

Sonoreperfusion Therapy for Microvascular Obstruction: A Review

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Abstract

Coronary artery disease is a major healthcare problem. Acute myocardial infarction is a leading cause of morbidity and mortality. Contemporary therapy is percutaneous coronary intervention, performed by balloon angioplasty and stent placement. Percutaneous coronary intervention is often complicated by microvascular obstruction (MVO), characterized by poor microvascular perfusion of myocardium distal to the site of primary occlusion. MVO is a multifactorial condition that is associated with adverse ventricular remodeling, arrhythmia, greater infarct size, and poor prognosis. Given the high prevalence and worsened outcomes associated with MVO, there is a clear need for an innovative approach to restore microvascular perfusion. Our group and others have investigated sonoreperfusion therapy for the treatment of MVO. This technique involves the application of both diagnostic imaging and therapeutic ultrasound pulses to the site of MVO in the presence of transiting microbubbles. This review will discuss the pathophysiology of MVO and the clinical translation of sonoreperfusion therapy.

Microvascular Obstruction

Coronary artery disease and acute myocardial infarction are a leading cause of morbidity and mortality. Contemporary therapy is percutaneous coronary intervention (PCI), performed by balloon angioplasty and stent placement. PCI is nearly ubiquitously accompanied by some degree of microvascular obstruction (MVO), and in many cases characterized by poor microvascular perfusion of myocardium distal to the site of primary occlusion. This phenomenon, also termed “no reflow”, was first documented in 1974 [1]. MVO is a powerful independent predictor of mortality, independent of age, infarct size, or ejection fraction [2]. Ultrasound targeted microbubble cavitation, termed “sonoreperfusion therapy”, is a promising treatment option to relieve MVO and reestablish microvascular perfusion. The purpose of this review is to discuss the pathophysiology of MVO and the clinical translation of sonoreperfusion therapy.

MVO is a multifactorial condition that is associated with adverse ventricular remodeling, arrhythmia, greater infarct size, and poor prognosis [3]. It occurs in up to 56% of ST-elevation myocardial infarction patients treated with PCI as measured by cardiac magnetic resonance imaging [2].

Bekkers et al. [4] postulated that endothelial cells and myocytes become edematous from osmotic overload in the setting of ischemia and subsequently occlude capillaries. This process promotes infiltration and activation of neutrophils and platelets, and promotes fibrin deposition. Adherence of activated neutrophils to the endothelium can drive increases in microvascular resistance. Lipowsky et al. [5] demonstrated that adherence of one white blood cell per high power field doubles microvascular resistance. Furthermore, coronary microembolization of atherosclerotic debris after PCI has been implicated in MVO pathophysiology [6]. All together, these phenomena result in increased microvascular resistance and reduced microvascular perfusion.

Sonoreperfusion Therapy

Contemporary therapy for MVO consists of medications such as nicardipine, nitroprusside, adenosine, etc. which provide microvascular vasodilation but also decrease systemic blood pressure. Often, however, patients undergoing PCI for acute myocardial infarction are hypotensive and therefore administration of these agents is contraindicated. Given the high prevalence of MVO and the accompanying worse outcomes, there is an

urgent unmet need for an innovative approach to restore microvascular perfusion. The use of ultrasound and microbubble therapy, referred to as sonothrombolysis, has been used to treat occlusive large thrombi in supplying vessels, including epicardial coronary arteries and larger cerebral arteries [7-12] and offers a unique approach to treating MVO.

Our group has investigated the therapeutic potential of ultrasound targeted microbubble cavitation, termed sonoreperfusion therapy (SRP), specifically to relieve microvascular obstruction and restore microvascular perfusion. This process involves the application of both imaging and therapeutic ultrasound (US) pulses to microbubbles (MBs) transiting through the microcirculation. This causes MB oscillations which generates mechanical forces and shear stress which disrupt microthrombi to relieve MVO. Additionally, this shear stress stimulates endothelial cells and acute release of nitric oxide (NO), a potent vasodilator. NO counteracts the numerous pathophysiological sequelae that emanate from MVO, namely inflammation, platelet activation, endothelial cell permeability and edema, and vasoconstriction. MBs are composed of a perfluorocarbon gas encapsulated in a lipid shell. Low frequency, high intensity US has been shown to effectively dissolve thrombi [13], while high frequency, low intensity US has been shown to enhance thrombus dissolution when combined with tissue plasminogen activator (tPa), a

clinically approved thrombolytic agent [13]. High intensity US causes inertial cavitation, non-linear microbubbles expansions and eventual collapse. Low intensity US causes stable cavitation, or cyclic microbubble vibrations. One major advantage of SRP is its potential for dual diagnostic and therapeutic functions in that contrast-enhanced US imaging can identify areas of MVO, with the same US transducer then being used to deliver therapeutic pulses.

Initial *in vitro* studies by our group identified US parameters that optimized microthrombus dissolution. Leeman et al. [14] assessed SRP efficacy in an *in vitro* model of MVO composed of a silicone-rubber flow phantom model. To model MVO, microthrombi made from venous blood were deposited onto a mesh with 40 μ m pores, resulting in increased resistance and upstream hydrostatic pressure. MBs were diluted in phosphate buffered saline and infused while therapeutic pulsed US was delivered to the mesh to induce MB oscillations adjacent to microthrombi. Upstream pressure was measured as a surrogate for thrombus burden. Figure 1 demonstrates upstream pressure over a 20 min therapy session at various acoustic pressures and pulse lengths [14]. More rapid and complete clot dissolution occurred with increasing pressure and pulse length. The most effective acoustic regime was 1 MHz, 1.5 MPa and 5000 cyc (5 msec) pulse length every 3 sec. This *in vitro* system provided an effective means to evaluate the pure mechanical effects of SRP.

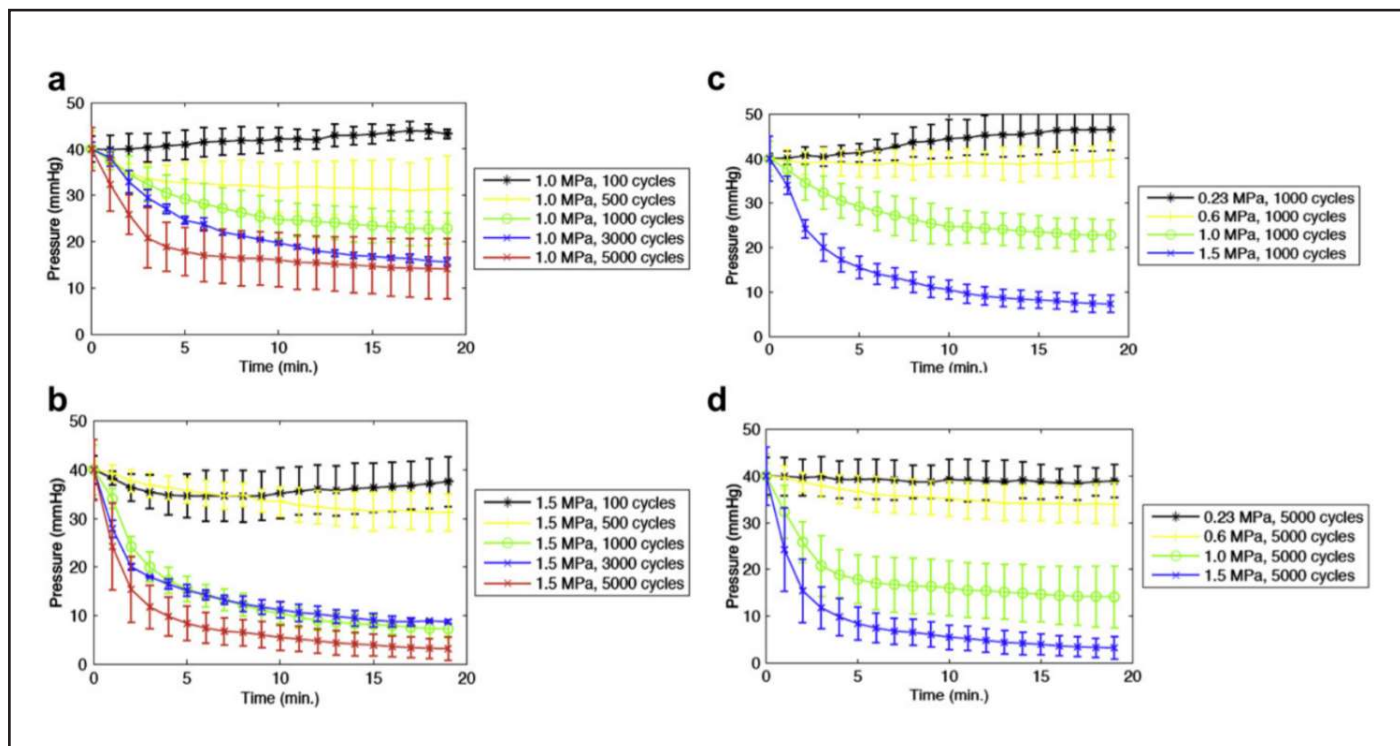


Figure 1: Upstream hydrostatic pressure over time during sonoreperfusion. Pressure upstream of site of microvascular obstruction represented thrombus burden. Ultrasound (1 MHz) pulse length was adjusted in (a) and (b). Ultrasound acoustic pressure was adjusted in (c) and (d). Longer pulse length and higher acoustic pressure yielded more effective thrombolysis.

In order to test the effects of viscosity on SRP efficacy, Roos et al. [15] used an identical system as was used in Leeman et al. For more clinical relevance, however, the authors used whole blood as the infusate. The viscosity of whole blood is ~4 times higher than that of phosphate buffered saline and the viscosity of the surrounding medium is known to affect MB oscillatory behavior [16]. The authors demonstrated that clot dissolution with venous microthrombi increased with pulse length, with the most effective thrombolysis occurring at 1.5 MPa and 5000 cycles (5 msec) pulse length every 3 sec. Addition of tPa further enhanced SRP efficacy. This study further supported clinical translation of high acoustic pressure, long pulse length US for SRP.

Black et al. [17] used this same *in vitro* model of MVO to study SRP on arterial thrombi, which are more representative of microthrombi associated with post-PCI MVO. The authors showed that for a given acoustic energy, there was greater dissolution of venous *vs.* arterial thrombi, but that arterial thrombi were successfully dissolved. They also demonstrated that increased viscosity of the surrounding medium was associated with less microbubble cavitation, particularly in whole blood. These results further supported the utilization of high mechanical indices and long pulse lengths for sonoreperfusion and restoration of microvascular blood flow (MBF).

Multiple studies have assessed both the mechanical and biologic mechanisms of MVO *in vitro* and *in vivo*. Chen et al. [18] optically characterized MB-microthrombus interactions in the presence of a short US pulse using a specialized ultra-high-speed microscopy imaging system, capable of imaging up to 25 million frames per second [19], to visualize acoustic behavior. With inertial cavitation, large amplitude MB oscillations caused thrombus deformation and pitting of the clot surface. These results illustrated the purely mechanical mechanism of SRP, brought upon by microjetting formed by MBs. Given the observed increased thrombolytic effect using long pulse length and high mechanical indices, Chen et al. [20] further investigated MB behavior under these US conditions. The authors showed that MBs underwent stable or inertial cavitation depending on the acoustic pressure and then formed gas filled clusters that continued to oscillate, fragment, and form new clusters. For the first time, they demonstrated that MB cavitation persisted throughout the 5 msec US pulse, providing new directions for future ultrasound system design.

Further studies were also conducted to evaluate the biological mechanism of SRP. Yu et al. [21] showed that MB oscillations activate the endothelial nitric oxide synthase (eNOS) pathway and contribute to increased perfusion via nitric oxide (NO), a potent vasodilator. Intramuscular NO and perfusion were monitored in response to US targeted MB cavitation (UTMC) in a rodent hindlimb

muscle. Following 2 minutes of UTMC, intramuscular NO increased at 6 min over 30 min and was higher than baseline after 13 min. Furthermore, contrast enhanced US imaging following UTMC showed increased perfusion flow rate. Importantly, enhanced flow persisted for ~4 hours. This prolonged effect raises the possibility that UTMC induces transcriptional changes and upregulation of eNOS. Other mechanisms that may also be implicated include sonoporation [22], or transient endothelial cell pore formation leading to intracellular calcium influx occurring in a similar time span as SRP. Of note, both NO and flow rate increases were blunted with LNAME, an NO inhibitor. Subsequent testing in an MVO rodent hindlimb model showed that perfusion rates did not return to baseline after SRP with LNAME pretreatment.

Preclinical and Clinical Translation of Sonoreperfusion Therapy

Promising early data regarding SRP efficacy in treating MVO [23,24] encouraged investigators to pursue further *in vivo* studies and human trials. To specifically address isolated MVO, Pacella et al. [25] developed a rodent hindlimb model of MVO to demonstrate SRP efficacy. In this model, an imaging transducer was placed on the biceps femoris muscle, perpendicular to a single element 1 MHz treatment transducer. Contrast enhanced US imaging was performed at baseline, after MVO, and after each of two successive US therapy sessions consisting of 5000 cycles, 0.33 Hz, PRF 5, 1.5 MPa for 10 min during continuous infusion of lipid encapsulated MBs. Control rats were injected with microthrombi and imaged at corresponding timepoints but did not receive therapeutic US. There was a 90% reduction in perfusion after MVO in both groups. Treatment rats demonstrated return of MBF to baseline, while control rats had persistently blunted MBF. Overall, this study demonstrated the clear effectiveness of a high mechanical index and long pulse length in restoring MBF after MVO.

Istvanic et al. [26] investigated the therapeutic potential of two clinically available US systems, the Philips Sonos 7500 *vs.* the Philips EPIQ. The same rodent hindlimb model as previously described [25] was used with changes in the therapeutic US delivery system. The EPIQ was modified to deliver long pulse lengths (1 msec) as was shown to be most effective in prior *in vitro* studies [14,15,17] and in the rodent hindlimb model [25]. The therapeutic efficacy of SRP utilizing various acoustic pressures and acoustic intensities was also assessed. The US parameters for each of the four experimental groups were as follows: (1) Sonos 7500 single-frame: 1.3 MHz, 1.3 MPa (MI=1.1), 27 μ s pulse length (total per line), time triggered, 1 frame every 3 sec; spatial peak temporal average acoustic intensity (ISPTA) 0.5 mW/cm²; (2) Sonos 7500 multi-frame: 1.3 MHz, 1.3 MPa (MI=1.1), 27 μ s pulse length (total per line), time

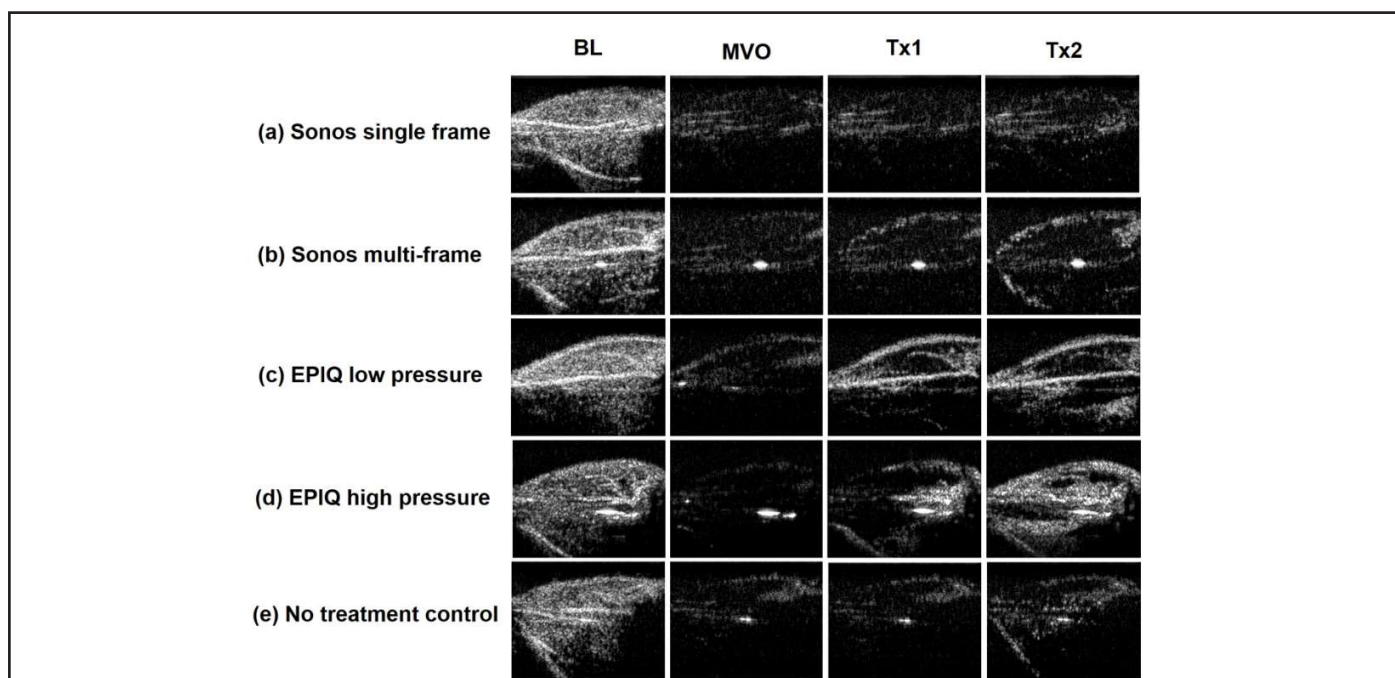


Figure 2: Selected still-frames from contrast enhanced ultrasound (US) perfusion imaging during sonoreperfusion using modified clinical ultrasound systems. The US parameters for each experimental group were as follows: (a) Sonos 7500 single-frame: 1.3 MHz, 1.3 MPa (MI=1.1), 27 μ s pulse length (total per line), time triggered, 1 frame every 3 sec; spatial peak temporal average acoustic intensity (ISPTA) 0.5 mW/cm²; (b) Sonos 7500 multi-frame: 1.3 MHz, 1.3 MPa (MI=1.1), 27 μ s pulse length (total per line), time triggered, 23 frames every 3 sec; ISPTA 11.6 mW/cm²; (c) EPIQ low pressure: 1.6 MHz, 0.5 MPa (MI=0.40), 1 ms pulse length, time triggered, 8.3 frames every 3 sec; ISPTA 23.0 mW/cm²; (d) EPIQ high pressure: 1.6 MHz, 1.44 MPa (MI=1.1), 1 ms pulse length, time triggered, 1 frame every 3 sec, ISPTA 23.0 mW/cm²; (e) no treatment control. Imaging at baseline showed uniform distribution of microbubbles throughout the microvasculature. Imaging 10 min after microthrombi injection showed persistent microvascular obstruction. Imaging after Tx2 showed significantly more blood flow in the (d) EPIQ high pressure, long pulse group relative to all other groups.

triggered, 23 frames every 3 sec; ISPTA 11.6 mW/cm²; (3) EPIQ low pressure: 1.6 MHz, 0.5 MPa (MI=0.40), 1 ms pulse length, time triggered, 8.3 frames every 3 sec; ISPTA 23.0 mW/cm²; and (4) EPIQ high pressure: 1.6 MHz, 1.44 MPa (MI=1.1), 1 ms pulse length, time triggered, 1 frame every 3 sec, ISPTA 23.0 mW/cm². The EPIQ high pressure condition was most effective and was the only treatment to restore MBF to baseline. This result is consistent with prior *in vitro* and *in vivo* findings that long pulse, high pressure US is associated with the most effective SRP. The EPIQ low pressure and Sonos multi-frame conditions resulted in significantly improved MBF after treatment but below baseline levels. The Sonos 7500 single-frame and control conditions showed no improvement in MBF. Figure 2 shows contrast enhanced US perfusion still-frame images at four timepoints for each experimental group [26]. Figure 3 shows microvascular blood volume at four timepoints for each experimental group [26]. Importantly, this study demonstrated that a clinically available US system is readily capable of delivering therapeutic long pulses. Though previous studies have demonstrated SRP efficacy using high mechanical indices from diagnostic US transducers in STEMI patients, as detailed below, they did not assess SRP efficacy using a modified clinical system capable of delivering long pulses. Moreover, they

did not address isolated, pure MVO, as would be seen post PCI; they focused on restoring epicardial coronary artery patency.

Roos et al. [27] attempted to apply long pulse, high mechanical index US in STEMI patients in a phase 2 clinical trial. However, the study was aborted after 6 patients were enrolled. 3 patients with acute myocardial infarction developed coronary vasoconstriction of the culprit artery that was deemed unresponsive to nitroglycerin by the investigators. To further investigate this isolated and newly described event, coronary artery diameters were measured in five pigs. Diameters distal to the injury site decreased after (1) balloon injury, (2) thrombus injection, and (3) administration of US. The authors concluded that long pulse US may cause coronary vasoconstriction. Mathias et al. [28] applied a similar US pulse regime in 10 patients and reported no occurrence of coronary vasoconstriction.

Yu et al. [29] assessed UTMC cavitation in rodent hindlimb microvascular perfusion using nitrite, an NO donor. Several preclinical and clinical studies have demonstrated the cardioprotective effect of nitrite [30,31]. Nitrite has increased bioactivity in the hypoxic and acidotic conditions characteristic of MVO. UTMC and nitrite co-therapy

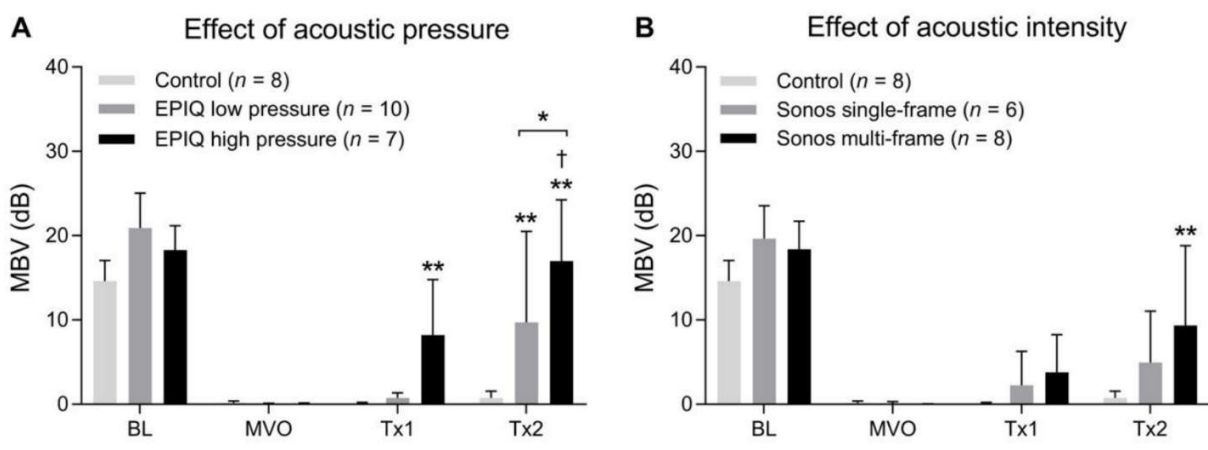


Figure 3: Microvascular blood volumes (MBV) during sonoreperfusion using clinically-approved ultrasound systems. MBV for EPIQ are shown in (A) and for Sonos are shown in (B). MBV in the EPIQ high pressure group significantly improved after Tx1 and returned to baseline after Tx2. Double asterisks (**) indicates significant difference from MVO state. Dagger (+) indicates no statistical difference from baseline (BL). Single asterisk indicates significant difference for the indicated comparison. Error bars represent standard deviation.

increased MBF and NO levels in rodent microvasculature. High mechanical index, long pulse length (5 msec) US was used in this study. Importantly, UTMC with nitrite yielded

no evidence of vasoconstriction as was observed in Roos et al. [27]. Figure 4 shows changes in microvascular blood volume and NO concentration after UTMC therapy [29].

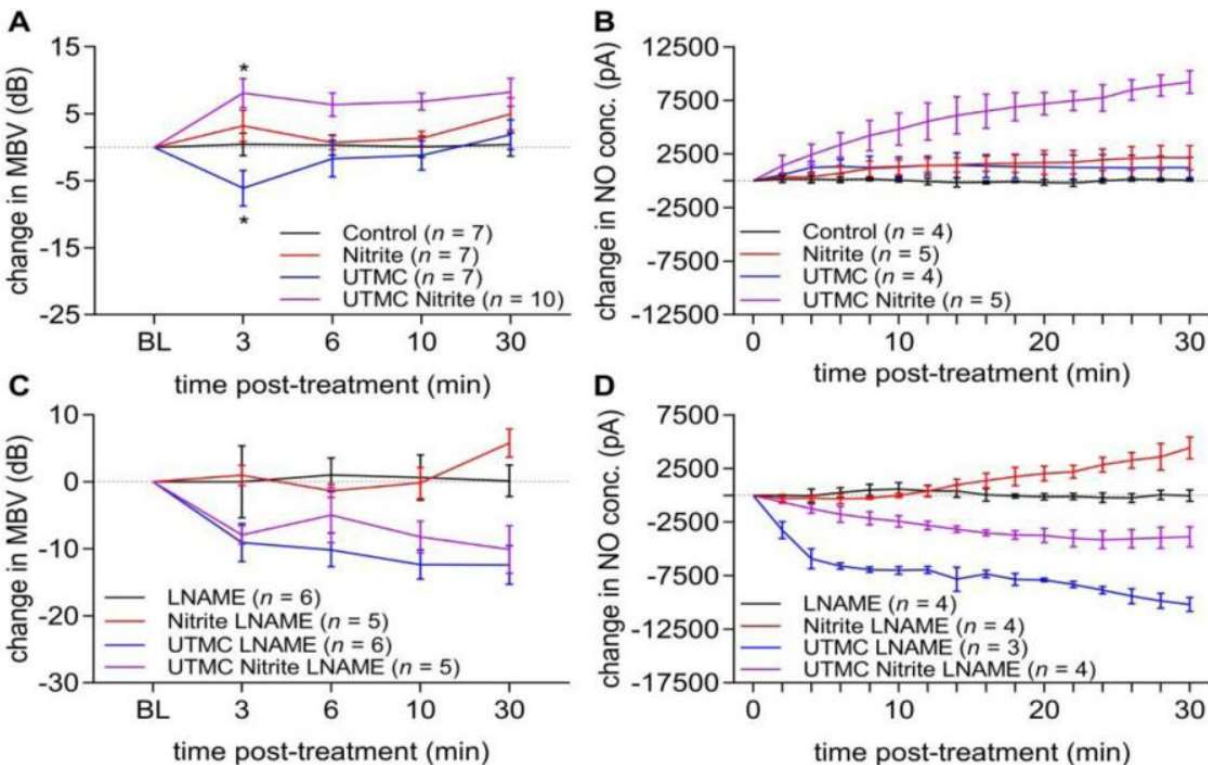


Figure 4: Microvascular blood volumes (MBV) and nitric oxide (NO) concentrations after ultrasound-targeted microbubble cavitation (UTMC) therapy. MBV are shown in (A) and (C). NO concentrations are shown in (B) and (D). The transient decrease in MBV in the UTMC only group at 3 min corresponded to vasospasm. No vasospasm was observed with the addition of nitrite to UTMC. Asterisk indicates $p < 0.0001$ compared to control. All error bars indicate standard deviation.

Recent human trials have also been conducted to promote clinical translation of SRP. Mathias et al. [28] analyzed the application of high mechanical index impulses from a diagnostic US transducer in STEMI patients. Results showed a smaller proportion of MVO at 1 month and overall improvement in LVEF. Of note, the US parameters used in the study are standard features of diagnostic US systems. Up to 20 usec pulse lengths were used. It is important to note that this study did not assess pure adjunctive SRP therapy immediately following PCI. SRP was administered at variable times and durations before and after PCI, with variable US regimes and contrast doses, making it impossible to identify optimal therapeutic approach. Additionally, more than twice as many patients in the treatment group had complete restoration of coronary blood flow prior to stenting, making it impossible to determine whether post-PCI adjunctive SRP conferred any additional benefit.

Mathias et al. [32] subsequently conducted a randomized control trial using high mechanical index SRP plus PCI vs PCI only in STEMI patients. <5 usec pulse length was used. In the high mechanical index plus PCI group, ST segment resolution occurred in 32% before PCI (4% in PCI only), angiographic recanalization was evident in 48% (20% in PCI only), infarct size was reduced, and LVEF increased immediately after PCI and remained higher at 6 months. Finally, the need for ICD was only 5% compared to 18% in PCI only group. Of note, this work also did not assess the efficacy of SRP as a pure adjunct following PCI, as a significant portion of patients received ultrasound therapy prior to PCI. This study was also done at a center with a very high volume of STEMI patients (Sao Paulo, Brazil) where patients often wait for cath lab availability and subsequently sometimes experience delays in door to balloon time. Importantly, our group is involved in an ongoing multicenter trial in the United States to test the effect of SRP on post-PCI MVO specifically.

Conclusions/Future Directions

In conclusion, high mechanical index and long pulse US is effective at restoring MBF. Further studies in porcine models using nitrite co-administration are underway and will allow for additional characterization of SRP efficacy. In addition to continuing clinical translation of SRP, further investigation of adjunctive agents is warranted. Prior *in vitro* studies have demonstrated increased thrombolytic efficacy of tPa when coupled with sonothrombolysis [14,27,33-35]. This approach can promote targeted drug delivery of tPa to sites of vascular obstruction and thus limit systemic exposure to tPa. Clinical trials in acute ischemic stroke patients treated with sonothrombolysis and adjunctive tPa have demonstrated safety and increased recanalization rates [36,37]. These trials support further investigation of adjunctive low-dose tPa during

SRP in porcine models of MVO. Overall, SRP presents an innovative and promising method to treat MVO.

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