CLINICALTRIALS.GOV Title: Physiologic Effect of Topical Nitroglycerin on Microcirculation Capacity in Patients With Circulatory Shock.

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Study Protocol and Statistical Analysis Plan

Patient selection

Adult patients (age>18 years) receiving elective cardiac surgery requiring cardiopulmonary bypass were screened for eligibility from August-October 2021. Written informed consent was obtained from eligible patients prior to surgery. Patients were excluded if they had a nitroglycerin allergy, were taking oral phosphodiesterase inhibitors, were unable to tolerate microcirculatory flow image acquisition or did not have a pulmonary artery catheter for continuous cardiac output monitoring after surgery. Patients only received the study drug if they met the clinical criteria for circulatory shock, defined as having postoperative vasopressor-dependent hypotension or low cardiac output requiring inotropic support, with signs of end-organ injury (capillary refill time > 3 seconds, lactate > 2, SvO2 < 60%). A second cohort of patients were enrolled to serve as a preoperative control, which were matched to the experimental group for age, co-morbidities, and planned operation. Study data were collected and recorded using a well-established clinical database tool (REDCap, Vanderbilt University, Nashville, TN) hosted at the University of Pennsylvania[1,2].

Study design and drug administration

This investigator-initiated, open-label study was conducted at the Hospital of the University of Pennsylvania, an urban quaternary academic medical center. The study was approved by the University of Pennsylvania's institutional review board, registered with ClinicalTrials.gov (NCT05102734), and conducted in accordance with the principles of the Declaration of Helsinki. Sample size was determined based on an anticipated 30% increase in TVD, and setting a one-sided α of 0.1, and ß of 0.8. A 1% topical nitroglycerin solution was prepared immediately prior to enrollment by reconstituting 400 mcg of nitroglycerin (Pfizer Pharmaceuticals, New York, NY, USA) in 4mL of sterile water within a dropper bottle that would administer the solution in 0.05mL aliquots. Two drops (0.1 mL) of the 1% nitroglycerin solution (5 mcg or $2.27*10^{-2}$ μmol per drop)

were applied to the sublingual space after obtaining baseline post-operative microcirculation images. A microdosing (approximately 1/50th of the lowest therapeutic sublingual dose) was administered to avoid any systemic effects.

Microcirculatory imaging and analysis

Sublingual microcirculation imaging was performed using HVM (CytoCam, Braedius Medical BV, the Netherlands) in the preoperative staging area (for controls) or within 2 hours of arriving to the intensive care unit after surgery by a trained member of the investigative team (JCG or FMT)[3]. The sublingual microcirculation was measured at 3 time points: baseline, 3 minutes post-nitroglycerin, and then 30 minutes later. Images were obtained by gently placing the videomicroscope under the subject's tongue until an adequate view of the microcirculation was obtained. A series of six second video clips (120 frames) duration were taken at each time point with attention to quality factors, especially the absence of pressure artifact, excess saliva, and proper location in accordance with the accepted consensus for microcirculation analysis. Image quality was assessed using the 6-factor Massey quality score, which scores each video for appropriate illumination, duration, focus, content, stability, and pressure. Images were only included in the final analysis if the Massey quality score was < 10 [4]. Acceptable clips were deidentified and coded for analysis after enrollment was complete.

Microcirculation videos were exported using commercial software (CCTools 2, Braedius Medical BV, the Netherlands) and manually analyzed using the validated Automated Vascular Analysis software (AVA 3.2; Microvision Medical B.V., the Netherlands). Video analysis was performed by trained investigators (JCG and FMT) who were blinded to the conditions of the subject. Only microvessels $\leq$ 20 $\propto$m in diameter were included in the calculation of TVD and PVD.

Red blood cell flow velocity was calculated within appropriate venules 20-30 ∝m in diameter by manually measuring the slope of individual RBC movement within software generated space-time diagrams [5]. Microcirculatory function was quantified according to the current best practice guidelines for microcirculation imaging[5]. Blood flow within each microvessel was graded using a semiquantitative scale, based on the vascular flow pattern ranging from 0-3 (0=no flow, 1=intermittent, 2=sluggish, or 3=continuous flow). Vessel perfusion was dichotomized as nonperfused (no flow or intermittent flow) or perfused (continuous or sluggish) for the calculation of PPV and PVD.

Individual pharmacodynamic response to the nitroglycerin challenge

An exploratory analysis was planned to identify the incidence of a patient specific pharmacodynamic response to the nitroglycerin challenge. A PD response threshold was set as a post-administration PVD > 1.8 standard deviations from the baseline, which was calculated by averaging the intra-patient variance between the three baseline PVD video sequences, which was then pooled across the entire cohort. This 1.8SD threshold was set to achieve 90 percent confidence interval (assuming a one-sided alpha, since there was only an anticipated increase in PVD). This method has been used in previous Phase 0 trials to provide a statistically rigorous threshold for individualized PD responses to novel therapeutic interventions[6].

Patient data and safety monitoring

Subject demographics, pre-operative risk score (euroSCORE II) and medical history were collected during initial screening. Cardiac index (CI), central venous pressure (CVP), pulmonary artery pressure (PAP), and mixed venous oxygen saturation (SvO_2) were continuously monitored by pulmonary artery catheter (Edwards Lifesciences LLC, Irvine, CA, USA). Arterial blood pressure was measured continuously using a standard invasive arterial line. Systemic hemodynamic data, vasopressor infusion doses, and mixed venous oxygen saturation were

recorded at baseline then every 3 minutes for 30 minutes after application of the topical nitroglycerin solution[7]. The vasopressor-inotrope score (VIS) was calculated to summarize the degree of post-operative vasoactive support was required at the time of the study[8]. Norepinephrine equivalents were calculated for each patient at each time point[9].

Availability of data and materials

The dataset supporting the results of this report is available via the Zenodo research data repository[10].

Statistical Analysis

Data normality was assessed using the D'Agostino-Pearson omnibus normality test. Global hemodynamic and microcirculation variables are reported as mean ± SD. Variables that were not normally distributed are reported as median with interquartile range [25^{th} – 75^{th} percentiles]. Student t-test was used to test for differences between normally distributed variables. Mann-Whitney U test was used to analyze non-normal data. Repeated measure one-way ANOVA with Tukey's post-hoc testing was used to assess for changes in microcirculatory function during the nitroglycerin challenge. Inter-rater reliability between coders was assessed in 10% of the videos using a Bland-Altman plot. Statistical analysis was conducted using Prism v 8.0 (Graph-Pad Software, San Diego, CA). Statistical significance was assumed at $p < 0.05$.

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