The Physiological Effects of Various Vasoactive Agents on Mouse Ear Microvasculature

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Jillian Bracaglia
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Technical Project Team Members
Julia Riedy

On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

Signature _____ _____ Date __4/26/20__
Jillian Bracaglia

Approved ____ _____ Date __4/26/20__
Patrick Cottler, Department of Biomedical Engineering

The Physiological Effects of Various Vasoactive Agents on Mouse Ear Microvasculature

Jillian L. Bracaglia, Julia C. Riedy

Abstract

Vasoactive agents are utilized by plastic surgeons in transplantations and reconstructive surgeries. A successful reconstruction or transplantation depends partly on ensuring the vascular vessels, or pedicles, supplying the tissue are active and attached well. The rapidly acting vasoactive agents chosen for this study generally are applied to the pedicle vessels that need to be reattached to the functional vasculature. The slower longer lasting agents chosen for this study are applied to the peripheral vessels in the entire tissue flap to ensure they are open to provide adequate blood flow while healing. This pilot study provides preliminary data and a basis for an experiment model. There is a lack of studies in the field on the exact details of the kinetics of these vasoactive agents and a lack of experiment models well suited for studying their effect on microvasculature.

Keywords: reconstructive surgery, vasoactive agents, vasculature

Introduction

Tissue flaps can be used in reconstructive surgery to recreate structures such as the breast. A flap is a transfer of tissue that still has a persistent blood supply from a major artery and vein to a recipient area. The transferred tissue can contain multiple types of tissue, and the higher volume of tissue transferred will be compromised if the arterial supply or venous drainage is interrupted during or following the transfer of the tissue[1]. During surgery, intraoperative vasospasms can have potentially devastating effects, while after surgery inadequate blood flow can lead to rejection of the newly implanted tissue[2]. Vasospasms can lead to decreased blood flow or clotting and is estimated to have significant effects on 5-10% of procedures[3]. After breast reconstructive surgeries, skin flap necrosis is a common complication and can occur in 5-30% percent of procedures[4,5]. Skin flap necrosis can occur if the network of blood vessels that supply blood to the tissue were damaged and there isn't enough blood flow to the skin, which causes the breakdown of tissue known as necrosis[6].

A vasoactive agent is an administered drug that either constricts or dilates the blood vessels[7] and can be injected during procedures or topically applied post-operatively as possible ways to pharmacologically prevent these complications. The injectable vasoactive agents are utilized to decrease vasospasms of the pedicle to ensure newly established flow at the anastomosis is maintained. In addition, the topical agents are used to ensure that perfusion of the tissue flaps distal to the pedicle is maintained. Agents being used today are known to cause vasodilation, however there is very little known of the exact kinetics in terms of duration and magnitude of vasodilation effect.

The purpose of our pilot study was to gain preliminary data on the magnitude and timeframe of activity of the vasoactive agents nitroglycerin, diltiazem, and lidocaine. These come in liquid solution forms, for fast action, as well as paste-like gel forms, for slower longer action or a delayed effect to correspond with healing processes.

Nitroglycerin is converted to nitric oxide in the body. Nitric oxide activates a phosphorylation pathway that ultimately results in the dephosphorylation of myosin light chains in smooth muscle fibers, causing the relaxation of smooth muscle within blood vessels[8]. Lidocaine acts on sodium ion channels and locks the channels in the open state, preventing nerve depolarization[9]. Diltiazem is a calcium channel blocker, and specifically it inhibits the influx of extracellular calcium ions across the myocardial and vascular smooth muscle cell membranes during depolarization[10].

To fulfill this research, we developed a model to observe the vasoactivity of each drug in the microvasculature of a mouse ear; which is a well-established model for viewing microcirculation[11]. The mouse ear is an established model because its relatively thin structure, about 500 micrometers thick, allows for non-invasive imaging of blood vessels as well as it being almost identical to human skin structure except for sweat ducts and glands[12]. The imaging modalities used in the model were sidestream dark field microscopy, two-dimensional laser Doppler perfusion imaging, and photography with ImageJ analysis. We worked under the guidance and advice of Dr. Cottler and Dr. Campbell, who both work in UVA's Plastic Surgery Department.

We aimed to create an experimental animal model that would allow for repeated measures of blood flow to determine the time course and effect of a collection of vasoactive agents. This animal model would provide an avenue to determining the specific characteristics of the agents tested and could be further applied to any other vasoactive agents not included in our study or to future innovations that enter the field.

There is a lack of research in specific characteristics of vasoactive agents in the reconstructive surgery field. Some studies have been done on preventing vasospasms in animal and in vitro models but few have been in a controlled fashion or on clinical subjects[2]. Once this study is completed in its full scope the model and results can be compared to the current standard of care to measure how representative it is. This study could be used to determine whether the way clinicians are applying these agents can be further optimized or whether there is a need to change approaches or agents to achieve the desired effects.

Results

Due to the timing of the COVID-19 epidemic in 2020, we were only able to obtain 3 trials of a lidocaine injection, 1 trial of a diltiazem injection, 3 trials of a nitroglycerin topical application, and 1 trial of a lidocaine topical injection. This is summarized in Table S1. Based off of our preliminary data we were able to observe changes in perfusion over various periods of time and potential peak effect time points.

Three mice were injected with 0.1 ml of a 4% lidocaine solution into their right ear at the base of the feeding vessel of the ear. The left ear of each mouse was left untreated to provide a control. The aggregate of the data obtained through two-dimensional laser Doppler perfusion imaging showed a slow increase up to 40% past baseline with a peak occurring 60 minutes post injection, as shown in Figure 1. There was an over 20% decrease by the 4 hour follow-up.

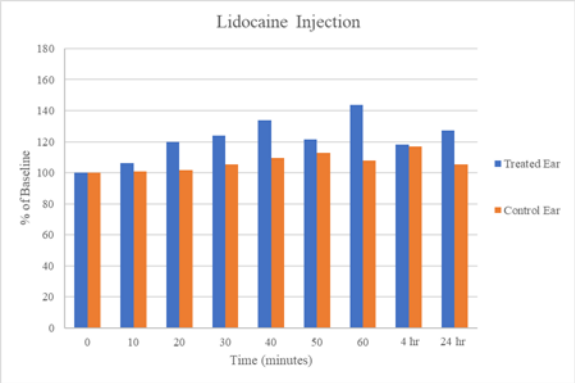

Figure 1. Percent of Baseline - Lidocaine Injection. Mice were injected with 0.1 ml of a lidocaine solution and imaged over time. The values were obtained from averaging data points from replicate trials and normalizing to the baseline point.

One mouse ear was injected with 0.1 ml of a 2% diltiazem solution. The data obtained showed minimal perfusion change in the first 10 minutes post injection and then a peak, of about 50% past baseline, occurring 20 minutes post injection, as shown in Figure 2. There was increased perfusion occurring 30, 40, and 50 minutes post-injection with a decrease back past baseline by 4 hours post injection.

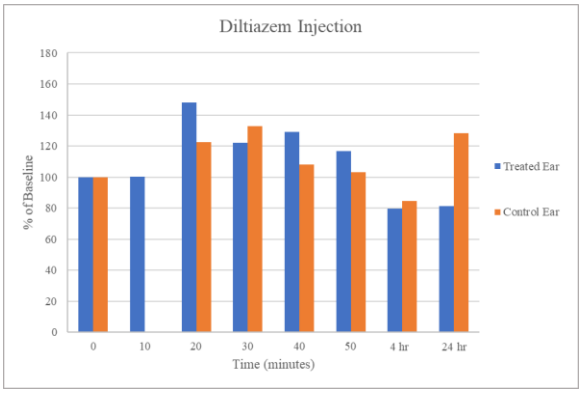

Figure 2. Percent of Baseline - Diltiazem Injection. Mice were injected with 0.1 ml of a diltiazem solution and imaged over time. The values were obtained by normalizing to the baseline point.

One mouse had the lidocaine containing gel applied to its right ear that was then wrapped with Tegaderm while one mouse had a lidocaine containing gel applied to its right ear with no subsequent wrapping. Both mice left ears had no application and no wrapping to serve as controls. Both ears showed about a 10-15% increase past baseline at the two hour mark post application with a return to relative

baseline by the six hour mark post application, as shown in Figure 3.

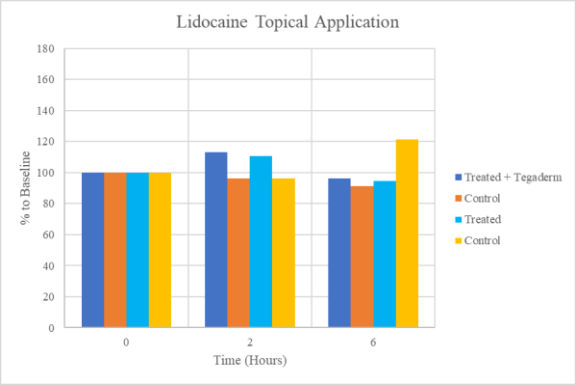

Figure 3. Percent of Baseline - Lidocaine Topical. One mouse had a lidocaine containing gel applied to its right ear that was then wrapped with Tegaderm. One mouse had a lidocaine containing gel applied to its right ear with no wrapping.

Three mice had a nitroglycerin containing paste applied to their right ears with a Tegaderm wrapping, and three mice had a nitroglycerin containing paste applied to their right ears with no subsequent wrapping. The aggregate of the data showed the greatest increase past baseline overall occurred in the Tegaderm wrapped ears with an increase of about 60%. The peak of the Tegaderm wrapped ear occurred at the 6 hour mark, as shown in Figure 4. The peak of the non-wrapped ear occurred at the 24 hour follow-up at about 15% past baseline. The Tegaderm wrapped ear began to drop back to baseline by the 24 hour follow-up time point.

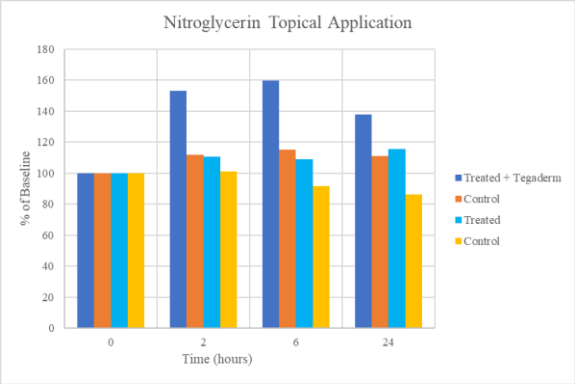

Figure 4. Percent of Baseline - Nitroglycerin Topical. Three mice had a nitroglycerin paste applied to the right ear and wrapped with Tegaderm. Three mice had a nitroglycerin paste applied to the right ear with no wrapping.

Discussion

This pilot study has provided preliminary data indicating injected vasoactive agents' peak effects occur within an hour of injection. The trials run on the topically applied agents indicated the peak effects occur multiple hours after application. This is consistent with the indications for these different application forms. Injected vasoactive agents are indicated for fast action during surgery through direct application to pedicle vessels. Topically applied vasoactive agents are indicated for slower absorption into the healing skin and tissue after a surgery through application to the impacted area including the distal edges of the newly transplanted tissue flap. This pilot study demonstrates the potential of this trial model for studying vasoactive agents within microvasculature and can be built upon to expand the data pool and draw further conclusions. Limitations in this pilot study included difficulty in mimicking patient experience, barriers to optimizing the imaging systems, and the difference in scale between mice and humans. Topically applied vasoactive agents are utilized after breast reconstruction surgeries on the newly planted tissue, especially the distal edges of the new tissue. The area is then covered with Tegaderm. Patients are told to keep the covering in place for a few days until their follow-up appointment. There was difficulty in mimicking this practice while obtaining images and data. To provide as much information as possible to be built upon, we had two experiment set ups for topical applications. One setup was to apply the topical application and then cover the ear with Tegaderm and reapply both the application and covering after each imaging. The other setup was to apply the topical application and leave the ear uncovered. This setup did not have any reapplication. Steps were taken to gently remove the Tegaderm for imaging, but it is possible the removal of the Tegaderm increased perfusion in the ear prior to imaging. There were some limitations as well due to our imaging model. The Braedius probe provided a useful view of the vessels themselves, shown in Figure 5, however, we were unable to measure the architectural details of a consistent vascular network, including specific vessels to serially monitor diameter changes. Another limitation of this study is the lack of knowledge of the change in perfusion needed to achieve the desired clinical outcome.

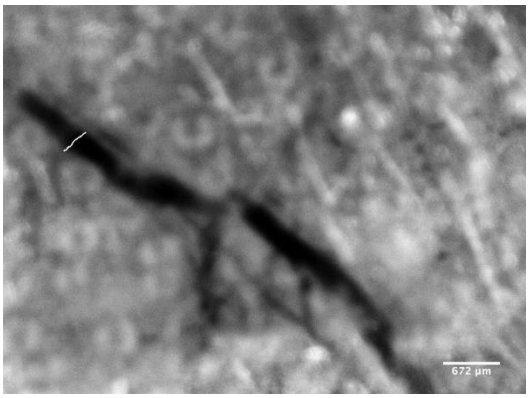

Figure 5. Braedius image of a mouse ear vessel 10 minutes post injection.

Figure 6. Positioning of mouse for imaging.

Several of these limitations can be worked on to create a stronger model for studying these vasoactive agents. We have shown the two-dimensional laser Doppler perfusion imaging can provide data to map perfusion change over time. The sidestream dark field microscopy through the Braedius probe has potential for measuring vessel diameter change. A step for future work would be creating a stationary structure for the handheld probe with a mapping system to enable observing and following one particular vessel through an application or injection.

Materials and Methods

Animal experiments were performed under a protocol approved by the University of Virginia Institutional Animal Care and Use Committee (Animal Welfare Assurance #A3245-01) in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Mice were housed in an AAALAC-accredited facility. 10 female mice C57BL/6 (10 weeks old) were included in the study. The mice were all anesthetized with inhaled isoflurane (1.5-2%), had their eyes covered with ophthalmic ointment to maintain ocular hydration, tails marked with a specifying number, and their ears shaved to remove hair from the ear and surrounding area and then wiped down with ethanol. Mice were laid down on their stomach with their nose placed in a cone to inhale isoflurane and oxygen. Each ear was placed on a small petri dish and lightly held down with aquagel, shown in Figure 6. Each mouse was imaged pre-application or pre-injection to obtain a baseline.

Topical Applications of Agents

For the topical applications of the vasoactive agents, the products used were, a nitroglycerin containing paste (2%) (Nitro-Bid TM, Savage Laboratories, Melville, New York), and 4% Lidocaine HCL, (BurnJel TM, Water-Jel Technologies L.L.C., Carlstadt, New Jersey) a gel. About one square centimeter that was 0.5 millimeter thick was applied to the whole ear using a cotton swab application. Tegaderm (Tegaderm TM, 3M, St. Paul, Minnesota), a transparent occlusive dressing, was cut to form a cover over both sides of the ear. This was applied to mimic clinical conditions as well as to prevent possible agent removal by mice. Mice were placed in individual cages once they regained consciousness. After two hours the mice were re-anesthetized with 1.5% inhaled isoflurane, the Tegaderm was removed, and both ears were imaged again using laser Doppler imaging. The agent and Tegaderm was then reapplied to the treated ears and mice were recovered. The mice were again placed in individual cages once they regained consciousness, and imaged at the six hour time point post initial application. The mice were put under and imaged once more 24 hours post initial application.

Injected Applications of Agents

For the injected applications of the vasoactive agents, the products used were 4% lidocaine and 2% diltiazem. Using a 25-gauge needle, 0.1 ml of solution was injected and fanned at the base of the ear, subdermal, near the main feeding vessel, shown in Figure 7 and Figure S1. The experimental ear and control ear were both imaged prior to the drug administration and every ten minutes for sixty minutes. The mice were then placed in cages with regained consciousness. The mice were put under and imaged four hours and 24 hours post injection.

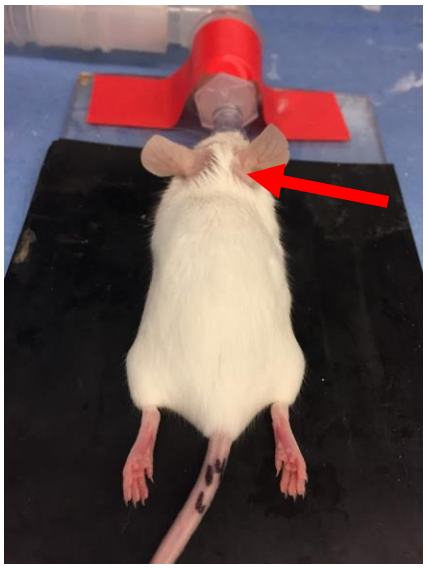

Figure 7. Mouse set up for application. The red arrow indicates the site of injection.

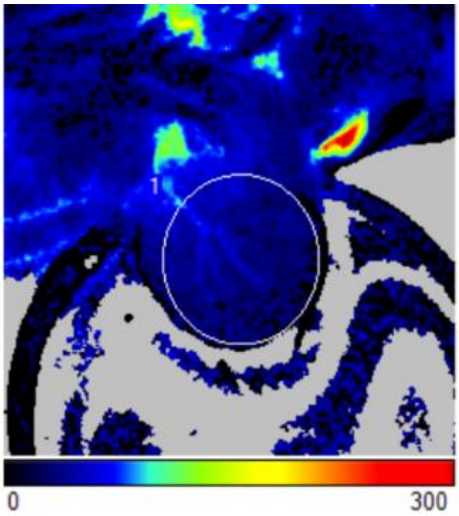

Figure 8. Image created by PimSoft of mice ear microvasculature during an injection experiment. The white circle is the region of interest. The color scale bar demonstrates the range of values picked up by the system.

Imaging Modalities

The two-dimensional laser Doppler perfusion imaging was done with a PeriScan PIM 3: Blood Perfusion Imager and PimSoft software for data analysis. The resolution was 0.09 mm, an average of 3 images were taken per time point, a duration of about 3 seconds, and the measurement area was a 3 cm x 3 cm square with a region of interest defined as a circle encompassing the ear, as shown in Figure 8. The laser Doppler imaging was used to estimate the movement of red blood cells for correlation to surface perfusion. The system emits a laser at a specific wavelength. This laser has a shallow penetration, and the beam is returned at a different wavelength. This difference is due to the amount of particles moving under the laser and the magnitude of the movement. The return wavelength is converted to a voltage for each pixel. The voltage is proportional to the relative perfusion. The voltage value for each pixel in the image within the region of interest was averaged and then normalized to the initial averaged value before any application. The color scale bar shows the voltage values from 0 to 300, the units were arbitrary.

The sidestream dark field microscopy was done with a CytoCam (TM, Braedius Medical, Netherlands) video microscope. It is a digital handheld video microscopy camera that is coupled with specialized software designed to capture and analyze video images. The optical system was designed for the purpose of microcirculation imaging. The imaging device consists of a pen-like probe incorporating incident dark field illumination with a set of high-resolution lenses projecting images on to a computer controlled image sensor synchronized with very short pulsed illumination light[13]. Illumination is provided by concentrically placed light-emitting diodes [14]. In this study, it was used to assess the peripheral and distal vessel prominence and observe the response of the vessels starting from the main branch of the vessels to the most distal small branches. The purpose of including this imaging modality was to be able to measure vessel diameter from the main feeding vessel to the distal branched vessels and quantify the vessel diameter change over time.

End Matter

Author Contributions and Notes

J.B. and J.R. designed research, J.B. and J.R. performed research, J.B. and J.R. analyzed data; and J.B. and J.R. wrote the paper.
The authors declare no conflict of interest.

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15. Fig 2. Two types of vasculature are in mouse ear. (A) Observation... *ResearchGate* https://www.researchgate.net/figure/Two-types-of-vasculature-are-in-mouse-ear-A-Observation-point-B-Whole-mount_fig6_279989775.

Supplementary Material

Table S1. Summary of Trials and Mice completed for each agent.

Drug	Number of Trials	Number of Mice
Lidocaine Injection	3	3
Diltiazem Injection	1	1
Nitroglycerin Topical	3	6
Lidocaine Topical	1	2

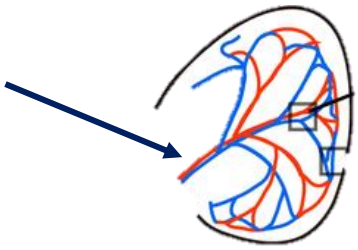

Figure S1. Basic schematic of vasculature of mouse ear for reference. Arrow is pointing to main feeding vessels that branches out into the ear.[15]