Sympathetic Regulation in Normal Wound Healing

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Sympathetic mediators could constrict arteries in the skin to prevent excessive blood loss. In intact skin around the wound, sympathetic fibers are crucial in maintaining physical properties of the skin. Without normal regulation, sweating skin can become dry and vulnerable to infections.

diabetes mellitus wound healing sympathetic system norepinephrine

1. Introduction

Soft tissue healing, like any acute inflammation, has several stages: vessel dilatation and permeabilization, proliferation and reparation [1]. The autonomic nervous system regulates all stages of wound healing in the wound area, in intact skin near the wound and in the whole body. Sympathetic mediators could constrict arteries in the skin to prevent excessive blood loss. In intact skin around the wound, sympathetic fibers are crucial in maintaining physical properties of the skin. Without normal regulation, sweating skin can become dry and vulnerable to infections. In the wound area catecholamines, acetylcholine and neuropeptides modulate leukocyte activity, reepithelization, wound contraction, and other important processes.

2. Inflammation and Immune Cells

In the first days after wounding, acute inflammation decides the fate of the healing process. In minutes after initial damage injured cells, resident immune cells and tissue debris affect local blood vessels and attract granulocytes from blood stream. Vasodilatation and increased vessel permeability enhance leukocyte transcytosis. Sympathetic fibers modulate acute inflammation in several ways with different neurotransmitters and receptors.

As any serious trauma usually acts as a stressor, the catecholamine level in blood quickly increases, simultaneous with local sympathetic fibers' discharge. Catecholamines increase skin arterioles' resistance, shunting more blood to vital organs. This effect could delay leukocyte migration, but it also prevents excessive blood loss. Some older works have stated that catecholamines also increase capillary permeability [2][3]. Later research contradicted this finding. Lack of sympathetic activation, for example in diabetic skin, rather increased capillary leak through lower vessel tone [4][5]. While most cytokines and inflammation mediators in the wound area increased capillary permeability, catecholamines act as antagonists.

Catecholamines effectively modulate leukocytes' activity [6]. Adrenoceptors have been found on all types of leukocytes with different density, most abundant are β_2-adrenergic receptors [7]. Information about other types of adrenergic receptors is controversial in different species and cell types. Next to β_2 by quantity are a_1, then a_2 and β_1-receptors, respectively [8]. a-adrenoceptors and β-adrenoceptors enhance opposite processes in leukocytes, but they have different affinity and abundance. β_2 adrenergic regulation of immune response is the most studied and probably the most prevalent and important. β_2-agonists decrease TNFa and other pro-inflammatory cytokines release, leukocyte migration and chemotaxis [9]. IL-10 concentration is elevated rapidly after β_2-AR activation leading to immunosuppression or localized inflammation [10]. Possible mechanisms include protein kinase A (PKA)—mediated NF-kB suppression and β-arrestin 2 protein synthesis [10][11]. A. Gosain et al. studied norepinephrine effects on activated neutrophils and macrophages, isolated from rat wounds. Both types of cells have shown significantly reduced phagocytic activity by PKA activation. Macrophages were inhibited both by physiological and pharmacological NE doses, while neutrophils were inhibited only by pharmacological NE dose [12][13].

Less data is available about a-adrenoceptor's role in soft tissue wound healing. In most cases, they have shown pro-inflammatory properties. a-adrenergic agonists increase pro-inflammatory cytokines production, immune progenitor proliferation and reactive oxygen species production [14][15]. In other inflammatory situations a-adrenoceptors stimulation increases many pathological reactions. In systemic inflammation, a_1-adrenergic stimulation increases the release of TLR-driven cytokines from macrophages [16]. a_{1b} receptors also have shown ability to form heterodimeric complexes with chemokine receptors and regulate their activity [17][18].

Overall, acute catecholamines release reduce inflammation through β_2-adrenergic receptors. This is interesting, because inflammation is vital in normal wound healing [19][20]. Despite thousands of works it is still impossible to predict when inflammation becomes deleterious or when anti-inflammatory agents become harmful. For example, glucocorticoids have been shown to prevent wound healing or even to induce wounding in different situations [21][22]. On the other hand, in some wound models, glucocorticoids improve healing [23]. Similarly, β-AR affecting drugs are very inconsistent in different inflammation models and diseases [24][25][26][27][28][29][30][31][32]. In simple wounding model cell proliferation rate, neutrophiles and mast cells migration, myofibroblast density and the blood vessels volume density were increased by a beta-blocker, while healing was delayed overall [33]. To the contrary, after propranolol administration in streptozotocin-induced rat diabetes model, the wound area was smaller 7 and 14 days after wounding in propranolol group, and inflammatory cells number and MMP-9 level were reduced [34].

Therefore, we should study pro- and anti-inflammatory agents in more detail to find finer switching mechanisms. Because β_2-AR related effects are dominant, Pongratz et al. hypothesize that sympathetic system nerve endings prevent deleterious inflammation spreading and tissue damage. β-adrenergic receptors bind NE with lower affinity, than a-adrenergic receptors. Sympathetic nerve terminals release NE, and in proximity it binds with both β- and a-adrenergic receptors on leukocytes with subsequent β_2-AR induced IL-10 release. Farther from NE source, a high-affinity a-adrenergic receptor binds more mediator, than β-adrenergic receptors and TNFa release prevails over IL-10 production. Therefore, intact sympathetic fibers reduce inflammatory response in intact wound margins and increase closer to the wound's center. Possible repulsion of sympathetic fibers from the inflammation area would

also positively modulate inflammation [35]. This concept only includes catecholamines, though other sympathetic transmitters in the skin also could be important for net inflammation balance.

As sudomotor sympathetic nerves release acetylcholine, they could trigger acetylcholine responses aside from vasodilation. As ubiquitous paracrine agent, acetylcholine is an important immune system mediator. Acetylcholine has been revealed as a pro-inflammatory mediator in many studies [36][37]. Leukocytes produce different types of cholinergic receptors: M_1–M_5 muscarinic receptors, a_3, a_5, a_7, a_9, a_{10} nAChR subunits [38][39]. Both cholinergic receptor types inhibit cytokine secretion from leukocytes, though mAChR are better-researched [40][41][42]. Considering that, acetylcholine release also could decrease inflammation in wound area. Contrary to catecholamines, ACh promotes vasodilation that stimulates leukocyte extravasation [43][44].

Vasoactive intestinal peptide (VIP) is characteristic for cholinergic neurons, including sympathetic sudomotor neurons. In the acute phase of inflammation, VIP induces histamine and bradykinin release from mast cells. Therefore, VIP itself and vasoactive inflammatory mediators induced by its actions promote vasodilatation in wound margins. In later stages, VIP could realize its anti-inflammatory properties. In different circumstances VIP could increase Treg cells level and protect them from apoptosis, inhibit TNFa and IL-6 secretion [45][46][47].

Neuropeptide Y (NPY) is produced in skin primarily by vasomotor sympathetic nerve endings. Its role in soft tissue wound healing is not completely understood. In other pathologies NPY acts as pro-inflammatory agent [48][49][50][51]. NPY induces cytokine production in leukocytes through Y1 and Y5 receptors, but other types of NPY receptors with other roles also were scarcely described [50][52].

Among typical skin sympathetic neurotransmitters, only NPY can possibly act as a pro-inflammatory without immunosuppressive functions in normal wound healing. To focus inflammation near the wound's margins immune cells need to repel nearby sympathetic fibers. Indeed, inflammatory sympathetic repulsion is a well-known phenomenon. A special class of semaphorin molecules called nerve repellent factors regulates neurite outgrowth. They include semaphorin 3F(SEMA3F), plexin-A2, neuropilin-2 and other factors [53]. In inflamed tissues, including diabetic Charcot foot, semaphorin 3C is highly expressed with lower sympathetic fibers' density [54]. S. Clatt et al. tested whether cytokines and hormonal factors released in inflamed tissue also have repellent properties. TNF-α repelled nerve fibers with moderate to strong effects (0–100%). High concentrations of dopamine and norepinephrine (10^{-6} M) induced weak but significant nerve fiber repulsion (up to 20%). Stimulation with low concentrations of 17β-estradiol (10^{-10} M, but not 10^{-8} M) repelled SNFs [55]. Systemic inflammatory responses in sepsis also induced nerve repulsion in primary immune organs. D. Hoover et al. have found that in patients with sepsis there are about 16% of normal sympathetic fibers. While spleen innervation provides ambiguous immune regulation, it's dysfunction could both decrease inflammation specificity and increase detrimental effects [56]. Major aspects of sympathetic neurotransmitters' interaction with immune cells were placed in **Table 1**.

Table 1. Overview of sympathetic neurotransmitter interactions with immune cells.

Neurotransmitters	Norepinephrine/Epinephrine	Acetylcholine	VIP	NPY
Primary effect	Immunosuppressive	Immunosuppressive	Immunosuppressive	Pro-inflammatory
Source	Vasomotor fibers/keratinocytes	Sudomotor fibers/keratinocytes	Sudomotor fibers/keratinocytes	Vasomotor fibers/keratinocytes
Wound healing role—inflammation	Low concentration in wound area, high in healthy tissue around	Not clear	Not clear	Not clear
Primary receptor blockade	Switch to pro-inflammatory, better healing or hyperinflammation	Inflammation increase, better healing?	Inflammation increase, better healing?	Studied in some hyperinflammatory conditions only

3. Keratinocytes

Keratinocytes (KC) are activated after wounding among other cells through the first minutes and hours. Keratinocytes are characterized by keratin expression profile, which defines the KC phenotype. In healthy skin there are basal KC, that proliferate and replenish lost corneocytes through different maturation stages. After wounding basal KC and some mature KC switch their phenotype to activated or contractible. Activated KC can migrate, proliferate and are crucial for re-epithelialization. Contractible KC pull the extracellular matrix to make wound area smaller. Without any kind of epidermis, the wound has a great risk of becoming infected or chronic [57][58].

Because intact epidermis cells synthetize NE, they could affect each other and prevent inflammatory activation. In vitro β_2-adrenergic receptors activation inhibits keratinocyte migration [59][60]. Epinephrine is a more potent keratinocyte migration inhibitor than norepinephrine [61]. Cellular events after keratinocytes β_2-AR activation include serine/threonine phosphatase PP2A activation, extracellular signal-related kinase (ERK) dephosphorylation and promigratory signaling cascade blockade [62]. Keratinocyte proliferation is also inhibited by β_2-AR agonist isoproterenol [63].

Unlike in immune cells, keratinocytes β_2-AR contribute to pro-inflammatory cellular response as well. Epinephrine increases interleukin production, and β_2-AR—TLR crosstalk significantly augments inflammatory response [64]. Because epidermis is always in contact with the outer world and its germs, cytokines could be important in maintaining skin defenses. While β_2-AR blocks KC activation, an increased cytokines level possibly could overcome this effect [65]. For now, this question remains open.

Wound modeling in keratinocytes culture leads to rapid norepinephrine release and persistent downregulation of β_2-AR protein and catecholamine synthesis enzymes gene expression [66]. Keratinocyte culture scratch wounding downregulated expression of β-adrenoceptors genes, tyrosine hydroxylase and PNMT genes. While β_2-adrenoceptors functional activity remained depressed, their gene expression returned to the baseline. With decreased β_2-AR stimulation, keratinocytes produced more norepinephrine, which impaired their migration activity in wound edges. Effects were diminished by β_2-AR selective antagonist ICI-118,551, β_1-AR selective antagonist

bisoprolol did not change them [66]. R. Sivamani et al. in vitro and in mouse burn model have found detrimental role of β_2-AR after epinephrine exposure. In vitro keratinocytes blockade was achieved by physiological stress-induced epinephrine concentrations [67].

As with immune cells, alpha-adrenoceptors have opposite effects after KC activation. In studies, a_{2A}/a_{2C}-adrenoceptors knockout transgenic mice have shown accelerated wound contraction and re-epithelialization. On the other hand, a_2-adrenoceptors on the presynaptic membrane reduce catecholamine release, therefore external a_2-AR sympathetic activation could improve wound healing through inhibited NE release and lower β_2-AR activation [68]. In vitro a_2-ARs increase keratinocyte migration. Under low norepinephrine concentration a_2-ARs overcome β_2-adrenoceptors and their stimulation induces rapid migration [69]. Probably, a-adrenoceptors activation prevents KC from switching back to a stable basal or mature phenotype.

Acetylcholine also is abundant in epidermis and can act on the keratinocytes directly via cell receptors. Muscarinic receptors of five molecular subtypes and nicotinic receptors were found in keratinocytes and melanocytes [70]. Combined acetylcholine receptors blockade in vitro leads to the complete organotypic skin culture growth and proliferation inhibition. The nAChR receptors blockage led to less prominent changes than did the mAChR blockage in terms of culture thickness and maturation marker genes' expression [71][72]. Important acetylcholine function is a keratinocytes cohesion stimulation [43][73]. Like NE, ACh also increases cytokine synthesis in keratinocytes. We propose that, by the same logic, cytokine stimulation can compensate direct inhibition [74]. In some reports M_3 mAChR activation inhibits KC migration, while M_4 mAChR activates migration [75]. In vitro experiments establish that a_9 nAChR is important for migration start; without it KC remained attached to the surface. A huge number of receptors with opposite effects reduced the chances to successfully target cholinergic system in wound healing [76][77]. Neuropeptides' interactions with KC are less researched. VIP induces keratinocytes migration and proliferation, and probably it is one of the most promising targets for study [78][79][80]. NPY receptors Y1 and Y4 were detected in all epidermal layers of the human skin [81]. Interestingly, while CGRP and VIP activate cAMP in keratinocytes culture, leading to increased cell proliferation, NPY downregulates cAMP with the opposite effects. It is probable that NPY blockers also could have some useful implications [82]. Major aspects of sympathetic neurotransmitters' interaction with keratinocytes were placed in **Table 2**.

Table 2. Overview of sympathetic neurotransmitter interactions with keratinocytes.

Neurotransmitters	Norepinephrine/Epinephrine	Acetylcholine	VIP	NPY
Primary effect	Inhibits activation, stimulates cytokines	Mixed through different receptors, stimulates cytokines	Activates KC	Inhibits activation
Source	Vasomotor fibers/keratinocytes	Sudomotor fibers/keratinocytes	Sudomotor fibers/keratinocytes	Vasomotor fibers/keratinocytes
Wound healing role—re-	Stimulates KC to produce cytokines, probably to stop	Stimulates KC to produce cytokines,	Stimulates KC	Inhibits KC

Neurotransmitters	Norepinephrine/Epinephrine	Acetylcholine	VIP	NPY
epithelialization	migration	to start migration		
Primary receptor blockade	Ambiguous data	Ambiguous data	Worse healing?	Better healing?

4. Fibroblasts

In later stages of the wound healing process, fibroblasts became key cellular elements. Their growth factors terminate inflammation, and their work defines the degree of scarring and functional restoration [83][84].

Fibroblasts produce almost all types of adrenergic and cholinergic receptors. In proliferation phase, β_2-receptors become more beneficent, as they activate fibroblasts. In zebrafishes and porcine skin wound model β_2-AR agonists inhibit contraction and fibrosis, reduce scar area, and improve scar quality [85]. β_2-AR activates fibroblasts migration and attenuates cAMP-dependent matrix contraction [86][87]. After the beta-adrenoceptors blockade wound contraction and epidermal healing were delayed, decreased hydroxyproline levels, collagen density and neo-epidermal thickness were in evidence [33]. After propranolol administration in streptozotocin-induced rat diabetes model, the wound area was smaller 7 and 14 days after wounding in propranolol group; MMP-9 level was reduced and cell proliferation, mast cells number, collagen deposition, blood vessels density and nitric oxide levels were increased [34]. In Pullar et al. work, β_2-AR antagonism increased angiogenesis, fibroblast functions, re-epithelialization [88]. Therefore, real data is inconsistent and more high-quality research is needed.

Less data is available about alpha-adrenoceptors, but in most cases, they have shown pro-inflammatory properties. Alpha-adrenergic agonists increase pro-inflammatory cytokines production, immune progenitor proliferation and reactive oxygen species production, as well as TGFb synthesis [14][15]. Dermal fibroblasts also express several acetylcholine receptors: a_3b_2, a_5, a_7, a_9 nAChRs, M_2, M_4, and M_5 mAChRs [43][73]. Cellular effects were not properly studied, but mostly, acetylcholine receptors activation promotes matrix formation or remodeling [89].

5. Blood Vessel Cells

Finally, fast, and functional restoration requires increased blood supply. All sympathetic neurotransmitters and neuropeptides affect angiogenesis. As this topic is described in many reviews, we briefly discuss several points [90][91][92][93][94][95].

Both in murine wound models and in human skin wounds, β_2-AR activation prevents phospho-ERK cytoskeleton remodeling and delays wound re-epithelialization and healing [96]. Interestingly, the same effects could be seen in vascular smooth muscle cell culture [97][98]. β_2-AR activation also decreases angiogenesis, and endothelial cells' migration via cAMP-dependent mechanisms [99]. It is possible that a_1-AR gene overexpression in vascular cells could lead to altered circulatory dynamics. Indirectly, these alterations could contribute to dysfunctional keloid scars that maintain high a_1-AR production [100].

High SNS activity leads to stable NPY increase, hence vascular tone is permanently elevated, like in arterial hypertension. Diabetes mellitus is often accompanied by lower NPY production in skin [101]. NPY released by sympathetic nerve fibers stimulates endothelial cells proliferation and migration [102][103]. NPY Y2 receptors' deletion in mice delays the wound healing by an angiogenesis blockade [101].

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