Selective Cryolysis of Sebaceous Glands

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Acne vulgaris is a nearly universal cutaneous inflammatory disease. Excess sebum production is an integral part of disease pathogenesis. Medical therapies that reduce sebum excretion result in clinical improvement of acne. Given the preferential susceptibility of lipid-containing cells to cold, we investigated the hypothesis that controlled local skin cooling causes preferential injury to sebaceous glands, in murine and swine models using a range of temperatures as low as −10 °C, and then on the backs of human subjects. In mouse ears, peak histologic damage occurred 72 hours after treatment; eosinophilic necrotic plugs formed within sebaceous glands, and the number of glands was significantly reduced up to 1 week post treatment. Cooling disrupted sebocyte cell membranes, alkaline phosphatase activity, and significantly reduced sebocyte lipid content. In human volunteers, cooling damaged sebaceous glands and reduced sebum output for 2 weeks, with minimal injury to surrounding tissues. Selective cryolysis of sebaceous glands is achievable through brief, non-invasive skin cooling, suggesting that controlled cooling could be developed as an effective treatment for acne vulgaris.

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INTRODUCTION

Acne vulgaris affects 80–95% of adolescents and often persists into adulthood. The pathogenesis is multifactorial, involving aberrant follicular keratinization, increased sebum excretion, proliferation of *Propionibacterium acnes*, and inflammation (Knutsen-Larson *et al.*, 2012; Zaenglein and Thiboutot, 2012). Many current therapies for acne—including isotretinoin (Nelson *et al.*, 2008), anti-androgens (Katsambas and Dessinioti, 2010), and photodynamic therapy (Sakamoto *et al.*, 2010)—reduce acne, at least in part, by damaging sebaceous glands and/or decreasing sebum excretion, and a 30–50% sebum reduction is correlated with improvements in clinical acne measures (Janiczek-Dolphin *et al.*, 2010). However, most current therapies are associated with significant side effects (Tripathi *et al.*, 2013); therefore, alternative methods of attenuating sebaceous glands are desirable.

Cryotherapy has a long history in dermatology. Solid carbon dioxide (−78 °C) and liquid nitrogen (−195 °C) have been used for decades for the nonspecific destruction of epidermal lesions such as actinic keratosis or verruca vulgaris (Cooper and Dawber, 2001). Our laboratory previously found that localized cooling can be used to selectively remove fat tissue, with minimal injury to surrounding tissues. The putative mechanism involves crystallization of cytoplasmic lipids at temperatures higher than the freezing point of tissue water (Manstein *et al.*, 2008). This noninvasive technology, called cryolipolysis, is now widely used for local reduction of unwanted subcutaneous fat. As sebaceous glands have high (~30%) lipid contents (Sakamoto *et al.*, 2012), we reasoned that they may also be preferentially susceptible to cold injury. Sebaceous gland damage in animals after localized cryoinjury was first reported decades ago (Gage *et al.*, 1979), but the potential and conditions for selective effect on sebaceous glands are unknown. We conducted the current study to establish the feasibility of selectively damaging sebaceous glands by localized skin cooling, first in animal models and then in a pilot clinical trial with human volunteers.

RESULTS

Murine model

By varying cooling parameters such as temperature, exposure time, cooling rate, and the number of cooling cycles (Table 1), we identified conditions at which sebaceous glands were selectively damaged, with little to no injury of surrounding tissues. One such combination—cooling at a device temperature of −7 °C for 10 minutes—was explored more extensively and is presented below.

In the days following the cooling procedure, sebaceous glands in the treated regions exhibited a progressive loss of cellular structures, including intracellular lipid granules, nuclei, sebocyte membranes, and intercellular septa, concurrent with the accumulation of an eosinophilic precipitate (Figure 1a).

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Table 1. Cooling parameters for selective cryolysis of sebaceous glands in murine model

Experimental group	Constant	Variable				
1	Duration = 10 min maximal cooling rate	**Temperature**				
		0 °C	**−5 °C**	**−7 °C**	**−10 °C**	
		−	±	+	+	SG
		−	−	−	+	NS
2	Temperature = −7 °C maximal cooling rate	**Duration**				
		0 min	**5 min**	**10 min**	**15 min**	
		−	−	+	+	SG
		−	−	−	−	NS
3	Temperature = −7 °C duration = 10 min	**Rate**				
		Cooling rate[1]				
		Max	**2 min**	**Max**	**2 min**	
		Thawing rate[2]				
		Ambient	**Ambient**	**2 min**	**2 min**	
		+	−	+	−	SG
		−	−	+	−	NS
4	Temperature = −7 °C	**Freeze-thaw cycles**				
		One 10-min cycle	**Two 10-min cycles**	**Two 5-min cycles**	**Five 2-min cycles**	
		+	+	+	+	SG
		−	+	+	+	NS

Abbreviations: NS, non-specific injury; SG, sebaceous gland injury. +, = histologic signs of injury. −, no sign of injury.
Different cooling parameters were explored in four experimental groups: (1) The cold plate was cooled to different target temperatures, whereas cooling duration was held constant and maximal cooling rate (i.e., cold plate was pre-cooled to target temperature prior to beginning treatment) was used; (2) Different treatment durations were used, at constant target temperature and cooling rate; (3) Different cooling and thawing rates were used, at constant target temperature and duration; and (4) Different numbers of cooling cycles were used, with thawing between each cycle, and the target temperature held constant. Selective damage to sebaceous glands, with minimal non-specific damage to surrounding tissues, was achieved under certain cooling parameters, as shown in this table.
[1]Max cooling rate denotes that the cold plate was pre-cooled to target temperature prior to contact with tissue. For a 2 min cooling rate, the cold plate was initially heated to 37 °C and then after contact with the tissue gradually cooled to target temperature over 2 min.
[2]"Ambient" thawing rate denotes that the tissue was exposed to room temperature (~25 °C) immediately after cooling treatment. For a 2 min thawing rate, after cooling treatment the cold plate was gradually heated to 37 °C over 2 min, and then the treated tissue was removed.

These histologic signs of cellular damage were maximal on the 3rd post-treatment day, present in over 60% of the glands. In contrast, <5% of the glands in untreated control mouse ears showed any histologic evidence of damage. The treated glands gradually recovered their normal morphology, and by 1 week <10% of the glands remained damaged (Figure 1b). There was transient swelling in the ear after cooling, but otherwise no gross or histologic evidence of nonspecific damage to surrounding skin or cartilage tissues was observed. Of note, there was a small but significant ($P<0.001$) reduction in the absolute number of glands in the treated samples, which persisted after 1 week, despite nearly complete recovery of the histological features in the remaining glands, suggesting that some glands may have been irreversibly damaged by cooling (Figure 1c).

Propidium iodide (PI), a membrane-impermeable dye, was used to label cells with compromised membranes. PI staining was minimal in untreated controls—most glands showed no staining, whereas a small number of glands had positive staining in the area immediately adjacent to the hair follicle (Figure 2a and c), consistent with the normal degeneration of secreted sebocytes. In contrast, post-cooling glands showed extensive PI staining throughout the glands, co-localized with both the nuclei and lipids in the glands (Figure 2b and d). Alkaline phosphatase (ALP) activity, normally prominent in sebaceous glands (Figure 2e), was substantially diminished (Figure 2f) at the time point corresponding to maximal histologic damage (3 days post cooling). At the same 3-day time point, cooling did not disrupt the expression of key protein markers associated with sebaceous glands (Figure 2i), including Keratins 5 and 15, Ki67, MUC1 (Mucin 1, Cell Surface Associated), MC5R (melanocortin receptor-5), and PPARγ (Peroxisome proliferator–activated receptor gamma). However, lipid contents in the treated glands were substantially reduced and in some cases completely abolished (Figure 2h).