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The effects of social isolation on wound healing mechanisms in female mice

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HIGHLIGHTS

- Social isolation reduces baseline circulating corticosterone concentrations in female mice.
- Isolation impedes dermal wound closure while decreasing initial wound bacterial load.
- Isolation decreases mRNA of pro-keratinocyte and -angiogenic factors in wound tissue.

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ABSTRACT

Various stressors impair wound healing in humans and rodents. For example, social isolation delays wound closure in rodents, but the healing mechanisms that underlie this delay have yet to be identified. Here, the effects of three weeks of social isolation on hypothalamic–pituitary–adrenal axis responses and healing factors involved in the inflammatory and proliferative phases of wound healing were assessed in adult female hairless mice. Social isolation reduced basal circulating corticosterone concentrations and increased body and thymus weights compared with group-housed controls. Isolation impaired dermal wound closure by up to 30% and reduced initial total wound bacterial load relative to controls. Inflammatory gene expression in the wounds was not affected by the observed differences in wound bacterial load. However, isolation reduced wound gene expression of keratinocyte growth factor and vascular endothelial growth factor, which are involved in keratinocyte proliferation/migration and angiogenesis during the proliferative phase of healing. These data indicate that social isolation induces healing impairments that may be attributed to reductions in growth factors necessary for proper skin cell proliferation and blood vessel growth during healing. This healing impairment occurred in the absence of both high wound bacterial load and elevated circulating glucocorticoids, which have previously been hypothesized to be required for stress-impaired healing in mice.

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1. Introduction

Many factors (e.g., stress, health, environment, and genetics) influence the process of wound healing [1] by modulating one or more of the tightly-orchestrated, overlapping phases: hemostasis/inflammation, proliferation, and remodeling [2,3]. Briefly, the initial inflammatory phase is characterized by clot formation and the recruitment of neutrophils and monocytes from the blood to the wound site. In addition to clearing the wound site of pathogens, these cells produce cytokines that stimulate keratinocytes, fibroblasts, and angiogenesis, which together mark the initiation of the proliferative phase. This second phase is characterized by re-epithelialization of the wound opening, wound contraction, collagen deposition, and angiogenesis. After wound closure, the final remodeling phase is characterized by blood vessel pruning, restructuring of the extracellular matrix, and scar formation [2]. In rodent models of dermal wound healing, inflammation occurs primarily from 0 to 3 days post-wounding, re-epithelialization occurs from days 3–7, and subsequent remodeling lasts months or more [4]. In humans, impairments or abnormalities in tissue repair can lead to dangerous and costly infections, poor surgical outcomes, extended hospital stays, and non-healing wounds [5].

In humans and other animals, loneliness or psychosocial stress (e.g., social isolation) have each been shown to have numerous detrimental health consequences [6,7], including the impairment of wound

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healing [8]. For example, caring for relatives with dementia prolongs dermal wound closure by 24% compared with non-caregiver controls [9]. Social isolation (i.e., individual housing), which is commonly used to model psychosocial stress, of hamsters, rats, and *Peromyscus* mice also delays dermal wound closure [10,12]. However, the healing mechanisms by which social isolation impairs wound closure remain to be determined.

Restraint, a nonsocial stressor, also impairs dermal wound closure in mice. Specifically, restraint dysregulates the initial inflammatory phase of dermal wound healing by delaying the inflammatory cascade and increasing wound bacterial load [13,14]. This healing impairment is, in part, mediated by increased circulating glucocorticoids in restrained mice [13,14]. However, social stressors have inconsistent effects on the hypothalamic–pituitary–adrenal (HPA) axis output [15,17] and the phases of tissue repair that are impaired by social isolation have not yet been delineated. Using a standardized model of wound healing in mice, the goal of this study was to determine which components of wound healing are affected by social isolation, specifically in the inflammatory and proliferative phases, and to identify potential mechanisms by which this occurs. The physiological consequences of isolation relevant to healing were measured in the first experiment and the specific healing phases affected by social isolation were determined in the second experiment. All previous studies of the effects of isolation on wound healing have used male rodents, except one [9], even though sex differences in immune function are abundant [18]. Therefore, female mice are used in these experiments to broaden the understanding of how social stress affects healing in both sexes. Mechanistic differences in the inflammatory and proliferative phases were also assessed via wound bacterial quantification and quantitative gene expression.

2. Materials and methods

2.1. Animals

Virus-antibody-free, outbred SKH-1 female mice (8 weeks of age) were obtained from Charles River, Inc. (Wilmington, MA, USA). SKH-1 mice were chosen because their skin is virtually hairless (similar to human skin) and the healing process has been well characterized in this strain [14,19,20,21]. Mice were housed in the vivarium at 21 ± 1 °C under a 14:10 h light:dark cycle (lights off at 6 pm) and had access to food (Harlan 7912 rodent chow) and water ad libitum. The vivarium is accredited by The American Association for the Accreditation of Laboratory Animal Care (AAALAC) and all procedures were approved by the Office of Animal Care and Institutional Biosafety at the University of Illinois at Chicago. Upon arrival, mice were isolated for three weeks prior to wounding and throughout healing or remained group-housed (5/cage). This time period was chosen based on a pilot study which determined that three weeks of isolation induces consistent, significant healing impairments. Isolation consisted of single-housing in individual cages with barrier lids to minimize scent communication among mice.

2.2. Somatic responses to isolation

One cohort of mice was used to determine the effects of isolation and wounding on the hypothalamic–pituitary–adrenal (HPA) axis, body mass, and relevant tissue masses. For this experiment, adult female mice were isolated for 3 weeks ($n = 10$) or remained group housed (5/cage; $n = 10$). Mice were weighed weekly and then daily following wounding. Corticosterone responses to an acute restraint stressor were measured after 3 weeks of isolation (see below). Two days after the acute stressor, mice were wounded (described below). On day 5 post-wounding, wet tissue weights of the adrenal glands (paired), thymus, and spleen were obtained and trunk blood was collected for post-wounding corticosterone concentration assessment.

2.3. Corticosterone responses to acute restraint

Within 2 min of isoflurane anesthetization, retro-orbital blood samples (100 μl) were collected before a 30-min bout of physical restraint (baseline), immediately after restraint (post-stressor), and 70 min after the end of restraint (recovery). All mice in a cage were anesthetized simultaneously. Mice were returned to their homecage between the collection of the post-stressor and recovery blood samples. For restraint, mice were enclosed in well-ventilated 50-ml polypropylene tubes for 30 min. Corticosterone was measured in duplicate in all blood samples via EIA according to the manufacturer's instructions (Enzo Life Sciences, Plymouth Meeting, PA, USA) after 1:30 dilution. Intrassay variation was 11% and interassay variation for the four plates was <10%.

2.4. Wound healing

In a second cohort of mice, the effects of social isolation on dermal wound healing rates ($n = 15–20$/treatment/day), wound bacterial load ($n = 5$/treatment/day), and genes relevant to the inflammatory and proliferative phases of healing were examined ($n = 10$/treatment/day). Following three weeks of being isolated or group-housed, mice were anesthetized (100 mg/kg ketamine mixed with 10 mg/kg xylazine, i.p.), the dorsal skin was cleaned with alcohol, and two full-thickness skin wounds were excised just below the scapulae using a sterile 3.5 mm biopsy punch (Miltex Instrument Company, Plainsboro, NJ, USA). Mice were then returned to a clean cage and bedding was not changed again during healing. Wound closure was assessed via daily photographs of the wounds (through day 5 post-wounding) alongside a standard 3-mm circle to control for variation in capture angle and distance. Image analysis was used to determine the ratio of the wound area to the standard area, and was then expressed as a ratio to the original wound size (from day 0; using Canvas 9 software) [19]. Wounds were considered to be healed when the wound opening was closed (i.e., 0% of the initial wound size). On day 1, 3, or 5 after wounding, wounds were harvested by sterile 6.0 mm biopsy punches following terminal anesthetization (as described above). One wound from each mouse was used to quantify total wound bacteria and the other was used for genomic comparison of factors known to regulate wound healing using quantitative RT-PCR (qRT-PCR).

2.5. Bacterial quantification (in vitro)

Wounds harvested for total bacterial quantification were weighed and then homogenized on ice in PBS. The homogenates were serially diluted 1:10 six times with PBS, and 100 μl of each dilution was plated in duplicate on brain–heart infusion agar (Becton Dickinson, Downers Grove, IL, USA). Colonies were counted after overnight incubation at 37 °C with 5% CO_2 and used to determine initial colony forming units (CFU) per gram of wound tissue [22].

2.6. Inflammatory and proliferative wound gene expression (qRT-PCR)

Wounds harvested for qRT-PCR were immediately placed in Trizol (Invitrogen, Grand Island, NY, USA) flash frozen in liquid nitrogen, and stored at −80 °C. Total RNA was extracted from wound tissue as described in detail elsewhere [13]. RNA concentrations were measured and 260/280 ratios were determined (ThermoScientific, Wilmington, DE, USA). Total RNA was reverse transcribed using SuperScript First-Strand kits (Invitrogen) according to the manufacturer's protocol. RNase H (1 μl) was added to each sample and incubated at 37 °C for 20 min and then 1 μl cDNA was diluted 1:5 with RNAse-free water and stored in −20 °C for qRT-PCR.

Six genes that peak during the inflammatory phase of healing and three genes that peak during the proliferative phase of healing were measured on days 1 and 3 or days 3 and 5 post-wounding,

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