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In vivo, micro-morphological vascular changes induced by topical brimonidine studied by Dynamic optical coherence tomography

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Abstract

Background Brimonidine is a selective α2 adrenergic receptor agonist with potent vasoconstrictive activity topically used for treatment of facial flushing and erythema caused by rosacea. Direct evidence for the *in vivo* morphology changes in skin vessels induced by topical application of brimonidine is limited. Dynamic optical coherence tomography is a novel technology that combines conventional OCT with information on flow and thereby provides supplementary information about the microvasculature. Dynamic OCT is non-invasive and creates high-resolution *in vivo* images of skin to a depth of maximum 2 mm.

Objective The objective of this study was to examine and describe micro-morphological skin vessel changes in normal skin exposed to brimonidine gel using Dynamic OCT.

Materials and methods A total of 35 healthy subjects from three European clinical dermatology centres were included in the study. A normal skin area on the cheek was marked and clinically photographed. Brimonidine gel 0.33% was applied on the area and chromaticity measurements; laser speckle measurements and Dynamic OCT images were acquired at baseline and 60 min after application. The images were subsequently described in detail and quantitatively analysed.

Results All the measurement tools (chromaticity, laser speckle, Dynamic OCT showed highly significant ($P < 0.001$) quantitative differences in blood flow before and after the application of brimonidine. In 58% of the subjects the Dynamic OCT images showed notable changes in the morphology of the blood vessel network after application of brimonidine including a marked reduction in the abundance and the diameter of the blood vessels.

Conclusion The vascular constriction induced by topical brimonidine gel 0.33% was visualized *in vivo* by Dynamic OCT and confirmed by quantitative measurements and analyses. This study shows that Dynamic OCT can detect characteristic morphological changes in the vessels and may potentially aid the monitoring of treatment effects on skin vessels following topical or laser treatment.

Conflicts of interest

None.

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Introduction

Brimonidine is a selective agonist for the α2 subtype adrenergic receptors. Stimulation of peripheral post-synaptic endothelial a2 receptors induced by brimonidine has been shown to cause vasoconstriction *ex vivo* and clinically.[1,2] In 2013, the topical use of brimonidine 0.33% gel was approved by the US Food and Drug Administration for treatment of facial erythema and rosacea.[2] Several studies have looked at the effects of topical brimonidine gel focusing primarily on patients with facial erythema. Outcome measures for the effect have primarily been

clinician's assessment of erythema and patient's self-assessment.[3–5] Only few studies have objectively measured brimonidine gel's effect on vasculature and blood flow, and direct evidence for the *in vivo* vasoconstrictive effect on skin vessels is limited.[1,6,7]

Optical coherence tomography (OCT) is a non-invasive imaging tool that was first introduced in dermatology in 1997. OCT uses infrared light to visualize skin structures in a manner analogous to ultrasound, but with better resolution and contrast.[8] OCT creates real-time, high-resolution, cross-sectional and en face images of skin to a depth of up to 2 mm. A wide range of skin disorders has been studied with OCT and the capability of OCT to image skin lesions, including non-melanoma skin cancer, is now well established.[9,10] The vessel structures in *in vivo* scar tissue, non-melanoma skin cancer and telangiectatic vessels have previously been studied using OCT,[11–13] however, a novel angiographic variation of OCT, named 'Dynamic OCT' (or speckle variance OCT), has recently been developed to specifically extract structural information regarding the microvasculature of skin. The Dynamic OCT technique is sensitive to the minute movement of blood cells and is non-invasively able to image the dermal microvasculature.

The aim of this study was to investigate the skin vessel morphology changes in normal facial skin exposed to brimonidine 0.33% gel using dynamic OCT.

Materials and methods

The study was carried out in three European clinical dermatology centres from September 2014 to February 2015. The institutional review board of each centre approved the study and all subjects provided written informed consent in accordance with the Declaration of Helsinki.

A total of 35 healthy subjects aged >18 years and without active skin disease were included in the study. A round skin area was marked on the cheek with a waterproof marker and clinically photographed. The subjects then received a single thin layer of brimonidine gel 0.33% (Mirvaso®, Galderma, Sophia Antipolis, France) applied to the marked area. Skin temperature measurements, skin colour measurements (Chroma meter CR-400, Konica Minolta, Inc.,Tokyo, Japan laser speckle measurements (moorFLPI-2, Moor Instruments Ltd., Devon, UK) and Dynamic OCT images (VivoSight, Michelson Diagnostics Ltd., Kent, UK) of the marked skin area were acquired at baseline and 60 min after application of the brimonidine gel. To ensure reproducible results from all the clinical centres, the study procedures were rehearsed at a joint meeting before the outset of the study. All data from the three study centres were sent to the study coordinator at Roskilde Hospital, Denmark for data analysis.

Dynamic OCT

For this study we used the VivoSight OCT scanner (Michelson Diagnostics). It is a multi-beam Swept-Source Frequency Domain OCT system that uses a centre wavelength of 1305 nm and operates at an A-line rate of 20 kHz. The optical resolution is <7.5 μm laterally and <5 μm axially (in tissue), the scan area is 36 mm^2 and the *in vivo* imaging depth is maximum 2 mm. The OCT skin images are captured in both cross-sectional and en face mode and for this study we used the multi-slice scan modality consisting of 120 B-scans (in the *x-y* plane) separated by 50.4 microns in the y direction. The pixel size is 4.44 μm and the voxel size is 4.44 × 4.44 × 50.4 μm (for a 120 slice en face scan).

The *in vivo* images of the microcirculation in the skin were acquired using the built-in Dynamic OCT/speckle variance option in the VivoSight system. For the speckle variance calculation, rapidly repeated OCT scans were acquired in the same location and analysed to detect changes between these successive scans.[14] Postprocessing of the images resulted in the detection of blood vessels in the imaged skin area. The Dynamic OCT images were acquired by placing the hand-held probe on the skin. The 120-slice Dynamic OCT scan (scan area: 36 mm^2) took 30 s to acquire and there was no off-line postprocessing. The skin surface was not prepared before the OCT scan and no oil was used with the device. Users already familiar with the specific OCT system performed the OCT scans.

Dynamic OCT data analysis

To extract a quantitative measure of the speckle variance signal in the Dynamic OCT images, a proprietary software tool (Michelson diagnostics Ltd.) was used to analyse the images. The tool provides an indirect measure of blood flow in the Dynamic OCT images by measuring the amount of speckle variance signal across all frames at different depths below the skin surface. The speckle variance signal data are presented numerically in an arbitrary unit at each depth. To optimize signal strength and minimize noise, speckle variance signal was measured in 0.05 mm intervals at a depth of 0.1–0.35 mm below the skin surface, corresponding to the papillary dermis. The average numerical value from the depth interval (0.1–0.35 mm) was calculated for each dynamic OCT scan and the results were compared.

Micro-morphology and vascular pattern were assessed in en face Dynamic OCT images before and after the application of brimonidine gel. To avoid normal anatomical variation in vessel morphology (depth-dependent differences in vasculature) the same depth was chosen for all images. For this, we utilized the software tool 'OCTFITTER' (University of Modena and Reggio Emilia, Modena, Italy) that detects and follows the curved surface of the skin and thereby ensures that the whole area of the en face image (36 mm^2) is viewed at the same depth below the skin surface. To identify the most appropriate skin depth to evaluate and compare the vessel morphology, we plotted all of the speckle variance measurements against the depth below the skin surface and from this we calculated the maximum slope for each graph

indicating the highest variation in speckle variance signal. The correlated depths were identified and the average depth was calculated to be 0.33 mm below the skin surface. Two of the authors (LT and GJ) performed blinded evaluations of the randomized Dynamic OCT images at a skin depth of 0.33 mm. The blood vessels identified at this skin depth are located in the upper part of the dermis. The number of visible vessels on each frame was manually counted and the vascular pattern was classified qualitatively according to overall vessel size and vessel network (see Table 1).

Skin colour measurements

The Chromameter is a hand-held instrument for the determination of colour, based on the CIE L*a*b* colour system. In this study, we used the CR-400 Chroma meter (Konica Minolta Sensing). The a* parameter represents changes along a red/green axis with changes from +60 for a red surface to −60 for a green surface, in this way erythema and skin blanching could be quantified by the increase/decrease of the a* parameter. In this study, the changes in the a* parameter were used as an indirect measure of blood flow changes in the skin. The instrument was calibrated every day using a white calibration plate. Two chromameter measurements (each consisting of the mean value of three readings) were performed each time and the mean from these were then calculated. The skin measuring area of the chromameter was 8 mm in diameter and the probe was applied perpendicular to the skin surface.[15]

Laser speckle contrast imaging (LSCI)

LSCI is a non-invasive, non-touch laser based technique for imaging the microcirculation. LSCI measures average blood flow velocity and concentration of moving red blood cells and data are presented in an arbitrary unit called flux. In this study, we used the moorFLPI-2 system (Moor Instruments Ltd.) which has an imaging depth of maximum 1 mm measuring only superficial blood flow but with a fast measurement time, detecting changes in microcirculation over an area of up to 15 cm × 20 cm.[16] LSCI does not give any information on vessel morphology. During the study period, calibration of the system was performed every 2–4 weeks. We recorded LSCI measurements of the marked area on the skin over a time period of 30 s. The data were then analysed using moorFLPI review software (Moor Instruments Ltd.) and the average flux in the marked area on the cheek over the recorded time period of 30 s was calculated for each measurement.

Statistics

Correlation analysis of the blood flow measurement methods used was performed using Pearson's correlation coefficient.

To calculate the difference within each pair of before-and-after brimonidine quantitative Dynamic OCT, LSCI and skin colour measurements, we used the paired samples *t*-test. This statistical test determines the mean of the before-and-after brimonidine changes with exact 95% confidence intervals, and reports whether this mean of the differences is statistically significant. A P-value < 0.05 was regarded as statistically significant and a $P < 0.001$ was regarded as statistically highly significant. The analyses were carried out using (IBM SPSS Statistics for Macintosh version 22.0, IBM Corp, Armonk, NY, USA).

Results

A total of 35 healthy participants (9 men, 26 women) from three European clinical dermatology centres were included in the study. The median age was 30 years (23–60) and the skin types varied from I to IV on the Fitzpatrick scale. The included participants did not have active skin disease.

Correlation analysis (Pearson's) of the measurement tools showed that the OCT speckle variance (SV) signal measurements were significantly correlated with the laser speckle flux measurements $r = .40$, 95% CI [.152,.592], $P = 0.001$, and chromaticity a* measurements $r = .55$ [.319,.725], $P < 0.0001$; the flux measurements were also correlated with the chromaticity a* measurements $r = .71$, 95% CI [.577,.816], $P < 0.0001$.

All the measurement tools showed highly significant quantitative differences in blood flow before and after application of

Table 1 Results from the quantitative measurements and manual vessel count

	n	Mean value before topical brimonidine	Mean value 60 min after topical brimonidine	Mean difference	95% confidence interval	*P*-value
Quantitative measurements						
Dynamic OCT (speckle variance signal)	35	0.19	0.16	0.029	0.018–0.040	<0.0005
Chroma meter (a* value)	35	13.3	8.9	4.39	3.7–5.1	<0.0005
Laser speckle (flux value)	32	174.8	107.2	67.6	46.5–88.7	<0.0005
Number of vessels – manual count						
Observer 1	31	13.3	7.6	5.6	2.7–8.5	<0.0005
Observer 2	31	11.3	6	5.3	3.6–7.0	<0.0005

n, number of subjects in the analysis.

brimonidine. The results of the quantitative measurements of blood flow differences are summarized in Table 1.

Dynamic OCT

In four of the Dynamic OCT images, we experienced difficulties in processing the images using the 'OCTFITTER' and therefore we were only able to analyse the vessel morphology in 31 subjects.

In 19% of the subjects (6/31) the application of brimonidine led to a complete clearance of visible vessels (see Fig. 1).

In 39% of the subjects (12/31) the Dynamic OCT images showed a distinct change in the morphologic pattern of the facial vessels before-and-after brimonidine: The images showed that after the application of brimonidine, the smaller vessels ceased to be visible leaving only the large- and medium-sized vessels to be recognized. However, these remaining larger-sized vessels clearly had a reduced vessel diameter (see Fig. 2).

In 35.5% of the subjects (11/31) distinct changes in the size of the vessel network or the vessel morphology could not be detected. However, out of these 11 subjects 7 still showed a numerical reduction in visible vessels.

In 6.5% (2/31) of the subjects no distinct vessels were visible neither before nor after the application of brimonidine.

The results from the two observers' manual count of visible vessels in the Dynamic OCT images at a skin depth of 0.33 mm showed a highly significant decrease in visible number of blood vessels after brimonidine (see Table 1).

The quantitative results of the Dynamic OCT measurements of speckle variance signal also showed a highly significant decrease in blood flow 60 min after the application of brimonidine (speckle variance signal mean difference: 0.029). Looking at the results individually, two of the subjects showed an increase in speckle variance signal after brimonidine and in one subject no difference was registered.

Skin colour measurement

The skin colour measurements showed a significant overall decrease in redness (a* mean difference: 4.39) 60 min after the application of brimonidine. Looking at the subjects individually, all of them showed a varying decrease of redness (a*) after brimonidine.

LSCI

Measurements showed a highly significant overall decrease in blood flow (flux mean difference: 67.6). In two of the subjects a small increase in flux after brimonidine was registered. In one of the subjects the LSCI recordings appeared overly exposed and with multiple artefacts because of improper positioning of the subject, these recordings were therefore discarded. In two of the subjects some of the LSCI recordings had by mistake not been saved and these could therefore not be analysed leaving data from 32 subjects for analysis.

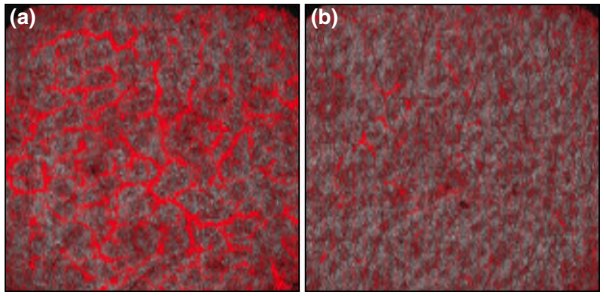

Figure 1 Dynamic OCT image, en face view, of normal skin located on the cheek before [image (a)] and 60 min after application of topical brimonidine [image (b)] (each image is sized: 6 × 6 mm). Image (a) shows a conspicuous network of blood vessels. In image (b) the application of brimonidine has led to an almost complete clearance of visible vessels.

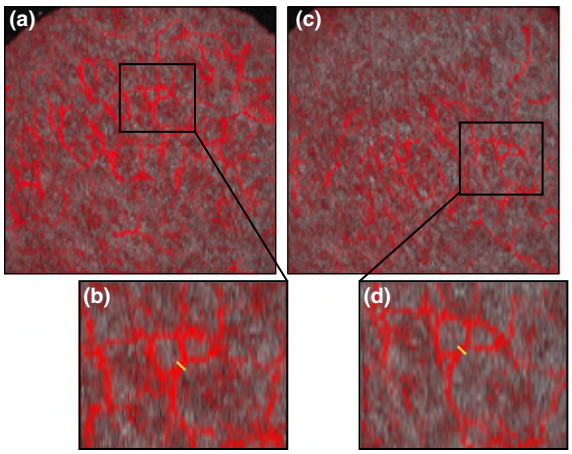

Figure 2 Dynamic OCT image, en face view, of normal skin located on the cheek before [image (a)] and 60 min after application of topical brimonidine [image (c)] [image (a) and (c) are sized: 6 × 6 mm]. The images show that there are a reduced number of vessels after the application of brimonidine, however, many of the medium to large-sized vessels are still visible (c). Image (b) and (d) are enlarged clippings of the white dashed areas seen in image (a) and (c). They show the exact same vessels in the Dynamic OCT images, both at baseline and after brimonidine application. In the (d) image the vessels seem to have a reduced diameter. The yellow bars indicate the location where measurements of vessel diameter were performed. The measurements in this example showed a reduced vessel diameter of 42% after the application of brimonidine (diameter at baseline: 0.08 mm; diameter after brimonidine application: 0.046 mm).

Discussion

In this multicentre study we investigated the effects of brimonidine 0.33% gel on *in vivo* facial skin in healthy subjects using Dynamic OCT.

In 39% of the subjects the Dynamic OCT images showed a distinct morphological pattern where the smaller vessels ceased

to be visible after the application of brimonidine, leaving only the large and medium sized vessels. These results are consistent with the results of a previous study by Piwnica *et al.* where brimonidine was found to be a highly potent vasoconstrictor on human isolated *ex vivo* subcutaneous arteries smaller than 200 μm in diameter. The potency and efficacy of brimonidine was found to decrease as the vessel diameter increased.[1] In four of the subjects, we were able to identify and directly compare the exact same large-sized vessels in the Dynamic OCT images both at baseline and after brimonidine application (an example of this is seen in Fig. 2). In one of these cases we could measure a reduced vessel diameter of 42% after the application of brimonidine (diameter at baseline: 0.08 mm; diameter after brimonidine application: 0.046 mm). The fact that some of the larger-sized vessels at a skin depth of 0.33 mm did not have a complete response to the topical brimonidine 0.33%, whereas the smaller-sized vessels readily disappeared in the Dynamic OCT images after brimonidine application, confirms that topical brimonidine is mostly efficacious in the smaller-sized vessels. However, the reduced diameter of the larger-sized vessels implies that brimonidine 0.33% does have a vasoconstrictive effect, but that it exerts an inadequate constriction of larger-sized vessels leaving the lumen remaining open. A previous case report described cross-sectional OCT imaging of a rosacea patient treated with topical brimonidine 0.33%,[17] but in this case the authors found no significant change in vessel lumen diameter before and after topical brimonidine treatment. The en face mode and the speckle variance signal displayed in the Dynamic OCT system makes it easier to visualize individual vessels and vascular networks.

By using non-invasive Dynamic OCT imaging, we were able to focus on the morphologic changes of the skin vessels to a depth that included the dermis (facial skin is ~1 mm thick[18]). The results of the manual vessel count also confirm the results of the quantitative measurement of blood flow in the Dynamic OCT images. The observations are supported by the use of quantitative measurements of Dynamic OCT speckle variance signal as an indirect measure of blood flow. These indicate a highly significant decrease (0.029, $P < 0.005$) in skin blood flow 60 min after the application of topical brimonidine.

The results were in agreement with the results of skin colour measurements and LSCI, both of which are validated non-invasive techniques for measurement of skin blood flow.[15,16]

Most studies on topical brimonidine have used subjective outcome measures such as erythema rating scales to evaluate the efficacy of the drug[3] and only a few previous studies have objectively tried to evaluate the effects of topical brimonidine by using laser Doppler perfusion monitoring, chromameter measurements or examination of histology sections.[1,6,7] These studies were, however, performed on either rat/mouse models or *ex vivo* human skin models and thus do not provide information on the effects of brimonidine on *in vivo* human skin. Topical brimonidine is thought to be effective in decreasing facial erythema and inflammation by inducing vasoconstriction and increasing vascular tone[1,2,17] although, to our knowledge, no studies have previously been able to show the direct effect that brimonidine exerts on skin vessels *in vivo*.

Limitations of the Dynamic OCT technique

The primary limitation of the Dynamic OCT technique is that OCT imaging is limited in depth penetration, and noise becomes dominant at about 500 μm. Therefore, it is not suitable for measurements of very thick skin, nor can it penetrate to measure the deep vascular dermis. Its resolution may also be insufficient to detect and measure flow in the finest blood capillaries (diameter ~10 μm).

A full en face Dynamic data set (of 120 frames) takes 30 s to acquire, meaning that any changes faster than this cannot be observed in the en face data sets. In our study, we were interested in physiological changes occurring over 60 min, so the 30 s capture timeframe was thought to be sufficiently fast to detect the vascular changes in this study. Further Dynamic OCT studies are needed to investigate the regional vascular differences in the skin and the impact of, e.g. age and photo damage in the skin. Improvement of the underlying methodology and algorithms are required to fully characterize the limits of Dynamic OCT.

Conclusion

In this study we have shown that Dynamic OCT imaging is able to visualize vascular changes induced by topical brimonidine 0.33% gel. Furthermore, the measurements of the speckle variance signal are a useful quantitative measure of blood flow changes in the skin. Our study only looked at very short-term effects (60 min.) induced by topical brimonidine 0.33% gel and we therefore suggest the need for studies on Dynamic OCT monitoring of topical brimonidine treatment with longer follow-up periods. Future Dynamic OCT studies of the effects of brimonidine might bring new knowledge about the pathophysiological mechanisms of unwanted effects associated with the use of topical brimonidine gel, such as *acute paradoxical erythema* and *exaggerated recurrence of erythema*. Long-term Dynamic OCT monitoring could also be used to examine the effects of prolonged daily use of topical brimonidine.[2]

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