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Dialysis solution containing hyaluronan: Effect on peritoneal permeability and inflammation in rats

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Background. Hyaluronan (HA), a high molecular weight mucopolysaccharide found in interstitial tissues and fluid, is lost from the peritoneal cavity during peritoneal dialysis. In order to determine the role of HA in peritoneal function, we investigated the effects of exogenous HA on peritoneal permeability, markers of intraperitoneal inflammation, and peritoneal morphology in rats exposed to peritoneal dialysis solution for four weeks.

Methods. Wistar rats were infused intraperitoneally, twice daily, with conventional, hypertonic dialysis solution (Dianeal 3.86%; control) or Dianeal solution containing 10 mg/dL of high molecular weight HA. Peritoneal permeabilities and clearances of solutes and protein were determined using a modified peritoneal permeability test (peritoneal equilibration test) at the beginning and the end of the treatment. Peritoneal volume and ultrafiltration were determined using a macromolecular marker and by gravimetric methods. Peritoneal inflammation was determined by cell counts and differential and by the measurement of cytokine concentrations in the dialysate effluent. Peritoneal thickness and HA content were determined in liver and mesentery biopsies taken at the end of the experiment.

Results. After four weeks of exposure to the dialysis solution, transperitoneal protein equilibration was significantly lower in HA-treated rats compared with rats treated with Dianeal alone (46% lower for albumin, $P < 0.001$; 33% lower for total protein, $P < 0.001$). The total drained volume after a four hour dwell was 29% higher in the HA group compared with the control ($P < 0.001$), yielding a positive net ultrafiltration in the HA group versus a negative net ultrafiltration in controls. Peritoneal clearances of urea and creatinine tended to be elevated in HA-treated rats, while clearances of total protein and albumin tended to be lower. Dialysate effluent from rats exposed to HA contained a lower percentage of neutrophils (8.8 ± 6.7 vs. 22.8 ± 9.5%, $P < 0.01$) and lower levels of the cytokines, tumor necrosis factor-α (11.2 ± 14.7 vs. 42.3 ± 35.3 pg/mL, $P < 0.05$) and monocyte chemoattractant protein-1 MCP-1 (72.0 ± 86.5 vs. 402.4 ± 258.3 pg/mL, $P < 0.02$), compared with rats treated with Dianeal alone. The thickness of the peritoneal interstitium showed a similar increase in both groups, but mesenteric tissue from the HA group contained more HA (48%, $P < 0.01$) than tissue from control animals.

Conclusions. The addition of HA to peritoneal dialysis solution decreases protein permeability, increases ultrafiltration, and decreases cytokine levels and the proportion of peritoneal neutrophils in dialysate from rats exposed to hypertonic dialysis solution. These results suggest that exogenous HA may help to protect the peritoneal membrane during exposure to dialysis solutions. These benefits, if sustained in the clinical setting, could lead to improvements in the therapy of peritoneal dialysis.

Key words: peritoneal dialysis, dialysate, membrane permeability, mucopolysaccharide.

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In uremic patients treated with peritoneal dialysis, the morphology and function of the peritoneal membrane gradually deteriorate in a manner proportional to the duration of dialysis [1, 2]. These changes are believed to be due to the bioincompatibility of dialysis solutions or to chronic inflammation induced by dialysis therapy [3, 4]. In extreme cases, ultrafiltration failure and reduced small-solute clearance may lead to inadequacy or even termination of peritoneal dialysis.

In recent years, several modifications of the composition of peritoneal dialysis solutions, such as neutral pH and the elimination of glucose degradation products, have been proposed to improve the biocompatibility of dialysis solutions. Results of in vitro experiments with such new solutions are promising [5, 6], and verification of the advantages of these solutions is eagerly awaited in the clinic. Nevertheless, repeated intraperitoneal infusions of PD solutions, even those that show high biocompatibility when tested in vitro, may produce nonspecific irritation of the peritoneum and a subsequent inflammatory response, potentially leading to impaired ultrafiltration and a loss of efficiency of peritoneal dialysis.

We have previously demonstrated that exposure of

rats to a once-daily intraperitoneal infusion of dialysis solution supplemented with glycosaminoglycans improved net ultrafiltration when compared with animals treated with standard dialysis solutions [7, 8]. Glycosaminoglycans are macromolecules that regulate various processes within connective tissues and interstitial spaces [9]. Hyaluronan (HA) is a high molecular weight glycosaminoglycan produced in great amounts within the peritoneal cavity of continuous ambulatory peritoneal dialysis patients [10]. Both peritoneal mesothelial cells and fibroblasts produce HA, and HA synthesis is increased during episodes of peritonitis [11, 12]. HA is known to play an important role during both acute and chronic inflammation within connective tissue, modulating the intensity of that process [13, 14]. In peritoneal dialysis patients, Yamagata et al observed a positive correlation between the duration of dialysis and the amount of HA in the dialysate effluent, suggesting a relationship between HA synthesis and chronic exposure to peritoneal dialysis solutions [15].

To investigate the effects of exogenous HA during repeated exposure of the peritoneum to dialysis solutions, we infused rats twice daily for four weeks with a standard hypertonic dialysis solution or with the same solution supplemented with 10 mg/dL HA. Our results suggest that exogenous HA, added to the dialysis solution, may improve peritoneal ultrafiltration and reduce markers of peritoneal inflammation or injury during chronic peritoneal dialysis.

METHODS

Experiments were performed on male Wistar rats (average weight of 325 $\pm$ 26 g). Initially, under ether anesthesia and sterile conditions, the abdominal cavity was opened, and an omentectomy was performed. A peritoneal catheter with two dacron cuffs was inserted into the peritoneal cavity, and one dacron cuff was attached with sutures to the abdominal muscle. The distal part of the catheter was tunneled subcutaneously to the back side of the rat's neck and exteriorized at that site. The external dacron cuff of the catheter was sutured to the skin of the neck. During the following three days, the animals were infused once daily with Dianeal peritoneal dialysis solution containing 1.36% glucose (Baxter Healthcare Corporation, Deerfield, IL, USA). Infused solution was left to be absorbed from the abdominal cavity.

Experimental procedure

Four days after insertion of the peritoneal catheters, 12 rats were randomly divided into two groups of six rats each. Control animals were infused twice a day with 20 mL of Dianeal containing 3.86% glucose (Baxter Healthcare Corporation), and those in the treatment group were infused with 20 mL of Dianeal containing 3.86% glucose supplemented with 10 mg/dL HA (Genzyme Pharmaceuticals, Cambridge, MA, USA). The HA used in this study had an average molecular weight ranging between 1.8×10^6 and 2.4×10^6 D. This molecular weight was selected because high molecular weight HA, in contrast to low molecular weight HA, is known to have anti-inflammatory properties [16]. High molecular weight HA also appears to exert greater effects on ultrafiltration in acute rat studies [17]. The concentration of HA (10 mg/dL) was chosen based on our previous experiments [8]. Dialysis solutions in each group were also supplemented with antibiotics (5 mg/L gentamicin and 50 mg/L cefuroksin). Infused solutions were allowed to dwell and to be absorbed gradually from the peritoneal cavity.

Evaluation of peritoneal permeability

Peritoneal permeability was evaluated using a four-hour peritoneal equilibration test (PET). PETs were performed in all rats four days after implantation of the peritoneal catheter (PET 1) and again after four weeks of treatment with the control and test solutions (PET 2). On the day preceding the PET, animals were infused with the dialysis fluid only once, in the morning, to ensure its complete absorption from the peritoneal cavity prior to the start of the PET. At each PET, animals were infused with 30 mL of Dianeal solution containing 3.86% glucose (without HA). Dialysate samples (1 mL) were taken at time 0 (immediately after infusion), 30, 60, 120, and 240 minutes after infusion for measurement of urea, creatinine, glucose, albumin, and total protein. Blood samples were taken from the tail vein, under ether anesthesia, at the beginning and at the end of the PET. During the PET, animals were awake and had free access to water and food.

Concentrations of urea, creatinine, and glucose in serum and dialysate were measured using enzymatic methods (Analco, Warsaw, Poland). Albumin concentrations were measured using bromcocresol green (Sigma Chemical Co., St. Louis, MO, USA), and total protein was measured by the method of Lowry et al [18]. Peritoneal permeability of urea, creatinine, albumin, and total protein was expressed as the dialysate to serum ratio (D/S). Glucose permeability was expressed as the ratio of glucose concentration in the dialysate at a designated time to its concentration in the infused dialysis solution (D/D_0). Peritoneal clearances for urea, creatinine, albumin, and total protein were calculated by multiplication of the total drained volume (including residual volume) by the D/S ratio obtained at four hours during PET 2.

Evaluation of peritoneal volume

For PET 1, dialysate was drained at the end of the four-hour dwell by gravity, and its volume was measured by weight. For PET 2, intraperitoneal dialysate volume

was determined at designated time periods by calculation of the dilution of a macromolecular marker, after correction for absorption from the peritoneal cavity. The volume marker, blue dextran (Sigma Chemical Co.), was added to the infused dialysis solution at a concentration of 100 mg/dL, and the concentration of blue dextran in the collected dialysate samples was measured colorimetrically at 620 nm by comparison of data with a standard curve. Dialysate volume at each time period (n) was calculated according to the following formula:

$$V_n = \frac{C_0 \times V_0}{C_n} - Abs \times t_n$$

where V_n is the dialysate volume at time n; C_0 is the concentration of the macromolecular marker in the dialysate at time 0; V_0 is the dialysate volume at time 0; Abs is the absorption rate of the macromolecular marker from the peritoneal cavity; t_n is time n; and C_n is the concentration of the macromolecular marker in the dialysate at time n.

At the end of PET 2, after collecting the last dialysate sample, animals were sacrificed by an overdose of ether anesthesia, and the peritoneal cavity was opened. Residual dialysate was collected from the peritoneal cavity with a Pasteur pipette, and its volume was measured by weight. The peritoneal cavity was then infused with 20 mL of Dianeal containing 3.86% glucose, and after mixing for one minute, a sample was drawn for measurement of blue dextran concentration. The volume of residual dialysate was calculated from the dilution of blue dextran in the wash fluid according to the following formula:

$$V_{residual} = \frac{C_{wash} \times 20}{C_{240min} - C_{wash}}$$

where C_{wash} is the concentration of blue dextran in the dialysis fluid infused for washing the peritoneal cavity, and C_{240min} is the concentration of blue dextran in dialysate at 240 minutes of the dwell.

The total drained volume at the end of PET 2 was calculated from the sum the volume obtained by weight and the residual volume.

The absorption rate of the macromolecular marker from the peritoneal cavity was calculated for each animal from the difference between the absolute amount of the marker in the collected dialysate after the four-hour dwell and the initial amount infused into the peritoneal cavity, divided by the time of the dwell (240 min). It was assumed that absorption of the macromolecular marker from the peritoneal cavity was constant during the entire time of the dwell.

Evaluation of markers of peritoneal inflammation

Markers of peritoneal inflammation (cell counts, cell differential, and cytokine levels) were evaluated in dialysate samples obtained during PET 1 and similar samples obtained on the day preceding PET 2. Peritoneal cell counts were determined using a Neubauer chamber, and the cell differential was determined in cell smears following Giemsa staining of the cytospinned cell pellets. Total cell counts were approximated from cells per milliliter and the total drained volume, which was not corrected for residual volume. Peritoneal cell counts and differential were also determined in rats that were not exposed to dialysis solutions. In these animals, the peritoneal cavity was opened after sacrifice and filled with 20 mL of Hank's solution. After one to two minutes of mixing, a sample of fluid was drawn for cell counts and differential. Cytokine levels [tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), and interleukin-10 (IL-10)] were measured in dialysate samples (100 μL) using enzyme-linked immunosorbent assay kits (ELISA) from Biosource Europe S.A. (Fleurus, Belgium). Detection limits for the ELISA were 4, 8, and 5 pg/mL for TNF-α, MCP-1, and IL-10, respectively. The dialysate nitrite concentration was measured colorimetrically with Griess reagent after reducing nitrates to nitrites with nitrate reductase (Boehringer Mannheim, Mannheim, Germany) [19].

Morphometry of the peritoneal membrane

After collection of residual dialysate at the end of PET 2, biopsies of the liver were taken for light microscopy. Similar biopsies were taken from rats never exposed to dialysis solution ($N = 8$ animals). All liver biopsies were taken from the edge of the right lobe of liver. Peritoneal thickness was determined in hematoxylin and eosin-stained tissue specimens by measurement of the combined thickness of the mesothelium and submesothelial interstitium, lying between the mesothelium and liver tissue. Twenty measurements were taken and averaged for each specimen.

Measurement of hyaluronan content in peritoneal tissues

Mesenteric biopsy specimens, each weighing approximately 100 mg, were incubated in digestion buffer containing 0.05 mol/L Tris-HCl, 0.01 mol/L $CaCl_2$ (pH 7.2), and 2.4 U/mL pronase (Sigma Chemical Co.) for 24 hours at 55°C. Tissue digestion was stopped by boiling at 100°C for five minutes. Vials with digested tissue samples were centrifuged at 10,000 r.p.m. for one hour. After discarding the lipid layer from the surface of fluid in each vial, the HA concentration in each sample was measured by radioimmunoassay (Pharmacia AB, Uppsala, Sweden). The concentration of HA in each sample was expressed per mg of wet tissue.

Statistical analysis

Results are presented as mean ± SD. Statistical analysis was performed using analysis of variance with post

Table 1. Transperitoneal permeability to small solutes and macromolecules at the peritoneal permeability test 1 (PET 1) and after four weeks of exposure to the dialysis solution (PET 2)

	PET 1 (D/S)		PET 2 (D/S)		Δ PET 2–PET 1	
	Control	HA	Control	HA	Control	HA
Urea	0.984 ± 0.020	0.992 ± 0.020	0.930 ± 0.160	0.905 ± 0.160	−0.055 ± 0.178	−0.087 ± 0.157
Creatinine	0.797 ± 0.120	0.790 ± 0.141	0.850 ± 0.137	0.796 ± 0.065	0.053 ± 0.252	0.005 ± 0.122
Albumin	0.103 ± 0.023	0.098 ± 0.024	0.143 ± 0.043	0.066 ± 0.026[a]	0.043 ± 0.045	−0.032 ± 0.022[b]
Total protein	0.065 ± 0.007	0.075 ± 0.013	0.076 ± 0.013[a]	0.050 ± 0.015[a]	0.019 ± 0.010	−0.025 ± 0.020[b]

Values are expressed as dialysate/serum concentration (D/S) obtained at 4 hours during the PET. Negative numbers in the right hand columns indicate a decrease in peritoneal permeability from PET 1 to PET 2.

Abbreviation HA is hyaluronan.

[a] $P < 0.05$ vs. PET 1

[b] $P < 0.005$ vs. the control group

hoc analysis using the Kruskal–Wallis test, multivariate analysis of variance with post hoc analysis using the Newman–Keuls test, Mann–Whitney, or Wilcoxon test, as appropriate. A P value of less than 0.05 was considered significant.

RESULTS

Peritoneal permeability

Transperitoneal permeabilities to urea, creatinine, glucose, albumin, and total protein were similar in both groups of rats at PET 1. After four weeks of exposure to dialysis solution, permeability to albumin and total protein increased slightly in the control group, whereas both albumin and total protein permeability decreased significantly in animals exposed to solution containing HA (Table 1). Changes in protein permeability from PET 1 to PET 2 were significantly different between groups ($P < 0.01$ and $P < 0.005$ for albumin and total protein, respectively). During PET 2, permeability to albumin was significantly lower in the HA-treated group at four hours ($P < 0.001$; Fig. 1B), and total protein permeability was significantly lower at 0.5, 2, and 4 hours ($P < 0.05$, $P < 0.01$, and $P < 0.01$, respectively; Fig. 2B). There were no significant changes in the permeability to urea or creatinine from PET 1 to PET 2 in either group, and there were no differences between groups in small solute permeability during PET 2 (Table 1). Absorption of glucose from the dialysate was similar in both groups during PET 1 and PET 2 (data not shown).

Peritoneal volume, ultrafiltration, and clearances

Peritoneal volume, determined during PET 2 by dilution of blue dextran and at the end of PET 2 by gravimetric methods, was significantly greater for rats treated with HA compared with Dianeal solution alone. Peritoneal volumes determined by macromolecular volume marker during PET 2 were significantly greater for the HA-treated group at 120 minutes ($P < 0.005$) and 240 minutes ($P < 0.001$; Fig. 3). The mean volume of drained dialysate

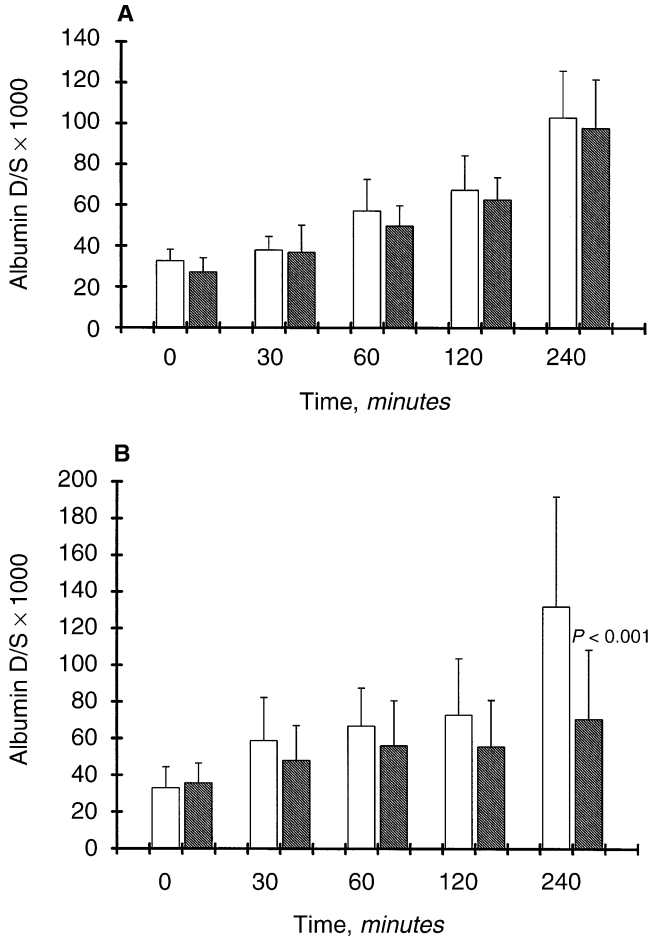

Fig. 1. Transperitoneal equlibration (D/S) of albumin for rats implanted with a peritoneal cathether and exposed briefly to peritoneal dialysis solution (*A*, PET 1) and for rats exposed for four weeks (*B*, PET 2) to a hypertonic dialysis solution (control; □) or a solution supplemented with 10 mg/dL hyaluronan (HA; ■).

at the end of PET 2 (determined gravimetrically with a correction for residual volume) was 34.2 ± 3.8 mL for the HA-treated group versus 26.6 ± 5.2 mL for the control group ($P < 0.001$). Net ultrafiltration was therefore

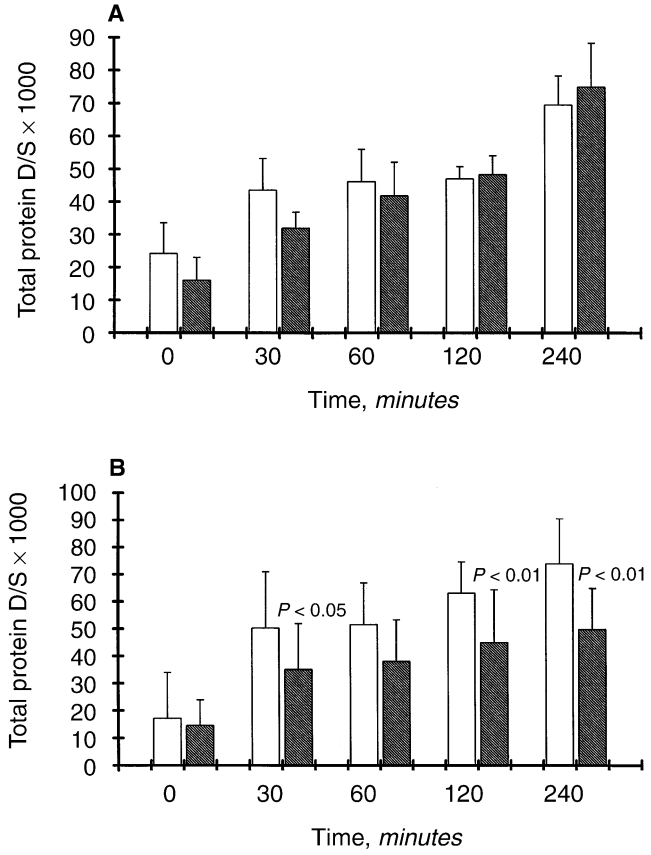

Fig. 2. Transperitoneal equlibration (D/S) of total protein for rats implanted with a peritoneal cathether and exposed briefly to peritoneal dialysis solution (*A*, PET 1) and for rats exposed for four weeks (*B*, PET 2) to a hypertonic dialysis solution (control; □) or a solution supplemented with 10 mg/dL hyaluronan (HA; ■).

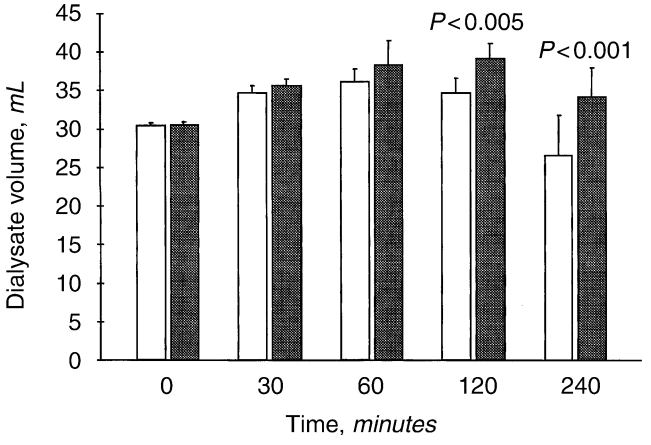

Fig. 3. Dialysate volumes during PET 2 calculated from the dilution of a macromolecular volume marker (blue dextran; discussed in the Methods section). Symbols are: (□) control; (■) hyaluronan.

Table 2. Differential of peritoneal leukocytes at baseline (PET 1) and after four weeks of infusion of dialysis solutions (just prior to PET 2)

	PET 1		PET 2	
	Control	HA	Control	HA
Macrophages	38.7 ± 4.0	40.0 ± 5.7	36.0 ± 3.7	49.2 ± 2.9[a]
Neutrophils	18.3 ± 3.1	15.2 ± 1.9	22.8 ± 9.5	8.8 ± 6.7[a]
Eosinophils	18.8 ± 3.7	16.7 ± 2.3	18.8 ± 9.9	15.5 ± 5.3
Monocytes	8.8 ± 1.3	10.0 ± 2.3	6.3 ± 2.0	10.3 ± 2.7[a]
Lymphocytes	14.0 ± 4.2	16.8 ± 1.8	15.2 ± 5.3	14.7 ± 4.0
Basophils	0.8 ± 0.8	0.8 ± 0.4	0.5 ± 0.5	0.3 ± 0.5
Mast cells	0.5 ± 0.5	0.5 ± 0.5	0.3 ± 0.5	1.2 ± 1.0

Values are the percentage of total cells. Approximate total cell counts are provided in the text.
[a] $P < 0.05$ vs. the control group

negative (−3.4 mL) in the control group and positive (4.2 mL) in animals treated with HA.

There was a trend toward higher peritoneal clearances of urea (control 25.1 ± 9.3 and HA 30.9 ± 5.3 mL/4 h) and creatinine (control 22.6 ± 5.7 and HA 27.3 ± 4.2 mL/4 h) and lower clearances of albumin (control 3.4 ± 1.5 and HA 2.4 ± 1.1 mL/4 h) and total protein (control 2.0 ± 0.6 and HA 1.7 ± 0.4 mL/4 h) in animals treated with HA, but these differences were not significant.

Dialysate cell counts and cytokine levels

In untreated rats ($N = 6$), the total number of peritoneal cells (harvested by washing the peritoneal cavity with Hanks solution) was $24.1 \times 10^6 \pm 5.7 \times 10^6$, of which macrophages accounted for 36.3 ± 10.9%, neutrophils 1.2 ± 0.8%, eosinophils 28.3 ± 6.0%, monocytes 14.7 ± 5.9%, lymphocytes 16.5 ± 5.9%, and mast cells 3.0 ± 3.1% of the total cells. In rats implanted with a peritoneal catheter and exposed to dialysis solution for four days, total cell counts increased to approximately $71 \times 10^6 \pm 40 \times 10^6$ cells in the control group and approximately $70 \times 10^6 \pm 48 \times 10^6$ cells in the group randomized to receive HA, respectively. The percentage of neutrophils in the dialysate also increased from 1.2 ± 0.8% to 18.3 ± 3.1% in the control group and 15.2 ± 1.9% in the group to receive HA ($P < 0.05$ vs. untreated rats; Table 2). There was no difference in cell counts or differential between the two groups at PET 1.

After four weeks of exposure to dialysis solutions, dialysate cell counts obtained from four-hour effluents (obtained prior to PET 2) were lower in both groups (approximately $47.3 \times 10^6 \pm 21.7 \times 10^6$ cells in the control group and approximately $59.4 \times 10^6 \pm 24.0 \times 10^6$ cells in the HA group). In contrast to the control group, in which the proportion of neutrophils increased, there was a marked reduction in the proportion of neutrophils in dialysate samples obtained from rats exposed to solution containing HA (8.8 ± 6.7 vs. 22.8 ± 9.5% in control, $P < 0.01$; Table 2). The proportions of macro-

Table 3. Dialysate concentration of cytokines and nitrites at baseline (PET 1) and after four weeks (PET 2) of intraperitoneal infusion of Dianeal 3.86% (Control) or Dianeal 3.86% containing 10 mg/dL hyaluronan (HA)

	PET 1		PET 2		Δ PET 2−PET 1	
	Control	HA	Control	HA	Control	HA
TNF-α *pg/mL*	4.4 ± 1.4	3.8 ± 1.6	42.3 ± 35.3	11.2 ± 14.7[a]	38.0 ± 34.9	7.4 ± 15.4[a]
MCP-1 *pg/mL*	14.1 ± 2.8	14.1 ± 4.3	402 ± 258	72.0 ± 86.5[a]	388 ± 260	57.9 ± 88.8[a]
II-10 *pg/mL*	15.7 ± 8.0	18.2 ± 9.1	87.0 ± 30.3	45.4 ± 49.7	71.4 ± 34.6	27.2 ± 53.8
Nitrites *nmol/mL*	4.5 ± 0.8	3.6 ± 1.1	12.5 ± 10.6	5.3 ± 2.8	8.1 ± 10.6	1.6 ± 3.1

Mean changes in dialysate cytokines (Δ PET 2-PET 1) are also presented.
[a] $P < 0.05$ vs. the control group

phages and monocytes were correspondingly higher in the HA-treated group.

Concentrations of cytokines and nitrites in the four-hour effluent dialysate were similar in both groups at PET 1. Interestingly, after four weeks of exposure to dialysis solutions, cytokine and nitrite levels increased in effluents from rats exposed to hypertonic solution without HA, whereas these increases were markedly attenuated in animals exposed to solution containing HA (Table 3). Concentrations of TNF-α and MCP-1 were 73% ($P < 0.05$) and 82% ($P < 0.02$) lower, respectively, in dialysate samples obtained at week 4 from the HA-treated rats compared with rats exposed to Dianeal alone. There was also a trend toward lower IL-10 and nitrite levels in dialysate from HA-treated rats as compared with the control group (Table 3).

Peritoneal morphometry

Peritoneal thickness (mesothelium plus interstitium) from liver biopsies was significantly greater in animals exposed for four weeks to dialysis solutions compared with animals never exposed to dialysis solutions (12.0 ± 4.8 μm for the Dianeal control group; 11.2 ± 7.7 μm for the HA group; vs. 3.01 ± 0.73 μm for untreated rats, $P < 0.05$ for untreated vs. both treated groups). There was no difference in peritoneal thickness between the control and HA-treated groups.

Peritoneal hyaluronan content

In rats treated with Dianeal containing HA, the HA content in mesenteric tissue samples was 48% higher than that from rats exposed to Dianeal alone (0.187 ± 0.026 μg/mg wet tissue compared with 0.126 ± 0.028 μg/mg for the control group, $P < 0.01$). Both groups of rats exposed to dialysis solution had significantly greater peritoneal HA content than untreated animals (0.105 ± 0.009 μg/mg wet tissue, $N = 6$, $P < 0.05$ vs. both treated groups; Fig. 4).

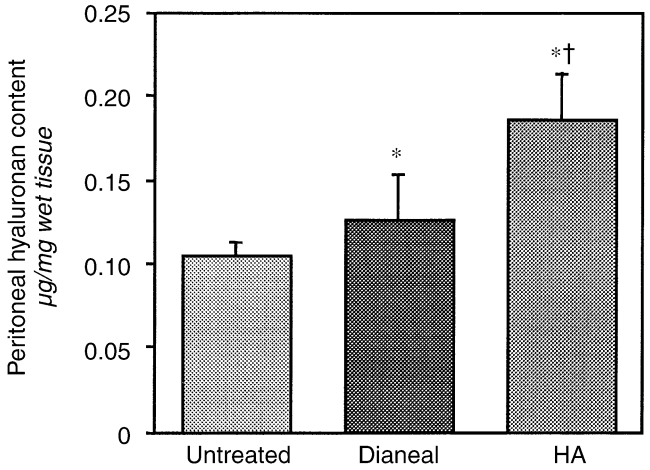

Fig. 4. Hyaluronan content of peritoneal tissue (mesentery) in untreated rats and rats exposed to dialysis solution without (Dianeal control) or with 10 mg/dL HA. *$P < 0.05$ vs. untreated rats; †$P < 0.01$ vs. control rats.

DISCUSSION

Hyaluronan is known to modulate both acute and chronic inflammatory reactions and to enhance tissue repair [13, 16, 20]. Inflammatory reactions in connective tissue are followed by increased local production of HA, and HA synthesis is up-regulated by proinflammatory cytokines [11, 21, 22]. HA may protect against tissue damage during inflammation by scavenging free radicals [23] or by inhibiting elastase release from activated leukocytes [24]. HA has also been shown to prevent the excessive growth of connective tissue, and the presence of hyaluronic acid in wound fluid is associated with healing of fetal wounds without scar formation [25]. Preliminary studies of the effect of HA on scar-free wound healing in adults have been promising [26, 27].

In patients treated by peritoneal dialysis, intraperitoneal production of HA increases dramatically during episodes of peritonitis [10, 11], consistent with a role for HA in modulating peritoneal inflammation and in peritoneal wound healing following peritonitis. HA concentrations in the dialysis effluent also increase with the duration of dialysis [15], possibly related to the chronic exposure of the peritoneal membrane to dialysis solutions. During peritoneal dialysis, locally produced HA is removed from the peritoneal cavity at each fluid ex-

change. As a result, HA may never achieve its optimal concentration for prevention of tissue damage in the peritoneal cavity. Thus, we hypothesized that exogenous HA, added to the dialysis solution, may protect the peritoneal membrane from inflammation and damage caused by the chronic exposure to peritoneal dialysis solutions.

In our experiments, rats treated with dialysis solution containing HA had significantly fewer peritoneal neutrophils, despite similar numbers of total peritoneal cells, when compared with rats exposed for four weeks to hypertonic dialysis solution alone. In addition, dialysate levels of proinflammatory cytokines, TNF-α and MCP-1, were significantly lower when HA was added to the dialysis solution. These data suggest that exogenous HA may reduce peritoneal inflammation or leukocyte activation that is associated with exposure to dialysis solutions in this model. Our study did not demonstrate a significant difference in the thickness of the peritoneal interstitium when control animals were compared with rats treated with HA. However, we examined only one peritoneal biopsy from each animal, and the length of exposure to dialysis solution may have been too short to observe significant differences between groups. Further studies are required to determine the effects of HA administration on peritoneal morphology in this model.

Hyaluronan has been shown to increase net ultrafiltration when administered with the dialysis solution during acute exchanges in rats [17, 28]. This increase in ultrafiltration can be explained by a decrease in fluid reabsorption, which, in turn, has been explained by a decrease in the hydraulic conductivity of the connective tissue in the peritoneal interstitium [17, 28–30]. According to this hypothesis, the decrease in hydraulic conductivity of the peritoneal interstitium impedes fluid reabsorption, which depends on fluid reuptake into the venular end of the microvessels, but does not interfere with water flow into the peritoneal cavity induced by the osmotic gradient created by glucose. Although HA was not included in the dialysis fluid during PET 2 in this study, we observed an increase of ultrafiltration and peritoneal volumes during the dialysis exchange in animals that had been previously exposed to solution containing HA. Thus, the ability of HA to improve ultrafiltration appears to be preserved after its removal from the dialysis solution. It is possible that the increase in tissue HA may be responsible for this effect, as HA concentrations in peritoneal tissues were 48% greater in the HA-treated animals. A higher net ultrafiltration in exposed to HA could not be explained by slower absorption of glucose from the dialysate because it was similar as in control group.

Increased HA content in the peritoneal interstitium may also explain the slower transperitoneal equilibration of albumin and other proteins observed in this study. Negatively charged glycosaminoglycans have been shown to be important in reducing transport of protein across the glomerular basement membrane [31, 32]. We have also previously observed in rats that treatment with dialysis fluid supplemented with glycosaminoglycans seemed to reduce peritoneal protein loss [7, 8]. Importantly, Bazzato et al have demonstrated a decrease in peritoneal protein loss following dialysis of continuous ambulatory peritoneal dialysis patients for one month with a solution containing a mixture of heparan and dermatan sulfate [33]. Although not significantly different between groups, clearance of small solutes (urea, creatinine) trended to be higher for rats exposed to solution containing HA, whereas clearance of macromolecules (that is, proteins) tended to be lower. These opposite directions in the peritoneal clearances of small and large molecules, both of which are favorable for peritoneal dialysis therapy, imply that the lower concentrations of proteins in the effluent dialysate from HA-treated rats are due not only to their dilution with increased dialysate volumes, but also reflect decreased permeability of the peritoneum to charged macromolecules.

Ultimately, long-term supplementation of the dialysis fluid with HA may prevent peritoneal damage and improve the efficiency of peritoneal dialysis by improving ultrafiltration and decreasing peritoneal permeability to protein. By replacing HA lost during repeated dialysis exchanges, this approach may mimic natural homeostatic mechanisms that operate within the peritoneal cavity, in which locally produced HA may modulate inflammation and enhance the process of wound healing during peritoneal dialysis. Supplementation of the dialysis solution with exogenous HA may help to maintain this homeostasis and thereby decrease the intensity of intraperitoneal inflammation and reduce the damage induced by peritonitis or chronic exposure to dialysis solutions.

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