respectively (P < 0.01). The peritoneal absorption rate (estimated as the RISA elimination coefficient, K_E) tended to be lower in the HA1 group (36 ± 11 μl/min) and was significantly lower in the HA2 group (28 ± 9 μl/min) compared with the control group (43 ± 6 μl/min, P < 0.01) (Figure 2). There were no significant differences in the lymphatic flow rate as estimated by K_{EB} among the three groups: 7.5 ± 1.8, 6.8 ± 1.5, and 7.5 ± 1.9 μl/min for the HA1, HA2, and control groups, respectively. Therefore, the differences in K_E were mainly due to differences in the fluid reabsorption to peritoneal tissue, K_{ET}, which was 28 ± 10, 21 ± 9, and 35 ± 5 μl/min for the HA1, HA2, and control groups, respectively (P < 0.01, Figure 2).

Transport of Solutes

D/D_0 of glucose was initially lowered (only at 3 and 15 min of the dwell) in the HA1 and HA2 groups compared with the control group (P < 0.05), but no significant difference was found between the two hyaluronan groups (Figure 3). After 30
Figure 1. Intraperitoneal volume versus time. □, control group; ○, 0.005% hyaluronan group; ◇, 0.01% hyaluronan group (mean ± SD). The intraperitoneal volume was significantly higher in the two hyaluronan groups compared with the control group (two-way ANOVA with repeated measurements, \( P < 0.01 \)), but there was no significant difference between the two hyaluronan groups.

Figure 2. The radiolabeled human serum albumin (RISA) elimination rate from the peritoneal cavity. \( K_E \), total RISA elimination rate representing the fluid absorption rate from the peritoneal cavity; \( K_{EB} \), RISA elimination rate to the blood from the peritoneal cavity representing the peritoneal lymphatic absorption; \( K_{ET} \), RISA elimination rate to peritoneal tissue. □, control group; ■, 0.005% hyaluronan group; △, 0.01% hyaluronan group (mean ± SD). *\( P < 0.01 \).

Figure 3. (A) Dialysate glucose concentration to fresh dialysate glucose concentration (D/D₀) ratio versus dwell time. (B) Dialysate to plasma urea concentration (D/P Urea) ratio versus dwell time. (C) Dialysate to plasma protein concentration (D/P Protein) ratio versus dwell time. Symbols as in Figure 1.

between the two hyaluronan groups. There were no significant differences in \( K_{AD} \) values for any of the investigated solutes among the three groups. The urea clearance was significantly higher in the HA1 group (76 ± 9 \( \mu l/min \)) and HA2 group (88 ± 9 \( \mu l/min \)) compared with the control group (68 ± 13 \( \mu l/min \), \( P < 0.01 \)). There was no significant difference in the protein clearance among these three groups when estimated for the period between 3 min and 240 min: 3.3 ± 1.1, 4.1 ± 2.3, and 2.5 ± 1.1 \( \mu l/min \) for the HA1, HA2, and control groups,
Discussion

Our results showed that adding hyaluronan to dialysis solution significantly increased the net fluid removal by reducing the peritoneal fluid absorption. Fluid transport from the peritoneal capillaries to the peritoneal cavity occurs by transcapillary ultrafiltration caused mainly by the osmotic pressure gradient (21). During the dwell, fluid also simultaneously leaves the peritoneal cavity via various pathways such as direct lymphatic and interstitial entry (22). Fluid absorption from the peritoneal cavity adversely affects the efficacy of peritoneal dialysis by reducing the potential for net fluid removal by 40 to 50% and by reducing small solute clearances by 15 to 20% (23). The rate of fluid absorption has been shown to increase with time on dialysis (24). The dialysis procedure itself and the composition of dialysis solution may contribute to this change (25). New evidence from other studies (13,26,27), as well as the present one, suggests that hyaluronan content in the peritoneal interstitium (19) and at the mesothelial cell surface may play an important role in the peritoneal fluid absorption. It has been reported that during peritoneal dialysis, a large amount of hyaluronan is removed from dialysate, especially in high transporters and in patients with peritonitis (8,9,28) in whom the rate of peritoneal fluid absorption is high. In the present single-dwell study, the removal of hyaluronan in the dialysate in the control group was found to be in the order of 0.3 mg/L, which is similar to the reported "normal" values in the dialysate effluents of continuous ambulatory peritoneal dialysis patients (9).

Several mechanisms may be involved in the increased hyaluronan removal during peritoneal dialysis. First, increased hydrostatic pressure and loading with dialysis solutions during continuous peritoneal dialysis may lead to increased hyaluronan efflux from peritoneal interstitium through lymphatic flow, which is the main route for elimination of hyaluronan in tissue interstitium (29). Townsley et al. found that both increased hydrostatic pressure and saline loading increased hyaluronan efflux from canine lung (30). We found that after 10 d of continuous exposure to dialysis fluid, the peritoneal lymphatic absorption was significantly increased in rat (25). This increased lymphatic absorption may result in increased removal of hyaluronan from peritoneal interstitium. Second, hyaluronan may be directly removed through dialysis exchanges into the dialysate effluent. This removed hyaluronan may mainly come from newly synthesized hyaluronan from peritoneal mesothelial cells (8,31). Yung et al. (8) found that the major part of hyaluronan in dialysate effluents was produced locally. The hyaluronan in the culture medium of mesothelial cells was found to have molecular size similar to hyaluronan isolated from continuous ambulatory peritoneal dialysis fluid (8). Third, the peritoneal hyaluronan may be degraded by the increased production of free radicals within the peritoneum after some time of dialysis (32,33), which may contribute to the loss of hyaluronan from the peritoneum.

There are several possible mechanisms by which hyaluronan could decrease the peritoneal fluid reabsorption as found in the present study. First, the molecular weight of hyaluronan used in this study has been reported to be of M_r 549,000 to 774,000 (34). The size of the hydrated hyaluronan would prevent it from reaching the interstitium of the peritoneum during the dwell. This is also supported by the low absorption of hyaluronan (<10%) in the two hyaluronan groups. Because peritoneal fluid absorption (bulk flow) is driven mainly by intraperitoneal hydrostatic pressure and exists during the whole dwell (14), a layer of sieved hyaluronan molecules (filter "cake") may thus build up at the peritoneal cavity–membrane interface (Figure 4). Because the resistance to flow through the hyaluronan molecular domain is high, formation of a hyaluronan molecular filter "cake" should impede efflux of the peritoneal fluid. A similar observation has been reported by McDonald and Levick, who found that hyaluronan retarded the fluid absorption from the joint (34). This effect was more obvious with high intra-articular hydrostatic pressure. The increasing resistance to flow in the presence of hyaluronan was believed to be caused by partial molecular sieving of hyaluronan by the small porosities of the synovial interstitial matrix, leading to accumulation of a restrictive filter "cake" of hyaluronan chains at the tissue–cavity interface (34).

Second, adding high molecular weight hyaluronan exogenously may stabilize the endogenous hyaluronan, which forms a stagnant layer at the mesothelial cell surface (27). It has been suggested that this stagnant layer containing hyaluronan plays an important role in peritoneal transport (27,35). Stirring the stagnant layer at the dialysate–peritoneum interface has been found to increase the peritoneal small solute transport and decrease the fluid removal (36). Although the presence of hyaluronan receptors (e.g., CD44) has not been proven in the peritoneal mesothelial cell surface, Heldin and Pertoft (37) have shown that normal human mesothelial cells in vitro surrounded themselves with a pericellular matrix, or "coat", containing mainly hyaluronan. The function of the hyaluronan-containing coat is not fully understood, but it has been proposed to protect the cells from viral infections and the cytotoxic effect of lymphocytes and to regulate cell differentiation (37). It is possible that during peritoneal dialysis, especially in high transporters and during peritonitis, this stagnant layer may be damaged and the coat deteriorated through increased shedding of hyaluronan into the dialysate. Exogenously added hyaluronan would presumably stabilize the meshwork, as it may form bridges between endogenous chains (37). Further studies are needed to elucidate any possible interaction between hyaluronan and peritoneum mediated by hyaluronan receptor(s), especially during long-term peritoneal dialysis when mesothelial cells may undergo transformation (38).

Glucose absorption was unexpectedly slightly enhanced in the two hyaluronan groups during the initial part of the dwell; however, the mechanism is not clear. It has been reported that the diffusion of glucose in a matrix gel containing hyaluronan was significantly increased (39). Hadler suggested that this increased diffusivity might be due to the interaction between...
Free hyaluronan in dialysate

Peritoneal cavity

Mesothelial cells

Fluid absorption

Stagnant layer
(hyaluronan coat)

Interstitial
hyaluronan

Capillary

Figure 4. Schematic drawing of the possible mechanism by which hyaluronan may affect peritoneal fluid absorption.

Figure 4. Schematic drawing of the possible mechanism by which hyaluronan may affect peritoneal fluid absorption.

Glucose and the hyaluronan domain that facilitates glucose movement (40). Whether the observed transient increase in glucose absorption in the two hyaluronan groups was a result of increased diffusivity of glucose in the (postulated) hyaluronan layer (filter "cake") remains to be answered. Also, our results do not agree with a previous study (13) in that hyaluronan in our study did not significantly decrease the peritoneal transport of protein. On the contrary, we found that dialysate protein concentrations were higher in the two hyaluronan groups during the whole dwell. It is unlikely that the higher protein concentration was due to an increased transport of protein from the blood because the difference in dialysate protein appearance was observed during the initial 3 min only. In separate experiments, we did not find any significant difference in white blood cell counts in the effluents by adding hyaluronan to the dialysis fluid compared with the control solution, suggesting that adding hyaluronan to the dialysate did not induce local inflammation. In addition, hyaluronan is an effective anti-inflammation substance, and it has been shown that hyaluronan could inhibit acute and chronic inflammation (41). The accuracy of the protein analytical assay was found to be unaffected by the presence of hyaluronan in vitro (data not shown). Therefore, we speculate that the increased protein concentration found during the initial part of the dwell may be due to a competition of hyaluronan with surface proteins for binding onto the mesothelial cell surface. It is much more probable that hyaluronan competes for binding sites than nonspecifically sterically excludes proteins. The sterical exclusion effect should be far too small to show up as an increase in protein concentration in the dialysate.

In summary, the present study suggests that hyaluronan, possibly through its unique physical-chemical characteristics, could increase the peritoneal fluid removal mainly by decreasing the peritoneal fluid absorption, resulting in increased peritoneal urea clearance. Additional studies are warranted to evaluate the potential clinical application of hyaluronan as an additive to dialysis fluid to enhance peritoneal fluid removal.

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References

4. Wang T, Heimbürger O, Waniewski J, Bergström J, Lindholm B: Increased peritoneal permeability in CAPD results in decreased