derived cells were unlikely to be involved in spontaneous skin wound healing.

Sustained systemic and local clodronate depletion affected both circulating monocytes and WAM. While the clodronate treatment resulted mainly in a defect of Ly6c^{low} circulating monocytes regardless of their level of MHCII (Fig. S1), it affected more drastically the MHCII[hi] WAM populations, regardless of Ly6c level of expression. This dichotomy between circulating and infiltrating populations could be explained by the fact that recruited monocytes (especially Ly6c^{hi}) may significantly change their phenotype once exposed to the wound's complex environment (10,16). The fact that the clodronate depletion reduced collagen transcription and affects to a larger extent the MHCII[hi] WAM may suggest that MHCII[hi] macrophages have a more prominent role in controlling collagen transcription during the early stages of healing, although this remains to be more specifically tested. (10). The molecular mechanism of the control by macrophages of Col 1 α2 transcription in wounds needs to be further investigated. The direct or indirect production or promotion of TGF-beta could be incriminated for Col 1α2 transcription by competent cells (17). However, other factors that may induce profibrotic mediators by intermediate cell types could also be considered (5,18–20). Naturally, because of the central role of macrophages in wound healing biology, these hypotheses are not mutually exclusive.

In conclusion, our data reveal that Col 1α2 transcription starts early during the healing process, only 2 days after wounding. Moreover, our data indicate that this early transcription of Col 1α2 can be controlled by macrophage depletion and more specifically by targeting MHCII[hi] macrophages. Further studies need to elucidate the underlying molecular mechanisms driving the macrophage–mesenchyme crosstalk.

Acknowledgements

Author's contribution
KK and MPR designed the research study; JMDL and MPR performed the research; JMDL, KK and MPR analysed the data; GB provided essential tools; MPR wrote the manuscript; KK, JMDL and GB edited the manuscript.

Conflict of interests
The authors have no conflict of interest to disclose.

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Supporting Information
Additional Supporting Information may be found in the online version of this article:

Data S1. Experimental design.

Figure S1. Effect of clodronate treatment on circulating monocyte.

Figure S2. Effect of macrophage depletion on wound fibrosis: representative picture of Masson's Trichrome staining of wound sections from mice treated with (a) PBS and (b) Clodronate. (c) Quantitative analysis of the relative surface area of blue (collagen) staining in mice treated with PBS ($n = 8$ sections from four mice) and Clodronate ($n = 4$ sections from two mice). Results show as mean ± SD. ***<0.01 (Mann Whitney), sa, Scar Area.

The biodisposition and hypertrichotic effects of bimatoprost in mouse skin

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Abstract: Studies on bimatoprost were performed with two objectives: (i) to determine whether bimatoprost possesses hair growth-stimulating properties beyond eyelash hypertrichosis and (ii) to investigate the biodisposition of bimatoprost in

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skin for the first time. Bimatoprost, at the dose used clinically for eyelash growth (0.03%) and given once daily for 14 days, increased pelage hair growth in C57/black 6 mice. This occurred as a much earlier onset of new hair growth in shaved mice and the time taken to achieve complete hair regrowth, according to photographic documentation and visual assessment. Bimatoprost biodisposition in the skin was determined at three concentrations: 0.01%, 0.03% and 0.06%. Dose-dependent C_{max} values were obtained (3.41, 6.74,

12.3 μg/g tissue), and cutaneous bimatoprost was well maintained for 24 h following a single dose. Bimatoprost was recovered from the skin only as the intact molecule, with no detectable levels of metabolites. Thus, bimatoprost produces hypertrichosis as the intact molecule.

Background

Bimatoprost was originally designed as an ocular hypotensive and has been extensively used for treating glaucoma (1,2). Eyelash hypertrichosis was observed as a side effect, and bimatoprost effects on eyelash growth have now been studied in detail (3–5). Because bimatoprost effects on other hair types have not been reported, we conducted studies on mouse pelage skin (6,7). The cutaneous biodisposition of bimatoprost was also investigated because the presence of substantially intact bimatoprost would be indicative of prostamide receptor involvement (8–13).

Questions addressed

1. Do the hypertrichotic properties of bimatoprost extend beyond eyelashes, for example mouse pelage hair (6,7)?
2. Is bimatoprost metabolically converted in skin? Substantial levels of intact bimatoprost would indicate prostamide receptor mediation (8–13).

Experimental design

Pelage hair growth was studied in C57/black 6 mice. The animals were shaved, and hair growth was evaluated by photographic documentation and visual assessment as (1) the time for onset of hair regrowth and (2) the time taken to completely cover the shaved area with new hair. Bimatoprost, at the dose used clinically to treat eyelash hypotrichosis, was given once daily for 14 days. The duration of the experiment was 42 days.

Bimatoprost studies on skin biodisposition were conducted at 0.01%, 0.03% and 0.06% doses. Blood samples were also collected for analysis. Two biodisposition studies were performed, one for 24-h duration and the other for 21-day duration.

Results

The effects of once-daily bimatoprost on pelage hair growth are summarised in Fig 1. The time of onset of hair regrowth was essentially halved (Fig. 1a). More importantly, the time taken to cover the shaved back with regrown hair was dramatically and highly significantly reduced (Fig. 1b). Interestingly, bimatoprost appeared to produce a uniform regrowth of hair over the shaved area, rather than radiating out from a central locus as was observed for the control group.

The cutaneous drug levels of graded doses of bimatoprost are depicted in Fig. 2. High concentrations of bimatoprost were rapidly achieved in the skin, and these remained relatively well maintained for 24 hr at about 1 μg/g tissue. In a subchronic 21-day study, no substantial drug accumulation was apparent, but a clear dose–skin concentration relationship was apparent. Supplementary tables provide the C_{max} values, areas under curve and absolute concentrations for both blood and skin. Bimatoprost was found

only as the intact molecule in both skin and blood, with no evidence of hydrolytic conversion to 17-phenyl $PGF_{2\alpha}$.

Conclusions

These studies show, for the first time, the biodisposition of bimatoprost in skin together with its effects on hair growth. The effects of bimatoprost on mouse pelage hair growth were investigated at the same dose as that employed for treating eyelashes (3,4). Cutaneous levels of bimatoprost achieved were dose dependent and were well maintained over a 1-day period. Results from a 21-day study provided no evidence for bimatoprost accumulation on repeated dosing with a 0.01% dose, but some accumulation was apparent for the 0.03% and 0.06% doses. Bimatoprost remained as the intact molecule, indicating that it exerts its effects on hair growth by stimulating prostamide receptors (8–13).

Bimatoprost essentially remained as the intact molecule in mouse skin; the putative enzymatic hydrolysis product (17-phenyl $PGF_{2\alpha}$) was only detected twice in a total of 270 separate analyses of different skin and blood samples. Previously, in mouse eyes, it was shown that bimatoprost remains intact (14). Similarly, $PGF_{2\alpha}$-ethanolamide (prostamide $F_{2\alpha}$) remains without significant hydrolytic degradation in mouse blood (15). On comparing mouse skin and monkey ocular tissue bioavailability (8), it appears that bimatoprost accesses cutaneous tissue more readily and the tissue levels are better maintained than in ocular tissues. These data suggest that once-daily administration to the skin should be adequate to obtain optimal hair growth. This contention presumes that there is a relatively homogeneous distribution of bimatoprost between the hair follicle and the skin layers. A further consideration is that bimatoprost was applied once daily for only 14 days in the hair growth experiment, as an expedient based on limited and overextended manpower resources. It follows that the dosing regimen used in these present studies may have underestimated the effect of bimatoprost on hair growth.
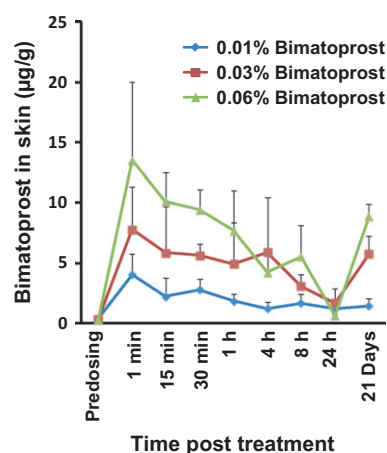
Bimatoprost has long been established as potently effective as the intact molecule, with a pharmacological profile distinct from prostanoid FP receptor agonists and their ester prodrugs (8,9,12,16). The pharmacology of bimatoprost closely resembles that of prostamide $F_{2\alpha}$ (9–11). Further pharmacological characterisation has been achieved by designing selective prostamide antagonists (17–19) and structural elucidation of the prostamide receptor (13). The results herein indicate hair growth as a further prostamide-mediated effect that may be mimicked by bimatoprost.
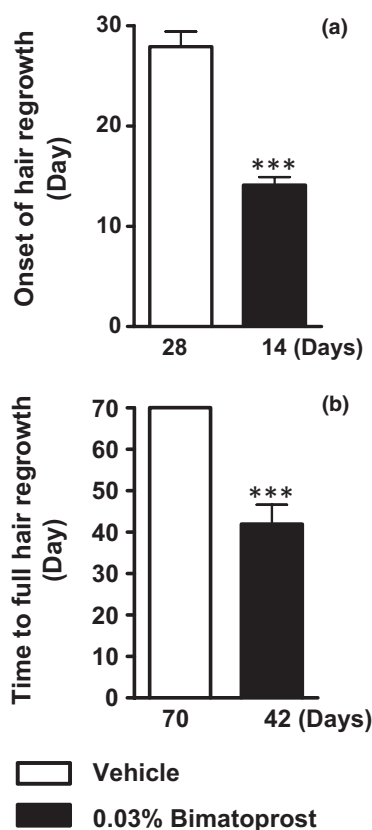
Figure 1. The effect of topically applied 0.03% bimatoprost on (a) the onset of hair regrowth and (b) the time for full hair regrowth to cover the shaved area. Open histogram blocks represent animals that received vehicle; filled histogram blocks represent animals that received 0.03% bimatoprost once daily for 14 days. Values are mean ± SEM, $n = 10$, ***$P < 0.001$ according to Student's non-paired t-test.

Figure 2. The biodisposition of graded doses of bimatoprost (0.01%-blue, 0.03%-red, 0.06%-green) in mouse skin. This graphical depiction combines the results from a single-dose, 1-day study and a once-daily, 21-day study where only the 21-day skin drug levels were determined. Points represent mean values ±SEM, $n = 5$.

Bimatoprost was almost invariably found in blood samples from mice that received topical bimatoprost on shaved skin. Blood levels were about one-thousandth of those present in skin. Although bimatoprost was detected in pharmacologically active levels in mouse blood, this would be greatly 'diluted' in the blood by humans as they are about 5000 times heavier/larger. The human scalp area of coverage would be about 10–50 times greater than that of the shaved mouse skin. Presuming similar penetration characteristics for bimatoprost in mouse and human skin, the likely blood concentration in humans would be in the range of 50 pg/ml. Anticipated human blood levels of 10–100 pg/ml are beneath the pharmacologically active levels for bimatoprost (20).

In summary, bimatoprost stimulates the growth of mouse pelage hair. Bimatoprost was found as the intact molecule in mouse skin and blood, indicating that it stimulates hair growth by interacting with prostamide-sensitive receptors (8–13). These studies indicate that the ability of bimatoprost to stimulate hair growth may extend beyond eyelashes, but effects on human scalp hair growth, for example, cannot necessarily be predicted from mouse pelage hair experiments. The human hair follicle expresses the prostanoid FP receptor (21), but expression of altFP4 (13) is required to predict a positive outcome with bimatoprost.

Author contribution

DW designed the mouse pelage hair growth study and cowrote the paper; ET performed the mouse pelage hair growth study; MA collected and analysed the drug metabolism data from the former Allergan facility in France; JW plotted the graphs, analysed the data, designed the mouse pelage hair growth study and cowrote the paper.

Conflict of interests

David F. Woodward, Elaine S.-H. Tang, Mayssa Attar and Jenny W. Wang are employees of Allergan.

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Supporting Information

Genetic alterations in RAS-regulated pathway in acral lentiginous melanoma

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Abstract: Studies integrating clinicopathological and genetic features have revealed distinct patterns of genomic aberrations in Melanoma. Distributions of *BRAF* or *NRAS* mutations and gains of several oncogenes differ among melanoma subgroups, while 9p21 deletions are found in all melanoma subtypes. In the study, status of genes involved in cell cycle progression and apoptosis was evaluated in a panel of 17 frozen primary acral melanomas. *NRAS* mutations were found in 17% of the tumors. In contrast, *BRAF* mutations were not found. Gains of *AURKA* gene (20q13.3) were detected in 37.5% of samples, gains of *CCND1* gene (11q13) or *TERT* gene (5p15.33) in 31.2% and gains of *NRAS* gene (1p13.2) in 25%. Alterations in 9p21 were identified in 69% of tumors. Gains of 11q13 and 20q13 were mutually exclusive, and 1p13.2 gain was associated with 5p15.33. Our findings showed that alterations in RAS-related pathways are present in 87.5% of acral lentiginous melanomas.

Key words: acral lentiginous melanoma – *AURKA* – melanoma – *MLPA* – *NRAS*

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Introduction

Molecular studies have revealed the existence of different biological subsets of melanomas based on the patterns of alterations identified (1–3), some of which correlated with degree of chronic sun-induced damage and site of origin (3). Furthermore, such molecular differences could result in clinical and histopathological differences among lesions (4,5).

Melanomas classified as acral lentiginous melanomas (ALM) develop on volar skin, usually unexposed to UV radiation and are characterized by the presence of an atypical lentiginous proliferation. ALM carry a high number of genomic alterations compared with other melanoma subtypes and most of them account for a smaller proportion of genome (1,3). The molecular hallmarks of ALM are *CCND1* amplifications (1,6,7) or somatic mutations in *c-KIT* (8).

Deletions in the 9p21 region where the *CDKN2A* gene is located are widely detected (9–11). However, other genes from this region could be implicated in melanoma because retention of the *CDKN2A* locus has been found in tumors with deletions at one or both sides of *CDKN2A* (10). Other reported aberrations include large amplifications of 12q (1,3), 7q or 20q and gains localized at 5p15, 11q13, 11q14 (3) and 22q11-13 (1).

Questions addressed

To characterize acquired molecular genomic alterations in a set of ALM from Spanish patients. The study was focused on specific chromosomal regions where genes involved in signalling pathways, cell cycle progression and apoptosis are located.

Experimental design

Seventeen fresh-frozen histopathologically confirmed primary ALMs based on Clark's classification were included. Sampling was guided by *ex vivo* dermoscopy (12) and documented by photography without altering the specimen, immediately fixed in formalin and embedded in paraffin (FFPE) for conventional histopathological diagnosis following the step-sectioning protocol for melanoma. Clinical data are described in Table S1. Genomic characterization of the *BRAF*, *NRAS*, *CDKN2A* and *MC1R* genes was performed by PCR-direct sequencing. Deletions of the 9p21 region and gains of