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Review Article

THE ROLE OF SEROTONINERGIC SYSTEM IN SKIN HEALING

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ABSTRACT

Wounds are a disruption to the continuity of cells that is repaired through well-coordinated steps including inflammation, proliferation and extra-cellular matrix (ECM) remodeling. Often these processes are dysregulated, resulting in either impaired wound healing seen in chronic diabetic wounds, or excessive healing seen in hypertrophic scarring. The serotonergic system is historically known for its action in the central and peripheral nervous systems, but its role in wound healing is recently coming to light. Serotonin (5HT) has an important role in the promotion of wound healing, particularly in the inflammatory and proliferative stages. In this review, we discuss the role of serotonergic agents and serotonin receptor antagonists in wound healing. Moreover, we discuss the potential mechanisms of actions, and the advantages and limitations of these drugs in the treatment of acute wounds, chronic wounds, and hypertrophic scarring. Since the effects of the serotonergic pathway in the context of wound healing is largely unexplored, we also discuss where future research in the field is warranted.

Keywords: Serotonin, 5HT receptors, Selective serotonin reuptake inhibitors (SSRIs), Ketanserin, Wound healing, Chronic wounds, Hypertrophic scarring.

INTRODUCTION

The healing process of cutaneous wounds involves restoration of the integrity and continuity of skin cells that is disrupted due to nocuous or traumatic stimuli. Proper wound healing response requires achieving hemostasis, activation of the innate and adaptive immune

responses, infiltration and proliferation of fibroblasts to the wound site, adequate collagen deposition and remodeling, and ultimately keratinocyte migration and restoration of the epidermis (Guo, *et al.*, 2010 and Portou, *et al.*, 2015). Wound healing is deficient especially in the case of chronic diabetic wounds (Blakytyn, *et al.*, 2006) or excessive in the case of hypertrophic scarring, which is very common in burn wounds (Finnerty, *et al.*, 2016). The advancement in the understanding of the pathways contributing to normal, deficient and excessive healing will aid in the development the appropriate pharmacological management of these conditions. One such novel pathway is the serotonergic system.

Serotonin is most commonly known for its association with psychiatric conditions including major depressive disorder (MDD); however, serotonin also plays an important role in hemostasis and tissue healing (Duerschmied, *et al.*, 2009). Serotonin and its receptors are widely found outside the nervous system, including platelets, immune cells, heart, lungs, liver and skin cells. Serotonin's healing potential in several organs systems has been well-established (Sadri, *et al.*, 2016 and Welsh, *et al.*, 2004). For instance, platelet-mediated serotonin plays an important role in liver regeneration, such that blocking serotonin signaling by antagonizing serotonin receptors (5HTR) inhibits liver regeneration (Lesurtel, *et al.*, 2006). Although, serotonin signaling is important for initiating inflammation and post-wound healing, alteration in its signaling can also result in aberrant healing and fibrosis in several organs including the liver and lungs (Mann, *et al.*, 2013). However, studies are still emerging regarding serotonin's role in skin healing. Recently, there is a growing interest to study the role of common modulators of serotonergic system in the context of wound healing, including psychiatric medications including serotonin reuptake inhibitors (SSRIs), serotonin receptor antagonists such as ketanserin and serotonin itself.

CUTANEOUS WOUND HEALING

Normal Stages of Wound Healing

Wound healing involves restoration of epidermal integrity that is disrupted due to an acute or chronic insult. This proceeds in four major stages: homeostasis, inflammation, proliferation and remodeling (Singer, *et al.*, 1999). During the homeostasis phase activation of clotting factors and activation of platelets result in the formation of provisional wound matrix consisting of fibrin and fibronectin, which acts as a scaffold to direct leukocytes, fibroblasts and endothelial and epidermal cells to the wound (Clark, *et al.*, 1982; Olczyk, *et al.*, 2014 and Xue, *et al.*, 2002) (Figure 1).

Platelets activation and aggregation occurs after disruption of skin and endothelial continuity results in the exposure to collagen fibers and von Willebrand Factor (vWF). Upon activation, platelets secrete a number of pro-inflammatory mediators and cytokines such as bradykinin, prostaglandins, platelet derived growth factor (PDGF), transforming growth factor- β (TGF- β), and vascular endothelial growth factor (VEGF) causing inflammation and

allowing inflammatory cells to home to the site of injury (Golebiewska, *et al.*, 2015). Among other pro-inflammatory factors released by platelets, platelets also release serotonin from dense granules, which also enhances the recruitment of neutrophils to sites of acute inflammation and injury (Duerschmied, *et al.*, 2013) (Figure 1).

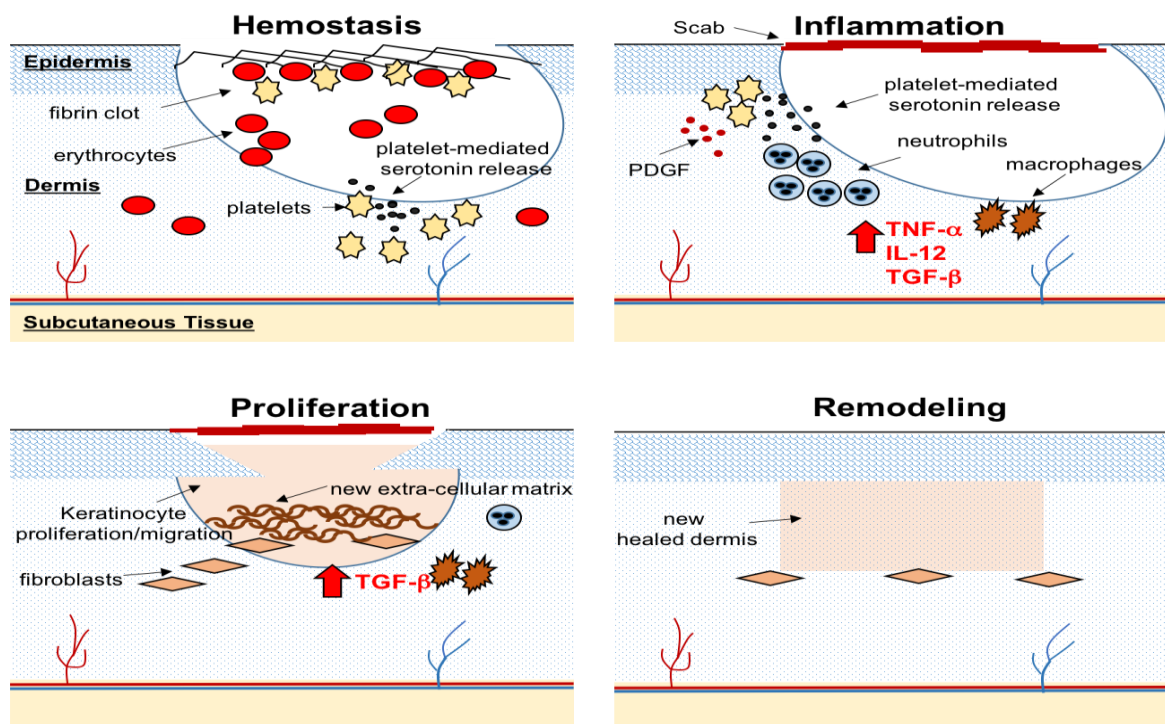


Figure 1: Stages of wound healing.

Immediately following injury, activated platelets initiate hemostasis by clot formation. Serotonin is released by activated platelets, which enhances platelet aggregation and activation. In the early phases of the inflammatory phase, platelets secrete serotonin and platelet-derived growth factor (PDGF), which promote the recruitment and activation of neutrophils and macrophages. Activation of neutrophils and macrophages results in the upregulation of key inflammatory cytokines including TNF- α and IL-12 that further perpetuate the inflammatory phase. The proliferative phase is marked by increased differentiation, proliferation and migration of keratinocytes and fibroblasts. Upregulation of TGF- β by inflammatory cells promotes fibroblast mediated collagen deposition. During the remodeling phase, type III collagen fibers are replaced by mature type I collagen fibers. Light black arrows are used for labelling purposes.

During the inflammatory phase, neutrophils and natural-killer (NK) cells enter the wound as first-line of defense against microbes and foreign debris (Portou, *et al.*, 2015 and Schneider, *et al.*, 2011), followed by macrophages becoming the most dominant inflammatory cell in the wound 3 days post-injury (DiPietro, *et al.*, 1995) (Figure 1). Apart

from providing defense against microbes, macrophages release several pro-inflammatory cytokines including tumour necrosis factor α (TNF- α) and interleukin 1 (IL-1), and several growth factors including, basic fibroblast growth factor (bFGF), keratinocyte growth factor (KGF), VEGF, and TGF- β (Rodero, et al, 2010). The adaptive immunity cells such as T-helper 1 (Th1) cells also contribute by stimulating macrophage, fibroblasts and keratinocyte function (Eming, et al, 2009 and Stout, *et al.*, 1997). This leads to the proliferative phase of wound healing, which is defined by epithelialization, proliferation of mesenchymal-like fibroblasts, deposition of type III collagen and angiogenesis.

TGF- β 1 promotes chemotaxis of fibroblasts to the site of injury, fibroblast-mediated deposition of type III collagen, and also promotes their differentiation to myofibroblasts (Penn, *et al.*, 2012 and (Poon, *et al.*, 2009). Myofibroblasts express α -smooth muscle actin (α -SMA) and contribute to wound contraction and closure. Epithelialization occurs with the differentiation and subsequent proliferation and migration of keratinocytes (Pastar, *et al.*, 2014). Lastly, the eventual remodeling of granulation tissue and organization of immature type III collagen into mature type I collagen commences (Xue, *et al.*, 2015). With collagen fibers tightly packed and cross-linked together, the scar thickness reduces and the tensile strength of the wound increases (Xue, *et al.*, 2015).

ABNORMAL WOUND HEALING RESPONSE

Prolongation of inflammatory and proliferative phases in wound healing can contribute to hypertrophic scarring, whereas a constant hyper-inflammatory state is seen in chronic non-healing wounds. Hypertrophic scars are defined by excessive deposition of extracellular matrix components such as collagen resulting in a greater than normal scar thickness. Collagen synthesis in hypertrophic and keloid scars has been reported to be 7 times and 20 times higher than normal respectively (Xue, *et al.*, 2015). Some types of injuries, especially burns, are more prone to hypertrophic scarring due to prolonged inflammatory phase resulting in high expression of TGF- β 1 (Penn, *et al.*, 2012). Increased fibroblast activity and excessive collagen deposition seen in burn correlates with increased expression of TGF- β 1 (Penn, *et al.*, 2012). Interestingly, overexpression of TGF- β 1 has also been implicated in the pathogenesis of other prolific scarring disorders such as keloids (Lee, *et al.*, 1999).

Chronic wounds such as diabetic ulcers result from dysregulated hyper-inflammatory response. This is noted by the sustained abundance of neutrophils and macrophages seen in diabetic ulcers resulting in excessive proteolytic environment leading to tissue extra-cellular matrix destruction (Menke, *et al.*, 2007 and Wetzler, *et al.*, 200). Moreover, aberrant expression of key inflammatory cytokines such as TNF- α and IL-1 in chronic wounds, which has been shown to be about 100 fold greater than acute wounds, perpetuates the inflammatory response (Tarnuzzer, *et al.*, 1996).

SEROTONINERGIC PATHWAY

Serotonin or 5-hydroxytryptamine (5HT) is a monoamine neurotransmitter biochemically derived from L-tryptophan. In the CNS, tryptophan hydroxylase (TPH) 1 and 2 are involved in the rate-limiting step of serotonin synthesis (Lv, *et al.*, 2017). It has long been implicated in the etiology and treatment of depression. In the central nervous system (CNS), the initiating event of serotonin signaling pathway involves the release of serotonin from the presynaptic neurons, thereby increasing serotonin concentration in the synaptic cleft (**Figure 2**). Serotonin then binds to several post-synaptic membrane-bound 5HT receptors (5HTR) classified into seven classes from 5HTR1 to 5HTR7 receptors (Hoyer, *et al.*, 2010). 5HT receptors are G-protein coupled receptors (GPCR), and upon binding to serotonin can have differing pharmacologic effects (Hoyer, *et al.*, 2010). However, the duration of serotonin's action is limited by serotonin reuptake transporters (SERT), that transport serotonin along its concentration gradient back into the presynaptic neuron, thereby decreasing its presence in the synaptic cleft (**Figure 2**). Furthermore, monoamine oxidases (MAO) also limit serotonin availability by breaking down serotonin in the mitochondria.

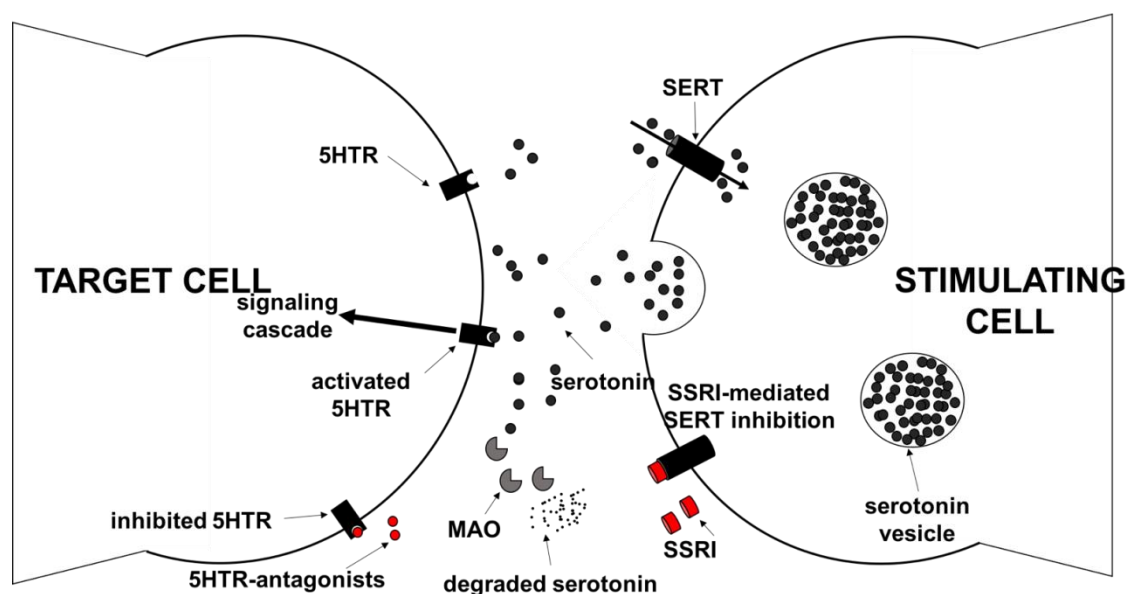


Figure 2: Major proteins involved in the serotonin signaling pathway.

A stimulating cell, such as a pre-synaptic neuron in the CNS or platelets in the blood, secrete serotonins stored in vesicles to the extracellular space following a stimulus. Serotonin reuptake transporters (SERT) promote the uptake of serotonin from the extracellular space to the intracellular space, decreasing the extracellular serotonin concentrations. Monoamine

Oxidase (MAO) degrades serotonin in the extracellular space. Selective serotonin reuptake inhibitors (SSRI) inhibit SERT transporters thus prolonging the duration of extracellular serotonin. The target cells respond to serotonin via serotonin receptor (5HTR), which are activated upon binding to serotonin. Selective inhibitors of 5HTRs, such as 5HTR2A inhibitor ketanserin, inhibit 5HTR signaling cascade. Light black arrows are used for labelling purposes.

SEROTONINERGIC PATHWAY IN PERIPHERAL TISSUES

Apart from the CNS and peripheral nervous system (PNS), serotonin is synthesized in the gastrointestinal mucosa (Stahl, *et al.*, 1998) and various peripheral tissues. For instance, tryptophan hydroxylase 1 (TPH1), a key peripheral serotonin synthesizing enzyme is expressed in several lymphocytes including macrophages, mast cells, and T lymphocytes (Ahern, *et al.*, 2011) (**Table 1**). Furthermore, SERT and 5HTRs are also found in various lymphocytes including macrophages, dendritic cells, B cells and T cells (Ahern, *et al.*, 2011). In the plasma, serotonin is taken up in platelets by the action of SERT and then subsequently released upon platelet activation to cause vasoconstriction and to enhance platelet aggregation for hemostasis (Duerschmied, *et al.*, 2009). Platelet-mediated serotonin secretion is also implicated in the pro-inflammatory response to acute injury, especially the recruitment of neutrophils and proliferation of lymphocytes (Mauler, *et al.*, 2016).

Table 1: The serotonergic system in the cellular components of wound healing in humans.

Stages of Wound Healing	Cellular Components	Serotonin Synthesis	Receptors/Transporters Expression
Hemostasis	Platelets	No (Mercado, <i>et al.</i> , 2010)	SERT and 5HTRs (Mercado, <i>et al.</i> , 2010)
Inflammation	Neutrophils	Yes (Ahern, <i>et al.</i> , 2011)	SERT and 5HTRs (Ahern, <i>et al.</i> , 2011)
	Macrophages	Yes (Ahern, <i>et al.</i> , 2011)	SERT and 5HTRs (Ahern, <i>et al.</i> , 2011)
	Mast Cells	Yes (Ahern, <i>et al.</i> , 2011)	SERT and 5HTRs (Ahern, <i>et al.</i> , 2011)
	Dendritic Cells	No (Ahern, <i>et al.</i> , 2011)	SERT and 5HTRs (Ahern, <i>et al.</i> , 2011)
	T lymphocytes	Yes (Ahern, <i>et al.</i> , 2011)	5HTRs (Ahern, <i>et al.</i> , 2011)
Proliferation/Remodeling	Fibroblasts	Yes (Slominski, <i>et al.</i> , 2002)	SERT and 5HTRs (Slominski, <i>et al.</i> , 2002)
	Keratinocytes	Yes (Slominski, <i>et al.</i> , 2002)	SERT and 5HTRs (Slominski, <i>et al.</i> , 2002)

In human skin, serotonin reuptake transporters (SERT) and 5HTRs are expressed on various skin cells including keratinocytes, melanocytes, and dermal fibroblasts (Slominski, *et*

al., 2003) (**Table 1**). Thus, along with platelets, lymphocytes, and macrophages that release serotonin in inflammatory phase during acute injury, these cells are involved in modulating the serotonergic communication in the skin. The biosynthetic pathways of serotonin including the expression of TPH1 and serotonin transporters have also been found in cutaneous human fibroblasts and keratinocytes (Slominski, *et al.*, 2002 and Slominski, *et al.*, 2005) (**Table 1**). Interestingly, the expression of MAO-A in human skin fibroblasts, a subtype of MAOs that has a higher affinity for serotonin and norepinephrine, highlights the presence of serotonin metabolism pathways in skin (Denney, *et al.*, 1999). Furthermore, inhibition of MAO in the murine skin results in diminished production of the byproducts of serotonin metabolism further highlights the existence of the complete serotonergic system in skin (Slominski, *et al.*, 2003).

ROLE OF SEROTONINERGIC PATHWAY IN SKIN

Serotonergic Pathway in Skin Pathology

Although the role of serotonin and 5HTRs in skin still largely remains elusive, their role in normal skin physiology and pathology is emerging with time. Studies have shown that aberrant overexpression serotonin signaling in skin may contribute to various pruritic inflammatory skin conditions such as allergic contact dermatitis and atopic dermatitis (Huang, *et al.*, 2004; Lundeberg, *et al.*, 1999; McAloon, *et al.*, 1995 and Morita, *et al.*, 2015). However, as in the CNS, activation of the serotonergic system has different effects based on the type of receptor activated. For instance, buspirone, a 5HTR1a agonist, has been shown to promote anti-inflammatory effects by inhibiting leukocyte infiltration and reducing tissue swelling in contact hypersensitivity reactions (McAloon, *et al.*, 1995), whereas ketanserin, a 5HTR2a receptor antagonist, inhibits contact hypersensitivity reactions (Ameisen, *et al.*, 1989). The expressions of 5HTR has also been implicated in psoriasis, where 5HT1a-positive and 5HTR3-positive cells are lower and 5HTR2a-positive cells are more abundant in both lesional and non-lesional psoriatic skin compared to skin of healthy controls (Nordlind, *et al.*, 2006). Moreover, in psoriatic skin, 5HTR1a are localized to mast cells and melanocytes, whereas 5HTR2a are primarily expressed in lymphocytes, suggesting a pro-inflammatory role for 5HTR2a in the disease (Nordlind, *et al.*, 2006). Moreover, SERT has also been implicated in inflammatory skin diseases. SERT expression in psoriatic skin dendritic cells is elevated compared to normal skin (Thorslund, *et al.*, 2009), and the expression positively correlates with disease severity (Thorslund, *et al.*, 2013). Thus, drugs targeting 5HTR and SERT could have implications for future therapies for skin conditions.

SEROTONINERGIC PATHWAY IN WOUND HEALING

Serotonin's healing potential is known in several organs such as the lungs and liver (Lesurtel, *et al.*, 2006; Martinez-de *et al.*, 1997 and Sadri, *et al.*, 2016), and recent studies are

shedding light on its potential role in cutaneous wound healing. In regards to wound healing, serotonin induces the release of vWF from endothelial cells, thus playing a role in promoting platelet activation and hemostasis (Schluter, *et al.*, 1999). Moreover, platelet-induced serotonin release generates a pro-inflammatory response to acute injury, thus playing a major role in the inflammatory stage of wound healing (Duerschmied, *et al.*, 2013 and (Mauler, *et al.*, 2016). Dendritic cells also enhance the inflammatory response by sequestering serotonin released from activated T lymphocytes via SERT, and subsequently exposing naïve T cells to promote their proliferation and differentiation (O'Connell, *et al.*, 2006).

Apart from enhancing the immune response in acute injuries, serotonin has also been shown to modulate the release of pro-inflammatory cytokines and inhibit apoptosis in human monocytes (Durk, *et al.*, 2005 and (Soga, *et al.*, 2007), suggesting its possible role in chronic inflammation or hypertrophic scarring. The presence of hyper-inflammatory and proteolytic microenvironment in chronic wounds such as diabetic ulcers has been well-established (Menke, *et al.*, 2007 and Wetzler, *et al.*, 2000). On the opposite spectrum, hyperplastic changes in the skin are due to a sustained inflammatory phase leading to excessive fibroblast proliferation and collagen deposition. Although it is possible that aberrant expression of serotonin in wounds could lead to a hyper-inflammatory microenvironment that is detrimental to skin healing; however, there is more evidence on serotonin's role in excessive wound healing and fibrosis. For instance, upon serotonin administration in normal skin *in vivo* epidermal fibroblasts undergo proliferation and hyperplasia leading to skin fibrosis (Macdonald, *et al.*, 1958). Interestingly, serotonin has also been shown to promote *in vitro* fibroblast proliferation in a dose-dependent manner, which suggests its role in the proliferative and remodeling phase of wound healing (Slominski, *et al.*, 2003). Furthermore, TPH1-null mice with deficient serotonin synthesis showed significantly delayed wound closure when compared to normal controls (Duerschmied, *et al.*, 2013). Serotonin, via 5HTR2A and 5HTR2B receptor signaling, also promotes myofibroblast differentiation (Lofdahl, *et al.*, 2016 and (Mann, *et al.*, 2013), which further highlights the serotonergic pathway's role in achieving wound closure.

Since serotonin plays an important role modulating all stages of wound healing, especially inflammation and fibrosis, drugs targeting 5HTR and SERT could have enormous implications for promoting wound healing or treating hypertrophic scarring conditions.

SEROTONIN REUPTAKE INHIBITORS (SSRI)

Several drugs that modulate the serotonergic pathway have been used in the treatment of major depressive disorder (MDD). These include non-specific ones such as monoamine oxidase inhibitors (MAOI), that inhibits the breakdown of monoamine neurotransmitters including serotonin, epinephrine, and norepinephrine. Other non-specific drugs include tricyclic anti-depressants (TCA) and serotonin-norepinephrine (Feighner, *et al.*,

1999) reuptake inhibitors (SNRI) that target reuptake proteins to increase a high presence of neurotransmitters in the synaptic cleft (Feighner, et al, 1999). More specific drugs belonging to the selective serotonin reuptake inhibitors (SSRI) family, such as citalopram, paroxetine, fluoxetine and fluvoxamine, decrease the affinity of serotonin binding to SERT thus preventing intracellular reuptake of serotonin (Stahl, et al, 1998).

SERT plays a crucial role in the platelet-mediated inflammatory response. Platelets do not synthesize serotonin but use SERT to uptake serotonin released by the gastrointestinal tract into the plasma, which is subsequently stored in dense granules (Mercado, et al, 2010). As SERT plays a crucial role in maintaining platelet intracellular stores of serotonin (Mercado, et al, 2010 and Skop, et al, 1996), inhibition of SERT hinders the ability of platelets to exert serotonergic effects. Consequently, SSRIs are associated with decreased platelet function and increased risk of bleeding (McCloskey, et al, 2008 and Tseng, et al, 2010) (**Figure 3**). Moreover, diminished serotonin secretion by platelets may also attenuate platelet-induced inflammatory response in wounds. Furthermore, dendritic cells use SERT to sequester serotonin released from activated T lymphocytes and subsequently exposing naïve T cells to serotonin, thereby activating them and amplifying the adaptive immune response (O'Connell, et al, 2006). Thus, SSRIs administration could diminish the amplification and prolongation of the inflammatory response in skin.

SSRIs are known to have systemic anti-inflammatory effects, which are particularly demonstrated by fluoxetine's ability to ameliorate graft-versus-host disease by T cell immunosuppression (Gobin, et al, 2013). However, to examine the anti-inflammatory effects of SSRIs in skin it is necessary to observe their effects on chronic inflammatory skin conditions like atopic dermatitis (AD) and psoriasis. Interestingly, two commonly prescribed SSRIs, paroxetine, and fluvoxamine have been shown to reduce pruritus in patients with atopic dermatitis (AD) (Jiang, et al, 2007 and Ständer, *et al.*, 2009). Furthermore, when mice treated with 2,4-dinitrochlorobenzene (DNCB), a known inducer of AD-like pathology in mice skin, had significantly reduced skin lesions and scratch behavior after co-treatment with fluoxetine delivered intra-peritoneally (Li, et al, 2016). Moreover, fluoxetine also suppressed the DNCB-mediated upregulation of inflammatory cytokines including IL-4 and interferon- γ (IF- γ) in the spleen, demonstrating the anti-inflammatory effects of the drug (Li, et al, 2016). Additionally, it has been suggested that SSRI use may attenuate psoriasis symptoms. For instance, a population-based cohort study involving 69,830 psoriatic patients found that SSRI use was associated with significant decrease in the need of systemic psoriatic treatment (Thorslund, et al, 2013), thus further highlighting the potential anti-inflammatory effects of systemic SSRI therapy. Other non-specific anti-depressants that also inhibit SERT also show amelioration of psoriasis symptoms.

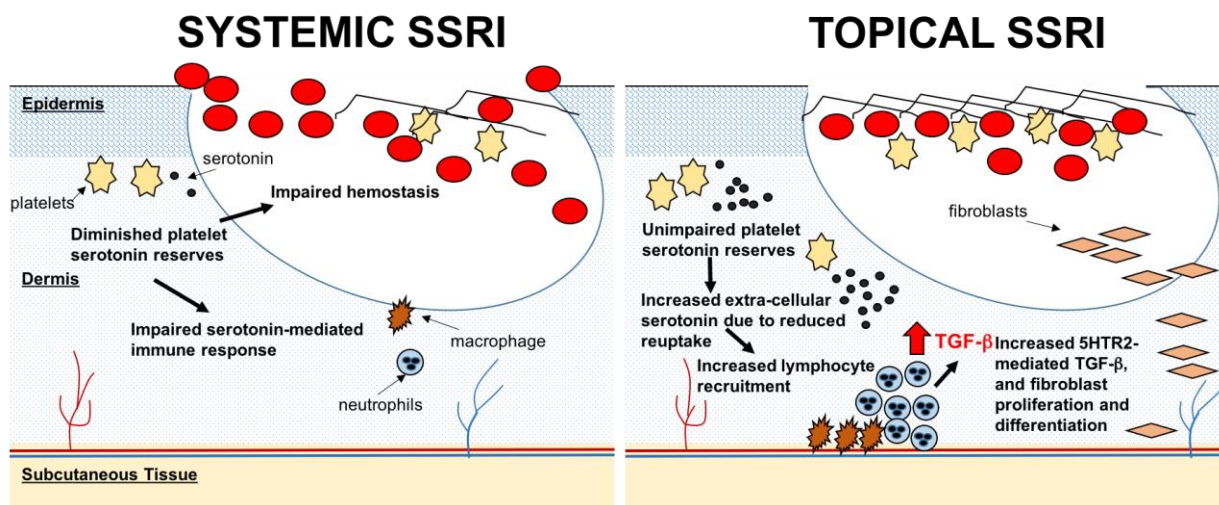


Figure 3: The proposed effects and mechanism of action of systemic and topical SSRI treatment on wound healing.

Since platelets rely on serotonin reuptake transporter (SERT) to uptake and maintain their intracellular serotonin reserves, systemic administration of SSRIs will diminish platelet serotonin action. Consequently, this may result in impaired platelet aggregation and impaired immune response. Whereas, topical administration of serotonin may not diminish platelet serotonin reserves. Perhaps, diminished locally reuptake of serotonin would increase extracellular serotonin levels and enhance pro-inflammatory serotonergic effects. The dense black arrows represent sequence of events. The light black arrows are used for labelling purposes.

Previously it was suggested that SSRIs could have a role in wound healing on the assumption that inhibition of SERT may prolong the extracellular concentration of serotonin like in the CNS (Malinin, *et al.*, 2004). However, considering that platelet and dendritic cell serotonin signaling is impaired by SSRIs, systemic administration of SSRIs may not promote wound healing (**Figure 3**). The key difference between SSRI effects in CNS and peripheral tissues is that CNS neurons are able to synthesize serotonin via tryptophan hydroxylase (TPH) (Lv, *et al.*, 2017). Studies have also shown that SSRIs administration results in enhanced TPH expression and serotonin synthesis (Kim, *et al.*, 2002). Whereas in platelets, intracellular serotonin levels are depleted to less than 2% of baseline after SSRI administration (Javors, *et al.*, 2000) (**Table 2**).

Furthermore, systemic SSRI administration significantly impairs the platelet-mediated serotonergic effects in the periphery (Duerschmied, *et al.*, 2013; McCloskey, *et al.*, 2008 and Tseng, *et al.*, 2010) that may affect in wound healing (**Table 2**).

Table 2. Comparative summary of the effects of systemic SSRI treatment on central nervous system neurons and platelets, a key peripheral tissue serotonin regulator.

Systemic SSRI Effects on:	CNS	Platelet
Serotonin Synthesis	TPH-mediated serotonin synthesis pathway is not impaired after SSRI administration.	Platelets do not synthesize serotonin, and rely on serotonin reuptake transporter (SERT) to maintain intracellular stores.
Concentration of Serotonin	Increased concentration in the extracellular synaptic cleft due to decreased pre-synaptic reuptake	Diminished platelet intracellular serotonin concentration due to inhibition of serotonin uptake from plasma. Increased overall plasma serotonin concentration due to decreased platelet uptake.
Result	Increased post-synaptic serotonin receptor occupancy	Decreased platelet-mediated serotonergic effects.
Functional outcome	Anti-depressant effects	Decreased platelet aggregation and homeostasis, and reduced platelet-induced inflammation. Reduced lymphocyte inflammatory cytokine secretion.

Interestingly, systemic SSRI-induced impairment of inflammation and skin wound healing has been previously documented and is comparable to that of TPH1-null mice that lack peripheral serotonin (Duerschmied, et al, 2013). The similarity in the functional outcome to acute injury in absence of peripheral serotonin and SSRI-induced platelet dysfunction highlights the importance of platelets in mediating serotonergic pathway in peripheral tissues (Duerschmied, et al, 2013).

SEROTONIN RECEPTOR (5HTR) MODULATORS

Furthermore, a previous study has shown that systemic SSRI administration in rats did not affect the healing of acute and chronic wounds (Yuksel, *et al.*, 2014). Since studies have shown that bioavailability of topical fluoxetine is only 10% of that of oral route (Ciribassi, *et al.*, 2003), topical administration of SSRIs on wounds could avoid undesirable systemic effects such as diminished platelet serotonin stores. Whether the amount of serotonin synthesized by the local or recruited cells in the wound milieu is abundant enough that will be affected by topical administration of SSRI is subject for further exploration. A

theoretical mechanism by which topical SSRIs could promote wound healing is by prolonging local extracellular serotonin concentration leading to increased serotonin-5HTR interaction just like in the neuronal synaptic cleft (Schloss, et al, 1998) (**Figure 3**). However, studies need to be done to evaluate the pharmacodynamics of topical SSRIs in wounds. Moreover, due to its skin anti-inflammatory effects, systemic SSRIs may have a role in the treatment of hypertrophic scarring and keloids, which result from prolonged inflammation. Thus, future studies are warranted to investigate the effects of topical and systemic SSRIs on wounds and hypertrophic scarring.

As mentioned previously, 5HTR2a is implicated in the promotion of serotonin's inflammatory effects. Consequently, 5HTR2a antagonists disrupt the pro-inflammatory serotonergic signaling pathways and thus are a great way to decipher the role of the serotonergic pathway in wounds. Interestingly, topical administration of ketanserin, 5HTR2a-specific antagonist, has shown efficacy in the healing of chronic venous and diabetic ulcers in human patients, demonstrated by accelerated wound closure and formation of healthy granulation tissue (Apelqvist, et al, 1990; Janssen, *et al.*, 1989; Martinez-de *et al.*, 1997 and Quatresooz, et al, 2006) (**Table 3**). Similar to SSRIs, in the context of acute wounds, ketanserin administration did not show significant effects on wound healing rate (Lawrence, *et al.*, 1995). Serotonin promotes the production of pro-inflammatory cytokines through macrophages (Bischoff, et al, 2009). However, ketanserin administration suppresses the activation of macrophages and inhibits a key pro-inflammatory transcription factor nuclear factor- κ B (NF- κ B) and its downstream inflammatory cytokines such as TNF- α and IL-12 in macrophages (Xiao, et al, 2016). The mechanism behind the efficacy of 5HTR2a inhibition in chronic wounds is likely due to the attenuation of the hyper-inflammatory microenvironment that perpetuates chronic wounds (**Figure 4**). Whereas, inhibition of pro-inflammatory pathways should not promote wound healing in acute settings, as inflammatory response is an essential part of wound healing. Further studies are warranted to investigate the biochemical composition of wounds after ketanserin application to better understand its mechanism of action.

Out of all 5HTRs, 5HT₂ receptors are mostly strongly associated with fibroproliferative changes. 5HTR2a activation results in the induction of TGF- β 1 signaling and fibrotic changes in cardiac, hepatic, renal and pulmonary tissues. Not surprisingly, inhibiting these signaling pathways results in the reversal of these effects. 5HTR2a antagonists have also shown efficacy in reducing inflammation and fibrosis in the liver. Ketanserin inhibits the secretion of pro-inflammatory cytokine TGF- β 1 by liver fibroblasts, resulting in decreased fibroblast proliferation and collagen deposition. Similar effects of ketanserin have been noted in cardiac fibroblasts, where ketanserin administrated inhibited TGF- β 1 secretion and migration of cardiac fibroblasts of cardiac fibroblasts. 5HTR2b is also associated with promoting pulmonary fibrosis.

Table 3: Human and animal trials testing the efficacy of serotonergic drugs in the treatment of diabetic chronic wounds.

Drug	Study	Population	Sample Size	Outcome Measure	Clinical Efficacy	Ref
Topical Ketanserin (5HTR antagonist)	Randomized single-blind - controlled study	Non-insulin dependent diabetic human patients	140	Reduction of ulcer area over 12 weeks	Average ulcer reduction of 87% by 12 weeks (vs. 63% placebo)	(Martinez-de <i>et al.</i> , 1997)
Topical Ketanserin (5HTR antagonist)	Randomized double-blind intra-comparative controlled study	Insulin-dependent human diabetics with two similar leg ulcers	12	Reduction in ulcer area over 8 weeks	Average ulcer reduction of 94% by 8 weeks (vs. 32% placebo)	(Quatresooz, <i>et al.</i> , 2006)
Topical Ketanserin (5HTR antagonist)	Double-blind controlled study	Human diabetics with foot ulcers and severe peripheral vascular disease (systolic toe pressure <45mmHg)	40	Number of patients with >50% reduction in ulcer area and gangrene prevention in 3 months	58% patients achieved >50% reduction in ulcer (37% control). 56% of patients with toe pressure <30mmHg achieved >50% reduction (vs. 11% control). Incidence of gangrene: 10.5% (vs. 31.5% in control)	(Apelqvist, <i>et al.</i> , 1990)
Systemic Paroxetine (SSRI)	Animal randomized controlled trial (rats)	Rats with streptozotocin-induced diabetes	64	Wound epithelialization	No significant difference with control	(Yuksel, <i>et al.</i> , 2014)

Interestingly, bleomycin-induced pulmonary fibrosis is prevented with 5HTR_{2b} receptor antagonism by SB215505. As mentioned previously, fibroproliferative disorders of the skin such as hypertrophic scarring and keloids are also associated with overexpression of TGF- β 1 leading to excessive fibroblast proliferation and differentiation, and collagen deposition. Apart from fibroblasts, macrophages also play an important role in the secretion

of TGF- β 1 and fibrosis. Since macrophages are an important mediator of inflammation in wounds its excessive activation is implicated in the pathogenesis of hypertrophic scars (Zhu, *et al.*, 2016). Since 5HTR2a and 5HTR2b antagonism promotes anti-fibrotic microenvironment, it is possible that ketanserin and SB215505 may also have a role in the prevention or treatment of fibroproliferative disorders of the skin (**Figure 4**).

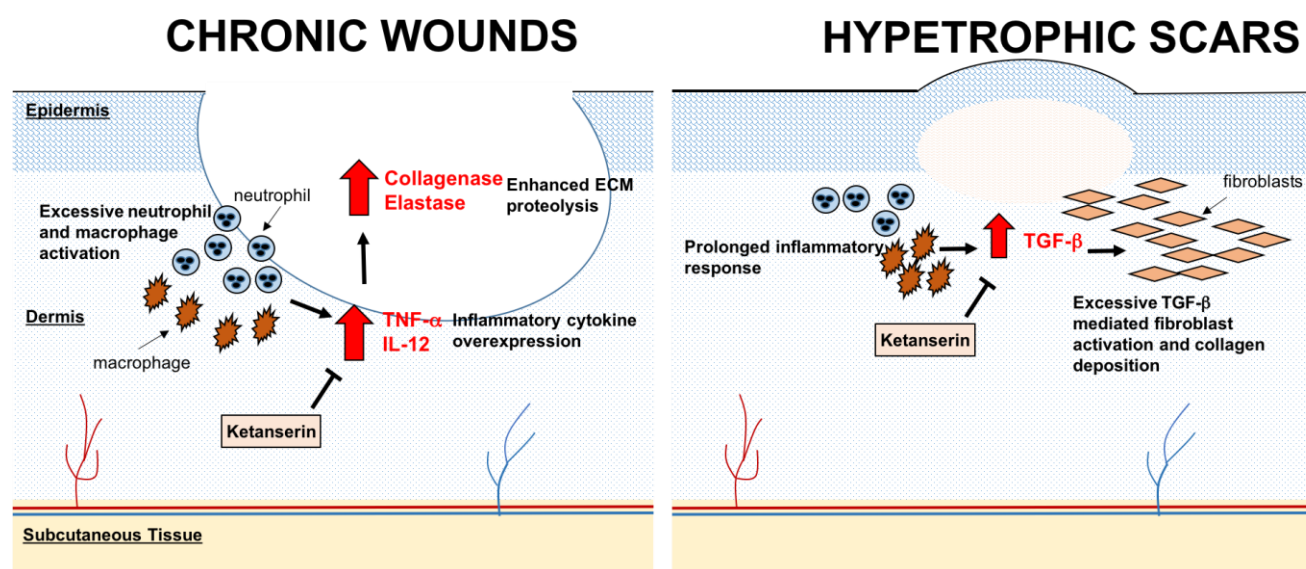


Figure 4: The proposed effects and mechanism of action of ketanserin in the context of chronic wounds and hypertrophic scarring.

Chronic wounds are perpetuated by hyperinflammatory response resulting in a highly proteolytic environment. Ketanserin has known anti-inflammatory effects such as inhibiting macrophage mediated pro-inflammatory cytokine production. The observed efficacy of ketanserin in chronic wounds in human diabetic patients might be due to its anti-inflammatory effects. In hypertrophic scarring, excessive fibroblast activation and collagen deposition results from overexpression of TGF- β levels. Ketanserin also inhibits TGF- β concentration and thus may have efficacy in context of scarring. Light black arrows are used for labelling purposes. Dense black arrows represent sequence of events. The T symbol represents an inhibitory action.

In contrast to 5HTR2, 5HTR1-mediated signaling has been associated with anti-inflammatory effects such as reducing levels of a key pro-inflammatory cytokine TNF- α , reducing leukocyte infiltration in inflammatory conditions like contact hypersensitivity, and having anti-fibrotic effects. The use of 5HTR1 agonists has not been investigated in the context chronic wounds or hypertrophic scarring and warrants future investigation.

SEROTONIN MODULATION OF ESSENTIAL SIGNALLING PATHWAYS DURING WOUND HEALING

Wnt/ β -Catenin and Notch signaling are implicated in the wound healing process. However, Notch signaling is comparatively less explored than Wnt/ β -Catenin signaling pathway in the context of wound healing. The importance of Wnt/ β -Catenin signaling during wound healing is well established. Wnt/ β -Catenin signaling has not only an essential role during re-epithelialization of injured skin, but its activity also affects the formation of granulation tissue, particularly during proliferative phase of skin healing. β -Catenin level regulate scar size and can modulate the differentiation of mesenchymal cells such as Pax7-positive stem cells to dermal fibroblasts. Recently, the role of serotonin pathway on Wnt/ β -Catenin signaling is coming to light, especially in the context of cancer therapeutics. For instance, 5HT administration has been shown to enhance the expression of β -Catenin-mediated activation of target genes and hepatocellular carcinoma (HCC) cell proliferation *in vitro* (Fatima, *et al.*, 2016). Studies that examined the effects of non-specific 5HTR2a receptor antagonizing agents such as olanzapine and spiperone showed inhibition of the Wnt/ β -Catenin signaling pathway (Lu, *et al.*, 2009). However, because these drugs are non-specific, and also have effects on dopamine receptor 2 (Gundlach, *et al.*, 1984), it is hard to conclude based on these results whether reducing in Wnt/ β -Catenin is due to 5HT2a antagonism alone. Moreover, it has been shown that selective 5HT1 and 5HT2 antagonism does not inhibit Wnt activation, thus suggesting a limited role of Wnt/ β -Catenin pathway in the context of 5HT1 and 5HT2 receptor function.

In the context of fibroproliferative disorders, Wnt/ β -Catenin signaling could be an important mediator since macrophages deficient in β -Catenin have impaired production of TGF- β 1 (Amini *et al.*, 2014). Moreover, our group has shown that TGF- β 1 stimulation increases the expression of β -Catenin *in vivo* (Amini *et al.*, 2007), suggesting that both TGF- β 1 and β -catenin are important in promoting the expressions of one another. Since increased serotonin levels has been shown to enhance Wnt/ β -Catenin signaling (Fatima, *et al.*, 2016), perhaps topical SSRI administration could have a similar effect by inhibiting the reuptake of extracellular serotonin. Theoretically, it is possible that topical SSRIs could lead to enhanced wound closure but may increase the likelihood of hypertrophic scarring. Therefore, future studies should also investigate the propensity of hypertrophic scarring in the topical administration of topical SSRI treatment in acute wounds.

DISCUSSION

Serotonin plays an important role in key stages of wound healing including achieving homeostasis, activating an inflammatory response in wounds, and promoting the differentiation and proliferation of fibroblasts. Platelet-mediated serotonin release acts as a

major immunomodulator, resulting in the recruitment of neutrophils to the site of injury. Serotonin also results in the amplification of the adaptive immune response. Serotonin also promotes fibroblast proliferation and myofibroblast differentiation via upregulating pro-inflammatory cytokines such as TGF- β 1, thus playing a crucial role in wound healing and closure. Other important players in the peripheral serotonergic system are local dendritic cells, keratinocytes, T lymphocytes, and mast cells that are known to express key serotonin synthesizing proteins, serotonin receptors (5HTR), and serotonin reuptake transporters (SERT).

Since serotonin promotes inflammation and fibroproliferative changes (Welsh, et al, 2004), modulators of serotonin signaling pathway may have enormous implications in the context of wound healing. Among them are SSRIs that act by inhibiting SERT, thereby prolonging the extracellular signaling action of serotonin. However, SERT are necessary for platelet's serotonin stores because platelets cannot synthesize serotonin (Mercado, et al, 2010). SERT inhibition will consequentially lead to diminished platelet-mediated serotonin signaling that is necessary for promoting hemostasis inflammatory response in sites of acute injury. Supporting this concept, systemic SSRI treatment does not promote wound healing in rats (Yuksel, *et al.*, 2014). However, to avoid anti-inflammatory systemic side effects of SSRIs, topical SSRIs applied locally to wound sites could be an alternative. We speculate that topical application of SSRIs could prolong serotonin-5HTR signaling by inhibiting intracellular uptake of serotonin by local lymphocytes, keratinocytes and fibroblasts. Future studies need to investigate whether treatment of topical SSRIs in local wounds enhances 5HTR1 and 2 signaling during the inflammatory phase. Moreover, investigations are warranted to determine the efficacy of topical serotonin and SSRI treatment in acute wounds.

Although SSRIs have shown to have some anti-inflammatory effects, SSRI treatment has not been found efficacious in promoting chronic wound healing (Yuksel, *et al.*, 2014). Whereas, 5HTR2A receptor antagonist, ketanserin, accelerates wound healing and closure in human chronic wounds (Apelqvist, *et al.*, 1990; Janssen, *et al.*, 1989; Martinez-de *et al.*, 1997 and Quatresooz, *et al.*, 2006). This may suggest that to ameliorate the hyper-inflammatory conditions in chronic wounds, specific inhibition of serotonin's pro-inflammatory signaling cascade is more effective. 5HTR2A receptors are associated with pro-inflammatory effects of serotonin, and thus its inhibition is more effective than SERT inhibition. Interestingly, ketanserin does not show efficacy in promoting acute wound healing (Lawrence, *et al.*, 1995), supporting the idea that its efficacy in chronic wounds may primarily be due to its anti-inflammatory effects, which do not play a role in acute wound healing.

Furthermore, 5HTR2 antagonists may have a role in preventing hypertrophic scarring. Hypertrophic scars are common post-burn injuries, and are associated with prolonged inflammatory response and heightened TGF- β 1-mediated fibrosis. 5HT2b antagonists are associated with diminishing myofibroblast differentiation and fibrotic changes (Lofdahl, *et*

al., 2016). Thus, by inhibiting 5HT_{2a}-mediated inflammation and TGF- β 1 upregulation, ketanserin may be able to prevent the formation of hypertrophic scarring post-burn. While 5HT₂ is associated with inflammation and fibrosis, 5HT₁ is associated with the reversal of these effects (McAloon, *et al.*, 1995; Raghunathan, *et al.*, 2014 and Sharifi, *et al.*, 2015). Therefore, 5HT₁ agonists like buspirone, should be investigated along with 5HT₂ antagonists in the context of chronic wounds and hypertrophic scarring.

CONCLUSION

Serotonin has an important role in the promotion of wound healing, particularly in the inflammatory and proliferative stages. Drugs involved in the serotonin pathway especially 5HT₂ antagonists, such as ketanserin, may have an important role in mitigating the hyper-inflammatory response seen in chronic wounds and ameliorating TGF- β 1-mediated fibroproliferative response leading to hypertrophic scarring. Systemic SSRIs lead to diminished platelet-mediated serotonin signaling and have not shown to promote wound healing. Topical SSRIs still remain a possible candidate for wound healing as it may act by enhancing local serotonin-5HT signaling by inhibiting serotonin intracellular uptake.

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