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Modulation of Pre-capillary Arteriolar Pressure with Drag Reducing Polymers: A Novel Method for Enhancing Microvascular Perfusion

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Abstract

Objective—We have shown that drag reducing polymers (DRP) enhance capillary perfusion during severe coronary stenosis and increase RBC velocity in capillaries, through uncertain mechanisms. We hypothesize that DRP decreases pressure loss from the aorta to the arteriolar compartment.

Methods—Intravital microscopy of the rat cremaster muscle and measurement of pressure in arterioles (diameters 20–132 μ m) was performed in 24 rats. DRP (polyethylene oxide, 1 ppm) was infused i.v. and measurements were made at baseline and 20 minutes after completion of DRP infusion. In a 10 rat subset, additional measurements were made 3 minutes after the start, and 1–5 and 10 minutes after completion of DRP.

Results—Twenty minutes after the completion of DRP mean arteriolar pressure was 22% higher than baseline (from 42 ± 3 to 49 ± 3 mmHg, $p < 0.005$, $n = 24$). DRP decreased the pressure loss from the aorta to the arterioles by 24% (from 35 ± 6 to 27 ± 5 mmHg, $p = 0.001$, $n = 10$). In addition, there was a strong trend towards an increase in pressure at 10 minutes after the completion of DRP ($n = 10$).

Conclusions—DRP diminishes pressure loss between the aorta and the arterioles. This results in a higher pre-capillary pressure and likely explains the observed DRP enhancement in capillary perfusion.

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Disclosures

Conflict of Interest: None declared for all authors.

Keywords

Drag reducing polymers; arterial pressure; microvascular blood pressure; servonull method

Introduction

During increasing coronary stenosis, myocardial arterioles progressively dilate to maintain coronary flow. When the stenosis becomes severe, and the arterioles cannot further dilate to reduce resistance, flow cannot increase to match demand. In canines with flow limiting coronary stenosis, we have previously shown that drag reducing polymers (DRP) further decrease coronary microvascular resistance, despite maximal arteriolar vasodilation, thus increasing microvascular perfusion, and potentially offering a novel means for treatment of acute coronary syndromes [15].

DRP are long-chain, soluble macromolecules with molecular weights greater than 1000 kD. Drag reduction in fluid flow with linear, high molecular weight polymers is a well-described hydrodynamic phenomenon [19], known as the Toms effect, which was used originally in industrial hydraulics [3, 17]. Although initially discovered in turbulent flow, it also occurs in certain types of disturbed laminar flow [5, 14, 11]. When added to the blood circulation, nanomolar concentrations of DRP are thought to provide a “liquid scaffold” which reduces resistance by organizing blood flow and suppressing the flow disturbances and flow separations which occur at vascular branch points [9, 8].

We previously studied the effects of DRP on myocardial perfusion during severe coronary stenosis in canines.[15] We demonstrated using both myocardial contrast echocardiography (MCE) and radiolabeled microsphere flow analysis that DRP restore myocardial perfusion to baseline. Intravital microscopy studies in the rat cremaster muscle using fluorescently labeled red blood cells (RBCs) demonstrated that intravenously administered DRP increased RBC velocity in all three microvascular compartments, particularly in the capillaries [16].

The intravascular mechanism of action of DRP is incompletely understood. One posited mechanism is that DRP suppress flow disturbances and separations, thus reducing pressure loss along the arterial tree [9, 8]. In the current study, we focused on downstream microvascular hemodynamics and hypothesized that DRP decrease pressure loss from the aorta to the arteriolar compartment. We tested this hypothesis by directly measuring the pressure difference between the aorta and arterioles before and after infusion of DRP.

Methods

Surgical preparation

The experimental protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Wistar male rats (n=24) weighing 120–150 gram were anesthetized with pentobarbital sodium (50 mg/kg I.P.) and maintained at 25 mg/kg/hr I.M. Tracheostomy and intubation were performed. The right external jugular vein was cannulated for DRP infusion. The cremaster muscle was prepared for intravital microscopy as previously described [2], mounted on a custom-designed microscope stage, and

continuously irrigated with 35°C HEPES buffer. A heating pad was used to maintain body temperature at 37°C. Animals were given an aspirin suppository (1 mg/kg) immediately prior to microvessel cannulation.

We measured aortic pressure (P_{Ao}) with a microtip catheter (Millar, Inc) positioned in the abdominal aorta via the left femoral artery in the last 10 of the 24 rats.

Intravital microscopy of the rat cremaster muscle

Microscopic observations were made with a compound upright microscope (Olympus BX51) linked to a high-resolution digital color camera (Olympus, DP-71). Various-sized arterioles within the cremaster muscle were imaged using trans-illumination.

Servonull technique for measurement of arteriolar pressure

A pneumatic servonull micro-pressure system was used (900A, World Precision Instruments, Inc) for direct intra-arteriolar pressure measurement. Glass micropipettes (tip 900A, World Precision Instruments, Inc) with tip diameters of approximately 2 μm were used. The pipettes were submerged in filtered, de-aired, 2M NaCl solution at 25°C and then secured into the pipette holder, while submerged, to avoid the introduction of air into the system. The pipette holder and pipette were positioned in a three degree-of-freedom motorized micromanipulator (Burleigh, Inc). The pipette was positioned in the holder at a 25–30 degree angle with respect to the cremaster tissue. Arterioles (diameter 20–132 μm) with branch points were identified. Pressure in the arteriole of interest was measured by cannulating the immediately adjacent branch, maintaining the pipette tip within the branch, to avoid altering flow conditions within the parent arteriole. The pipette was brought into focus directly above the neighboring branch of the arteriole and aligned with the long axis of the microvessel. The pipette was positioned such that after cannulation of the side branch, the final position of the tip was in direct proximity to the main branch, and then lowered vertically onto the vessel with the micromanipulator, until vessel blanching was noted. Then the pipette was advanced into the vessel. The damping and sensitivity of the system were adjusted to achieve a smooth pressure signal, and baseline pressure was recorded. DRP was infused while maintaining pipette position in order to measure arteriolar pressure ($P_{\text{arteriole}}$) 20 minutes after DRP infusion. The pressure difference between the aorta and arterioles was calculated as $P_{Ao} - P_{\text{arteriole}}$.

Experimental protocol for intravital microscopy

Baseline arteriolar pressure was recorded after confirmation of a stable pressure signal. DRP solution, polyethylene oxide (PEO, Polyox WSR-301, Dow Chemical Co) with an average MW of ~4500 kD and prepared as previously described [15], was then infused intravenously over 6 minutes to a final blood concentration of 1 ppm. The volume (mL) to be infused to obtain a concentration of 1 ppm was determined by: $([\text{rat weight (grams)} \times 0.07]/50 \text{ ppm})$. Twenty minutes thereafter, the arteriolar and aortic pressures were recorded. In addition to measuring $P_{\text{arteriole}}$ at baseline and 20 minutes after DRP infusion, in a subset of 10 rats, to study the temporal course of DRP-induced pressure change, we were able to maintain the pipette position to obtain $P_{\text{arteriole}}$ measurements at several additional time points including: 1) 3 minutes after the start of DRP infusion; 2) 1–5 minutes after completion of DRP

infusion; and 3) 10 minutes after completion of DRP infusion. In an additional four placebo rats, an equivalent volume of saline was intravenously administered over 6 minutes, and post-injection pressures were recorded at 20 minutes.

Data analysis

Data are expressed as mean $\pm$ SEM. Paired *t*-tests were used to assess for statistically significant differences in pressures before and 20 minutes after injection of DRP. In the subset of 10 animals in which we obtained arteriolar pressure at other time points during and after DRP infusion as outlined above, repeated measures ANOVA was performed to determine differences in pressure as a function of time. Any differences detected were assessed post hoc using Tukey testing to adjust for multiple comparisons. Significance was defined as $p < 0.05$.

Results

In the overall group of 24 DRP-treated rats, there was a significant increase from baseline (22 %) in mean arteriolar pressure 20 minutes after completion of DRP infusion (from 42 ± 3 to 49 ± 3 mmHg, $p < 0.005$) (Figure 1). In the 10 rats with simultaneous aortic pressure recordings, aortic pressure decreased slightly from 75 ± 3 to 74 ± 4 mmHg, after infusion of DRP ($p < 0.05$).

DRP decreased the pressure loss from the aorta to the arterioles by 24% (from 35 ± 6 to 27 ± 5 mmHg, $p = 0.001$, $n = 10$) (Figure 2). Figure 3 represents a typical tracing from one study in which DRP was associated with an increase in arteriolar pressure (from 47 to 55 mmHg) and a slight decrease in aortic pressure.

In our four placebo-control studies, there was no change in arteriolar or aortic pressure after an equivalent volume of saline infusion (Figure 1 and 4).

In the 10 rats with arteriolar pressure data at other time points during and after DRP infusion, relative to baseline, there was no change in arteriolar pressure at 3 minutes after the start of the 6 minute DRP infusion and at 1–5 minutes after completion of the infusion. Ten minutes after completion of the DRP infusion, there was a trend toward an increase in arteriolar pressure relative to baseline, and by 20 minutes, there was a statistically significant increase in arteriolar pressure compared to baseline (Table 1).

Discussion

This study is the first to show that intravenous DRP reduces the pressure loss between the aorta and the arteriolar compartment, resulting in a higher pre-capillary driving pressure. This finding suggests a novel chemically inactive approach for the enhancement of microvascular perfusion, and has important clinical implications for the treatment of acute coronary syndromes.

Pressure loss occurs progressively as blood traverses the arterial tree, predominantly at the level of the small arteries and arterioles. Along with vessel geometry and blood viscosity, an important contributor to the arterial pressure reduction across the arterial tree is the presence

of the flow separations and vortices at vessel bifurcations. DRP were previously shown to reduce flow separations in models of vessel bifurcations in vitro [9, 8]. An explanation for our present findings could be that DRP-diminish pressure loss and provide higher pre-capillary driving pressure by reducing hydraulic energy loss. Most pharmacologic therapies to improve coronary flow in patients with unstable or acute coronary syndromes are limited to antiplatelet and/or antithrombotic strategies [20]. However, the most definitive and symptom-relieving therapy requires anatomic revascularization. DRP offer a fundamentally different alternative/adjunctive therapy for improving microvascular blood flow, which can be used in cases where immediate revascularization is not possible.

In our previous canine study [15], we found that capillary blood volume decreased during severe left anterior descending artery (LAD) stenosis, consistent with the explanation by Jayaweera et al that capillaries derecruit in the face of decreased upstream arteriolar pressure [7] in order to maintain capillary hydrostatic pressure (30 mmHg) [18, 12, 7]. Jayaweera and colleagues used myocardial contrast echocardiography to measure intramyocardial capillary volume in canines with experimental non flow limiting coronary stenosis. In this model, it was found that when coronary blood flow becomes pressure-dependent during adenosine-induced maximal arteriolar vasodilation, intramyocardial blood volume decreases, resulting in maintenance of capillary hydrostatic pressure, even at the potential cost of myocardial oxygen delivery. These novel findings suggested that capillaries actively participate in the regulation of coronary blood flow when the autoregulatory limit is exceeded. In the presence of severe flow limiting LAD stenosis, we found, using myocardial contrast echocardiography, that DRP restored capillary blood volume (15), leading us to hypothesize that DRP restore pre-capillary arteriolar pressure. In the current study, using the servonull technique, we measured pressure in arterioles ranging from 20 to 132 μ m in diameter. We found that intravascular DRP was associated with a significant increase in arteriolar pressure (Figure 1), that was not associated with an increase in aortic pressure, resulting in a net reduction in pressure loss (Figure 2) between the aorta and arterioles. These data confirm that DRP reduces pressure loss along the arterial tree (Figure 2), which would allow a higher driving pressure to increase blood inflow to the cremaster capillary bed.

The potential role of vasodilation in the mechanism of action of DRP has been previously studied. To exclude vasodilation as the direct mechanism of DRP action, Grigorian et al [6] perfused ex vivo rat lower limb vessels to induce maximal vasodilation. After adding DRP to the perfusate, flow further increased, suggesting that DRP reduce hydraulic resistance in the vascular system independent of vasodilation. We recently investigated whether DRP have in vivo vasoactive properties [16]. Systemic hemodynamics after DRP infusion were studied in rabbits during adenosine induced maximal hyperemia [16]. We found that during maximal vasodilation using adenosine infusion into the superficial femoral artery, DRP administration resulted in a further reduction in femoral artery resistance. Furthermore, during blockade of endogenous nitric oxide with L-NAME in rats, we demonstrated that DRP caused an additional reduction in vascular resistance [16]. Both of these findings suggest that flow augmentation occurred through a means other than simple vasodilation. We also showed that DRP had no measurable effect on whole blood viscosity at the concentrations used in our previous studies (1–2.5 ppm) [15].

The DRP tested (PEO) in our experiments reduced resistance, but not under conditions of turbulent flow, as is most often associated with DRP, as this does not occur in the rat vascular tree. The maximum Reynolds numbers in rats are approximately 500–600 in the aorta and they drop down very quickly with branching of the arterial vessels to values of 1–5 in the terminal arteries and large arterioles [22]. As the original discovery/description of the Toms effect was in turbulent flow regimes, the mechanisms of the intravascular effects of DRP in non-turbulent conditions present in the rat vascular tree are not completely understood. However, multiple *in vivo* studies by us and others have demonstrated enhanced vascular perfusion in non-turbulent conditions with DRP, giving rise to several hypotheses for alternative mechanisms of action: Flow separations and secondary flows occurring at bends and bifurcations of the vasculature are widely described as part of the flow dynamics in the cardiovascular system and are potential areas of development of atherosclerosis [21, 13, 4]. Thus, it can be posited that the DRP intravascular mechanisms may be related to the fact that these polymers reduce flow separations at vessel bifurcations [9, 8] and other disturbances of blood flow in the vascular system. Our previous *in vitro* tests with models of bifurcating vessels demonstrated that DRP reduce the size of separation zones at Reynolds numbers ranging from ~50 to 400. Furthermore, DRP were found to reduce the near-wall cell free layer in microvessel models (*in vitro*), which is a potentially beneficial rheologic effect, as it may enhance RBC microvascular distribution [10].

Study Limitations

This study was performed in cremaster tissue without a stenosis. Since the same fluid dynamic concepts pertaining to pressure loss at vessel bifurcations apply in normal tissue as during compromised perfusion, these effects on arteriolar pressure would also be expected to occur under conditions of a severe stenosis.

One can hypothesize that a DRP-induced rise in microvascular resistance between the arterioles and venules could explain our finding of increased arteriolar pressure. However, given our previous findings outlined above, in which we found a DRP induced increase in capillary blood volume and hence a reduction in capillary resistance [15], as well as a previously reported increase in number of functioning capillaries following DRP injections [6], an increase in microvascular resistance between the arterioles and venules is unlikely.

Perspectives

This is the first study to demonstrate that DRP reduce the pressure losses which normally occur between the aorta and the arterioles. This results in a higher pre-capillary pressure and likely explains the enhanced capillary perfusion seen in our previous canine studies of flow limiting coronary stenosis and in other studies which demonstrated an increase in tissue perfusion by DRP. There is no other therapy that specifically targets alteration of microvascular blood flow hydrodynamics to enhance capillary perfusion. As such, DRP may have therapeutic value, in the setting of acute coronary syndromes, or any condition that results in compromised microvascular perfusion.

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Abbreviations and Acronyms

Ao	Aorta
DRP	Drag reducing polymer
LAD	Left anterior descending
MCE	Myocardial contrast echocardiography
P	Blood pressure
PPM	Part per million
PEO	Polyethylene oxide
SEM	Standard error of the mean

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Figure 1. Plot of mean arteriolar pressure (mmHg) measured using the servonull technique at baseline (light gray) and 20 minutes after completion of infusion of DRP (left) or placebo (right). n=24.

Figure 2.

Plot of pressure difference between the aorta and arterioles (mmHg) at baseline (light gray) and 20 minutes after completion of DRP infusion (black). n=10.

Figure 3. Servonull tracing of aortic (top) and arteriolar (bottom) pressure at baseline (left) and 20 minutes after completion of DRP infusion (right). DRP was associated with an increase in arteriolar pressure and a slight decrease in aortic pressure.

Figure 4.

Servonull tracing of aortic (top) and arteriolar (bottom) pressure at baseline (left) and 20 minutes after completion of saline placebo infusion (right), showing no change in aortic or arteriolar pressure.

Table 1

Mean arteriolar pressure measured at baseline, during and after DRP infusion.

TIME POINT	MEAN ARTERIOLAR PRESSURE (mmHg)
Baseline	41±4
3 minutes after starting DRP infusion	42±5
1–5 minutes after completion of DRP infusion	42±5
10 minutes after completion of DRP infusion	45±5
20 minutes after completion of DRP infusion	47±4*

*
p=0.014 versus baseline