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Microvascular Effects of Pulsed Dye Laser in Combination with Oxymetazoline

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Abstract

Background and Objective.—Oxymetazoline, an alpha-1A agonist, is approved by the United States Food and Drug Administration (FDA) for treatment of persistent facial erythema associated with rosacea and induces vasoconstriction by interacting with alpha receptors. The objective of our study was to study the microvascular effects of oxymetazoline and pulsed dye laser (PDL).

Materials and Methods.—A dorsal window chamber was surgically installed on 20 mice. Each animal was assigned to one of four experimental groups: saline alone, oxymetazoline alone (10 μL applied once daily x 7 days) , saline + PDL [saline applied five minutes before PDL irradiation (10 mm spot, 1.5 ms pulse duration, 7 J/cm^2 delivered to epidermis)], or oxymetazoline + PDL (10 μL oxymetazoline applied five minutes before PDL and then once daily x 7 days). Brightfield and laser speckle imaging were performed for seven days to monitor vascular architectural and functional changes.

Results.—We observed persistent blood flow in all of the saline-only and oxymetazoline-only experiments. A higher rate of vascular shutdown was observed with oxymetazoline + PDL (66.7%) compared to saline + PDL alone (16.7%). Oxymetazoline application increased venule diameter at 5 min post-application and decreased both arteriole and venule diameters at 60 min post-application.

Conclusion.—The combination protocol of oxymetazoline + PDL induces persistent vascular shutdown observed 7 days after irradiation. This result may be associated with the acute vascular effects of oxymetazoline. Oxymetazoline + PDL should be evaluated as a treatment for cutaneous vascular disease, including rosacea and port wine birthmarks.

Keywords

oxymetazoline; dorsal skinfold; laser speckle contrast; phototherapy

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Introduction

Rosacea affects roughly 16 million Americans and can have significant psychosocial impacts on a patient's quality of life [1]. There are multiple treatments, but many patients do not find significant relief from available options. Erythematotelangiectatic rosacea, which presents with vasodilation and telangiectasias, can be particularly challenging to treat [1]. Pulsed dye laser (PDL) treatment can be utilized, but many patients require multiple treatments, and recurrence is frequent.

Topical medications have also been utilized to treat rosacea. For instance, alpha agonists induce vasoconstriction via interaction with alpha receptors. Brimonidine is an alpha-2 agonist that was approved for persistent facial erythema associated with rosacea in 2013. While efficacy of this medication was demonstrated, some patients stopped application due to adverse effects including worsening of facial erythema [2]. Oxymetazoline, an alpha-1A agonist, was more recently approved by the United States Food and Drug Administration (FDA) for treatment of persistent facial erythema associated with rosacea. This medication appears to have an improved side effect profile [3,4].

Ryan et al. [5] evaluated two patients with persistent facial erythema for 7 hours after the application of oxymetazoline. With optical coherence angiography, they noted that at approximately 5 minutes after application, the vascular area was increased, followed by a subsequent decrease of the vascular area by 60 minutes and a return to baseline levels at 1-2 days post-treatment. Hence, oxymetazoline appears to induce acute vascular changes.

Combination treatments with laser and topical medication can provide an enhanced result, achieving a better effect than either treatment modality alone [6,7]. Here, we evaluate the preclinical potential of combined oxymetazoline and PDL for the treatment of cutaneous vascular disease, using a translational preclinical *in vivo* model of the microvasculature. We hypothesized that combining oxymetazoline with PDL would result in increased rates of persistent vascular shutdown, compared to saline with PDL.

Materials and Methods

Animal model.

This study was approved by the Institutional Animal Care and Use Committee at University of California, Irvine. Twenty adult (20-25 g) C3H mice were used in this study. Figure 1 provides a timeline of procedures. For each experiment, a rodent dorsal window chamber was installed (Figure 1A) [8]. Before the surgery, each mouse was anesthetized with isoflurane (2%, balance oxygen) followed by i.p. injection of a cocktail of ketamine (90 mg/kg) and xylazine (10 mg/kg). The dorsum was shaved and the remaining hair removed using a depilatory cream (Nair). The dorsal skin was pulled upwards, and two biocompatible titanium plates were installed with screws and sutures on to each side of the skinfold. One ~10 mm diameter, full-thickness region of skin was removed with surgical scissors and excess fascia gently dissected from the subdermal side of the remaining full thickness of skin. Sterile saline was applied on to the subdermal side and a circular glass coverslip placed

on top of the subdermal side to maintain hydration and mitigate the risk of infection. A metal retaining clip was placed on top of the coverslip to maintain its position. The body temperature was maintained using a heating pad and monitored throughout the procedure. After the window chamber surgery was completed, the animal was allowed to recover for two days.

Wide-Field Optical Imaging.

To monitor structural and functional changes to the microvasculature, we used a combination brightfield/laser speckle imaging (LSI) device described previously [9]. A scientific CCD camera (Chameleon, FLIR Systems) equipped with a macro lens (Nikon) was used for imaging. Each animal was positioned so that the subdermal side of the window chamber was proximal to the camera. For brightfield imaging, a white-light source equipped with a green filter was used to transilluminate the window chamber. The filter was used to maximize visualization of the microvasculature, using hemoglobin absorption as the primary source of contrast. For LSI, a 30 mW, 633 nm HeNe laser (Edmund Optics) was used to transilluminate the window chamber. For all LSI images, the camera exposure time was set to 10 ms and a sequence of 30 images collected.

Experimental Design.

The animals were divided into four experimental groups: saline alone (n=3), oxymetazoline alone (n=5), saline + PDL (n=6), and oxymetazoline + PDL (n=6). On day 0 (i.e., two days post-surgery), brightfield imaging and LSI was performed before the application of either oxymetazoline or saline. Ten μL of oxymetazoline or saline was applied to the epidermal side of the window. The oxymetazoline or saline was applied to all experimental groups at approximately the same time each day for 7 days after the initial application. The mice from the saline alone and oxymetazoline alone groups were imaged again at 5 minutes and 60 minutes post-application. For the saline + PDL and oxymetazoline + PDL groups, PDL irradiation (10 mm spot; 1.5 ms pulse duration; 7 J/cm^2 radiant exposure) was applied to the epidermal side at 5 minutes post-application of oxymetazoline or saline, in accordance with a previously reported observation [5] of increased vascular volume at this time point. These mice were then imaged at 5 minutes and 60 minutes post PDL. LSI was repeated at 5 minutes post-application on days 1, 2, 3, and 7; and 60 minutes post-application on days 2 and 7 (Fig. 1B).

Image Analysis.

To determine changes in arteriole and venule diameter in response to oxymetazoline or saline application, we selected five regions of interest within segments of arterioles and venules in the brightfield images. From these regions, vessel diameters were estimated. The same regions of interest were characterized at each subsequent time point. To determine changes in blood flow in response to oxymetazoline or saline application, we converted each raw speckle image of a given sequence into a speckle contrast image, using a 7x7 sliding window, as previously described [10]. Each speckle contrast image was converted to a Speckle Flow Index (SFI) image, commonly used as a map of blood flow. A mean SFI image was calculated from each SFI image in the sequence. The same ROIs selected in a corresponding brightfield image were applied to the mean SFI images to calculate a single

SFI image of each region of interest. Statistical analysis included parametric (paired t-test) and non-parametric tests (Wilcoxon matched-pairs signed rank test) performed in Prism (Version 8, GraphPad Software) to test the null hypotheses that there is no change in vessel diameter or blood flow with oxymetazoline application.

Results

We observed persistent blood flow in all of the saline alone (n=3) and oxymetazoline alone (n=5) experiments (Fig. 2A). Most (5/6) of the saline + PDL experiments resulted in a significant decrease in perfusion 5 minutes post PDL on Day 0. On Day 7, 5/6 experiments exhibited at least partial reperfusion of the window chamber (Fig. 2B). In contrast, 4/6 of the oxymetazoline + PDL experiments had persistent vascular shutdown (Fig. 2C), 1/6 had reperfusion on day 7, and 1/6 exhibited substantial vascular remodeling (Fig. 2D), with smaller, tortuous vessels indicative of angiogenesis. These data collectively suggest that persistent vascular shutdown can be achieved with a combined oxymetazoline + PDL treatment protocol.

To elucidate the mechanism of the differences in shutdown rates seen in the various treatment groups, we analyzed changes in vessel diameter and blood flow. Venule diameter increased ($p=0.03$) at 5 minutes post-application of oxymetazoline and was smaller ($p= 0.002$) at 60 minutes post-application (Fig. 3). At 5 minutes post-application, arteriole diameter was unchanged at 5 minutes (Fig. 3), but arteriole flow was higher ($p= 0.04$) (Fig. 4). Arteriole diameter also was smaller ($p= 0.02$) at 60 minutes post-application (Fig.3). Both arteriole diameter ($p=0.0001$) and blood flow ($p=0.03$) were increased at day 7 (Fig. 5).

Discussion

Our data suggest that the oxymetazoline + PDL protocol resulted in more consistent vascular shutdown than any of the other study groups. Saline + PDL did result in an initial decrease in flow, but reperfusion occurred over the 7-day observation period; this result is in agreement with our previous findings using similar PDL settings. As expected, saline alone and oxymetazoline alone did not result in vascular shutdown.

Oxymetazoline alone did affect the vasculature. After oxymetazoline application, we observed an increase in venule diameter at 5 minutes post-application (but no change in arteriole diameter) and a decrease in both arteriole and venule diameters at 60 minutes post-application. These trends are in agreement with optical coherence angiography measurements of blood volume in human subjects receiving topical oxymetazoline [5]. Previous research reported that different subtypes of alpha 1 receptors vary in their effects on arterioles versus venules. Alpha 1A receptors, which are targeted by alpha 1A agonists such as oxymetazoline, play a more critical role in the maintenance of blood pressure in arterioles than in venules [11]. This observation suggests that we would have observed a more significant effect on arterioles than on venules. However, results from other studies indicate that while oxymetazoline has a high affinity to alpha 1A receptors, it has a lower affinity to alpha 2B receptors, which are primarily involved in the contraction of arterioles [12,13]. Also, a constriction response in venules is mediated primarily by alpha 1B and

alpha 2D adrenergic receptors [12]. Ligands often recognize more than one adrenoceptor subtype, which may explain the effect that oxymetazoline alone had on both venules and arterioles [11]. The effects of oxymetazoline need to be further investigated to understand better the mechanisms associated with the persistent vascular shutdown that we observed in the oxymetazoline + PDL group.

Our data do not fully explain the improved shutdown observed with combined oxymetazoline + PDL. However, we did observe an increased venule diameter at 5 minutes and decrease at 60 minutes, which together likely contributed to the observed effect. An increase in vessel diameter would increase the local volume of hemoglobin, which in turn would increase the volumetric heat generation resulting from PDL irradiation. The decrease in arteriole and venule diameter at 60 min may facilitate the ensuing photo-induced coagulation effect [14]. This demonstration of persistent vascular shutdown with oxymetazoline + PDL suggests that this combination protocol may offer improved results for the treatment of cutaneous vascular disease, including rosacea and vascular malformations such as port wine birthmarks. Clinical trials are required to determine the safety and efficacy of the combination treatment for these indications.

Limitations exist in our study. Despite the statistically significant results and trends identified in this study, the power of these findings is limited due to a small sample size. Also, the skin and vasculature of the mouse dorsal window chamber differ from humans. Although the preclinical model serves as a common model for translational studies of phototherapies [15,16], the parameters used here may differ from those with therapeutic efficacy on human subjects. We would expect that the increased flow at 5 minutes followed by vasoconstriction that we observed, would occur in humans as well, but this will need to be confirmed in clinical studies.

CONCLUSIONS

The combination protocol of oxymetazoline + PDL induces persistent vascular shutdown observed 7 days after irradiation. This result may be associated with the acute vascular effects of oxymetazoline, including a pre-PDL increase in venule diameter followed by vasoconstriction observed in arterioles and venules at 60 min post PDL. Oxymetazoline + PDL should be evaluated as a treatment for cutaneous vascular disease, including rosacea and port wine birthmarks.

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Abbreviations Used:

PDL	Pulsed dye laser
LSI	Laser speckle imaging

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(b)

Day	Procedure
-2	Dorsal window chamber surgery
0	Brightfield and laser speckle imaging
	Application of either oxymetazoline or saline
	Wait 5 min
	PDL (if applicable)
	Brightfield and laser speckle imaging (t = 5 min)
	Brightfield and laser speckle imaging (t = 60 min)
1-6	Application of either oxymetazoline or saline
7	Brightfield and laser speckle imaging

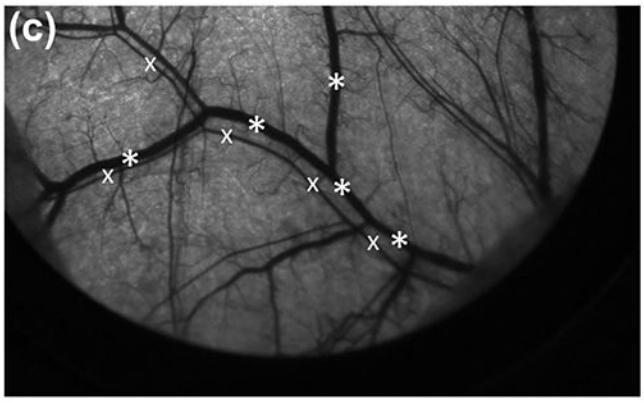

Figure 1.

Workflow of experiments. (**a**) Rodent dorsal window chamber model. (b) Chart listing the workflow for experiments. (**c**) Example of selection process for regions of interest. Asterisks (*) denote selection of a venule region while x's (x) denote selection of an arteriole region.

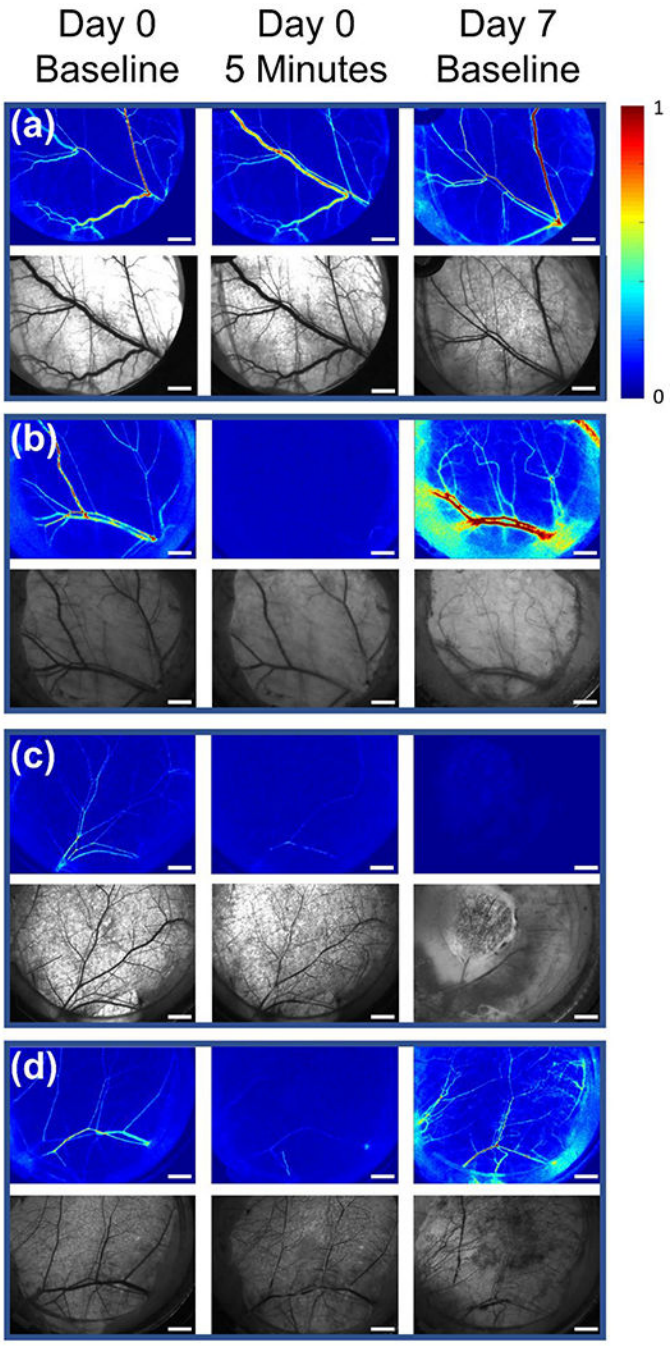

Figure 2.

Representative images of changes in vasculature after seven days. The top (colored) panels are Speckle Flow Index Maps, and the bottom (greyscale) panels are Brightfield Images. (**a**) Application of only oxymetazoline showed no signs of vascular shutdown. (**b**) Pulsed Dye Laser (PDL) with saline showed immediate shutdown after initial treatment, but exhibited reperfusion after the seven-day observation period. (**c**) PDL with oxymetazoline resulted in persistent vascular shutdown over the seven days. (**d**) One of the six PDL with oxymetazoline experiments displayed remodeling of vasculature with tortuous vessels.

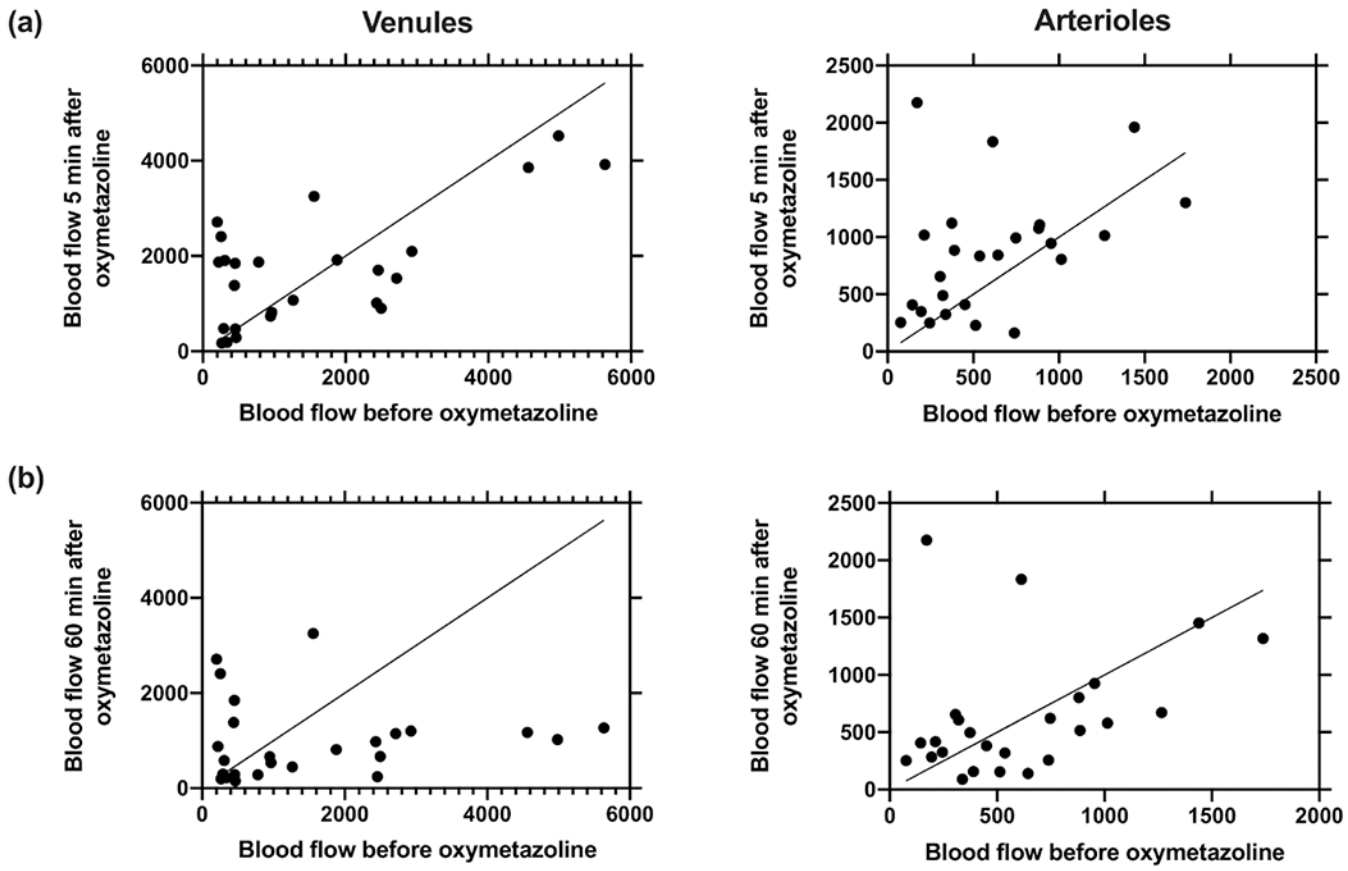

Figure 3.

Acute effect of oxymetazoline on vessel diameter. (**a**) Immediate (t = 5min) effect of oxymetazoline on (left) venule and (right) arteriole diameter. Diameters of vessels (five vessels/animal) identified with transillumination green-light imaging. There is a significant increase in venule diameter (p=0.032, n=5) but no change in arteriole diameter (p=0.98, n=5). (**b**) Short-term (t = 60min) effect of oxymetazoline on (left) venule and (right) arteriole diameter. There is a significant decrease in venule diameter (p=0.018, n=5) and arteriole diameter (p=0.016, n=5). The solid line in each graph represents no change in vessel diameter.

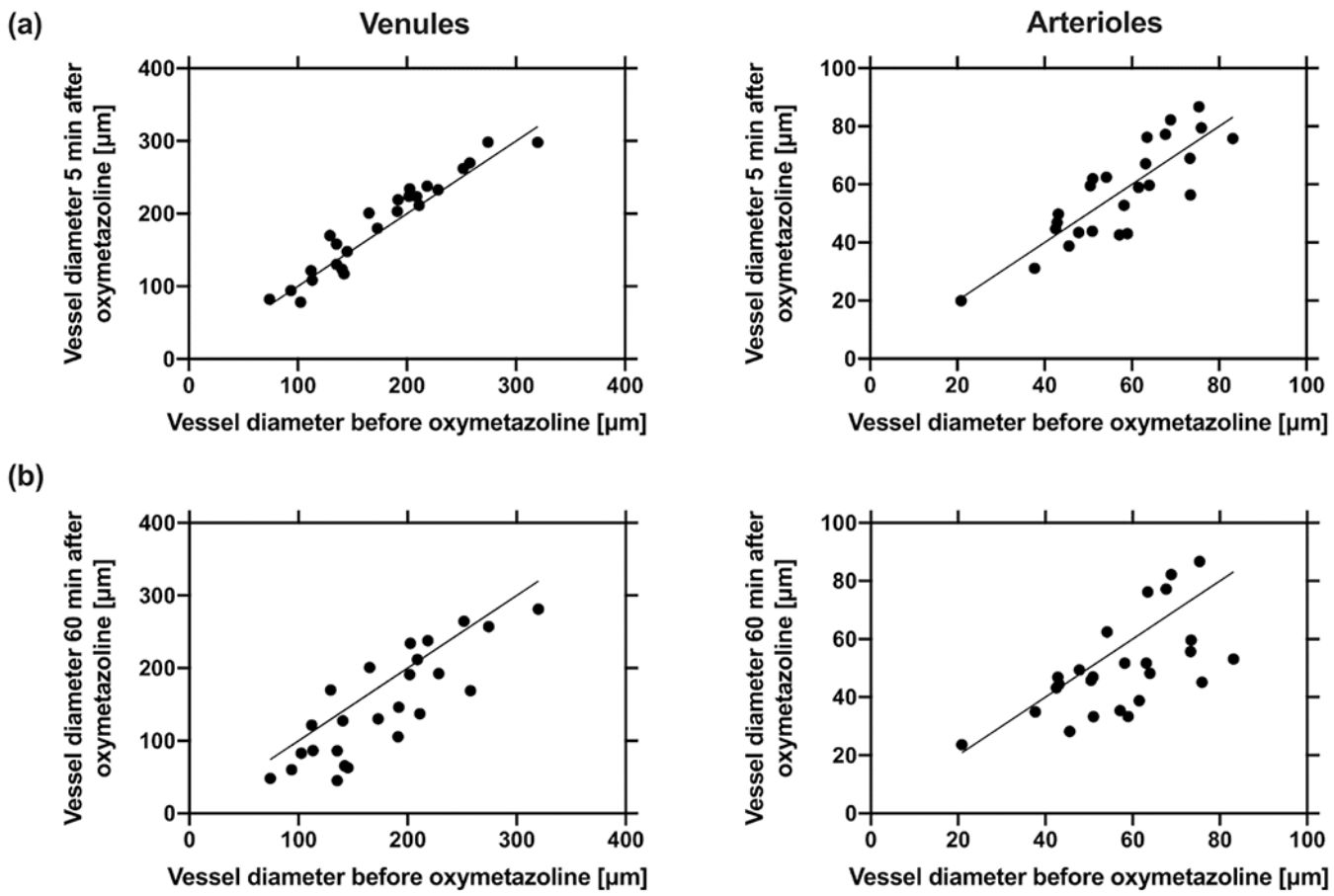

Figure 4.

Acute effect of oxymetazoline on blood flow. (**a**) Immediate (t = 5min) effect of oxymetazoline on (left) venule and (right) arteriole blood flow. Speckle Flow Index of vessels (five vessels/animal) identified with transillumination green-light imaging. There is a significant increase in arteriole blood flow (p=0.039, n=5) but no change in venule blood flow (p=0.55, n=5). (**b**) Short-term (t = 60min) effect of oxymetazoline on (left) venule and (right) arteriole blood flow. There is no significant change in venule (p=0.11, n=5) or arteriole blood flow (p=0.43, n=5). The solid line in each graph represents no change in vessel diameter.

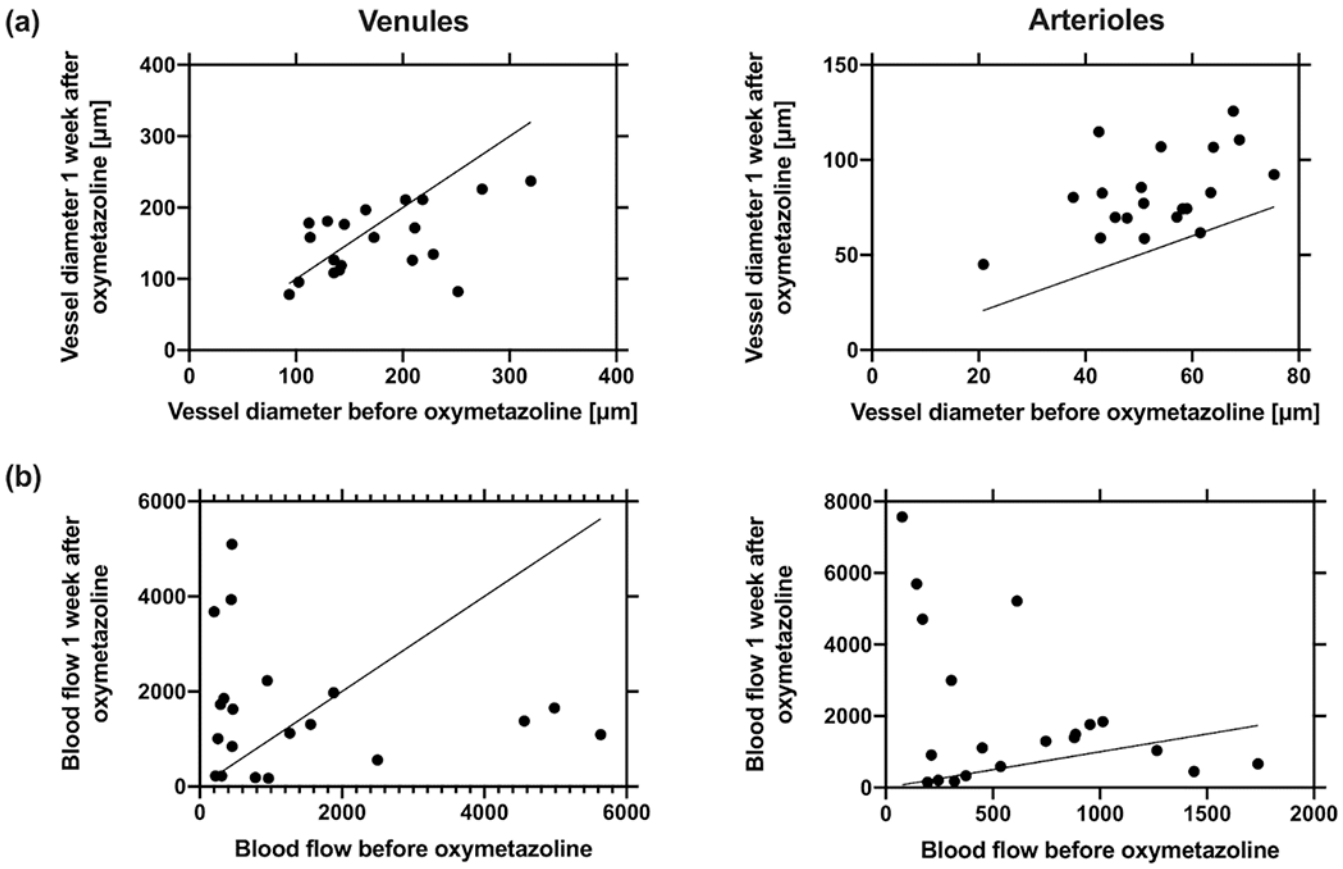

Figure 5.

Longer-term (7 day) effect of oxymetazoline on (**a**) vessel diameter and (**b**) blood flow. (**a**) Vessel diameter of vessels (five vessels/ animal) identified with transillumination green-light imaging. There is no change in venule diameter ($p=0.11$, $n=4$) but a significant increase in arteriole diameter ($p<0.0001$, $n=4$). (**b**) One week change in blood flow. There is no significant change in venule blood flow ($p=0.75$, $n=4$). A significant increase in arteriole blood flow ($p=0.027$, $n=4$) was observed. The solid line in (**a**) represents no change in vessel diameter, and in (**b**) represents no change in vessel blood flow.