Mucosal Wound Healing Is Impaired by Examination Stress

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Objective: Impairment of wound healing is a well-recognized sequelae of conditions that alter immune function, including diabetes, jaundice, and advanced age. There is also growing evidence that psychological stress has adverse consequences for immune function. This study addressed the effects of a commonplace stressor on wound healing. *Method: Two* punch biopsy wounds were placed on the hard palate of 11 dental students. The first wound was timed during summer vacation, whereas the second was placed on the contralateral side 3 days before the first major examination of the term; thus, each student served as her or his own control. Two independent methods assessed healing (daily photographs and a foaming response to hydrogen peroxide). *Results:* Students took an average of 3 days longer to completely heal the 3.5-mm wound during examinations, i.e., 40% longer to heal a small, standardized wound. Production of interleukin 1β (IL-1β) messenger RNA (mRNA) declined by 68% during examinations, providing evidence of one possible immunological mechanism. These differences were quite reliable: No student healed as rapidly or produced as much IL- 1β mRNA during examinations as during vacation. *Conclusions:* These data suggest that even something as transient, predictable, and relatively benign as examination stress can have significant consequences for wound healing. Key words: oral, wound repair, interleukin 1, psychological stress, surgery.

ANOVA = analysis of variance; IL-1 = interleukin 1; LPS = lipopolysaccharide; MANOVA = multivariate analysis of variance; mRNA = messenger RNA; PSS = Perceived Stress Scale.

INTRODUCTION

Oral wounds are relatively common mucosal wounds; healing in these tissues can reflect the susceptibility of other mucosal tissue to repair and infection. Reestablishing the barrier function provided by intact epithelium protects the host from infection after wounding and requires the orchestration of both recruited inflammatory and immunological cells and indigenous tissue cells (1, 2). As demonstrated by several animal and human studies, stress can alter multiple aspects of immune function, including the production of pro-inflammatory cytokines important for wound repair (3-8). Because neutrophil function is also impaired by stress (9), distressed individuals face additional risks from infection after wounding.

These immunological changes have clinical implications; an earlier study from our laboratory showed that severe and prolonged psychological stress was associated with poorer wound healing (6). Thirteen women who were engaged in a chronically stressful activity (caring for a parent or spouse with dementia) took an average of 24% longer to completely heal a small, standardized wound than 13 matched control subjects. In addition, the mononuclear cells of caregivers produced lower levels of the pro-inflammatory cytokine IL-1β in response to LPS stimulation. Inasmuch as IL-1 plays a critical role in wound healing by regulating inflammatory cell recruitment and activation, metabolism of matrix components, and production of growth factors early in the wound healing process, lowered IL-1 responses may be an important mechanism for delayed healing (10). Although these were provocative data, the sample was small; moreover, these caregivers typically report high levels of stress as they attempt to cope with the problematic behaviors of their relative, and caregiving normally extends over a period of years (6, 8).

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In this study, we sought to determine whether a brief commonplace stressor, academic examinations, would be associated with alterations in mucosal wound repair. This project was based on data that demonstrated that cellular immune responses during examinations differ from those measured in lower stress periods (11). Natural killer cell activity, mitogen responsiveness, γ-interferon production by peripheral blood leukocytes stimulated with concanavalin A, and the percentage of peripheral blood T lymphocytes expressing the interleukin-2 receptor were all lower during examinations compared with lower stress periods (11, 12). Thus, this relatively mild stressor can modulate a range of immunological activities. Here we assess the possibility that academic examinations modulate mucosal wound healing and down-regulate IL- l gene expression.

METHOD

Subject Characteristics and Timing of Wounds

Healthy dental students (9 men, 2 women, mean age 24.36, SEM = 1. 11), were recruited from The Ohio State College of Dentistry by an announcement placed in their mailboxes. The Ohio State Biomedical Research Review Committee approved the project; before participation, all subjects gave written informed consent. The first wound (the side of the hard palate chosen by coin toss) was placed in mid-August at the end of summer vacation, just before the beginning of classes. Approximately 6 weeks later, the second wound was placed on the contralateral side 3 days before the first major academic examination of the term.

Wound Creation and Assessment

The hard palate was anesthetized using 2% lidocaine and a 3.5-min wound was outlined between the palatal roots of the first and second molars, using an oral punch. A scalpel was then used to remove the surface epithelium and underlying superficial connective tissue, leaving a wound approximately 1.5 mm deep. The wound was not dressed. The subjects were instructed not to use rinses or other special home care other than normal oral hygiene.

As before, the wounds were assessed daily in two ways (6). The size of the wound was assessed by capturing a videograph of the wound, using an intraoral fiberoptic camera. A standard-sized label placed adjacent to the wound was used to account for magnification and angulation errors (6) and the wound images were transferred to a Macintosh computer. The images were blind coded, and the wound and the standardized labels were measured. As the wound approached closure, as determined by visual inspection (after at least day 5), the area was blotted dry with a gauze sponge and 3%, hydrogen peroxide was applied to the wound. The wound was considered negative for peroxide bubbling if it was negative for 2 consecutive days.

The peroxide test for wound healing measures the quality of the

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epithelial barrier. It is based on the fact that the underlying connective tissue contains the enzyme catalase. Catalase liberates oxygen gas and water from hydrogen peroxide. If the epithelial barrier is intact, then the hydrogen peroxide does not diffuse into the connective tissue and little oxygen is liberated. If the barrier is incomplete, then bubbles of oxygen are visible on the surface of the wound. We have used this in the skin model (6), a mouse model (13), and the present study; it correlates well with wound size measurements.

Whole Blood Assay for IL-1β

Heparinized blood (5 ml) was drawn on both wound dates. The blood was diluted with an equal volume of saline and was treated with 1 μg/ml of *Salmonella typhimurium* lipopolysaccharide (Sigma, St. Louis, MO) for 2 hours at 37°C in 5% carbon dioxide. Them RNA was then isolated and purified and mRNA samples isolated from vacation samples and examination samples were treated by electrophoresis together on the same gel, blotted, and then probed for IL-1β, and autoradiographed (14). The samples were also stained with ethidium bromide for 18 seconds as a loading control. Data from two subjects were lost because of technical problems. Video images were captured and analyzed, using National Institutes of Health (NIH) Image 1.58, and expressed as a ratio of IL-1β density to ethidium bromide.

Data and Health-Related Behaviors

The 10-item Perceived Stress Scale (15), administered at the time of each biopsy (wound) and again 3 days later, measured the degree to which subjects perceived their daily life during the previous week as unpredictable, uncontrollable, and overloading. Subjects rated each item from 0 (never) to 4 (very often).

Health-related behaviors assessed at each time point included recent medication use, and caffeine and alcohol intake (16). Subjects were also asked how many hours they had slept in the preceding 3 days, as well as in the last 24 hours.

Statistical Methods

Multivariate and univariate analyses of variance (MANOVAs and ANOVAs) were used to assess within-subject change over time (17).

RESULTS

Figure l shows differences in wound size between vacation and examinations with the size of the wound expressed as a percentage of the initial (day 1) wound for each set. A within-subjects MANOVA that compared wound size across the first 5 days before any subject had healed showed significant differences between vacation and examination wound size, $F(1,10) = 67.65$, $p < .001$.

Students took an average of 7.82 days (SEM = 0.62) to heal completely during vacation, compared with 10.91 days (SEM = 0.69) during examinations, $F(1,10) = 28.47$, $p < .001$. This difference translates into 40% longer to heal a small, standardized wound, as measured by the absence of response to hydrogen peroxide. The effect was remarkably robust: No student healed as quickly during examinations as he or she did while on vacation (Figure 2). Thus, convergent data obtained by two independent procedures (wound photographs and peroxide response) demonstrated that students healed comparable wounds more slowly during stressful examinations.

Consistent with the large differences in wound healing times, IL-1β declined dramatically during examinations, $F(1,8) = 38.19$, $p < .001$, with a mean of 2.43 (SEM = 0.30) for the first sample, compared with 0.70 (SEM = 0.10) 3 days before examinations. The average student's IL-1β declined an average of 68% during examinations. These changes were not a function of alterations in monocytes, because the number of monocytes did not differ between the two samples, $F(1,8)$ _ 0.09, $p = .77$, NS.

Psychological Data and Health-Related Behaviors

Consistent with other studies on academic stress, students reported significantly more stress on the Perceived Stress Scale during examinations than a month earlier, $F(1,10) = 11.16$, $p < .01$. Scale means were 7.36 (SEM = 1.21) and 6.27 (SEM = 1.35) at the time of the first wound and 3 days later; in contrast, scores increased sharply before and during examinations, 16.00 (SEM = 1.66) and 20.91 (SEM = 1.96), respectively. By way of comparison, the mean during examinations was more than 1.0 SD above the mean from a national sample, i.e., within the upper 10% of the population, whereas the mean during vacation was roughly 1.0 SD below the population mean (15).

Health-related behaviors differed in expected ways between vacation and examinations. Alcohol intake showed a

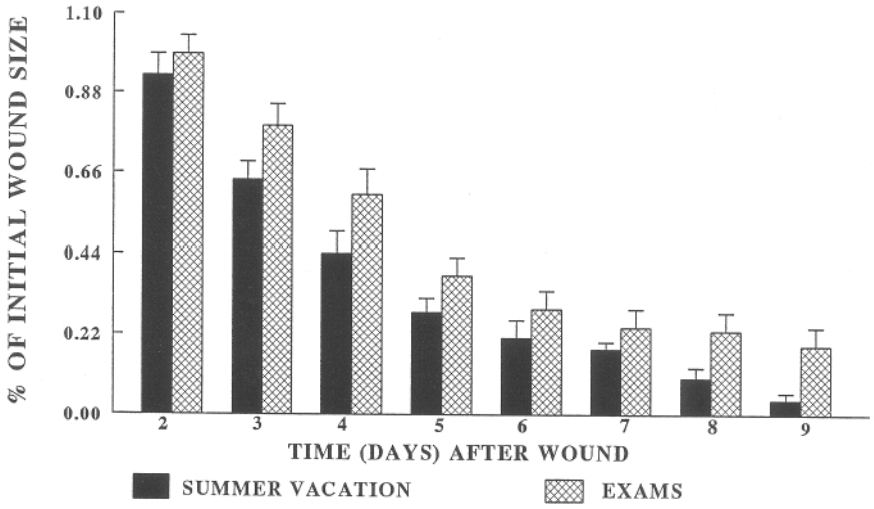

Figure 1. Wound size measurements. The average wound size was measured using videographs captured each day after wounding for the first 9 days of the study for each subject who had not yet healed.

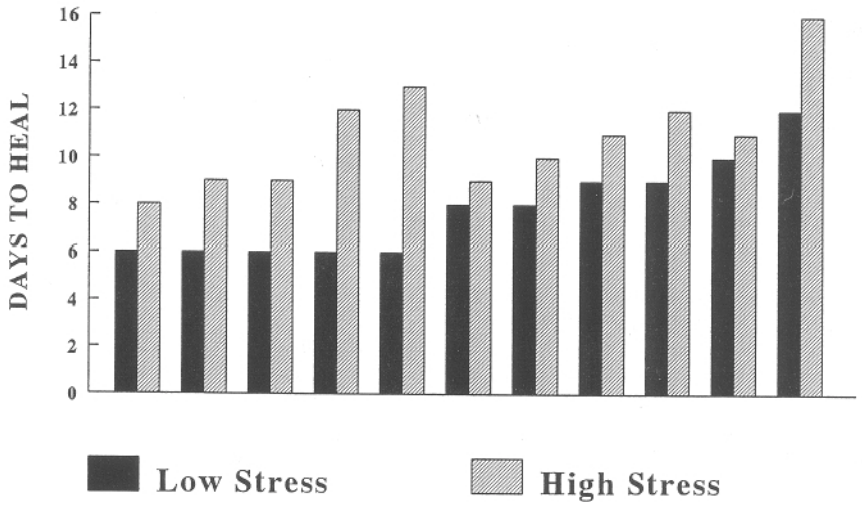

Figure 2. Healing time (as measured by response to hydrogen peroxide) is shown for each of the 11 subjects for the two time periods, summer vacation and examinations.

modest and nonsignificant diminution over time, $F(1,10) = 1.06$, $p = .33$, with students reporting an average of two to three drinks over the preceding 3 days during vacation, compared with one to two drinks in the same interval during examinations. Not surprisingly, students reported greater caffeine intake during examinations, $F(1,10) = 6.48$, $p < 0.05$, jumping from an average of just over one cup per day during vacation to four cups during examinations. Similarly, hours of sleep in the preceding night decreased precipitously, $F(1,10) = 17.33$, $p < .01$, with means of over 7 hours during vacation and 3 days after the first wound, 6.18 hours before the second wound, and a low of 2.82 hours on the night before the examination. However, correlations between caffeine or sleep and wound size or time to heal showed no consistent relationships in this small sample; moreover, dividing subjects by hours of sleep showed no differences, with mean wound sizes, times to heal, and IL-1β data that were virtually identical between those who were above or below the median. All subjects were within normal range on total lymphocyte counts. One student smoked cigarettes, but did so at both time points. One student took Zantac during vacation, but not during examinations. Thus, we found no differences in health-related behaviors that were reliably related to poorer healing.

DISCUSSION

In this study, healthy young adults who were undergoing a transient everyday stressor had uniform wounds placed in mucosal tissue. Wounds placed 3 days before examinations healed an average of 40% more slowly than those made during summer vacation, and the differences were quite reliable: No student healed as rapidly during examinations as during vacation. Our previous study demonstrated stress-related differences between well-matched groups of older women (6). These new data confirm and extend those previous findings in several important ways.

Perhaps most important was the demonstration of how a relatively mild and transient stressor can have sizable effects on wound repair. Like most professional students, these dental students were "experts" at taking tests-they had long histories of performing well under these same conditions. Thus, these data suggest that other everyday stressors that elicit comparable emotional responses may produce similar deficits in wound repair. Moreover, because each subject served as his or her own control, the study suggests that the effects are much larger than those documented in previous research (6).

Although these studies have obvious relevance to other mucosal lesions, other literature suggests that the early events in the wound healing process are "virtually identical" for oral and dermal wounds (2). Although injury to mucosal tissue is very common and wound healing normally proceeds rapidly without scarring in the oral cavity, any delay in healing may subject the host to potential infection and other adverse sequelae.

These data suggest that there was a significant delay in the earliest stages of wound healing, with differences observable within 24 hours; such findings implicate the pro-inflammatory response, and are consistent with the lowered IL-1β. IL-1β is induced within 12 hours of wounding and plays multiple roles in wound healing (10). This cytokine up-regulates adhesion molecules on endothelium and induces the expression of chemokines and, thus, is important in inflammatory cell recruitment (18). IL-1 also activates fibroblasts to produce keratinocyte growth factor, which induces keratinocyte proliferation and migration, key steps in reepithelialization of the wound (19). Furthermore, it induces the production and activation of metalloproteinases, which are required for initiation of keratinocyte migration and in remodeling of the wound (20, 21). Glucocorticoids have been shown to regulate IL- l expression and impair wound healing in the mouse model (10).
Glucocorticoids also down-regulate induction of keratinocyte growth factor (22). Accordingly, an important mechanism by which stress is likely to alter wound healing is through the ability of stress to disregulate normal glucocorticoid circadian rhythms.

These data show that something as transient, predictable, and relatively benign as examination stress can have significant consequences for wound healing. Both the delayed wound healing and the magnitude and reliability of the decrease in IL-1 production (i.e., across every subject) additionally implicates a central role for 1L-1 down-regulation in

delayed wound healing as a result of stress. Stress-related defects, such as those observed here, could have important implications for surgical recovery.

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