Kidney International, Vol. 53 (1998), pp. 496–502

Hyaluronan prevents the decreased net ultrafiltration caused by increased peritoneal dialysate fill volume

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Hyaluronan prevents the decreased net ultrafiltration caused by increased peritoneal dialysate fill volume. In the present study, we investigated (*1*) the effect of an increase in dialysate fill volume on peritoneal fluid and solute transport using a 1.36% glucose solution, and (*2*) the effect of intraperitoneal administration of hyaluronan on peritoneal transport characteristics when different fill volumes were used. A four-hour dwell study with frequent dialysate and blood sampling was performed in 26 male Sprague-Dawley rats with ^{131}I albumin as the intraperitoneal volume marker. Each rat was injected intraperitoneally with 25 ml (group Con25, $N = 6$) or 40 ml (group Con40, $N = 7$) of 1.36% glucose dialysis solution alone or 25 ml (group HA25, $N = 6$) or 40 ml (Group HA40, $N = 7$) of 1.36% glucose dialysis solution with 0.01% hyaluronan. The peritoneal transport of fluid, glucose, urea, and total protein as well as the intraperitoneal hydrostatic pressure (IPP) with different fill volumes were evaluated. We found that IPP and peritoneal fluid absorption rate significantly increased with the increase in fill volume ($P < 0.01$), and therefore the net ultrafiltration volume was significantly lower in the Con40 group compared to the Con25 group despite a higher transcapillary ultrafiltration rate in the Con40 group. The addition of hyaluronan to dialysate significantly ($P < 0.01$) decreased the peritoneal fluid absorption rate (by 22% in HA25 vs. Con25 and by 29% in HA40 vs. Con40) and thus significantly increased the net peritoneal fluid removal. The diffusive mass transport coefficients for glucose, urea and total protein did not differ between the Con25 and Con40 groups or between the two hyaluronan groups as compared to their respective control groups. The peritoneal clearance of urea increased significantly in the high fill volume group (by 58% in Con40 vs. Con25) and in the two hyaluronan groups (by 21% in HA25 vs. Con25 and by 16% in HA40 vs. Con40). We conclude that: (*1*) An increase in dialysate fill volume using 1.36% glucose dialysis solution results in higher intraperitoneal hydrostatic pressure and higher peritoneal fluid absorption rate, and therefore lower net ultrafiltration. (*2*) Intraperitoneal addition of hyaluronan significantly decreases the peritoneal fluid absorption rate, and the decreasing effect is even more marked when a high fill volume is used. (*3*) Small solute clearances increase markedly with increases in fill volume, and then further increase by adding hyaluronan to the dialysate due to the increase in drainage volume. Thus, intraperitoneal administration of hyaluronan during a single peritoneal dialysis exchange may significantly increase the peritoneal fluid and solute removal by decreasing peritoneal fluid absorption, and may thereby prevent the decreased net ultrafiltration caused by an increase in dialysate fill volume.

Key words: ultrafiltration, hyaluronan, peritoneal dialysate, volume.

Received for publication May 30, 1997
and in revised form September 17, 1997
Accepted for publication September 24, 1997

It has recently been recommended that the dialysate fill volume be increased from 2 liters to 2.5 liters or 3 liters to achieve adequate peritoneal dialysis in all patients, especially in large, anuric patients [1]. However, it has been demonstrated that increases in intraperitoneal volume would increase the intraperitoneal hydrostatic pressure (IPP) [2, 3]. This increase in IPP consequently increased peritoneal fluid absorption [3, 4]. In fact, several studies found that increased IPP is associated with poor fluid removal (decreased net ultrafiltration volume) [5–8]. Thus, great concerns have surfaced about the possible decrease in net ultrafiltration volume due to increased fluid absorption rate associated with high fill volume and high intraperitoneal hydrostatic pressure [9–11]. We have previously reported that the decreased net ultrafiltration volume associated with higher fill volume (due to higher IPP and higher peritoneal fluid absorption) could be avoided if hypertonic (3.86%) glucose solutions are used [3]. However, the use of hypertonic glucose solution may be limited by their adverse side effects [12]. In fact, dialysis fluid with low glucose concentration is the most commonly used solution. Krediet et al found that the net ultrafiltration using 1.36% glucose dialysis solution was significantly lower in the 3 liter group compared to the 2 liter group during a four-hour dwell study, mainly due to an increase in peritoneal fluid absorption in the 3 liter solution group [13]. Therefore, reducing the peritoneal fluid absorption should be a conceivable and potentially effective way to improve the adequacy (as regards to the removal of both small solutes and fluid) of peritoneal dialysis, especially when high fill volumes are used.

Hyaluronan is a long polysaccharide chain that is made up of repeating disaccharide units of N-acetylglucosamine and glucuronic acid. Hyaluronan is found in most tissues in the body [14, 15] and in the drainage dialysis fluid during peritoneal dialysis [16, 17]. Hyaluronan plays an important role in tissue hydraulic conductivity. It has been shown that hyaluronan exhibits a high resistance against water flow and can thus act in tissue as a barrier against rapid changes in water content [18–20]. In the interstitium, hyaluronan decreases the water permeability of the membrane [21, 22]. In a previous study, we have demonstrated that adding 0.01% hyaluronan to peritoneal dialysis fluid in rats could increase the peritoneal fluid removal mainly by decreasing the peritoneal fluid absorption, resulting in increased peritoneal urea

clearance [23]. We speculated that the observed effect of hyaluronan on peritoneal fluid absorption may be due to the accumulation of a restrictive filter "cake" of hyaluronan chains at the tissue-cavity interface [23]. As peritoneal fluid absorption is mainly driven by IPP, it is conceivable that raising IPP would transiently increase convective transport into the boundary, thereby raising the concentration and thickness of the hyaluronan layer, which could increase the resistance of the outflow of fluid from the peritoneal cavity. If such effects on peritoneal fluid absorption could be demonstrated by an addition of hyaluronan to peritoneal dialysis fluid, it could conceivably improve the efficiency (both regarding fluid and solute removal) of peritoneal dialysis with increased dialysate fill volume, especially when using low glucose (1.36%) solution.

In this study, we made a detailed investigation of peritoneal fluid kinetics and solute transport in rats receiving different dialysate fill volumes using 1.36% glucose solution. In the second part of the study, we then made a detailed analysis of the effect of intraperitoneal hyaluronan on the peritoneal fluid and solute transport characteristics in rats when the different fill volumes were used.

METHODS

Twenty-six male Sprague-Dawley rats with an average body wt of 290 g (range 280 to 300 g) were divided into four groups. Each rat was anesthetized with a single intraperitoneal injection of 50 mg/kg pure pentobarbital sodium (Pharmacia, Sweden). This anesthesia was reported not to alter the peritoneal transport in rats [9]. The fur over the abdominal wall was clearly shaved. The animal was laid in a supine position and was kept at 37°C with a heating pad (CMN/Microdialysis, Stockholm, Sweden). Isotonic saline, 1 ml/hour, was injected subcutaneously to prevent hypovolemia. A multiholed silastic catheter (Venoflon, 0.8 mm internal diameter; Helsingborg, Sweden) was inserted percutaneously in the left lower quadrant of the abdomen for dialysis fluid infusion and sampling. The experiment was started by giving an intraperitoneal injection of 25 ml (group Con25, $N = 6$) or 40 ml (group Con40, $N = 7$) of sterile 1.36% glucose dialysis fluid. In the other two groups, 0.01% sterile hyaluronan (protein and endotoxin free; average molecular wt 500,000; Hyal, Toronto, Canada) was added to 25 ml (group HA25, $N = 6$) or 40 ml (Group HA40, $N = 7$) of sterile 1.36% glucose dialysis fluid. This concentration of hyaluronan has been shown to protect the hyaluronan layer around the human peritoneal methothelial cells [24] and the peritoneum from injury caused by saline infusion [25], as well as to effectively decrease the peritoneal fluid absorption [23]. All the fluids were prewarmed to 37°C and mixed with 18.5 kBq ^{131}I-human serum albumin (RISA; Isopharma AS, Kjeller, Norway). A small dose (0.2 g/liter) of human albumin was added to the solution to minimize adhesion of tagged albumin to the surface of the catheter. The solution was administered via a three-way valve (Viggo, Connecta, Helsingborg, Sweden) and the catheter, over a period of about one minute, and allowed to remain in the peritoneal cavity for four hours. The intraperitoneal hydrostatic pressure was measured after the infusion using a water manometer connected to the peritoneal catheter, setting the reference level at the heart of the rat. Dialysate samples (0.4 ml) were taken at 0, 3, 15, 30, 60, 90, 120, 180 and 240 minutes after the dialysis fluid had been infused. Prior to 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 minutes from the tail artery. After 240 minutes, the peritoneal cavity was opened and the dialysate was collected using a syringe and preweighted gauze tissue.

Dialysate samples (0.1 ml) and blood samples (0.1 ml of plasma) were analyzed for RISA activity on a Gamma Counter (Packard Instrument Company, Meriden, CT, USA) for 10 minutes each. Dialysate and plasma concentrations of urea (urease-glutamate dehydrogenase method), protein (Coomassie Brilliant Blue dye binding method) and glucose concentration (hexokinase method) were analyzed using a Multistat Autoanalyzer (Instrumentation Laboratory, Spokane, WA, USA).

The intraperitoneal dialysate volume was estimated from the dilution of RISA with corrections made for the elimination of the RISA from the peritoneal cavity. The total peritoneal fluid absorption rate as assessed by RISA elimination coefficient (K_E ml/min) was calculated as described previously [26, 27]. The intraperitoneal volume change (net ultrafiltration) at time t was calculated as the intraperitoneal volume at time t (V_t) minus the infused volume (V_0). The transcapillary ultrafiltration rate (Q_u) was defined as the rate of intraperitoneal volume change plus the rate of fluid absorption (K_E) [27]. The direct lymphatic absorption of fluid from peritoneal cavity was assessed as the RISA elimination rate from the peritoneal cavity to the blood (K_{EB} ml/min). The K_{EB} was calculated from the rate of increase of RISA amount in plasma divided by the average intraperitoneal RISA concentration [8]. The plasma volume was set at 3.6 ml/100 g body wt [8]. 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}.

The dialysate-to-plasma-solute concentration ratios (D/P) for all the investigated solutes were calculated by dividing the dialysate concentration of a solute at a certain time with the aqueous concentration of the investigated solute in plasma [28]. If no blood sample was taken at the same time as a dialysate sample, then the blood concentration of solute was linearly interpolated from the blood sample taken before and after this moment [29]. 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). The clearance of the investigated solutes was calculated as the total amount of the solute in the drained dialysate at 240 minutes minus the infused amount and divided by the mean blood concentration of the solute and the dwell times.

The diffusive mass transport coefficients (K_{BD}, ml/min) were estimated using the modified Babb-Randerson-Farrell (BRF) model as described previously [30, 31]. The model describes the net change of the solute amount in peritoneal dialysate over time increment equal to the rate of solute flow between blood and dialysate due to combined diffusion, convective transport, and peritoneal absorption of the solute. In addition, in this study, we set the sieving coefficients (S) for glucose and urea to 0.55 and for total protein to 0.05 based on previous studies [3, 32].

Two-way ANOVA with repeated measurements and one-way ANOVA were applied to compare intraperitoneal volume, ultrafiltration rate, K_E, K_{EB}, K_{ET}, D/P ratios, and K_{BD}. When ANOVA showed a significant difference among the four groups, then Scheffe's F-test was used to compare the difference between different groups. Special attention was paid to compare the results between the Con25 group and the Con40 group, between the Con25 and the HA25 groups, and between the Con40 and the

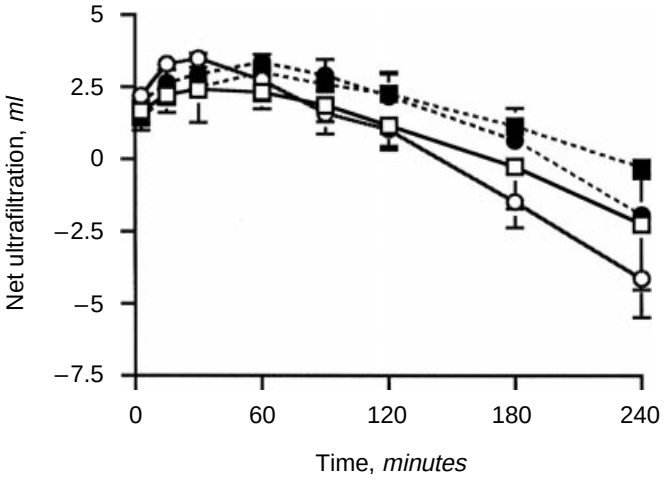

Fig. 1. Changes in intraperitoneal volume (net ultrafiltration) versus time. Symbols are: (□) 25 ml control group (Con25, $N = 6$); (○) 40 ml control group (Con40, $N = 7$); (■) 25 ml with 0.01% hyaluronan group (HA25, $N = 6$); (●) 40 ml with 0.01% hyaluronan group (HA40, $N = 7$). Data are mean ± SD. Significant differences ($P < 0.05$) were found between the Con40 and Con25 groups, between the HA25 and Con25 groups and between the HA40 and Con40 groups (Two-way ANOVA for repeated measures).

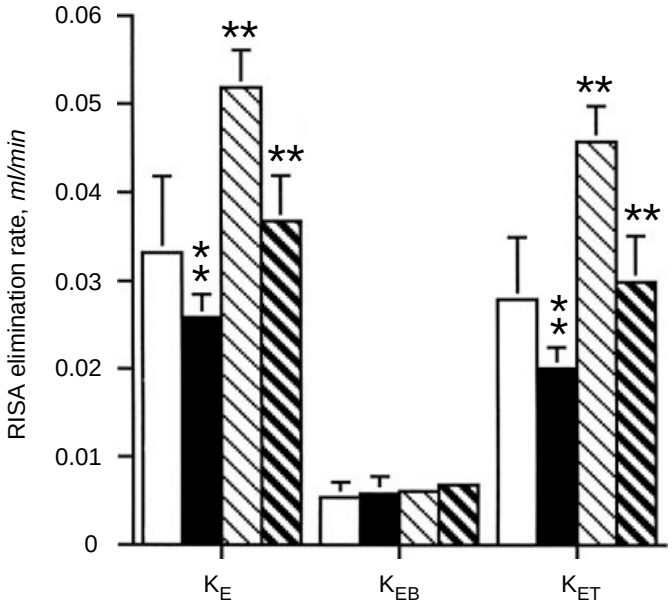

Fig. 2. The ^{131}I-human serum albumin (RISA) elimination rate from the peritoneal cavity. Abbreviations are: K_E, total RISA elimination rate representing the fluid absorption rate from the peritoneal cavity; K_{EB}, RISA elimination rate to the blood from the peritoneal cavity representing the peritoneal lymphatic absorption; K_{ET}, RISA elimination rate to peritoneal tissue. Symbols are: (□) 25 ml control group (Con25); (■) 25 ml with 0.01% hyaluronan group (HA25); ▨, 40 ml control group (Con40); ▨, 40 ml with 0.01% hyaluronan group (HA40). Data are mean ± SD. Significant differences are marked: $^{**}P < 0.01$ compared to their respective control groups, that is, between the Con40 and Con25 groups, between the HA25 and Con25 groups and between the HA40 and Con40 groups.

HA40 groups using the unpaired Student's *t*-test. The results are expressed as mean ± SD. A P value of less than 0.05 was considered significant.

RESULTS

Fluid transport

Fluid transport characteristics are shown in Figures 1 and 2 and Table 1. The increase in dialysate fill volume resulted in a significant increase ($P < 0.01$) in intraperitoneal hydrostatic pressure (IPP) from 1.7 ± 0.5 cm H_2O in the Con25 group to 5.1 ± 1.0 cm H_2O in the Con40 group (Table 1). No significant differences in IPP were found between the Con25 and HA25 groups or between the Con40 and the HA40 groups (Table 1). Although the transcapillary ultrafiltration rate (Q_u) between three minutes and 240 minutes of the dwell was higher in the Con40 group (0.024 ± 0.004 ml/min) as compared to the Con25 group (0.017 ± 0.004 ml/min, $P < 0.01$; Table 1), the net ultrafiltration volume was significantly lower ($P < 0.01$) in the Con40 group as compared to the Con25 group (Fig. 1 and Table 1), which was due to a significantly higher peritoneal fluid absorption rate (as assessed by the RISA elimination rate, K_E; Fig. 2 and Table 1).

The K_E values were significantly lower in the two hyaluronan groups as compared to their respective control group, that is between the HA25 group versus the Con25 group ($P < 0.05$) and between the HA40 group versus the Con40 group ($P < 0.01$; Table 1 and Fig. 2). Therefore, the ultrafiltration volume was significantly higher in the two hyaluronan groups as compared to their respective control groups (Table 1 and Fig. 1). There was no significant difference in the direct lymphatic absorption as assessed by K_{EB} among the four groups (Table 1 and Fig. 2), and thus the differences in K_E between the groups were mainly due to the differences in fluid absorption to the adjacent peritoneal tissue as assessed by K_{ET} (Fig. 2).

Glucose transport

Although the diffusive mass transport coefficient for glucose did not differ among the four groups (Table 2), the D/D_0 of glucose decreased markedly slower in the Con40 group as compared to the Con25 group (Fig. 3). However, the total absorbed amount of glucose during the dwell was significantly higher with high fill volumes than with low fill volumes (Fig. 3). The D/D_0 was initially lower and the total absorbed amount of glucose was initially higher (only at 15 min and 30 min of the dwell) in the HA25 group compared to the Con25 group (Fig. 3; $P < 0.05$). After 30 minutes of the dwell, D/D_0 as well as the total absorbed amount of glucose were not significantly different between the two groups. The D/D_0 of glucose also tended to be lower in the HA40 group as compared to the Con 40 group, although no statistical difference was found between the two groups (Fig. 3).

Transport of other solutes

The D/P for urea and protein were significantly lower in the Con40 group as compared to the Con25 group (Fig. 4; $P < 0.05$). There were no significant differences in D/P of urea between the Con25 and the HA25 groups or between the Con40 and the HA40 groups (Fig. 4). There were no significant differences in K_{BD} values for urea and for protein between the Con25 and Con40 groups as well as between the two hyaluronan groups and their respective control groups (Table 2). The urea clearance was significantly higher in the two high volume groups (Con40 and

Table 1. Fluid transport parameters and intraperitoneal hydrostatic pressure among the four groups (mean ± SD)

	N	IPP $cm\ H_2O$	$Q_{u3\text{-}240min}$ $\mu l/min$	Net UF ml	K_E	K_{EB}	K_{ET}
					$\mu l/min$		
Con25	6	1.7 ± 0.5	16.7 ± 4.4	−2.3 ± 2.3	33.2 ± 8.7	5.3 ± 1.8	27.8 ± 7.1
HA25	6	1.7 ± 0.3	16.0 ± 1.6	−0.3 ± 0.3[b]	25.8 ± 2.6[b]	5.8 ± 1.9	20.0 ± 2.4[b]
Con40	7	5.1 ± 1.0[a]	23.7 ± 3.7[a]	−4.2 ± 1.3[b]	51.8 ± 4.3[b]	6.1 ± 0.4	45.7 ± 4.0[b]
HA40	7	5.2 ± 0.9[a]	20.6 ± 2.6[a]	−1.9 ± 1.3	36.7 ± 5.2[c]	6.8 ± 0.7	29.9 ± 5.2[c]

Abbreviations are: IPP, intraperitoneal hydrostatic pressure after infusion; $Q_{u3\text{-}240min}$, mean transcapillary ultrafiltration rate between 3 min and 240 min; Net UF, net ultrafiltration volume at 4 hour of the dwell; 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; E_{ET}, RISA elimination rate to peritoneal tissue.

[a] $P < 0.01$ compared to the groups with the lower fill volume
[b] $P < 0.05$ compared to the Con25 group
[c] $P < 0.01$ compared to the Con40 group

Table 2. Diffusive mass transport coefficients, K_{BD} (ml/min), for glucose, urea, and total protein as well as peritoneal clearances, μl/min, for urea and total protein (mean ± SD)

	N	K_{BD} ml/min			Peritoneal clearance $\mu l/min$	
		Glucose	Urea	Total protein	Urea	Total protein[d]
Con25	6	0.24 ± 0.09	0.27 ± 0.10	0.003 ± 0.002	78 ± 4	3.2 ± 1.1
HA25	6	0.28 ± 0.05	0.29 ± 0.06	0.004 ± 0.002	94 ± 6[a]	3.2 ± 1.2
Con40	7	0.23 ± 0.06	0.34 ± 0.09	0.003 ± 0.001	123 ± 6[b]	3.0 ± 1.5
HA40	7	0.29 ± 0.04	0.43 ± 0.12	0.003 ± 0.001	143 ± 17[c]	3.4 ± 1.2

[a] $P < 0.05$ compared with the Con25 groups
[b] $P < 0.01$ compared with the Con25 group
[c] $P < 0.01$ compared with the Con40 group
[d] calculated for the time period 3–240 min

HA40) as compared to the two low volume groups (Con25 and HA25; Table 2). The addition of hyaluronan resulted in significantly higher urea clearance as compared to their respective control groups (Table 2). The dialysate protein concentration was significantly higher during the dwell in the two hyaluronan groups as compared to the control groups of the same volume, resulting in significantly higher ($P < 0.05$) D/P of protein in the two hyaluronan groups as compared to the respective control groups (Fig. 4). This was due to a markedly higher protein appearance in the dialysate during the initial three minutes of the dwell. There was no significant difference in the protein clearance among these four groups when estimated for the period between three minutes and 240 minutes (Table 2).

DISCUSSION

The present study shows that increased peritoneal dialysate fill volume of 1.36% glucose dialysis fluid results in *decreased* net fluid removal because of increased peritoneal fluid absorption. The addition of hyaluronan to the dialysis solution significantly increased the net fluid removal by reducing the peritoneal fluid absorption, and could thus prevent the decrease in net fluid removal associated with the higher fill volume of 1.36% glucose dialysis solution.

Effect of increased peritoneal fill volume

Peritoneal fluid absorption is mainly driven by the intraperitoneal hydrostatic pressure (IPP) [9, 33, 34]. The increased peritoneal fluid absorption rate associated with the increased IPP due to the higher intraperitoneal fill volume in the present study is in agreement with previous studies [3, 8, 9]. However, our results show that direct lymphatic absorption assessed as the rate of appearance of intraperitoneally administered macromolecules (that is, RISA) in the blood (K_{EB}) was not significantly increased with increases in fill volume (Table 1 and Fig. 2). A similar finding was reported in previous studies [3, 8, 9]. Therefore, the increase in peritoneal fluid absorption rate associated with higher fill volume was mainly due to increased fluid absorption rate into the adjacent tissues of the peritoneal cavity as assessed by K_{ET}.

Although the K_E was significantly higher in the Con40 group as compared to the Con25 group, the transcapillary ultrafiltration rate (Q_u) was also significantly higher in the Con40 group. This is in agreement with our previous study using 3.86% glucose solution [3]. The increase in Q_u was mainly due to a better maintenance of the glucose concentration gradient in the high fill volume groups (Fig. 3), in agreement with previous studies [2, 3, 13]. It is important to note that despite a higher transcapillary ultrafiltration rate in the higher fill volume groups, the net ultrafiltration volume was still significantly lower in the Con40 group (−4.2 ± 1.3 ml) than in the Con25 group (−2.3 ± 2.3 ml). This was due to the fact that the increased Q_u could not fully compensate for the increased K_E in the Con40 group when using 1.36% glucose dialysis solution. A similar finding was reported by Krediet et al, who found that the net ultrafiltration using 1.36% glucose dialysis solution was significantly lower when using 3 liters of fill volume compared to 2 liters of fill volume during a four-hour dwell study in CAPD patients, mainly due to increased peritoneal fluid absorption in the 3 liter solution group [13]. However, in a previous study in rats, we found that increases in dialysate fill volume using 3.86% glucose dialysis solution results in a higher net ultrafiltration despite a higher peritoneal fluid absorption rate,

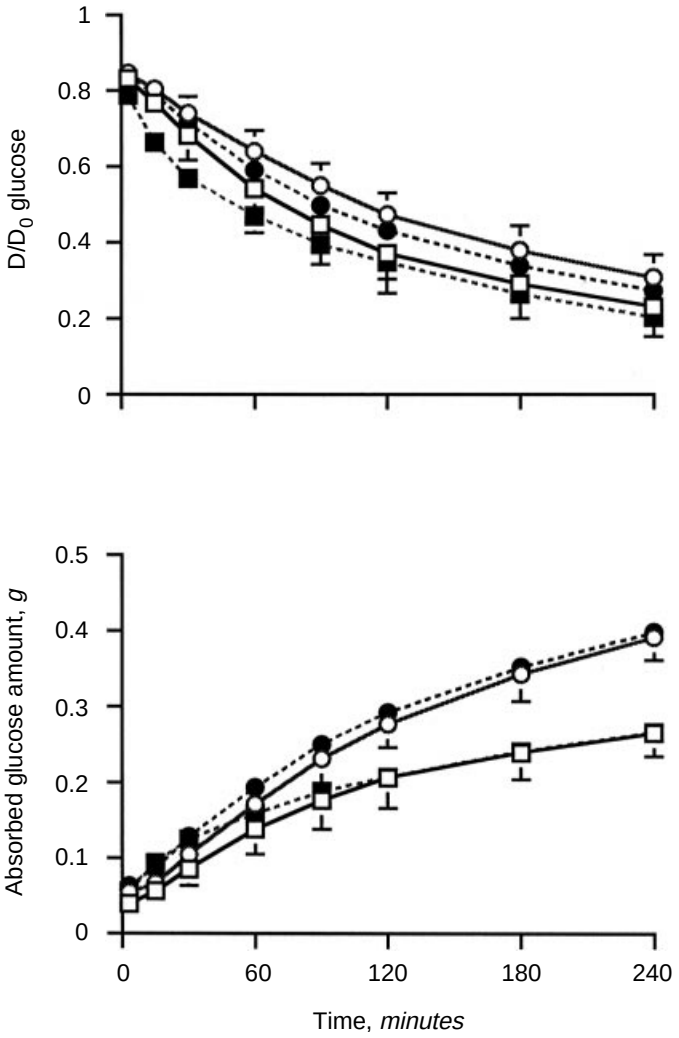

Fig. 3. Dialysate glucose concentration (D) to fresh dialysate glucose concentration (D_0) ratio (*A*) and total absorbed amount of glucose (*B*) versus dwell time. Symbols are: (□) 25 ml control group (Con25, $N = 6$); (○) 40 ml control group (Con40, $N = 7$); (■) 25 ml with 0.01% hyaluronan group (HA25, $N = 6$); (●) 40 ml with 0.01% hyaluronan group (HA40, $N = 7$). Significant differences ($P < 0.05$) were found between the Con40 group and the Con25 group for both D/D_0 and the total absorbed amount of glucose. The D/D_0 was significantly lower and the total absorbed amount of glucose was significantly higher in the HA25 group at 15 and 30 minutes of the dwell compared to the Con25 group.

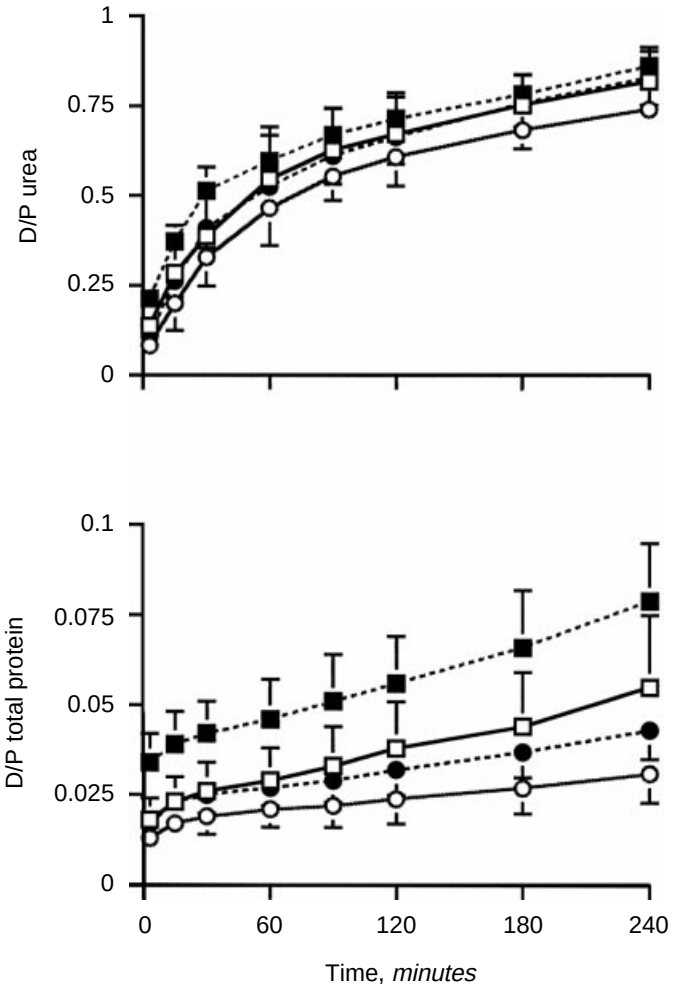

Fig. 4. Dialysate to plasma concentration ratio (D/P) for urea (*A*) and total protein (*B*) versus dwell time. Symbols are: (□) 25 ml control group (Con25, $N = 6$); (○) 40 ml control group (Con40, $N = 7$); (■) 25 ml with 0.01% hyaluronan group (HA25, $N = 6$); (●) 40 ml with 0.01% hyaluronan group (HA40, $N = 7$). Significant differences ($P < 0.05$) were found for total protein between the Con25 and Con40 groups as well as between the two hyaluronan groups and their respective control groups.

which was due to a higher Q_u with the 3.86% glucose solution counterbalancing the increase in K_E [3]. Therefore, we conclude that a decreased net ultrafiltration volume associated with higher dialysate fill volume (due to higher IPP and higher peritoneal fluid absorption) can be avoided if hypertonic glucose solutions are used.

Effect of hyaluronan on fluid transport

The significant decrease in K_E in the HA25 and HA40 groups compared to their respective control groups is in accordance with our findings in a previous study showing that an addition of hyaluronan to the dialysis fluid could markedly reduce peritoneal fluid absorption [23]. In the previous study, we speculated that the observed effect of hyaluronan on peritoneal fluid absorption may be due both to the accumulation of a restrictive filter "cake" of hyaluronan chains at the tissue-cavity interface [23] as well as to a stabilizing effect on endogenous hyaluronan at the mesothelial cell surface [35]. It is interesting to note that in the present study the magnitude of the decrease in peritoneal fluid absorption was much higher when the higher fill volume was used. The difference (0.15 ml/min) in K_E between the Con40 and the HA40 groups was much higher than the difference (0.07 ml/min) in K_E between the Con25 and the HA25 groups. As peritoneal fluid absorption is mainly driven by IPP, it is possible that raising IPP may transiently increase convective transport into the boundary, thereby raising the concentration and thickness of the hyaluronan layer, which could increase the resistance to the outflow of fluid from the peritoneal cavity. These results are similar to the observation made by McDonald and Levick, who found that an increase in the intra-articular pressure significantly increase the resistance of fluid outflow from the joint [36], the fluid outflow rate (from the joint) was in fact even lower when a significantly higher pressure

was induced compared to the outflow rate observed under low intra-articular pressure [36]. Under electron microscopy, they also observed that a hyaluronan membrane was formed at the tissue-cavity interface [36]. In the present study, the difference in peritoneal fluid absorption rate between the two hyaluronan groups and their respective control groups was almost entirely due to the difference in the fluid absorption rate to adjacent peritoneal tissues as assessed by K_{ET}, suggesting a specific effect of hyaluronan on K_{ET} (and not K_{EB}). The similar K_E between the Con25 and the HA40 groups in our study suggests that intraperitoneal addition of hyaluronan could prevent the decreased net ultrafiltration volume associated with higher dialysate fill volume of 1.36% glucose solution.

Solute transport

The significantly slower decrease in dialysate glucose concentration (as reflected by D/D_0) as well as lower D/P of urea and total protein in the Con40 group compared to the Con25 group are in agreement with our previous study [3]. This is a result of the principle governed by the geometry of diffusion stating that equilibration of a solute occurs rapidly when the dialyzed solute diffuses into or from a relatively small volume, whereas relatively slower equilibration occurs in association with diffusion into a larger volume. As the diffusive mass transport coefficients (K_{BD}) for glucose and urea did not significantly increase with the increase in fill volume, the rate of maximal diffusive transport (as estimated by K_{BD}) to the volume that should be cleared (fill volume) decreased with high dialysate volume, resulting in a slower decrease in the dialysate glucose concentration as well as in lower D/P of urea.

Glucose absorption was unexpectedly slightly enhanced in the two hyaluronan groups during the initial part of the dwell, which is similar to our previous observation [23]. The mechanism is not clear. However, it has been reported that the diffusion of glucose in a matrix gel containing hyaluronan was significantly increased [37]. Hadler suggested that this increased diffusivity might be due to the interaction between glucose and the hyaluronan domain that facilitates glucose movement [38]. However, further studies are needed to elucidate the mechanism(s) of the transiently increase in glucose absorption induced by the addition of hyaluronan to the dialysate.

The total protein concentrations in dialysate in the two hyaluronan groups were higher during the whole dwell as compared to their respective control groups. It is unlikely that the higher protein concentration was due to an increased transport of protein from the blood, as the difference in protein appearance in dialysate was observed during the initial three minutes 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 as compared to 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 [39]. The accuracy of the protein analytical assay was found to be unaffected by the presence of 0.01% hyaluronan *in vitro* (data not shown). There was no significant increase in protein clearance in the HA groups in our study as estimated from three minutes, which is in agreement with previous observations [3]. Therefore, we speculate that the rapid increase in protein concentration found during the initial three minutes of the dwell may be due to a competition of hyaluronan with surface proteins for binding onto the mesothelial cell surface.

The significant increase in urea clearance in the high fill volume groups is not unexpected. However, it is important to note that adequacy of dialysis is not only a matter of removing enough small solutes, but also a matter a removing enough fluid [40, 41]. In fact, inadequate fluid removal and inadequate blood pressure control are common in CAPD patients [42, 43], and may contribute to cardiovascular disease, which is the main cause of death in PD patients [43]. Therefore, if the increase in urea clearance with high dialysate fill volume is associated with a decrease in net fluid removal, this represents an important clinical problem. The addition of hyaluronan to dialysate may be a way to overcome this problem, as the addition of hyaluronan resulted in both increased fluid removal and increased urea clearances.

In summary, our results suggest that (*1*) An increase in dialysate fill volume using 1.36% glucose dialysis solution results in higher intraperitoneal hydrostatic pressure and higher peritoneal fluid absorption rate, and therefore in fact results in *lower* net ultrafiltration despite a higher transcapillary ultrafiltration rate. (*2*) Intraperitoneal addition of hyaluronan significantly decreases the peritoneal fluid absorption rate. The decreasing effect was more significant when high fill volume was used, reflecting perhaps a possible formation of a "hyaluronan filter cake" at the peritoneal cavity-tissue interface. (*3*) In general, the peritoneal diffusive mass transport coefficients did not change with different fill volume or by adding hyaluronan; however, small solute clearances increase markedly with increases in fill volume and by adding hyaluronan to the dialysate due to the higher drainage volume.

ACKNOWLEDGMENTS

This study was supported by a grant from Baxter Healthcare Corporation, McGaw Park, Illinois, USA. We thank Dr. Torvard C. Laurent for support and constructive criticism of this work, and Ms. Monica Eriksson for technical assistance.

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APPENDIX

Abbreviations used in this paper are: IPP, intraperitoneal hydrostatic pressure; Con25 and Con40, control groups with 25 ml and 40 ml glucose injection, respectively; HA25 and HA40, groups with 25 ml or 40 ml glucose + hyaluronan injection, respectively; RISA, ^{131}I-human serum albumin; K_E, rate of fluid absorption; V_t, intraperitoneal volume at time t; V_0, infused volume; Q_u, transcapillary ultrafiltration rate; K_{EB}, elimination rate from the peritoneal cavity to the blood; K_{ET}, fluid absorption to the peritoneal tissue interstitium and capillaries; D/P, dialysate-to-plasma-solute concentration ratio; D/D_0, dialysate glucose concentration divided by the glucose concentration in fresh dialysate; K_{BD}, diffusive mass transport coefficient.

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