Radiotherapy and wound healing

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ABSTRACT

This review article discusses basic radiation physics and effects of radiation on wounds. It examines various postulated hypothesis on the role of circulatory decrease and radiation-induced direct cellular damage. The new concept related to the radiation pathogenesis proposes that there is a cascade of cytokines initiated immediately after the radiation. Sustained activation of myofibroblasts in the wound accounts for its chronicity. Recent advances highlight that transforming growth factor β1 is the master switch in pathogenesis of radiation fibrosis. This articles overviews its role and summarises the available evidences related to radiation damage. The goal of this article was to provide its modern understanding, as future research will concentrate on antagonising the effects of cytokines to promote wound healing.

Key words: Complications • Fibrosis • Radiotherapy • Transforming growth factor β

Key Points

- this article examines various theories related to the radiation damage to wounds and highlights the essential changes observed in different stages of wound healing that are affected by the radiotherapy
- the direct effect of radiation on tissues ionizes DNA
- the indirect effect through hydrolysis is more harmful and causes two thirds of the total cellular damage
- mutation frequency is linearly related to the radiation dose
- consequences may include healing with no damage, complete loss or total rearrangement of genetic material
- injury to the irradiated tissue can be divided into acute or delayed
- both types of injury are time and dose dependent

INTRODUCTION

Despite continuous advances in the devices that deliver therapeutic radiation, complications are still frequent. The incidence of complications is in the order of 50% (1). Most of these are because of hypovascularity and fibrosis. The present article examines various theories related to the radiation damage to wounds. It highlights the essential changes observed in different stages of wound healing that are affected by the radiotherapy.

Ionising radiation can be electromagnetic or particulate (2). Electromagnetic radiation involves high-energy photons in the form of X-rays and gamma rays. X-rays are produced externally by electrical machines. Gamma rays are produced by the decay of radioactive isotopes, such as cobalt 60 (2).

In cell cycle, G2 and M phase are highly susceptible, and S phase is the most resistant. Canti and Spear observed the division delay in chick fibroblasts in vitro when exposed to various doses of radiation (3). Cells stop and attempt to repair the damage after G2 phase. The effect of radiation on tissues can be direct or indirect. The direct effect ionises DNA. It is the indirect effect through hydrolysis that is more harmful. High-energy electrons interact with water molecules producing highly reactive free radicals. Two thirds of the total cellular damage is because of these hydroxyl radicals (OH^-). Mutation frequency is linearly related to the radiation dose. Structural changes induced in chromosomes by radiation include single break, multiple breaks or chromosome clumping. Consequences may include healing with no damage, complete loss or total rearrangement of genetic material (2).

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EFFECTS OF RADIATION

Mammalian cells of all types are radiosensitive in the range of 110–240 rads. Severity of the effects is directly related to absorbed dose. Other factors include the volume of the irradiated tissue, type of radiation, protraction and the time interval between radiation and the observed clinical effects (4). Injury to the irradiated tissue can be divided into acute or delayed. Acute direct effects are associated with necrosis of rapidly dividing cells, such as skin, mucosa of intestine or epithelial lining of oropharynx. Delayed effects result from progressive stromal lesions, the actual impact of which comes from progressive

fibrosis. Although acute changes are more affected by timing, and chronic changes are more affected by total dose, both types of injury are time and dose dependent (2).

ACUTE AND CHRONIC EFFECTS ON SKIN AND MUCOSA

Early signs of epithelial damage involve erythema, warmth and tenderness. This is because of transient dilatation of vessels caused by vasoactive amines (2). The second phase of erythema is because of vessel damage seen 2–3 weeks after the radiation. The third and the final wave of erythema occurs 10–16 weeks after the initial injury and is related to the epidermal basal cell death. Sweat glands are radioresistant. Dry desquamation or hyperpigmentation may occur (5).

Chronic effect can be seen in 4–6 months after irradiation. Grossly, the skin becomes firm with thin epidermis, which leads to easy damage. A 'classic radiation wound' results from collective effect of fibrosis, necrosis, ulceration and fistula formation. Hyper or hypopigmentation of the skin results from increased activity of melanocytes or destruction of melanocytes. Alopecia or telengiectasis may appear.

ACUTE OR CHRONIC EFFECTS ON MUSCLES OR BONES

Adult bones are radioresistant, and changes in bone are vascular in nature. It is sometimes difficult to differentiate between myelitis of bone from radiation injury. Radiation-induced muscle damage is infrequent but atrophy can be seen at 2400–5000 cGy.

FRACTIONATION

Irradiated wounds are susceptible to infection and poor wound healing. Modern radiation therapy treatments are given in daily fractions over an extended period (up to 6 or 8 weeks), so that a high total dose is given to the tumour while ideally sparing normal tissues. The biological effects on tissue from this fractionation therapy depend on the four Rs of radiation biology. These are repopulation, redistribution, repair and reoxygenation. It is believed that development of fibrosis is less severe with fractionation (6). Conversely, Ozbek et al. showed reduced tensile wound strength after hyperfractionated radiotherapy in the early evaluation period as compared with the control group (7). They showed increased fibrosis histologically in the irradiated rats. It is important to note here that the surgery was performed 3 weeks after the radiation. In existent clinical setting, for example, in breast cancer surgery, surgery precedes radiotherapy (7). It is probable that entirely different mechanisms may be accountable for healing in preirradiated wounds.

HYPOTHESIS

According to Meyer, classic sequence in the pathogenesis of radiation damage was the product of radiation, trauma and infection. In early 1980s, Marx challenged this hypothesis (8,9). He studied 26 clinical cases of osteoradionecrosis of mandible and deduced that the infection was the result of contamination, and trauma was a result of breakdown. He emphasised that traumatic breakdown of the bone and infection were the outcome of radiation and not the result of aetiological factors.

Marx introduced 'three-H' principle for the pathogenesis. He implicated a sequence of radiation, hypoxic–hypocellular–hypovascular tissue, tissue breakdown and chronic non healing wound (8,9). Marx indicated that the body relies on normal cellular turnover to maintain a healthy function. This turnover is retarded after radiotherapy, and this in turn results in fibrosis and wound breakdown. This theory is supported by the absence of pus and classic inflammatory signs found at the site. Sublethal damage from radiation leads to endarteritis obliterans, which renders the area hypoxic and hypovascular. Fibrosis ensues following an irreversible damage. It is supported by an autopsy study of 17 cases of osteoradionecrosis of mandible performed by Bras et al. (10) It showed radiation-induced obliteration of inferior alveolar artery, which is the main blood supply to the mandible. The most characteristic lesion seen with the radiation change is eccentric myointimal proliferation of small arteries and arterioles (2).

Marx emphasised the significance of hypoxia and hypovascularity as major mechanism of radiation-induced damage (8,9). Thus, improving blood supply should result in enhanced clinical wound healing. Clinically, this is evident after postradiational abdominoperineal resections for rectal tumours, where reinforced vascularised rectus abdominis muscle flaps prevent the perineal wound complications and breakdown (11).

Key Points

- early signs of epithelial damage involve erythema, warmth and tenderness
- chronic effect can be seen in 4-6 months after irradiation; the skin becomes firm with thin epidermis, which leads to easy damage
- a 'classic radiation wound' results from collective effect of fibrosis, necrosis, ulceration and fistula formation
- adult bones are radioresistant, and changes in bone are vascular in nature
- it is believed that development of fibrosis is less severe with fractionation
- Marx indicated that the body relies on normal cellular turnover to maintain a healthy function
- this turnover is retarded after radiotherapy, and this in turn results in fibrosis and wound breakdown

Key Points

- Rudolph was the first to indicate that chronic radiation damage is a direct result of cellular effect and not of circulatory decrease
- he envisaged that it was the tension in the wounds along with malnutrition that contributed to poor healing
- differentiation of fibroblasts into myofibroblasts with resultant apoptosis are also features of tissue remodelling
- the new concept related to the radiation damage proposes that there is a cascade of cytokines initiated immediately after the radiation
- recent advances highlight the fact that TGF-b isoforms (b1, b2 and b3) play an essential role in tissue fibrosis and pathogenesis of radiation-induced damage
- the fibrotic process is not governed by a single cytokine but a complex network of other cytokines as well

Although Marx's theory of decrease in circulatory capacity is widely accepted and probably clinically more applicable, Rudolph was the first to indicate that chronic radiation damage is a direct result of cellular effect and not of circulatory decrease (12–17). He proposed this on the observation finding that irradiated tissues often bleed profusely. He showed the presence of myofibroblasts in radiation ulcers 14–54 weeks after radiotherapy in rats. He indicated that delayed wound contraction was the result of delayed onset of myofibroblast development, independent of blood supply. He showed good perfusion of ulcer margins with fluorescein. He measured transcutaneous oxygen pressure and showed no decrease in their levels, decades after the radiotherapy. He envisaged that it was the tension in the wounds along with malnutrition that contributed to poor healing. He suggested that avoidance of tension could lead to better surgical results.

Qu et al. showed similar results and decreased proliferation, adhesion and increased apoptotic ratio of fibroblast after whole body radiation (18). It is the traditional practice to avoid irradiation of skin grafts after oncologic reconstruction. Recent experience from Memorial Sloan-Kettering Cancer Center illustrated quite encouraging results. In a retrospective analysis of 30 patients, only one patient had complete and two had partial skin loss after radiotherapy (19). This clinical study supports Rudolph's experimental work.

NEW CONCEPT OF SUSTAINED MYOFIBROBLAST ACTIVATION AND ROLE OF GROWTH FACTORS

Since long time, fibrotic tissues have been considered as an irreversible dead scar. Recent evidences suggest that fibrosis is a dynamic process, which involves long-term fibroblast activation and continuous remodelling. Fibroblasts play a central role in fibrogenesis and wound contraction. In normal wound healing, fibroblasts are transiently activated into myofibroblasts to proliferate and deposit the extracellular collagen matrix. Differentiation of fibroblasts into myofibroblasts with resultant apoptosis are also features of tissue remodelling (20). When feedback mechanisms are downregulated, fibroblasts disappear by apoptosis at the completion of normal wound healing. In fibrosis, on the contrary, the feedback regulations are not observed. There is a sustained activation of myofibroblasts. The abnormal stimulating factors seem to be various cytokines and growth factors (21). Various cytokines are found to be deregulated following radiation exposure. They are transforming growth factor β (TGF-β), platelet-derived growth factor (PDGF), tumour necrosis factor (TNF)-α, interleukin-1 and interleukin-4.

The new concept related to the radiation damage proposes that there is a cascade of cytokines initiated immediately after the radiation. Cytokine activities continue to persist in clinically silent period and leads to the development of late damage. TGF-β is involved in a wide spectrum of biological activities. It stimulates connective tissue deposition through chemotaxis of fibroblasts and mast cells (22–24). It is involved in production of collagen and prevents its degradation. It is also implicated in the regulation of angiogenesis (25). Recent advances highlight the fact that TGF-β isoforms (β1, β2 and β3) play an essential role in tissue fibrosis and pathogenesis of radiation-induced damage. TGF-β binds to at least three membrane proteins called receptor types I, II and III (26). Type I and II receptors are serine–threonine kinases, and type III is a proteoglycan that has no signalling structure but acts so as to present TGF-β to the other receptors.

Excessive and sustained production of TGF-β is vital for tissue fibrosis (26). TGF-β1 is considered as a master switch for the fibrotic programme. It is believed that TGF-β1 is more important than TGF-β2 and TGF-β3 in radiation-induced enteropathy (25). Martin et al. showed rapid induction of TGF-β in skin within 6 hours of the gamma irradiation (21). He detected the presence of both the protein as well as the messenger RNA (mRNA). Their results suggested early induction of c-fos, c-jun and beta-actin mRNAs in irradiated skin. Biopsies obtained from patients 6 months to 20 years postradiation showed continued expression for collagens I, III and TGF-β, suggesting their enhanced activity (21).

The role of TGF-β is more complex. Local administration of TGF-β1 helps in tissue inflammation by increasing leucocyte adhesion and chemotaxis. Systemic administration opposes this effect. It decreases endothelial cell expression of adhesion molecules. This can be explained by the fact that the fibrotic process is

not governed by a single cytokine but a complex network of other cytokines as well. Also, TGF-β has a regulatory effect on other cytokines such as TNF and PDGF. However, TGF-β-signalling pathway plays a central role in radiation-induced damage.

Altered levels of TGF-β are reported to promote fibrosis and to suppress vascularisation during wound healing. Wehrhan et al. showed that neutralising of TGF-β1 activity led to increased expression of TGF-β III receptor and increased vascularisation (27,28). They suggested that TGF-β III receptor is associated with newly formed blood vessels during neovascularisation in wound healing. TGF-β plays a critical role in vascular compartment, involved in pathogenesis of chronic radiation injury. It downregulates the expression of thrombomodulin (29). Thrombomodulin is the measure of the endothelial function. Decreased thrombomodulin leads to hypercoagulation platelet aggregation. Platelets are abundant with TGF-β. Their aggregation releases more TGF-β. Decreased thrombomodulin also leads to autoinduction of TGF-β synthesis and upregulation of adhesion molecules and inflammatory cell chemotaxis. Wang et al. suggested that these self-perpetuating processes may be important in the chronicity of radiation-induced wound damage (25).

The role of cytokine is further supported by the study carried out by Chung et al. (30). It is the first study to examine the cytokine- and gene-modulatory effects of the inhibition of histone deacetylase (HDAC) inhibitor in cutaneous radiation syndrome. HDAC inhibitors (e.g. phenyl butyrate and valproic acid) promote healing of wounds caused by radiation. They also reduce skin fibrosis. Their effect correlated with the suppression of radiation-induced expression of TGF-β and TNF-α. They believe that the radiation-induced damage could be regarded as the genetic disorder of wound healing. HDAC inhibitors exhibit their effect by counteracting hyperacetylation. Histone hyperacetylation results in upregulation of cell-cycle inhibitors, downregulation of oncogenes and repression of inflammatory cytokines. HDAC inhibitors induce cell-cycle arrest, cell differentiation and fibrosis in inflammatory diseases. It is apparent that release of TNF-α and TGF-β is triggered by altered genetic programming of cell differentiation and proliferation from radiation. By associating results clinically, histologically and immunohistochemically, they have shown an alternative way of counteracting radiation-induced fibrosis.

Marx's hypothesis of decreased circulatory capacity and Rudolph's emphasis on cellular events are both equally important. Recent knowledge of cytokines may explain both the hypotheses. Decreased levels of thrombomodulins and identification of TGF-β III receptors in neovascularised vessels can explain the vascular effects of radiation. Although Marx labelled the radiated tissue as hypocellular, it has been confirmed that 'active remodelling' continues in dead scar tissue with the help of cytokines. Rudolph's experimental work is probably a beginning of the cellular research, explaining the important role of fibroblast in radiation-induced changes. Sustained activation of cytokines play the major role in these changes, and future research in this area will unfold many events that are currently beyond our understanding (31).

Anti-TGF-β treatment and HDAC inhibitors are still limited in their clinical use. Use of antibiotics, hyperbaric oxygen therapy, and free and pedicle flaps for revascularisation of ischaemic wounds play a key role in existing management of radiation wounds.

CONCLUSION

Complications following radiotherapy are still commonly seen. Acute changes are time dependent, and chronic effects are dose dependent. The most commonly accepted theories explaining the effects of radiation therapy focus on decreased vascularity and hypoxia of the tissue. More recently, new concept of sustained activity of myofibroblasts because of uncontrolled and excessive effect of cytokines especially TGF-β1 is gaining popularity. Future research will concentrate on antagonising the effects of cytokines to reverse or prevent the radiation-induced damage.

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Key Points

- it is apparent that release of TNF-a and TGF-b is triggered by altered genetic programming of cell differentiation and proliferation from radiation
- the new concept of sustained activity of myofibroblasts because of uncontrolled and excessive effect of cytokines especially TGF-b1 is gaining popularity
- future research will concentrate on antagonizing the effects of cytokines to reverse or prevent the radiation-induced damage

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