SHORT REPORT

Effects of 420-nm intense pulsed light in an acne animal model

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Abstract

Background Blue light in the 400–420 nm range has been shown to reduce the levels of Propionibacterium acnes (P. acnes) in the skin. P. acnes has been postulated to be a critical trigger for inflammatory acne. Thus, treatment with 420 nm-intense pulsed light should reduce inflammatory activity in acne.

Aim To evaluate the clinical and histological effects of 420 nm-intense pulsed light treatment on acne in animal model.

Method Inflammation acne animal model was constructed by intradermal injection of P. acnes of rat auricular. Levels of tumour necrosis factor alpha (TNF-α) and matrix metalloproteinase 2 (MMP-2), markers of inflammation implicated in acne, were assessed in treated and untreated animals by immunohistochemistry and quantitative polymerase chain reaction (PCR).

Result Treatment with 420 nm intense pulsed light led to marked improvement after 6 biweekly treatments. Immunohistochemistry and PCR showed that TNF-α and MMP-2 levels correlated with the extent of acneiform activity and were reduced by treatment with 420 nm light.

Conclusion A 420-nm intense pulsed light may exert its beneficial effects on inflammatory acne by reducing the levels of P. acnes and secondarily reducing inflammation induced by the bacteria.

Conflict of interest None declared.

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Propionibacterium acnes produces high amounts of intracellular porphyrins, mainly coproporphyrin, rendering the bacteria sensitive to light.1 Exposure to blue light in the range 407–420 nm has been shown to eradicate P. acnes,1 which appears to be a significant factor in inflammatory lesions of acne vulgaris.

The clinical efficacy of 420 nm IPL on patients with moderate to severe acne has been previously reported.2,3 The study by Xin et al. showed that 420 nm IPL effectively reduces the number of active inflammatory acne lesions. Intradermal injection of P. acnes induces histological and clinical changes similar to acne.4 That was the rational of this study, to observed the clinical, histological and immunohistochemical parameters in response to 420 nm pulsed light in a rat auricular inflammation model.

Materials and methods

P. acnes (ATCC6919) was cultivated in brain heart infusion medium, washed, and filtered. The concentration was adjusted to 60 000 000 organisms per mL prior to inactivation at 95° for 5 min. Thirty-two female Sprague-Dawley rats aged 3–5 weeks, weight 100–120 g, were obtained from Beijing Weitong Lihua Experimental Animal Technical Co. Ltd, Beijing, China. The animals were divided into four experimental arms of eight animals each. Group A served as untreated controls. Group B underwent a single intradermal injection of 50 μL sterile saline in the right ear pinna. Group C and D underwent a single intradermal injection of 50 μL P. acnes suspension in the right ear pinna. Group D group also began treatment with 420 nm IPL after 7 days; treatment was performed twice weekly at 13 J/cm², spot size 18 × 4 mm, for 3 weeks into total. Groups A, B and C were sacrificed and analyzed 7 days after injection; group D group rats were sacrificed after completion of treatment.

Rat ears were examined for general appearance before and after treatment. After sacrifice the pinna was submitted for routine for-
malin fixation and paraffin embedding. Five-micron slices were examined with routine haematoxylin and eosin staining as well as immunohistochemistry for tumour necrosis factor alpha (TNF-α) and matrix metalloproteinase 2 (MMP-2). Rabbit anti-rat TNF-α antibody, rabbit anti-rat MMP-2 antibody, second antibody and DAB display box were purchased from Shanghai Maisha Biotechnology Ltd, Shanghai, China. We hypothesized that intense pulsed light will lead to P. acnes TNF-α changes, while TNF-α will induce downstream MMP-2 changes, and explore its therapeutic mechanism.

Real-time polymerase chain reaction (RT-PCR) was performed with amplification kits purchased from Beijing Dongsheng Innovation Biotech Co. (Beijing, China) and Rotor-Gene RG-3000 thermal cycler (Corbett Research, Sydney, Australia). Total RNA was extracted according to the specifications of the kit. The upstream primer for TNF-α was 5'-CACGTCGTAGCAAACCACTGACCAA-3' and the downstream primer was 5'-GTTGGTTGTC-TTTGAGATCCAT-3', yielding a product of 100 bp. The upstream primer of MMP-2 was 5'-CTTCAGGTTCTCCAGCATGA-3', the downstream primer of MMP-2 was 5'-CACCTTGGCACACCTG-TATC-3', for a product of 131 bp. The upstream primer of the internal control GAPDH was 5'-CAAGGCTGAGAATGGGAAGACCG-3', the downstream primer of the internal control GAPDH was 5'-TGGTGAAGACGCGCATGAG-3', and the product was 131 bp.

After initial denaturation at 94°C for 15 min, amplification was performed for 50 cycles at 94°C for 15 s, 58°C for 15 s, 72°C for 10 min. The PCR products were diluted according to concentration gradient. The diluted products with 1/2, 1/4, 1/8, 1/16, 1/32 concentration were regarded as the standard template for the fluorescence quantitative PCR. Standard curves were generated from the reaction data generated from the standard samples, according to fluorescent real time monitoring data of the reaction and the concentration relationship of the standard samples by software Rotor-Gene 6.0.

SYBR Green I RT-PCR was carried out in a 20 μL reaction volume that contained 5 μL of 5 x buffer 1 μL of 10 mmol

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**Figure 1** Histology of rat ears skin before and after the irradiation of 420 nm IPL (HE, 20× original magnification). (a) Control group A. (b) P. acnes-injected group C. (c) 420 nm treated group D.

**Figure 2** TNF-α expression (immunohistochemistry, 20× original magnification). (a) Control group A. (b) P. acnes-injected group C. (c) Group D after six treatments.
Results

As expected, untreated rats in group A exhibited no changes in the pinna during the study. The ears of rats in group B, which received 50 µL intradermal saline, exhibited transient edema, representing the injected saline. In group C and D, which underwent injection of 50 µL P. acnes, erythema and edema began after 24 h and increased gradually. By day 5 acne-like lesions including comedones and pustules were seen. In group D, treatment with 420 nm IPL reduced the clinically apparent inflammatory lesions after six treatments.

Histological examination of the animals in groups C and D showed hyperkeratosis of epidermis, dilation of the hair follicle, diffuse infiltration of inflammatory cells and disappearance of sebaceous gland (Fig. 1). After six treatments with 420 nm IPL, group D showed reduction in hyperkeratosis and less inflammatory dermal infiltrate, as well as normalization of sebaceous glands.

Immunohistochemistry studies showed low levels of TNF-α and MMP-2 expression in the control group (Figs 2 and 3). After injection of P. acnes, both markers were strongly expressed. Both inflammatory markers returned to baseline levels after six treatments with 420 nm IPL.

PCR studies from ear skin substantiated the findings of immunohistochemistry. When normalized to baseline expression in control group A, no change was observed in saline treated group B, but significant increases in mRNA for TNF-α and MMP-2 were detectable in group at day 7 (TNF-α: 4.24x, MMP-2: 2.22x). (Fig. 4) The mRNA levels returned to near-baseline values after 6 treatments with 420 nm IPL (TNF-α: 1.27, MMP-2: 0.91). Both TNF-α and MMP-2 in the rat skin lesion of the model group showed high expression (TNF-α 4.24 ± 0.69, MMP-2 2.22 ± 0.35); the expression of TNF-α and MMP-2 were significantly down-regulated after the exposure of 420 nm intense pulsed light (TNF-α 1.27 ± 0.21, MMP-2 0.91 ± 0.14).

Discussion

The causes and mechanisms of acne are complex and not fully understood. The induction of an inflammatory response by P. acnes appears to be a central factor, and it has therefore been targeted by antibiotics that reduce the burden of P. acnes in the
sebaceous gland. Blue light therapy has also been used with success based in its photodynamic activation of the abundant endogenous porphyrins in *P. acnes* upon photoactivation, these porphyrins release singlet oxygen and lyse the bacteria, reducing the severity of inflammatory acne.1–3

To assess the tissue response to 420 nm IPL we applied the treatment to the validated rat auricular inflammation model, in which intradermal injection of *P. acnes* induces histological and clinical changes similar to acne.4 The current study demonstrates inflammatory changes in the skin after intradermal injection, which changes were reversed by six treatments with 420 nm IPL. TNF-α and MMP, mediators and markers of inflammation, were induced by the *P. acnes* injection, as evidence by an acute rise in their mRNA and protein levels, reflected by the results of the PCR and immunohistochemistry respectively. This is consistent with prior reports that *P. acnes* can stimulate focal secretion of TNF-α.5,6 It is also consistent with prior studies that show MMPs can be induced by *P. acnes* either directly or by *P. acnes*-induced elevation of TNF-α.7,8 The reduction in these inflammatory mediators as well as the reduction in clinical and histological evidence of inflammation after treatment with 420 nm IPL clearly indicate a role of the anti-inflammatory effect of the treatment. We surmise that the 420 nm IPL exhibits this anti-inflammatory effect by exerting an anti-*P. acnes* effect, thereby eliminating the triggers for inflammation.

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**References**