The Exudate of Pressure Ulcers Contains a Substantial Amount of Vascular Endothelial Growth Factor

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Pressure ulcers (PUs) are chronic wounds that occur as areas of tissue necrosis that result from external physical compression, shear forces, and friction. Recently, the efficacy of polyvinylidene film dressing (PVFD) for PUs without any agents promoting wound healing has been reported, suggesting that PUs have their own mechanism of spontaneous healing achieved by vascularization, synthesis of extracellular matrix, and re-epithelization. Since vascular endothelial growth factor (VEGF) 165 and fibroblast growth factor 2 (FGF-2) are released at traumatic or surgical wound sites and play major roles in vascularization and wound healing, we measured the concentrations of VEGF165 and FGF-2 in the exudate and fibrinous sloughs of PUs after PVFD. We collected 10 exudate samples and 3 samples of fibrinous sloughs from 10 PUs of 9 patients immediately after PVFD for 8 h and measured VEGF165 and FGF-2 by ELISA. All 10 exudate samples contained substantial amounts of VEGF165, from 2.79 to 13.27 μg g⁻¹, irrespective of the severity of the PUs. In contrast, we detected FGF-2 (0.21 and 2.03 μg g⁻¹) in only two exudate samples. Similarly, we detected VEGF165 (from 3.14 to 5.93 μg g⁻¹) and FGF-2 (less than 0.31 μg g⁻¹) in fibrinous sloughs of 3 PUs. These results demonstrate that the exudate and fibrinous sloughs of PUs contain considerable amounts of VEGF, which would contribute to the spontaneous healing of PUs by PVFD. The presence of VEGF165 in the exudate of PUs inspires us to reconsider the treatment strategy of PUs that enhances the spontaneous healing.

Keywords: fibroblast growth factor; pressure ulcer; vascular endothelial growth factor; wound healing


Recently, Takahashi et al. (2006) have reported the efficacy of polyvinylidene film dressing (PVFD), a procedure that was originally reported by Toriyabe et al. (1999) for the treatment of pressure ulcers (PUs). In the treatment of PU by PVFD, after cleansing with saline, the wound is first covered with the non-sterilized polyvinylidene film (PVF) and then the PVF is sealed with non-woven adhesive tape. Next, the PVFD is overlaid with gauze dressing to drain excess exudate. This procedure can be applied to PUs of any stage such as the acute inflammatory phase and proliferative phase.

The mechanism for the efficacy of PVFD remains unknown. It is well recognized that the control of moisture and drainage from the wound helps to provide an optimal wound environment for healing, and PVFD may provide this condition. PVFD does not use any skin cleansers or antiseptics, which are cytotoxic and retard epithelization (Witkowski and Parish 1996). Unexpectedly, however, PVFD did not increase the incidence of wound infection (Takahashi et al. 2006). PVFD appears to provide a favorable environment for the wound repair and cells.

Pufe et al. (2003) have demonstrated that vascular endothelial growth factor (VEGF) is expressed in the granulation tissue of chronic sacral PUs, suggesting that PUs themselves release factors to promote wound healing. Among the various pro-angiogenic mediators involved in wound healing, VEGF and fibroblast growth factor 2 (FGF-2) appear to be of primary importance. VEGF is an endothelial cell mitogen that promotes angiogenesis in vivo and renders the microvasculature hyperpermeable to circulating macromolecules. VEGF is chemotactic for monocytes and is a pro-coagulant (Ferrara et al. 2003). VEGF induced the mobilization of bone marrow-derived endothelial progenitor cells in an animal model (Asahara et al. 1999) and in human subjects (Kalka et al. 2000). Five VEGF isoforms of 121, 145, 165, 189, and 206 amino acids are produced by alternative splicing of a single species of the VEGF gene transcript (Neufeld et al. 1999). Among
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these VEGF isoforms, VEGF of 121 amino acids (VEGF121) and VEGF165 are the predominant forms and have potent angiogenic activity (Neufeld et al. 1999). FGF-2, also known as basic FGF (bFGF), is involved in angiogenesis by controlling the proliferation and migration of vascular endothelial cells, fibroblasts, and other types of cells and by inducing VEGF expression (Nugent and Iozzo 2000).

In this study, we measured the concentrations of VEGF165 and FGF-2 to clarify whether the exudate or sloughs of PUs treated with PVFD contain pro-angiogenic mediators that promote wound healing. We assumed that PVFD does not deprive PUs of this potential, since PVFD does not use any skin cleansers or antiseptics that might destroy potential bioactive substances that promote wound healing, in the exudate or sloughs of PUs. The results clearly demonstrated that the exudate and sloughs of PUs treated with PFVD contained a substantial amount of VEGF165.

Patients, Materials and Methods

Patients

Nine patients with PUs (mean age = 70 years; 3 men and 6 women) were recruited in this study. The PUs we examined in this study were classified as either stage III or IV (National Pressure Ulcer Advisory Panel guideline) (1989). We also assessed the severity of each PU by the DESIGN scale, a Japanese standard used as an assessment tool for the severity of PUs developed by the Japanese Society of Pressure Ulcers (Sanada et al. 2004). DESIGN is an acronym of six of the seven subscales used to classify the wound state — Depth, Exudate, Size, Infection, Granulation, Necrosis — plus pocket (undermining). The state of the wound is assigned a number according to the concrete definition of each subscale, and the scores are added up. The total score ranges from 0 to 28; the higher the score, the greater the severity. This scale was reported to show high reliability and validity. Using this scale, not only the wound size but also the qualitative changes in a PU, including signs of infection and amounts of exudate, pus and necrotic tissue, are evaluated quantitatively.

This study was performed in accordance with the Declaration of Helsinki and its amendments, and approved by the ethics committees of Osaki Citizen Hospital, Osaki, Japan. After providing a full explanation of the study, informed consent was obtained from the patients or family members when the patient’s communication was impaired.

Polyvinylidene film dressing (PVFD)

We treated the PUs of these patients with PVFD according to the procedure described by Takahashi et al. (2006). Namely, after cleansing with saline, the wound was simply covered with non-sterilized PVF, which was sealed with non-woven adhesive tape (Fig. 1). The excess exudate was absorbed by the overlaying gauze. This procedure was repeated twice every day.

Samples

We collected 10 samples of exudate from 10 PUs of 9 patients and 3 samples of fibrinous sloughs from 3 PUs of 3 patients after the last PVFD for 8 h. To collect exudate from PUs, we put a 1 cm² square filter paper on the bottom of PUs immediately after removing PVF and left it to absorb the exudate. We weighed the filter paper before and after absorbing the exudate and calculated their weight in the filter paper. We also weighed the fibrinous sloughs. After calculating the weight, we transferred the filter papers or the fibrinous sloughs into 2 ml cryotube vials and mixed them with 1.0 ml of physiological saline. After we stirred the vials by vortex for 1 min, we collected the supernatants and stored them at −80°C until use.

ELISA

We measured the concentrations of VEGF165 and FGF-2 in the supernatants by relevant immunoassay kits from R&D Systems, Inc. (Minneapolis, MN) for VEGF165 and from Invitrogen Corporation (Camarillo, CA) for FGF-2, according to the manufacturer’s instructions. The VEGF165 and FGF-2 levels were calculated using standard curves obtained with recombinant VEGF165 (from 15.6 to 1,000 pg mL⁻¹) and with recombinant FGF-2 (from 15.6 to 1,000 pg mL⁻¹). We calculated the concentration of each growth factor in the exudate or the sloughs by the following formula.

The concentration of each growth factor in the exudate (µg g⁻¹):

\[ = \frac{[\text{the concentration of VEGF165 or FGF-2 in the supernatants (µg mL}^{-1})] \times 1.0 (\text{mL})}{[\text{the weight of the exudate (g)}]} \]

The concentration of each growth factor in the sloughs (µg g⁻¹):

\[ = \frac{[\text{the concentration of VEGF165 or FGF-2 in the supernatants (µg mL}^{-1})] \times 1.0 (\text{mL})}{[\text{the weight of the sloughs (g)}]} \]

Fig. 1. Schematic illustration of polyvinylidene film dressing (PVFD) and a pressure ulcer treated with PVFD.

(A) A schematic representation of PVFD. After cleansing with saline, the wound is simply covered with non-sterilized PVF, which is further sealed with non-woven adhesive tape. (B) An example of PVFD. A pressure ulcer is treated with PVFD.
Results

We collected 10 exudate samples from PUs with DESIGN scores ranging from 3 to 19. Irrespective of the DESIGN score, the location of PUs, the gender or the age of patients, the 10 samples contained from 2.79 to 13.3 \( \mu g \ g^{-1} \) of VEGF165. In contrast, only two exudate samples had measurable amounts of FGF-2, 2.03 and 0.21 \( \mu g \ g^{-1} \), respectively (Table 1). Similarly, all the fibrinous sloughs from PUs with DESIGN scores ranging from 9 to 10 also contained VEGF165, from 3.14 to 5.93 \( \mu g \ g^{-1} \), while only 2 samples contained much less FGF-2, 0.31 and 0.07 \( \mu g \ g^{-1} \) (Table 2). Again, although the number of the examined cases was limited, the concentration of VEGF165 or FGF-2 in the sloughs did not seem to show any correlation with the DESIGN score or the location of the PUs.

Discussion

In this study, we have clearly shown that the exudate and sloughs of PUs contain a substantial amount of VEGF165, which is consistent with the observation by Pufe et al. (2003) demonstrating that the granulation tissue of PUs contains VEGF165 by immunohistochemistry and Western blotting. These studies indicate that the granulation tissue of PUs actively synthesizes and secretes VEGF165. Recently, Labler et al. (2009) have demonstrated that the exudate of traumatic soft tissue wounds contains 8,396.5 ± 762.7 (pg mL\(^{-1}\)) during vacuum-assisted closure (VAC) therapy and 5,364.4 ± 695.0 (pg mL\(^{-1}\)) during therapy using Epigard\(^{\text{TM}}\) dressing. Although the method to recover the exudate is different between their procedures and ours, the VEGF165 concentrations in the exudate of PUs were comparable to those of traumatic soft tissue injury. These data suggest that PUs are not deficient in the production of VEGF165.

A variety of stimuli including hypoxia, growth factors, cytokines and oxidative stress can increase VEGF expression in many cell types involved in wound healing (Ferrara et al. 2003). Among 5 different VEGF isoforms, with 121, 145, 165, 189, and 206 amino acids, only the splice forms VEGF121, VEGF145, and VEGF165 are secreted. The signaling tyrosine kinase receptors, such as VEGFR-1, bind VEGF121 and VEGF165, while VEGFR-2 additionally binds VEGF145 (in addition to certain VEGF-related peptides). Interestingly, these receptors mediate different biological effects of VEGF. Namely, the activation of VEGFR-1 and VEGFR-2 induces cell migration and cell proliferation, respectively (Neufeld et al. 1999). Since endothelial cells of small blood vessels in the PU granulation tissue express only VEGFR-2 (Pufe et al. 2003), VEGF165 secreted by the PU granulation tissue may mainly function as an endothelial cell mitogen. The crucial role of VEGF165 in wound healing has been demonstrated by the impaired wound healing in an animal model of diabetes.

<table>
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<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Location</th>
<th>VEGF (( \mu g \ g^{-1} ))</th>
<th>FGF-2 (( \mu g \ g^{-1} ))</th>
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<td>M</td>
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<td>F</td>
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<td>65</td>
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U indicates undetectable amount. R and L indicate right and left, respectively.

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leptin-deficient (db/db) mouse, in which the administration of a topical formulation of rhVEGF165 accelerated cutaneous wound healing (Galiano et al. 2004). Thus, the retention of VEGF165 in PUs treated with PVFD would be a benefit for wound healing.

In contrast to VEGF, the exudate of most PUs did not contain detectable amounts of FGF-2. Consistent with this finding, Labler et al. (2009) have shown that the FGF-2 concentration in the exudate of traumatic soft tissue wounds is much less than the VEGF165 concentration, namely 361.9 ± 43.9 (pg mL⁻¹) of FGF-2 during VAC therapy and 452.4 ± 76.9 (pg mL⁻¹) of FGF-2 during therapy using Epigard dressing. Moreover, Corral et al. (1999) reported that wounding the skin up-regulated VEGF mRNA expression 6- to 7-fold, irrespective of whether the wound was ischemic, while wounding alone increased FGF-2 mRNA levels less than 2-fold over normal skin in both ischemic and nonischemic wounds. Recently, however, analysis of the cytokine profile in wound drainage fluids, which were obtained after incisional hernia repair (Di Vita et al. 2006), liposuction, abdominoplasty and breast augmentation (Aiba-Kojima et al. 2007), has demonstrated that FGF-2 production is higher on postoperative day 1, decreasing thereafter, while VEGF production increases progressively after the operation. Since the PUs we examined in this study had existed at least for 10 days after the onset, the lack of FGF-2 in the exudate of PUs may reflect the dynamics of growth factor secretion in the process of wound healing. These data suggest that the VEGF165 production is greater than that of FGF-2, at least in chronic wound tissues.

The detection of less FGF-2 in PU exudate might be ascribable to the nature of FGF-2. FGF-2 is a polypeptide that exhibits a wide range of in vitro biologic activities, such as stimulation of cell mitogenesis and chemotaxis. The mitogenic effects of FGF-2 are directed primarily toward cells of mesodermal or neuroectodermal origin. In addition, FGF-2 is a potent mitogen and chemoattractant for capillary endothelial cells (Robson et al. 1992). One of the most striking features of FGF-2 is the lack of a consensus signal sequence for secretion, whereas significant amounts of the 18-kDa form of FGF-2 are found outside the cell. The mechanism of secretion of the 18-kDa FGF-2 remains unclear, although it has been suggested that FGF-2 is released from cells as the result of cell damage, death and non-lethal membrane disruptions. In addition, FGF-2 is found in the extracellular matrix and basement membranes bound to heparan sulfate proteoglycan (Nugent and Iozzo 2000). Therefore, it is conceivable that FGF-2 produced in PUs cannot be recovered entirely from the exudate or sloughs.

Our study demonstrating the presence of a substantial amount of VEGF165 in the exudate of PU inspires us to reconsider the treatment of PUs. The efficacy of PVFD for the treatment of PU can be explained by the fact that it does not reduce VEGF165 activity. To achieve spontaneous wound healing, our treatment should take care to preserve the biological activity of factors such as VEGF in the exudate of PUs. We should reevaluate dressing products and methods from molecular biological approaches, to enhance the innate ability of wound healing.

References
