

Review Article

Nicotine effects on skin: Are they positive or negative?

Misery L. Nicotine effects on skin: Are they positive or negative?
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Abstract: The adverse effects of tobacco on the skin are well known but the role of nicotine is more controversial. Nicotinic receptors are expressed in the skin, on keratinocytes, fibroblasts and blood vessels. Nicotine induces vasoconstriction associated with local hyperaemia. It inhibits inflammation through effects on central and peripheral nervous system and through direct effect on immune cells. It delays wound healing and accelerates skin aging. The role of nicotine on skin diseases remains unclear. Therapeutic effects of nicotine could be possible and this a new stimulating field of research.

Laurent Misery

Laboratory of Cutaneous Neurobiology,
Department of Dermatology, University Hospital,
Brest, France

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Pr Laurent Misery
Department of Dermatology
University Hospital
29609 Brest cedex
France

Tel.: +33 298 22 33 15

Fax: +33 298 22 33 82

e-mail: laurent.misery@chu-brest.fr

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The adverse effects of tobacco on the skin are well known. Changes in the rheologic characteristics of the blood, increased vasoconstriction, and damage to the epithelial layer of the vessel play an important pathogenic role in impaired wound healing, thromboangiitis obliterans, and peripheral arterial obstructive diseases. Interactions with collagen metabolism are of special significance in wound healing and skin aging. The immunological effects, such as the induction of an inflammation could play a role in palmoplantar pustulosis, psoriasis, atopic dermatitis, acne vulgaris, acne inversa, thromboangiitis obliterans, and lupus erythematosus. Tobacco consumption may contribute to the pathogenesis of human papilloma-virus infections, malignant melanoma, and epithelial tumors of the skin and neighboring mucous membranes (1). But tobacco is made of hundreds of substances. What are the effects of nicotine on the skin?

Nicotinic receptors in the skin

Acetylcholine has two types of receptors: nicotinic (nAChR) and muscarinic (mAChR). Nicotine is an agonist of acetylcholine. Its effects are mediated through nicotinic receptors (2). The nAChR is a 290 kDa protein, initially described at the nerve-muscle synapse but also expressed in the whole peripheral and central nervous system and on

non-neuronal cells (3). It consists of a ring of five similar subunits ($\alpha\beta\gamma\delta\epsilon$), delineating through the membrane a central pathway for the ions. Seventeen nAChR subunits have been identified and termed $\alpha 1$ – $\alpha 10$, $\beta 1$ – $\beta 4$, γ , δ and ϵ (4,5). The differences in subunit composition of nAChR determine the functional and pharmacologic characteristics of the ion channels formed. Nicotine acts as an agonist at all nAChR types, except for $\alpha 9$ -made channels (6). The subunit $\alpha 10$ fails to produce functional receptors alone: it promotes robust acetylcholine-evoked currents when co-injected with $\alpha 9$. The presence of $\alpha 10$ modifies the physiological and pharmacological properties of the $\alpha 9$ receptor indicating that the two subunits co-assemble in a single functional receptor (5).

In the skin, both mAChR and nAChR have been reported (7,8). Obviously, electrophysiological and immunohistochemical data indicate the functional expression of nicotinic acetylcholine receptors (composed of $\alpha 3$, $\alpha 5$, and $\beta 4$ but not of $\alpha 4/\beta 2$ or of $\alpha 7$ subunits) on the axonal membrane of unmyelinated human C fibers (9). But the expression of nAChR is not restrained to nerve endings.

Effects on keratinocytes

Human keratinocytes synthesize, secrete, and degrade acetylcholine (10). Activity of acetylcholine

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is mediated by mAChR and nAChR (7,8). There are approximately 35 400 binding sites of nAChR per cell on mature keratinocytes freshly isolated from human neonatal foreskins (7). These receptors contain $\alpha 3$, $\alpha 4$, $\alpha 5$, $\alpha 7$, $\alpha 9$, $\alpha 10$, $\beta 2$, and $\beta 4$ subunits (5,7,11–13).

Nicotine increases cell–substrate and cell–cell adherence of cultured keratinocytes and stimulates their lateral migration (7). Thus, it appears as a mediator for keratinocyte adhesion and motility. Nicotine exerts inhibitory effects on keratinocyte migration, and Ca^{++} serves as a second messenger in the signaling pathway (14). It could explain deleterious effects of nicotine on wound re-epithelialization and suggest that smoking may delay wound healing via nicotinic receptor-mediated pathway. The $\alpha 3$, $\alpha 9$, and M3 acetylcholine receptors play Key roles in regulating cell adhesion in a synergistic mode keratinocyte adhesion, most probably by modulating cadherin and catenin levels and activities (15) and by regulating desmosomal adhesion of keratinocytes by altering the level of expression of both desmoglein (Dsg) 1 and Dsg 3 and the phosphorylation status of Dsg 3 (16).

Nicotine is also involved in keratinocyte differentiation by stimulating calcium influx and enhancing the number of cells forming cornified envelope and expressing keratin 10, transglutaminase 1, involucrin, and filaggrin (17). ACh signaling through $\alpha 7$ nAChR channels controls late stages of keratinocyte development in the epidermis by regulating expression of the cell-cycle progression, apoptosis, and terminal differentiation genes. These effects are mediated, in part, by alterations in transmembrane Ca^{++} influx (13). Nicotine, at concentrations up to 100 $\mu\text{g}/\text{ml}$, is not an irritant but induces cornification of the skin (18). It induces squamatization (6,17–19).

Elimination of the $\alpha 7$ component of nicotinic signaling in keratinocytes decreased relative amounts of the pro-apoptotic Bad and Bax at both the mRNA and the protein levels, suggesting that $\alpha 7$ nAChR is coupled to stimulation of keratinocyte apoptosis (20). Terminally differentiated keratinocytes exert an apoptotic secretion upon secretagogue action of acetylcholine. A combination of a cholinergic nicotinic agonist and a muscarinic antagonist is required to trigger the apoptotic secretion. Analysis of the relative amounts of cholinergic enzymes and receptors expressed by keratinocytes capable of secretion and the pharmacological profiles of secretion regulation reveals an upward concentration gradient of free acetylcholine in epidermis which may provide for its unopposed secretagogue action via the

m1 muscarinic and the $\alpha 7$ and $\alpha 9$ nicotinic receptor types expressed by keratinocytes at the latest stage of their development in the epidermis (21).

In summary, nicotine enhances keratinocyte adhesion, differentiation, and apoptosis and inhibits keratinocyte migration.

The effects of nicotine on keratinocytes through nAChR are not limited to the skin but are also observed in the mouth. Thus, some pathobiologic effects of tobacco products in oral tissues may stem from nicotine-induced alterations of the structure and function of keratinocyte nAChRs responsible for the physiologic regulation of the cell cycle by the cytotransmitter acetylcholine (22).

Effects on fibroblasts

Specific nAChRs are expressed by fibroblasts: $\alpha 3$, $\alpha 5$, $\alpha 7$, $\beta 2$, and $\beta 4$ nAChR subunits are detected in human fibroblasts (23). Exposure of these cells to nicotine increases mRNA and protein levels of the cell-cycle regulators p21, cyclin D1, Ki-67, and PCNA and increases apoptosis regulators Bcl-2 and caspase 3 (23). Nicotine exposure also up-regulates expression of the dermal matrix proteins collagen type I $\alpha 1$ and elastin as well as matrix metalloproteinase-1 (23). In $\alpha 3$ nAChR knockout mice, there are alterations in fibroblast growth and function that are opposite to those observed in fibroblasts treated with nicotine (23). The skin of $\alpha 7$ knockout mice feature decreased amounts of the extracellular matrix proteins collagen 1 and elastin as well as the metalloproteinase-1 (20). Thus, some of the pathobiologic effects of tobacco products on extracellular matrix turnover in the skin may stem from nicotine-induced alterations in the physiologic control of the unfolding of the genetically determined program of growth and the tissue-remodeling function of fibroblasts as well as alterations in the structure and function of fibroblast nAChRs.

Effects on blood vessels

Smoking a single cigarette decreases the cutaneous blood flow in habitual smoker as well as in non-smoker subjects. Moreover, the slower recovery phase of smokers suggests that their microcirculation becomes inured to smoke (24). The damaging effects of smoking on the skin vasculature and on oxygenation have been documented in both human and animal models. Nicotine does not appear as the only one factor of vasoconstriction and hypoxemia (25). Nonetheless, the blood vessel network is under control of cholinergic system. NACHR are expressed on muscles and endothelial

cells, (26) and acetylcholine is released in synapses. Nicotine induces vasoconstriction, higher in skin than in gingiva, associated with local hyperaemia (27). Acetylcholine and nicotine seem to exert effects on endothelial cells, like a small facilitatory effect on the expression of intracellular adhesion molecule-1 (28). Acute exposure of human skin vasculature to nicotine has deleterious effects on endothelial function: amplification of norepinephrin-induced skin vasoconstriction and impairment of endothelium-dependent skin vasorelaxation (29).

Effects on inflammation

Nicotine inhibits inflammation through effects on central (30) and peripheral nervous system. *In vitro* studies have shown nicotine to be chemoattractant to neutrophils and in lower concentration (as in the plasma of smokers) to enhance the response to chemotactic peptides (31). Nicotine inhibits enzyme release from neutrophils and superoxide production (32), which has implications for defense against infection as well as antitumor activity. The nAChR $\alpha 7$ subunit is required for acetylcholine inhibition of macrophage tumor necrosis factor (TNF) release. Electrical stimulation of the vagus nerve inhibits TNF synthesis in wild-type mice but fails to inhibit TNF synthesis in $\alpha 7$ -deficient mice. Thus, the nAChR $\alpha 7$ subunit is essential for inhibiting cytokine synthesis by the cholinergic anti-inflammatory pathway (33). Nicotine also alters immune responses by directly interacting with T cells (34).

After transdermal application of nicotine (patches), the response to sodium lauryl sulfate is diminished, as well as the erythema response to UVB (35). But no effect on cutaneous blood flow is demonstrated, suggesting that they are related to other compounds of tobacco (36). These studies raise the possibility that transdermal nicotine could be used in inflammatory skin disease.

Nonetheless, inflammation of the buccal mucosa, gingiva, and periodontal tissues is a significant problem in users of nicotine. Nicotinic agents, acting at nAChRs contained on primary sensory neurons, could be capable of directly modulating the stimulated release of calcitonin gene-related peptide (CGRP) (37). This modulation could contribute to inflammatory processes within the oral cavity. But CGRP is also known for inhibiting antigen presentation (38). A better explanation could be the activation of COX-2 expression by nicotine, associated with a nicotine-induced cytotoxicity not directly via the induction of COX-2 expression (39). Nicotine treatment significantly increased IL-1 α concentrations in

cultured keratinocytes; however, PGE2 synthesis was not altered (40). Nicotine has different regional effects on small bowel and colonic cytokine mucosal levels, which might explain some of its opposite effects on small bowel and colonic inflammation (41). Such a differential effect could also be observed with mouth and skin.

Effects on skin aging and cutaneous homeostasis

Topical application of nicotine, by activating the calcium channel in neurons, delays the barrier repair after tape stripping (42). Light microscopy and electron microscopy observation shows delay of the exocytosis from keratinocytes. Nicotine exerts inhibitory effects on keratinocyte migration, Ca^{++} serving as a second messenger. These results also explain deleterious effects of nicotine on wound re-epithelialization (14). Nicotine stimulates calcium influx and enhances cell differentiation (17). The subunit $\alpha 7$ appears especially involved in Ca^{++} influx regulation (13). Like the nervous system, influx of calcium into epidermal keratinocytes through ionotropic receptors plays a crucial role in cutaneous barrier homeostasis.

Tobacco is known to accelerate skin aging. Nicotine exerts a specific role in this phenomenon. Changes in the rheologic characteristics of the blood, increased vasoconstriction, and damage to the epithelial layer of the vessel are probably involved. Interactions with collagen metabolism and keratinocyte differentiation and migration are also probably implied in skin aging.

Effects on wound healing

Tissue repair and remodeling are altered by tobacco, mainly through the effects of tobacco on structure and function of fibroblasts (43,44). Through its specific receptors, the role of nicotine appears central (20,23). The association between cigarette smoking and delayed wound healing is well recognized (45). Nicotine is a vasoconstrictor that reduces nutritional blood flow to the skin, resulting in tissue ischemia and impaired healing of injured tissue. Nicotine also increases platelet adhesiveness, raising the risk of thrombotic microvascular occlusion and tissue ischemia. In addition, proliferation of red blood cells, fibroblasts, and macrophages is reduced by nicotine. Nicotine is also involved in wound healing by inhibiting keratinocyte migration and differentiation, i.e. the second phase of wound healing: re-epithelialization (7,14,17). Nonetheless, a recent article brings contradictory results. Through an activation of angiogenesis mediated by endothelial

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nAChRs, nicotine would accelerate wound healing in genetically diabetic mice (46).

Effects of transdermal application of nicotine

Nicotine is brought not only by tobacco, but also by chewing gums or transdermal patches used for stopping tobacco consumption. Transdermal patches of nicotine are usually well-tolerated. The most frequently reported adverse effects are skin reactions, which include erythema, edema, pruritis, and burning sensation at application site (47,48). Most of these reactions are mild and short-lived and may be minimized to a great extent by changing the site of application daily. Nicotine, however, can act as a hapten and behave like a full antigen when bound to the proteins in the cutaneous tissue, and it can induce some sensitization reactions in a small number of patients, like urticaria (49) or contact allergic dermatitis (48).

Effects on skin diseases

Tobacco appears as an aggravating factor for diseases such as psoriasis, atopic dermatitis, acne, lichen, or lupus (1,50–52). But the role of nicotine is less clear.

Immunosuppression caused by nicotine consumption may contribute to the pathogenesis of human papilloma-virus infections, malignant melanoma, and epithelial tumors of the skin and neighboring mucous membranes (1). The carcinogenic role of nicotine is not demonstrated but nicotine facilitates tumor growth (53).

The induction of reactivation of herpes after systemic exposure to nicotine in rabbits has been demonstrated (54). On the contrary (55), the prevalence of recurrent herpes labialis in a general adult Swedish population during a period of 2 years was significantly lower among smokers, and especially among pipe smokers, compared to people with no tobacco habit. Is there a link with nicotine?

Considerable teratogenic effects of nicotine were observed histologically on newborn rat skin: increased mitotic activity in the basal cells, induction of hypertrophic epithelial cells in the epidermis (56).

A suggested role for nicotine in the pathogenesis of palmoplantar pustulosis (PPP) has been discussed. The target for the inflammation in PPP is the acrosyringium. In healthy controls, both nAChR $\alpha 3$ and $\alpha 7$ subtypes show stronger immunoreactivity in the eccrine glands and ducts than in the epidermis. The papillary endothelium was positive for both subtypes. Epidermal $\alpha 3$ staining was stronger and that of the coil and dermal ducts weaker in healthy smokers than

in healthy non-smokers. In involved PPP skin, granulocytes displayed strong $\alpha 3$ immunoreactivity. The normal epidermal $\alpha 7$ staining pattern was abolished in PPP skin and was replaced by strong mesh-like surface staining, most markedly adjacent to the acrosyringium, which in controls was intensely $\alpha 7$ positive at this level. Endothelial $\alpha 7$ staining was stronger in PPP skin than in the controls. Hence, smoking can influence nAChR expression (57). The altered nAChR staining pattern in PPP skin may indicate a possible role for nicotine in the pathogenesis of PPP.

Some inflammatory diseases of the skin such as rosacea and severe acne have been found to be more prevalent in non-smoking subjects (35). It seems possible that smoking has complex pharmacological and immune modulating effects, but the mechanisms by which cigarette smoking alters the expression of quite different diseases remains largely unknown.

Nicotine patches could be treatment for psoriasis because it could induce keratinocyte differentiation (17), but this concept needs to be confirmed by clinical studies. Traditional remedy for eczema in Bangladesh contains high amounts of nicotine (58).

Pemphigus vulgaris is an auto-immune disease. Antibodies against desmosomal proteins are responsible for the occurrence of blisters. But these lesions could be associated with autoantibodies to keratinocyte cholinergic $\alpha 9$ receptors (12), which are known to regulate cell adhesion (59). Pyridostigmine bromide, an analogue of nicotine, seems to be able to keep this disease in control (60,61). Hence, the improvement of pemphigus by cigarette smoking (62) is probably due to a direct effect of nicotine.

Some inflammatory skin diseases have been successfully treated by nicotine. But all these case reports remain anecdotal. A patient with malignant atrophic papulosis was successfully treated with nicotine patches (63). Skin disorders with prominent eosinophilic infiltration, such as Kimura's disease and erythema nodosum, could be treated by nicotine chewing gum (64). Other cases of healing with nicotine are reported in neutrophilic dermatoses, like pyoderma gangrenosum (65) and Behçet's disease (66).

Aphthous disease is known to be less frequent in smokers (67). Bittoun (68) investigated the effect of nicotine in the form of Nicorette[®] tablets on aphthous ulcers in three non-smoking patients. Lesions healed and new ulcers did not form during 1 month of nicotine therapy, although two of the three patients relapsed after having been weaned from the tablets.

Due to the presence of acetylcholine receptors on lice, nicotine is an effective adjunct to other insecticidal therapy for head lice (69).

Conclusions

In the skin, nicotine mimics effects of acetylcholine (6). The effects of nicotine on skin are ambivalent and often unclear (with contradictory results). They appear more negative than positive. They are negative on keratinocyte homeostasis, skin aging, wound healing, and in some dermatological diseases. Quitting of smoking and protection against nicotine and tobacco effects are necessary. Nonetheless, revealing the benefits of nicotine containing products (especially effects of nicotine on inflammation) is not intended to encourage people to smoke nor to spare them from the pressure to discontinue their habit. Studying the therapeutic effects of these products on skin diseases, however, is important as it might lead to a better understanding of their cause and pathogenesis. It might provide the seed from which new alternative treatments will grow in the future.

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