The transport barrier in intraperitoneal therapy

Michael F. Flessner
Division of Nephrology, Department of Medicine, University of Mississippi Medical Center, Jackson, Mississippi

Flessner, Michael F. The transport barrier in intraperitoneal therapy. Am J Physiol Renal Physiol 288: F433–F442, 2005; doi:10.1152/ajprenal.00313.2004.—The peritoneal cavity is important in clinical medicine because of its use as a portal of entry for drugs utilized in regional chemotherapy and as a means of dialysis for anephric patients. The barrier between the therapeutic solution in the cavity and the plasma does not correspond to the classic semipermeable membrane but instead is a complex structure of cells, extracellular matrix, and blood microvessels in the surrounding tissue. New research on the nature of the capillary barrier and on the orderly array of extracellular matrix molecules has provided insights into the physiological basis of osmosis and the alterations in transport that result from infusion of large volumes of fluid. The anatomic peritoneum is highly permeable to water, small solutes, and proteins and therefore is not a physical barrier. However, the cells of the mesothelium play an essential role in the immune response in the cavity and produce cytokines and chemokines in response to contact with noncompatible solutions. The process of inflammation, which depends on the interaction of mesothelial, interstitial, and endothelial cells, ultimately leads to angiogenesis and fibrosis and the functional alteration of the barrier. New animal models, such as the transgenic mouse, will accelerate the discovery of methods to preserve the functional peritoneal barrier.

peritoneum; membrane; dialysis; regional chemotherapy; diffusion; convection; osmosis

THERAPEUTIC USE OF THE PERITONEAL CAVITY

The importance of transport across the peritoneum is derived from the therapeutic use of the peritoneal cavity as in means of dialysis or drug delivery. The barrier to transport is complex and not easily understood in simple terms of a membrane model. The goal of this review is to update the physiological aspects of the peritoneal barrier and the effects that therapeutic uses of the cavity bring about on the transport barrier and its function.

Intraperitoneal Chemotherapy

Intraperitoneal (ip) chemotherapy attempts to maximize the transfer of solutes from the peritoneal cavity to targets in the underlying tissues (1, 28, 64). Regional drug delivery in the peritoneal cavity is most important for metastatic ovarian or colorectal cancer, which metastasizes to almost any surface of the peritoneum and results in strangulation of the bowel and major morbidity and mortality. In each peritoneal chemotherapy, antineoplastic agents are dissolved in 2–3 liters of solution and instilled into the cavity. The solution is allowed to be absorbed over 24 h because these patients typically have kidney function and can excrete the fluid. The goal of this therapy is to maximize the concentration in the subperitoneal tissue and, in particular, to target tumors without significant systemic toxicity.

Dialysis

Peritoneal dialysis (PD) is a therapeutic technique used by ~8.4% of the dialysis patients in the United States for the removal of waste metabolites and water from their bodies (10). Typically, glucose-based solutions are instilled into the peritoneal cavity four to five times a day and drained and replaced after several hours (41). Small solutes (<1,000 Da) transport chiefly by diffusion from the blood circulating in the subperitoneal microcirculation to the solution in the cavity. Convection of solutes is the second mechanism of transperitoneal solute transport and is particularly important for macromolecules. Water is extracted from the body by osmosis from the blood via solutions made hypertonic with glucose. The discovery of the family of aquaporins has shed new light on the mechanism of osmotic filtration during PD and will be discussed below in more detail.

Although the major direction of transport and the goals of these two clinical therapies are different, the underlying physiology of the transport relies on these same fundamental processes of passive diffusion and convection. Transport of small solutes across the peritoneum is equivalent in pathways and rates (27), and therefore substances placed into the dialysate fluid will transfer to the plasma at the same rate as the appearance of the same substance injected intravenously. Except for the normal cell maintenance functions affected by the cellular Na-K-ATPase, there is no active transport across the peritoneum. One major difference in the two therapies is the duration of the therapy, which lasts for days to weeks for ip chemotherapy but can be years to decades in dialysis. In patients after ~6 years of PD, it has been well documented that chronic fibrosis and vasculopathy occur in the subperitoneal tissue, resulting in major changes in the structure and transport function (46, 113). The mechanisms involved in this process will be discussed below.
Role of the Peritoneum

The normal peritoneal cavity exists as a potential space surrounding the major abdominal and pelvic organs. The peritoneum is a single layer of mesothelial cells overlying several layers of connective tissues. The most detailed dissection of the visceral peritoneum was performed by Baron (2), who demonstrated five layers of connective tissue and the layer of mesothelial cells for a total thickness of ~90 μm. There were very few blood vessels within the peritoneum, and those that existed were ~40 μm from the surface. The mesothelial cells play a major role in the secretion of lubricant solutions made of primarily phospholipids and glycosaminoglycans (21). This ensures a smooth sliding surface between the visceral and parietal peritoneum, which is not only important for gut motility but for distribution of the therapeutic solution. The mesothelium also plays a major role in host defense within the peritoneal cavity and even reacts to the instillation of currently used sterile dialysis solutions (113) (see below). In the discussion that follows, the nature of the functional barrier will be described in light of recent findings, and the interaction between the physiological transport barrier and the mesothelium and other highly reactive immunological cells will be discussed. While many clinicians believe that the mesothelium and underlying connective tissue, which together make up the peritoneum, are equivalent to a “membrane,” conclusive evidence has been obtained to the contrary.

THE PERITONEAL TRANSPORT BARRIER

The Anatomic Peritoneum is not a Major Transport Barrier

The peritoneal barrier is a complex, three-dimensional structure made up of the peritoneum, the underlying tissue space, which includes parenchymal cells, interstitial cells, interstitial matrix, pericytes, and the endothelial cells that form the microvessels that course through this tissue space (see Fig. 1). While many nephrologists call the barrier the “peritoneal membrane,” analogous to the membrane of the artificial kidney, this terminology is misleading when the fundamental physiology of the system is mechanistically studied. Studies in rodents and in dialysis patients have demonstrated that protein leaves the peritoneal cavity at rates 5–10 times the rate in which it appears in the blood (15, 34, 46, 86). Since the only route of protein transfer from the cavity to the central circulation is via lymphatics (83), this means that there must be some other mechanism responsible for the disappearance of protein from the peritoneal cavity. Through dissection of rodent tissues, it was shown that all of the protein that left the cavity but did not reach the blood was contained in the surrounding peritoneal tissues (34, 35). In subsequent experiments, it was shown that the concentration in abdominal wall tissue was not affected by the extent of the peritoneal resection (19a). Complete removal of the peritoneum in rodents demonstrated no effect on the osmotic filtration of fluid from the peritoneal tissue into a transport chamber, nor did it affect the transfer of small solutes from the chamber into the tissue (30). Although an analogous experiment has not been performed in human dialysis patients, recent observations in patients undergoing partial or total peritonectomy for treatment of peritoneal carcinomatosis confirm the findings in rats. The clearance of mitomycin C from the peritoneal cavity was not significantly affected by the extent of the peritoneal resection (19a).

From these studies, it is concluded that the anatomic peritoneum is not a significant barrier to small solutes or macromolecules. This leaves the blood vessel wall and the surrounding interstitium as the major barriers to transport. While the mesothelium and the associated connective tissue that make up the peritoneum are not a significant physical barrier to transport, the mesothelium plays an important role in the maintenance and transformation of the barrier during long-term therapy.

AQUAPORINS, OSMOTIC FILTRATION, AND TRANSENDOTHELIAL TRANSPORT

Pore Theory

While the peritoneum does cover a portion of specialized tissue such as the spleen and liver, most of the potential surface area covers smooth muscle of the gut or the skeletal muscle of the retroperitoneum or abdominal wall (14). Since it is likely that only a small fraction of the ~10% of total anatomic peritoneal area made up of the spleen and liver is in contact with the fluid (23, 90), most authors emphasize the properties of the peritoneum to be the major transport barrier.
of muscle capillaries and their distribution within subperitoneal tissue.

Transendothelial transport in the subperitoneum has been classically represented by the three-pore model of Rippe and colleagues (25, 82, 84, 87, 88). The theory hypothesizes that 1–2% of the total pore surface area (102) is made up of “transcellular pores” with a radius of 0.3–0.5 nm, which permit only water to cross. Of the pore surface area, 95% is made up of so-called “small pores,” with a radius of 4.0–6.0 nm. The remainder of the total pore area is made up of “large pores,” which are on the order of 20–30 nm, which allow protein or large molecular solutes to pass without restriction.

Aquaporins and Osmosis

The finding of aquaporins by Agre and colleagues (79) has opened up an exciting area in peritoneal research. Carlsson and colleagues (6) were the first to show that in vivo inhibition of aquaporin-1 channels with mercuric chloride during acute PD demonstrated a significant decrease in the volume of osmotically filtered fluid from the tissue. Computer simulations of peritoneal transport were compatible with a 66% inhibition of water flow through the aquaporins. These findings were corroborated by Yang and colleagues (118) in transgenic aquaporin-1 knockout mice. When these animals were dialyzed with a hypertonic solution, the filtration in the aquaporin-1 knockout mice was ~40% of that of normal mice. The absorption of isosmolar fluid was unchanged by removal of aquaporin-1. These experiments verified the early experiments with mercurials (6) and demonstrated the importance of aquaporin-1 channels in subperitoneal capillaries to the process of peritoneal ultrafiltration. While this clearly demonstrates that elimination of aquaporin-1 results in a marked decrease in water removal from the body, the correlation of the loss of osmotic filtration capability in a patient with a functional change in endothelial aquaporins is controversial. A recent study in 40 PD patients demonstrated that the volume of solute-free filtrate during dialysis was much lower in 11 patients who had failure of osmotic fluid transfer during hypertonic dialysis (often termed “ultrafiltration failure”) (95). While these results implied loss of functional “water-only aquaporins,” the case of a 67-yr-old man on PD for 11 years in whom ultrafiltration failure occurred was associated with an apparently normal expression of aquaporin-1 (40).

Influence of Nitric Oxide Synthase During Acute Inflammation

During normal dialysis with a hypertonic solution, the sodium concentration is often observed to decrease in the first few minutes (57). This has been shown to be due to the presence of aquaporins, which allow only water to transport across the endothelium without an accompanying solute. The nature of the capillary barrier in the vicinity of the aquaporin is therefore one of a perfect semipermeable membrane, which allows the solvent to pass through the channel without passage of the solute. The lowering of the sodium concentration has been observed to disappear with acute peritonitis (6, 106). Analogously, in a rat model of acute peritonitis, the loss of ultrafiltration and sodium sieving has been observed during the acute phase of peritoneal inflammation (12). The peritonitis induced a major increase in total nitric oxide (NO) synthase (NOS) isoforms of both the inducible and endothelial (e)NOS type. Aquaporin-1 expression was unchanged from normal in rats with peritonitis. The loss of sodium sieving during peritonitis was therefore not due to a loss of aquaporin-1 but likely due to changes in solute vascular permeability induced by NO. Subsequently, this group demonstrated in their rat model that inhibition of NOS during peritonitis significantly improved the observed ultrafiltration and corrected the permeability changes observed during peritonitis (24). Further experiments with eNOS knockout mice demonstrated a significant reversal of permeability, osmotic filtration, and structural changes in acute peritonitis (71). While there are still questions about the interaction of NO and the permeability of capillaries to water and solutes, the discovery of water channels and their influence on osmotic water transport and solute transport has added significantly to our understanding of peritoneal osmotic transport.

Nature of the Endothelial Barrier

While the existence of water-only channels in the capillary endothelium is now well established, the concept of solute pores is being replaced by one of a pore-matrix, in which a glyocalyx, 0.4–0.5 μm thick (38, 104), lines the space between cells and determines permeability. The glyocalyx was not observed in early histological preparations because the layer was destroyed in the fixation process. This glyocalyx is present on the luminal side of the endothelial cells and within the interendothelial space and restricts the passage of molecules across the endothelium according to molecular size, charge, and structure (105). Hyaluronan, a major component in the interstitium, has been found to make up a major part of the glyocalyx endothelial barrier; treatment with hyaluronidase results in a marked increase in permeability of 70- and 145-kDa FITC-dextrans (48). Cytokines, such as tumor necrosis factor-α (TNF-α), cause modification of the glyocalyx and result in increased permeability to macromolecules, even when TNF-α-enhanced white cell adhesion was inhibited (49). Modifications in the glyocalyx brought about an ischemia-reperfusion increase in microvascular permeability, but this can be reversed by pretreatment with adenosine (76, 77) and may be responsive to the NOS inhibitor Nω-nitro-l-arginine methyl ester (93). In mathematical terms, either the pore theory (85, 87) or the pore-matrix theory (38, 39) can be used to model transendothelial transport in tissue such as the subperitoneum. However, the fundamental findings of an endothelial coating that is responsive to cytokines and vascular mediators make the latter concept more realistic and amenable to current scientific findings.

EFFECTS OF INTRAPERITONEAL PRESSURE ON THE TRANSPORT BARRIER

Significance of the Interstitium

While many mathematical and conceptional models of the peritoneal barrier neglect the interstitium as a major player in the peritoneal barrier, transport between the blood and the peritoneal cavity is significantly affected by its presence. The importance of the interstitium is illustrated by studies of absorption of inert gases from the peritoneal cavity of pigs...
(11). If the interstitium were an insignificant barrier, as it is in the lungs, then the absorption of gases from the peritoneal cavity would occur at the same rate and be equivalent to the blood flow in the surrounding tissue. However, the study with 6 inert gases demonstrated a 100-fold range of peritoneal clearances, which correlated with the log of aqueous diffusivity of the gases. These results imply that the tissue interstitium limits the transport of these inert gases and further imply that all larger solutes are limited in their transfer by the tissue space that separates therapeutic solutions in the cavity from blood vessels in the subperitoneum (25, 27).

**Interstitial Matrix**

Recent findings have demonstrated that the interstitial matrix, which occupies the space between the cells, is a very ordered structure of the tissue (58). Collagen fibers are linked to interstitial cells and possibly to parenchymal cells through adhesion molecules such as β1-integrins (81, 92). Wrapped around the collagen fibers and perhaps attached to them are long molecules of hyaluronan, to which are bound proteoglycans that interact with surrounding cells (58, 91). Hyaluronan molecules are highly negatively charged, imbibe large amounts of water, and restrict the passage of negatively charged proteins (36). The proteins are restricted to ~50% of interstitial space (111, 112). This means, for example, that in muscle interstitial space, which is 12–20% of the total tissue space, only ~6–10% of that space will be for transport of large-molecular-mass proteins.

Transport coefficients depend on properties of the interstitium. The interstitial portion of the peritoneal barrier undergoes physiological responses that result in changes in the transport properties of the barrier. The processes of diffusion and convection in tissue are dependent on not only the forces of concentration and pressure that drive each transport but also the properties of the tissue. Diffusivity is typically modeled as (94)

\[
D_{\text{eff}i} = \frac{D_i \theta_i}{\tau_i}
\]

(1)

where \(D_{\text{eff}i}\) is the effective solute diffusivity of the tissue, \(D_i\) is the diffusivity of solute \(i\) in the space available to the solute, \(\theta_i\) is the fraction of tissue available to solute \(i\) (for water-soluble solutes, this would be the interstitial space), and \(\tau_i\) is the tortuosity of the space to solute \(i\) (equal to the ratio of the true path length between 2 points to the linear distance between the 2 points, ~2.5 for muscle) (94). For water-soluble substances, both \(\theta_i\) and \(\tau_i\) are dependent on the magnitude of interstitial pressure and the makeup of the interstitial matrix (59).

With regard to convection, the hydraulic conductivity \((K)\) is dependent on \(\theta_i\) and the concentrations of collagen \((C_c)\) and glycosaminoglycans \((C_{\text{gag}})\), which include hyaluronan and proteoglycans (59)

\[
K = K(\theta_i, C_c, C_{\text{gag}})
\]

(2)

From Eqs. 1 and 2, we can conclude that changes in the makeup of the interstitial matrix or the fraction of tissue that is the interstitium will alter \(D_{\text{eff}i}\) and \(K\) and result in changes in both diffusion and convection through the tissue space.

**Barrier Alterations due to Elevated IP Pressure**

During therapeutic procedures in which 2–3 liters of solution are infused into the cavity, significant changes occur in the tissue subjected to hydrostatic pressure of the fluid. Depending on the position and size of the patient and the flexibility of the abdominal wall (42, 100), ip pressures are typically raised from 0 to 2–20 mmHg. Relatively low pressures (2–6 mmHg) in the cavity result in marked increases in fluid flow into the abdominal wall. Studies in rats have demonstrated that at these pressures, the extracellular space doubles (120, 121) and the hydraulic conductivity can increase four to five times (119). Recent sampling of the rat subperitoneal interstitial fluid after 4 h of dialysis demonstrated a 50% reduction in the colloid osmotic pressure (89), which correlates well with the doubling of the interstitial volume during dialysis (120). Additional studies demonstrated that a constant ip pressure of 6 mmHg results in a translocation of hyaluronan from the peritoneal side of the abdominal wall toward the skin side (120). This was hypothesized to be responsible for the marked increase in hydraulic conductivity through this tissue. Subsequent experiments in rats showed that the acute elimination of hyaluronan in abdominal wall tissue with the application of hyaluronidase resulted in a marked increase in hydraulic conductivity (31). The expansion of the interstitial space and the changes in the interstitial matrix in the abdominal wall will result in marked increases in diffusion and convection. On the other hand, tissues that are surrounded by the peritoneal fluid, such as the gastrointestinal tract, may not experience the same changes and will likely have different transport coefficients and rates of transfer. Other researchers using a different experimental model have not demonstrated marked changes in overall dialytic capacity in the rat, subsequent to treatment with ip hyaluronidase (7).

**RATE OF MASS TRANSFER AND CONTACT SURFACE AREA**

**Simple Model of Mass Transfer**

The area of contact between the peritoneum and a therapeutic solution is an important variable in the rate of transfer. Figure 2 illustrates a conceptual model of peritoneal exchange that condenses the complex barrier of Fig. 1 into a single “barrier” structure, often termed the peritoneal membrane by clinicians. This concept permits a simpler mathematical approach toward the transfer of a small solute between the plasma and the ip solution, which is illustrated in the following equation

\[
\text{Rate of mass transfer} = \frac{d(V_{pc}C_{pc})}{dt} = \sum_i MTC_i \cdot A_i (C_{\text{plasma}} - C_{pc})
\]

(3)

where \(C_{pc}\) and \(C_{\text{plasma}}\) are solute concentrations in the peritoneal cavity and the plasma, respectively; \(V_{pc}\) is the volume in the peritoneal cavity and is assumed to remain constant; and the terms \(MTC_i\) and \(A_i\) are the mass transfer coefficient and the fluid contact surface area of each tissue element \(i\) of the transport system, respectively. The peritoneal volume is assumed to be a well-mixed compartment with uniform concentration. The total resistance of each tissue element has been
lumped into the term MTC, which is an engineering term equivalent to the overall permeability of the ith barrier of the transport system. The simpler model of Fig. 2 can be related mathematically to the concept of Fig. 1 by the following equation, where $D_{eff,i}$ represents the effective solute tissue diffusivity and $(pa)$, is equivalent to the capillary permeability-area density product (19).

$$\text{MTC}_i = \sqrt{D_{eff,i}(pa)}.$$

The term $p$ lumps together the permeabilities of all structures responsible for transendothelial transport of the solute. Equations 3 and 4 assume that there is no blood flow limitation at the tissue level. Equation 4 demonstrates that the MTC is proportional to the square root of the tissue diffusivity or the capillary permeability or the perfused capillary area/unit volume of tissue; therefore, one of these or the combined product of all the terms would have to increase four times to affect a doubling of the MTC. While Eq. 4 is still awaiting experimental verification, a recent biopsy study in dialysis patients clearly demonstrated an association between the density of stained vessels and solute transport rates (73).

**Increasing Solution Contact Area Improves Mass Transfer**

For each tissue in the system, Eq. 3 demonstrates that the rate of mass transfer is directly proportional to the peritoneal surface contact area of the tissue ($A_i$). There are some who argue that the only important area is the vascular surface. However, the vascular area does not come into play if the overlying surface is not in contact with the therapeutic fluid (see Fig. 1). The effect of the contact area was assessed by Keshaviah and colleagues (55), who determined the mass-transfer coefficient (MTAC) in 10 patients who were dialyzed with different solution volumes varying from ~0.5 liter up to ~3.5 liters. Since the surface contact area could not be easily separated from the mass transfer coefficient, the MTAC ($= \text{MTC} \cdot A$) was calculated in Eq. 3. This study demonstrated that there was a linear rise in the MTAC from 0.5 liter up to ~3 liters of dialysate, with subsequent flattening of the curve. The authors pointed to the fact that the solutions themselves should not change the intrinsic permeability of the peritoneum and that a greater surface contact area was likely present due to the larger volume of the solution. Subsequent studies in rodents and in patients have confirmed this. Large solution volumes in rodents have demonstrated not only a direct relationship between surface area and the MTAC but have also demonstrated that a larger surface area promotes the loss of protein from the underlying tissues to the dialysis fluid (32, 33). These studies in rodents were confirmed in patients by Chagnac and colleagues (9), who dialyzed PD patients with a contrast agent in the dialysate. They employed computed tomography with a radiographic contrast agent injected ip and calculated the surface contact area to be ~0.55 m², about one-third of anatomic peritoneal area that is equivalent to the body surface area (107). They demonstrated that an increase in ip volume from 2 to 3 liters of solution resulted in an 18% increase in contact area and an ~25% increment in the MTAC (8). The correlation of the increase in area with the increase in MTAC demonstrates the effect of a larger volume resulting in an increase in the contact area on total transperitoneal transport. The clinical importance of this finding is that one can maximize peritoneal transfer by increasing the contact surface area. However, before the use of surface-active agents to access more of the peritoneal surface, the long-term effects of the loss of protein and potential toxicity need to be considered (33).

**Importance of Contact Area to Chemotherapy**

The issue of contact surface area is actually more important to the patient undergoing ip chemotherapy. Since metastasis from colorectal or ovarian carcinoma can appear anywhere on the peritoneum, the entire peritoneum is the target, and the residence time of the solution against a particular surface determines the dose of antineoplastic agent delivered to the tissue. To maximize contact of the peritoneum with the solution, a substance such as diacetyl-sodium sulfosuccinate (DSS) has been shown to increase the contact surface area and to proportionally increase the rate of mass transfer (32, 33, 75). Unfortunately, DSS, which is commonly taken orally as a stool softener, is toxic when administered ip (33). While a large volume of ip solution covers only ~30% of the total surface area, over a 24-h period the entire surface area will come in contact with the solution at a particular time (33). However, there is no guarantee of the actual time of exposure of the particular surface to the solution. Therefore, the use of a nontoxic additive with the characteristics of DSS would be useful in chemotherapy.

An alternative technique to the use of a surface-active agent is to utilize a solution that maintains a constant peritoneal volume for up to 24 h or more to maximize the probability of contact with all targeted surfaces. Compared with a saline solution, which is fully absorbed within 12–24 h with a constantly diminishing contact area, a 4.0% icodextrin (~20-kDa starch) solution was shown to maintain the peritoneal volume constant for 48 h and to result in only 50% volume loss at 96 h (50). Either the 4.0 (50, 51) or 7.5% (54) icodextrin solution has been shown to be effective as a drug carrier to...
Inflammation and Peritoneal Transport

Functional Transport Changes with Chronic Dialysis

While most patients on PD maintain stable transport function for over 5–6 years, investigators have observed changes in a portion of the population (45). Database studies (16) have demonstrated that patients who transport small solutes rapidly on standardized tests such as the Peritoneal Equilibration Test (PET; high MTAC or high dialysate/plasma concentration for glucose or creatinine) drop out early from treatment. Observations in 22 patients continuously treated with glucose solutions for over 5 years demonstrated 13 with stable function, whereas 9 patients had a sustained increase in solute transport and an earlier loss of residual renal function; peritonitis rates were similar in both groups (17). Studies of comorbidity have demonstrated a quantitative effect on mortality that is independent of age, residual renal function, and transport status in PD patients; comorbidity is associated with increased solute transport on the start of PD (18). However, studies in anuric patients on automated PD (5) have shown no correlation of initial solute transport with survival; the initial fluid removal rate was associated with survival. None of these relatively small studies is conclusive, but it would appear that patients who have increased comorbidity and solute transport at initiation of dialysis experience changes in their dialysis technique and a higher mortality.

Cellular Integration Within the Peritoneal Barrier

The peritoneal barrier is more accurately represented by the integrated structure of the mesothelial cells, interstitium, and microcirculation in the tissues that surround the cavity. Figure 3 demonstrates the parts of the barrier that interact with each other through specific signaling mechanisms. Clinical observation (97) has also indicated that systemic inflammation in PD patients is correlated with a lower residual (<2 ml/min), and that both basic fibroblast growth factor (bFGF) and VEGF are lower in patients with lower rates of solute transport at dialysis initiation. Various hypotheses have been made concerning the cytokine cascade, which results from the interaction of resident macrophages and mesothelial cells with bacteria, foreign bodies, or nonbiocompatible solutions (99). Indeed, the installation of any commercially available sterile dialysis solution potentially can set off this cascade and result in marked changes in the subperitoneal tissue space. These processes typically involve cellular infiltrates, angiogenesis, and progressive fibrosis. Critical to an understanding of the peritoneal barrier is this integration of cellular and matrix constituents.

Dialysis Solutions are not Biocompatible

While the inflammation due to bacterial peritonitis has been shown in many studies to cause increases in inflammatory mediators such as TNF-α, interleukins, prostaglandins, and growth factors (3, 98, 123), which result in major changes in peritoneal transport and in the peritoneal barrier (37, 80, 103), recent research has shown that the solutions utilized in PD have properties that are not compatible with the biological structure of the peritoneal barrier (53, 66, 115). Conventional PD solutions typically have a low pH, a high lactate concentration, and very high concentrations of glucose. These properties result in a sustained and generalized vasodilation in the visceral subperitoneum that lasts up to 60 min (122). All of these factors have been shown to change mesothelial cells growing in vitro (43, 44, 110, 116). However, in vivo studies have disputed low pH as a major factor since it is buffered to 7.4 very rapidly by the body (53). Bicarbonate is the natural buffer in the mammalian body, and lactate may have a negative impact on the peritoneum. The high concentrations of glucose essentially create a state of diabetes in the peritoneum, and this has been shown to stimulate inflammatory cell function and interstitial cells that underlie the peritoneum and accelerate inflammatory changes (96). Most recent research has focused on glucose degradation products, which are a result of the heat sterilization process utilized in conventional PD solutions (114).

Chronic Changes in the Barrier

Changes in the barrier likely begin during the first exposure of the mesothelium to bioincompatible solutions. In an elegant study of cells and tissue recovered from noninfected PD patients, Yanez-Mo and colleagues (117) observed a transition of mesothelial cells from an epithelial phenotype to a fibroblastic, mesenchymal one. Fibroblasts have been increasingly seen to play a major role in the observed changes (74) and produce prostaglandins in response to stimulation (52). The fibrotic effect of TGF-β1 secretion by mesothelial cells in response to exposure to dialysis solutions (43, 44) was clearly demonstrated in the in vivo model in which the mesothelium was transformed to hyperssecrete TGF-β1 (63), which, in turn, stimulated secretion of tissue inhibitor of metalloproteinase-1.
(61). Selective depletion of fibroblasts in a transgenic model has been shown to preserve morphology and function of the mouse peritoneal barrier (74).

The state of inflammation in the peritoneal cavity and the resulting slow fibrotic changes over time have been illustrated by a study by Williams et al. (113). The authors examined biopsy samples from an array of patients, including those who had normal renal function, those undergoing kidney transplant who had been on PD, patients who had been treated only with hemodialysis, and patients with renal disease who had never been dialedized. The results showed clear indications of fibrosis and vasculopathy in the submesothelium; the changes were present even in patients who had not yet reached end-stage renal disease. Patients who were on hemodialysis clearly had a different peritoneum than patients who had normal renal function. The degree of fibrosis and thickness of the overlying structure above the peritoneum and the degree of vasculopathy were directly proportional to the number of years on PD. Unfortunately, the accompanying data for correlation of the peritoneal transport characteristics to structural changes were not available.

In a smaller study of 41 PD patients, Plum and colleagues (78) investigated functional transport changes with histological changes in the peritoneum. Peritoneal fibrosis correlated with the total glucose load over time, and patients with a rapid rate of small-solute transfer on PET had significantly thicker submesothelial fibrous layers. Patients on automatic PD, who are often exposed to the higher amounts of glucose, tended to have more fibrosis and an increased number of angiogenic vessels. This study confirmed the findings by Williams et al. (113) and linked the alterations in peritoneal histology with differences in function.

**Hypothesis on Reactive Carbonyl Species**

Miyata and colleagues (66) have proposed a hypothetical chain of mechanisms that leads from exposure in the uremic state to the transport dysfunction after years on PD. Reactive carbonyl species together with hyperosmolality/glucose in uremia promote the formation of AGEs/ALEs, which stimulate peritoneal cells to secrete NOS, VEGF, FGF2, and TGF-β. The NOS-induced vasodilation and VEGF/FGF2-induced angiogenesis result in increased perfused vascular area in the subperitoneum, which, in turn, accelerates the transport of solutes and water. Transendothelial permeability may be altered by changes induced in the glycocalyx by the release of these cytokines and vasoactive intermediates (49, 76, 77). bFGF2 and TGF-β stimulate mesothelial cells and fibroblasts to increase production of hyaluronan and collagen, leading to fibrosis and ultrafiltration failure (13, 43, 44, 61, 63), and increase secretion of tissue inhibitor of metalloproteinase-1, which downregulates metalloproteinase and helps to perpetuate the fibrotic process (61).

**Solutions for Prevention of Barrier Alterations**

Work is ongoing by a number of different groups on newer solutions, which have low quantities of these reactive carbonyl species. The recent “Balance” study (114) demonstrated a benefit after 3 mo with a solution that was adjusted to normal pH and had a very low content of glucose degradation products. With the use of the new solution, the levels of cancer antigen-125 increased, signifying either an increased number of mesothelial cells or increased production of the antigen by those cells. There were decreased levels of N-carboxy methyllysine and decreased levels of hyaluronan, indicating less oxidative stress and inflammation. The differences in transport were relatively minor. It is clear that the effects of these solutions must be demonstrated over much longer periods of time. While these solutions are promising, they are significantly more expensive than the current solutions and will be challenging for payors to support their use.

**CHALLENGES AHEAD**

**Mechanism of Transperitoneal Osmosis**

The true mechanism of removal of fluid from the subperitoneum during dialysis has not been fully elucidated. Clinicians often think of the peritoneal membrane as being a large capillary (with pores) that makes up a barrier for a hypertonic glucose solution and results in osmosis from the blood to the peritoneal cavity. However, the surface area of the vasculature adjacent to the peritoneum or within the peritoneum is relatively minor and likely cannot support the quantity of osmotic filtration that occurs. It has also been shown that the peritoneum or layer of mesothelial cells does not present an osmotic barrier (25). For vessels within the tissue that are >50 μm, it is not clear how water extracted from these blood vessels moves into the peritoneal cavity. Since the typical glucose concentration profile within the subperitoneal tissue decreases to ~10% of its value at the surface over the first 500 μm underneath the peritoneum (29), there is a diminishing osmotic effect as one moves more deeply into the tissue. It is not clear how the water, removed through osmosis from subperitoneal vessels to the interstitial space, flows into the cavity. The
microanatomy of the transport system needs to be better defined with respect to perfused blood vessels in the subperitoneum.

Inflammation and Therapeutic Solutions

Research concerned with inflammation and alterations in the peritoneal barrier continues, with parallel work on alternative solutions that will minimize the changes in the mesothelium and underlying tissue (108, 115). Integration of the many elements in the complex system of cells and matrix molecules that make up the transport barrier is essential to unlocking the secrets of this portal for therapeutic intervention. Angiogenesis alone or with a moderate amount of fibrosis cannot adequately explain all of the changes observed in long-term PD. Are all of the new vessels that are induced by VEGF functionally perfused? Are these new vessels altered in their permeability characteristics, particularly with respect to aquaporins? How does a relatively thick layer of fibrosis increase the rate of solute and fluid transport across the peritoneal barrier? Are these new vessels altered in their permeability?

Recent work with transplantation of transplanted mesothelial cells has opened the possibility of early discovery of mechanistic events in peritoneal inflammation, fibrosis, and vasculopathy and will lead to new methods to alter the new vessels that are induced by VEGF functionally perfused and enhanced expression of endothelial nitric oxide synthase in human peritoneum exposed to long-term peritoneal dialysis. J Am Soc Nephrol 11: 717–728, 2000.


Invited Review

TRANSPERITONEAL TRANSPORT

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