



Review

Stress, immunity and skin collagen integrity: Evidence from animal models and clinical conditions

V. Kahan^a, M.L. Andersen^{a,*}, J. Tomimori^b, S. Tufik^a^a Department of Psychobiology, Universidade Federal de São Paulo (UNIFESP) – São Paulo, SP, Brazil^b Department of Dermatology, Universidade Federal de São Paulo (UNIFESP) – São Paulo, SP, Brazil

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ABSTRACT

The skin is the largest organ of the human body and plays a major role in maintaining homeostasis and protection. As the main component of skin, collagen has a key role in providing integrity and elasticity to this organ. Several factors, including autoimmune disease, aging, and stress, can change the quantity and integrity of skin collagen. These factors impair collagen quality and consequently affect skin function. Stress seems to affect the integrity of skin collagen through glucocorticoid-mediated processes that alter its synthesis and degradation. Glucocorticoids also affect skin quality through modulation of the immune system. This review will briefly present comprehensive data from both animal and human studies delineating processes that modulate alterations in collagen in general, and will treat in more detail the consequences of stress on skin collagen.

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1. Introduction

Collagen represents a family of proteins present in all vertebrates and invertebrates. The size, function and tissue distribution of collagens vary considerably. Collagen is the main component of the surface of the sea cucumber (*Cucumaria frondosa*), the bones of an extinct reptile, and the skin and bones of human beings (Solomon and Cheah, 1981; Trotter et al., 1995). Collagen family members have various functions, are present in all organs, and provide rigidity and integrity to the bones and skin (Kühn, 1987; Myllyharju and Kivirikko, 2001). The diversity of these polypeptides indicates that collagen plays a key role in the structure and integrity of both organs and tissues.

Collagen fibers are assembled from glycoproteins (Junqueira and Carneiro, 2008). All members of the collagen family share a characteristic triple-helical structure composed of three α -chains (Kühn, 1987). Of the 28 different types of collagen described to date (Heino, 2007), type I is the most abundant in the human body and is the most studied. Type I collagen comprises 90% of bone mass and is the main component of human skin (80%), with collagen type III making up the remainder of skin collagen (15%) (Fleischmajer et al., 1990). In newborn rats, in contrast, collagen type

III comprises 70% of the skin, and collagen type I 30%. The preponderance of collagen type III disappears after the second week of life in mice; thereafter, collagen type I comprises 82% of total skin collagen and type III makes up the remainder of skin collagen (18%) (Klein and ChandraRajan, 1977).

Metalloproteinases (collagenase, gelatinase and stromelysin) can degrade collagen fibers, although collagen is relatively stable. Interstitial collagenase (MMP-1) produced by interstitial fibroblasts and inflammatory cells (macrophages and leukocytes) can degrade collagen types I and III; MMP-1 introduces a single break in each molecule of the triple α -chain, thus denaturing the collagen molecule. Metalloproteinases-9 (MMP-9) and metalloproteinases-2 (MMP-2) can degrade collagen IV (Gross and Lapiere, 1962). After the initial proteolytic cleavage, collagen can be degraded by other proteolytic enzymes (Horwitz et al., 1977; McCroskery et al., 1975). In general, this process is similar for all types of collagenase; however, differences exist in the susceptibilities of different types of collagen to various collagenases. For example, MMP-1 cleaves type I collagen more rapidly than it cleaves type III collagen (Horwitz et al., 1977).

Changes in the synthesis and degradation of collagen lead to many human diseases, such as Cushing's syndrome (Crapo, 1979). In this context, the quality and quantity of collagen in the skin is affected by both endogenous and exogenous factors, including disease, hormones, stress, aging, and treatment with topical or systemic glucocorticoids (GC) (Autio et al., 1994; Cohen et al., 1979; Minor, 1980; Oikarinen et al., 1992). These factors can

* Corresponding author. Address: Department of Psychobiology, Universidade Federal de São Paulo (UNIFESP), Rua Napoleão de Barros, 925, Vila Clementino, 04024-002 São Paulo, SP, Brazil. Fax: +55 11 55725092.

E-mail address: mandersen@psicobio.epm.br (M.L. Andersen).

interfere with collagen and its fibers. As an example, vitamin A modulates the activity of MMP-1 in humans and increases the strength of connections between collagen fibers in animals (Levenson et al., 1984). Deficiency in ascorbic acid results in bleeding gums, an impaired immune system, easy bruising and bleeding, and slowed healing of wounds and fractures (Fauci et al., 1998; Gross, 2000; Porto da Rocha et al., 2002). Indeed, ascorbic acid is essential for the hydroxylation of proline and lysine during the formation of collagen molecules.

The aim of the present article is to review data obtained from experimental animal models as well as clinical evidence concerning the effect of stress on collagen and skin integrity. Deeper knowledge of this relationship is not only important for our understanding of the basic physiology of the skin, but may also shed light on therapeutic approaches that can be adopted for patients with diseased or aged skin caused or exacerbated by stress.

2. Changes in collagen synthesis

2.1. Collagen changes caused by aging

Previous studies have shown that collagen synthesis varies in different stages of life. The two primary processes of skin aging, intrinsic and extrinsic, are controlled, respectively, by genetic variations and by extrinsic components including smoking, alcohol consumption, and chronic sun exposure (Uitto, 1997).

Changes in the production and formation of collagen and elastic fibers are common characteristics of an aging dermis. Photo-aged dermis contains disorganized collagen fibers and accumulated abnormal elastin (El-Domyati et al., 2002; Fisher et al., 1997). The relative proportion of the types of collagen in skin also changed with age. Young skin is composed of approximately 80% type I collagen and about 15% collagen type III. In aged skin, collagen fibers became thicker and there was a loss of collagen type I, which altered the ratio of collagen types (Oikarinen, 1990). Moreover, aged fibroblasts synthesized lower levels of collagen, both *in vitro* and *in vivo*, compared to young adult fibroblasts (Varani et al., 2006). Recently, Fisher et al. (2009) reported that dermal fibroblasts express higher levels of MMP-1 in aged human skin *in vivo*, when compared with fibroblasts from young skin. In addition, the annual loss of total collagen was approximately 1% from 50 years of age onward (for review, see Uitto, 2008). Senescent fibroblasts exhibit phenotypic changes, becoming wide and flat with irregular shapes and lobulated nuclei (Kletsas, 2003). These modified fibroblasts are unable to proliferate and display a proinflammatory phenotype. Both of these characteristics suppress the synthesis of collagen and lead to greater expression of metalloproteinases (Shelton et al., 1999).

In aged skin, there is an accumulation of elastosis and further degradation of the extracellular matrix due to the action of metalloproteinases (gelatinase and stromelysin), which decreased collagen levels (Yin et al., 2000). Recently, it was shown that smoking also resulted in changes in the components of the dermis and that it significantly decreased the amount of type I collagen in the skin of hairless-strain mice (Tanaka et al., 2007). Altogether, these data suggest that cigarette smoking can cause premature aging of the skin (Tanaka et al., 2007; Yin et al., 2000).

A large amount of evidence relates stress to changes in skin collagen. An important distinguishing characteristic of stress is its duration. An important indicator of the deleterious effects of chronic stress is dysregulation of the circadian cortisol/corticosterone rhythm (Dhabhar and McEwen, 1997; Sепhton et al., 2000). In response to stress, corticotrophin-releasing factor (CRF, which regulates the hypothalamic–pituitary–adrenal axis, HPA) initiates a cascade of events that culminate in the release of GC from the

adrenal cortex. The HPA axis is sensitive to negative feedback by GC, thus previously elevated levels of GC may inhibit further response to stress. However, in chronic stress conditions, the HPA axis often exhibits an inadequate response to subsequent stressors. This augmented level of GC that occurs under chronic stress conditions has strong immunomodulating effect that is mediated primarily by cytosolic GC receptors (for review, see Stahn and Buttgereit, 2008). In the short term, release of cortisol may play a key role in the survival of an organism; however, chronic exposure to cortisol can lead to a number of negative effects. Excess GC, whether endogenous or exogenous, has negative effects on nearly all tissues (Boscaro et al., 2001) and accelerates the aging process (Roupe, 2001). Several studies have linked the release of stress hormones such as GC to changes in collagen in the skin.

2.2. Sympathetic nervous system activation and skin integrity

Another response of organisms to stressful stimuli is activation of the sympathetic nervous system (SNS) (for review, see Elenkov and Chrousos, 2002). Sympathetic fibers innervate all tissues, release norepinephrine (NE) and acetylcholine as their main neurotransmitters, and can modulate immune function as well as the skin immune system (Luger, 2002). Sympathetic fibers innervate sweat glands and hair follicles and appear as single nerve fibers in the dermis and epidermis; also, sympathetic fibers terminate in close contact with lymphocytes in the lymphoid organs (Madden et al., 1995). The existence of distinct types of adrenoceptors, α and β , was first proposed by Ahlquist (1948). Further development in biochemical, molecular and genetic techniques has allowed the classification of nine subtypes of adrenoceptors to date (Guimaraes and Moura, 2001).

Human dermal fibroblasts, such as keratinocytes and melanocytes, express β 2-adrenergic receptors in their membranes (McSwigan et al., 1981; Steinkraus et al., 1996). Keratinocytes also have the capacity to synthesize NE, and β 1- and β 2-adrenoceptors play an important role in the physiological regulation of skin perfusion (Cassuto et al., 2005). Indeed, following the activation of the SNS after a traumatic injury, there is a massive release of catecholamines into the peripheral circulation (Woolf et al., 1992). Topical application of a β 2 receptor agonists (alprenolol and procaterol) into the flank of the hairless mouse delayed repair of the epidermal barrier after tape stripping until 6 h; 24 h after tape stripping there were no significant changes in these parameters in each and every one of the groups tested (Denda et al., 2003). In turn, application of a β 1,2 receptor antagonist (clenbuterol) and a β 2 antagonist (ICI-118 551) accelerated the recovery of the skin barrier (Denda et al., 2003). It was demonstrated that patients with atopic eczema exhibit decreased β 2-adrenoceptor binding in peripheral-blood lymphocytes caused by a point mutation in the β 2-adrenoceptor gene that impaired antagonist binding to β 2-adrenoceptor (Schallreuter et al., 2007) and that epidermal cells from psoriatic lesions show a low response to β 2-adrenoceptor activation (Eedy et al., 1990). Collectively, this evidence shows that β 2-adrenergic receptors are localized in the epidermis and that they play an important part in the barrier homeostasis of skin. In addition, β receptor antagonists (e.g., propranolol and atenolol) reduced wound contraction, reepithelialization, MMP-9 and MMP-2 levels, and collagen I and III deposition (Romana-Souza et al., 2009).

Dermal fibroblasts are actively involved in the wound healing process. These cells migrate to the wound site, proliferate and produce collagen and extracellular matrix components and form granulation tissue (Grinnell, 1994). Pullar and Isseroff (2005) reported that the activation of β 2-adrenoceptors in dermal fibroblasts was both pro-motogenic and pro-mitogenic.

A study investigating wound healing in a murine model of NE depletion specific to the peripheral nervous system (intraparito-

neal injections of hydroxydopamine) (Thoenen and Tranzer, 1968) found an increase in neutrophil recruitment to the wound in the first 72 h after injury in control mice; however, these levels equaled those in NE-depleted animals 120 h after wound injury. Moreover, intact control mice showed increased macrophage recruitment after the injury; nevertheless, after 120 h, macrophage recruitment to wounds in NE-depleted mice had far surpassed that seen in the control mice. These data indicate a deficit in the inflammatory phase of wound healing in NE-depleted animals. The failed recruitment of neutrophils and macrophages 24 h after injury suggests a role of NE in the wound healing process, since 120 h after injury the macrophage infiltration in the wounds was greater in NE-depleted animals than in controls (Gosain et al., 2006). This study also reported that NE-depleted animals exhibit lower reepithelialization. On the other hand, NE-intact mice display markedly lower levels of angiogenesis compared with NE-depleted animals, suggesting that NE promotes or suppresses many or all aspects of the proliferative phase (Gosain et al., 2006). Souza et al. (2005) have also described improvement in collagen arrangement, impairment of reepithelialization, mast cell accumulation and disturbed inflammatory responses in NE-depleted rats (Souza et al., 2005). Collectively, there is considerable data suggesting that psychological modulators can influence, albeit indirectly, activity of the various MMPs. Indeed, activation of the HPA and SNS axes can modulate levels of MMPs.

2.3. Stress-induced glucocorticoids and collagen

Excess GC result in several adverse effects. Skin tissue is greatly affected by stress and excess GC, which can lead to skin thinness (Josse et al., 2009). Excess GC can also hinder healing and accelerate the aging process (Boscaro et al., 2001; Oikarinen and Autio, 1991). As collagen is a major component of the skin, it is well accepted that GC can affect its synthesis.

Physiological stress is meant to indicate any type of stress that can trigger a constellation of physiological events, such as HPA axis dysregulation or release of neurotransmitters and hormones (Dhabhar and McEwen, 1997). Based on this definition, the magnitude of this type of stress can be measured by the level of released hormones or neurotransmitters or by other physiological changes, such as increased blood pressure and heart rate (Shapiro and Melhado, 1958). One of the most important changes related to the stress response is an alteration of basal concentrations of corticosterone in rodents (Dhabhar and McEwen, 1997) and cortisol in humans (Sephton et al., 2000). In addition to their immunosuppressive effects, there is also evidence that GC act as immunomodulators and immunopromoters (Wilckens and De Rijk, 1997).

The negative effects of GC on the skin are primarily mediated by inadequate function of fibroblasts and by immunosuppression. *In vitro* studies have shown that GC modify several parameters of homeostasis and cause changes in the extracellular matrix and in collagen (Autio et al., 1994; Oikarinen et al., 1998). Following treatment with GC, concentrations of the peptide precursors of collagen decreased considerably, resulting in decreased thickness of the skin (Autio et al., 1994). This effect is similar to that observed following topical treatment with a GC. Specifically, the concentrations of collagen I and III typically fall by 80% following topical GC treatment (Oikarinen et al., 1998). Collagen mRNA levels correlate with collagen synthesis. In cultured human fibroblasts, GC treatment results in up to 70% decrease in the levels of collagen type I and III mRNA (Oikarinen et al., 1998) and there is evidence that GC can regulate the promoter activity of the pro $\alpha 1(I)$ collagen gene (Meisler et al., 1995). The source of GC (natural or synthetic), other physiological factors (cytokines and neurotransmitters), and the immune status of the subject are all factors that are crucial in determining the nature of the effects of GC on a specific immune response.

2.4. Stress-related immunological changes and collagen

It is known that the immune response can be modulated by stress. Stress-induced changes in the immune response occur mainly through dysregulation of the HPA axis, which, in situations of chronic stress, increases the release of corticosterone in rodents and cortisol in humans and leads to a state of immunosuppression (Dhabhar, 2000). Acute stress can prime a positive physiological response directed at preventing imminent damage, such as increases in leukocyte trafficking with redistribution of leukocytes from the blood to organs such as bone marrow, lymph nodes and skin, enhancing the immune response. This redistribution of leukocytes may represent an evolutionarily and adaptive neuroendocrine-immune response which increases immune surveillance during times of stress. Activation by acute stress can prime a positive response from the body to prevent imminent damage, like increases leukocyte trafficking, with a redistribution of leukocytes from the blood to organs such as the bone marrow, lymph nodes and skin, enhancing the immune responses. This redistribution of leukocytes may represent an evolutionarily and adaptive neuroendocrine-immune course which increases immune surveillance during conditions of stress (reviewed by Dhabhar, 2003; Sanders and Kohm, 2002). However, repeated acute stress can create an environment in which the immune response is continually repressed (for review, see Godbout and Glaser, 2006). Repeated acute stress consists of an acute stress repeated several times with an interval. This protocol is very useful in determining habituation effects of stress (Kirschbaum et al., 1995). Mischler et al. (2005) submitted healthy volunteers to repeated acute stress when they applied the Trier Social Stress Test, which is comprised of a preparation time, a job interview and mental arithmetic to be presented in front of an audience. Subjects underwent the stress protocol three times with an interval of 1 week. At the third week, subjects exhibited a lower level of hematocrit and of erythrocytes compared to the first week throughout the time-points assessed (Mischler et al. (2005)). Indeed, GC treatment can affect a range of immunological parameters, including the levels of antigen presentation molecules and the proliferation and circulation of leukocytes (Butts and Sternberg, 2008). These factors may change the arrangement of molecules and the production of collagen.

There is evidence that the release of interleukin (IL)-1 increases the production of collagen type IV by epithelial cells in mice (Matsushima et al., 1985). IL-1 also stimulates the proliferation of fibroblasts and, consequently, their production of MMP-1, thus altering the production of collagen I and III. An increase in IL-1 β can induce a rapid redistribution of dimensional collagen, thus acting as a possible modulator for the accession of fibroblasts (Qwarnstrom et al., 1991). In addition, interferons (β and γ) may inhibit the production of collagen, but they did not inhibit the proliferation of fibroblasts (Qwarnstrom et al., 1991). IL-1 and TNF- α have been shown to up-regulate collagenase gene expression (Burrage et al., 2006). It also been shown that the expression of these immunological mediators are affected by physiological stress, which modifies the HPA axis (Heijnen et al., 1991).

In addition to stress resulting from major life events, another present stressor in modern life is sleep deprivation. Sleep deprivation can be caused by artificial light, shift work, sleep disturbances, or social life. The negative effects of sleep deprivation on the immune system have been well documented (Everson, 2005; Lange et al., 2003; Ruiz et al., 2007; Zager et al., 2007). For instance, 42 h of total sleep deprivation affects homeostasis of the skin barrier and increases the levels of IL-1 β and TNF- α and the activity of natural killer (NK) cells in women (Altemus et al., 2001). Sleep loss not only exerts negative effects on the immune system, but it also causes decreased cognitive and psychomotor performance and changes in mood and behavior (Andersen et al., 2006; Alvarenga

et al., 2008; Benedetti et al., 2008). There is evidence that lack of sleep can also impair the function of the epidermal barrier. For instance, rats subjected to a prolonged period of sleep loss developed ulcerative lesions on their paws and tails and a higher susceptibility to bacterial invasion (Everson and Toth, 2000; Kushida et al., 1989). Taken in total, there is strong evidence that stress, in its different forms, can interfere with the integrity of the skin via modulation of the HPA axis and the immune system.

Collectively, studies investigating the relationship between stress and the immune system have shown that increases in psychological stress are associated with various skin dermatoses, such as psoriasis and dermatitis, and with disruption of the epidermal barrier (Gupta and Gupta, 1996; Tausk and Nousari, 2001).

3. Stress and skin integrity

3.1. Humans

Dermatitis results from an abnormal function of the epidermal barrier. Since the 1960s, dermatitis has been known to be associated with stress. Indeed, psoriasis and dermatitis are highly correlated with psychological disorders (Gupta and Gupta, 1996; Tausk and Nousari, 2001). Recently, Gupta (2002) reported that, in contrast to other stress-reactive dermatoses (e.g., acne, alopecia areata, and atopic eczema), psychosocial stressors more frequently predate the onset or exacerbation of psoriasis, suggesting that psoriasis is more sensitive to stress than some other stress-reactive dermatologic disorders.

In order to investigate the role of stress in psoriasis, Arnetz et al. (1985) interviewed healthy men who had suffered from psoriasis for a mean time of 16 years. The volunteers stated that psychosocial stress provoked or aggravated their disease. During a stress period (forced mental arithmetic and color-word conflict), psoriatic volunteers showed higher levels of stress and of urinary adrenaline than the control group. Psoriatic volunteers also exhibited more substantial decreases in serum cortisol than the control group (Arnetz et al., 1985). In another study, five psoriatic volunteers were monitored for a 20-week period in order to investigate possible links between changes in mood and quality of life and to analyze the perception of psychological stress. During this period, the subjects underwent dermatological and psychological evaluations. The results indicated that psoriasis ratings were weakly correlated with the impact of adverse life events and with psychological distress (O'Leary et al., 2004). Taken together, these studies demonstrate that psoriatic patients perceive situations as more stressful and, consequently, excrete more adrenaline than normal subjects. The levels of perceived stress, although strongly associated with mood disorders and quality of life, were not associated with psoriasis severity (Arnetz et al., 1985; O'Leary et al., 2004).

Another study in young healthy volunteers showed changes in the recovery of the skin barrier after tape stripping during school ratings, which are considered to be a very stressful period (Garg et al., 2001). The subjects were evaluated at three time-points: the beginning of classes, a week before the final tests, and after the test period (low stress, high stress and recovery, respectively). The results indicated a statistically significant decline in the homeostatic barrier of the skin, as measured by transepidermal water loss, and a significant reduction in the recovery period of the skin of these volunteers during the high stress phase. These data provide a link between psychological status and skin function. Relevant to this topic, a study found that even short-term administration of a potent topical GC can interfere with collagen molecules and fibers. For instance, increased production of GC can inhibit the synthesis of lipids, resulting in a lower production

and secretion of lamellar bodies and impairing the integrity of the stratum corneum (Kao et al., 2003).

It has been reported that inhalation of certain odors can impact the homeostatic skin barrier. In one study, healthy men were asked to inhale three different substances: dimethoxymethylbenzene, (component of Bulgarian rose oil), citralva and one placebo. Dimethoxymethylbenzene and citralva are substances with anti-stress effects. Transepidermal water loss was then measured. The data show an acceleration of the recovery of the epidermal barrier when the subjects inhaled a substance with known sedative properties (Denda et al., 2000). This finding can be explained by either of two possible mechanisms: either each odorant substance directly accelerates the homeostatic response of the barrier, or the substances prevented damage to the barrier.

Altemus et al. (2001) compared the effects of three types of stress on various aspects of skin physiology and on some immunological parameters in young healthy women. The authors sought to compare the effects of a stress interview (as described by Kirschbaum et al., 1993), total sleep deprivation for 42 h, and three daily sessions of exercise. The stress caused by the interview resulted in a significant decrease in the recovery of skin barrier function after tape stripping, an increase in transepidermal water loss, and significant increases in cortisol, NE, TNF- α , IL-1 β , and IL-10 levels and the number of NK cells. Sleep deprivation resulted in a significant reduction in skin barrier function recovery, but no effect on transepidermal water loss or stratum corneum water content. Increases in plasma levels of IL-1 β and TNF- α , but no statistically significant differences in plasma levels of IL-10 were observed. Increases in NK cell function were also reported after sleep deprivation. Finally, after three sessions of exercise, no statistically significant differences were observed in any aspect of skin physiology; however, an increase in NK cell function and a decrease in circulating T cells were described (Altemus et al., 2001).

In order to examine the role of stress in human wound healing, Kiecolt-Glaser et al. (1995) studied women who were caring for a relative with Alzheimer's disease (mean age 62 years). The volunteers underwent a 3.5 mm punch biopsy wound; the healing process was evaluated by photographs and response to hydrogen peroxide. When compared to the control group, these women showed a lower production of IL-1 β mRNA by peripheral-blood leukocytes in response to *Salmonella typhimurium* lipopolysaccharide stimulation and the healing process was significantly longer (24%) in caregivers than in controls.

Similarly, Marucha et al. (1998) sought to investigate whether a brief commonplace stressor (academic examinations) is associated with alterations in mucosal wound repair. First, a punch biopsy wound was placed on the hard palate of healthy young adults (dental students) during summer vacation. Another wound was placed on the contralateral side 3 days prior to the first academic examination of the term. The wounds placed 3 days before the examinations healed, on average, 40% more slowly than those made during summer vacation. In addition, the average student's IL-1 β declined an average of 68% during examinations (Marucha et al., 1998). In another study, patients with inguinal hernia were given a standardized questionnaire assessing psychological stress and concern about the operation before undergoing open incision repair as described by Cohen and Williamson (1988). After the surgical procedure, the volunteers replied to another questionnaire which contained two visual analog scales, one for pain experienced since the operation and one for self-assessed surgical recovery. In addition, wound fluids and peripheral blood were collected. This study reported a lower level of IL-1 in wound fluids of patients who exhibited a higher level of psychological stress before surgery. Furthermore, patients who reported more concern about their upcoming surgery had lower levels of MMP-9 in the wound site (Broadbent et al., 2003).

Although animal studies involving hamsters, voles and monkeys have been performed (for review, see Gauthier, 1996), further studies on the mechanisms mediating skin barrier homeostasis, wound healing and skin integrity are warranted in order that a better understanding of how stress negatively interferes with these processes can be obtained. Such data would allow a more comprehensive parallel between the human conditions studied and data obtained from animal models, with respect to impairment of skin healing under stress paradigms.

3.2. Animal models

Although it is not possible to directly extrapolate data from animal model studies to disease in humans, studies using humans and rodents have shown that stress and GC are closely linked to skin integrity. Padgett et al. (1998) reported that mice subjected to restraint stress 3 days before and 5 days after a full-thickness wound to the dorsum show increased levels of corticosterone. Indeed, restraint stress dysregulate the HPA axis and slows the healing process in mice compared to unstressed animals, possibly by attenuating the early inflammatory response. In this study, in order to confirm the involvement of GC in the slowing of healing, a GC receptor antagonist (RU40555) was administered. The agonist restored the normal wound healing in restraint-stressed animals.

GC has also been thought to be responsible for stress-induced disturbances of homeostasis, and have been shown to impair the ability to control and eradicate a *Staphylococcus aureus* infection during healing (Rojas et al., 2002). Mice were subjected to restraint stress before being wounded in the dorsal area, where *S. aureus* was inoculated. Animals that were submitted to restraint stress had three times more *S. aureus* in their wounds when compared to non-stress group. To assess the impact of restraint stress on GC levels, the mice were treated with a GC receptor antagonist (RU486). A significant reduction in bacterial growth was observed, suggesting that GC play an important role in increasing the susceptibility to infection during the healing process (Rojas et al., 2002).

The healing process has been linked to a decrease in the expression of proinflammatory cytokines (IL-1 α and IL-1 β) and growth factors. Restraint stress could induce a downregulation of IL-1 β mRNA expression during the first day after wounding; on the third day, levels of IL-1 β mRNA are near control level. After 5 days, there was a higher level of IL-1 β mRNA when compared to the control group, suggesting that stress not only affects the kinetics of wound healing but could also impair the quality of the healing by altering immunological parameters (Mercado et al., 2002a). In order to investigate the role of GC in the release of IL-1 α and IL-1 β , *in situ* hybridization was employed. These experiments revealed that stress differentially regulates gene expression in targeted leukocytes, keratinocytes, and fibroblasts within the wound, which impairs the quality of the tissue undergoing repair (Mercado et al., 2002b).

Epithelial cells of mice showed an increase in the production of collagen type IV when stimulated by IL-1 (Matsushima et al., 1985). To confirm this effect, human monocyte-derived IL-1 was purified and was found to stimulate the production of collagen type IV in cultured cells. IL-1 also significantly increased the production of collagenase in cultured fibroblasts and resulted in the stimulation of fibroblast proliferation. The concentrations used in these experiments are equivalent to those required to stimulate mouse thymocyte proliferation (Schmidt et al., 1982). IL-1 at these concentrations can also alter the production of collagen in response to different stimuli by lymphocytes *in vitro*. Collagen type IV is a ubiquitous component of basement membranes at the dermal–epidermal junction (Sage, 1982); over-production of collagen IV can lead to renal injury (Ribaldo et al., 2009) and fibrosis in the gastric wall (Manetti et al., 2007). In the skin, an excess of collagen

IV causes a thickening of the basement membrane, one of the factors associated with psoriasis (Mondello et al., 1994).

Paradoxical sleep deprivation is one of the most common stressors applied in animal models. It results in significantly increased levels of GC (Andersen et al., 2005). Indeed, sleep deprivation in animals has been used to promote activation of the HPA axis. In the sleep deprivation procedure, mice or rats are placed inside a tiled water tank containing circular platforms with water up to within 1 cm of their upper surface. Once the animals reach the paradoxical phase of sleep, muscle atonia sets in, which results in the animals falling into the water and subsequently waking. The effects of this selective sleep deprivation method suggest suppression of the immune system (Everson, 2005; Ruiz et al., 2007; Zager et al., 2007) and deterioration in the barrier function of the skin and mucous membranes (Kushida et al., 1989). However, further studies are needed to confirm whether the sleep deprivation is able to impair the quality and quantity of skin collagen.

In 1989, Kushida and coworkers showed that prolonged sleep deprivation (total or paradoxical) using a disk apparatus resulted in ulcerative and hyperkeratotic lesions on the feet and tails of rats. These lesions could not be explained by water immersion, infection or necrosis. Later, Everson and Toth (2000) submitted rats to paradoxical sleep deprivation for up to 20 days and observed lesions on the animals' feet and tails. In addition, bacterial invasion of the lymph nodes and other tissues, resulting from immune suppression, occurred. The authors pointed out that the presence of bacteria in various tissues of the sleep deprived animals could be a sign of host defense malfunction.

In sum, there is strong evidence indicating that many factors affect the synthesis and degradation of various types of collagen molecules, and that stress and subsequent variations in the HPA axis play key roles in maintaining collagen integrity. Stress-mediated changes trigger a series of events that are mediated by the release of GC. Over-production of GC has negative effects on all tissues, including the skin, where it may slow healing and accelerate aging. Herein, we have reviewed evidence in support of a role for stress-induced GC release in the production and degradation of skin collagen. Investigations of the neurobiological mechanisms involved in the interplay between stress and collagen production and maintenance may be important to our understanding of the aging process.

4. Conclusions

There is much evidence that collagen is crucial for maintaining homeostasis of the skin. The events that occur during the formation of collagen throughout the lifespan of humans and other animals can be affected by endogenous and exogenous factors. Stress seems to have a significant effect on this balance, inhibiting the production or promoting degradation of dermal collagen.

In this review, we have presented evidence that stress can change the epidermal barrier by limiting healing, predisposing the skin to bacterial infections and reducing the quantity and quality of collagen in the skin. Different stressors activate the HPA axis, which, in turn, results in the release of GC. An excess of GC hampers the healing process. One of the side effects of topical GC therapy is decreased thickness of the skin. GC can further alter collagen molecules and their fibers by effecting changes in fibroblasts, which suppress the synthesis of collagen and increase the synthesis of metalloproteinases. Excess GC also results in immunomodulation, which can interfere with collagen synthesis. The release of IL-1 α , IL-1 β , and interferons can inhibit collagen production. These processes can act in a combinatorial manner to impair skin homeostasis. Stress resulting from major life events and from various experimental stressors can interfere with the integrity of the skin

and can change collagen in various ways. Expanding the knowledge of the machinery that constitutes the largest organ of the human body is essential for understanding homeostasis and protection. Understanding the mechanisms behind altered collagen synthesis and degradation due to stress is an important step towards the development of new therapeutic modalities.

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