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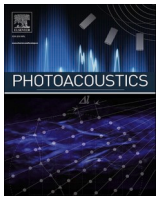

Quantitative volumetric photoacoustic assessment of vasoconstriction by topical corticosteroid application in mice skin

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ABSTRACT

Topical corticosteroids manage inflammatory skin conditions via their action on the immune system. An effect of application of corticosteroids to the skin is skin blanching caused by peripheral vasoconstriction. This has been used to characterize, in some cases relative potency and also as a way to compare skin penetration. Chromameters have been used to assess skin blanching—the outcome of vasoconstriction caused by topical corticosteroids—but do not directly measure vasoconstriction. Here, we demonstrate quantitative volumetric photoacoustic microscopy (PAM) as a tool for directly assessing the vasoconstriction followed by topical corticosteroid application, noninvasively visualizing skin vasculature without any exogeneous contrast agent. We photoacoustically differentiated the vasoconstrictive ability of four topical corticosteroids in small animals through multiparametric analyses, offering detailed 3D insights into vasoconstrictive mechanisms across different skin depths. Our findings highlight the potential of PAM as a noninvasive tool for measurement of comparative vasoconstriction with potential for clinical, pharmaceutical, and bioequivalence applications.

1. Introduction

Topical corticosteroids are indispensable in managing a spectrum of inflammatory skin disorders, including, but not limited to, psoriasis and atopic dermatitis [1,2]. They suppress inflammation primarily by inhibiting the production and release of inflammatory cytokines and dampening the activity of various cells and molecules involved in the inflammatory response [3]. Additionally, they constrict peripheral blood vessels leading to capillaries by suppressing the secretion of vasodilating substances, thereby reducing vascular permeability and limiting the migration of blood and cells to inflamed areas [4,5]. In contrast to therapeutic agents such as antifungals or antibacterials, topical corticosteroid products are categorized based on their potencies [6]. Traditionally, potency evaluation has relied on clinical observations and randomized comparative studies [7]. However, different classification systems are employed across countries, leading to differences in potency interpretation [8,9]. While vasoconstrictor assays (VCAs) have been employed for potency assessment, the literature is inconsistent regarding dose and duration parameters. Moreover, discrepancies in potency classification persist across published studies [10–12]. The VCAs have been integral to FDA's determination of bioequivalence for certain topical corticosteroids. Although the VCA typically involves the use of a chromameter, many assessments continue to be made through visual means.

To compare related compounds on a standardized molar basis, attempts using a chromameter have been made to rank the intrinsic potency of topical corticosteroids and to standardize doses [13]. However, it is important to note that the VCA using a chromameter only indirectly evaluates the result of vasoconstriction: it does not directly assess the vasoconstrictive mechanisms of topical corticosteroids. In addition,

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measurements may be problematic when there is chromatic interference, for example when used in human subjects with skin of color. Hence, we seek to introduce a novel methodology for direct vasoconstriction potency assessment.

Photoacoustic (PA) imaging is well suited for directly assessing the vasoconstrictive mechanisms of topical corticosteroids. This imaging technique capitalizes on the PA effect, wherein optically irradiated targets emit ultrasound waves through a series of energy conversions involving light absorption, temperature elevation, thermal expansion, and vibration [14–17]. The ultrasound waves generated upon the absorption of pulsed light energy by target materials are captured and then reconstructed into visual representations. An advantageous feature of PA imaging is its ability to visualize blood vessels noninvasively, leveraging the significant light absorption properties of hemoglobin, a naturally occurring chromophore in the body, in the visible and near-infrared optical ranges [18–31]. PA imaging does not require ionizing radiation or exogenous contrast agents for vascular imaging, and it has been extensively applied in diverse preclinical and clinical settings [32–48].

Previously, we disclosed the capability of photoacoustic microscopy (PAM) to precisely track the vasoconstrictive effects of hydrocortisone, a type of corticosteroid [49]. However, the methodology of the previous investigation, involving the injection of a hydrocortisone solution, lacked clinical or dermatological relevance. The topical corticosteroid product category must be more comprehensively examined to bridge the gap between preliminary research findings and practical clinical evaluations. In this study, we sought to ascertain the potential of PAM for discerning discrepancies in vasoconstriction induced by four topical corticosteroid products (clobetasol propionate 0.05 % ointment, mometasone furoate 0.1 % ointment, triamcinolone acetonide 0.1 %

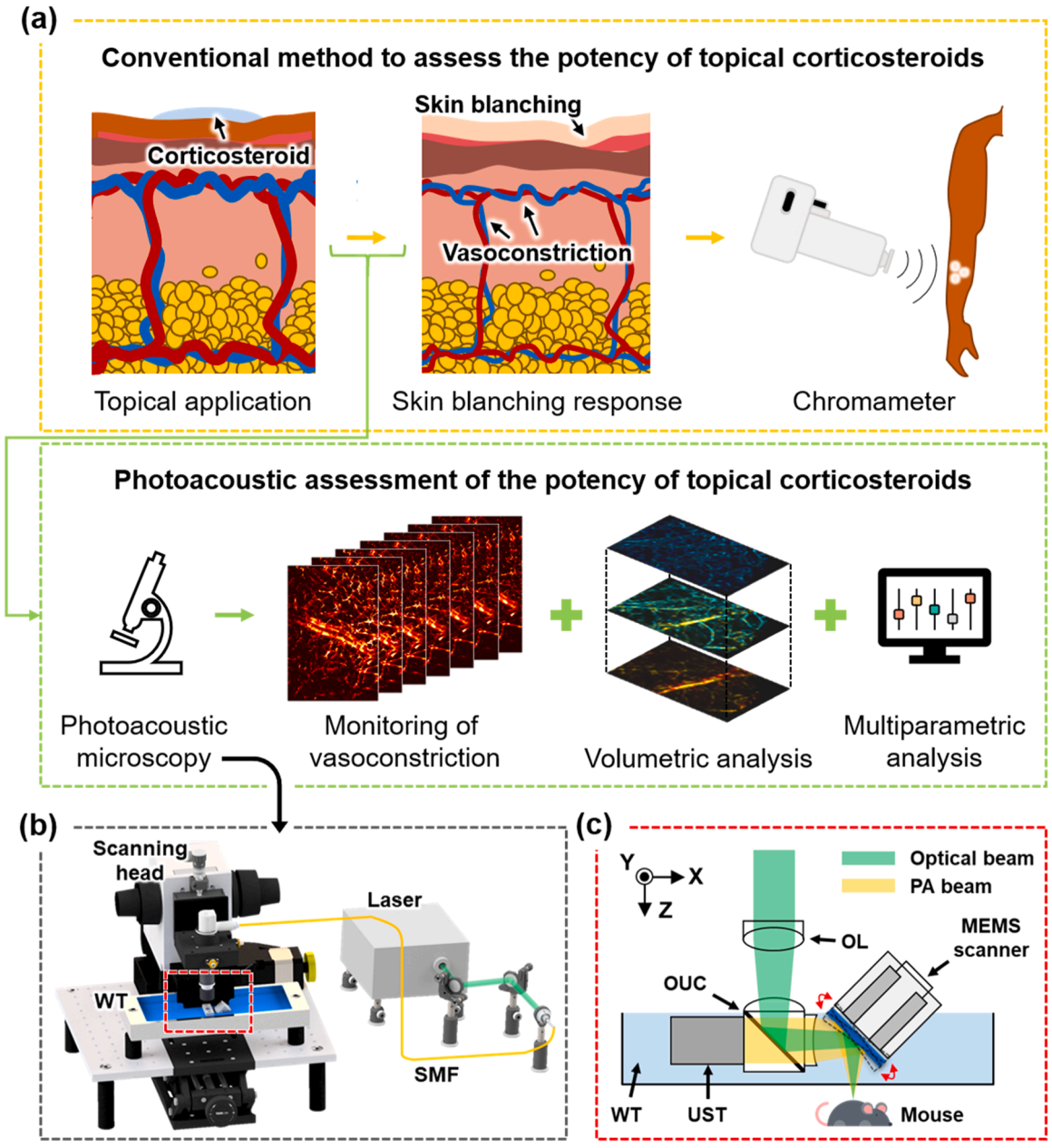

Fig. 1. Comparative representations of the proposed photoacoustic (PA) assessment and the conventional method for assessing the potency of topical corticosteroids. (a) Workflows of conventional chromameter-based assessment of the potency of topical corticosteroids and the proposed PA assessment of topical corticosteroids. (b) Schematic of the high-speed PAM system. (c) Schematic of the beam alignment based on an OUC module. WT, water tank; SMF, single-mode fiber; OL, objective lens; OUC, opto-ultrasound beam combiner; UST, ultrasound transducer; and MEMS, microelectromechanical system.

cream, and hydrocortisone 1 % lotion). Notably, we could distinguish the vasoconstrictive effect of varying potencies of the four topical corticosteroid products by analyzing vessel volume density, vessel skeleton density, and PA signal parameters. Moreover, PAM facilitated a detailed assessment of the degree of change across different skin depths, leveraging its volumetric imaging capabilities. Encouraged by these promising findings, we believe that PAM holds significant promise as a noninvasive approach for directly assessing potency, that could be used to facilitate the classification of topical corticosteroid products based on their vasoconstrictive mechanisms.

2. Materials and Methods

2.1. Photoacoustic assessment of topical corticosteroids via a high-speed photoacoustic microscopy system

Fig. 1a represents the comparative workflows of the proposed PA assessment and conventional chromameter-based assessment of skin blanching following the topical application of corticosteroids. Upon corticosteroid application, the secretion of vasodilators is suppressed and subcutaneous blood vessels constrict, blanching the skin. Historically, corticosteroid potency has been assessed by using a chromameter to measure the degree of skin blanching. In contrast, the proposed PA assessment directly monitors the corticosteroid-induced vasoconstriction process.

By integrating optical focusing and the temporal data from PA signals, high-speed PAM visualizes contrast distribution in 3D (Fig. 1b). This particular system provides lateral and axial resolutions of 5 μm and 30 μm, respectively, along with an imaging depth of approximately 1 mm in biological tissues [49]. The PA signal is received by a 50 MHz ultrasound transducer and digitized by a data acquisition card sampling at 500 MHz. Utilizing a 532 nm nanosecond pulsed laser to visualize hemoglobin, PAM allows direct observation of blood vessels, elucidating the vasoconstrictive mechanisms of topical corticosteroids. At 532 nm, hemoglobin absorbs light effectively, making it suitable for detecting vascular structures at the shallow depths typically associated with mouse skin, which is thinner compared to human skin. Consequently, PAM presents an alternative to quantifying skin blanching with a chromameter. Equipped with a high-speed microelectromechanical systems (MEMS) scanner operating unidirectionally, the PAM system rapidly images capillaries within tissues (Fig. 1c) [50,51]. Given the approximately 0.04 seconds required to acquire one B-scan, a volumetric image measuring $4 \times 6 \times 1$ mm^3 can be obtained by motorized scanning perpendicular to the B-scan in less than a minute. This speed allows the PAM system to monitor capillary changes over a period of only a few minutes, providing new insights even before blanching becomes detectable by conventional chromameter-based methods.

Following PA image acquisition, volumetric analysis provides depth-wise information. Skin layers can be segmented for independent analysis based on anatomical structure, and this segmentation also allows for assessment of vasoconstrictive mechanisms' speed in relation to the potency of topical corticosteroids. In contrast to surface-level information provided by chromameter-based methods, volumetric analysis using PAM offers literally deeper insights into the potency of topical corticosteroids and vasoconstrictive mechanisms, enhancing our understanding of their pharmacological effects. The high-speed PAM system, coupled with multi-parametric analysis capabilities, facilitates direct evaluation of the potency of topical corticosteroids. Fig. 1a shows the workflows of both PA assessment and traditional chromameter-based skin blanching assessment, clearly revealing the advantage of PA assessment, which directly visualizes topical corticosteroid mechanisms and also supports volumetric analysis and various parameter-based analyses.

2.2. Photoacoustic microscopy system

For vascular imaging of mice, we employed a high-speed PAM (OptichoM, Opticho, Republic of Korea) system with a water-immersible MEMS scanner (Fig. 1b and Supplementary Fig. S1). Operationally, 10-nanosecond pulses of light from a 532 nm pulsed laser (AWAVE532–1 W-10 K, Advanced Optowave, USA) were delivered to the PAM via a single-mode fiber (P1–460B-FC-1, Thorlabs, USA). The optical beam was then focused by an objective lens (AC127–050-A, Thorlabs, USA) and directed to the MEMS scanning module. Within the scanning module, the laser beam was reflected by an opto-ultrasound beam combiner (OUC) and a MEMS scanner to irradiate the imaging target, thereby generating PA waves. The resulting PA waves were detected by a 50 MHz ultrasound transducer (V214-BB-RM, Olympus NDT, USA), and amplified by a 50 dB amplifier (PE15A1013, Pasternack, USA) before being digitized by a waveform digitizer card (ATS9350, Alazar Technologies, Canada) with a sampling rate of 500 MHz. The high-speed PA imaging was facilitated by the MEMS scanner steering both light and US waves, aided by two motorized linear stages (L-509, Physik Instrumente, Germany) that extended the imaging area.

A multi-functional data acquisition (DAQ) board (PCIe-6321, National Instruments, USA) ensured fully synchronized timing sequences, with triggers initiated based on the reference signal's positive edge (Supplementary Fig. S1). Laser and digitizer triggers were enabled only during unidirectional scanning. The data acquisition and storage were managed using LabVIEW software (National Instruments, USA). The PAM system provided vascular images in a volume of $4 \times 6 \times 3$ mm^3 (along the X, Y, and Z axes, respectively) within 2 min. The optical energy was approximately 8 mJ/cm^2, well below the 20 mJ/cm^2 maximum permissible exposure (MPE) value set by the American National Standards Institute (ANSI). To ensure the accuracy and reliability of the comparative analyses, optical fluence and all scanning parameters were maintained consistently across all experimental groups throughout the study. From the acquired 3D vascular data, top-view blood vessel images (so called PA maximum amplitude projection (MAP) images) were generated using MATLAB (MathWorks, USA), alongside the 3D Photoacoustic Visualization Studio (PHOVIS) for processing and visualization.

2.3. Experimental procedures

All experimental procedures adhered to a protocol approved by the Institutional Animal Care and Use Committee (IACUC) of Pohang University of Science and Technology (POSTECH). Sixteen nude mice (6 weeks old, approximately 20 g each) were prepared, with four assigned to each experimental group. Prior to experimentation, the mice were anesthetized with a mixture of 0.5 L/min O_2 and 2 % isoflurane, using a vaporized anesthesia system (VIP 3000 Veterinary Vaporizer, Midmark, USA). Under respiratory anesthesia, the mouse's leg was positioned on a customized leg holder attached to the z-axis stage to reduce variations in imaging angles and depth penetration. To obtain pre-application data, the mouse's thigh was covered with ultrasound gel, then placed in contact with a thin plastic membrane at the bottom a water tank to facilitate the propagation of PA waves. We conducted all measurements in a controlled environment, maintaining consistent anesthesia levels and warmer's conditions for maintaining body temperatures to minimize physiological fluctuations that could affect vascular response. After acquiring pre-application data, the mouse stage was repositioned, and the ultrasound gel was removed. Approximately 0.03 g of the topical corticosteroid was applied and allowed to absorb into the skin for 20 min, after which any excess was wiped off before PA monitoring. Then the mouse's thigh was again covered with ultrasound gel and placed in contact with the thin plastic membrane. PA 3D data were collected at 10 min intervals for 1 h to monitor vascular changes over time.

2.4. Medications

In this study, we investigated four topical corticosteroids: clobetasol propionate 0.05 % ointment (Dermovate Ointment, Glaxo Operations UK Limited, UK), mometasone furoate 0.1 % ointment (Momenta Ointment, Theu PHARMACEUTICAL Co., Republic of Korea), triamcinolone acetonide 0.1 % cream (Tricort Cream, DongKwang Pharm Co., Republic of Korea), and hydrocortisone 1 % lotion (LactiCare HC Lotion, Koreapharma, Republic of Korea). The medications were purchased from Korean pharmacies shortly before each study, stored away from direct sunlight, and maintained at ambient room temperature, not exceeding 25 °C. A predetermined amount of each topical corticosteroid was applied using a cotton swab.

2.5. Postprocessing for multiparametric 3D PA images analysis

To quantify the vascular changes in the PA images, blood vessel segmentation was performed in MATLAB (Supplementary Fig. S2). The quantitative analyses of vasculatures involved two main aspects: volumetric hemodynamics and vessel skeletal morphology. Initially, blood vessels were enhanced using a Frangi filter and binarized via adaptive thresholding, aiding in the differentiation of vessels from background noise. Subsequently, the white pixel count of the binary map was divided by the total pixel count of the binary map to calculate the vessel volume density. For the analysis of vessel skeletal morphology, the centerlines of the vessels were extracted using the MATLAB function "bwskel". To refine the analysis and eliminate inaccurately extracted vessel segments, skeletons with branch lengths below five pixels were

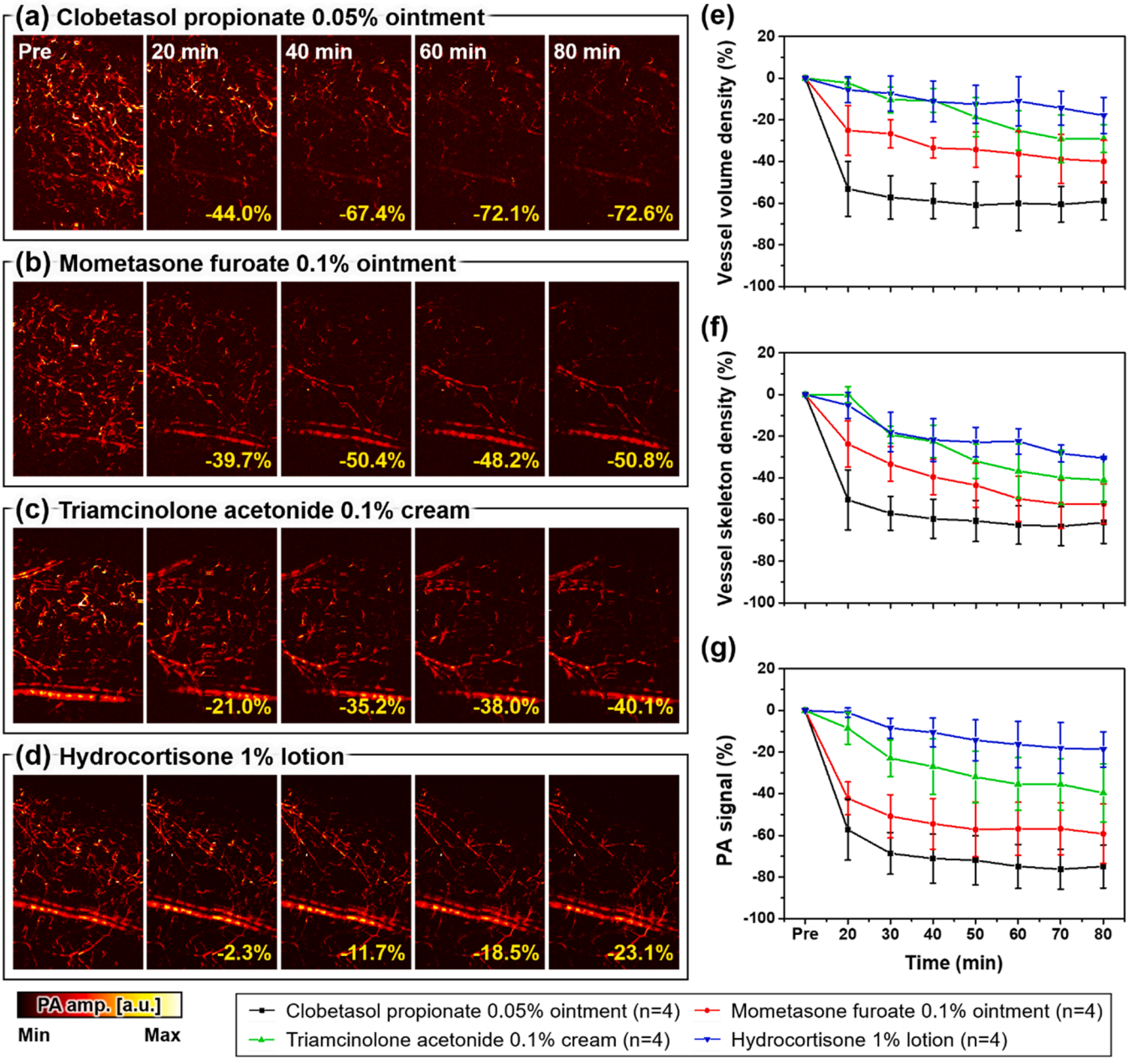

Fig. 2. Serial PA monitoring of vascular networks over an 80 min period after topical corticosteroid application, with measurements taken at 10 min intervals. Comparative PA maximum amplitude projection (MAP) vascular images after a topical application of (a) clobetasol propionate 0.05 % ointment, (b) mometasone furoate 0.1 % ointment, (c) triamcinolone acetonide 0.1 % cream, and (d) hydrocortisone 1 % lotion. Yellow numerical values indicate changes in the PA signal normalized to pre-application data. Quantitative changes in (e) vessel volume density, (f) vessel skeleton density, and (g) PA signal (mean ± standard error).

removed. Following this, the vessel skeleton density was calculated by dividing the white pixel count of the skeleton map by the total pixel count of the skeleton map.

3. Results

3.1. Time-dependent photoacoustic monitoring of vascular changes

To confirm whether vascular constriction induced by topical corticosteroids could be observed using the PAM system, we first compared an anesthetized control mouse without any application to mice that received a topical application. To ensure that the observed changes were not due to repositioning, the control mouse underwent identical experimental procedures to the topical application group, except for the topical application itself. MAP images in the B-scan direction revealed weakened PA signals or blood vessel constriction near the skin surface of a mouse with topical application of triamcinolone acetonide 0.1 % cream, unlike the control mouse without any application (Supplementary Fig. S3). Plotting these images as histograms in the depth direction quantitatively confirmed that topical application affects capillaries in the relatively shallow dermis.

Subsequently, we investigated whether differences in vasoconstriction depending on the potency of various topical corticosteroids could be significantly distinguished using the PAM system, utilizing clobetasol propionate 0.05 % ointment, mometasone furoate 0.1 % ointment, triamcinolone acetonide 0.1 % cream, and hydrocortisone 1 % lotion with varying potencies. During an 80 min PA imaging session, we acquired eight PA vascular images. Fig. 2a–d are time-dependent vascular images from the four topical corticosteroids. It is important to note that due to the high-speed scanning along the x-axis, varying optical beam path lengths occur at different x-axis positions within a single vessel. This variation can affect the beam shape, resulting in discontinuous intensity distribution within the vessels, as seen in Fig. 2, particularly in OR-PAM systems where the optical beam is tightly focused. Vasoconstriction is observed over time in all groups, with more pronounced changes in mice receiving higher potency corticosteroids such as the clobetasol propionate 0.05 % ointment. To quantify the differences in vascular changes between these groups, we employed three indicators: the vessel volume density from the PA binary map image, the vessel skeleton density from the PA skeletal map image, and, for intensity-based analysis, the change in PA signals within the field of view (FOV). The yellow numbers in Fig. 2a–d represent the changes in the PA signal normalized to the pre-application data. Fig. 2e–g present the quantified vessel volume densities, vessel skeleton densities, and changes in PA signal, respectively, obtained from four mice in each group, shown with means and standard errors. The image postprocessing and quantification methods are detailed in the Materials and Methods section.

In the clobetasol propionate group, the vessel volume density decreases by 59.0 ± 9.1 %, the vessel skeleton density decreases by 61.5 ± 10.0 %, and the PA signal decreases by 75.0 ± 10.3 % at 80 min after topical application. Quantifying the vessel skeleton density separately from the vessel volume density reduces the influence of changes in arteriovenous pairs located at relatively deep points. However, after topical corticosteroid application, significant vasoconstriction in the capillaries caused a substantial number of vessels to become too small to be detected by PAM. This phenomenon was particularly pronounced in the groups treated with relatively high potent corticosteroids, resulting in the reduction of vessel skeleton density paralleling the changes observed in vessel volume density. In the hydrocortisone group, the vessel volume density decreases by 17.9 ± 8.7 %, the vessel skeleton density decreases by 30.5 ± 0.42 %, and the PA signal decreases by 18.7 ± 8.4 % at 80 min after topical application. In the same parametric order, the mometasone furoate group shows changes of 40.0 ± 10.2 %, 52.5 ± 9.6 %, and 59.2 ± 14.3 %, and the triamcinolone acetonide group shows changes of 29.0 ± 6.7 %, 41.1 ± 10.5 %, and 39.6 ± 14.0 %. These findings regarding vasoconstrictive capacity, ranked in the order of clobetasol propionate 0.05 % ointment > mometasone furoate 0.1 % ointment > triamcinolone acetonide 0.1 % cream > hydrocortisone 1 % lotion, align with the established topical corticosteroid potency classifications by the National Psoriasis Foundation (Supplementary Table S1). Moreover, the quantitative results obtained from the PA MAP images indicate that the three PA multiparametric indicators can distinguish vasoconstrictive ability according to the potency of the particular topical corticosteroid.

3.2. Multi-layered analysis of vasoconstriction induced by topical corticosteroids

In assessing the potency of topical corticosteroids, using the PAM system to directly analyze vascular changes is intuitively more comprehensive compared to the conventional evaluation of skin blanching. Furthermore, using PAM for potency assessment allows volumetric image analysis. While chromameter information comes solely from the skin surface, the PAM system noninvasively and comprehensively acquires volumetric characteristics, as illustrated in Fig. 3 and Supplementary Movie S1. Fig. 3a–b show sectioned PA MAP images of different skin layers captured by imaging a mouse thigh before and 1 h after a topical application of triamcinolone acetonide 0.1 % cream. Capillaries can be clearly seen in the shallow dermal layer close to the skin, while thick pairs of arteries and veins appear in the deeper layers. For illustrative purposes, we have divided the skin layer into three sub-layers (0 – 300, 300 – 600, and 600 – 1000 μm) and quantified the changes in PA signals in each layer. To create volume slices at a constant distance from the skin profile, the layer division was conducted using 3D PHOVIS to reflect the non-flat structure of the mouse thigh, rather than using a Cartesian coordinate system [52].

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Fig. 3c presents the quantified changes in vessel volume densities, vessel skeleton densities, and PA signals of each layer in a serial manner, obtained from four mice in each group after the topical applications of various corticosteroids. The multi-layered analyses show that the clobetasol propionate group has the largest decrease in PA signal (81.0 ± 8.54 % in the layer ranging from 0 to 300 μm in depth, 67.2 ± 11.7 % in the layer ranging from 300 to 600 μm in depth, and 65.7 ± 13.4 % in the layer ranging from 600 to 1000 μm in depth), whereas the hydrocortisone group has the smallest PA signal decrease (27.9 ± 11.4 % in the layer ranging from 0 to 300 μm in depth, 21.9 ± 13.1 % in the layer ranging from 300 to 600 μm in depth, 9.5 ± 6.2 % in the layer ranging from 600 to 1000 μm in depth). These trends are very similar to the qualifications of vessel volume densities and vessel skeleton densities. Interestingly, all types of topical corticosteroids show the greatest change in the layer closest to the skin surface, and the decreases in PA signal, vessel volume density, vessel skeleton density are relatively less in the deeper layers, where the thick arterial and venous pairs flow at the timepoint evaluated during this study. These trends of results indicate that topical corticosteroids are gradually absorbed into the deeper skin after topical application. Further, PAM, unlike a chromameter, can more accurately detail the action of topical corticosteroids by dividing the skin into anatomical layers.

3.3. Statistical analysis across the products' vasoconstrictive abilities over time

As Fig. 2 shows, all the tested topical corticosteroids induced vasoconstriction over time. However, to effectively classify topical corticosteroid products based on their vasoconstrictive mechanisms, we wanted to assess potency directly. To examine between-group differences in various parameters, we used a two-tailed Student's *t*-test to evaluate the significant differences among the four experimental groups (clobetasol propionate 0.05 % ointment, mometasone furoate 0.1 %

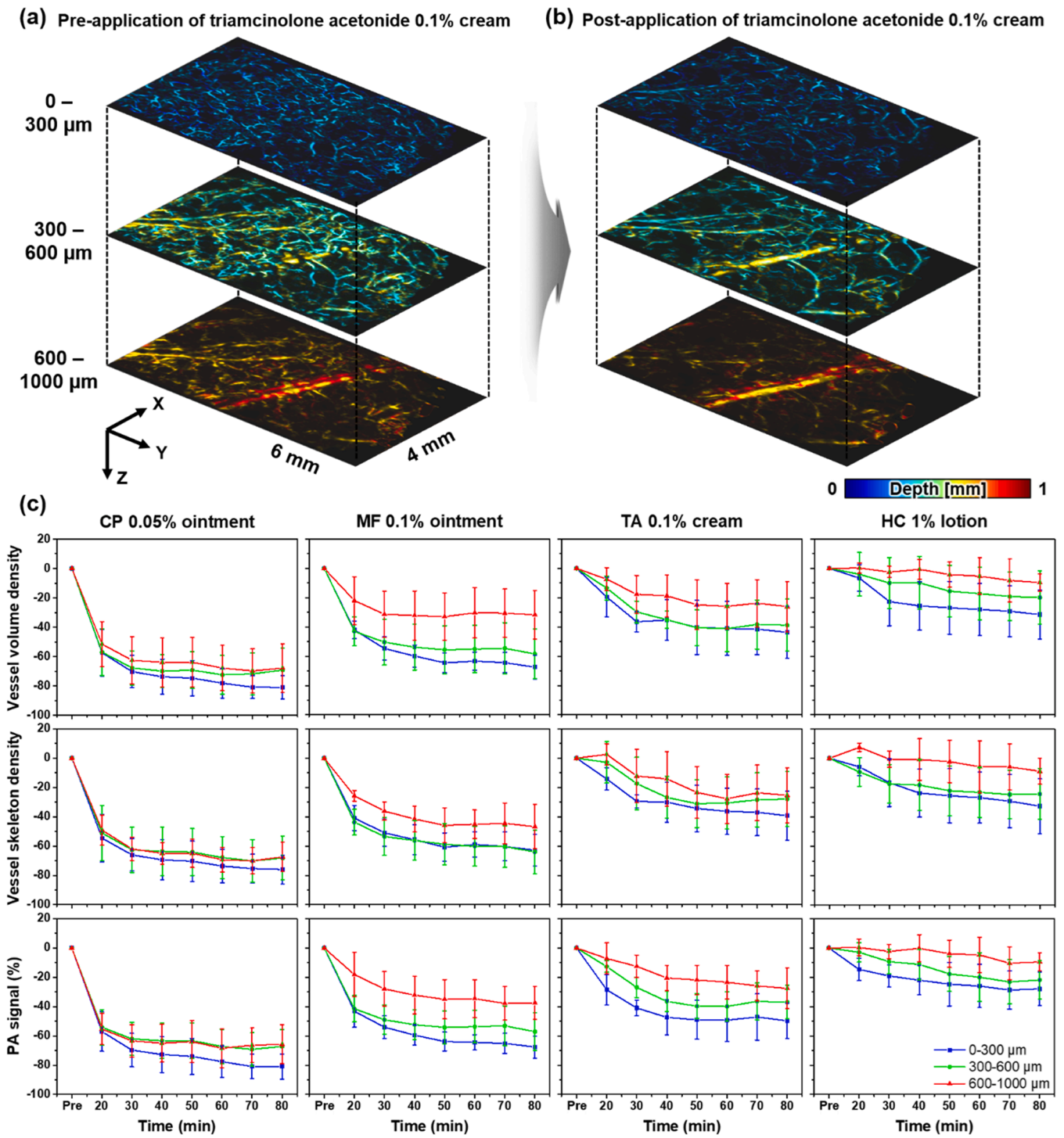

Fig. 3. Multi-layered analysis of vasoconstriction induced by topical corticosteroids. Vertically sectioned PA MAP images from different skin layers (0 – 300, 300 – 600, and 600 – 1000 μm) of a mouse thigh (a) before and (b) 1 h after a topical application of triamcinolone acetonide 0.1 % cream. (c) Depth-dependent serial changes in vessel volume densities, vessel skeleton densities, and PA signals after topical applications of clobetasol propionate 0.05 % ointment, mometasone furoate 0.1 % ointment, triamcinolone acetonide 0.1 % cream, and hydrocortisone 1 % lotion (mean ± standard error). CP, clobetasol propionate; MF, mometasone furoate; TA, triamcinolone acetonide; and HC, hydrocortisone.

ointment, triamcinolone acetonide 0.1 % cream, and hydrocortisone 1 % lotion; $n = 4$ for all groups) across the parameters of vessel volume density, vessel skeleton density, and PA signal intensity (Fig. 4). The resulting statistical output, expressed as mean ± standard error, has the following significance levels: $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. The statistical analyses were performed using the "T.TEST" built-in function in Excel (Microsoft, USA). Detailed data for all *t*-test statistical analyses are provided in Supplementary Table S2.

Two primary types of analyses were conducted: immediate changes within the 20 min period (Fig. 4a), which refer to the difference between the pre-application and the first PA MAP images after topical application. Longer term changes were those occurring over the 80 min period (Fig. 4b) between the pre-application and the last PA MAP images post-application. Interestingly, for the immediate changes over 20 min,

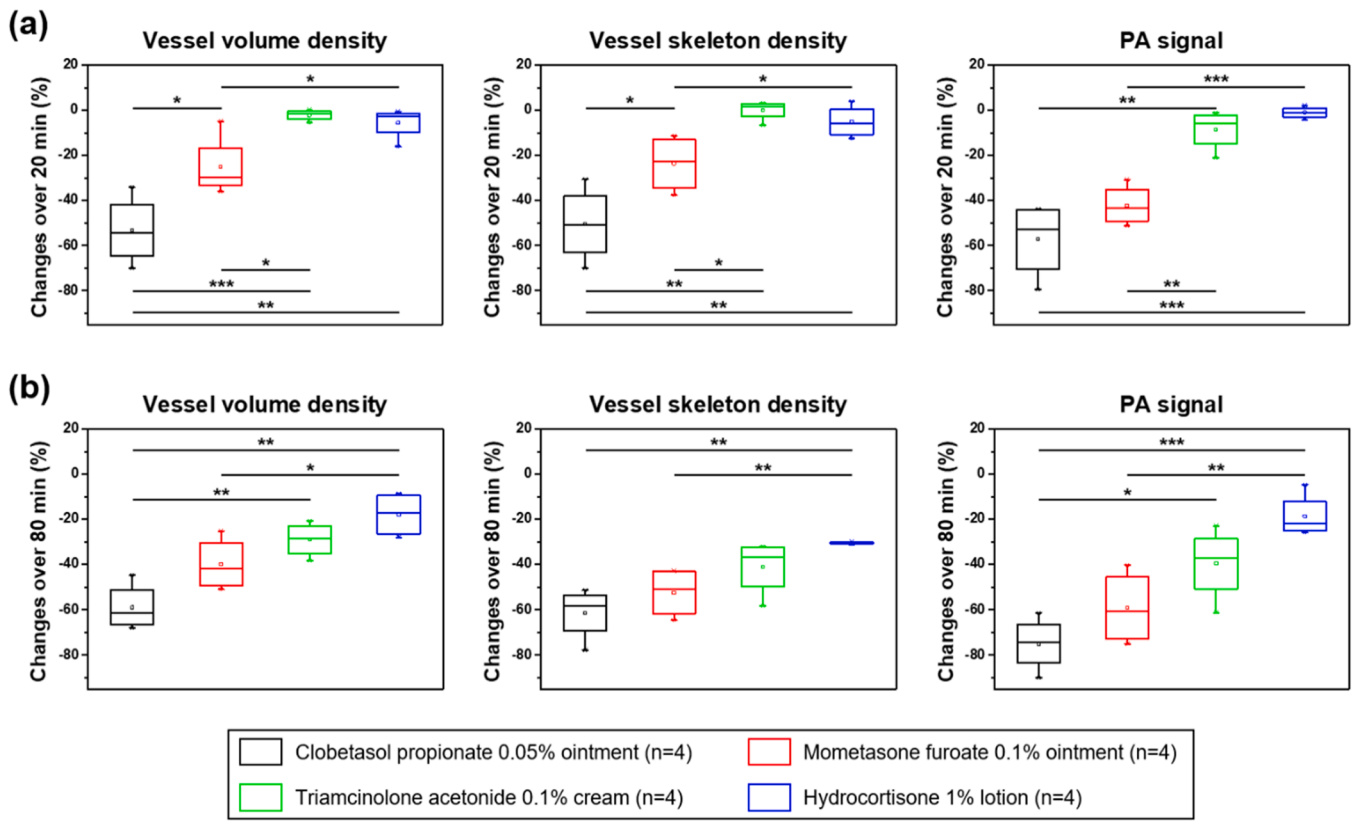

Fig. 4. Statistical analysis of the effects of topical corticosteroid products over time. (a) Immediate changes (within 20 min) observed between the pre-application PA MAP image and the first PA MAP image after topical application, focusing on the vessel volume density, vessel skeleton density, and PA signal intensity. (b) Longer term changes (over 80 min) between the pre-application PA MAP image and the last PA MAP image post-application, considering the vessel volume density, vessel skeleton density, and PA signal intensity. The *p* values indicating statistical significance were calculated from the two-tailed Student's *t*-test.

clobetasol propionate 0.05 % ointment and mometasone furoate 0.1 % ointment caused significantly more vasoconstriction than either triamcinolone acetonide 0.1 % cream or hydrocortisone 1 % lotion. Compared to the other two drugs, immediate vasoconstrictive abilities of clobetasol propionate 0.05 % ointment and mometasone furoate 0.1 % ointment are statistically higher in all three parameters (Fig. 4a). Therefore, these parameters for immediate change may serve as effective measures for primarily classifying topical corticosteroids with relatively high potency. Notably, significant differences in vessel volume density and vessel skeleton density were observed between the immediate vasoconstrictive effects of clobetasol propionate 0.05 % ointment and mometasone furoate 0.1 % ointment (Fig. 4a). After these immediate changes, the vasculature tended to stabilize rather than constrict further over 80 min in both groups. This apparent stability may appear to be due to the limited penetration depth of our PAM system, which cannot detect vasoconstriction beyond 1 mm depth. Conversely, triamcinolone acetonide 0.1 % cream and hydrocortisone 1 % lotion create little change immediately after topical application, but instead gradually induce vasoconstriction over time. This behavior intuitively suggests that their immediate vasoconstrictive abilities are relatively poor compared to those of clobetasol propionate 0.05 % ointment and mometasone furoate 0.1 % ointment. However, in the longer term, all of the studied medications, including triamcinolone acetonide 0.1 % cream and hydrocortisone 1 % lotion, cause vasoconstriction, as evidenced by a decrease in all of the vascular analysis parameters demonstrating the vasoconstrictive mechanism of topical corticosteroids at 80 min (Fig. 4b). These multiparametric information provided by the PAM offers a more comprehensive assessment than a single indicator can, such as color measured by a chromameter.

4. Discussion and conclusion

We have introduced a novel methodology utilizing PAM to directly assess the vasoconstrictive properties of topical corticosteroids. Conventional studies to assess the potency of topical corticosteroids utilize a chromameter to quantify skin blanching caused by vasoconstriction, rather than directly characterizing the vasoconstriction itself. Additionally, chromameter-based skin blanching quantification in humans typically monitors changes over several hours post-topical application [53,54]. In contrast, our direct PAM analysis of vascular changes offers a fast and intuitive approach to assessing vasoconstriction. This noninvasive and comprehensive approach provides volumetric vascular image data, whereas chromameter analysis provides only information about the skin surface. Furthermore, PAM imaging allows the mechanism of topical corticosteroids to be analyzed in greater depth, providing multiparametric information such as changes in vessel volume density, vessel skeleton density, and PA signal intensity, unlike the single metric of color provided by a chromameter.

The significance of our methodology is highlighted by the demographic limitations present in current clinical research. Many clinical studies on topical corticosteroids have predominantly involved participants with lighter skin (e.g., Caucasian), with literature suggesting that certain products may be less effective on darker skin tones [55,56]. For instance, despite the high prevalence of atopic dermatitis (AD) in skin of color populations, there is limited data on the efficacy of common therapies for AD in skin of color/ethnic groups [57]. This paucity of data is partly due to the underrepresentation of certain groups in clinical trials and the lack of subset analyses for skin of color [56]. Furthermore, topical corticosteroids—particularly those of high potency—can cause hypopigmentation in darker skin types, with changes in skin color being more noticeable in people with dark skin [58]. These issues underscore

the current challenges related to skin tone in clinical practice. PA imaging is also influenced by skin tone, as the concentration of melanin, a primary optical absorber in tissue, largely determines skin tone. Darker skin tones exhibit higher PA signals at the skin surface and reduced penetration depth compared to subjects with lighter skin tones, but there are studies aimed at compensating for these signal differences [59–61]. For example, the bias associated with darker skin tones can be corrected by formulating an equation that describes changes in PA signals as a function of Fitzpatrick skin types. If our methodology can allow for more accurate assessments across diverse populations, it would offer a substantial practical advantage.

Our optical-resolution PAM (OR-PAM) system had a tight optical focus, providing excellent spatial resolution within the optical diffusion limit that allowed the observation of small changes in capillaries [62, 63]. Despite these advantages, the current OR-PAM system has a limited penetration depth that restricts its applicability in human clinical settings. To transfer the technology from preclinical research to clinical practice, a new PAM system with greater penetration depth must be developed. Acoustic-resolution PAM (AR-PAM) is a suitable candidate that offers greater penetration depth due to its ability to capture PA signals from scattered photons [30,64]. Such a system can achieve an imaging depth sufficient to visualize deeper vascular structures within human tissues. Additionally, using a laser with a near-infrared wavelength, such as 1064 nm, instead of the 532 nm wavelength used in this study, can further improve the penetration depth due to the reduced tissue scattering at longer wavelengths [65].

In addition, although the current PAM system is less portable compared to conventional chromameters, recent advancements in handheld and freehand scanning PAM systems offer greater portability [66–68]. These developments will be crucial for making the system more suitable for clinical use, ensuring that it is not influenced by various differences between the mouse and the human skin, such as thickness, vascular structure, and response to corticosteroids, and ultimately translating the proposed methodology into a practical clinical tool. Furthermore, employing a high pulse repetition rate laser in the range of hundreds of kHz could improve imaging speed and make blood flow velocity measurements more feasible for clinical applications. This additional capability to analyze blood flow velocity holds great potential for achieving breakthroughs in pharmacological research. Additionally, optical coherence tomography (OCT) offers high-resolution 3D structural imaging, which enables more precise segmentation of skin layers. Recently, there has been increasing research on integrating PA and OCT techniques, leveraging the complementary strengths of both modalities in terms of contrast mechanisms, penetration depth, and spatial resolution [21,69]. Combining these two powerful imaging approaches in a dual-modal PA-OCT system could significantly enhance research outcomes, providing a more comprehensive understanding of vascular changes in response to corticosteroid application.

Directly assessing the vasoconstrictive properties of topical corticosteroids through the analysis of vascular changes captured by PAM represents a paradigm shift from traditional methods reliant on skin blanching. This innovative approach not only provides a nuanced understanding of the vasoconstrictive properties of topical corticosteroids but also facilitates the assessment of potential side effects, such as capillary dilation. Moreover, by leveraging the volumetric data capabilities of the PAM, we gain a richer dataset, allowing for comprehensive analysis beyond surface-level observations. While conventional techniques offer insights limited to the skin's surface, the PAM enables exploration of intricate vascular dynamics beneath the skin, promising enhanced clinical utility in assessing vasoconstriction by topical corticosteroid. Given these advantages, this methodology will be further expanded into clinical studies, incorporating long-term investigations to establish stronger clinical correlations. Furthermore, this technique holds substantial potential to advance research on the formulations of topical corticosteroids and offers valuable applications in drug development, providing a robust tool for evaluating the vascular effects as well as monitoring local inflammatory responses and tissue structural changes of new therapeutic agents.

CRediT authorship contribution statement

Chulhong Kim: Writing – review & editing, Supervision, Project administration, Conceptualization. **Donghyun Kim:** Data curation. **Priyanka Ghosh:** Writing – review & editing, Methodology, Conceptualization. **Markham C. Luke:** Writing – review & editing, Methodology, Conceptualization. **Donggyu Kim:** Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Data curation, Conceptualization. **Seungah Yoo:** Methodology. **Ji Hyun Lee:** Methodology. **Joongho Ahn:** Software, Methodology, Data curation. **Jin Young Kim:** Software, Methodology.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: J. Ahn, J. Y. Kim and C. Kim have financial interests in OPTICHO, which, however, did not support this work.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.pacs.2024.100658.

Data Availability

Data will be made available on request.

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