Molecular Mechanisms of Peritoneal Dialysis–Induced Microvascular Vasodilation

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Peritoneal dialysis (PD) solutions dilate microvessels by undefined mechanisms. This vasodilation directly affects ultrafiltration and solute exchange during a PD dwell and is thought to account for the variable mass transfer area coefficient for small solutes during a glucose-based hypertonic dwell. We hypothesized that PD-mediated vasodilation occurs by endothelium-dependent mechanisms that involve endothelium energy-dependent K^+ channels (K_{ATP}), adenosine A1 receptor activation, and NO release.

We used intravital videomicroscopy to study 3 levels of microvessels (A1 inflow arterioles about 100 μm diameter to pre-capillary A3 arterioles 10 – 15 μm diameter) in the terminal ileum of anesthetized rats under control conditions in vivo in a tissue bath. Ileum was bathed with hypertonic mannitol or 2.5% glucose-based PD solution (Delflex: Fresenius Medical Care North America, Waltham, MA, U.S.A.) with or without topical application of individual or combined specific inhibitors of the endothelium-dependent dilation pathways: NO (L-NMMA), prostaglandin I2 (mefenamic acid), endothelium hyperpolarizing factor (glibenclamide), and adenosine A1 receptor antagonist (DPCPX).

The mannitol and PD solutions induced rapid and sustained peritoneal vasodilation whose magnitude depended on microvascular level and osmotic solute. Combined inhibition of endothelium-dependent dilation pathways completely abolished the mannitol-induced hyperosmolality-mediated dilation at all microvascular levels, but selectively eliminated the PD solution–mediated A3 dilation. The K_{ATP} and adenosine receptor antagonists, individually or combined, remarkably attenuated dilation in the smaller pre-capillary arterioles; NO inhibition, alone or combined with K_{ATP} and adenosine receptor antagonists, eliminated the PD solution–induced dilation. The cyclooxygenase pathway is not involved in PD-induced dilation.

Solutions for PD dilate the visceral peritoneal microvasculature by endothelium-dependent mechanisms, primarily the NO pathway. Adenosine receptor–activated NO release and K_{ATP} channel-mediated endothelium hyperpolarization significantly contribute to vasodilation in the smaller peritoneal pre-capillary arterioles.

Key words
Hyperosmolality, vascular reactivity, adenosine receptors, intestinal microcirculation, vasodilation

Introduction

The effective capillary surface area available for exchange during peritoneal dialysis (PD) determines mass solute transport and ultrafiltration. In acute PD, this surface area is functionally recruited by the pre- to post-capillary resistance ratio that modulates the number of perfused capillaries. Changes in the resistance ratio are dictated by the prevailing vasoactivity (dilation or constriction) of those microvessels.

Numerous publications have shown that PD solutions dilate both the visceral and the parietal microvascular beds (1–5). The general consensus is that hyperosmolality, low pH, and glucose degradation products (GDPs) are the components that give PD solutions their vasoactive properties; however, ranking the vasoactive potencies of those components remains controversial (2–5). In addition, no studies are specifically investigating the molecular mechanisms of PD solution–induced vasoactivity.

White and colleagues (6,7) demonstrated that glucose-based PD solutions reverse a NO-inhibition-mediated vasoconstriction of the mesenteric arterioles and prevent venular leukocyte adhesion by a NO synthase (NOS)–independent pathway. Steenburgen and Bolen (8) showed that hyperosmolar sodium solutions

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perfused into the intestinal lymph produce vasodilation of submucosal arterioles through a mechanism partially mediated by a hyperosmolality-induced release of NO. Similarly, intravenous infusion of hypertonic galactose or mannitol solutions in pigs increased baseline hepatic blood flow, presumably by a mechanism attributed to osmotic stress (9). Other *in vitro* studies have found that suffusion of isolated, cannulated, and pressurized skeletal muscle arterioles with solutions made hypertonic by the addition of various osmotic solutes produces equal arteriolar dilation. That hyperosmolality-driven dilation was caused by endothelium-mediated mechanisms independent of the NOS or cyclooxygenase pathways, and was abolished by glibenclamide, an ATP-sensitive potassium channel (K_{ATP}) inhibitor (10). Similar results were obtained in coronary arterioles perfused *ex vivo* with either hypertonic glucose or sucrose solutions. In those vessels, only glibenclamide caused attenuation of the hypertonic solution–mediated vascular relaxation; in contrast, the inhibition of other potassium channels had no effect on the hyperosmolality-induced vascular reactivity (11,12).

Hein and colleagues (13,14) examined the direct effect of lactate or adenosine on retinal vascular tone. Lactate produces its microvascular effects predominantly through NO release that activates guanylyl cyclase, with subsequent opening of K_{ATP} for vasodilation. Adenosine dilates the microvasculature by activation of adenosine receptor subtypes and subsequent secondary release of NO and opening of K_{ATP}. This adenosine-mediated mechanism was confirmed in our recent intravital microscopy study of the visceral peritoneal microvasculature (5). In that study, we demonstrated that topical application of an isotonic glucose solution to the small intestine produces time-dependent vasodilation, preferentially of the smaller pre-mucosal precapillary arterioles (5). That vasodilation was not observed when the intestine was topically exposed to isotonic mannitol solution. Subsequently, we showed that this glucose-specific preferential vasodilation can be completely abolished by specific inhibition of the adenosine A1 receptor subtypes (5).

Collectively, our data and the cited literature suggest that the vasoactivity produced by PD solutions must occur by endothelium-dependent mechanisms, which are largely triggered by hyperosmolality. We therefore hypothesized that acute exposure of the visceral peritoneal microvasculature to glucose-based PD solutions produces vasodilation of the visceral peritoneal microvasculature by endothelium-dependent mechanisms that involve at least two signaling pathways:

- Hyperpolarization secondary to hyperosmolality-induced activation of a glibenclamide-sensitive K channel (K^{+}_{ATP})
- NO release secondary to energy-dependent intracellular glucose uptake and activation of adenosine receptors

Methods

General animal care and surgery

All animal care and experimental procedures conformed to the *Principles of Laboratory Animal Care* from the National Society for Medical Research and the *Guide for the Care and Use of Laboratory Animals* from the U.S. National Academy of Sciences as published by the National Institutes of Health (revised 1987) and were approved in advance by the institutional animal care and use committees of Hamad Medical Corporation and Qatar University.

Experiments were performed on rats (200 – 210 g) that had fasted overnight. Anesthesia was induced with intraperitoneal pentobarbital (60 mg/kg) and maintained with supplemental subcutaneous injections as needed to maintain a surgical plane of anesthesia. A rectal probe and servo-controlled heating pad were used to maintain body temperature at 37 ± 0.5°C. Surgery was carried out after loss of the blink and withdrawal reflexes. Tracheostomy was performed to reduce airway resistance, and the animals were allowed to spontaneously breathe room air. The right femoral artery was cannulated with a PE-50 catheter to provide continuous monitoring and online recording of arterial blood pressure.

Bathing solutions

All chemicals were purchased from the Sigma Chemical Company (St. Louis, MO, U.S.A.). The intestinal segment was continuously bathed during tissue preparation and equilibration with a non-vasoactive (glucose-free) modified Krebs solution (solution A) that contained 6.92 g/L sodium chloride, 0.44 g/L potassium chloride, 0.37 g/L calcium chloride, and 2.1 g/L sodium bicarbonate at a pH of

7.4 and an osmolality of 285 mOsm/L. To separate hyperosmolality from other potential vasoactive components of conventional PD solutions, a hypertonic 5% mannitol solution was studied. Mannitol was added to the Krebs solution to yield a final osmolality of 560 mOsm/L and a pH of 7.4. A conventional 2.25% dextrose-based heat-sterilized dialysis solution (Delflex: Fresenius Medical Care North America, Waltham, MA, U.S.A.) that contains 5.67 g/L sodium chloride, 3.92 g/L sodium lactate, 0.257 g/L calcium chloride, and 0.152 g/L magnesium chloride at a pH of 5.5 and an osmolality of 398 mOsm/L served as a second test solution. All solutions were filter-sterilized and warmed to 37°C before use.

The two hypertonic solutions with differing compositions and levels of osmolality were used in the present study by design. The hypertonic mannitol solution addressed two important issues:

- Isolation of hyperosmolality from other potential vasoactive components of PD solution (pH, lactate, GDPs), because hyperosmolality is the major vasoactive component shared by conventional and new PD solutions (15)
- Total exclusion of the solute (which is metabolically inert) from cells, thereby allowing for exclusion of the intracellular solute uptake signaling pathway, which is then unique for glucose (5)

Drugs

All chemicals were purchased from the Sigma Chemical Company unless otherwise indicated. The chemicals included K_{ATP} inhibitor (glibenclamide, 20 µmol/L); adenosine A1 receptor antagonist (DPCPX, 200 nmol/L); NOS inhibitor (L-NMMA, 100 µmol/L); cyclooxygenase inhibitor (mefenamic acid, 40 µmol/L).

The effective concentration of each of the specific antagonists used in the present study was determined from various sources, including the manufacturer, published data, and our previous studies. The DPCPX concentration used here and in our earlier studies represented at least 3 times the 50% effective inhibitory concentration for the adenosine A1 receptor as determined by information provided by the manufacturer. Acetylcholine hydrobromide and sodium nitroprusside were used to evaluate the integrity of the vascular endothelium and to determine the maximal endothelium-independent response at the end of each experimental protocol. In the tissue bath, isoproterenol was topically applied to retard peristalsis without unwanted effects on the contractile machinery of the vascular smooth muscle (16).

Experimental procedure

The peritoneal cavity was exposed through a midline abdominal incision (1.5 cm), and a 2-cm to 3-cm segment of the terminal ileum was gently withdrawn from the peritoneal cavity with its neurovascular supply intact. Using electrocautery, the segment was opened along its antimesenteric border. The enteric contents were gently removed from the mucosal surface, and the animal was positioned on a specially-designed polyurethane board. Using 4-0 silk sutures, the opened ileum was suspended, serosal side up, over a viewing port in a tissue bath. The non-vasoactive bathing solution was maintained at 37°C and was bubbled with nitrogen and carbon dioxide to maintain a pH of 7.4. Isoproterenol was added to the bathing solution in a very dilute concentration (0.01 µg/mL) to retard peristalsis. This dose of isoproterenol was orders of magnitude below the threshold at which vascular smooth muscle tone is altered (16).

The animal board was positioned on the stage of a trinocular microscope for direct *in vivo* intravital microscopy. Microvascular images were transmitted through the microscope to a photodiode array in an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M University, College Station, TX, U.S.A.) to measure centerline red blood cell velocity for the calculation of blood flow (in nanoliters per second) in the intestinal A1 inflow arteriole (FA1):

$$FA1 = (V / 1.6) (\pi r^2) (0.001),$$

in which V is the centerline red blood cell velocity (in millimeters per second), r is the A1 arteriole radius (in micrometers), 1.6 is a correction factor that converts centerline velocity to average cross-sectional velocity, and 0.001 is a conversion factor to express flow in nanoliters per second (17). This equation assumes a parabolic flow velocity and a circular conduit. Studies have identified 1.58 – 1.60 as the ideal correction factor for a wide range of microvessels (17).

The microvascular images were then transmitted to a digital camera (Model K-P D51: Hitachi Denshi,

Tokyo, Japan), which provided 30 images per second to a computer. The digitalized microvascular images were stored as streamline video in the computer hard drive for later measurement of microvascular diameters with calipers.

Criteria for an acceptable microvascular preparation during intravital microscopy included a baseline mean arterial pressure greater than 90 mmHg, a centerline red blood cell velocity in a first-order arteriole of more than 20 mm/s, and active vasomotion in the intestinal pre-mucosal A3 arterioles. We used a standard nomenclature for the intestinal microvessels (Figure 1), as originally described by Bohlen and Gore (16). Briefly, first-order arterioles (A1) arise from a mesenteric arcade artery to traverse the mesenteric border of the bowel wall and then penetrate through the muscle layers to the submucosal layer. In the submucosal layer, second-order arterioles (A2) arise from the first-order arterioles to run along the longitudinal axis of the bowel. First and second-order venules (V1, V2) parallel the first and second-order arterioles. The A2 arterioles give rise to branching second-order arcade vessels as well as to smaller third-order arterioles (A3). The A3 vessels branching at a right angle from the A2 arterioles are termed proximal pre-mucosal A3 arterioles; they eventually form distal A3 (dA3) arterioles, which terminate in the mucosa as central villus arterioles.

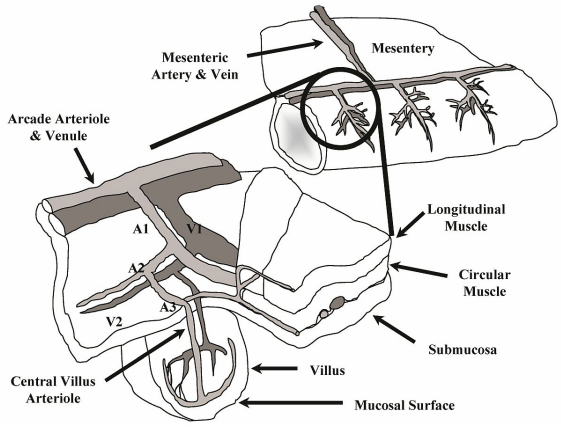

FIGURE 1 Anatomy of the intestinal (terminal ileum) microcirculation of the visceral peritoneum. A1 = first-order inflow arteriole; V1 = first-order venule; A2 = branching second-order arteriole; V2 = second-order venule; A3 = pre-mucosal third-order arteriole.

Experimental protocol and measurements

Figure 2 depicts the timeline of the experimental protocol. After surgical preparation, 40 minutes were allowed for the intestinal segment to equilibrate in the tissue bath. During that time, the segment was continuously bathed in the non-vasoactive Krebs solution. Blood pressure, heart rate, rectal and bath temperatures, and bath pH were continuously monitored (Digi-Med Signal Analyzers: Micro-Med, Louisville, KY, U.S.A.) and recorded every 5 minutes during the equilibration period.

Microvascular data consisted of A1, pA3, and dA3 arteriolar diameters and centerline red blood cell velocity in the inflow A1 arteriole. Baseline measurements were considered valid when the variability between two successive measurements within a 10-minute interval was less than 5%. After baseline measurements, the designated inhibitor was added topically to the non-vasoactive Krebs solution in the tissue bath for 10 minutes, and microvascular diameters and flow data were recorded. Next, the non-vasoactive Krebs solution was aspirated from the tissue bath, and the test solution with the designated inhibitor was instilled into the tissue bath according to protocol. Microvascular data points were first measured at 5 minutes after addition of the test solution and then at 20-minute intervals during the subsequent 60 minutes. At the conclusion of the experiment, one dose of acetylcholine hydrobromide (10^{-4} mol/L) was topically administered in the tissue bath, and microvascular data were measured at 1-minute intervals for 10 minutes to assess endothelial cell function and endothelium-dependent receptor-dependent vasodilation after acetylcholine hydrobromide. Finally, a single dose of sodium nitroprusside (10^{-4} mol/L) was topically administered in the tissue bath to assess the endothelium-independent maximal dilation capacity. It should be emphasized that no sequential addition of solutions or inhibitors was given in any anesthetized animal.

Experimental groups

The experimental groups were assigned to assess all 4 endothelium-dependent dilation pathways for individual antagonists or combinations thereof:

- NO pathway
- Endothelium-dependent hyperpolarizing factor (EDHF)

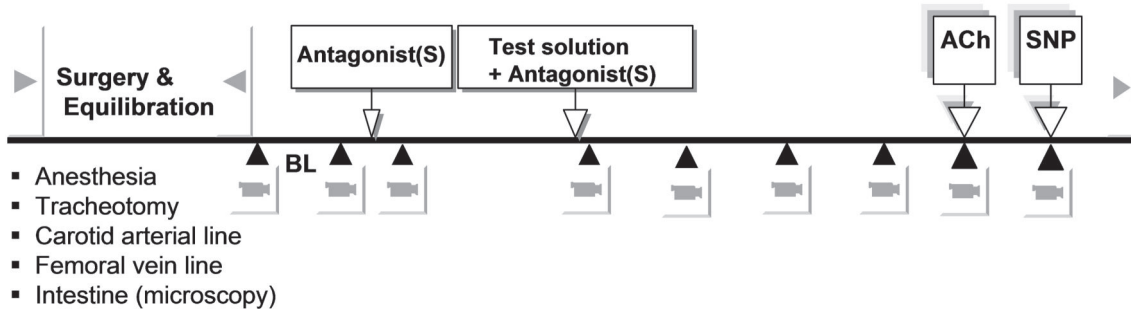

FIGURE 2 Timeline of the experimental protocol. Bold arrowheads mark the times at which microvascular images and hemodynamic data were acquired. BL = baseline measurements; ACh = acetylcholine; SNP = sodium nitroprusside.

- Cyclooxygenase pathway (PGI2)
- Adenosine signaling pathway (because the adenosine signaling pathway is unique for glucose, the specific adenosine A1 receptor antagonist DPCPX was not used individually in the mannitol series)

Animals were randomly assigned (7 per group) to a mannitol series (6 groups) or a PD solution series (6 groups):

- Group I: 5% mannitol
- Group II: 5% mannitol plus glibenclamide
- Group III: 5% mannitol plus glibenclamide plus DPCPX
- Group IV: 5% mannitol plus glibenclamide plus L-NMMA
- Group V: 5% mannitol plus glibenclamide plus DPCPX plus L-NMMA
- Group VI: 5% mannitol plus L-NMMA
- Group VII: PD solution
- Group VIII: PD solution plus glibenclamide
- Group IX: PD solution plus DPCPX
- Group X: PD solution plus glibenclamide plus DPCPX
- Group XI: PD solution plus glibenclamide plus DPCPX plus mefenamic acid
- Group XII: PD solution plus glibenclamide plus DPCPX plus L-NMMA
- Group XIII: PD solution plus L-NMMA

Data analysis and statistics

All data are presented as mean ± standard error of the mean. Microvascular diameter data are normalized and presented as percentage change from baseline. Percentage change of the vessel diameter from baseline was assessed by one-way analysis of variance, and the Dunnett multiple-range test was used to evaluate change from baseline in the same animal. Two-way analysis of variance and Bonferroni post-tests were used to assess the relationship between vascular reactivity and arteriolar type, and to evaluate the effects of inhibition of the endothelium-dependent dilation pathways and vascular response. Statistical significance was set *a priori* for the probability of a type I error at $p < 0.05$.

Results

Baseline hemodynamics were not significantly different in the experimental groups (Table I). Instillation of PD solution alone or with the inhibitors in the tissue bath did not cause any significant change in mean arterial pressure from baseline in any of the animals investigated in the present study (data not shown). Intestinal A1 blood flow increased significantly in concert with the vessel diameter change produced by the test solution or by the designated endothelium-dependent pathway inhibition (Figure 3). Venous outflow in the V1 venule mirrored the changes in the corresponding A1 arteriolar flow (data not shown).

Study I: mannitol series

In the first series of experiments, mannitol was used as an osmotic solute to isolate and assess the molecular mechanisms of hyperosmolality-induced vasodilation, without the potential confounding vascular effects from glucose, low pH, lactate, and GDPs. The hypertonic mannitol solution produced

instantaneous and sustained vasodilation of the large A1 arteriole [10.7% ± 1.2%, $n = 7$, $p < 0.001$ vs. baseline; Figure 4(A)]. Inhibition of NO had no effect on the A1 mannitol-induced dilation [Figure 4(A); $n = 7$, $p > 0.1$]; however, the dilation was partially attenuated by K_{ATP} inhibition (–36.4% ± 1.2%, $n = 7$, $p < 0.05$) and by combined adenosine A1 receptor and K_{ATP} inhibition (–30.4% ± 2.1%, $n = 7$, $p < 0.05$). In addition, mannitol-mediated dilation was markedly attenuated by combined K_{ATP} and NO inhibition (–72.2% ± 3.4%,

TABLE I Summary of baseline values

Group	*Pathways and antagonists*	*MAP (mmHg)*	*Diameter (μm)*			*A1 flow (nL/s)*
			A1	*pA3*	*dA3*	
I	5% Mannitol (no inhibitor)	115±4	76.1±5.6	15.0±0.5	10.1±0.4	578±11
II	K_{ATP} (glibenclamide)	113±5	79.2±5.0	13.8±0.7	9.8±0.3	585±12
III	K_{ATP} (glibenclamide) + AdoA1 (DPCPX)	114±4	89.0±2.6	13.4±0.7	10.1±0.7	666±6
IV	K_{ATP} (glibenclamide) + NO (L-NMMA)	110±5	85.1±3.9	14.7±0.5	11.0±0.6	587±10
V	K_{ATP} (glibenclamide) + A1 (DPCPX) + NO (L-NMMA)	114±6	98.2±2.0	13.3±0.5	9.0±0.4	763±6
VI	NO (L-NMMA)	117±3	75.6±6.5	14.3±0.7	10.8±0.5	463±10
VII	2.5% Delflex (no inhibitor)	117±4	85.1±4.9	14.2±0.8	9.7±0.5	595±9
VIII	K_{ATP} (glibenclamide)	112±5	88.1±2.8	15.1±0.5	10.9±0.3	587±4
IX	AdoA1 (DPCPX)	116±8	91.5±3.6	12.1±0.7	9.1±0.8	550±6
X	K_{ATP} (glibenclamide) + AdoA1 (DPCPX)	116±8	95.3±3.6	13.1±1.1	9.5±1.0	654±8
XI	K_{ATP} (glibenclamide) + AdoA1 (DPCPX) + cyclooxygenase (MA)	—	98.2±4.9	12.9±0.7	9.2±0.2	683±8
XII	K_{ATP} (glibenclamide) + A1 (DPCPX) + NO (L-NMMA)	117±2	90.3±4.1	13.2±0.7	9.4±0.7	552±5
XIII	NO (L-NMMA)	109±5	71.8±4.0	15.4±0.5	10.2±0.5	457±7.0

MAP = mean arterial pressure; A1 = inflow arteriole; pA3 = proximal pre-mucosal arteriole; dA3 = distal pre-mucosal arteriole; K_{ATP} = endothelium energy-dependent K+ channels; AdoA1 = adenosine A1 receptor activation; DPCPX = dipropylcyclopentylxanthine; L-NMMA = *NG*-monomethyl-L-arginine; MA = mefenamic acid.

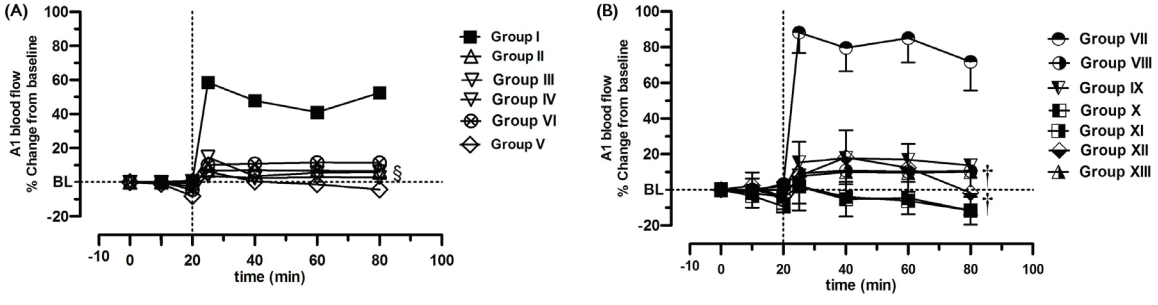

FIGURE 3 Intestinal A1 (inflow arteriole) blood flow. (A) Group I: 5% mannitol, no antagonists; Group II: 5% mannitol plus glibenclamide (a specific ATP-dependent potassium channel blocker); Group III: 5% mannitol plus glibenclamide plus DPCPX (an adenosine A1 receptor antagonist); Group IV: 5% mannitol plus glibenclamide plus L-NMMA (*NG*-monomethyl-L-arginine, a NO inhibitor); Group V: 5% mannitol plus glibenclamide plus DPCPX plus L-NMMA; Group VI: 5% mannitol plus L-NMMA. (B) Group VII: 2.5% glucose peritoneal dialysis (PD) solution (Delflex: Fresenius Medical Care North America, Waltham, MA, U.S.A.), no antagonists; Group VIII: Delflex plus glibenclamide; Group IX: Delflex plus DPCPX; Group X: Delflex plus glibenclamide plus DPCPX; Group XI: Delflex plus glibenclamide plus DPCPX plus mefenamic acid (a cyclooxygenase pathway inhibitor); Group XII: Delflex plus glibenclamide plus DPCPX plus L-NMMA; Group XIII: Delflex plus L-NMMA. *$p < 0.001$ compared with baseline by two-way analysis of variance and Bonferroni posttest.

$n = 7, p < 0.01$) and completely abolished by combined adenosine A1 receptor, K_{ATP}, and NO inhibition (–97.6% ± 1.6%, $n = 7, p < 0.001$).

Hypertonic mannitol-mediated vasodilation of the smaller pA3 and dA3 pre-mucosal precapillary arterioles was 21.7% ± 2.0% and 26.5% ± 2.2% from baseline [Figure 4(B,C)], which is double the magnitude of the dilation seen in the larger inflow arterioles, suggesting a dependency of hyperosmolality-induced vascular reactivity on vessel level. Inhibition of only K_{ATP} caused significant attenuation of mannitol-induced vasodilation (–48.5% ± 1.6% and –51.1% ± 1.2% in the pA3 and dA3 arterioles respectively, $n = 7$, $p < 0.001$). Similar attenuation of mannitol-induced dilation in the pA3 (–66.2% ± 1.4%, $n = 7$, $p < 0.001$) and dA3 arterioles (–63.3% ± 1.7%, $n = 7$, $p < 0.001$) was obtained with combined inhibition of K_{ATP} and the adenosine A1 receptor. However, NO inhibition alone or in combination with inhibition of K_{ATP} or adenosine A1 receptor completely abolished mannitol-mediated dilation in the pA3 and dA3 pre-mucosal precapillary arterioles alike ($n = 7, p < 0.001$).

Study II: PD solution series

The 2.5% glucose-based heat-sterilized PD solution produced instantaneous and sustained vasodilation at all intestinal visceral peritoneal microvascular levels [Figure 5, showing the (A) A1, (B) pA3, and (C) dA3 intestinal arteriole results]. That dilation was double the magnitude of the mannitol-induced dilation (Figure 4) at all arteriolar levels and was more remarkable in the smaller pre-mucosal precapillary arterioles.

Those findings suggest that the PD solution has vasoactive components other than hyperosmolality and that the magnitude of the vasoactivity depends on vessel level. In the larger inflow arterioles, PD solution produced an average dilation of 18.5% ± 3.2% from baseline ($n = 7$, $p < 0.01$). That dilation was not affected by adenosine A1 receptor inhibition (–14.3% ± 3.6%, $n = 7, p > 0.05$). However, inhibition of NO or K_{ATP} attenuated the dilation by –45.2% ± 3.2% ($n = 7$, $p < 0.05$) and –50.0% ± 2.2% ($n = 7$, $p < 0.05$) respectively. Combined inhibition of the adenosine A1 receptor, K_{ATP}, and NO pathways or of adenosine A1 receptor and K_{ATP} attenuated the PD solution–induced dilation in the inflow A1 arteriole by –60.0% ± 2.6% and –61.6% ± 3.2% respectively ($n = 7$, $p < 0.05$), which is similar to the attenuation produced by NO or K_{ATP} inhibition.

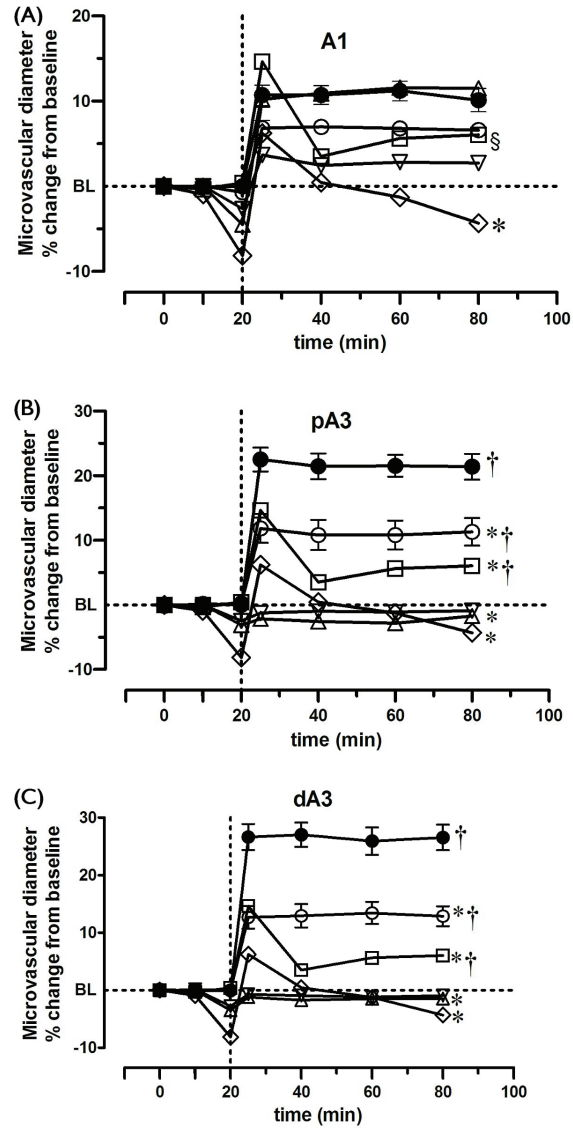

FIGURE 4 Effects of endothelium-dependent antagonists on mannitol-induced vasodilation in (A) inflow (A1) arterioles, (B) proximal pre-mucosal (pA3) arterioles, and (C) distal pre-mucosal (dA3) arterioles. Group I (solid circles): 5% mannitol, no antagonists; Group II (open circles): 5% mannitol plus glibenclamide (a specific ATP-dependent potassium channel blocker); Group III (open squares): 5% mannitol plus glibenclamide plus DPCPX (an adenosine A1 receptor antagonist); Group IV (open inverted triangles): 5% mannitol plus glibenclamide plus L-NMMA (*NG*-monomethyl-L-arginine, a NO inhibitor); Group V (open diamonds): 5% mannitol plus glibenclamide plus DPCPX plus L-NMMA; Group VI (open triangles): 5% mannitol plus L-NMMA. *$p < 0.001$ compared with the NO inhibitor group (Group I). †$p < 0.001$. §$p < 0.05$ compared with baseline, by two-way analysis of variance and Bonferroni post-test. BL = baseline.

The PD solution–mediated vasodilation of the smaller pA3 and dA3 pre-mucosal precapillary arterioles was 35.3% ± 3.2% and 46.1% ± 5.1% (n = 7, $p < 0.001$) from baseline [Figure 5(B,C)], which is approximately twice the magnitude of the dilation seen in the larger inflow arterioles, suggesting dependency of the PD solution–induced vascular reactivity on vessel level. Inhibition of only K_{ATP} caused significant attenuation of PD solution–induced vasodilation to –64.6% ± 3.6% and –65.9% ± 3.2% in the pA3 and dA3 arterioles respectively (n = 7, $p < 0.001$). Selective inhibition of the adenosine A1 receptor significantly attenuated the PD solution–mediated dilation by –37.8% ± 3.2% and –59.3% ± 4.4% in the pA3 and dA3 arterioles respectively. Marked attenuation of PD solution–induced dilation in the pA3 (–66.7% ± 2.4%, n = 7, $p < 0.001$) and dA3 arterioles (–80.5% ± 3.7%, n = 7, $p < 0.001$) was obtained with combined K_{ATP} and adenosine A1 receptor inhibition.

Addition of cyclooxygenase inhibition to the combined blockade of the K_{ATP} and adenosine A1 receptor pathways caused no further attenuation of the PD solution–mediated dilation at any microvascular level, but selective inhibition of the NO pathway alone completely abolished the PD solution–mediated precapillary A3 dilation (n = 7, $p < 0.001$). Similarly, NO inhibition, alone or in combination with inhibition of the K_{ATP} and adenosine A1 receptor pathways, completely abolished the PD solution–mediated dilation in the pA3 and dA3 pre-mucosal precapillary arterioles alike (n = 7, $p < 0.001$).

Study III: isotonic Krebs series

In a separate series of control experiments, the intestinal visceral peritoneal microvasculature was bathed with a non-vasoactive isotonic Krebs solution, and microvascular diameter and flow were assessed after inhibition of the NO pathway or combined blockade of the NO, K_{ATP}, and adenosine A1 receptor subtypes. As seen in Figure 6, NO inhibition alone or in combination with inhibition of K_{ATP} and adenosine A1 receptor—and in the absence of the vasoactive components of PD solution—produced marginal vasoconstriction of the inflow A1 and precapillary A3 arterioles. This vasoconstriction produced a modest decline in A1 blood flow (n = 7, $p < 0.05$).

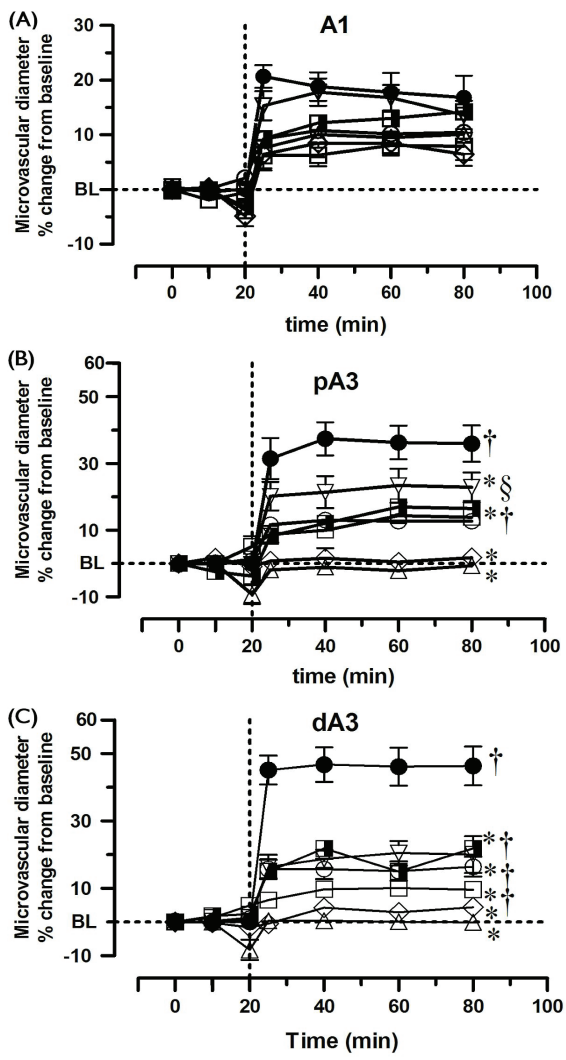

FIGURE 5 Effects of endothelium-dependent antagonists on 2.5% Delflex (conventional peritoneal dialysis solution from Fresenius Medical Care North America, Waltham, MA, U.S.A.)–induced dilation in (A) inflow (A1) arterioles, (B) proximal pre-mucosal (pA3) arterioles, and (C) distal pre-mucosal (dA3) arterioles. Group VII (closed circles): Delflex, no antagonists; Group VIII (open circles): Delflex plus glibenclamide (a specific ATP-dependent potassium channel blocker); Group IX (open inverted triangles): Delflex plus DPCPX (an adenosine A1 receptor antagonist); Group X (open squares): Delflex plus glibenclamide plus DPCPX; Group XI (half-closed squares): Delflex plus glibenclamide plus DPCPX plus mefenamic acid (a cyclooxygenase pathway inhibitor); Group XII (open diamonds): Delflex plus glibenclamide plus DPCPX plus L-NMMA (*NG*-monomethyl-L-arginine, a NO inhibitor); Group XIII (open triangles): Delflex plus L-NMMA. *$p < 0.001$ compared with the NO inhibitor group (Group VII). †$p < 0.001$. §$p < 0.05$ compared with baseline by two-way analysis of variance and Bonferroni post-test. BL = baseline.

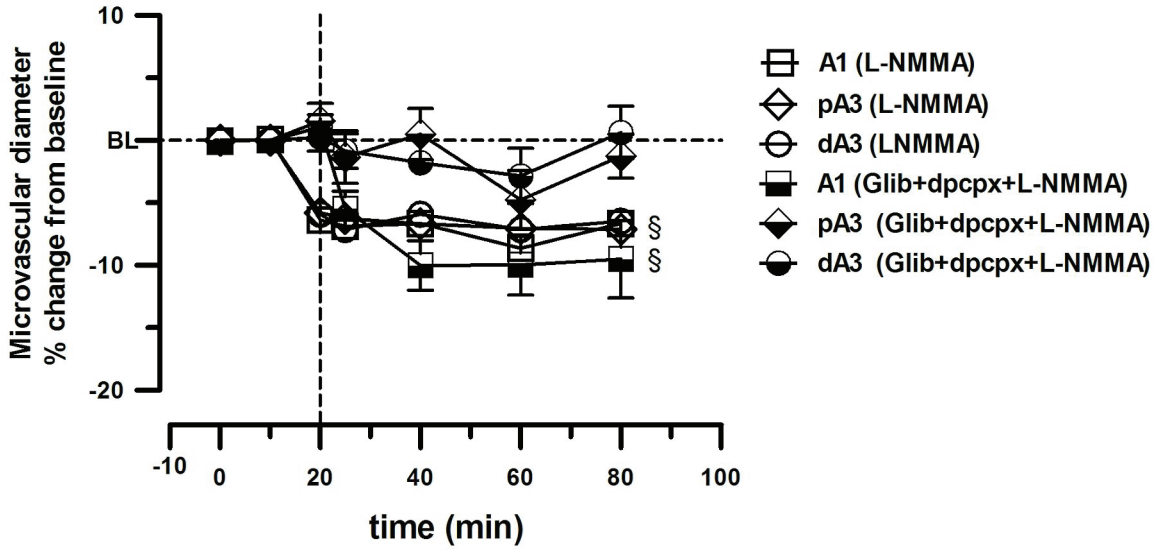

FIGURE 6 Microvascular diameters of visceral intestine (isotonic Krebs series). $^{\S}p < 0.05$ compared with baseline by two-way analysis of variance and Bonferroni post-test. BL = baseline; A1 = inflow arteriole; L-NMMA = *NG*-monomethyl-L-arginine, a NO inhibitor; pA3 = proximal pre-mucosal arteriole; dA3 = distal pre-mucosal arteriole; Glib = glibenclamide, a specific ATP-dependent potassium channel blocker; dpcpx = an adenosine A1 receptor antagonist.

Discussion

The present study is the first to define the molecular mechanisms of conventional PD solution–induced dilation of the visceral peritoneal microvasculature. Such dilation is rapid, sustained, and dependent on the vascular endothelium. The endothelium signaling pathways involved in this dilation are determined by the anatomic level of the microvessel in the microvascular tree. In the larger inflow A1 arterioles (about 100 μm in diameter), NO and K_{ATP} channels are moderately involved in PD solution–mediated vasodilation, and the contribution of the adenosine A1 receptors is minimal. In the smaller pre-mucosal precapillary pA3 arterioles (about 15 μm in diameter) and the dA3 arterioles (about 9 μm in diameter), the NO pathway accounts for all of the PD solution–mediated vasoactivity. Those findings are not surprising, because depending on the vascular bed, microvessels of similar size are well known to use different mechanisms for endothelium-dependent regulation of vascular tone (18). Similarly, the relative contributions of agonist-stimulated NO and EDHF to endothelium-dependent relaxation appears to vary by sex (19), by arteriolar size within the same vascular bed (20), and by vascular bed (18,21,22).

Inflow arteriole reactivity

Intestinal visceral inflow arterioles (70 – 100 μm) are first-order (A1) branches from the mesenteric arcade (16). As shown in the present study, mannitol solution and PD solution both produced rapid and sustained vasodilation in A1 arterioles. Compared with downstream dilation, the A1 arteriole dilation was significantly less in magnitude. The A1 arteriole dilation was also greater with PD solution than with hypertonic mannitol solution despite the remarkably greater osmolality of the mannitol solution.

Interpretation of the different results with mannitol and with PD solution is based both on physiologic principles and on biochemical differences between the two solutions. Physiologically, the vascular control mechanisms of the larger inflow A1 arterioles are quite different from the mechanisms that control vascular tone in the smaller downstream precapillary arterioles. Intestinal A1 vascular control is predominantly neurogenic: the neurohormonal reflexes are centrally driven through preganglionic sympathetic fibers of the intermediolateral area of the spinal cord. This neurogenic input to the large mesenteric arteries and smaller A1 arterioles is primarily sympathetic, with the α-adrenergic (vasoconstriction) influence

predominating over the β-adrenergic (vasodilation) influence (23,24). In contrast, vascular control of the smaller intestinal A3 precapillary arterioles is exclusively local, using metabolic and paracrine mechanisms (25).

Biochemically, conventional glucose PD solution has vasoactive components other than hyperosmolality (15) and a dilation mechanism that is unique to glucose and therefore not available to mannitol. However, as demonstrated in the present studies, the fractional contribution of the adenosine pathway to the PD solution–induced A1 dilation is particularly trivial, because there is nearly no distribution and expression of adenosine receptors in the A1 arterioles (26).

In recent studies, we demonstrated that lactate (the buffer anion system of PD solution) produces a rapid and transient pH-dependent submaximal dilation in the intestinal visceral peritoneal microvasculature. Glucose degradation products also contribute to the dilation (5,15). Lactate at low pH and GDPs are both components of the Delflex solution but not of the mannitol solution used in the present study. Those differences explain the difference in the magnitude of A1 dilation between the mannitol and Delflex solutions.

Precapillary arteriole reactivity

Intestinal visceral precapillary A3 arterioles (10 – 15 μm) are third-order branches from the second-order transition A2 arterioles (16). Vascular tone regulation of these small precapillary arterioles is exclusively autoregulatory and locally derived through metabolic and paracrine mechanisms within the microenvironment (25). The vasoactivity of PD solution is attributed to its hyperosmolality, glucose, lactate, and low pH (1–3,5). Those vasoactive components of PD solution must exert their vascular effects through metabolic and paracrine pathways. The mannitol series in the present study was designed to selectively isolate hyperosmolality from other vasoactive components of PD solution. Results from the mannitol series confirmed that hyperosmolality is the major vasoactive component of PD solution and that it exerts its A3 arteriolar dilation effects predominantly through K_{ATP}. It is likely that hyperosmolality-mediated opening of K_{ATP} channels is associated with the release of energy substrates such as adenosine (27), which explains the remarkable attenuation of mannitol-induced hyperosmolality-mediated dilation with combined inhibition of K_{ATP} channels and adenosine A1 receptors (experimental group III). The magnitude of the PD solution–mediated A3 dilation, which was double that seen in the A1 arterioles, was markedly attenuated by individual paracrine and autocrine antagonists. In particular, the NO pathway fully accounts for the PD solution–mediated A3 dilation. However, the remarkable contribution to precapillary A3 vasodilation of the adenosine receptor–stimulated NO release mechanism and of the opening of K_{ATP} was also observed. The PD solution–induced A3 dilation is predominantly caused by hyperosmolality, but other vasoactive components of the solution (such as lactate, low pH, and GDPs) contributed significantly. Data from the present study suggest that NO is the second messenger involved in the signal transduction pathways for vasodilation caused by all vasoactive components of conventional PD solutions.

In the present study, the finding that inhibition of the NO pathway completely abolishes the dilation response does not refute the contribution of K^+_{ATP} and adenosine A1 receptor activation to the observed dilation response. The interaction between the endothelium-dependent dilation pathways is well established in the literature. To summarize, hyperosmolality and glucose are well recognized to separately affect intercellular calcium (Ca^{2+}) homeostasis and mobilization (28–30). In turn, intracellular Ca^{2+} mobilization is known to activate constitutive NOS to generate *de novo* NO from the endothelial cell. In addition, NO is generated by lactate at high extracellular H^+ concentrations, which is the typical microenvironment in the presence of PD solution. And yet the intracellular signal transduction of this lactate-driven, pH-dependent NO release is still undetermined. However, data from studies of the small retinal arterioles suggest that lactate uptake by vascular cells through monocarboxylate transporters causes retinal arteriolar dilation predominantly by stimulation of NOS and subsequent activation of guanylyl cyclase and cyclase/cGMP signaling that triggers opening of K_{ATP} channels for vasodilation (31). Furthermore, NO is also produced after active glucose transport into cells, which results in generation of adenosine (32,33). Generated adenosine binds to its G-protein-coupled receptor, which activates adenylate cyclase to increase levels of cAMP. The cAMP mobilizes Ca^{2+}, which activates constitutive

NO release from endothelial cells and causes opening of K_{ATP} channels (13). For the endothelium-derived NO to elicit vasodilation, NO diffuses to the vascular smooth muscle to activate guanylate cyclase and release soluble cGMP. There are two pathways by which cGMP causes vascular smooth muscle relaxation:

- cGMP phosphorylates and activates K_{ATP} to elicit vasodilation secondary to cell membrane hyperpolarization.
- Kinase activation by cGMP elicits relaxation directly by lowering Ca^{2+}.

In control studies, Delflex PD solution was instilled into the peritoneal cavity while the small segment of the terminal ileum in the tissue bath continued to be bathed with the non-vasoactive isotonic Krebs solution. Surprisingly, no change was observed in the ileal microvascular diameter at any level. Those observations clearly demonstrate that, for PD solution–mediated dilation to occur, contact must be established between the intraperitoneal PD solution and the tissue. Our earlier studies demonstrated that, during the first hour of a dwell, a large intraperitoneal fill volume makes contact with only about 40% of the anatomic peritoneum (34). The suggestion is that, during conditions of peritoneal transport studies in small, quiescent, anesthetized animals, a small anatomic peritoneal surface area (about 40%—which includes a relatively larger effective vascular surface area produced by vasodilation) accounts for the high clearance of small solutes during the initial phase of a dwell (0 – 30 minutes). The data also imply that, during short dwells, a larger anatomic peritoneal surface area (about 60% of the anatomic peritoneum) that is not in contact with the PD solution encompasses a much larger microvascular area that, in effect, remains a potential site for dialysis enhancement. Potential clinical support for those hypotheses is obtained from a comparison of automated PD and of long dwells with icodextrin solution. Although the mechanisms of water removal are different in those PD modalities, relatively smaller contact and vascular areas are probably used in automated PD than in icodextrin dwells.

Conclusions

Peritoneal dialysis solutions produce instantaneous and sustained vasodilation of the visceral peritoneal microvasculature. The magnitude of the dilation response depends on vessel level and is mediated by endothelium-dependent mechanisms, primarily the NO pathway. The NO release resulting from adenosine receptor activation and K_{ATP} channel-mediated endothelium hyperpolarization significantly contribute to vasodilation in the smaller precapillary arterioles.

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Disclosures

The authors declare that no financial conflict of interest exists.

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