ORIGINAL ARTICLE

Mucosal Wound Healing

The Roles of Age and Sex

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Hypothesis: It remains unclear whether aging delays wound healing, as past human studies have not adequately controlled for confounding factors such as morbidity and medications. Furthermore, although dermal wounds heal more quickly in women than in men, clinical observations suggest that the opposite may be true for mucosal healing. We assessed age and sex differences in mucosal wound healing, and we hypothesized that aging delays healing and sex modulates healing independent of age.

Design and Setting: Clinical experimental study performed from June 2000 to August 2003 involving younger and older adult volunteers from the general community.

Participants: Two hundred twelve male and female volunteers aged 18 to 35 years (n=119) or 50 to 88 years (n=93).

Intervention: Standardized 3.5-mm circular wounds were placed on the oral hard palates of volunteers.

Main Outcome Measure: Wound videographs were taken daily for 7 days after wounding to assess wound closure.

Results: Wounds healed significantly more slowly in older adults compared with younger adults ($P<.001$) regardless of sex. This remained true even when individuals receiving medication and/or having a coexisting medical condition were excluded. Mucosal wounds healed more slowly in women than in men ($P=.008$) regardless of age. These effects were independent of demographic factors such as ethnicity, alcohol or nicotine use, or body mass index.

Conclusions: Wound closure in older individuals was clearly delayed even when eliminating potential age-related confounds, indicating that aging does slow wound healing. Wound closure in women was also delayed, suggesting that wound healing is modulated by different mechanisms depending on tissue type. These findings may help target patients with increased surgical risks and greater need for postsurgical care.

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THE FASTEST-GROWING POPULATION in Western countries is the elderly population (aged ≥65 years). In the United States, elderly persons account for 12.4% (35.9 million) of the total population and will likely compose 20% (71.5 million) of the population by 2030.[1] Presently, the treatment of impaired healing costs United States health services more than $9 billion per year.[2] Much of this has been attributed to age-associated delays in wound closure that, in turn, relate to higher rates of infection and medical complications. Thus, the role of aging in wound healing is an important issue, as it severely impacts national health care costs.

It has commonly been reported that people heal more slowly with increasing age.[3-5] A chief criticism of such findings, however, is that studies have not adequately controlled for confounding factors that are more common in aged persons, such as medication use and morbidity.[6-9] Other studies[9-11] report no difference in the healing rates of older vs younger adults. Thus, it remains controversial whether aging per se delays wound healing in humans.[6-9] The aim of the present study was to examine the effects of age on the healing of standardized mucosal wounds while accounting for such factors.

See Invited Critique at end of article

It is well accepted that skin morphology changes with aging.[6] For instance,

older skin has been associated with reductions in vascularization,[10] collagen density or production,[10-12] granulation tissue,[10] and elastin.[9,13] However, many of these changes that occur in aged skin are due to extrinsic aging factors such as sun exposure.[9,12] Mucosal tissue is not exposed to the sun and thus may provide a better assessment of the effects of intrinsic aging on wound healing. Mucosal wounds occur frequently, and the healing of the mucosa is important in most surgical outcomes. Oral wounds are a common type of mucosal wound and are comparable to other mucosal tissues in both repair rates and susceptibility to infection. However, little is currently known regarding the effects of aging on the healing of mucosal tissue.

Sex has been implicated as a factor in wound healing, and a number of studies[14-16] clearly show a female advantage in human healing rates. However, these findings are mainly derived from dermal wound studies, and a female advantage in the healing of nondermal wounds is unapparent. A number of clinical studies[17-19] observed no sex difference in the healing of the oral mucosa. Other studies that examined the healing of mucosal tissues after third-molar surgery found that women healed significantly more slowly[20,21] and needed additional postsurgery treatment[21,22] compared with men. Taken together, aging and sex may additively or synergistically affect the healing of mucosal wounds.

This study examined age and sex differences in the healing of standardized mucosal wounds created on the oral hard palate. Potentially confounding effects, such as individuals reporting morbidity or medication usage, were eliminated by the exclusion of these subjects from specific follow-up analyses. Using this rigorous approach, our results indicate the following: (1) older adults heal mucosal wounds more slowly than young adults, even when factors such as medication use and morbidity are eliminated; and (2) women heal mucosal wounds more slowly than men, independent of age. These findings further indicate that older women are at the highest risk for delayed healing of mucosal tissue.

METHODS

PARTICIPANTS

This study involved 212 volunteers aged 18 to 35 years (52 men; 67 women) or 50 to 88 years (33 men; 60 women). All of the individuals gave written informed consent and received monetary compensation for their participation. Questionnaires were used to determine demographics, health behaviors, a detailed history of health, and current medication use (type, dose, frequency, and purpose) for each individual. Participants were excluded from the study only if they had an oral disease needing emergency treatment or medical problems that made them a high surgical risk (eg, unstable angina or the presence of an infectious disease, such as active hepatitis, tuberculosis, or AIDS). These limited exclusion criteria allowed for a broad subject base to enhance the generalization of the results. All of the wounding was performed by a periodontist (P.T.M.). All of the procedures were carried out in the College of Dentistry Clinical Research Center, The Ohio State University, Columbus, and met with institutional review board and ethics committee approval by The Ohio State University.

PROCEDURES

Subjects arrived and were seated in the dental clinic between 9:30 AM and 10:30 AM. Wounds were created between the first and second molar approximately 3 mm from the marginal gingiva. The wound site was anesthetized with 2% lidocaine, after which the wound was outlined using a 3.5-mm tissue punch. A scalpel was then used to remove the surface epithelium and superficial connective tissue, creating a uniform wound 1.5 mm deep. Afterward, wounds were not dressed and participants were instructed not to change their normal hygiene procedures with the exception of abstaining from alcohol-based mouthwash. There were no differences in oral hygiene between age or sex groups (ie, the frequency of toothbrushing or the use of mouthwash or floss).

Wounds were videographed at 24-hour intervals for 7 days after wounding or until considered healed by visual inspection. A standard 6-mm label was placed around the wound to account for variation due to magnification and angulation. Wound images were then transferred to a Macintosh computer (Apple Computer, Inc, Cupertino, Calif), blind coded, measured (for area), and expressed as a ratio of the wound size to the standard label size. All of the wound ratios were determined by a single investigator (April Logue, MS). Changes in health status and medication use were assessed each postwounding day by questionnaires.

STATISTICAL ANALYSIS

Data were analyzed using a mixed-design analysis of variance that treated the wound sizes for 7 days after wounding as a within-subject factor and sex and age as between-subject factors. Separate analyses of variance were used for post hoc comparisons. Correlations were determined using Pearson product moment correlations (r), and χ^2 tests were used to assess between-group differences in healing rates. Demographic analyses consisted of analyses of variance and correlations. Age was treated as a covariate when determining sex differences within younger or older groups. All of the hypothesis tests were 2-tailed and used $\alpha = .05$ to determine significance. Data were analyzed using SPSS version 11.5 for Windows (SPSS, Inc, Chicago, Ill).

RESULTS

Healing rates were unrelated to ethnicity, body mass index, exercise, alcohol consumption, or nicotine use (although only 31 subjects smoked).

AGE EFFECTS

Younger participants displayed a different pattern of wound healing than older participants ($F_{1,208} = 31.67$; $P < .001$). Although all of the wounds were initially the same size, younger individuals had smaller wounds by day 2; this difference continued to the end of the study ($P < .001$ for each day) (**Figure 1**A). In addition, the proportion of individuals who were considered healed (wounds >90% closed) was significantly higher in younger subjects than in older subjects on days 5 through 7 (Figure 1B).

Studies that have assessed healing in aged persons have been criticized because analyses have not accounted for the higher occurrences of medication use and morbidity in elderly individuals. In this study, 4 sets of analyses that excluded the following individuals were performed: (1) those receiving any type of medication

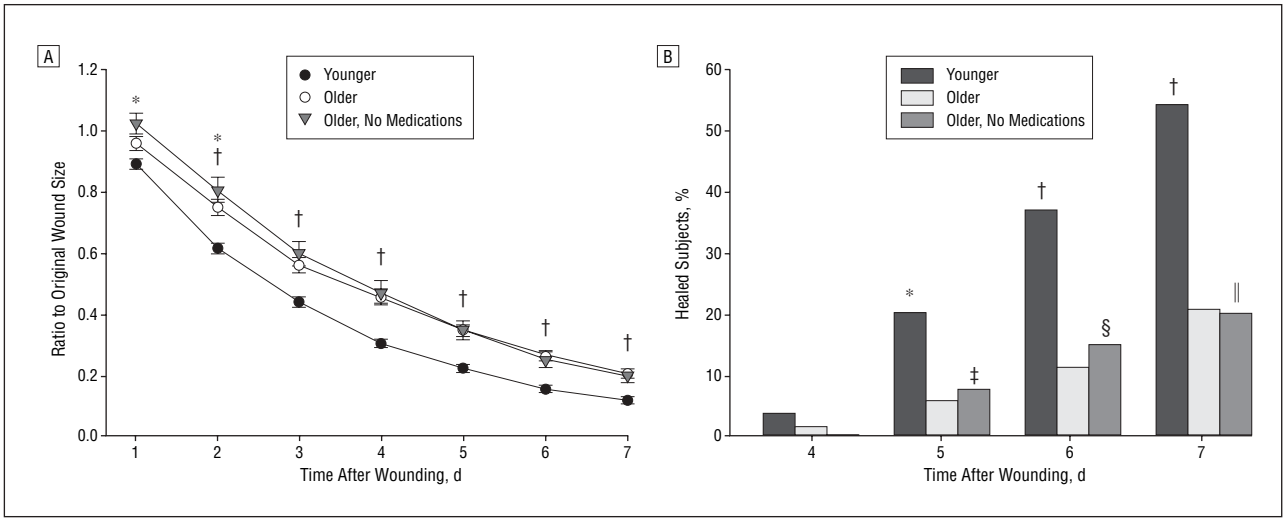

Figure 1. Age-related changes in wound healing parameters. Statistical significance was determined with 2-tailed *t* tests and χ^2 tests. A, Wound sizes for 7 days after wounding. Compared with younger individuals, older individuals had larger wounds on all days, even when excluding individuals receiving medication (younger group, n=104; older group, n=41). Error bars indicate SEM. *$P<.05$ (older group receiving medications [n=51] vs older group not receiving medications [n=41]). †$P<.001$ (younger group vs older group). B, Percentage of subjects considered healed (wounds <10% of original size) on days 4 through 7. Compared with the younger group, a lower proportion of the older group was considered healed on days 5 through 7, even when excluding individuals receiving medication. For the younger group vs the older group: *$P<.01$; †$P<.001$. For the younger group vs the older group not receiving medications: ‡$P<.05$; §$P<.01$; ‖$P<.001$.

(excluding allergy medication, birth control, or nutritional supplements [eg, vitamins]); (2) those who presently have or in the past had a serious medical condition (eg, diabetes, cancer, stroke, heart disease, hypertension, hypothyroidism, arthritis, irritable bowel disease, bacterial meningitis, psychopathological abnormalities); (3) those who reported to not be in good overall health; or (4) those who fit all of these criteria. After applying these exclusion criteria, older individuals still exhibited significantly larger wounds, and thus slower healing, than younger individuals for all of the analyses (see Figure 1 for results that excluded individuals receiving medication).

Interestingly, within the older group, medication produced a different pattern of wound healing ($F_{6,540}=4.40$; $P<.001$). Individuals receiving medication (as defined earlier) had significantly smaller wounds on days 1 and 2 ($P\leq.02$) than those not receiving medication (Figure 1A). Wound sizes on subsequent days were similar. Subjects receiving medication had no differences in oral hygiene or in general health behaviors (exercise, alcohol or nicotine use) from subjects not receiving medication (data not shown). Common classes of medication included α- and β-blockers, diuretics, vasodilators, calcium blockers, and others. No individual classes of medication were found to significantly alter wound closure on their own.

SEX EFFECTS

Men exhibited a different pattern of wound healing than women ($F_{1,208}=7.13$; $P=.008$). Although all of the wounds were initially the same size, at 24 hours after wounding, men had significantly smaller wounds; this difference was apparent until day 5 (**Figure 2**A). In addition, the proportion of individuals considered healed was significantly higher for men than for women on days 5 and 6 (Figure 2B).

AGE AND SEX

The effects of age occurred regardless of sex, as older subjects healed significantly more slowly than younger subjects for both women ($F_{1,125}=24.13$; $P<.001$) and men ($F_{1,83}=10.79$; $P=.001$). Wounds were significantly larger in older women compared with younger women on all 7 days ($P\leq.002$). Within men, a significant day × age interaction occurred ($P<.001$), as wound sizes were similar on day 1 but significantly larger in older men than in younger men on days 2 through 7 ($P\leq.02$) (data not shown). Compared with the older subjects, the proportion of individuals considered healed was significantly higher in younger women on days 5 through 7 ($P\leq.007$) and in younger men on days 6 and 7 ($P\leq.009$ each day) (data not shown).

The effects of sex occurred regardless of age, as there were significant day × sex interactions within both younger ($F_{6,696}=3.36$; $P=.003$) and older ($F_{6,540}=2.95$; $P=.008$) subjects. Women had significantly larger wounds (ie, slower healing) than men on days 3 through 5 in the younger group ($P\leq.02$) and on day 1 in the older group ($P=.002$) (data not shown). Also, within each age group, a higher proportion of men than women were considered healed. In younger subjects, this effect approached significance on day 5 ($P=.08$) and was significant on day 6 ($P=.02$); in older subjects, this effect approached significance on days 5 and 6 ($P=.05$ and .09, respectively) (data not shown).

The magnitudes of these age and sex effects on wound healing were substantial. For example, observing values obtained 5 days after wounding, wounds were 56% larger in older subjects as compared with younger subjects, and younger individuals were 3.7 times more likely to be considered healed than older individuals. Similarly, the wounds of women were 27% larger than those of men, and men were 2.5 times more likely than women to be

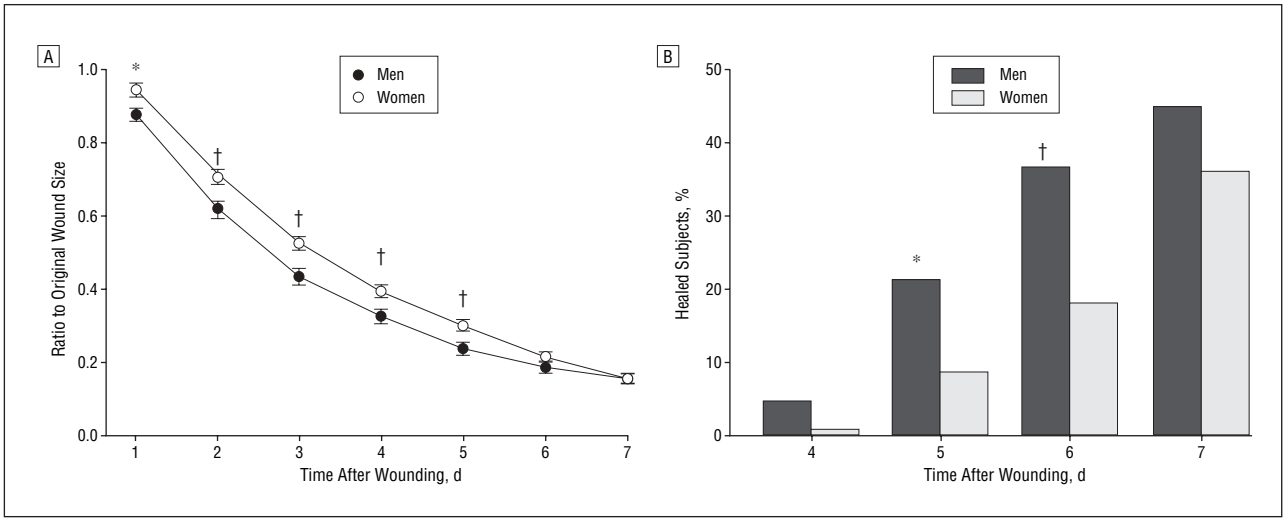

Figure 2. Sex-related changes in wound healing parameters. Statistical significance was determined with 2-tailed *t* tests and χ^2 tests. A, Wound sizes for 7 days after wounding. Men had smaller wounds than women on days 1 through 5. Error bars indicate SEM. B, Percentage of subjects considered healed (wounds <10% of original size) on days 4 through 7. Compared with men, a lower proportion of women were considered healed on days 5 and 6. *$P<.05$ (men vs women). †$P<.01$ (men vs women).

considered healed. Older women were the slowest to heal, and their wounds as compared with those of younger men were 95% larger by 5 days after wounding. Thus, older women appear to be at the greatest risk for delayed wound closure.

COMMENT

A chief strength of this study was that all of the wounds were created under the same experimental conditions and were standardized for size, depth, site, and time of placement. The results were also independent of common demographic factors (eg, ethnicity), allowing for a more clear determination of the roles of age and sex in wound healing.

In this study, older adults (aged ≥50 years) healed oral mucosal wounds significantly more slowly than younger adults (aged 18-35 years). This remained true even when individuals who were receiving medication and/or had a coexisting medical condition were removed from the analyses. A common criticism of past studies is that the inclusion of such individuals may exaggerate or even account for age-associated healing impairments.[6-9] Surprisingly, the exclusion of individuals receiving medication strengthened our findings (Figure 1), and within the older group, those receiving medication had an improved pattern of healing. This suggests that age-associated delays in wound healing are not generally exaggerated by medication use. Furthermore, the deleterious effects of age on healing may be stronger than previously suspected.

Much of the current literature[14-16] on wound healing stems from dermal wounds, and a clear sex difference in favor of women is evident. However, the current study indicates that men heal oral mucosal wounds more quickly than women. This male advantage in mucosal healing is a robust effect, and we have recently replicated this finding in another clinical study using young adults (n = 193; mean ± SD age, 20.14 ± 0.15 years; age range, 18-31 years). These results suggest that there are fundamental differences between the healing of mucosal vs dermal wounds. Compared with skin, mucosal epithelial turnover is more rapid and its tissue is more vascularized. As a result, it takes less time to recruit inflammatory cells to a mucosal wound site. Furthermore, immunomodulating compounds such as growth factors are more readily supplied to the site via mucosal secretions (ie, saliva), and lower levels of neutrophils, macrophages, and their associated cytokines have been reported in mucosal wounds as compared with dermal wounds.[23] These differences all likely contribute to the divergent healing rates seen in these tissues and may help to explain why the sexual dimorphism in these rates varies between tissue types.

Sex hormones likely modulate oral mucosal wound healing, as they have been shown to play a role in both dermal wound healing[2,14,15] and periodontal disease.[24,25] We hypothesize that sex hormones modulate both oral mucosal and dermal wound healing but do so differentially, perhaps driving healing in opposite directions. Lower inflammatory responses have been associated with faster wound healing[26,27] and inflammation appears to be substantially reduced in mucosal wounds as compared with dermal wounds,[23,28] possibly explaining why mucosal wounds heal more rapidly.[23,28] Given that testosterone has potent anti-inflammatory qualities[29,30] and is abundant in saliva and other mucosal fluids,[31] testosterone is a putative mechanism for explaining the faster healing of mucosal wounds observed in men in this study.

CONCLUSIONS

Our results indicate that women heal oral mucosal wounds more slowly than men, which is the opposite of the sex effect reported in dermal wound healing. Often, dermal and mucosal wounds are equated and direct comparisons are commonly made between their underlying healing processes. However, our findings suggest that heal-

ing in these tissues is differentially modulated and that direct comparisons between dermal and mucosal tissues may be inappropriate. These findings also indicate, to our knowledge for the first time, that older adults heal standardized wounds more slowly than younger adults, even when factors such as medication use and morbidity are removed from the analyses. Thus, both age and sex appear to be influential factors in mucosal wound healing, and older women may be at the highest risk for delayed healing following oral or mucosal surgery or injury.

With an increasing number of surgical procedures being performed owing to the rising age of the population and for aesthetic purposes, a greater emphasis needs to be placed on expediting the healing process. Determining the mechanisms that underlie these age and sex differences will help target treatment strategies to reduce postsurgical recovery times. This, in turn, will decrease the risk of infection and improve ultimate healing outcomes.

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