January 2018

Arterial And Venous Impacts Of Transdermally Administered Vasodilators On The Local Microvasculature

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Arterial and venous impacts of transdermally administered vasodilators on the local microvasculature

A Thesis Submitted to the

Yale University School of Medicine

in Partial Fulfillment of the Requirements for the

Degree of Doctor of Medicine

by
George Guo

2017

ABSTRACT

ARTERIAL AND VENOUS IMPACTS OF TRANSDERMALLY ADMINISTERED VASODILATORS ON THE LOCAL MICROVASCULATURE

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Microcirculatory function is an important component of cardiovascular pharmacology as related to cardiovascular dysfunction. We used photoplethysmography (PPG) to compare the microcirculatory effects of transdermal patches of rivastigmine (Exelon, Novartis), nicotine, nitroglycerin (NTG) and topically applied EMLA (eutectic mixture of lidocaine/prilocaine). We anticipate that this initial pilot comparison of single doses of each medication will catalyze future multi-dose comparisons of the various vasodilatory features of these and other drugs.

Methods: With IRB approval, 10 healthy volunteers were monitored with PPG at the time each transdermal patch was applied and every 8 minutes afterwards for a total of 40 minutes. 1x1 cm portions of patches of rivastigmine, nicotine, and NTG were placed and monitored on different sites of the forehead. Another site was isolated and pretreated 6 hours earlier with EMLA, since this drug requires many hours to induce vasodilation.[1] All voltage changes were changed to ACmults, i.e., in multiples of the change in voltage associated with delivery of the stroke volume to the given site under resting conditions[2]. A linear mixed model was used to compare patch effects on

maximum change in AC, DC, and ΔAC/ΔDC. This model accounts for the variance that can be attributed to an individual's multiple measurements within an unstructured covariance matrix. A p-value <0.05 was given to be statistically significant. Data were expressed as mean within a 95% confidence interval.

Results: The max ΔAC change for each drug were significantly different from that of its control while only the max ΔDC of NTG was significantly greater than that of its control. Changes in the ΔAC/ΔDC ratio were found to be inconsistent. Rivastigmine and control had significantly lower ΔAC/ΔDC values at 8 minutes compared to that of EMLA; the differences were not significant at following time points.

Discussion: These results may provide some insight into the cardiovascular effects of the study agents used. NTG, a direct NO donor, caused significant increases in AC (arteriolar) and DC (venous) values. Acting at the pre/post ganglionic junction of local parasympathetic pathways to the region of the precapillary sphincter, nicotine caused a significant increase in AC, whereas the change in DC was not found to be significant. Rivastigmine, which inhibits the metabolic degradation of acetylcholine, caused a selective increase in AC. The local anesthetic (EMLA) caused a significant increase in AC. Rivastigmine caused significantly lower ΔAC/ΔDC ratio at 8 minutes when compared to EMLA (which had been on for 6 hours previously). At $\geq$16 minutes, the ΔAC/ΔDC values of rivastigmine and EMLA did not differ significantly, findings that may reflect the time needed for acetylcholine to to increase over time via the inhibition of its breakdown as opposed to an immediate introduction of additional agonist via the nicotine or NTG patch. Future studies using different and/or additional drug

combinations may help give further insight into the convoluted physiology of the microvasculature.

**The above abstract was presented at the American Society of Anesthesiologists 2016 annual meeting.*

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my research mentor, Dr. David G. Silverman, for his guidance and support. It is an honor to receive valuable instruction from such a passionate and tireless researcher. I would also like to express my thanks to Katherine Chuang, Nitin Sukumar, Feng Dai, Dr. Aymen Alian, Dr. Kirk Shelley, and Dr. Paul Heerdt. Without their contributions, this project would have been impossible. Moreover, my gratitude goes to Ms. Jacki Fitzpatrick for her continued help and encouragement.

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INTRODUCTION

Photoplethysmography (PPG) is a simple and low-cost optical technique that is used non-invasively at the surface of the skin to detect blood volume changes in microcirculation [1]. Photoplethysmography was first rendered in 1938 by Hertzman of the United States and Matthes and Hauss of Germany and has since been extensively studied and used in a wide range of commercially available medical devices for measuring oxygen saturation, blood pressure and cardiac output, assessing autonomic function and detecting peripheral vascular disease [2, 3].

Since its conception, the PPG has contributed to improvements in clinical practice but has also continued to mystify physicians and researchers. Though the origins of the PPG signal are not fully understood, it is generally accepted that they can provide valuable information about the cardiovascular system [4]. In the 1970s, a major advancement in the clinical use of a PPG-based technology came when its utility as a non-invasive method for monitoring patients' arterial oxygen saturation was realized [5]. Since then, the PPG has been used in various other clinical applications and is now in several commercially available medical devices - pulse oximeters, vascular diagnostics, and digital beat-to-beat blood pressure measurement systems. This success has been possible despite the characteristics of the PPG waveform from being thoroughly studied and understood; more research needs to be done to uncover the myriad of physiologically relevant information contained in the PPG.

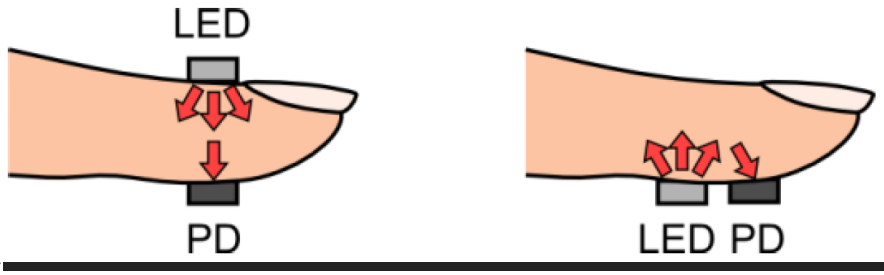
This study will build on previous work done in our laboratory that converts changes in PPG voltage to changes in blood flow in the local environment secondary to

locally targeted vasodilators. We will use PPG monitoring to see if any differences can be seen in arterial and venous blood flow between different vasodilatory drugs.

PHOTOPLETHYSMOGRAPHY

PPG uses a light source to illuminate tissue (usually the skin) and a photodetector that measures small variations in light intensity associated with changes in perfusion in the local microvasculature. It is often used at the red or near infrared wavelength to facilitate measurement of blood flow. The photodetector converts light energy into an electrical current, which is connected to low noise electronic circuitry that includes a transimpedance amplifier and filtering circuitry [3].

There are two configurations of photoplethysmography that can be utilized in practice. The most common modality is "transmission mode" in which tissue is placed between a light-emitting diode and a photodetector, e.g. across a fingertip or earlobe (see Figure 1A). A pulse of blood increases both the optical density and path length through the illuminated tissue, a process that decreases the light intensity at the photodetector. In "reflectance mode," the photodetector is placed alongside the light-emitting diode (see Figure 1B) and records the light that returns after being reflected by non-hemolyzed red blood cells [6].



A. B.

Figure 1. Configurations of PPG in transmission mode (A) and reflection mode (B).

The quantitative relation between blood perfusion through an irregular network of vessels, and the fraction of photons that pass through the tissue or is reflected, is complicated. The Beer-Lambert law gives us a way to understand the PPG waveform in more simplistic terms; in other words, assuming a uniform and diffuse layer of blood perpendicular to a beam of light and taking into consideration only light absorption (not scatter, refraction, reflection), the Beer-Lambert law states that light intensity decays as a function of distance exponentially [6].

There are two components to parcel out with regard to the PPG waveform. The baseline level of the photoplethysmogram is referred to as the direct current (DC) and is a relative index of skin vascularity that is affected by perturbations in thermoregulation, respiration, metabolic state, drugs, central and local regulatory mechanisms [6]. This baseline is not steady but contains low-frequency oscillations due to changes in recruited capillary densities and venous volume fluctuations. These oscillations have been found even distal to an arterial tourniquet, which may represent sympathetically mediated shifting of blood between different compartments of the peripheral microcirculation [7]. The pulsatile waveform attributed to changes in blood volume generated by individual heart beats is referred to the alternative current (AC) and is a manifestation of cardiac stroke volume typically seen around 1 Hz (See Figure 2). This is, as stated, superimposed on top of the slowly varying DC baseline which relates more to tissue (See Figure 3) including skin, bone, muscle and average blood volume [3].

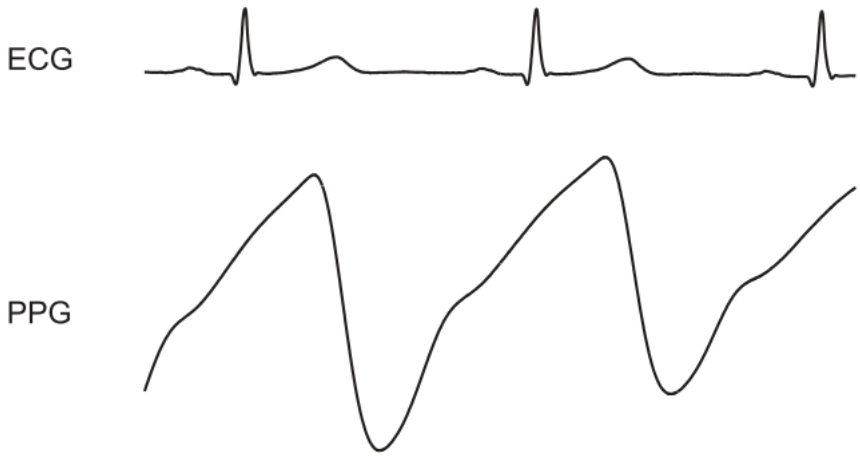


Figure 2. The pulsatile (AC) component of the PPG and corresponding electrocardiogram (ECG)

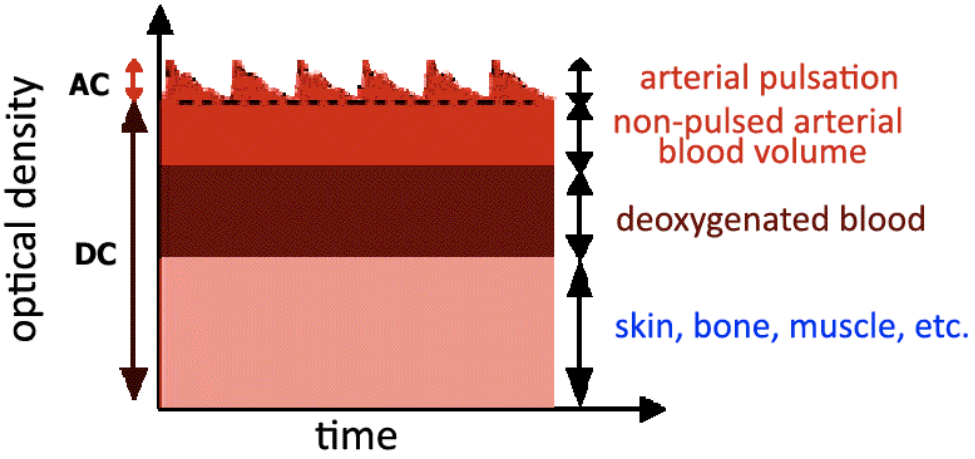


Figure 3. AC & DC components of the PPG

With regard to most commercial pulse oximeters however, the baseline PPG waveform usually has been electronically processed to remove baseline vacillations and reduce signal distortions to yield a photoplethysmogram that appears "cleaner." There is no one standard method for removing oscillations in the DC baseline; in fact, many processing algorithms vary among manufacturers and are often proprietary. Some also "autoscale" the PPG waveform so that the amplitude of the signal is adjusted to fit

neatly in the display window. Unfortunately, this processing removes much of the useful information in the PPG waveform.

ASSESSMENT OF ENDOTHELIAL FUNCTION

The endothelium is the layer of thin, single layer of cells that line the interior surface of blood vessels to form an interface between the circulating blood and the vessel wall. These cells line the entire circulatory system from the heart to the capillary. Endothelial cells are involved in many aspects of vascular functionality, including vasoconstriction and vasodilatation, blood clotting, angiogenesis, atherosclerosis, and inflammation [3]. Endothelial dysfunction is considered to be an early event in atherosclerosis and is associated with major risk factors for cardiovascular disease. Traditionally, endothelial function is assessed non-invasively by an ultrasound to measure the brachial artery diameter before and after several minutes of blood flow occlusion in the arm [8]. The change in arterial diameter gives an indication of flow mediated endothelium-dependent vasodilatation. Unfortunately, the technique is operator dependent and requires a high degree of skill, which could limit its usefulness for routine clinical assessments. PPG has also shown potential for the assessment of endothelial function but is much less costly than the ultrasound approach. The potential for PPG assessments to assess vasodilatation has been demonstrated using hemodynamic responses to nitroglycerin (NTG) therapy [9]. Understanding the physiology of the microvasculature is a vital part of understanding endothelial dysfunction, a key component of cardiovascular disease. As nitroglycerin is but one of many types of vasodilatory drugs, our laboratory aims to use NTG and other drugs

(rivastigmine, nicotine and an eutectic mix of local anesthetics, i.e. EMLA) to further refine the PPG as an instrument to better understand what may be occurring at the level of the microvasculature in response to specific, locally-targeted vasodilatory drugs. This is especially interesting within the context of testing more than one drug at the same time and seeing potential additive effects on the local microvasculature.

To date, sildenafil, commonly known as Viagra, is the most commercially successful drug ever launched. As a potent vasodilator, it is prescribed to increase the flow of blood to the penis to treat erectile dysfunction. Despite its efficacy or perhaps because of it, many have reported on its interaction with NTG, a potent vasodilator commonly prescribed to treat heart disease, to produce potentially lethal hypotension. Sildenafil, a phosphodiesterase-5 inhibitor (PDE5), blocks the enzyme responsible for the breakdown of cyclic GMP. NTG is an exogenous organic nitrate that releases nitric oxide (NO) near vascular smooth muscle cells. Nitric oxide activates soluble guanlyl cyclase, an enzyme that catalyzes the formation of cGMP from GTP. Thus, by inhibiting the breakdown of cGMP, the effect of cGMP-dependent protein kinase G (PKG) is potentiated and induces greater vascular smooth muscle relaxation through the phosphorylation of myosin light chain kinase (See Figure 4).

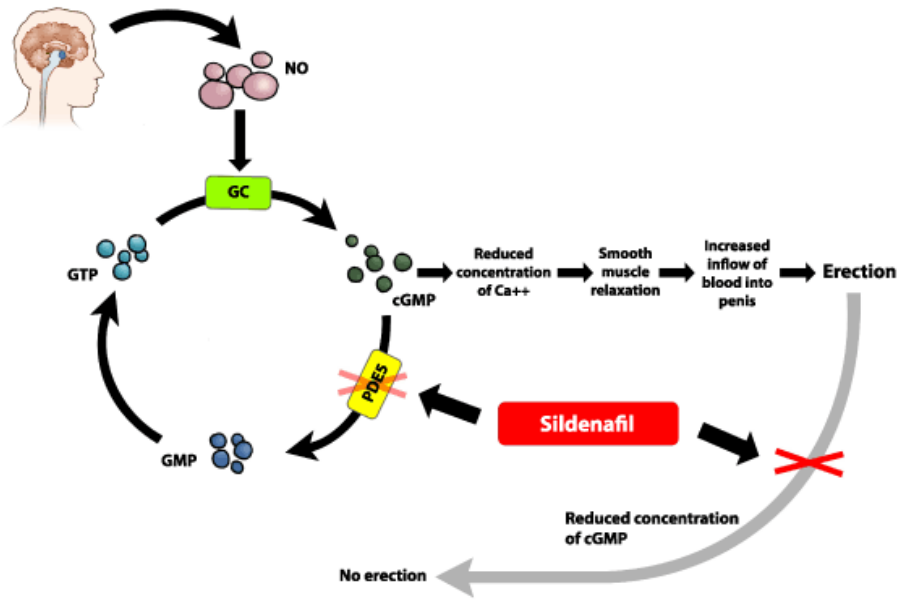


Figure 4. Mechanism whereby sildenafil induces greater vascular smooth muscle relaxation

Because of the potentially lethal risks involved in testing adverse drug reactions, effective studies utilizing normal human physiology is difficult. Webb et al found the coadministration of sildenafil and isosorbide mononitrate resulted in a significantly greater decrease in both sitting and standing blood pressure than that of the coadministration of sildenafil and placebo [10]. A double-blind, randomized study led by Parker et al also found that doses of intravenous NTG (between 5ug/min to 80ug/min) infused after 100mg of sildenafil resulted in a 4 to 6mmHg hypotensive effect as compared with placebo [11]. However, it would be unwise to extend the results of this study to interactions of sublingual NTG with sildenafil or other PDE5 inhibitors. It is therefore important to develop a standard model of study that can be used safely and consistently in human subjects.

PURPOSE & HYPOTHESIS

Our laboratory has developed a dual-platform drug model that utilizes "micropatches" as a way to test and compare the direct effects of a variety of drugs on the level of the microvasculature in conjunction with a drug taken systemically. Such a method allows any potential drug interactions to be seen in a localized and controlled environment that minimizes systemic risk. Furthermore, activation of multiple systemic responses and reflexes, many of which may modulate the effect at the target site, is minimized. Thus, the dual-platform drug model is a promising way to evaluate the effects of NTG and sildenafil taken conjointly, as well as other drugs which may have a different propensity for inducing vasodilation and hypotension.

Our study is divided into two phases. As NTG, rivastigmine, nicotine and EMLA induce vasodilation via different physiological mechanisms, the first phase of our study focuses on comparing their vasodilatory effects on the peripheral microvasculature without systemic perturbation. In the second phase of our study, we will use the dual-platform drug model to determine how these effects change with systemic administration of sildenafil. This thesis will focus its attention primarily on the first phase as it represents my contribution to a larger study.

In this study, PPG is used as a noninvasive monitor of arterial and venous microcirculation. We compared the effects of transdermal patches of nicotine, rivastigmine (dependent upon autonomic pathways and intact endothelium) and NTG (endothelium-independent) with topically applied EMLA. Because these drugs act through differing pathways, we hypothesize that specific differences in their local vasodilatory effects can be captured by information extracted from the PPG waveform.

MATERIALS & METHODS

With IRB approval, 10 healthy volunteers were enrolled in a double-blind crossover study conducted over two sessions with a one-week washout period in between in which subjects were randomized between two days and given either a placebo pill or a sildenafil pill. Subjects were 10 healthy medical students. All subjects received verbal and written descriptions of the study protocol with associated risks. An informed consent approved by the IRB was signed by all volunteers. Though the full protocol will be described here, this paper will focus its analysis exclusively on sessions where a placebo pill was used.

During one session, the subject received transdermal translucent patches of nitroglycerin, rivastigmine, nicotine and EMLA in conjunction with a placebo pill; on the other day, the subject received the same set of patches in conjunction with sildenafil. Sildenafil (100mg capsule) and placebo pills were produced and blinded by an external research pharmacist. Both pills were designed to look and feel identical and were given to experimenters and subjects in the same fashion in order to preserve double-blinding. The blinding was broken only after data analysis.

In order to minimize information bias, the sites at which the patches were administered were randomized between subjects. Because sildenafil no longer interacts with nitroglycerin 24 hours after sildenafil administration (and may be gone as early as four hours after sildenafil intake), we anticipated that repeating the study a week later to be a sufficiently long enough washout period to minimize any carryover effect.

Two hours after sildenafil or placebo administration, we obtained baseline readings of microvasculature dilation with PPG. 1x1 cm transdermal patches of

nitroglycerin, rivastigmine, nicotine were then placed and monitored on different forehead sites randomized between the two sessions, along with a control site that was also monitored without any drug. A specific template demarcating this area was utilized that allowed for adequate spacing between all drug sites (See Figure 5). New sites on the forehead that have not had any drug exposure were then utilized the week after to minimize potential confounding and carryover effect. In order to compare data points from the two days (placebo vs sildenafil) for each subject, the same measuring probes were used to minimize measuring differences that may exist between probes. Of note, EMLA came prepackaged as a topical cream; an additional 1x1 cm area of the forehead was pretreated with EMLA for 6 hours and removed before PPG monitoring as this drug had a longer prodromal phase in comparison.

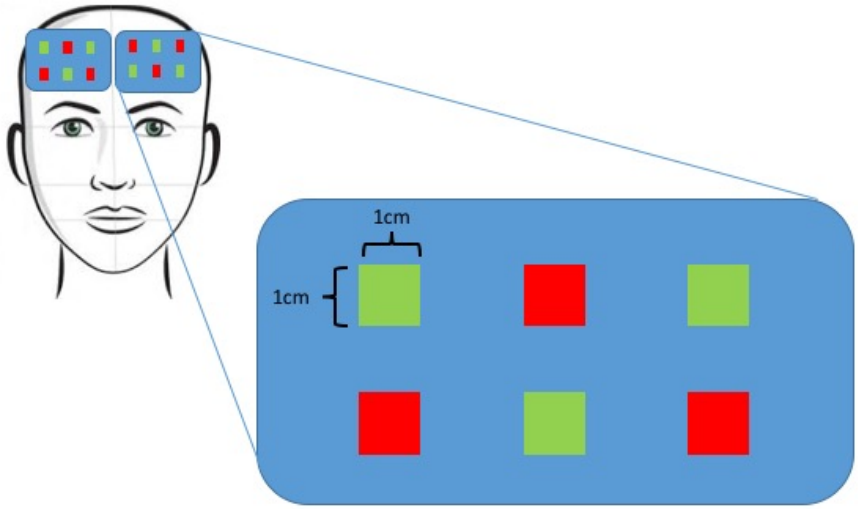


Figure 5. Template for patch application on subject forehead. Red boxes were utilized during first session. Green boxes were used during second session to minimize potential confounding and carryover effect of drugs.

Our research subjects were monitored with fixed gain reflective PPG (ADI probe: Novametrix Medical Systems Inc) interfaced via bridge amplifer to data acquisition system, LabChart (ADInstruments, Boulder CO), at the time of application and every 8 minutes afterwards for 40 minutes with sampling frequency of 200 Hz (See Figure 6). Continuous monitoring was not performed in order to minimize any confounding risk of probe heating on vasodilation.

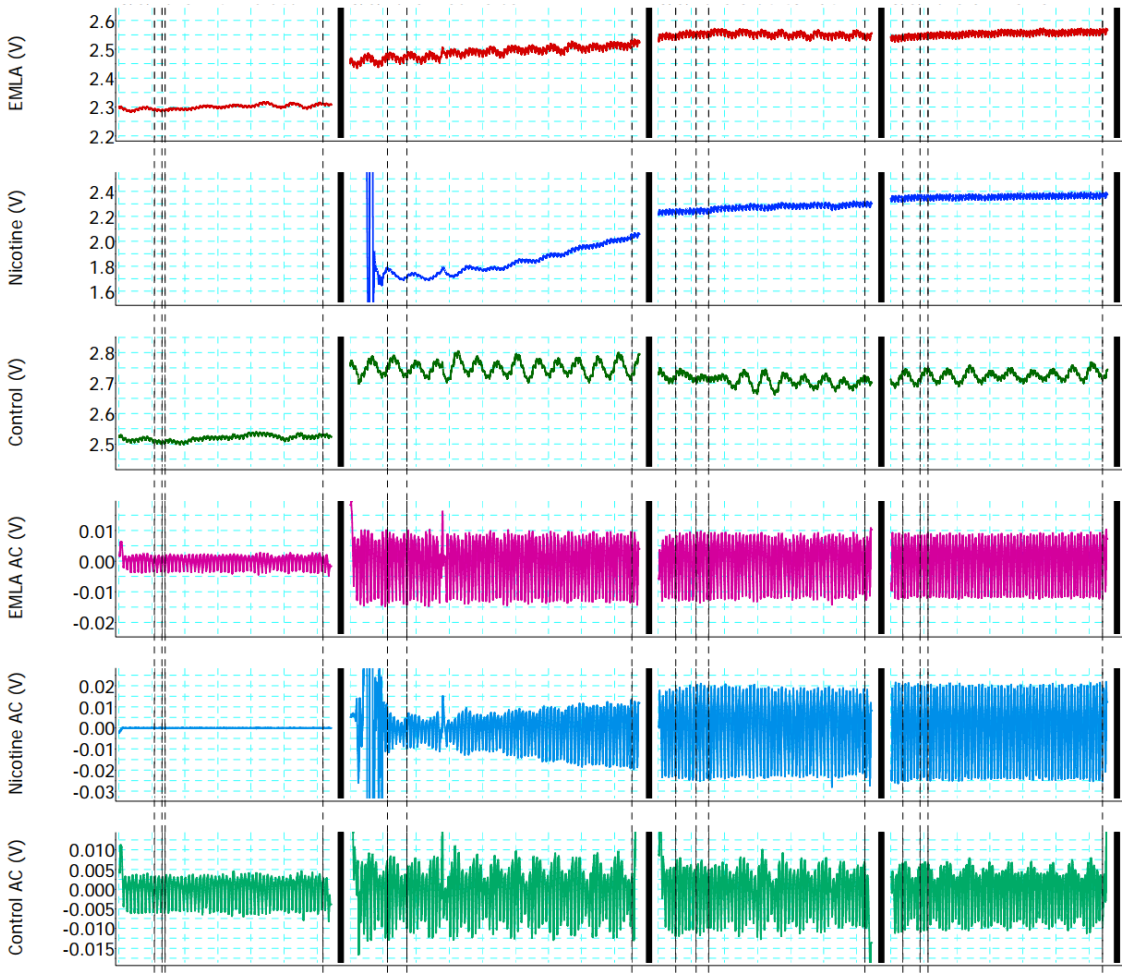


Figure 6. PPG sample of a research subject undergoing the experimental protocol for nicotine, EMLA and control. Upper three panels show the raw PPG signal. Lower three panels show the filtered AC signal. From left to right, columns designate pre-testing

baseline, baseline at 0 minutes at initial patch application, 8 minutes afterward, and 16 minutes afterward.

Previous studies from our laboratory have shown that a voltage to volume conversion based upon pulsatile volume at rest allows volume monitoring using a PPG when resting stroke volume is known (measured or estimated). That is to say, one is able to distinguish between arterial and venous components of the PPG signal under certain assumptions. Our previous work has shown that PPG monitoring at a site relatively devoid of vasoconstrictive activity (e.g., forehead) reveals relative degrees of pulsatile and nonpulsatile blood volume similar to the 3000/125 (~24/1) relationship between circulating venous volume and stroke volume. Thus, all voltage changes can be expressed as "AC multiples at rest," or "AC equivalents," i.e., in multiples of the change in voltage associated with the pulsatile delivery of the stroke volume to the given site under resting conditions [12]. The AC multiple at rest is associated with an AC voltage at rest; this voltage then becomes the calibrating voltage that one can then use to make sense of changes in AC and DC in terms of multiples of "stroke volume." This estimation is used because AC is independent of background and normalizing to the AC found at rest eliminates impact of attenuation. Thus, when a baseline stroke volume is available (e.g., 125 ml as measured by echo or estimated from population average), change in volume (in ml) can be calculated from the PPG based upon the following conversion:

Stroke volume at rest = 125 ml = 1 [AC multiples at rest] ~ AC calibrating voltage = [AC voltage at rest].

Change in volume (in ml) can be calculated in one of two ways.

= 'Given reading in [AC multiples at rest]' x '125 ml/1 [AC multiples at rest]'

or

= 'Given readings in volts' x '125 ml/[AC voltage at rest]'

ANALYSIS

All PPG waveforms were extracted by ADInstruments LabChart. A linear mixed model was used to compare the patch effects on maximum change in AC, maximum change in DC, and maximum change in ΔAC/ΔDC. This model accounts for the treatment group, day of study, repeated subjects, the sequence of treatment, and the variance attributable to an individual's multiple measurements with an unstructured covariance matrix. Sequence of treatment refers to which order the subject received sildenafil and placebo on day 1 and day 2 of the study. Subjects were treated as random effects in the model. A p-value <0.05 was considered significant. Analyses was conducted on the assumption that controls can be pooled together.

From the raw data sets, the median values of the first 20 data points at each time segment for each patch on each day of study were calculated. Median was chosen as the statistic to use due to the fact that the sample mean can often be unduly influenced by outliers. The 0-minute time segment AC value for each patch on each day of study were used as denominators to calculate AC equivalents for all corresponding median AC and

DC values. The ΔAC and ΔDC values were calculated as changes in raw and AC equivalents from the 0-time segment values for each patch.

Maximum ΔAC and ΔDC values for each patch on each day of study were determined from the 0 to 40-minute data for patches NTG, nicotine, Exelon and its associated controls. Magnitude comparisons of changes in AC and DC in terms of absolute AC equivalents between patches were not done because drug patches weren't dose equivalent. Thus, max changes in the ratio between AC and DC were also obtained in similar fashion for each patch since the comparison of a ratio nullifies the dose effects of each patch, i.e., the apparent dose of drug that induced a certain change in AC should be of the same factor as that which induces the change in DC as well.

For patches EMLA and its associated control, maximum ΔAC and ΔDC values for each patch on each day of study were assumed to be at time segment 0, which was compared to a baseline taken 6 hours prior to application.

RESULTS

As shown in table 1, within the placebo session, the maximum ΔAC (in AC equivalents) was 1.211 units higher in Exelon compared to its paired control ($p=0.0223$). The maximum ΔDC (in AC equivalents) was -5.099 compared to its paired control, but this was not statistically significant. The maximum Δ(ΔAC/ΔDC) is 0.174 higher in Exelon compared to its paired control; this is a marginally significant difference ($p=0.0554$).

The maximum ΔAC (in AC equivalents) was 1.387 units higher in NTG compared to its paired control ($p=0.0002$). The maximum ΔDC (in AC equivalents)

was 37.479 compared to its paired control (p=0.0037). The maximum Δ(ΔAC/ΔDC) is 0.001 higher in NTG compared to its paired control; this was not statistically significant.

The maximum ΔAC (in AC equivalents) was 2.352 units higher in EMLA compared to its paired control (p<0.0001). The maximum ΔDC (in AC equivalents) and the maximum Δ(ΔAC/ΔDC) was not significantly different compared to its paired control.

The maximum ΔAC (in AC equivalents) was 2.581 units higher in nicotine compared to its paired control (p=0.0002). The maximum ΔDC (in AC equivalents) and the maximum Δ(ΔAC/ΔDC) was not significantly different compared to its paired control.

Table 1. Comparison of drug patch to control with regard to max ΔAC, max ΔDC, max Δ(ΔAC/ ΔDC) within the placebo session **(**in terms of AC equivalents)

	Max ΔAC (AC eq.)		Max ΔDC (AC eq.)		Max Δ(ΔAC/ΔDC)	
Patch	Estimate [95% CI]	p-value	Estimate [95% CI]	p-value	Estimate [95% CI]	p-value
Exelon	1.211 [0.217, 2.206]	0.0223 *	-5.099 [-29.378, 19.180]	0.6460	0.174 [-0.005, 0.353]	0.0554
NTG	1.387 [0.862, 1.911]	0.0002 *	37.479 [15.648, 59.309]	0.0037 *	0.001 [-0.223, 0.225]	0.9926
EMLA	2.352 [1.466, 3.238]	0.0002 *	10.757 [-28.937, 50.451]	0.555	0.031 [-0.799, 0.862]	0.9335
Nicotine	2.581 [2.098, 3.063]	<.0001 *	34.270 [-5.117, 73.658]	0.0806	-0.224 [-0.666, 0.217]	0.2799

*Denotes a p-value that is statistically significant

When comparing the effects of different drugs, the max change in ΔAC/ΔDC ratio was used as a way to control for differing dosing equivalents of drug patches. As shown in Table 2, comparisons between all possible combinations of drug patches were calculated. Some values are repeated albeit in the opposite direction (e.g., -0.262 vs 0.262 with regard to EMLA vs Exelon) depending on which drug patch was set as the reference of comparison during analysis. As can be seen, there are no significant differences between drug patches within the placebo session with regard to max Δ(ΔAC/ΔDC).

Table 2. Comparison of max change of ΔAC/ΔDC between drug patches within the placebo session

	Max Δ(ΔAC/ΔDC)	
Patch Comparison	Estimate [95% CI]	p-value
vs Exelon		
EMLA	-0.262 [-0.719, 0.195]	0.2275
NTG	-0.075 [-0.208, 0.058]	0.2348
Nicotine	-0.016 [-0.193, 0.161]	0.8414
vs Nicotine		
EMLA	-0.246 [-0.605, 0.113]	0.1558
Exelon	0.016 [-0.161, 0.193]	0.8414
NTG	-0.059 [-0.152, 0.034]	0.1873
vs NTG		
EMLA	-0.187 [-0.563, 0.189]	0.2902
Exelon	0.075 [-0.058, 0.208]	0.2348
Nicotine	0.059 [-0.034, 0.152]	0.1873
vs EMLA		
Exelon	0.262 [-0.195, 0.719]	0.2275
NTG	0.187 [-0.189, 0.563]	0.2902
Nicotine	0.246 [-0.113, 0.605]	0.1558

Table 3 compares EMLA at its max effect, which is specifically found at time 0 (PPG monitoring directly after removal of EMLA status post pretreatment of 6-hour duration), against all other drug patches at 8 minutes, 16 minutes and 24 minutes during the placebo session. Again, because of dose equivalency considerations, ΔAC/ΔDC ratios were utilized. EMLA was used as the reference group.

Table 3A. Comparison of ΔAC/ΔDC ratios at EMLA time 0 and time 8 minutes for other patches during the placebo session.

	ΔAC/ΔDC within Placebo	
Patch	Estimate [95% CI]	p-value
Exelon	-.07875 [-.13149,-.02600]	0.0082*
NTG	-.03114 [-.07383,.011554]	0.1334
Nicotine	.020799 [-.08800,.129594]	0.6756
Control	-.07716 [-.14914,-.00518]	0.0383*

*Denotes a p-value that is statistically significant

As shown in Table 3A, during the placebo session, the ΔAC/ΔDC ratio was -0.079 units lower in Exelon at 8 minutes compared to EMLA at 0 minutes (p=0.008). The ratio of the control at 8 minutes was 0.08 units lower compared to the ratio of EMLA at 0 minutes (p=0.04).

Table 3B. Comparison of ΔAC/ΔDC ratios at EMLA time 0 and time 16 minutes for other patches during the placebo session.

	ΔAC/ΔDC within Placebo	
Patch	Estimate [95% CI]	p-value
Exelon	.033609 [-.08899,.156211]	0.5505
NTG	-.03177 [-.07981,.016262]	0.1688
Nicotine	-.03484 [-.10243,.032744]	0.2735
Control	-.05746 [-.11836,.003445]	0.0616

As shown in Table 3B, there were no significant differences between the EMLA ΔAC/ΔDC ratio at 0 minutes and the ΔAC/ΔDC ratio for other patches at 16 minutes during the placebo session.

Table 3C. Comparison of ΔAC/ΔDC ratios at EMLA time 0 and time 24 minutes for other patches during the placebo session.

	ΔAC/ΔDC within Placebo	
Patch	Estimate [95% CI]	p-value
Exel	-.03907 [-.10351,.025375]	0.2035
NTG	-.03257 [-.07733,.012184]	0.1341
Nico	-.02888 [-.08427,.026507]	0.2684
CtrlN	-.03895 [-.25341,.175500]	0.6908

As shown in Table 3C, there were no significant differences between the EMLA ΔAC/ΔDC ratio at 0 minutes and the ΔAC/ΔDC ratio for other patches at 24 minutes during the placebo session. Across these comparisons, EMLA tends to have the highest ΔAC/ΔDC ratio, but as mentioned, most of these differences are not significant.

DISCUSSION

The PPG is a powerful tool replete with information that has yet to be fully understood. Given its ability to observe changes in blood circulation in local cutaneous tissue, it is a great tool to understand the mechanisms and effects of different drugs at the level of the microvasculature. In our study, we aim to understand the differences between four locally targeted vasodilators including NTG, nicotine, rivastigmine, and EMLA with regard to changes in venous and arterial microcirculation. This study attempts to lay the foundational groundwork for future phases and studies in which additive drug combinations are "layered" systemically on top of locally targeted drugs. This is the concept of the "dual platform" drug model to assess pharmacological interactions in a localized and controlled manner that minimizes systemic risk.

As seen in our results, all four drugs resulted in significant increases in the AC component of the PPG as compared to control. For example, in terms of AC equivalents or stroke volume equivalents, nicotine showcased the largest AC increase with 2.581 AC equivalents versus rivastigmine with an increase of 1.211 AC equivalents. If we take resting stroke volume to be 125ml, a 2.581 AC equivalent increase is estimated to be an increase in 322.625 ml versus 151.375 ml with regard to rivastigmine. Though these increases seem to be vastly different, we must remember that our patches are not dosed equivalently. A direct comparison of patches that take into consideration dosing differences using changes in the ratio of AC and DC does not show the patches to be statistically significantly different. This again extends not just to nicotine versus EMLA, but to all possible drug comparison combinations in our study. While this conversion is

not relevant to local changes in the microvasculature, it may prove valuable in the context of challenges and changes in systemic volume.

Interestingly, NTG was the only drug that resulted in significantly increased changes in DC along with significantly increased changes in AC. It is known that nitrates predominantly cause the dilation of peripheral veins, and in higher doses, cause the dilation of peripheral arteries [13]. As an exogenous organic nitrate, it likely releases nitric oxide (NO) near vascular smooth muscle cells both before and after the pre-capillary sphincter, acting in conjunction on both the arterial and venous side within the local microvasculature.

Parasympathetic ganglion and post-ganglionic parasympathetic fibers have been considered as potential mediators of vasodilation in the peripheral microvasculature [14]. Since the arteriole-capillary-venule microvasculature is not a homogeneous system, differences in the extent of autonomic innervation, where endogenous NO is produced and where NO can act may influence the extent of vasodilation measured. In contrast to NTG, acetylcholine (ACh) and nicotine administered peripherally via micropatches are likely activators of postganglionic parasympathetic fibers, which induce the release of ACh [15]. Nitric oxide synthase is constitutively present in endothelial cells; ACh induces endothelium-dependent vasodilation and stimulates nitric oxide synthase to produce endogenous NO, producing vasodilation in areas with vascular smooth muscle tissue [16]. Thus, a transdermal acetylcholinesterase inhibitor like rivastigmine (or nicotine directly) will be able to prolong the effects of ACh within the parasympathetic ganglion and induce vasodilation through endothelium-dependent processes. Our study seems to show that these postganglionic parasympathetic fibers

may be more predominantly concentrated at (rather than beyond) the pre-capillary sphincter, thus resulting in more of an arterial vasodilatory effect.

On the other hand, EMLA (an eutectic mix of local anesthetics), which blocks all sodium channels in nerves, would block transmission of all electrical impulses in the periphery [17]. Since blood vessels are held under a certain level of constant vasoconstriction through sympathetic activity, the release of this constriction via EMLA produces vasodilation where sympathetic activity was seen previously. Though we are not able to discern or isolate the potential for off-target effects that EMLA may produce within the local area, our study seems to indicate that the primary measurable vasodilatory effect was isolated to the arterial microcirculatory system before the pre-capillary sphincter.

When we use EMLA as a reference for comparison, interesting temporal relationships are observed. Because of the time it takes for EMLA to permeate through the upper epidermal layer, we pretreated a designated site 6 hours before our main experimental protocol. This 6-hour timeframe was determined through repeated experimentation as the minimum amount of time needed for measurable effects to be seen via PPG. Thus, at “time 0” when the other drug patches were placed and the EMLA cream removed before monitoring (due to the cream being opaque in nature), PPG measurements of the EMLA site should demonstrate maximal vasodilation, an effect confirmed by our experimental protocol. As expected, the vasodilatory effects of a “control patch” was significantly lower than that of EMLA at its peak effect as measured by changes in the AC to DC ratio. Of note, the vasodilatory effects of rivastigmine at 8 minutes were also significantly lower than that of EMLA at time 0;

this difference disappeared at 16 minutes and beyond. It is also important to note that this phenomenon was not observed in any other drug patch, i.e., the effects of other drug patches were not significantly different from that of EMLA at 8 minutes or beyond. Rivastigmine is unique among the drugs that we tested as the sole acetylcholinesterase inhibitor [18]; it prolongs the effect of of ACh within the parasympathetic ganglion by preventing its breakdown and thereby induce vasodilation through endothelium-dependent processes. We hypothesize that this particular temporal "delay of effect" with regard to rivastigmine at 8 minutes represents a sort of "enzymatic lag" that may reflect the time needed for acetylcholine to "build-up" in the synaptic cleft via the inhibition of its breakdown as opposed to an immediate introduction of additional agonist via the nicotine or NTG patch. Further repeated testing in the future will be conducted to confirm this hypothesis.

A main limitation of this study revolves around the use of the ADI probe, a research PPG device that unfortunately causes local tissue warming and thus, potentially introduces a source of external vasodilation unrelated to the drug patches themselves. We tried to remedy this issue by using the probe only in one minute intervals (every 8 minutes) to minimize probe heating; still, it is uncertain how much variability this may have introduced into the study. Development of a probe that can provide a DC signal without relying on a research probe that causes local tissue warming would be ideal.

As stated, this paper represents the first phase of study in which we lay the foundation for understanding the complex physiology of the microvasculature. Future analysis will explore this environment with different and/or additive drug combinations

to further elucidate the physiological interaction between several different drug combinations in a safe and reproducible manner, particularly in conjunction with sildenafil taken systemically. Evidence indicates that Alzheimer's disease may be derived from a vascular pathology characterized by cerebral hypoperfusion and that pharmacotherapy that improves cerebral perfusion often lowers neurodegenerative symptoms [19]. If it is possible to find a drug combination that can maximally induce vasodilation within the microvasculature while minimizing systemic hypotensive risk, clinical applications may exist for Alzheimer's and other more well known cardiovascular diseases.

CONCLUSION

In summary, the PPG may be used as a non-invasive monitor of arterial and venous microcirculation. We aimed to better elucidate the effects of various transdermal vasodilators on the forehead microvasculature and understand the underlying mechanisms at work for each drug. Such a study lays the groundwork for future experiments exploring potential drug interactions in a safe and reproducible manner devoid of systemic adverse effects.

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