Late Dermal Effects of Breast Cancer Radiotherapy

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Riitta Riekki (*right*) defended her thesis on November 24th, 2006 in the University of Oulu, Finland. The thesis work was supervised by Professor Aarne Oikarinen (*middle*), and Faculty Opponent was Docent Sirkku Peltonen (*left*) from the University of Turku, Finland.

Radiotherapy is used in the treatment of breast cancer to reduce the local recurrence rates. Radiotherapy may induce both acute and late side effects. However, treatment alternatives for cancer are limited. The late side effects of radiation occur from a few months to several years after treatment. Fibrosis is a common late side effect of radiotherapy treatment for cancer. Fibrosis is characterized by a loss of flexibility and impaired function of the involved tissue. Other side effects of radiotherapy, such as neuropathy, pain and lymphoedema, are often associated with fibrosis. The pathogenetic mechanisms of radiation-induced fibrosis are not yet clear. In skin, the effects of radiation can be readily investigated.

Changes in collagen metabolism apparently have an important role in the formation of fibrosis. Collagens are the most abundant proteins of the extracellular matrix. They give tissues structural integrity and tensile strength. Over 20 distinct types of collagens have been characterized so far. Each collagen type has distinct tissue distribution, function and properties. In skin, collagen comprises 70-80% of its dry weight. The most abundant dermal proteins are type I and III collagens, which are produced by fibroblasts. Type I collagen comprises 80-85%, and type III collagen 10-15% of skin collagen. Type I and III collagens are synthesized as precursor molecules that have propeptides at both aminoterminal and carboxyterminal ends. These propeptides are cleaved off during the extracellular stages of collagen biosynthesis. Normally the balance between extracellular matrix protein synthesis and degradation is sustained by a complex system of cellular and cell-matrix interactions. Alterations in collagen synthesis are seen during ageing, in various pathological conditions, such as scleroderma, and in association with several medical therapies.

Matrix metalloproteinases (MMPs) are a group of enzymes capable of degrading virtually all extracellular matrix components. The MMPs have an essential role in collagen degradation. The proteolytic activity of MMPs can be inhibited by specific tissue inhibitors of matrix metalloproteinases, the TIMPs. The effects of irradiation of MMPs and their inhibitors, TIMPs, are not well documented.

While collagens provide tensile strength, the recoil properties of skin are largely due to elastin. Dermal elastin is synthesized by fibroblasts. In previous studies, elastic fibres in radiotherapy-treated human skin have not been analysed.

Tenascin is a large glycoprotein of the extracellular matrix. In most adult tissues its expression is restricted or absent. In skin, tenascin has been associated with wound healing and various malignant lesions. Tenascin expression in radiotherapy-treated human skin has not been previously studied.

Mast cells in the dermis may have a role in escalating fibrosis. Several lines of evidence suggest that mast cells increase fibroblast proliferation and collagen synthesis.

The purpose of this thesis study was to examine the effect of breast cancer radiotherapy on human skin collagen metabolism and on certain other components of skin extracellular matrix, such as elastic fibres and tenascin. The histological findings and physio-mechanical properties of radiotherapy-treated skin were compared with those of non-treated skin. Wound regeneration in radiotherapy-treated skin was studied using a suction blister model. The possible role of mast cells in radiation-induced fibrosis was also studied.

Skin collagen synthesis was found to be markedly increased as a consequence of radiotherapy. The level of aminoterminal propeptide of type procollagen (PINP) in suction blister fluid reflecting the actual local type I collagen synthesis in vivo was increased 2.8–3.8 fold in irradiated skin compared to non-treated skin. The level of aminoterminal propeptide of type III procollagen (PIIINP) in suction blister fluid, reflecting local type III collagen synthesis in skin, was 2.2–3.4 fold in radiotherapy-treated skin compared to control skin. These findings are in accordance with previous results (Autio et al. 1998). The amount of PINP measured from skin biopsies was also significantly increased in irradiated skin, and these values correlated markedly with the level of PINP in suction blister fluid samples. The levels of circulating procollagen propeptides remained within normal range, further indicating that radiotherapy caused only a local increase of skin type I and III collagen synthesis.

The number of PINP-positive fibroblasts was also significantly increased in irradiated skin, indicating increased type I collagen production in fibroblasts as a result of radiotherapy. Intracellular PIIINP could not be detected immunohistochemically. This could be due to the fact that the intracellular level of PIIINP is much lower than that of PINP.

Type I and III collagen synthesis was also studied on the RNA level using in situ hybridization. A significantly increased number of fibroblasts positive for type I and III collagen mRNA was found in irradiated skin. There was a significant correlation between the number of type I collagen mRNA positive fibroblasts and the level of PINP in suction blister fluid. These findings suggest that type I and III collagen synthesis was increased in radiotherapy-treated skin on both protein and transcriptional levels. Also, skin thickness and skin stiffness were found to be increased on the radiotherapy-treated side, which could be due to an accumulation of dermal connective tissue components as a result of irradiation. It should also be noted that an increase of protein content also results in an increase of protein-bound water in skin. This may also contribute to thickening of skin after radiotherapy (Lahtinen et al. 1999).

The concentration of type I collagen degradation marker (SP4) was increased in the soluble biopsy extracts of irradiated skin. There was also a significant correlation between SP4 and the marker of type I collagen synthesis (PINP), indicating an increased turnover of type I collagen in irradiated skin.

Measured from insoluble tissue digests, an increase of cross-linked type I collagen in irradiated skin could be found. The main cross-link type in irradiated skin was HHL (histidinohydroxylynonorleucine), similarly to that in normal skin (Mechanic et al. 1987). This finding suggests that the microstructure of collagen fibres in irradiated skin is conserved. It has also been shown that during normal ageing the content of HHL-cross-linked type I collagen increases in skin (Yamauchi et al. 1988). The findings in irradiated skin thus resemble those seen in the ageing process of skin. A similar increase in concentration of HHL-cross-linked type I collagen has also been found in skin samples of scleroderma patients (Ishikawa et al. 1998).

The levels of MMP-9, MMP-2/TIMP-2 complex, TIMP-1 and TIMP-2 were determined in both SBF and tissue extracts (Öberg et al. 2000). In irradiated skin, the level of MMP-2/TIMP-2 complex was found to be increased in SBF. Measured in tissue extracts, however, the concentration of MMP-2/TIMP-2 complex was lower in irradiated skin compared to non-treated skin. In addition, no significant difference could be found in the levels

of TIMP-1 and TIMP-2 measured in tissue extracts, but in suction blister fluid the level of TIMP-1 was higher in treated than in non-treated skin. This suggests that no significant alteration of collagen degradation capacity in irradiated skin could be detected using the aforementioned markers.

No difference in the morphological appearance or proportional area of elastic fibres could be found between radiotherapy-treated and non-treated skin. The reason for higher values in irradiated skin measured by elastometer could thus be an accumulation of connective tissue, especially collagens, as a result of irradiation.

The amount of tenascin immunostaining was markedly increased in irradiated skin. The exact role of tenascin in vivo is not clearly defined. In normal adult human skin, the expression of tenascin is fairly low (Lightner et al. 1989). An increase of tenascin expression has previously been shown in several pathological conditions, such as scleroderma (Lacour et al. 1992), and also in wound healing (Latijnhouwers et al. 1996) and after topical retinoid treatment (Haapasaari et al. 1997). In addition, upregulation of tenascin expression has previously been found in pig skin as a result of irradiation (Geffrotin et al. 1998). Accumulation of tenascin could be due to and induction of cytokines by irradiation. TGF-β is a cytokine that has been shown to be induced by irradiation (Barcellos-Hoff 1993), and also to increase tenascin deposition by fibroblasts (Gentilhomme et al. 1999).

The number of blood vessels in irradiated skin was slightly increased, and the increase was more pronounced in upper parts of the dermis. The mechanism behind this is not known. These findings could be due to the activation of angiogenetic factors, such as TGF-β, as a consequence of irradiation, or due to the vascular wall damage caused by irradiation. Despite the increase in their number, all the blood vessels in irradiated dermis may not be functional. In blood flow measurements using Laser-Doppler, no difference between radiotherapy-treated skin and non-treated skin could be found.

No difference could be detected in restoration of epidermal barrier function rate between radiotherapy-treated and non-treated skin areas, as measured by the reduction of TEWL as well as by measurements of skin blood flow, skin colour and erythema in suction blister bases. This suggests that the epidermal regeneration rate is not affected by the changes in collagen synthesis, and that the epidermal regeneration rate reflecting keratinocyte migration and differentiation remained intact in irradiated skin. This finding is in agreement with previous results on scleredema skin, where collagen synthesis was markedly increased while the restoration of epidermal barrier function was intact (Haapasaari et al. 1996).

The number of mast cells positive for tryptase, chymase and Kit receptor was significantly increased in the upper dermis of radiotherapy-treated skin compared to non-treated control skin. This finding suggests that mast cells may be one factor contributing to the increased collagen synthesis and fibrosis induced by radiotherapy. This is in line with previous studies. Previously, mast cells have been found to be involved in the pathogenesis of scleroderma and in wound healing, and several mast cell cytokines such as TGF-β, have been associated with fibrogenesis (Irani et al. 1992, Trabucchi et al. 1988, Kanbe et al. 2000, Hermes et al. 2001). The most abundant proteins in mast cells, tryptase and chymase, have been shown to induce collagen synthesis and to stimulate fibroblast proliferation (Abe et al. 1998). Histamine, a mast cell mediator, is also able to induce collagen synthesis in skin fibroblasts (Hatamochi et al. 1991).

The findings of the present study offer new information on the mechanisms of radiation-induced fibrosis, which would be necessary in order to find methods to prevent or treat such conditions.

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