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Stress-related modulation of matrix metalloproteinase expression

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Abstract

Matrix metalloproteinases (MMPs) and the tissue inhibitors of metalloproteinases (TIMPs), whose expression can be controlled by cytokines, play a role in extracellular matrix remodeling in physiological and pathological processes. Using a blister chamber wound model on UV-B-exposed human forearm skin, we examined whether stress or mood-associated neuroendocrine alteration is sufficient to modulate MMP and TIMP expression. We did not find evidence that depressive symptoms were reliably associated with modulation of either MMP or TIMP expression. However, we did find that activation of the hypothalamic–pituitary–adrenal (HPA) and sympathetic–adrenal medullary (SAM) axes can modulate levels of MMPs. A positive association between plasma norepinephrine levels and MMP-2 protein levels, and a negative correlation between plasma cortisol levels and MMP-2 levels were found. The data suggest that activation of the HPA and SAM axes, even in individuals within the normal range of depressive symptoms, could mediate MMP levels and wound healing in blister wounds.

Keywords: Stress; Depressive symptoms; Matrix metalloproteinases; Neuroendocirine hormones; Blister chamber wound model; UV-B irradiation

1. Introduction

Matrix metalloproteinases (MMPs) belong to a family of more than 20 structurally related zinc-dependent endopeptidases that, together, are capable of degrading most extracellular matrix molecules (Nagase and Woessner, 1999; Henney et al., 2000). Work in the past four decades have implicated these enzymes in the degradation of the extracellular matrix in several physiological processes (e.g. wound healing and regeneration, embryonic development, bone remodeling, and angiogenesis) and pathological processes (e.g., photoaging, arthritis, cardiovascular diseases, tumor progression and metastasis, periodontal disease, dermal ulcers, and fibrotic diseases) (Nagase and Woessner, 1999; Berneburg et al., 2000; Yang et al., 1999; Cockett et al., 1998; Galboiz et al., 2001; Henney et al., 2000; Khasigov et al., 2001; McCawley and Matrisian, 2001; Stetler-Stevenson and Yu, 2001; Ravanti and Kahari, 2000). MMP activity has been shown to be regulated at the level of transcription and translation, and through the activation of the inactive zymogen by proteolytic cleavage. Furthermore, their activities can be inhibited by their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs). Progression of the physiological processes listed above is achieved by the balance between levels of MMPs and TIMPs, thus maintaining the balance between synthesis and degradation of extracellular matrix components. On the other hand, disruption of this balance can result in pathological conditions.

Previous work from our laboratory and others have shown that dysregulation of cytokine production can result from stress-induced activation of the hypothalamic–pituitary–adrenal (HPA) and sympathetic–adrenal medullary (SAM) axes (Glaser and Kiecolt-Glaser, 1998; Maes et al.,

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1998; Marshall et al., 1998). This cytokine dysregulation can have negative effects on health of the individual, including susceptibility to viral infections and delayed wound healing (Cohen et al., 1999; Kiecolt-Glaser et al., 1995; Cohen and Rabin, 1998; Bonneau et al., 1998; Cruess et al., 2000; Leserman et al., 1999; Vedhara et al., 1999).

Recently, our laboratory has shown that psychological distress associated with such stressors as taking academic examinations or caregiving for a family member with dementia can result in delays in wound healing ranging from about 24% to 40% (Kiecolt-Glaser et al., 1995; Marucha et al., 1998). For example, women who provided care for a spouse or parent with Alzheimer's disease took 9 days longer to completely heal a 3.5-mm punch biopsy than well-matched noncaregivers (Kiecolt-Glaser et al., 1995). Also, compared to controls, caregivers' peripheral blood leukocytes (PBLs) exhibited a decreased ability to express the IL-1β gene in response to lipopolysaccharide stimulation in vitro; IL-1β is a proinflammatory cytokine that plays an important role in the early stages of wound healing. Similar results were obtained in a follow-up study showing an effect of academic stress on the rate of healing of a mucosal wound (Marucha et al., 1998). Additionally, results obtained using a mouse model for stress and wound healing suggest a possible link between delayed wound healing and immune/cytokine dysregulation (Padgett et al., 1998).

In further work, a blister chamber wound model was employed to elucidate the mechanism of stress-associated delay in wound healing. This model system allowed us to study the effect of stress on the production of two important proinflammatory cytokines, IL-1α and IL-8, directly at the wound site (Glaser et al., 1999; Kuhns et al., 1992). Women who reported more stress produced significantly lower levels of IL-1α and IL-8 at these wound sites. In addition, subjects who had lower levels of both cytokines 24 h after wound creation had higher levels of salivary cortisol than those subjects who had higher cytokine levels (Glaser et al., 1999). Together, these data suggest that a possible mechanism by which psychological stress affects the local wound environment is through the dysregulation of proinflammatory cytokine production.

As several cytokines, including IL-1 and IL-8, have been implicated in the transcriptional control of MMP and TIMP expression (Alexander et al., 1998; Burger et al., 1998; Azuma et al., 1997; Bond et al., 1998; Schonherr and Hausser, 2000), we hypothesized that the stress-related delay in wound healing observed in these studies is mediated (at least in part) by stress-associated modulation of MMP and TIMP levels. We also hypothesized that stress-induced activities of the HPA and SAM would mediate MMP and TIMP levels. In addition, since UV irradiation has been shown to upregulate MMP expression (Fisher et al., 2001; Fisher et al., 1996; Fisher et al., 1997), we also explored whether psychological distress could affect the production of MMPs and TIMPs in blister wounds created in skin after UV-B irradiation, and that activation of the HPA and SAM axes can cause changes in the expression of MMPs in UV-irradiated human skin.

2. Materials and methods

2.1. Subjects

The mean age of the 51 subjects (31 females, 20 males) was 41.72 (S.E.M. = 2.42) years (range 20–74). The subjects had an average of 15.4 (S.E.M. = 0.36) years of education. Participants were recruited from newspaper advertisements, notices posted in the community, local newsletters, and referrals from other participants. Healthy subjects were selected and classified based on skin type in order to determine the amount of exposure to the UV-B sunlamp required to generate erythema; preliminary screening during a phone interview determined eligibility. We focused on skin types II and III (Azizi et al., 1988). The Ohio State University Biochemical Research Review Committee approved the project; all subjects gave written informed consent prior to participation.

2.2. Psychological measures (Beck Depression Inventory)

The Beck Depression Inventory (BDI), a 21-item self-report measure, was employed as a measure of psychological distress. This inventory is sensitive to mild to moderate levels of depression. Scores correlate highly with clinicians' ratings of depression (Beck and Beck, 1972).

2.3. UV-B irradiation of skin and the induction of blister wounds

Subjects were selected based on skin type as defined by the ability to "always burn sometimes tan" (Skin Type II) and "sometimes burn and always tan" (Skin Type III) (Skov et al., 1998). The time required to produce a detectable reddening of the skin was defined as one minimum erythema dose (MED). The time to produce one MED was determined for each subject prior to wounding. A UV-B lamp Model SEL-002 (National Biological, Twinsburg, OH), which emitted a spectrum with wavelengths between 280 and 320 nm, was used as the source of UV-B irradiation. The volar surface of the nondominant forearm of each subject was exposed to 2 MED. Four 8-mm suction blisters were raised 6 h after UV-B irradiation; two blisters were raised in irradiated skin and two in nonirradiated skin (Glaser et al., 1999). Procedures for production of suction blisters have been discussed previously (Kuhns et al., 1992). Once the blister roofs were removed, a plastic template containing chambers was placed over the blister wounds. The chambers were filled with a mixture of 70% autologous serum in Hank's balanced salt solution. Eighteen hours later, fluid from the chambers were removed using a syringe and centrifuged to remove cells from the chamber fluid. The chamber fluids were stored at −80 °C

until used for analysis of TIMP-1, MMP-2, MMP-8, and MMP-9 protein levels.

2.4. TIMP-1, MMP-2, MMP-8 and MMP-9 ELISAs

The Biotrak assays for TIMP-1, MMP-2, MMP-8, and MMP-9, used to assay levels in chamber fluids, are based on a two-site enzyme-linked immunosorbent assay (ELISA) "sandwich" format (Amersham Pharmacia Biotech). The TIMP-1 assay recognizes both free TIMP-1 and TIMP-1 complexed to MMPs. The assay can detect TIMP-1 complexed to MMP-1, MMP-2, MMP-3, MMP-9, and pro-MMP-9. The MMP-2 assay recognizes pro-MMP-2 that is both free and complexed to TIMP-2. There is no cross-reactivity to MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, and membrane-type MMP (MT-MMP). The MMP-8 assay recognizes both pro- and active MMP-8. There is no cross-reactivity with MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-13, or MT1-MMP. The MMP-9 assay recognizes both free and complexed pro-MMP-9. There is no cross-reactivity to MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, and MT-MMP.

Chamber fluids from UV-B-irradiated and nonirradiated skin wounds were diluted with the appropriate amounts of sample dilution buffers provided with the kits. The amounts of MMPs and TIMP-1 in the chamber fluids were assayed as specified for plasma according to the manufacturer's instructions. All tests were performed in duplicate for each sample. The reaction was terminated by addition of 1 M sulphuric acid, and absorbance was measured at 450 nm using a Titertek Multiskan plate reader. The MMP and TIMP-1 concentrations in the samples were determined by interpolation from standard curves using standards provided with the kits.

2.5. Neuroendocrine measures

Assays for cortisol and norepinephrine were performed using plasma from blood collected in EDTA tubes prior to suction blister induction. Plasma cortisol levels were tested using a fluorescent polarization technique (TDX-Abbott Lab, Chicago, IL). This assay has intra- and inter-assay coefficients of variation of less than 10% (Malarkey et al., 1995). Plasma norepinephrine levels were determined by high performance liquid chromatography using a Waters system with an electrochemical detector. Alumina was used to extract the samples before they were placed on columns. The sensitivity of this system for norepinephrine is 20 pg/ml. The assay has intra- and inter-assay coefficients of variation of 7% for norepinephrine, and the internal standard to monitor percent recovery averaged over 80% (Kiecolt-Glaser et al., 1998).

2.6. Statistical analyses

Statistical analyses were conducted using the SPSS statistical package. General Linear Model (GLM) procedures were used to analyze UV-B irradiation, depressive symptoms, and neuroendocrine levels as predictors of levels of MMPs and TIMP-1 in the chamber fluid. UV-B irradiation (irradiated vs. nonirradiated) was included as a within-subjects factor, and continuous measures of depressive symptoms and neuroendocrine levels were included in analyses as covariates. Accuracy of parametric statistical tests requires that a distribution of scores meets the assumption of normality. Many types of physiological data are not distributed normally, violating the assumption of normality. Most of the MMPs and TIMP distributions were significantly skewed. We subjected the data to a $\log_{10}$ transformation before conducting statistical tests in order to reduce skewness and to avoid violating the assumption of normality. For example, the skewness statistic for the distribution of MMP-8 protein concentration values for non-exposed blisters was 2.56. After the transformation, skewness was reduced to 0.011. The figures in Section 3 are presented using transformed data to be consistent with the analyses. Figures based on transformed values show the same pattern of findings as those that depict the findings using raw values. Because not all participants had all neuroendocrine measures, sample sizes vary for analyses including neuroendocrine variables.

3. Results

3.1. Depression and the effect of UV-B irradiation on mmp production

MMPs and TIMP-1 values were subjected to a mixed design GLM procedure that included UV-B exposure (UV-B-irradiated vs. nonirradiated) and the Beck Depression Inventory (BDI) scores as predictors. The mean score on the BDI was 5.41 (S.E.M. = 0.65), with scores ranging from 0 to 21. Results show a significant effect of UV-B irradiation on MMP-2 protein levels when Beck scores were included in the model, $F(1,49) = 8.69$, $p < 0.01$, partial $\eta^2 = 0.15$. Although UV-B irradiation produced higher MMP-2 levels in the chamber fluid than nonirradiated skin, this effect was qualified by a significant UV-B irradiation by depressive symptoms interaction, $F(1,49) = 13.30$, $p < 0.001$, partial $\eta^2 = 0.21$. The effect of UV-B irradiation was influenced by depression. Specifically, results suggested that at the highest level of depression observed in the sample, UV-B irradiation was associated with lower MMP-2 protein levels (not shown). However, close inspection of the data indicated that this finding was primarily attributable to the presence of a single individual who exhibited the highest Beck score, as well as the lowest MMP-2 level in response to irradiation. When this individual's values were removed from the data set and the analysis repeated, the effect of UV-B irradiation remained significant, $F(1,48) = 4.82$, $p < 0.05$; however, the interaction did not produce a significant effect ($F < 1$) (not shown). Thus, given that the statistically significant inter-

action was found to be attributable to a single influential observation, the bulk of the data indicate no significant main effect or interaction involving the Beck scores.

3.2. Norepinephrine and MMP-2 protein levels

Log_{10}-transformed values of MMP protein were subjected to a mixed design GLM procedure with UV-B irradiation and norepinephrine values as predictors. There was a significant main effect of norepinephrine (mean = 354.25, S.E.M. = 25.34) on MMP-2 protein levels, $F(1,34) = 4.71$, $p < 0.05$, partial $\eta^2 = 0.12$. This relationship is shown in Fig. 1, with the regression line indicating a positive relationship wherein higher levels of norepinephrine were associated with higher MMP-2 protein concentrations. The values of MMP-2 protein concentrations in blister chamber fluids obtained from blisters in both UV-B-irradiated and nonirradiated skin were averaged and are presented in Fig. 1. One participant had an extremely low MMP-2 protein concentration level in UV-B-irradiated skin. Excluding this participant's data did not influence the statistical significance of the results.

3.3. Plasma cortisol and MMP-2 protein levels

Log_{10}-transformed MMP protein levels were also subjected to a mixed design GLM procedure, examining UV-B irradiation and plasma cortisol levels as predictors. There was a significant effect of plasma cortisol levels (mean = 15.19, S.E.M. = 1.15) on MMP-2 protein levels in the chamber fluids, $F(1,34) = 7.00$, $p < 0.05$, partial $\eta^2 = 0.17$. Higher levels of plasma cortisol were associated with lower levels of MMP-2 protein, as shown in Fig. 2. As in Fig. 1, MMP-2 values in Fig. 2 represent the mean value for each individual across the two categories of UV-B irradiation.

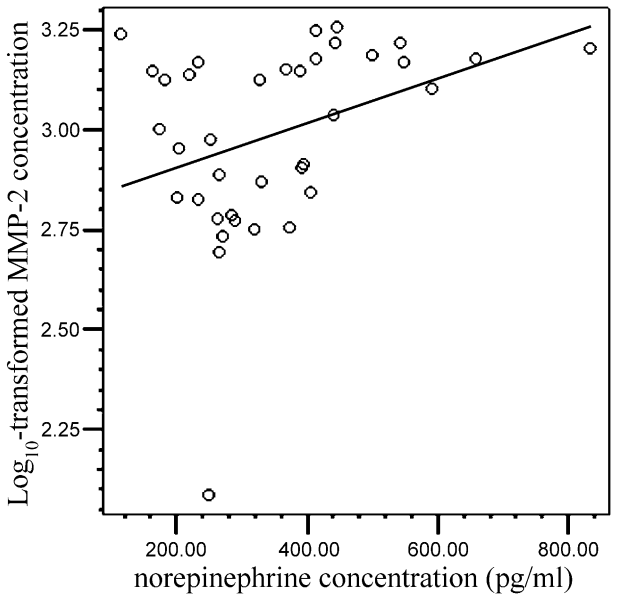

Fig. 1. The association of plasma norepinephrine (NEPI) levels and MMP-2 protein levels in chamber fluid 24 h post-irradiation ($n = 36$).

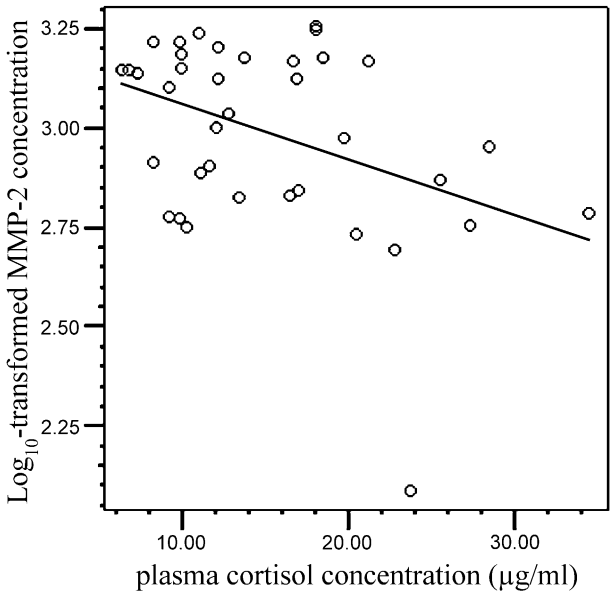

Fig. 2. The association of plasma cortisol levels and MMP-2 protein levels in chamber fluid 24 h post-irradiation ($n = 36$).

3.4. The effect of UV-B irradiation on MMP production

To examine the effect of UV-B irradiation alone on MMP production, MMPs and TIMP-1 values were subjected to a repeated measures GLM procedure that included UV-B exposure (UV-B-irradiated vs. nonirradiated) as a predictor. There was not a statistically significant effect of 2 MED doses of UV-B irradiation on levels of MMP-2, $F(1,50) < 1$, or MMP-9 protein levels, $F(1,50) = 2.03$, $p < 0.20$.

There was a marginally significant effect of UV-B exposure on MMP-8, $F(1,50) = 3.65$, $p < 0.10$, $\eta^2 = 0.07$, with the UV-B-irradiated site showing higher MMP-8 protein levels (mean = 3.01, S.E.M. = 0.06) than the nonirradiated site

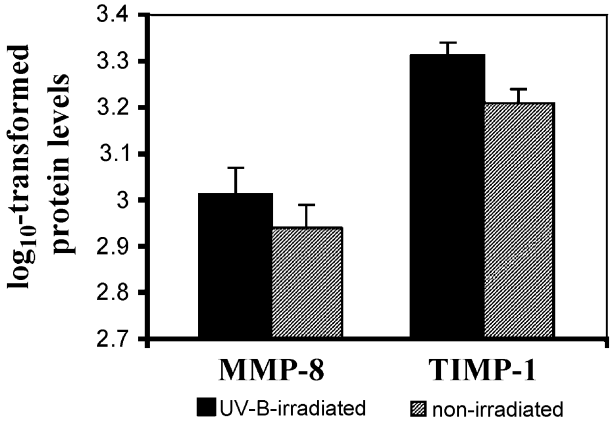

Fig. 3. Effect of 2 MED UV-B irradiation on protein levels of MMP-8 and TIMP-1 in chamber fluid 24 h post-irradiation ($n = 51$). MMP-8 and TIMP-1 levels were measured using ELISA as described.

Table 1
Descriptive statistics for MMP and TIMP concentrations

Measure	*n*	Mean	S.E.M.
MMP-8			
Nonirradiated	49	1307.29	182.24
UV-B irradiated	48	1452.97	181.24
TIMP-1			
Nonirradiated	49	1841.14	132.00
UV-B irradiated	48	2341.33	175.99

(mean = 2.94, S.E.M. = 0.05) (Fig. 3). UV-B-irradiated skin significantly differed from nonirradiated skin in chamber fluid levels of TIMP-1, $F(1,50) = 20.84$, $p < 0.001$, $\eta^2 = 0.29$. UV-B irradiation was associated with higher levels of TIMP-1 (mean = 3.31, S.E.M. = 0.03) than no UV-B exposure (mean = 3.21, S.E.M. = 0.03) (Fig. 3) (Table 1 shows corresponding results of raw values of MMP-8 and TIMP-1 and show that the pattern seen in Fig. 1 for log-transformed measures also hold for untransformed measures). The changes observed in the levels of MMP-8 and TIMP-1 in blister chamber fluid obtained from skin which were exposed to UV-B irradiation are consistent with changes observed in previous studies (Fisher et al., 2001; Fisher et al., 1996) and provide evidence that our model represent what occurs under these conditions.

4. Discussion

Our data suggest that activation of the HPA and SAM axes is associated with modulation of MMP-2 expression in blister. Analyses of our data suggest no significant relationship between the level of depressive symptoms and the level of MMP-2 protein in chamber fluids in response to UV-B irradiation. It is important to note that none of the subjects had high enough BDI scores to be categorized as clinically depressed. However, we have shown, in a previous study, that even in individuals who have normal ranges of stress, it is still possible to observe evidence for activation of the HPA and SAM axes. This relationship was evidenced in a down-regulation of IL-8 and IL-1α in blister wound fluids; both of these proinflammatory cytokines are known to be important for the wound-healing process (Glaser et al., 1999). It is, therefore, likely that these differences would be magnified in patients who are clinically depressed.

We did not find associations between depressive symptoms and MMP-8, MMP-9, and TIMP-1. It is certainly possible that plasma cortisol levels in the subjects with higher levels of depressive symptoms were not high enough to modulate the expression of the MMP-8, MMP-9, and TIMP-1 genes because these individuals were not clinically depressed. Little is known about the relationship of plasma cortisol levels and MMP-8, MMP-9, and TIMP-1 regulation. There are data consistent with our results that suggest that the expression of MMPs may not be integrated or linked (Galboiz et al., 2001). Further studies will need to be performed to clarify this issue.

Many of the studies on the effects of cytokines, glucocorticoids, and catecholamines on expression of MMPs have addressed pathological conditions or used in vitro methods. In this more naturalistic study, we addressed the question of whether changes in the HPA and SAM axes, as measured by norepinephrine and plasma cortisol levels, are related to changes in the expression of MMPs and TIMPs at a wound site. In order to explore possible neuroendocrine mechanisms, we measured plasma norepinephrine and cortisol levels. Higher norepinephrine levels were associated with higher levels of MMP-2. These data are consistent with the results from a study showing that higher levels of norepinephrine were associated with higher MMP-2 levels in patients with idiopathic dilated cardiomyopathy (Yokoseki et al., 2000). On average, we also found that subjects with higher plasma cortisol had lower MMP-2 protein levels. These results are in agreement with observations suggesting that glucocorticoids can down-regulate the expression of several MMPs (Bosse et al., 1999; Harkness et al., 2000; Oikarinen et al., 1998).

Even though our subjects did not show a relationship between BDI scores and MMP levels in this relatively nondistressed sample, the hypothesis that psychological stress can affect MMP activity is supported by our data showing HPA and SAM activation and MMP-2 levels. The hypothesis is also supported by recent studies in mice. Using social isolation as a stressor, the mRNA levels of MMP-2, MMP-9, matrix-type matrix metalloproteinase-1 (MT1-MMP), and urokinase-type plasminogen activator were higher in the tumor and liver tissues of the isolated mice than in control mice (Wu et al., 1999). Furthermore, a recent study has shown that restraint stressing mice causes an increase in expression of the plasminogen activator inhibitor-1, another key player in the plasminogen/plasmin enzyme system (Yamamoto et al., 2002). As these aforementioned proteins have roles in MMP activation, these data suggest that psychological modulators can influence, albeit indirectly, the activities of the various MMPs. Furthermore, as MMPs have recently been implicated in the degradation of non-matrix proteins (McCawley and Matrisian, 2001), their function is no longer viewed as being restricted to the release of cells from the extracellular matrix but now includes, among other things, activation of cytokines and growth factors or their receptors, thereby modulating cellular behavior. Therefore, stress-related modulation of MMP and TIMP balance may have broader consequences.

The Biotrak MMP-2 ELISA kit we utilized in this study detects the inactive pro-MMP-2 and not the active enzyme. MMP-2 is constitutively expressed in a wide variety of cell types and is, therefore, hypothesized to function as a "housekeeping" gene. However, although transcriptional control is lacking in MMP-2 expression, activation of the pro-MMP-2 is vital to the regulation of its activity in the different cell types in which it is found. Activation of pro-

MMP-2 has been shown to be achieved through its cleavage by other proteases, including MT1-MMP, kallikrein, and plasmin (Murphy et al., 1999). Our study cannot distinguish between a change in synthesis of the MMP-2 protein, activation of pro-MMP-2, or both. Molecular studies, including real-time RT-PCR, on gene expression of MMP-2 and other MMPs are underway in order to distinguish among these possibilities.

In conclusion, the current study suggests that activation of the HPA and SAM axes can modulate levels of MMPs. Since stressors activate both the axes, we hypothesize that such interactions could impact on the level of one or more MMPs. Our observations that plasma levels of cortisol and norepinephrine are associated with changes in levels of MMP-2 in UV-B-irradiated skin will contribute to the understanding of how stress and mood modulation can have negative effects on health. Despite the fact that we did not show a direct correlation between MMP-2 protein levels and the rate of wound healing, ample data exist implicating this enzyme in several developmental processes, including tumorigenesis and cutaneous wound healing (Ågren et al., 2001; Makela et al., 1999; Cockett et al., 1998; John and Tuszynski, 2001; Varani et al., 2000; Parks and Mecham, 1998; Berneburg et al., 2000). Although its role in these processes is not fully understood, recent data suggest that the specific cleavage of laminin-5 by MMP-2 exposes a cryptic site that promotes cell migration (Seftor et al., 2001). A balance between all the MMPs and TIMPs are critical for wound healing, studies on gene expression, proenzyme activation, and the precise contribution of the different cell types within the wound are necessary in order to completely understand the mechanism of stress-mediated effects on cutaneous wound healing and other developmental processes.

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References

Ågren, M.S., Mirastschijski, U., Karlsmark, T., Saarialho-Kere, U.K., 2001. Topical synthetic inhibitor of matrix metalloproteinases delays epidermal regeneration of human wounds. Exp. Dermatol. 10, 337–348.

Alexander, J., Samples, J., Acott, T., 1998. Growth factor and cytokine modulation of trabecular meshwork matrix metalloproteinase and TIMP expression. Curr. Eye Res. 17, 276–285.

Azizi, E., Lusky, A., Kushelevsky, A.P., Schewach-Millet, M., 1988. Skin type, hair color, and freckles are predictors of decreased minimal erythema ultraviolet radiation. J. Am. Acad. Dermatol. 19, 32–38.

Azuma, M., Motegi, K., Aota, K., Hayashi, Y., Sato, M., 1997. Role of cytokines in the destruction of acinar structure in Sjogren's syndrome salivary glands. Lab. Invest. 77, 269–280.

Beck, A.T., Beck, R.W., 1972. Screening depressed patients in family practice: a rapid technique. Postgrad. Med., 81–85.

Berneburg, M., Plettenberg, H., Krutmann, J., 2000. Photoaging of human skin. Photodermatol. Photoimmunol. Photomed. 16, 239–244.

Bond, M., Fabunmi, R., Baker, A., Newby, A., 1998. Synergistic upregulation of metalloproteinase-9 by growth factors and inflammatory cytokines: an absolute requirement for transcription factor NF-kappa B. FEBS Lett. 435, 29–34.

Bonneau, R., Zimmerman, K., Ikeda, S., Jones, B., 1998. Differential effects of stress-induced adrenal function on components of the herpes simplex virus-specific memory cytotoxic T-lymphocyte response. J. Neuroimmunol. 82, 191–199.

Bosse, M., Chakir, J., Rouabhia, M., Boulet, L., Audette, M., Laviolette, M., 1999. Serum matrix metalloproteinase-9: tissue inhibitor of metalloproteinase-1 ratio correlates with steroid responsiveness in moderate to severe asthma. Am. J. Respir. Crit. Care Med. 159, 596–602.

Burger, D., Rezzonico, R., Li, J., Modoux, C., Pierce, R., Welgus, H., Dayer, J., 1998. Imbalance between interstitial collagenase and tissue inhibitor of metalloproteinases 1 in synoviocytes and fibroblasts upon direct contact with stimulated T lymphocytes: involvement of membrane-associated cytokines. Arthritis Rheum. 41, 1748–1759.

Cockett, M., Murphy, G., Birch, M., O'Connell, J., Crabbe, T., Millican, A., Hart, I., Docherty, A., 1998. Matrix metalloproteinases and metastatic cancer. Biochem. Soc. Symp. 63, 295–313.

Cohen, S., Rabin, B., 1998. Psychologic stress, immunity, and cancer. J. Natl. Cancer Inst. 90, 3–4.

Cohen, S., Doyle, W., Skoner, D., 1999. Psychological stress, cytokine production, and severity of upper respiratory illness. Psychosom. Med., 175–180.

Cruess, S., Antoni, M., Cruess, D., Fletcher, M., Ironson, G., Kumar, M., Lutgendorf, S., Hayes, A., Klimas, N., Schneiderman, N., 2000. Reductions in herpes simplex virus type 2 antibody titers after cognitive behavioral stress management and relationships with neuroendocrine function, relaxation skills, and social support in HIV-positive men. Psychosom. Med., 828–837.

Fisher, G., Datta, S., Talwar, H., Wang, Z., Varani, J., Kang, S., Voorhees, J., 1996. Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 379, 335–339.

Fisher, G., Wang, Z., Datta, S., Varani, J., Kang, S., Voorhees, J., 1997. Pathophysiology of premature skin aging induced by ultraviolet light. N. Engl. J. Med. 337, 1419–1428.

Fisher, G., Choi, H., Bata-Csorgo, Z., Shao, Y., Datta, S., Wang, Z., Kang, S., Voorhees, J., 2001. Ultraviolet irradiation increases matrix metalloproteinase-8 protein in human skin in vivo. J. Invest. Dermatol. 117, 219–226.

Galboiz, Y., Shapiro, S., Lahat, N., Rawashdeh, H., Miller, A., 2001. Matrix metalloproteinases and their tissue inhibitors as markers of disease subtype and response to interferon-beta therapy in relapsing and secondary-progressive multiple sclerosis patients. Ann. Neurol. 50, 443–451.

Glaser, R., Kiecolt-Glaser, J., 1998. Stress-associated immune modulation: relevance to viral infections and chronic fatigue syndrome. Am. J. Med. 105, 35S–42S.

Glaser, R., Kiecolt-Glaser, J., Marucha, P., MacCallum, R., Laskowski, B., Malarkey, W., 1999. Stress-related changes in proinflammatory cytokine production in wounds. Arch. Gen. Psychiatry 56, 450–456.

Harkness, K., Adamson, P., Sussman, J., Davies-Jones, G., Greenwood, J., Woodroofe, M., 2000. Dexamethasone regulation of matrix metalloproteinase expression in CNS vascular endothelium. Brain 123, 698–709.

Henney, A., Ye, S., Zhang, B., Jormsjo, S., Whatling, C., Eriksson, P., Hamsten, A., 2000. Genetic diversity in the matrix metalloproteinase

family. Effects on function and disease progression. Ann. N.Y. Acad. Sci. 902, 27–37.

John, A., Tuszynski, G., 2001. The role of matrix metalloproteinases in tumor angiogenesis and tumor metastasis. Pathol. Oncol. Res. 7, 14–23.

Khasigov, P., Podobed, O., Ktzoeva, S., Gatagonova, T., Grachev, S., Shishkin, S., Berezov, T., 2001. Matrix metalloproteinases of normal human tissues. Biochemistry 66, 130–140.

Kiecolt-Glaser, J.K., Marucha, P.T., Malarkey, W.B., Mercado, A.M., Glaser, R., 1995. Slowing of wound healing by psychological stress. Lancet 346, 1194–1196.

Kiecolt-Glaser, J., Glaser, R., Cacioppo, J., Malarkey, W., 1998. Marital stress: immunologic, neuroendocrine, and autonomic correlates. Ann. N.Y. Acad. Sci. 840, 656–663.

Kuhns, D., DeCarlo, E., Hawk, D., Gallin, J., 1992. Dynamics of the cellular and humoral components of the inflammatory response elicited in skin blisters in humans. J. Clin. Invest. 89, 1734–1740.

Leserman, J., Jackson, E., Petitto, J., Golden, R., Silva, S., Perkins, D., Cai, J., Folds, J., Evans, D., 1999. Progression to AIDS: the effects of stress, depressive symptoms, and social support. Psychosom. Med., 397–406.

Maes, M., Song, C., Lin, A., De Jongh, R., Van Gastel, A., Kenis, G., Bosmans, E., De Meester, I., Benoy, I., Neels, H., Demedts, P., Janca, A., Scharpe, S., Smith, R., 1998. The effects of psychological stress on humans: increased production of pro-inflammatory cytokines and a Th1-like response in stress-induced anxiety. Cytokine 10, 313–318.

Makela, M., Larjava, H., Pirila, E., Maisi, P., Salo, T., Sorsa, T., Uitto, V., 1999. Matrix metalloproteinase 2 (gelatinase A) is related to migration of keratinocytes. Exp. Cell Res. 251, 67–78.

Malarkey, W.B., Pearl, D.K., Demers, L.M., Kiecolt-Glaser, J.K., Glaser, R., 1995. Influence of academic stress and season on 24-hour mean concentrations of ACTH, cortisol, and β-endorphin. Psychoneuroimmunology 20, 499–508.

Marshall, G.J., Agarwal, S., Lloyd, C., Cohen, L., Henninger, E., Morris, G., 1998. Cytokine dysregulation associated with exam stress in healthy medical students. Brain Behav. Immun. 12, 297–307.

Marucha, P., Kiecolt-Glaser, J., Favagehi, M., 1998. Mucosal wound healing is impaired by examination stress. Psychosom. Med., 362–365.

McCawley, L., Matrisian, L., 2001. Matrix metalloproteinases: they're not just for matrix anymore! Curr. Opin. Cell Biol. 13, 534–540.

Murphy, G., Stanton, H., Cowell, S., Butler, G., Knauper, V., Atkinson, S., Gavrilovic, J., 1999. Mechanisms for pro matrix metalloproteinase activation. APMIS 107, 38–44.

Nagase, H., Woessner, J.J., 1999. Matrix metalloproteinases. J. Biol. Chem. 274, 21491–21494.

Oikarinen, A., Haapasaari, K., Sutinen, M., Tasanen, K., 1998. The molecular basis of glucocorticoid-induced skin atrophy: topical glucocorticoid apparently decreases both collagen synthesis and the corresponding collagen mRNA level in human skin in vivo. Br. J. Dermatol. 139, 1106–1110.

Padgett, D.A., Marucha, P.T., Sheridan, J.F., 1998. Restraint stress slows cutaneous wound healing in mice. Brain Behav. Immun. 12, 64–73.

Parks, W.C., Mecham, R.P., 1998. Matrix Metalloproteinases. Academic Press, San Diego.

Ravanti, L., Kahari, V., 2000. Matrix metalloproteinases in wound repair (review). Int. J. Mol. Med. 6, 391–407.

Schonherr, E., Hausser, H., 2000. Extracellular matrix and cytokines: a functional unit. Dev. Immunol. 7, 89–101.

Seftor, R., Seftor, E., Koshikawa, N., Meltzer, P., Gardner, L., Bilban, M., Stetler-Stevenson, W.G., Quaranta, V., Hendrix, M., 2001. Cooperative interactions of laminin 5 gamma2 chain, matrix metalloproteinase-2, and membrane type-1-matrix/metalloproteinase are required for mimicry of embryonic vasculogenesis by aggressive melanoma. Cancer Res. 61, 6322–6327.

Skov, L., Hansen, H., Allen, M., Villadsen, L., Norval, M., Barker, J., Simon, J., Baadsgaard, O., 1998. Contrasting effects of ultraviolet A1 and ultraviolet B exposure on the induction of tumour necrosis factor-alpha in human skin. Br. J. Dermatol. 138, 216–220.

Stetler-Stevenson, W., Yu, A., 2001. Proteases in invasion: matrix metalloproteinases. Semin. Cancer Biol. 11, 143–152.

Varani, J., Hattori, Y., Chi, Y., Schmidt, T., Perone, P., Zeigler, M., Fader, D., Johnson, T., 2000. Collagenolytic and gelatinolytic matrix metalloproteinases and their inhibitors in basal cell carcinoma of skin: comparison with normal skin. Br. J. Cancer 82, 657–665.

Vedhara, K., Cox, N., Wilcock, G., Perks, P., Hunt, M., Anderson, S., Lightman, S., Shanks, N., 1999. Chronic stress in elderly carers of dementia patients and antibody response to influenza vaccination. Lancet 353, 627–631.

Wu, W., Yamaura, T., Murakami, K., Ogasawara, M., Hayashi, K., Murata, J., Saiki, I., 1999. Involvement of TNF-alpha in enhancement of invasion and metastasis of colon 26-L5 carcinoma cells in mice by social isolation stress. Oncol. Res. 11, 461–469.

Yamamoto, K., Takeshita, K., Shimokawa, T., Yi, H., Isobe, K., Loskutoff, D.J., Saito, H., 2002. Plasminogen activator inhibitor-1 is a major stress-regulated gene: implications for stress-induced thrombosis in aged individuals. Proc. Natl. Acad. Sci. 99, 890–895.

Yang, E., Gardiner, D., Carlson, M., Nugas, C., Bryant, S., 1999. Expression of MMP-9 and related matrix metalloproteinase genes during axolotl limb regeneration. Dev. Dyn. 216, 2–9.

Yokoseki, O., Yazaki, Y., Suzuki, J., Imamura, H., Takenaka, H., Isobe, M., 2000. Association of matrix metalloproteinase expression and left ventricular function in idiopathic dilated cardiomyopathy. Jpn. Circ. J. 64, 352–357.