psoriasis. Thus, partially effective and even clinically ineffective therapy may still cause profound signaling changes in psoriasis. The future of psoriasis treatment may involve assessing signaling pathways in psoriatic lesions with a partial or no response, showing the additional therapies that require targeting. Sequential or combination therapies may become the norm for psoriasis treatment. As a final goal, we should accept no less than long-term remission.

CONFLICT OF INTEREST

JLA is inventor of technology U.S. Patents 9289414 (carbazole) and 9592226 (ceramide derivatives-solenopsin).

ACKNOWLEDGMENTS

Supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases grant number RO1AR 47901.

REFERENCES


See related article on pg 994

A Modeling Conundrum: Murine Models for Cutaneous Wound Healing

Sharon Elliot¹, Tongyu C. Wikramanayake², Ivan Jozic² and Marjana Tomic-Canic²

The complexity of the cutaneous wound healing process and its impairment in disease states, combined with the urgent clinical need for new therapies demand well-defined preclinical models that facilitate translation of research findings to clinical use. Many murine wound models are well suited for studying mechanisms of various aspects of wound healing, but they have shown limited utility for translating research findings to human conditions, thereby impeding therapeutic developments. Ansell et al. underscore the need for standardization of preclinical murine models by showing the impact of duration of diabetes, animal sex, and assessment methods on healing outcomes in the streptozotocin-induced diabetes mellitus rat model. Recognizing and understanding the limitations and advantages of preclinical murine wound models will facilitate more effective translation of experimental results to improved treatment of experimental results to human chronic wounds.


¹DeWitt Daughtry Family Department of Surgery, University of Miami Leonard M. Miller School of Medicine, Miami, Florida, USA; and 2Wound Healing and Regenerative Medicine Research Program, Department of Dermatology and Cutaneous Surgery, University of Miami Miller Medical School, Miami, Florida, USA

Correspondence: Marjana Tomic-Canic, Wound Healing and Regenerative Medicine Research Program, Department of Dermatology and Cutaneous Surgery, University of Miami Miller School of Medicine, 1600 NW 10th Avenue, RMSCB, Room 2033-A, Miami, Florida 33136, USA. E-mail: mtcanic@med.miami.edu

© 2017 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology.
Clinical Implications

- Many variables need to be considered when choosing preclinical models to support clinical testing, including age, sex, duration of diabetes, types of wounds, and wound locations.
- Preclinical models should be validated before proceeding with testing.
- Well-defined and validated studies that involve standardized murine models in preclinical testing will facilitate more successful translation of research findings into clinical use.

Cutaneous wound healing proceeds through several overlapping phases that require precise spatiotemporal control and contributions from multiple cell types (platelets, keratinocytes, fibroblasts, endothelial cells, neutrophils, macrophages, local and circulating progenitors, etc.) that orchestrate the myriad cellular processes that ultimately result in wound closure and restoration of the barrier (Eming et al., 2014). This remarkable process is evolutionarily conserved and can be studied in numerous in vivo animal models, ranging from models involving corals, hydras, Drosophila species, zebrafish, and chickens to those using mice and pigs. Over the past several decades, use of wound healing models has contributed to major discoveries, advancing the knowledge and understanding of the molecular and cellular events that contribute to this process (Eming et al., 2014). Despite advances in the biology of wound healing, understanding of the pathophysiology of chronic human wounds (diabetic foot ulcers, venous leg ulcers, and pressure ulcers) is limited, resulting in a paucity of efficacious therapies for patients. Despite the urgent clinical need, development of effective new therapies has been limited, in part, because of difficulties translating results from preclinical models to clinical use. Adaptation of animal models that were designed to study biology and mechanisms to preclinical testing has resulted in numerous variations of models that have not been standardized and validated, making it very difficult to interpret findings or to potentially perform comparative analyses of existing data.

This is a major problem because this extremely complex biological process is influenced by many variables including age, sex differences, metabolic underpinnings, hair cycling, and microbiome diversity. Thus, we encounter a conundrum. On one hand, the field is required to use animal models to perform preclinical testing as a prerequisite for therapeutic development. On the other hand, we are faced with the limited predictive value of findings obtained in preclinical testing to clinical implementation. Underpinning both the limitations and advantages of various models and the methods of assessment are essential prerequisites to choosing appropriate models for preclinical testing. A model that is very useful for studying mechanisms is not automatically appropriate for preclinical testing. Ansell et al. (2018) show an approach to testing important biological variables, sex differences, and duration of diabetes, underscoring the necessity of validating and characterizing the wound healing phenotype in the model of choice before proceeding with preclinical testing.

Murine models of wound healing

Murine models that test wound healing may be excisional (2–15 mm in diameter), incisional, splinted, punch, polyvinyl alcohol sponge inserted, magnet-pressure induced, redox manipulated, bleomycin induced, ischemic, diabetic, aging, infected, humanized, or xenografted and may involve dorsal, ear, or tail skin (Eming et al., 2014; Wong et al., 2011). Assessment of gross wound size, planimetry, presence of the scab, re-epithelialization by histology, tensile/breaking strength, tensile stiffness, fibrosis, cellular infiltrate, microbial composition, granulation tissue formation, and transcutaneous water loss are among the methods used to evaluate wound healing. Even if one chooses to ignore the obvious structural differences between human and mouse skin (Figure 1) and does not take into account additional biological variables (age, sex, microbiome, location), it becomes clear that reproducibility and extrapolation of data, as well as comparison among published studies, is very challenging, especially when details of experimental design are missing (Ansell et al., 2018). As an example, recombinant human platelet-derived growth factor-BB, which has received U.S. Food and Drug Administration approval for treatment of diabetic foot ulcers, shows variable efficacy in preclinical murine models, ranging from improved wound closure of 1.5-cm excisional diabetic mouse wounds (Greenhalgh et al., 1990), to significant increase of granulation tissue without improvement in the time to wound closure (and only in the 1.5-cm but not in 0.6- to 1.0-cm–diameter excisional wounds) (Chan et al., 2006), to no effect on re-epithelialization in a murine splinted diabetic wound model (Park et al., 2014). It is increasingly evident that consistency and, in some form, standardization of preclinical murine models, as well as validated methods of assessment, are urgently needed. Because each model can provide specific aspect(s) of useful information, a combination of models and multiple assessment methods may provide advantage in preclinical testing designed to support clinical trials.

Murine models of diabetic wound healing

Genetic diabetic models, including db/db (a point mutation in the leptin receptor gene) and obese ob/ob (leptin deficient) mice, are widely used as animal models of type II diabetes, whereas streptozotocin (STZ)-induced diabetes (featuring destruction of insulin-producing cells in the pancreas) and non-obese diabetic mice (with polymorphisms in the Idd3 locus, which are linked to IL-2 production, and a mutation in exon 2 of the CTLA-4 gene) are used as experimental models of type I diabetes. Although cutaneous wounds heal in all these diabetic murine models, healing is delayed and delays are more pronounced in larger excisional wounds.
Diabetic murine models are often used in combination with additional inducing factors that delay healing. For example, inhibition of two major antioxidant enzymes, catalase and glutathione peroxidase in db/db mice induces chronicity (Dhall et al., 2014). Moreover, the introduction of *Staphylococcus aureus*-specific biofilms into splinted excisional wounds delays epithelialization and wound closure in db/db mice (Nguyen et al., 2013), whereas wounds in aged db/db mice heal with reduced stiffness and breaking load, along with reduced deposition of granulation tissue that is independent of glycemia (Brem et al., 2007). Ansell et al. (2018) discuss the lack of experimental details reported in the literature when the STZ model is used, particularly with regard to the impact of duration of diabetes on healing outcome. They examine this model more closely by evaluating...
multiple assessment methods: wound photographs (planimetry), histology, granulation tissue, re-epithelialization, inflammation, collagen deposition, and angiogenesis. The data show that 3 weeks after STZ induction of diabetes, which is similar to diabetes mellitus, mellitus is too early to capture wound healing delay, whereas at 6 weeks some parameters (planimetry, re-epithelialization, macrophage count), but not all (collagen deposition, granulation tissue, wound width), show impaired healing phenotype, further supporting the notion that duration of diabetes and multiple assessment methods are necessary for model validation. The use of a single wound time point (5 days after wounding) is a limitation of the study, because it is likely that a minimum of 6–8 weeks of STZ-induced diabetes should occur before initiation of wounding experiments.

Microbiome and murine wound models
It is increasingly evident that both commensal and pathogenic microorganisms can influence outcomes of wound healing in mice and humans. Modeling the human condition in mice represents a major challenge, and the field is in the early stages of developing relevant models that include polymicrobial biofilms using bacterial isolates from patients’ wounds. Regardless, given the variability of mouse models, one has to take into the account the environment in which preclinical testing is performed. Mice are housed in the constant presence of fecal bacteria, even if specific-pathogen–free mice or a pathogen-free animal facility is used. Another option is germ-free mice. Although germ-free murine models provide a unique environment and very valuable insights into the cellular and molecular mechanisms of skin immunity, they do not reflect a clinically relevant environment necessary for preclinical testing for wound healing. In humans, every wound is considered colonized, regardless of the absence of clinical signs of infection. A more dynamic wound microbiome is associated with better healing (Loesche et al., 2017). Therefore, polymicrobial experimental murine wound models may provide better insights that are more relevant to the human wound environment. Furthermore, the types of dressings that are used to cover wounds and hair status (plucked, clipped, or shaved) (Stojadinovic et al., 2011) are among additional variables that can affect microbial environment and that may influence healing outcomes.

Age, sex, gonadal hormones, and wound healing
Age plays a major role in wound healing, but it is often neglected in clinical testing, resulting in very limited knowledge regarding mechanisms by which age slows healing in adults (Gould et al., 2015). In addition, very few studies focus on aging in preclinical rodent models, as reported by Ansell et al. (2018) (see Table 1 in the Ansell et al. study). Aging is intertwined with the effects of steroid hormones, because murine models in which sex hormone signaling is blocked (e.g., ovariectomized, castrated, or treated with selective estrogen receptor modulators) are often used as surrogate models of aging. In humans, many of the age-related skin changes observed in females result from a decline in estrogens, but surprisingly this is true in males as well (Gilliver et al., 2010). Microarray-based profiling of genes that are differentially expressed in wounds from young and elderly men identified 78% as estrogen regulated, whereas only 3% were age associated (Hardman and Ashcroft, 2008). Ansell et al. found delayed wound healing in young diabetic rats, although the magnitude of the delay was less evident in female rats, suggesting a possible protective role for estrogen.

Estrogen has been shown to modulate all phases of wound healing (Thornton, 2013). In human skin, evidence indicates that estrogen receptor (ER)-β signaling suppresses skin aging, whereas stimulation of ER-α has little effect (Thornton, 2013). To that end, both topical and systemic estrogens increase the rate of wound healing in elderly men and women (Ashcroft et al., 1999). There is less consensus in rodent models, in part because of inconsistencies in the models that are used. Sixteen-week-old female db/db mice that were treated with 17β-estradiol had accelerated wound healing compared with those receiving control treatment, and 17β-estradiol stimulated ER-α expression without an effect on ER-β expression (Pincus et al., 2010). STZ-induced diabetes increased the expression of ER-β in the skin of male wild-type mice, and ER-β-knockout mice had a wound healing response that was similar to that of wild-type mice (Sunkari et al., 2014). The divergence in estrogen response between males and females requires additional investigation, because factors including sex, age, diabetes, and/or strain differences may play a role.

Less data has been compiled regarding the effect of gonadal hormone testosterone on wound healing. Several studies suggest that testosterone inhibits human wound healing, and testosterone has been reported to contribute to a slower barrier development in the fetus (Fimmel and Zouboulis, 2005; Greenough, 1996). A single report on STZ-induced diabetic rats suggested that 5α-dihydrotestosterone (i.e., DTH) improved wound healing compared with sham-treated wounds (Goncalves et al., 2016). Unfortunately, the authors did not clarify the sex of the animals used. In contrast, others have shown that use of a topical androgen antagonist promoted wound healing in male C57BL/6 mice (Toraldo et al., 2012). To date most studies on gonadal hormones, wound healing, and aging have focused on 17β-estradiol (or estrogen). Less data is available on the role of androgens, which may play a role in age-related wound healing, especially because androgens can be converted to estrogens. Further studies are warranted to investigate estrogen and androgen signaling and potential downstream effects.

Taken together, murine models should be carefully chosen and should incorporate assessment of parameters that will accurately reflect the wound response in the context of potential mechanism of action. Furthermore, additional variables that include duration of diabetes, aging, gonadal hormone components, and microbial environment should all be considered and incorporated into the experimental design. Specific details of the model should also be provided in publications to facilitate comparative analyses of the data. Finally, some type of consensus is needed from the scientific community
to provide a more standardized approach to preclinical testing.

CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES


