Psychological Stress Impairs Early Wound Repair Following Surgery

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Objective: Laboratory studies have demonstrated that psychological stress is associated with slower healing of small superficial wounds. The application of this finding to the clinical environment has not yet been undertaken. In order to do this, we investigated the relationship between psychological stress and wound repair in patients following routine surgery. **Methods:** Forty-seven adults with an inguinal hernia were given a standardized questionnaire assessing psychological stress and worry about the operation before undergoing open incision repair. Wound fluid was collected from 36 participants over the first 20-hour postoperative period. Wound healing was assessed by levels of interleukin-1, interleukin-6, and matrix metalloproteinase-9 in the fluid. Other outcome measures included patient self-reports of recovery, as well as cytokine response to lipopolysaccharide stimulation of peripheral blood. **Results:** Greater preoperative perceived stress significantly predicted lower levels of interleukin-1 in the wound fluid ($\beta = -0.44$, $p = 0.03$). Greater worry about the operation predicted lower levels of matrix metalloproteinase-9 in the wound fluid ($\beta = -0.38$, $p = 0.03$) as well as a more painful ($\beta = 0.51$, $p = 0.002$), poorer ($\beta = -0.36$, $p = 0.04$), and slower recovery ($\beta = 0.43$, $p = 0.01$). **Conclusions:** Psychological stress impairs the inflammatory response and matrix degradation processes in the wound immediately following surgery. This finding generalizes previous laboratory research to surgical patients and expands the known influence of stress to connective tissue matrix remodelling processes. These results suggest that in clinical practice, interventions to reduce the patient's psychological stress level may improve wound repair and recovery following surgery. **Key words:** stress, surgery, wound healing, interleukin-1, smoking, metalloproteinases.

ELISA = enzyme linked immunosorbent assay; **IL-1** = interleukin-1; **IL-6** = interleukin-6; **LPS** = lipopolysaccharide; **MMP-9** = matrix metalloproteinase-9.

INTRODUCTION

Previous laboratory work with both animal and human subjects has shown that stress decreases the speed of wound healing. Stressed mice produce higher levels of glucocorticoids and display less inflammation in the first 3 days after wounding, impaired bacterial clearance, and slower healing than their nonstressed counterparts (1, 2). Laboratory studies have also shown that psychological stress impairs the healing of small puncture wounds in humans. Women caring for relatives with Alzheimer's disease reported greater stress and demonstrated 24% slower healing of dermal punch biopsy wounds than did matched control subjects (3). Similarly, punch biopsy wounds in the hard palates of students healed on average 40% more slowly during an examination period than in the same students during vacation time (4). Both of these studies found that higher stress was associated with a poorer IL-1 response to LPS-stimulated peripheral blood. This is consistent with the hypothesis that stress impairs wound healing via immune processes.

Wound repair involves a number of progressing stages. In the initial stages the pro-inflammatory cytokines, including IL-1 and IL-6, attract phagocytes to the wound which remove infectious agents and prepare the site for the growth of new tissue (5). The cytokines also regulate the production and activation of matrix metalloproteinase enzymes, which are involved in the degradation of collagen. MMP-9 facilitates cellular invasion and migration in the wound by degrading basement membranes (6). This is necessary to allow the recruitment of cells involved in tissue regeneration. Measuring cytokine and metalloproteinase concentrations in the wound provides an effective way to monitor repair processes. Previous research has found that women who report higher psychological stress have lower levels of IL-1 and IL-8 in laboratory-induced blister wounds than do other women (7), suggesting that stress impairs the inflammatory stage of wound repair.

Wound healing is a critical outcome in surgery. Poor healing can result in wound infections or complications, as well as prolong hospital stays, increase patient discomfort, and delay return to activity. While previous research has established the deleterious effects of stress in laboratory settings, there has previously been no work examining the effect of stress on wound healing in clinical settings. The aim of this study was to investigate the effects of stress on wound repair in patients following surgery. Based on previous research, we hypothesized that higher levels of stress would be associated with reduced inflammatory and matrix degradation processes in the early stages of repair.

METHODS

Participants

Participants were inguinal hernia patients booked for elective open surgery at North Shore Hospital, Auckland, New Zealand. Excluded from the study were those whose hernia was bilateral or recurrent because larger operations and those performed on previous operation sites could adversely affect healing. Also excluded were those who were on any form of medication that affected their immune status and patients who had any illnesses known to influence immune function or impair cognitive ability. Consecutive sampling of patients older than 16 years referred to the preoperative clinic was used. Fifty-five patients who met the inclusion and exclusion criteria were invited to participate in the study. Four patients declined to take part in the research (93% participation rate). Refusers did not differ from participants on any demographic variables. Four patients who were enrolled in the study had their operation postponed beyond the completion date of the study. The participants receiving surgery comprised 41 males and 6 females, aged between 16 and 86 years (mean = 63.36 years, SD = 16.42 years). They were predominantly European and two were from other racial groups. Twelve of the participants were smokers.

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Of the 47 patients who received surgery, wound fluid data were not available from eight participants because wound drains were inadvertently not inserted by the surgeon. Three patients' wound fluids were excluded from the analysis: two due to blockage in wound drains and one due to a myocardial infarction while recovering in hospital, which has been shown to affect cytokine levels (8). These patients did not differ from other participants on any variables. The final sample size used in wound fluid analyses was therefore 36. Forty-two participants attended their scheduled follow-up visit and returned their postoperative questionnaires (89%).

The study was granted ethics approval by the Ministry of Health Ethics Committee. Recruitment began in mid-April and continued until mid-October 2001.

Procedures

Patients with inguinal hernia were admitted through the surgical unit by a standard protocol of preadmission and anesthetic assessment. At the preadmission clinic 1 week before surgery, participants completed a preoperative questionnaire and gave a blood sample. The surgical procedure followed a standard protocol in which the groin was exposed through a skin crease incision measuring approximately 6 cm. The external oblique muscle was divided, and the ilioinguinal and iliohypogastric nerves were preserved. The hernial sac was excised and a nontension mesh repair using prolene mesh was carried out. The mesh was sutured to the conjoint tendon and the deep aspect of the inguinal ligament with prolene. The muscles were repaired with nonabsorbable suture and the skin closed with subcuticular absorbable suture. Before closure, a manovac drain (Medinorm, French gauge 6, 40 ml) was inserted above the outer muscle layer to drain the wound. No significant local or general complications occurred in study participants. Pain was controlled by a standard range of narcotic and nonnarcotic analgesics. In the first 6 hours intravenous morphine by patient-controlled pump (dosage range 1–3 mg/ml) was used. Thereafter until discharge, nonsteroidal anti-inflammatory agents and paracetamol were used. Thirty-seven of the operations were performed under general anesthetic and 10 were performed using spinal anesthetic.

Patients stayed overnight in the ward where the drains were removed 20 hours following surgery. Patients attended an outpatient clinic 1 week after surgery. At this clinic, the research surgeon took a blood sample and gave the participants a postoperative questionnaire to return by mail.

Preoperative Questionnaire

The 10-item Perceived Stress Scale (9) was used to assess the degree to which participants found their daily lives over the past 4 weeks to be unpredictable, uncontrollable, and overloading. Subjects rated their responses from "never" (0) to "very often" (4). Cronbach's alpha for the scale was 0.87 (mean = 13.14, SD = 6.66).

Respondents rated how worried they were currently feeling about their operation on a 100-mm visual analogue scale ranging from "not at all worried" (0) to "extremely worried" (100), (mean = 28.45, SD = 28.24).

The Mental Health Index (10) was used as an indicator of negative affect. Five items asked how frequently participants experienced depression, anxiety, behavioral/emotional control, and positive mood states over the past month on a six-point Likert scale from "none of the time" to "all of the time." Higher scores indicate lower levels of negative affect. Cronbach's alpha for the scale was 0.86 (mean = 74.65, SD = 21.29).

Data were also collected on whether participants smoked, their alcohol consumption in the past 3 months, the amount of strenuous exercise they did in an average week, and the amount of sleep they obtained in the past week because these variables all have the potential to influence immune parameters (11). Age, gender, and ethnicity were recorded from medical records.

Postoperative Questionnaire

Outcome variables included two 100-mm visual analogue scales for pain experienced since the operation (ranging from "no pain" to "extreme pain") and self-assessed surgical recovery (ranging from "poor" to "excellent"). Patients were also asked to estimate how many days it would take them from that day forward to feel like they were back to normal.

Peripheral Blood Analysis

Blood samples were collected in 4-ml heparinized tubes. The blood was divided into ten 250-μl aliquots and each was added to a 20-μl aliquot of 13.5 mg/l LPS in a 96-well microtiter tray (Nunclon brand, cat. no. 163320, batch no. 057743) and incubated for 24 hours at 37°C (3% CO_2 and 96% humidity). Culture supernatants were collected, pooled, and frozen at −20°C until the end of data collection when they were analyzed for their IL-1β and IL-6 content using a standard sandwich ELISA technique using monoclonal capture antibodies and polyclonal detection antibodies (R&D Systems, Minneapolis, MN).

Wound Fluid Analysis

Wound drain fluid was transferred to 10-ml plastic tubes, centrifuged at 2000 rpm for 5 minutes, and the plasma recovered and stored frozen at −20°C. When all the samples had been collected, they were assayed for MMP-9 using an ELISA system (Amersham Pharmacia Biotech UK Ltd, code RPN 2614, batch no. 185867) and for their IL-1β and IL-6 content using a standard sandwich ELISA technique.

Statistical Analysis

The data were analyzed using SPSS version 10 software. Means and reliabilities of scales were calculated and data checked for normal distributions. The relationships between variables were investigated using Pearson correlation coefficients, and scatter plots were run to check for linearity of relationships. Hierarchical multiple linear regression analyses were run to assess how stress and worry affected wound repair and self-rated surgical outcomes, after first controlling for possible confounding factors.

Within the distributions of the cytokine data, five cases were identified as outliers. Each outlier was on a different cytokine variable, and each was a different participant. Thus cytokine data were log-transformed to create normal distributions (12).

RESULTS

Hierarchical multiple regression analyses were conducted to predict the immune markers of wound repair from perceived stress and worry about surgery after controlling for age, gender, exercise, alcohol intake, sleep, smoking, and type of anesthetic (Tables 1 and 2). These control variables did not significantly predict any variance in IL-1 concentration in the wound fluid. Perceived stress significantly accounted for 17% of the variance in IL-1 in the wound fluid over and above the control variables. Higher stress predicted lower IL-1 in the wound fluid as shown in Figure 1.

The concentration of MMP-9 in the wound fluid increased in a linear relationship with IL-6 ($r = 0.59$, $p < 0.01$), indicating that matrix remodelling was linked to inflammatory processes. The hierarchical multiple regression analysis showed that after entering the control variables and perceived stress, worry about surgery significantly predicted 12% of the variance in MMP-9 concentration over and above the previous steps. As shown in Table 2, nonsmoking patients and those with greater worry about surgery had a significantly lower concentration of MMP-9 in their wound fluid.

A hierarchical regression analysis to predict IL-6 in the wound fluid from control variables, perceived stress, and worry about surgery was not significant (all p values >0.05). Similar regression analyses conducted to predict the concentrations of IL-1 and IL-6 in LPS-stimulated blood samples were also not significant (all p values >0.05).

Three hierarchical multiple regression analyses were run to

TABLE 1. Multiple linear regression analysis for the prediction of log IL-1 in the wound fluid From perceived stress and worry controlling for other factors

Step	Predictor	R^2	R^2 Change	*F* Change	Significant *F* Change	β	Significance
1	Gender[a]					−.24	.18
	Age					.16	.34
	Smoking[b]					.10	.57
	Exercise					.10	.58
	Sleep					.26	.13
	Alcohol					−.10	.59
	Anesthetic type[c]					.17	.38
		.17	.17	.79	.60		
2	Perceived stress	.34	.17	6.47	.02	−.44	.03
3	Worry	.36	.02	.77	.39	−.15	.39

[a] Male = 1; female = 2.
[b] Nonsmoker = 1; smoker = 2.
[c] General = 1; spinal = 2.

TABLE 2. Multiple linear regression analysis for the prediction of MMP-9 in the wound fluid from perceived stress and worry controlling for other factors

Step	Predictor	R^2	R^2 Change	*F* Change	Significant *F* Change	β	Significance
1	Gender[a]					−.17	.33
	Age					.05	.76
	Smoking[b]					.44	.02
	Exercise					−.16	.36
	Sleep					.13	.42
	Alcohol					−.08	.65
	Anesthetic type[c]					−.12	.55
		.26	.26	1.34	.27		
2	Perceived stress	.26	.00	.10	.76	.02	.91
3	Worry	.38	.12	4.99	.03	−.38	.03

[a] Male = 1; female = 2.
[b] Nonsmoker = 1; smoker = 2.
[c] General = 1; spinal = 2.

predict self-rated postsurgical pain, recovery, and time to return to normal from perceived stress and worry about surgery, after controlling for age, gender, type of anesthetic, and negative affect. The regression analyses showed that none of the control variables nor perceived stress were significantly related to self-report outcomes. However, worry about surgery significantly predicted 23% of the variance in postsurgical pain [R^2 change = 0.23, *F* change (1,33) = 11.97, $p = 0.002$], 12% of the variance in quality of recovery [R^2 change = 0.12, *F* change (1,33) = 4.60, $p = 0.04$], and 16% of the variance in recovery time [R^2 change = 0.16, *F* change (1,33) = 7.00, $p = 0.01$], over and above the control variables. Greater worry predicted greater pain ($\beta = 0.51$, $p = 0.002$), poorer self-rated recovery ($\beta = -0.36$, $p = 0.04$), and longer recovery time ($\beta = 0.43$, $p = 0.01$). The correlations between the physiological outcome variables and the self-reported outcome variables were not statistically significant.

DISCUSSION

This study found that higher reported psychological stress before surgery predicted lower cellular wound repair processes in the early postoperative period. Patients who reported greater perceived stress for the month before surgery had lower levels of IL-1 in their surgical wounds. Furthermore, patients reporting greater worry about their upcoming surgery had lower levels of MMP-9 in the wound site. These findings are consistent with earlier laboratory research that has shown stress to impair inflammatory processes in the wound (7) and extend the influence of stress to the matrix degradation stage of wound repair.

Consistent with earlier research (6), this study found a positive correlation between MMP-9 and IL-6 in the wound fluid. This link with the inflammatory response provides theoretical support for the association between worry about the operation and lower MMP-9 levels. Stress-induced glucocorticoid secretion may nonspecifically impair the inflammatory response, which in turn may impair MMP-9 production, although this argument is weakened by the lack of relationship between IL-1 and IL-6, and between IL-1 and MMP-9. It is also possible that glucocorticoids may directly influence the production of MMP-9, although there is currently little literature on this proposed relationship. Some evidence suggests

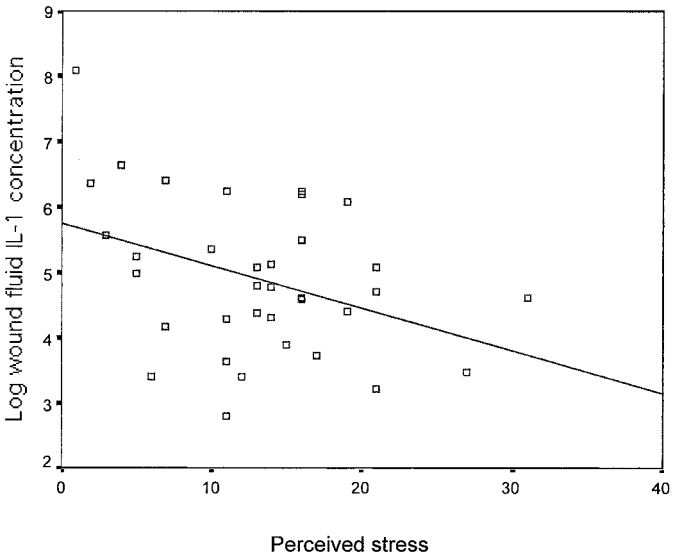

Fig. 1. Scatter plot of perceived stress and log IL-1 concentration in the wound fluid.

that norepinephrine and cortisol can modulate levels of another metalloproteinase involved in wound healing, MMP-2 (13). There may be different effects on immune function from acute worry (about the operation) and more longer-term stress.

The finding that worry was associated with lower levels of MMP-9 is further evidence for the ability of psychological processes to impair wound healing because MMP-9 is an enzyme critical to tissue reconstruction. However, the implications of this finding may extend to tissue remodelling in other processes not associated with wound healing, such as airway remodelling (14). The impact of worry on MMP-9 may therefore affect medical conditions outside of surgical wound healing.

The finding that MMP-9 concentration was higher among smokers is also consistent with earlier work. Airway macrophages in smokers have been found to produce more MMP-9 at baseline and in response to IL-1β and LPS than those of nonsmokers (14). Smoking may be associated with higher baseline levels of MMP-9 in a number of body tissues and therefore prolonged high levels of MMP-9 in the wound. Prolonged elevation of MMP-9 levels has been associated with chronic nonhealing of wounds (15, 16). Slower wound repair has been well recognized in smokers on a clinical level for many years, and studies have found that smokers have an increased incidence of skin sloughing after face-lift surgery, a higher rate of skin-flap and breast surgery complications, and worse scarring after surgery than nonsmokers (17). The mechanisms remain largely unknown, but nicotine is known to increase catecholamine release, which causes vasoconstriction and reduces oxygen flow to the wound, and to reduce fibroblast and macrophage production. Recent work in this area has found smokers to have significantly higher levels of MMP-8 and lower collagen synthesis in blister wounds than nonsmokers (18).

It is important to recognize that levels of cytokines from LPS-stimulated blood cultures may not necessarily reflect what is happening at a local wound site. This is borne out by the stronger relationship of stress to wound fluid markers than with blood-derived measures. However, the small sample size may have limited the ability of the study to detect significant effects in blood-derived measures. In previous work, higher preoperative stress has been associated with a lower lymphocyte response and lower lymphocyte counts in the blood following surgery (19).

In addition to lower wound remodelling processes, presurgical worry was also associated with greater postoperative pain, poorer self-rating of recovery, and a longer recovery time. The finding that worry about the procedure predicted these self-reported recovery outcomes while general stress and negative affect did not do so suggests that it is not stress per se that causes people to experience greater pain and distress after surgery but more specifically it is concern about the operation. This may be related to previous work that suggests a mediating role for self-focused attention in symptom reporting (20). Patients who are more worried may pay more attention to their wounds and notice signs of pain and discomfort more than their less worried counterparts.

A limitation of this study is that participants' use of pain medication was not recorded and controlled for in the analyses. It is possible that the more stressed individuals used more pain medication, which could have influenced immune responses. However, we consider this unlikely as reported postsurgical pain was not significantly related to either perceived stress or to any of the immune variables.

This study extends earlier laboratory wound healing research to an everyday clinical surgical setting. By examining IL-1, IL-6, and MMP-9 (an enzyme not previously studied in stress research), the data provide further support for the influence of stress on wound healing at the cellular wound repair level. This current study suggests that interventions designed to reduce presurgical stress, which have previously been found to shorten length of hospital stay, and to reduce postoperative complications, pain, and distress (21), may also improve wound repair.

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