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Vasoconstriction and anti-inflammatory properties of the selective α-adrenergic receptor agonist brimonidine

David Piwnica *, Carine Rosignoli, Séverine Thibaut de Ménonville, Thierry Alvarez, Marlene Schuppli Nollet, Olivier Roye, André Jomard, Jérôme Aubert

Research, Galderma, Sophia Antipolis, France

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ABSTRACT

Background: The facial erythema of rosacea is recognized as the most prevalent and most difficult manifestation of rosacea to treat. A recent approach in patients with rosacea has been to reduce this erythema through vasoconstriction of cutaneous blood vessels by selectively targeting α_2-adrenergic receptors with brimonidine.
Objective: To further investigate the pharmacodynamic profile of brimonidine, its vasoconstrictive effects and its anti-inflammatory properties.
Methods: The potency for the α_{1A}, α_{1B}, α_{2A}, α_{2B} and α_{2C} receptors of brimonidine was measured, as well as performing a large target profiling study in order to determine the target selectivity profile of brimonidine. The vasoconstrictive effects of brimonidine were measured using *ex vivo* wire myography and human skin biopsy neuroinflammation models. The anti-inflammatory properties of brimonidine were measured using two *in vivo* mice ear inflammation models.
Results: Brimonidine was found to be highly selective for the α_{2A} adrenoreceptor (EC_{50} 0.45 nM) over the other α-adrenoreceptors. Additionally, the large target profiling study demonstrated the high selectivity of brimonidine with minimal off-target effects. The *ex vivo* wire myography model showed that brimonidine is a potent vasoconstrictor of human subcutaneous vessels with a diameter of less than 200 μm (EC_{50} 0.4 nM). The *ex vivo* human skin biopsy neuroinflammation model demonstrated that brimonidine completely inhibited vasodilation induced by capsaicin. Both *in vivo* mouse ear inflammation models highlighted that brimonidine inhibited ear edema (up to 76%) when compared to vehicle.
Conclusion: The selectivity, vasoconstrictive and anti-inflammatory properties of brimonidine that have been described in these studies are in agreement with the benefits observed with this compound in the treatment of facial erythema in rosacea.

1. Introduction

Rosacea is a chronic skin disorder with an unknown etiology, characterized by transient or persistent erythema, telangiectasia, papules and pustules, that primarily affects the central facial region (cheeks, nose, chin and forehead) [1–3]. Persistent facial erythema is most commonly associated with subtypes I and II of rosacea, namely erythematotelangiectatic (ETR) and papulopustular rosacea (PPR) [2,3]. Rosacea, in particular facial erythema, is often triggered by a wide range of environmental or lifestyle factors including, but not limited to: sun exposure, emotional stress, temperature, exercise and alcohol consumption [4]. The facial erythema of rosacea is recognized to be the most prevalent and also the most difficult manifestation to treat [4–6].

The pathophysiology of facial erythema of rosacea is relatively unknown. It is suggested to result from dysregulation of cutaneous vasomotor responses, which causes abnormal, involuntary and persistent dilation of facial blood vessels [7–10]. The severity of rosacea has also been linked to prolonged vasodilation of small blood vessels (<200 μm), with evidence to suggest that vasodilation may result from inflammatory mediators released during the early phase of rosacea [7,9,11,12]. These characteristics of rosacea are similar in nature to the main features of neurogenic inflammation, a condition caused by the local release of inflammatory mediators from sensory neurons [13]. The link between rosacea and neurogenic inflammation is further strengthened by a

* Corresponding author. Tel.: +33 1492383026.
E-mail address: David.Piwnica@galderma.com (D. Piwnica).

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number of recent studies that demonstrate the release of vasoactive peptides by sensory neurones in skin upon exposure to known triggers [7,11,14], and co-localization between facial sensory nerve endings and blood vessels in patients with rosacea [7,11,13]. One recent study has shown an increase of markers, which are also present in neurogenic inflammation such as mast cells, neuropeptides (substance P and $CGRP_{\alpha}$) as well as matrix remodeling, in patients with rosacea [15]. In addition, patients with rosacea have been associated with a higher prevalence of migraine, a condition also thought to involve neurogenic inflammation [16,17]. Neurovascular and neuroimmune interactions in rosacea have revealed the involvement of a number of genes including adrenergic receptors [13].

Novel approaches are being assessed for the treatment of facial erythema of rosacea. One recent approach has been to reduce erythema through vasoconstriction of cutaneous blood vessels by targeting both α-adrenergic receptors with oxymetazoline [18,19] and brimonidine [10,20]. Oxymetazoline is an agonist of the α_{1A}-adrenoceptor and a partial agonist of the α_{2A}-receptor agonist; it is currently in clinical development (Phase IIb) for the treatment of facial erythema in rosacea [18,19]. Brimonidine is a highly selective α_2-adrenergic receptor agonist with potent vasoconstrictive activity that has been approved for the treatment of open-angle glaucoma for almost 20 years [21–24]. Brimonidine has also recently been approved as a promising, new therapy for the topical treatment of persistent (nontransient) facial erythema of rosacea in adults 18 years of age or older (Mirvaso®, Galderma R&D, Sophia Antipolis, France) [25]. Recent Phase II and III studies with topical brimonidine 0.33% gel (once daily) demonstrated its efficacy and safety [10,20] for periods of up to one year [26].

The aim of this study was to further investigate the pharmacodynamic profile of brimonidine, its vasoconstrictive effects on subcutaneous arteries and veins and its anti-inflammatory properties *in vivo*, in comparison with oxymetazoline.

2. Materials and methods

2.1. Target-based assays

An adrenoreceptor assay selectivity profile study was conducted at Cerep (Poitiers, France) with human α_{1A} (Cerep Cat 1500), α_{1B} (Cerep Cat 1901), α_{2A} (Cerep Cat 2A2558), α_{2B} (Cerep Cat 1813) and α_{2C} (Cerep Cat 1736) receptors selected to evaluate the EC_{50} potency of oxymetazoline (Sigma–Aldrich, France) and brimonidine (Sigma–Aldrich, France). Both compounds were dissolved in DMSO then diluted in HEPES sodium salt buffer. Both compounds tested over a dose range of 1 pM to 10 μM.

A large target profiling study of brimonidine (Bioprint® profile) was performed at Cerep (Poitiers, France) with the panel mainly based on target diversity. The study included 104 binding assays (non-peptide, peptide and nuclear receptors, ion channels and amine transporters) and 32 enzyme assays (including 10 kinases, 9 proteases and 5 phosphodiesterases). The full assay list can be found in the supplementary information. Brimonidine response was normalized to the maximum signal detected in each assay.

2.2. In vitro *wire myography*

Subcutaneous blood vessels were isolated from human abdominal plastic surgery. Healthy human skin was placed in cold physiological saline solution (PSS) and stored at 4 °C until required. The subcutaneous blood vessels were removed from the skin using forceps and scissors under stereomicroscope and maintained in PSS at pH = 7.4.

Blood vessels were cut into ring segments, roughly 2 mm in length, and stored in cold PSS. The 2 mm-segments were then mounted using two fine tungsten wires (40 μm in diameter) into a four chamber myograph (EMKABath4, EMKA Technologies). Data were recorded using the Iox 2.8 software. The vessel segments were then stretched to the tension corresponding to a physiological pressure of 90 mmHg for arteries and 18 mmHg for veins, and lumen diameter was measured using Normalize software from EMKA Technologies.

Vessel segments were maintained in a preheated PSS solution gassed with a mixture of 5% CO_2, 20% O_2 and 75% N_2 (pH = 7.4) for 1 h before being used. During this period, the bath solution was changed every 20 min. The viability of blood vessel segments was assessed by measuring the contraction obtained after two successive stimulations with the PSS solution containing 80 mM of KCl. Then, the smooth muscle vascular wall function was evaluated using a vasoconstrictor compound such as prostaglandin F2α at 10 μM, whereas the endothelium function was assessed using acetylcholine at 1 μM. Oxymetazoline and brimonidine were dissolved in DMSO. Both compounds were added in a range of concentrations directly to the chamber and the modulation of vascular tones was recorded.

2.3. Ex vivo *human skin model*

Healthy human skin was obtained from 10 different donors who underwent abdominoplasty. Skin biopsies (1 cm^2) were obtained and placed in culture with the epithelium on top using an insert with 0.45 μm polycarbonate membrane (Fig. 1). Inserts were then set in 12-well plates pre-filled with culture medium (DMEM supplemented with FCS and antibiotics). Thus, this *ex vivo* organ culture system maintained the skin at the air–liquid interface and fed the dermis and epidermis by nutrient diffusion across the insert. Biopsies were then placed in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. 20 μL/cm^2 of 0.33% of brimonidine (Mirvaso®, Galderma R&D, Sophia Antipolis, France, 0.33 g brimonidine in 100 mL of gel equivalent to 11.3 mM) or vehicle gel (corresponding vehicle gel to Mirvaso®, Galderma R&D, Sophia Antipolis, France), was added topically to the skin biopsies 2 hours before addition of capsaicin and capsaicin was added at 10 μM to the culture medium for 4 h. Skin

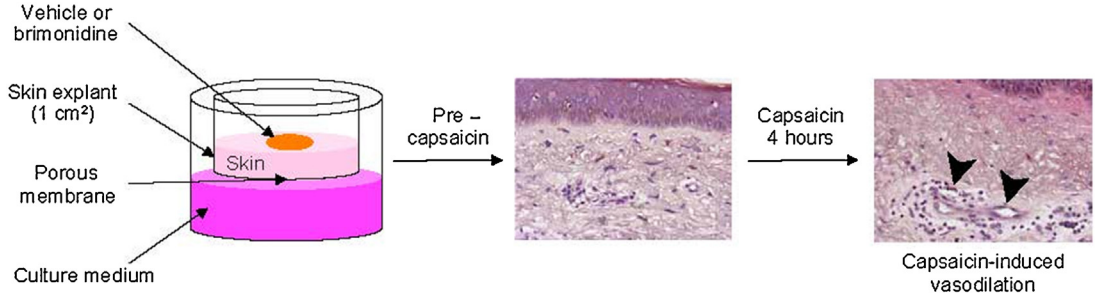

Fig. 1. Schematic diagram of the *ex vivo* human skin model used to measure the vasoconstrictor activity of brimonidine. Black arrowheads show vasodilation induced by capsaicin application.