β-blocking wounds

By Tim Fulmer, Senior Writer

Researchers at the **University of California, Davis** think they may have identified a new indication for β-blockers: wound healing.¹ Although the safety profile of the drugs in cardiovascular disorders provides an incentive to repurpose them, there still remains lack of clarity on how β-blockers actually work in wound repair and how many wound types could be addressed by this approach.

Wound healing is a highly organized, stepwise process that comprises three basic phases: clotting and inflammation, new tissue formation and tissue remodeling. Each phase involves multiple cell types and signaling pathways that could potentially be targeted to accelerate wound repair.

Despite the plethora of therapeutic intervention points (see **Box 1, “Skin-deep target”**), standard care for the three types of chronic wounds—diabetic foot ulcers, venous ulcers and pressure ulcers (bedsores)—remains a combination of daily dressing changes and the use of devices to periodically remove weight and pressure from wounded areas.

There are no small molecules approved for wound healing, and the lone biologic on the market is Regranex becaplermin, a gel formulation of recombinant platelet-derived growth factor-β (PDGF-β) sold by **Johnson & Johnson** to treat diabetic foot ulcers. Skin substitute products have also been approved as devices to help treat chronic wounds.

The UC Davis researchers have been focused on new tissue formation. This phase of wound repair is characterized by the parallel migration of three different cell types into the wound gap: keratinocytes to generate new epidermis, fibroblasts to generate new dermis and endothelial cells to generate new blood vessels.

The researchers previously found that activating adrenergic receptor β₂ (ADRB2) delayed migration of keratinocytes *in vitro*,² whereas antagonizing ADRB2 with β-blockers increased keratinocyte migration.³ In their new paper published in *PLoS Medicine*, they investigated whether β-blockers could accelerate wound healing *in vivo*.

Systemic delivery of a β-blocker to mice subjected to a full-thickness burn wound significantly increased epidermis regeneration (re-epithelialization) compared with that seen using saline control at 5 and 10 days post-injury (*p*<0.001 for both).

Additional studies revealed that the stress hormone epinephrine—an ADRB2 agonist—decreased the migratory rate of cultured

keratinocytes and inhibited the epidermis regeneration of *ex vivo* cultured human skin wounds.

The findings suggested a mechanism in which a burn or chronic wound increases systemic and local levels of epinephrine, which subsequently activates ADRB2 on keratinocytes to slow their migration and impair wound healing. Preventing activation of ADRB2 using β-blockers could therefore have the opposite effect and accelerate wound repair.

Fleshing out the mechanism

Despite the positive results in the burn model, it remains unclear whether β-blockers could work in other wound types.

“The paper makes a good case for using β-blockers in patients with elevated levels of stress hormones that occur with burn wounds. However, there is not as much evidence out there that chronic wounds are necessarily associated with elevated levels of those hormones,” said Jeffrey Davidson, professor of pathology at the **Vanderbilt University School of Medicine** and a senior research

career scientist at the **U.S. Department of Veterans Affairs**. “Thus, a key next step will be looking at the β-blockers in chronic wound models.”

“The *PLoS Medicine* paper shows that β-blockers can induce wound re-epithelialization, which suggests they may have value restoring the epidermis and treating more superficial wounds,” said Damien Bates, VP of medical affairs at **Organogenesis Inc.** “However, accelerating the repair of deep wounds, where the dermis is partly or entirely missing, will require

inducing the migration of fibroblasts and vascular endothelial cells in addition to keratinocytes.”

Bates therefore recommended the use of a porcine excisional wound model to evaluate the β-blockers. “The skin of larger mammals like pigs is more structurally similar to human skin than that of mice and therefore is better for evaluating a potential wound healing therapy,” he said. “Moreover, an excisional wound model—which involves cutting out portions of the epidermis and the dermis—is ideal for evaluating the approach in deep tissue wounds that occur in the chronic wound and third-degree burn setting.”

Organogenesis markets Apligraf, a skin substitute made of bovine type I collagen plus human fibroblasts and keratinocytes, to treat diabetic foot ulcers and venous leg ulcers.

Once a model is chosen, it will be important to look at later time points in the healing process beyond wound re-epithelialization, said George Cotsarelis, associate professor of dermatology at the **University of Pennsylvania**. “That should help show how the β-blockers influence the complete process of wound repair, up to and including wound closure and scarring,” he said.

Bates agreed. “Any wound healing strategy should be evaluated on three critical endpoints: incidence of wound closure, reduction in time

“If possible, large-scale retrospective studies of wound healing in patients already on β-blockers could provide some insight into the usefulness of the strategy in the chronic wound and burn setting.”

—Hynda Kleinman,
The George Washington University

Box 1. Skin-deep target

A study in *Nature*⁵ suggests a strategy for boosting regrowth of the epidermis in patients with chronic wounds by targeting caspase-8, a protease usually associated with apoptosis. Such a treatment would likely have to be coupled with treatments targeted at regrowing the dermis.

“We found that downregulation of caspase-8 recapitulates multiple phases of a wound healing response: inflammation, stem cell proliferation and tissue remodeling,” said Colin Jamora, assistant professor of biology at the **University of California, San Diego**. “Available therapies that solely stimulate one phase of this process lack this coordination that makes a complete response.”

Jamora’s team knocked out caspase-8 in the epidermis of mice and found that the mice had higher transcription of proinflammatory genes than wild-type controls—a pattern also seen in actively healing wounds. Compared to the skin of wild-type controls, the skin of the knock-outs had high levels of IL-1 α , a proinflammatory cytokine needed for wound healing. The skin also had higher numbers of proliferating, migrating keratinocytes, a hallmark of wound healing.

Jamora told *SciBX* that caspase-8 could potentially be targeted with topical small interfering RNA therapeutics.

However, because the work wasn’t done in standard burn or chronic wound models, the effect of caspase-8 deletion

on *bone fide* healing is uncertain. Indeed, other researchers worried that knocking out caspase-8 simply led to excess inflammation as opposed to the beneficial inflammation related to wound healing. As a result, they cautioned that knocking the enzyme down might lead to other dermatological problems.

“It’s now appreciated that IL-1 α is a key mediator of healing of various injuries,” said Jeffrey Davidson, professor of pathology at **Vanderbilt University School of Medicine** and a senior research career scientist at the **U.S. Department of Veterans Affairs**. “However, excess IL-1 α is unfavorable to the healing process. You may be able to harness some of the positive response for wound healing, but this kind of phenotype is reminiscent of things going out of control.”

For example, the caspase-8 knock-outs reported in the *Nature* article developed excessively flaky, thickened skin that, according to Davidson, resembled that found in psoriasis.

George Cotsarelis, associate professor of dermatology at the **University of Pennsylvania**, agreed. He noted that the keratinocyte proliferation observed by Jamora’s team could be a consequence of inflammation rather than of wound healing.

“It’s not unusual to see inflammation when you knock out a gene,” said

Cotsarelis. “They’re seeing proliferation, but I’m not sure if it’s analogous to wound healing.”

Davidson and Cotsarelis think the best case scenario for caspase-8-inhibiting therapeutics would be as adjuvants to dermis-regenerating therapy, because during the healing of deep wounds, the underlying dermis must first grow back to provide a surface for the epidermis to cover.

Marketed wound healing drugs like **Johnson & Johnson’s** Regranex becaplermin, a recombinant platelet-derived growth factor- β (PDGF- β), work by stimulating regrowth of the underlying dermal layer.

“The epidermis is activated pretty early on in the wound healing process, but the keratinocytes need to have something to travel on,” said Cotsarelis. “Chronic wounds don’t have a good dermis, so the keratinocytes bunch up at the edges.”

All three researchers agreed that the next step is to test the effect of knocking down caspase-8 in chronic wound models such as diabetic mice, which have constitutively low epidermal IL-1 α levels and poor wound healing compared with wild-type mice.

Meanwhile, Jamora and the University of California are determining whether to patent the findings, which are not yet available for licensing.

—Lev Osherovich, Senior Writer

to wound closure and quality of wound closure,” he said.

Roslyn Rivkah Isseroff, corresponding author on the *PLoS Medicine* paper and professor of dermatology at UC Davis, told *SciBX* her next steps include “looking at stages of the wound healing process that occur after re-epithelialization—in particular, dermal healing and scar formation. To accomplish that, we plan to use an established model of chronic wounds such as the rabbit ear dermal ulcer model.”

Informing trials

The good news for any company looking to repurpose a β -blocker is that retrospective data from the clinic supplement the preclinical findings.

For example, Isseroff and David Margolis have conducted a retrospective study of more than 400,000 patients in the U.K.’s General

Practice Research Database (GPRD) who received β -blockers and β -agonists for a wide variety of medical conditions. They found that the blockers protected certain subgroups against development of venous leg ulcers.⁴ Margolis is a professor of dermatology and director of the Cutaneous Ulcer Program at the University of Pennsylvania.

“If possible, large-scale retrospective studies of wound healing in patients already on β -blockers could provide some insight into the usefulness of the strategy in the chronic wound and burn setting,” said Hynda Kleinman, adjunct professor at **The George Washington University** and guest researcher at the NIH.

Kleinman is the original developer of RGN-259, a synthetic version of thymosin β 4, a naturally occurring 43-amino-acid peptide that has been in-licensed by **RegeneRx Biopharmaceuticals Inc.** and is in Phase II testing to treat pressure and venous stasis ulcers.

Cotsarelis wanted to see prospective studies. He suggested following patients on β -blockers who undergo some type of surgical incision to determine if the drugs accelerate wound repair.

Delivering the goods

A key question for any trial of β -blockers in wound healing will be the optimal mode of delivery.

"If systemic delivery of β -blockers leads to local modulation of wound healing in humans, as the *PLoS Medicine* paper shows in mice, that could offer an advantage over topical delivery approaches" such as Regranex, said Margolis. "In the chronic wound setting, topical delivery often requires multiple applications and high doses because the wound environment can potentially degrade and oxidize therapeutics. An orally or systemically available small molecule would potentially get around that problem by not having to enter the wound bed from the skin surface."

Margolis is taking a different tack on the delivery issue. His group is using adenoviral gene therapy to deliver the gene expressing PDGF- β directly to cells in the wound bed.

The gene therapy completed a Phase I safety trial in venous leg ulcers late last year. According to Margolis, immunological staining of patient wound biopsies confirmed that fibroblasts local to the injection site produced increased quantities of PDGF- β .

Tissue Repair Co., a subsidiary of **Cardium Therapeutics Inc.**, has a similar product in development. The company's Excelsiorate (GAM501), an adenoviral gene encoding PDGF- β formulated in a topical bovine type I collagen matrix, is in Phase IIb testing to treat diabetic foot ulcers. The formulation allows wound repair cells to migrate into the matrix, where they take up the gene and produce PDGF- β .

Isseroff told *SciBX* that she and colleagues already have plans for clinical trials in patients with burns and chronic wounds. They are looking for industry or university partners to help finance and run the studies. Isseroff and colleagues have submitted a patent application for the use of ADRB2-targeting compounds to modulate wound repair.

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