Acute Peritoneal Dialysis in Rats Results in a Marked Reduction of Interstitial Colloid Osmotic Pressure

BERT-INGE ROSENGREN,*† BENGT RIPPE,* OLAV TENSTAD,† and HELGE WIIG†
*Department of Nephrology, Lund University, Lund, Sweden; and †Department of Biomedicine, Section of Physiology, University of Bergen, Bergen, Norway

Abstract. The aim of this study was to investigate whether the interstitial colloid osmotic pressure (COPi) of peritoneum is of importance for peritoneal fluid reabsorption during peritoneal dialysis (PD). For testing this hypothesis, a method to isolate interstitial fluid from the peritoneal membrane and to measure the interstitial COPi in the normal peritoneum and directly after the initiation of PD needed to be developed and validated. Eighteen female rats were anesthetized subcutaneously in the neck region with fentanyl-midazolam (1:1). Nylon wicks were implanted postmortem by means of a plastic catheter in the tissue just underneath the peritoneal surface. The characteristics of this fluid were compared with that isolated from wicks that were implanted in intermuscular spaces in hindlimb muscle and back subcutis. All wicks were removed after 20 min and centrifuged. The wick fluid was collected and analyzed in a colloid osmometer constructed for submicroliter samples, and interstitial fluid COP was compared with that of plasma. PD was initiated by injecting 20 ml of 3.86% glucose-containing PD fluid (Dianeal) into the peritoneal cavity, over a dwell time of 4 h. Control rats received no PD. The ratio of COPi to that of plasma (COPp) during control was 0.65 ± 0.05 in peritoneum, 0.53 ± 0.04 in muscle, and 0.59 ± 0.05 in skin. After a PD dwell, the ratio was 0.29 ± 0.03 in peritoneum, 0.54 ± 0.08 in muscle, and 0.41 ± 0.06 in skin. Thus, the COP ratio in peritoneum fell by 55% (P = 0.014) and in skin by 30% (P = 0.03), whereas the COP ratio in muscle was unchanged. The results suggest that acute PD results in a marked fall of the COPi in the peritoneal membrane, shifting the Starling equilibrium toward an absorptive state. The effect was restricted to the peritoneal membrane and, to some extent, the skin. It is speculated that the increase in peritoneal hydrostatic pressure after PD causes an increase in interstitial tissue volume, with primarily a dilution of interstitial colloids.
Experimental Interventions (PD)

In PD, a hyperosmotic solution is instilled into the PC to remove excess fluid and metabolites from the blood of patients with impaired renal function (12). In this study, PD was performed by injecting 20 ml of 3.86% Dianeeal (Baxter Health Care, Castlebar, Ireland), which catheter was withdrawn. Two to three parallel wicks were inserted in the peritoneal wall in this manner. The peritoneal wall was covered quickly with plastic film and mineral oil to prevent dehydration.

All wicks were removed after 20 min and their ends with any sign of bloodstains were cut off, and they were centrifuged in special-made Eppendorff tubes. Funnels were made from cut bottoms of Eppendorff tubes and making a hole in the bottom by means of a heated syringe. The funnels then were put in siliconized Eppendorff tubes used for wick centrifugation. After centrifugation, the wick fluid could be collected from the bottom of the siliconized Eppendorff tubes. Wicks from the same tissue were pooled. The characteristics of the peritoneal wick fluid were compared with that isolated from wicks that were implanted in intermuscular spaces and from back subcutis.

All wick implantation and handling of wick fluid were done in an infant incubator kept at 100% relative humidity. Care was taken at all times to avoid dehydration of wicks and of peritoneal tissues by using mineral oil and covering exposed tissues with plastic film.

Characterization of Wick Fluid

To evaluate the level of cellular water flux, which may dilute the wick fluid, we performed separate experiments. A catheter was inserted in the left jugular vein in four rats, and both kidneys were tied off via flank incisions. A bolus dose of 51Cr-EDTA was then given through the catheter and was allowed to equilibrate for 1 h. The partitioning of the marker should be equal in extracellular fluid and plasma after equilibration; otherwise, dilution of wick fluid by cellular water may have occurred. At the end of the equilibration period, blood samples were collected by cardiac puncture, and the rats then were killed. Wicks were implanted as described above, and the plasma and wick fluid were analyzed in an LKB gamma counter (model 1282; Compugamma, Turku, Finland).

For evaluating the level of direct leakage of proteins from plasma to wick fluid, 125I-labeled albumin was given as a bolus through the jugular vein catheter during the last 5 min of the equilibration period of 51Cr-EDTA. The concentration of 125I-albumin in wick fluid then was compared with that in plasma. Furthermore, additional wick fluid was collected from rats in the same manner as described above for analysis by HPLC using a Superose 12 size exclusion column (Pharmacia-Biotech, Uppsala, Sweden). After the implantation period, the wicks were transferred to preweighed vials that contained 500 μl of 0.02% azide in 0.15 M saline and then were vortexed and left overnight for elution. After shaking, the wicks were removed and dried to calculate the wick fluid content. A total of 100 μl of eluent was injected onto the HPLC system using a Gilson 234 autoinjector (200-μl loop). A constant flow of 1 ml/min was obtained by a Spectraseries P2000 pump (Thermo separation products), and protein was detected by UV at 280 nm (Spectraseries UV100). The UV signal was digitized, sampled at 2 Hz, and computer-analyzed using ChromoQuest (version 2.51; ThermoQuest).

Materials and Methods

Experiments were performed on 18 female Wistar-Møller rats that had an average body weight of 250 g. The rats had free access to food and water until the day of experiment. Body temperature was kept at 37 to 38°C during experiments using a heating lamp. Anesthesia was induced by subcutaneous injections of fentanyl-midazolam (1:1) in the neck of the rats. Thus, any side effects of the anesthesia on the peritoneal membrane was minimized. Blood samples were collected by cardiac puncture, and the rats were killed by intracardiac injection of saturated KCl. All experiments were performed in accordance with the recommendations given by the Norwegian State Commission for Laboratory Animals and were approved by the local ethical committee.

Wick Implantation Method

Nylon wicks were washed in acetone, ethanol, and distilled water. After being washed, the wicks were dried and were kept in a box at 100% relative humidity. Wicks were implanted postmortem in the back subcutis (two double strands each 4 to 5 cm long, Ø ~ 0.60 mm) by means of a surgical needle as described in detail elsewhere (9) and in the intermuscular spaces in left and right medial hindlimb muscle as detailed in a previous publication (10).

For isolation of interstitial fluid from peritoneum, an alternative approach was used. Our method was modified from that described for hindlimb muscle (10). Thinner wicks (Ø ~ 0.36 mm, 2 to 4 cm long) were implanted carefully postmortem by means of a siliconized plastic catheter (PE50) in the tissue just underneath the peritoneal membrane after carefully cutting a fold of the abdominal wall and exposing the inner side. The mesothelium was lifted carefully using a forceps, and a small hole was cut. The catheter was inserted through the hole and was proceeded gently 3 to 5 cm parallel to the fibers of M. obliquus internus abdominis and was then pushed through the mesothelium. The wicks then were inserted into the catheter, and the
essentially is a lactated Ringer solution that contains glucose as osmotic agent, into the PC. This procedure will result in an increase in IPP of 3.6 mmHg (13). The dwell time was set at 4 h, which according to previous experiments [e.g., (14)] results in a net fluid removal of 1.5 ml. Control rats were not dialyzed. The dialysis fluid was prewarmed to 37°C in a heating bath before infusion.

Morphology
In one control rat, extraperitoneal wicks were inserted as described above. Thereafter, the whole abdominal wall was excised with the wicks and fixed overnight in paraformaldehyde. For showing the position of the wicks, the abdominal wall was cut into smaller sections, which were routinely processed for freeze sectioning and cut into 30-μm sections that were stained with hematoxylin and eosin for later light microscopy analysis.

Statistical Analyses
All data are expressed as means ± SEM. Statistics were obtained using the Mann-Whitney test. All statistical calculations were made using the computer program SPSS for Macintosh release 10.0.7a (SPSS Inc., Chicago, IL).

Results
On average, we were able to collect 1 to 2 μl of wick fluid from extraperitoneal wicks, 6 to 7 μl of fluid from intermuscular wicks, and 20 to 25 μl of interstitial fluid from wicks implanted in subcutis. The absolute values of COP are presented in Figure 1. There was a trend of increasing plasma COP after a 4-h PD dwell, from 20.2 ± 1.2 (SEM) to 24.5 ± 1.5 mmHg (P = 0.073; NS), whereas the peritoneal COP was decreased from 12.1 ± 0.7 to 6.9 ± 0.5 mmHg (P = 0.014). No major changes in muscle or skin COP were found with regard to the absolute values. In Figure 2, the COP relative to plasma COP are presented. There was a clear fall (P = 0.014) in relative COP of the peritoneum, from 0.65 ± 0.05 to 0.29 ± 0.03, and a fall in the relative COP of skin from 0.59 ± 0.05 to 0.41 ± 0.06 (P = 0.03). There were no statistical changes in relative COP of muscle. After a single PD dwell, the relative COP of the peritoneum thus decreased by 55% and in the skin by 30%.

The relative concentration of 51Cr-EDTA in wick fluid relative to concentration in plasma was on average 1.1 ± 0.1 (not different from 1.0), indicating that the wick fluid was not contaminated by cellular water. The relative concentration of 125I-albumin in wick fluid compared with plasma concentration was on average 0.008 ± 0.003, indicating low leakage of plasma protein from capillaries to wick fluid.

The elution pattern of wick fluid was similar to that of plasma (Figure 3). There were, however, lower levels of macromolecules larger than albumin in wick fluid but slightly more small molecules in wick fluid than in plasma.

The peritoneal wicks were found just underneath the mesothelial cell layer (Figure 4). Most of the wick fibers were lost in the process of preparing the sections, but the empty space that remained in the sections represents the site that the wick was occupying in the abdominal wall. No inflammatory cells were observed in the sections, and the mesothelium seemed intact, suggesting that the induced trauma was negligible.

Discussion
The present results validate and describe a method to sample extraperitoneal interstitial fluid in rats. Furthermore, the results demonstrate that an acute PD dwell markedly alters the COP in the peritoneal tissue, thereby contributing to a shift in the Starling equilibrium from a filtrative to an absorptive state. The effect was restricted to the peritoneal membrane, because the COP in leg muscle was unchanged and that in skin was changed to a much lesser extent. We speculate that the increase in peritoneal hydrostatic pressure after exposure to dialysis fluid during PD causes an acute increase in the interstitial tissue volume, with dilution and/or washout of colloids.

The present article describes a new method to sample extraperitoneal interstitial fluid using a modified wick technique. Because our method may be characterized as invasive, inflam-
ation may be a problem as it is in all such procedures. To minimize this problem, we performed all wick insertion post-mortem, thereby avoiding the vascular component of the inflammatory response and thus the possible local edema formation. To validate the method, we measured the level of protein (albumin) derived directly from plasma. Less than 1% of the protein found in wick fluid was coming directly from plasma, showing that the contamination of wick fluid by plasma was negligible. To validate the method further, we allowed an extracellular marker (51Cr-EDTA) to equilibrate with the interstitium and compared the tracer concentrations in wick fluid and plasma. A difference in the concentrations could suggest that cellular water has contaminated the wick fluid (lower concentration of 51Cr-EDTA in wick fluid), whereas a higher concentration of the tracer in wick fluid would suggest that dehydration of wicks has occurred. However, we found almost identical concentrations of 51Cr-EDTA in plasma and wick fluid. Furthermore, we analyzed plasma and wick fluid on HPLC. Plasma and wick fluid had a similar chromatographic pattern (Figure 3) except for a lower content of proteins larger than albumin in wick fluid and a higher content of smaller proteins in wick fluid. Importantly, the amount of nonplasma proteins was very low in wick fluid. The method also responded to perturbations (PD) as discussed below. Thus, we conclude that the method of isolating extraperitoneal wick fluid is valid.

The extraperitoneal wick position is illustrated in Figure 4. The wicks were positioned just underneath the mesothelial cell layer. Flessner (15) evaluated how deep into the peritoneal membrane the exchanges during a PD dwell occur and found that up to 500 μm of the peritoneal membrane is involved in the exchanges of fluid and (small) solutes. Macromolecules and water may be exchanged across the entire thickness of the peritoneum (~1 mm). Thus, the wicks can be expected to collect interstitial fluid that is involved in the exchanges that occur during the dwell.

Very few previous attempts have been made to measure the COP of peritoneal interstitium. In rabbits, Negrini et al. (8) measured the COP in intermuscular spaces of the abdominal wall. The authors registered values very close to the present (14 versus 12 mmHg) in the control animals. However, Negrini et al. did not validate the method or dialyze the animals. Zakaria and Rippe (16) were able to show that elevated IPP led to fluid loss from the PC, and a major part of the fluid could be detected in tissues within or surrounding the PC. The elevated IPP did not lead to a shift in the transcapillary Starling forces of an extent that was originally thought, because the raised IPP was to a large extent transmitted retrogradely to the capillaries via large-vein compression. However, the edema formation as a result of fluid loss into peritoneal tissues may have lowered the peritoneal COPi (not measured by Zakaria and Rippe) as in the present study. Furthermore, Zakaria et al. (17,18) determined the in vivo effect of varying hydrostatic and osmotic pressures on the tissue water of the anterior abdominal wall.
muscle in rats that underwent PD. The authors were able to show that a significant increase in tissue water was observed, regardless of peritoneal fluid tonicity (including isotonic fluid). The tissue expansion was observed primarily in the interstitium, which doubled from control when IPP was raised from 1.7 to 4 mmHg. Washout of hyaluronic acid from interstitial tissue to the surrounding subcutaneous space was also observed. In the same dialysis model as the present, Zhu et al. (12) measured the IPP after infusing different fluid volumes into the PC of rats. The IPP was found to be slightly negative (−1.77 mmHg) in control (anesthetized) rats, whereas infusion of 20 ml of dialysis fluid resulted in a pressure of −1.8 mmHg, suggesting that the same changes occurred in our study. Carlsson et al. (19) measured the extracellular volume in the muscular portion of the peritoneal wall at different times during PD in rats using 51Cr-EDTA as an extracellular volume marker. The authors found that the extracellular space increased by 60% after 4 h of dialysis using 1.5% glucose-containing PD fluid (Gambrosol). These previous results correspond well with those in the present article. In the present study, after a single PD dwell, the COP of the extraperitoneal tissues decreased by 55% (Figure 2). This seems to reflect an expansion of the peritoneal interstitial volume with or without washout of macromolecules.

During the initial 2 to 4 h of a PD dwell in humans, there is a net transport of fluid from plasma to the PC driven by glucose-induced osmosis. After 2 to 4 h, when the osmotic gradient in the peritoneal tissues has dissipated, the transport direction is reversed, resulting in a net fluid absorption from the PC. Fluid then is transported across the peritoneum directly back into the capillaries, driven by the transcapillary Starling forces with a lesser contribution from lymphatic absorption. This is provided that the COP is very low.

Previously, Leypoldt and Mistry (20) as well as Rippe et al. (12) estimated how much the Starling forces have to change during PD to account for the observed volume changes. The authors calculated that the COP in the peritoneal tissues needs to fall from ~10 mmHg to 1 to 2 mmHg to account for such changes. Indeed, the present study demonstrates a high COP in “normal” peritoneal tissue and, furthermore, that the COP is markedly reduced already during the very first dwell in PD. In the present study, it thus dropped by 5 to 6 mmHg during a 4-h PD dwell. It is conceivable that the COP will drop even further during the subsequent dwells, as a result of washout of colloids, to provide an even larger shift in the Starling equilibrium in chronic PD. Theoretically, a 7- to 8-mmHg drop in COP would be sufficient to promote an effective absorptive force during the late phase of the PD dwell (12,20).

Because macromolecules exhibit convection-dominated transport, driven by the capillary hydrostatic pressure, the transport from plasma to dialysate is of a unidirectional nature [although there may be some back-diffusion of albumin across the small pores, as shown by Haraldsson (21)]. Thus, removal of large solutes from the PC is largely by way of lymphatic absorption. Under normal conditions, lymphatic absorption thus seems to function as an “overflow valve,” returning back to the bloodstream the fluid and plasma proteins that have been filtered across the capillaries, thus preserving steady state. During PD, however, only a minor portion (20 to 30%) of the total fluid and solute reabsorption seems to occur by the lymphatics (4–7). True lymphatic absorption from the PC occurs from the peritoneal tissues and also via the lymphatic lacunae in the diaphragm (22,23). The latter have the ability to remove small particles up to the size of 10 µm, thus even erythrocytes. Of the total lymphatic absorption, 60% is by way of diaphragmatic lymph vessels, 25 to 30% is by way of visceral lymphatics, and 10 to 15% is by parietal lymphatics (7).

The COP in skin and muscle are of similar magnitude as in previous studies (10). The fall in relative skin COP after dialysis was unexpected, whereas the absolute value of skin COP did not change. The fall in relative COP of skin may just reflect that plasma COP increased after dialysis, whereas skin COP was unchanged. The increased plasma COP favors reabsorption of interstitial tissue fluid from muscle and skin, but given the relatively higher density of capillaries in muscle and the high muscle mass (nearly 50% of the body weight), fluid may be recruited primarily from muscle as compared with skin.

The main clinical implication of our findings is that the fall in peritoneal COP gives enough driving force to explain how dialysis fluid can be reabsorbed after peak time during a PD dwell. In the long term, we speculate that the peritoneal COP will be decreased even further as a result of washout of colloids, giving rise to a higher osmotic gradient and, thus, absorptive force. Thus, the reabsorption of fluid does not need to involve lymphatic absorption to a large extent. This gives useful information with respect to parameters involved in computer programs that are designed to calculate peritoneal fluid transport in patients and to prescribe the correct dialysis dose to patients. A quantitative understanding of the driving forces that govern fluid transport may lead to strategies to minimize fluid loss to the patient’s body and to improve fluid recovery at the end of a PD dwell. This also can assist in our understanding and improvement of intraperitoneal therapies that are designed to administer macromolecular drugs to intraperitoneal tumors.

In summary, we were able to develop and validate a method to collect interstitial fluid from the parietal peritoneum. Using this method, we found that after a single acute PD dwell, the COP of the peritoneal interstitium fell dramatically by 55%, thus shifting the Starling equilibrium to a state that favors peritoneal fluid reabsorption. In part, the decreased peritoneal COP may reflect an increased interstitial tissue volume after PD, but some degree of washout of colloids may also contribute. The latter may be more important in chronic PD.

Acknowledgments

This study was supported by a Marie Curie Host Fellowship (contract no QLK5-CT-2001-60039) and grants from the Swedish Medical Research Council (grant 14X-08285), from Gambro Lundia Corporation and Locus on Circulatory Research, University of Bergen.

Part of this study was presented as an abstract (24). Fruitful discussions with Dr. Christina Gyenge are greatly appre-
ciated. The technical assistance by Gerd Salvesen and Wibeke Skytterholm is gratefully acknowledged.

References


