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Review article

The radiotherapeutic injury – a complex 'wound'

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Abstract

Radiotherapeutic normal tissue injury can be viewed as two simultaneously ongoing and interacting processes. The first has many features in common with the healing of traumatic wounds. The second is a set of transient or permanent alterations of cellular and extracellular components within the irradiated volume. In contrast to physical trauma, fractionated radiation therapy produces a series of repeated insults to tissues that undergo significant changes during the course of radiotherapy. Normal tissue responses are also influenced by rate of dose accumulation and other factors that relate to the radiation therapy schedule. This article reviews the principles of organised normal tissue responses during and after radiation therapy, the effect of radiation therapy on these responses, as well as some of the mechanisms underlying the development of recognisable injury. Important clinical implications relevant to these processes are also discussed.

Keywords: Radiation injury; Wound repair; Early and late effects

1. Introduction

Radiotherapeutic injury is a complex process that occurs in *organised* tissues, i.e. tissues which comprise a large number of interacting, mutually dependent cellular lineages, as well as a multitude of biologically active extracellular molecules. This perspective is in some contrast to the more traditional (minimalist) approach that considers injury to individual cell lines that can be modelled by cell culture. All organised tissues are capable of mounting reparative responses to injury. This review examines some of these responses and draws attention to some unique phenomena that occur as a result of *repetitive* injuries – the series of exposures to ionising radiation that make up a course of radiotherapy.

The response of normal tissues to radiotherapy can be viewed as comprising two partially interacting components, each of which is very complex. The first is a process that in many, but not all, respects resembles the healing of traumatic wounds, while being subject to perturbation by the radiation treatment. The second is a set of specific injuries that affect virtually all cellular and extracellular components within the irradiated volume, and that may be responsible for the progression of injury over a period of many years.

The radiotherapy 'wound' differs in interesting ways from acute traumatic, thermal or chemical wounds, in which structural tissue damage occurs instantaneously, or nearly so. In contrast to these types of injury, exposure to ionising radiation produces a burst of free radicals, which, while obviously not re-arranging tissue components immediately, not only causes DNA damage, but also alters proteins, lipids, carbohydrates, and complex molecules. While the amount of energy deposited is minimal, each exposure inflicts considerable injury. Another important characteristic of radiation therapy is that it inflicts a *series* of small tissue insults as each fraction is delivered. In many tissues, each fraction thus contributes to accumulating inflammatory cell recruitment as well as to the accumulation of direct tissue injury. Furthermore, each fraction affects tissue that already exhibits a dynamic spectrum of cellular injury, ongoing repair, inflammation, and other pathophysiologic responses. Therefore, with repetitive radiation exposure, many cellular and molecular responses will be substantially exacerbated, suppressed, or substantially altered compared to the situation after a single exposure to radiation or traumatic injury.

Rate of dose accumulation (RDA) is important to all of these processes and sometimes quite independently of fraction size. First, the timing and magnitude of the inflammatory response to radiotherapy depends on RDA, since inflammatory responses do not 'fade' (or cease) within hours of each

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radiation exposure as 'sublethal' cellular damage generally does. Therefore when a course of radiotherapy involves rapid dose accumulation (i.e. is 'dose intense'), regardless of how it is fractionated, the inflammatory response also accumulates quickly. This may be important because aspects of the inflammatory response are capable of greatly amplifying radiation-induced microvascular injury. Second, suppression of reparative tissue responses to injury depends on RDA, since such responses, be they re-epithelialisation or the formation of 'granulation' tissue, include vigorous proliferation of several cell lineages. Therefore, the more rapidly dose is delivered, regardless of fraction size, the more effective the suppression is.

While what appears as full 'healing' of the sub-acute radiotherapy injury may ensue, perturbation of the reparative processes affect the integrity of the repair. It is well known that healed traumatic wounds 'remodel' continuously for years following injury. In contrast, the viability of irradiated tissues and/or their capacity to remodel is often compromised by lasting cellular dysfunction or changes in the supporting (mesenchymal) stroma. Inflammation may further stress irradiated epithelial lined tissues if failure of the reparative process results in insufficient epithelial barrier function. Coupled with this, and sometimes compounded by self-perpetuating 'reactive' fibrosis, progressive parenchymal cellular depletion with 'replacement' fibrosis completes a picture that is recognised as delayed radiation injury.

The main part of this review deals with organised tissue responses to radiotherapy, the effect of radiotherapy on these responses, and the development of recognisable injury. The final part of the review discusses some important clinical implications of these observations. To assist readers with some of the analogies and comparisons that are drawn in this review, a simplified schematic diagram of processes involved in healing of traumatic wounds is presented in Fig. 1. While it will be immediately apparent that, there is little requirement for haemostasis and tissue closure by scar formation in radiotherapy injury as there is in a traumatic wound, it will be equally obvious that many processes that contribute to healing of traumatic wounds are also involved in early and delayed normal tissue responses to radiation.

Examination of radiotherapeutic injury from this perspective is helpful because it provides explanations both for the clinical and pathological features of early and delayed injuries. An understanding of the mechanisms that contribute to organised tissue injury together with an appreciation of the reparative processes that occur in response to this injury, may assist in the development of avoidance and prevention strategies.

2. Part 1 – normal tissues responses to radiotherapy

2.1. The induction of a 'wound healing' response

Higher vertebrates respond to traumatic tissue injury by initiating a sequence of overlapping events that includes activation of the coagulation system, inflammation, epithelial regeneration, granulation tissue formation, and matrix deposition and remodelling. This complex process is orchestrated by a large number of interacting molecular signals, including cytokines, chemokines, and growth factors.

While the response to radiotherapeutic injury of normal tissues differ in many ways from a traumatic wound healing response, many processes are similar and/or occur in a similar sequence. For example, as indicated above, an important difference between radiation injury and most traumatic wounds is the accumulating and repetitive nature of the former. Because of ongoing cellular regeneration and increasing inflammation *during* a course of radiation therapy, the 'normal' tissue that is included in the radiation field changes dramatically from the time of delivery of the first fraction to the delivery of the last fraction. In other words, the normal tissue that is irradiated at the beginning of the radiotherapy course is qualitatively very different from the 'normal' tissue that is irradiated towards the end.

The following sections describe aspects of the response of normal tissue to radiation injury, focusing primarily on similarities and differences between the responses to radiation injury and the response seen after physical trauma.

2.2. Endothelial cell (EC) changes and activation of the coagulation system

Activation of the coagulation system is the initial response to virtually all forms of traumatic injury. While radiation injury does not physically disrupt blood vessels, the coagulation system may also become activated in structurally intact vessels by direct functional radiation effects. For example, radiation-generated reactive oxygen species may cause immediate inactivation of thrombomodulin (TM) on the EC surface, a process which is greatly potentiated in the presence of inflammation [1,49]. Subsequent endothelial effects of radiation include apoptosis [117], and altered expression of adhesion molecules, tissue factor, von Willebrand factor, prostacyclin, angiotensin converting enzyme, plasminogen activator, thromboxane, and TM [57,73,134,161,171,172,186]. Down-regulation of EC nitric oxide synthase (NOS), TM, and plasminogen activator (PA) activity may be permanent, thus creating a permanently pro-coagulant EC surface. These processes, in concert with other endothelial effects, may initiate and 'drive' the processes recognised as delayed radiation injury.

Activation of the coagulation system increases the formation of the serine protease, thrombin. Thrombin, plays a central role in coagulation by removing fibrinopeptides A and B from fibrinogen and activating platelets, thus forming the fibrin–platelet clot. However, thrombin is also an important regulator of cell proliferation, inflammation, and tissue remodelling. For example, thrombin regulates endothelial permeability [26], chemotaxis of neutrophils and monocytes

[10,16], and TGF-β1 production [182] by mechanisms that are independent of coagulation. Specific inhibitors of thrombin decrease smooth muscle cell proliferation, migration, and collagen production in vitro and in vivo [112,123]. Most of the 'non-coagulant' thrombin effects are mediated through activation of receptors that belong to the family of protease-activated receptors (PAR), of which PAR-1 is the best studied and likely the most relevant biologically.

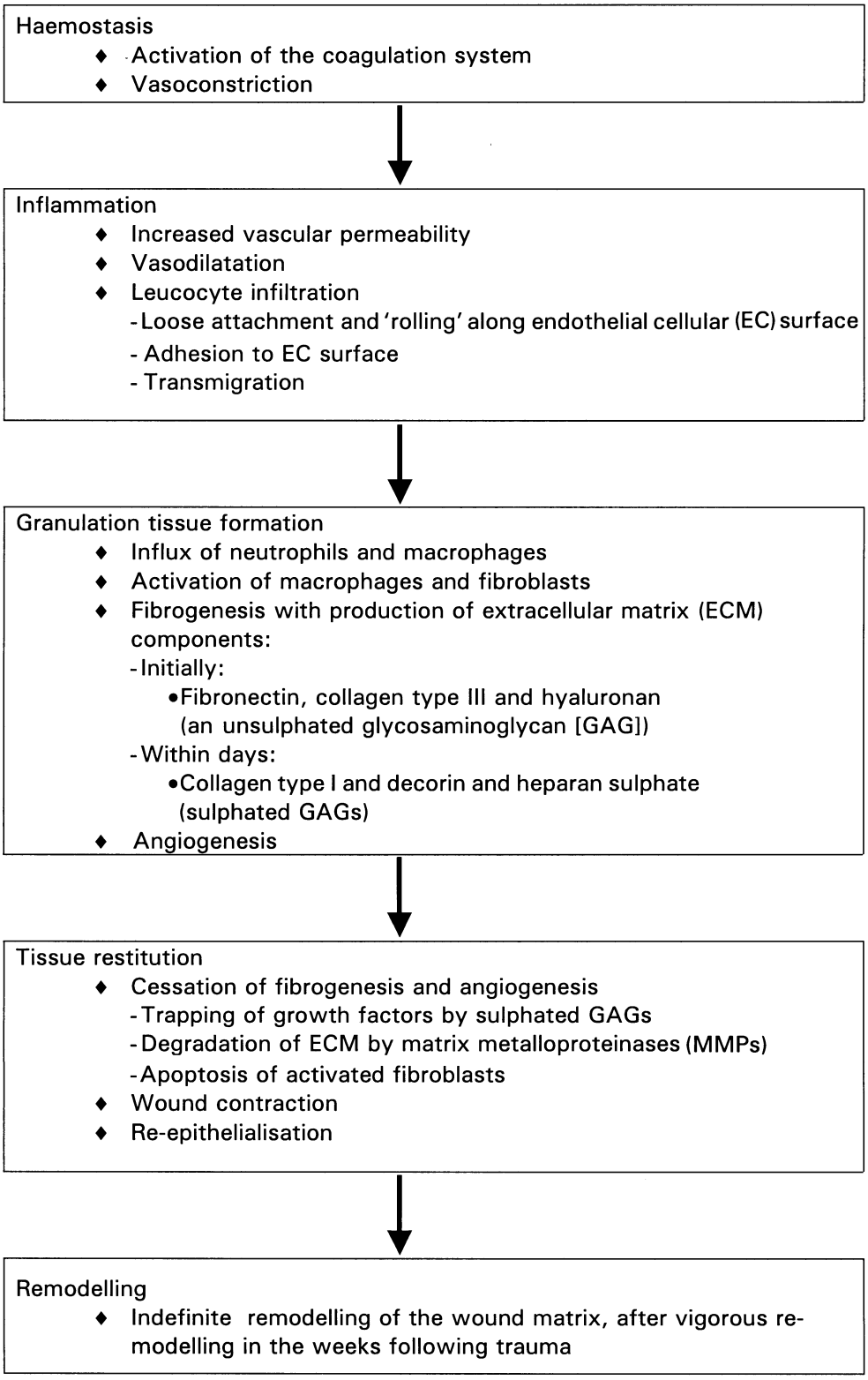

Fig. 1. Overview of normal wound healing processes (using acute trauma as an example).

Table 1
Important direct non-lethal effects of radiation on endothelial cells

Plasma membrane effects:
- Production of reactive oxygen intermediates (ROIs) [109] leads to:
  - Irreversible inactivation of thrombomodulin
  - Inhibition of circulating 5HT removal by pulmonary ECs;
  - Increased phospholipase A2 (PLA2) activity, leading to:
    - ➡ Release of arachidonic acid (AA)
    - ➡ Generation of prostanoids including thromboxane (TXA_2)
    - ➡ Initial decrease in prostacyclin (PGI_2) production in first 4 h of exposure followed by a temporary increase

  Accumulation of diacylgycerol (DAG)
- Activation of tyrosine–specific protein kinases [109] leads to:
  - ➡ Activation of protein kinase C (PKC) (see below)
- Decrease in NOS and PGI_2 activity [148] leads to:
  - ➡ Failure to oppose vasoconstricting influences and platelet aggregation;
  - ➡ Failure to inhibit smooth muscle cell (SMC) proliferation
- Decrease in transglutaminase activity [85] has implications for:
  - ➡ Cell growth and differentiation
- Downregulation of thrombomodulin [127] promotes:
  - ➡ A failure to inhibit intravascular coagulation hence induces a 'procoagulatory' environment
- Downregulation of plasminogen activator activity [155] leads to:
  - ➡ A decrease in fibrinolytic activity and promotes a procoagulatory environment

Intracellular effects:
- Activation of protein kinase C (PKC) [61,142] leads to:
  - ➡ Transcription of tumour necrosis factor α (TNF-α) gene;
  - ➡ Transcription of early response genes C-jun and C-fos
- Activation of early response genes [81] leads to:
  - ➡ Activation of the AP-1 and nuclear factor κB (NFκB) transcription factors
- Activation of NFκB [12,58,108] leads to:
  - ➡ Cell surface expression of leucocyte adhesion molecules P-Selectin, E-Selectin and ICAM-1, etc.
- Luminal release of:
  - ➡ Von Willebrand factor (vWF) [59];
  - ➡ Platelet derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) [181]

Table 1 summarises some of the important direct non-lethal effects of radiation on ECs.

2.3. Inflammation

2.3.1. Direct inflammatory effects

Some aspects of radiation-induced inflammation result from direct non-lethal radiation effects. An example is the first phase of the erythematous skin reaction in humans, which commences in a matter of hours after relatively small radiation exposures a vasodilation often undetectable by the naked eye can be measured using sensitive instrumentation [144]. The magnitude of the effect varies across species [74,124].

The mechanisms by which radiation causes increased vascular permeability and vasodilation are becoming better understood. These include direct radiation effects on mast cells [118] and ECs resulting in the generation of thrombin and the release of histamine and prostaglandins I_2 and E_2 (PGI_2 and PGE_2), facilitated by neutrophil adhesion to the endothelial surface in the hours following radiation exposure [38,104,116,121]. Activation of the complement and kinin cascades may also contribute [22]. Cobra venom factor depletion of complement, for example, has been shown to suppress both increased vascular permeability and subsequent fibrosis in the skin [158].

After physical trauma, the acute inflammatory response is triggered by activation of stress-sensitive kinases and transcription factors that control the synthesis of pro-inflammatory cytokines, such as TNF-α, IL-1, IL-8, and IFN-γ. Subsequently, termination of inflammation occurs as a result of the short half-life of pro-inflammatory cytokines and by production of anti-inflammatory cytokines, such as, IL-4, IL-10, IL-13, and TGF-β [83]. Situations, in which inflammation does not adequately resolve, such as radiation injury, appear to involve aberrant cytokine pathways or chronic overproduction of certain cytokines, resulting in uncontrolled matrix accumulation and fibrotic sequelae.

During radiation therapy, many of the inflammatory phenomena that evolve in response to each radiation fraction does not dissipate within 24 h, thus leading to an accumulating response ('fractionated inflammatory insult'). Furthermore, the processes involved in leucocyte adhesion appear to be both dose and dose-rate dependent. In an in vivo rodent model Molla et al. found that P-selectin and intercellular adhesion molecule-1 (ICAM-1) expression were increased to a greater extent in rats irradiated at high dose-rate than in rats irradiated at medium dose-rate to the same total dose [104]. Ross et al. found that expression of

interleukin-1β (IL-1β) and IL-6 mRNAs were increased at high but not at low dose rates [132]. Hallahan et al. demonstrated that neutrophil adhesion is promoted by the expression of the adhesion molecule E-Selectin at low fractional doses (<2 Gy), but by ICAM-1 at higher fractional doses (~5 Gy) [58,60].

The main (or secondary) erythematous reaction in most epithelial surfaces is likely due to an inflammatory response to a combination of lethal, functional, and indirect cellular injury. At the light microscopic level, acutely irradiated human tissues exhibit margination of neutrophils and perivascular infiltration [41,48,102,110,126,136,145]. Radiation-induced EC apoptosis may cause increased permeability and microvascular thrombosis [42,135]. Leucocytes are chemotactically drawn to the site of injury, adhere to the endothelium, transmigrate into the tissue, and release proteolytic enzymes and reactive oxygen species. Apoptosis or necrosis of other cells and subsequent inflammation of these cell layers also contribute to increased vascular permeability and vasodilation. Inflammation further exacerbates the radiation response by amplifying endothelial dysfunction and by increasing the levels of cytokines and growth factors, such as transforming growth factor β (TGF-β), thus delaying the process of re-epithelialisation.

As already noted, the rate at which increases in vascular permeability and vasodilation dissipate after each fractional dose is relevant to the clinical manifestations that appear during a fractionated course of radiation. Single (large) dose studies, such as those cited above, indicate that the rate of dissipation is to be measured in many hours, perhaps days, rather than the few hours taken for sub-lethal radiation damage to repair. The issue of how the vasodilation and increased permeability accumulates during a course of radiation is a complex one. Using daily fractions near 2 Gy, erythema in human skin measured using reflectance spectrophotometry approximately parallels accumulating (biologically effective) dose. The accumulation does not proceed at a steady rate, however, because vasoconstrictive influences of uncertain mechanism oppose the process of vasodilation in the second and third weeks of radiotherapy [144]. More important, at fractional doses both below and above 2 Gy/day, the level of maximal erythema measured during or after radiotherapy considerably exceeds the predictions of the linear quadratic (LQ) formula. Therefore, RDA is quite clearly an important determinant of inflammation and microvascular injury.

Relatively little laboratory research has focused directly on the effects of fraction size and overall time on the magnitude of the overall inflammatory response. Hauer-Jensen's group has provided indirect evidence that both are important in the intestine [128]. Major increases in TGF-β expression at both 2 and 26 weeks occurred when fraction size was doubled, but overall time was maintained. Even more dramatic increases were observed, however, when overall time was halved, but fraction size was maintained. While a very much less dramatic simultaneous trend in the same direction was observed for histological radiation injury score, previous work from this group has shown unequivocally that both overall time and fraction size affect the level of structural injury [62,63,92,93].

2.3.2. Suppression of the inflammatory response

As Trott and Kamprad have pointed out in reviewing the anti-inflammatory effects of radiation [153] the pro- and anti-inflammatory effects of radiotherapy are strongly dose- and schedule dependent. While suppression of inflammatory processes occurs at low radiation doses, the doses that comprise typical high dose fractionated courses of radiotherapy given with the intention of tumour control provoke inflammation, but, as stated earlier, suppress some of the normal reparative processes that occur in response to injury. As Trott and Kamprad have also pointed out, however, the precise circumstances that determine whether inflammatory response is predominantly down or up-regulated have not been fully elucidated and certainly deserve further research.

For example, the suppressive effects of a course of fractionated radiotherapy on the radiosensitive inflammatory infiltrate remains unresolved. A reduction in macrophage activity and suppression of early wound matrix formation might be expected [99,129]. However, the leucocytic infiltrate component of the inflammatory response is derived from the circulation and therefore replenished during regional radiotherapy. Any suppression of the inflammatory process will therefore be limited. Of interest is the recent discovery that the inflammatory leucocytic infiltrate includes circulating 'fibrocytes" capable of expressing collagen type 1 as well as the cytokines and chemokines necessary to amplify the inflammatory response [20].

In addition, regional radiotherapy produces a lymphopenia as early as the first week of the treatment course due to killing of lymphocytes that circulate through the irradiated region during irradiation. However, the effects of lymphopenia on the acute inflammatory response, the subsequent reparative processes in which lymphocytes play a role or, indeed, any of the possible chronic inflammatory processes that occur after radiation are unknown at present.

2.4. Immediate effects of radiotherapy on epithelial surfaces

Cells in the basal and suprabasal layers in the epithelial surfaces are rapidly killed during radiotherapy. However, the evolution of clinically visible injury is determined by inflammatory responses in underlying supportive tissues and by the accelerated repopulative response in surviving epithelial stem cells.

2.4.1. Epithelial injury and repopulation

The vigorous repopulation of the epithelial surface that commences during radiotherapy appears to start after an initial period of growth arrest, which for the human oropharygeal mucosa is approximately 7 days in patients treated at

2 Gy/day [35]. The trigger to acceleration of regeneration is unknown, but work from Trott and Kummermehr indicates that it may be related to a decrease in the density of epidermal cells below a threshold of approximately 60% [154]. Expression of connexin 43 (a gap junction protein) in mouse skin [98] which changes as keratinocyte density decreases may play a role in the regenerative response in this tissue. Clinical studies of cellular repopulation in response to radiotherapeutic injury confirm that the mucosal surface of the upper aerodigestive passage exhibits a remarkable capacity to regenerate. In at least one-third of cases, the process appears to offset cell killing inflicted at a rate of 2 Gy/day [32]. The mechanisms that enable such a massive increase in proliferation are uncertain. Dörr has suggested that the normal restriction to 'asymmetric' stem cell division is lifted and a switch to symmetrical division brings about the major increase in growth fraction that is necessary [34]. In addition cells normally destined to exfoliate without division, or make only one division, regain the capacity to divide twice or thrice. The mechanisms that regulate continued proliferation during radiotherapy remain unclear. In the mouse tongue the processes appear highly regulated, enabling proliferation to exactly match the continued depletion caused by daily fractions of different sizes [36]. Human rectal mucosal biopsy data from Hovdenak et al. [72] also seem to suggest a well-orchestrated response capable of maintaining limited barrier protection.

At dose accumulation rates exceeding conventional fractionation, i.e. 2 Gy/day, 10 Gy/week, the clinical manifestations of epithelial cellular depletion appear earlier and the severity of the clinical reaction is greater. This is because the rate at which surviving epithelial cells are killed exceeds the maximum rate at which replenishment of the cellular population by regeneration can take place. Epithelial denudation will become prolonged if cellular depletion is profound and if disturbance of barrier function adds to the underlying inflammation or further inhibits the ongoing reparative processes. Pre-clinical experiments with accelerated fractionation of rat intestine (2.8 Gy once daily versus twice daily versus thrice daily) also showed results consistent with this notion [62].

As shown in Fig. 1 wound contraction can hasten the process of re-epithelialisation of traumatic wounds by reducing the area requiring epithelial coverage. Suppression of the evolving wound matrix by radiation would not be expected to assist the process of contraction. Indeed Yanase et al. [183] noted disruption of the actin microfilaments within wound fibroblasts of their model following radiation and suggested that this could lead to impaired wound contraction.

2.4.2. Injury to the epithelial basement membrane zone (BMZ) and loss of barrier function

Destruction of the BMZ substantially retards re-epithelialisation [147] and therefore prolongs the period of compromise to the normal barrier function of the epithelial surface. This exposes already injured and inflamed underlying structures to further injury, contributing to further inflammation and fibrosis. Severe acute injury with ulceration that fails to heal completely and therefore becomes chronic has been dubbed 'consequential' injury, by Peters et al. [119]. However, data suggest that 'consequential'-type injury may occur after acute injury that does not lead to (detectable) non-healing epithelial injury. While the 'threshold' and 'ceiling' levels for 'consequential'-type injury has not been established, disruption of the epithelial basement membrane and breakdown of the barrier function of the epithelium substantially increases the risk. An example of temporary breakdown of the basement membrane and epithelial barrier leading to late mucosal effects came from the Trans-Tasman Radiation Oncology Group (TROG) 91.01 head and neck trial [31]. In this trial 70 Gy in 35 daily 2 Gy fractions over 7 weeks was compared with 59.4 Gy in 33 twice daily 1.8 Gy fractions over 3.5 weeks. It was anticipated that patients treated on the conventional arm would experience significantly more late mucosal effects because they had received higher doses. Although this expectation was confirmed in patients experiencing the shortest acute confluent reactions, no difference between treatment arms was observed amongst patients experiencing the longest confluent acute reactions. This suggested that additional mechanisms contributed to late effects in the accelerated arm. Direct evidence of the importance of the mucosal barrier was provided by Hauer-Jensen and coworkers [64,166,169], who showed that a reduction of both early and delayed rat ileal radiation injury was achieved by the surgical or pharmacologic ablation of pancreatic secretion.

Acute injuries that do not result in epithelial denudation also activate mechanisms that contribute directly to delayed injury in some instances [27]. In the enteropathy model referred to above, delayed injuries in animal groups experiencing minimal acute epithelial injuries were also fractionation insensitive and strongly treatment time dependent. In addition, there are limited clinical data suggesting that acute injuries that do not involve serious disruption of the basement membrane can lead to 'consequential' late effects. Bentzen and Overgaard drew attention to an increased rate of skin telangiectasia in patients treated post-operatively for breast cancer who developed moist desquamation [13,14]. Turesson et al. confirmed this observation but found that patients who had experienced severe erythema, but **not** moist desquamation, also had increased rates of telangiectasia [156].

2.5. Effects of radiotherapy on the early wound matrix

High dose radiotherapy does not evoke the rapid granulation tissue response that occurs in the context of acute traumatic wounds. First, the gross tissue destruction, haemorrhage, hypoxia, and bacterial infiltration that promote granulation tissue formation after traumatic injury

are much less evident after radiotherapy injury. Furthermore, fibrogenesis and angiogenesis are inhibited by radiation in a dose- and fractionation-dependent manner. Indeed, suppression of the granulation response compromises surgical wound healing and has been an important consideration in designing appropriate surgery/radiotherapy combinations [150]. While normal granulation tissue is rarely seen in histological sections of tissues undergoing 'acute' radiation reactions, it may be seen occasionally in delayed epithelial ulcers (Fajardo 2001, personal communication).

The early wound matrix in humans is particularly vulnerable to radiation in the first 5 days after traumatic wounding, and irradiation during this period significantly increases the risk of wound healing complications [5,114,150]. In contrast, experimental studies in mice and guinea pigs [15,50], as well as clinical studies [66,115], have shown that the wound healing process is affected little if radiation is delayed for 7 or more days after the wound. Depletion of fibroblasts by radiation prior to wounding also has an important negative impact on wound healing. Eighteen Gray delivered before surgical wounding in a mouse model had the same deleterious effects on wound breaking strength whether delivered 1 h or 95 days prior to wounding [50]. However, the impact was reduced by fractionation. Wound healing following pre-operative radiation is compromised by single doses of 10 Gy administered shortly before surgery [80] but well fractionated courses using total doses of 45–50 Gy, 4–6 weeks prior to radiation do not [75,105,159,178].

In vitro, ECs are as radiosensitive as smooth muscle cells [17] and radiation inhibits EC proliferation [45]. In vivo, radiation induces EC apoptosis and inhibits angiogenesis in normal tissues [86,122]. Archambeau et al. [6,7] found that the ECs of the subpapillary capillary plexus are sensitive to radiation in a dose dependent manner. Abrupt decreases in cellularity follow only days after epithelial regeneration after single radiation doses and this is associated with increases in vessel diameter and occlusion of the lumen. After a conventionally fractionated course, however, microvascular changes follow 6 weeks later, indicating that fractionation and/or RDA is important to the expression of this type of injury. Archambeau found no evidence of microvascular endothelial proliferation in response to radiation. The rat superficial epigastric vascular pedicle model of Doyle et al., [33] however, has provided some of the most direct data on the effects of radiation on the neovascular process. Two doses of 3 Gy at 0 and 24 h after implantation had little effect on neovascularisation, but a third at 48 h had a significant effect. Subsequent doses up to 30 Gy caused limited additional early suppressive effect. It is of interest that, in this model, three daily fractions of 3 Gy prior to implantation did not suppress the neovascular process significantly.

2.6. Processes contributing to delayed injury

The mechanisms that lead to delayed, or 'chronic', radiation injury likely involve depletion of epithelial and stromal cells in combination with perturbation of the reparative processes mentioned above. Hence, there is little reason to believe that the injurious effects of radiotherapy are mediated exclusively by cellular depletion. Indeed, significant non-lethal effects on ECs and fibroblasts may persist long after the early normal tissue reactions have regressed. In addition, as already noted, damage to the normal barrier function of irradiated epithelial surfaces may also contribute to ongoing sub-epithelial inflammation and fibrosis.

The respective contributions of 'reparative' ('replacement') and 'reactive' processes to the pathogenesis of radiation fibrosis remain uncertain in most instances. While replacement fibrosis occurs in response to parenchymal cell loss and undoubtedly contributes to radiation fibrosis in many situations, the molecular events that mediate this process have not been fully characterised. Fajardo has pointed out repeatedly that there is a distinct lack of inflammatory infiltrate in many human radiotherapy fibroses. 'Reactive' processes may make the strongest contribution to the development of post-radiation fibrosis in tissues where a breakdown of the epithelial barrier is an important feature and some degree of chronic inflammation persists.

The clinical evidence of a dissociation between the presence of telangiectasis and subcutaneous fibrosis in patients undergoing radiotherapy after mastectomy reported by Bentzen and Overgaard [13,14] is interesting. It indicates that the mechanisms responsible are not common to both and that there is not a single genetic basis for both sets of processes. The observation that telangiectasis is far more common after acute radiation injuries in which the BMZ and epithelial barriers are disrupted is relevant in this respect. Coupled with the findings by Turesson and Thames that radiation-induced telangiectasia of human skin is treatment time dependent [156,157], these observations suggest that inflammatory damage to the microvasculature during acute injury have an important role in the development of telangiectasia. However, the same mechanisms, which are also associated with suppression of normal reparative responses, play a limited role in the post-irradiation fibrosis seen at many sites.

At the present time, the exact nature of the radiation-induced non-lethal cellular effects and their role in contributing to delayed normal tissue injury in vivo are incompletely understood. Some of the proposed mechanisms by which non-lethal (functional) cellular effects may contribute to delayed radiation injury are summarised in Table 2.

2.6.1. Endothelial cells

Vascular sclerosis was recognised as a characteristic feature of late radiation injury only 4 years after the discovery of the X-ray [48]. At the microscopic level, a variety of long term changes have been recognised in heavily irradiated vessels of all sizes [124]. The capillary network is particularly vulnerable to radiotherapy. Best seen in sequential observation studies are obstruction of the capillary

Table 2
Proposed (non-replacement) mechanisms of radiation fibrosis

1	Repeated tissue exudates lead to fibrin deposition, which does not resolve due to deficiency in tissue plasminogen activator (PA) [40]
2	EC injury leads to plasma exudate which stimulates collagen synthesis [2,94,95]
3	Detachment of ECs leads to bFGF activation and loss of mitogenic control of SMCs, which then overproduce collagen [56]
4	Radiation-induced EC expression of TNF-α [109] and PDGF [180] which stimulate SMC proliferation and production of collagen
5	Downregulation of EC NOS activity allows unopposed SMC proliferation [148]
6	Downregulation of EC thrombomodulin, enables SMC activation by thrombin with assistance of TGF-β [185]
7	Prolonged epithelial barrier breakdown leads to chronic subepithelial inflammation, including TGF-β production which drives fibroblast and SMC proliferation [91] TGF-β activation is promoted by mast cell hyperplasia in the gut [52,53,103]
8	Proliferation of alveolar macrophages and type II pneumocytes which express TGF-β, leading to pulmonary fibrosis [138]
9	Permanent phenotypic alterations induced in fibroblasts by radiation lead to overproduction of matrix [24,139]
10	Alteration of the normal fibroblast population profile by radiation leads to an accumulation of post-mitotic fibrocytes which produce matrix elements [130]

lumen by swelling of the EC cytoplasm [124,125,164,187], EC detachment of localised groups of proliferating ECs [69,89], thrombosis, rupture of the capillary wall, and loss of entire capillary segments [39]. Telangiectasia is a common late phenomenon that may be due to loss of capillary pressure control resulting from smooth muscle cell (SMC) loss in proximal or distal vessels, or to changes in the surrounding extracellular matrix. Loss of ECs may also contribute to telangiectasia, as reflected by an onion skin like appearance of new basement membrane laid over the previous membrane [135]. Subendothelial and adventitial fibroses with partial or complete replacement of the media by an acellular acidophilic material known as 'hyaline' [136,141,143,187] are the most frequently observed lesions in arterioles and small diameter arteries. In medium sized arteries intimal fibrosis is the most common lesion, resulting in variable degrees of concentric or eccentric luminal narrowing. Fibrotic plaques occur in segments throughout the irradiation region and are often associated with some degree of medial and adventitial fibrosis. Almost pathognomic of radiation injury are the presence in the intima of lipid laden 'foam cells'.

Law [94] was among the first to propose that vascular sclerosis and radiation fibrosis were related to EC damage. Subsequent research has provided substantial evidence supporting the notion that radiation, in addition to inducing apoptosis at high doses, also induces long-standing phenotypic changes in ECs and that these changes cause, or at least contribute to, further pathological changes. As mentioned previously, radiation induces a pro-coagulant and pro-inflammatory environment. However, these changes not only promote clotting and leukocyte recruitment, but also induce a plethora of receptor-mediated cellular changes. For example, thrombin, by activating protease-activated receptors on a variety of cell types, potently upregulates the expression of adhesion molecules and chemokines [77]; increases the production of inflammatory and fibrogenic cytokines and growth factors, including TGF-β [182]; increases proliferation of fibroblasts and smooth muscle cells [19]; promotes fibroblast-mediated collagen lattice contraction [120]; and increases collagen expression [21].

Given the heterogeneity of the vascular tree, it is likely that the nature and consequences of EC dysfunction will differ in vessels of different sizes and at different sites, as many structural observations suggest. In addition to the many coagulant, mitogenic, pro-inflammatory, and pro-fibrogenic effects of locally generated thrombin, vessel wall injury and interstitial fibrosis may also be influenced by circulating vasoactive and potentially toxic molecules such as angiotensin II (Ang II) and low density lipoprotein (LDL)-cholesterol. In fact, the foam cells referred to above are only seen in the presence of hypercholesterolaemia [4,43,44,82,90,97]. Ang II upregulates the TGF-β2 receptor in many cell lineages, which may be an important mechanism by which it contributes to vascular sclerosis and interstitial fibrosis [175].

Despite the very obvious compromises to the vasculature that radiotherapy produces, which for many years were considered to be responsible for the varied late radiotherapeutic injuries that occur in all organised tissues [137], it is still not known whether tissue ischaemia results in or is a consequence of radiation injury. Hopewell et al. cite evidence from various studies that atrophy, fibrosis, and necrosis in the rat brain and the pig skin are preceded by vascular injury [68] but aside from the increased risk of myocardial infarction that accompanies mediastinal irradiation, ischaemic tissue injury in humans appears limited. A recent study by Vujaskovic et al. suggest that radiation-induced hypoxia may contribute to the perpetuation of delayed radiation pneumonitis [163].

The extent to which revascularisation in heavily irradiated tissues is possible is uncertain at present. Radiation-damaged ECs may respond unsatisfactorily to angiogenic stimuli and subsequent wound healing is compromised. It is conceivable that neo-angiogenic responses originate in ECs of larger vessels rather than in the microvasculature (Archambeau 2000, personal communication).

2.6.2. Fibroblasts and myofibroblasts

The term 'reactive' fibrosis is frequently used to describe phenomena that occur during wound matrix formation and

chronic inflammation. A number of factors, including thrombin and activated macrophages, indirectly promote deposition of collagen and other matrix elements by recruiting, transforming, and stimulating fibroblasts, myofibroblasts, and smooth muscle cells. In addition, high radiation doses permanently affect fibroblasts and other cells involved in tissue repair. The significance of sub-lethal damage of local fibroblasts is illustrated by the observation that injection of syngeneic fibroblast into irradiated tissue restores wound healing to normal [51,87]. Clinical studies corroborate these experimental findings. Skin fibroblasts obtained up to 18 years after radiotherapy exhibit reduced growth rates compared to fibroblasts obtained from unirradiated control areas [140,146]. Rudolph et al. examined ultrastructural changes in skin biopsies from patients with long term radiation-induced fibrotic skin sequelae [139]. In their study, irradiated wounds bled normally and vascular density appeared to be normal. However, most fibroblasts exhibited ultrastructural changes, suggesting that persisting radiation-induced genetic abnormalities in these cells may be involved in the mechanisms of fibrosis. The cells appeared to be myofibroblasts phenotypically, an observation subsequently supported by Delanian et al. [24]. While these myofibroblasts did not overexpress TGF-β or tissue inhibitor of metalloproteinase, they had shorter lifespan, reduced growth on stimulation, and lower superoxide dismutase and catalase activity levels than 'healthy' fibroblasts [24]. In addition to skin, myofibroblasts are found in many other irradiated tissues, especially in areas of heavy collagen deposition [170]. As in the healing of traumatic wounds, myofibroblasts in irradiated tissues express TGF-β and collagen [166,170], as well as PAR-1 [168] and PAR-2 [167], consistent with a role for thrombin and mast cell proteases in the mechanisms of fibrosis.

The fact that significant fibrosis occurs in heavily irradiated regions in which the fibroblast population is both dysfunctional and depleted and that subsequent wound healing is compromised in these regions is intriguing. The work of Rodemann and Bamberg [130] may provide clues to this apparent paradox, suggesting that TGF-β1, which is overexpressed in irradiated tissues, induces fibroblast proliferation via an expansion of the progenitor fibroblast pool as well as a premature differentiation of progenitor fibroblasts into post-mitotic fibrocytes. These fibrocytes have the capacity to produce extracellular matrix components in far greater quantity than progenitor fibroblasts. If fibroblasts in heavily irradiated tissues, including the myofibroblasts described by Rudolph and Delanian, are post-mitotic but retain the capacity to produce collagen in abundance, it would help explain their ability to produce replacement fibrosis while being unable to proliferate in response to a new wound.

Radiation fibrosis may be considered a form of injury response where there is a continuous signal for connective tissue deposition and/or failure of the down-regulatory processes that normally serve to terminate fibrogenesis. The pig model of Martin et al. provides some insights into these processes [100,101,162,176,177]. In their model, a very high single dose of radiation is used to create a radionecrotic cutaneous ulcer. While the relevance of this model to normal tissue fibrosis in humans is debatable, it has produced data compatible with the existence of prolonged abnormalities in multiple cell types and processes. Studies in this model also suggest the presence of two distinct fibrotic 'compartments'. The peripheral 'compartment', situated at the periphery of the fibrotic mass, contains inflammatory infiltrates of macrophages and neutrophils, actin-positive (myo-)fibroblasts, and pronounced neo-angiogenic activity [162]. The extracellular matrix from this 'compartment' is rich in type III collagen and fibronectin [177]. The fibroblasts cultured from these areas, in contrast to those grown from unirradiated skin or from radiation-damaged human skin, appear capable of indefinite division [101]. The second (central) 'compartment' is much less active and is well vascularised. It is also less cellular and is composed of morphologically normal fibroblasts. Mature collagen (type I) and the sulphated proteoglycans, heparan and dermatan sulphate, make up the extracellular matrix in the central 'compartment' [177].

2.6.3. TGF-β and mast cells

There is strong evidence linking TGF-β to radiation fibrosis in many organs [11,47,88,128,165]. TGF-β1 stimulates mesenchymal cell proliferation and collagen production, and inhibits epithelial cell proliferation. TGF-β1 also acts as a potent immunosuppressor by inhibiting the proliferation and/or function of T-cells, B-cells, and natural killer cells [78,79,131], and by inhibiting the expression of monocyte chemoattractant protein (MCP)-1 and TNF-α receptors on ECs. TGF-β is also the strongest chemotactic factor known for mast cells [55] and observation which may have mechanistic relevance in radiation fibrosis.

Mast cell hyperplasia frequently accompanies radiation fibrosis, temporally follows TGF-β overexpression, and has been quantified in radiation pneumonitis [160,173] and enteropathy animal models [113,128]. The relative significance of various mast cell mediators in specific fibrotic processes in vivo, particularly in the context of radiation fibrosis, is largely unknown. Histamine, TGF-β, fibroblast growth factor (FGF), IL-4, and TNF-α are released by activated mast cells and may stimulate fibroblast proliferation and collagen formation. Direct and indirect effects of heparin, generation of angiotensin II by chymase, and activation of PAR-2 by tryptase are other mechanisms with potential relevance to radiation fibrosis.

Hauer-Jensen's group has provided direct evidence for the mechanistic involvement of both mast cells [184] and TGF-β [185] in intestinal radiation fibrosis. Their studies also provided evidence of important crosstalk between mast cells and TGF-β during radiation fibrosis development. While intestinal radiation fibrosis was significantly attenuated in mast cell deficient rats compared to mast cell compe-

tent littermate controls, TGF-β expression levels were similar in the two types of animals [184]. Conversely, scavenging active TGF-β by administration of a soluble TGF-β type II receptor fusion protein significantly attenuated intestinal radiation fibrosis, but did not affect the level of mast cell hyperplasia [185].

2.7. Impact of other chronic pathological processes on normal tissue responses to radiotherapy

The ability of radiotherapy to induce endothelial dysfunction and apoptosis may explain the frequent clinical observations of increased late radiation injury in chronic medical conditions in which endothelial dysfunction is a feature. For example, diabetes, peripheral vascular disease, hypertension, and obesity are associated with increased likelihood of delayed radiation-induced bowel morbidity [18,65]. Clinical observations also suggest that development of vascular disease *after* radiation therapy may exacerbate subclinical chronic radiation injury and thereby trigger its clinical presentation. Experimental studies addressing the influence of vascular injury on radiation toxicity have largely addressed phenomena rather than mechanisms. Radiation-induced spinal cord injury [8,9] and fatal brain injury [71] are increased in rats with renovascular hypertension. It is of interest that radiation, while aggravating atherosclerosis [84,97], induces myointimal fibrosis but not the development of foam cells [4,82,90,97] or atheroma [43,44] in the absence of hypercholesterolaemia.

Patients with scleroderma and other 'collagen vascular diseases' such as rheumatoid arthritis and systemic lupus erythematosus also appear to have an increased incidence and severity of delayed normal tissue radiation toxicity [25,106,133]. The mechanisms underlying these interactions have not been identified.

3. Part 2 – clinical implications

The recognition that non-lethal functional cellular injuries and tissue responses such as inflammation, reparative processes, and fibrosis contribute to delayed radiation injury over and above the contribution made by progressive cellular depletion leads to a revised framework within which radiation injury may be classified [28] (see Table 3).

This recognition is compatible with the recent observation by Jung et al. [76] that the rate of development of late effects does *not* plateau out over time. However, it does enable additional processes to be targeted for therapeutic intervention.

Table 3
Pathological mechanisms of radiation injury

1	Direct cytocidal effects ('primary')
2	Direct dysfunctional cellular effects ('primary')
3	Indirect phenomena ('secondary')
	All three are expressed after a short or long delay

If cell killing was exclusively responsible for delayed injury, as suggested by the 'target cell theory' [179] then efforts to avert it would rely entirely on: (1) identification of 'radiosensitive' individuals; (2) restriction of radiation target volumes through dose sculpting techniques; (3) altered fractionation schedules designed to minimise normal tissue injury; and (4) replenishment or enhancement of stem cell numbers through growth factor administration. The identification of additional targets for intervention is therefore extremely important for the many patients in whom these strategies are of limited or no value.

As the preceding discussion indicates, several additional processes may be amenable to modulation: (1) inflammation; (2) epithelial barrier breakdown and re-epithelialisation; (3) other early reparative processes; (4) endothelial dysfunction; (5) fibrosis; and (6) the impact of intercurrent pathological processes.

3.1. Experimental and clinical interventions to date

The most important lesson to learn from experiments addressing the pharmacologic modification of radiation injury is that various pharmacological strategies can, in specific instances, prevent or ameliorate established early and delayed injuries. The literature is well reviewed by Moulder et al. [107], and Ward et al. [171]. As Ward et al. have pointed out pharmacological agents with activity in in vitro models do not necessarily have activity in in vivo models and vice versa. Indeed inter-species and inter-site differences are common. In addition, the systematic testing of either single agents or combinations in the clinic has been very limited. Ward et al. noted that most successful experimental interventions have targeted aspects of endothelial function. The success of steroids and non-steroidal anti-inflammatories in several experimental injuries implicate an inflammatory component in the immediate endothelial response. The effectiveness of antioxidant and superoxide dismutase preparations in some situations suggest that reactive oxygen intermediates contribute to the inflammatory reaction and, perhaps, to the development of fibrosis.

The pig fibrosis model of Martin et al., referred to earlier, is interesting in that the experimental changes can be partially reversed by the administration of superoxide dismutase preparations [96] as well as by pentoxifylline (PTX) when used in conjunction with the antioxidant vitamin E (VitE) [23]. What is more interesting still is that these preparations have also produced successful results in clinical trials in patients with established radiation fibrosis [23]. How these substances stop the self-perpetuating fibrosis that characterises this particular model is unclear. Delanian et al. [24], themselves, believe that the PTX–VitE combination may reverse the abnormal fibroblast phenotype that perpetuates the fibrotic process. Clearly more work to identify the mechanisms of these therapeutic actions are necessary.

The activity of the Ang II inhibitors suggest that Ang II has a role in the development of some fibroses although whether this is always mediated by ECs or other cellular lineages is uncertain. As indicated earlier mast cells also have a role in the development of fibrosis and the activity of various anti-histamines is well summarised by Graham and Peterson [54]. Useful lessons may come from research from other disciplines into fibroses that are not induced by radiation. For example the inhibition of chemokine activity may be a promising new approach to the prevention of fibrosis [174]. The interested reader is also directed to Franklin [46], which is a helpful overview of pharmacological strategies aimed at the prevention or treatment of these fibroses.

3.2. Relevance to the modelling of injury

Although it is beyond the scope of this review it is pertinent to include some discussion of the relevance of the mechanisms discussed here to the quantitative framework of parameterisation that has been carefully documented in academic radiotherapy circles over many years [149]. The major relevance relates to: (1) a set of parameters that attempt to describe the reparative tissue responses that occur during radiotherapy, collectively known as the 'time factor'; (2) the description of delayed injury by the target cell concept; (3) the concept of equal effect per fraction; and (4) the volume effect.

In many clinical radiotherapy schedules there is a co-dependence of fraction size and overall treatment time (i.e. the shorter the overall time the larger the fraction size). As a result the parameters that relate fraction size to measured effect may also be influenced by overall treatment time. One of the problems with the nominal standard dose formulation (NSD) was its characterisation of the time factor as an exponent of overall treatment time. This characterisation was more ethereal than biological and, in the absence of a better framework, the LQ model, which embodies the target cell concept of tissue injury, omitted the time factor altogether. This, too, has been a problem because it is impossible to equate the acute effects of different fractionation schedules using the LQ model without using a time factor [32].

Since many of the endpoints used to derive fractionation parameters reflect manifestations of the mechanisms described in this review as well as the lethal cellular events as described by the LQ model, it is to be expected that derivation of the LQ fractionation parameters will depend on these mechanisms. Delayed mucosal injury provides an example of where difficulties in quantifying fractionation parameters can arise. If, for example, inflammatory processes induced by fractionation schedules employing a rapid RDA contribute to (i.e. increase) mucosal injury in a proportion of patients treated, it would be expected that mucositis would develop earlier during treatment in such patients. Also it would be expected that mucositis and compromise of the mucosal barrier would be prolonged for those patients and that more severe late injury would result. Indeed this is exactly what is observed in the clinic, as seen in the TROG 91.01 trial referred to earlier [31]. Problems would have become apparent if an attempt had been made to derive LQ fractionation parameters for delayed mucosal injury from this trial, which compared accelerated (twice daily 1.8 Gy) with conventional fractionation (to total doses of 59.4 and 70 Gy, respectively). This is because different fractionation parameter values would be derived for different patient subgroups. Since early mucosal reactions vary enormously in their duration, the patients in each arm of the trial could have been divided into subgroups based on the duration of their early mucosal reactions. For patients experiencing the shortest early mucosal reactions the incidence of late mucosal injury was observed to be dependent on total dose. Late effects were significantly more frequent amongst patients treated by conventional fractionation to 70 Gy and the incomplete repair variant of LQ model using a low α/β ratio associated with a repair half time in the range of 2–4 h, but without a time correction factor, might have fitted the experience of these patients quite satisfactorily. However, for patients with the longest duration early mucosal reactions late mucosal injury did not relate to total dose in the same way. In this subgroup of patients late mucosal injuries were more frequent than expected from predictions of the LQ model using the same parameter values. In fact the rates of late mucosal injury were quite similar in both treatment arms and the LQ formula using a low α/β ratio associated with a *very long* repair half time (>4 h) would have fitted the experience of this subgroup of patients. This is not the only approach that can be adopted to fitting the scenario described, however. It has been recognised for some time that consequential late effects (CLE) may be fitted by the same parameters that fit early effects, i.e. high α/β ratios and a time correction factor [37]. In the sub-group of patients who were treated on the accelerated fractionation trial arm, and who experienced the longest early mucosal reactions, it is likely that late mucosal effects occurred as a direct consequence of the prolonged barrier breakdown that accompanied the early reaction (i.e. these late effects could have been called 'consequential late effects'). Indeed it was found that late mucosal effects in these patients could also have been fitted with a high α/β ratio and a time correction factor. Obviously it is unlikely that mucosal DNA repair half time or fractionation sensitivity could vary so dramatically within a population of head and neck cancer patients, and better models enabling inter-patient variation in inflammatory and proliferative responses to be described effectively are needed.

It may be seen, therefore, that some of the mechanisms referred to in this review can have a profound influence on the endpoints used to derive LQ model parameter values and that sometimes misleading values can result. While this does not mean that the published parameter values should

be doubted for tissues where these mechanisms are not strong contributors to injury, such as the spinal cord, it does mean that derivations for epithelial lined structures should be viewed with caution, especially if no form of time factor correction has been considered at all. In particular the fractionation sensitivity of these structures may be *lower* than the published values, especially when dose intense regimens are used [30].

The characterisation of an appropriate time factor is problematic in itself. A simple exponential time function is unlikely to reflect the complex and highly regulated process of epithelial proliferation. The initiation of the proliferative response is likely to be related to the accumulation of epithelial injury and be linked to a specific level of cellular depletion [152]. For the mucosal lining of the human oropharynx this level is likely to be reached within the first week of a conventionally fractionated course of treatment [35]. In addition, the magnitude of the proliferative response is limited by the proportion of cells remaining that can undergo division and by cell cycle length constraints [32,34]. Two recent studies have investigated how the time correction factor should be structured when used in conjunction with the LQ model. One study addressed epithelial proliferation in the rat small gut model of Hauer-Jensen and coworkers [27]. The other looked at radiation-induced tumour cell repopulation for human squamous cancers of the head and neck [29]. In both scenarios fitting of the data was unsuccessful unless the initiation of the proliferative response was linked to accumulated damage and a ceiling to the magnitude of the response was applied. It was acknowledged, however, that even this apparently simple approach to the development of a time correction factor involves the use of enough additional parameters to challenge the validity of the successful models due to 'over-parameterisation'.

As has been pointed out earlier in this review, epithelial cellular depletion and compensatory proliferation are not only processes that occur in a normal tissue during radiotherapy, and the time courses of these processes may differ markedly. However, it may be seen that the initiation of the reparative response and the rate at which injury accumulates after the reparative response has commenced, are directly dependent on the RDA. This, in turn, explains why RDA is a more important and direct determinant of tissue injury than overall treatment time. It is also apparent that efforts to model the time factor will meet with limited success until the underlying processes can be parameterised successfully. The importance of this issue is demonstrated when RDA is escalated during treatment (e.g. when applying a 'concomitant boost'). Although overall treatment time is identical if a concomitant boost is delivered at the beginning or at the end of the treatment course, the normal tissue consequences can differ dramatically. In Hauer-Jensen's rat small gut model delayed injury was significantly greater in rats who received the concomitant boost at the beginning of therapy [3]. This finding was thought to be due to impairment of the epithelial proliferative response before it had become established and provides strong support for the thesis that normal tissue response to radiotherapy is not the same at the beginning and at the end of the treatment course. Clearly equal effects are not produced by each fraction in a normal tissue that mounts a reparative response during the treatment course.

Mention has been made already of the difficulty of determining what LQ model parameters are for delayed injuries. However, this review makes it clear that killing of target cells is not the only process that contributes to the manifestations of delayed injury. Processes like fibrosis, which in some instances may be reversed by medication, are a case in point. The target cell concept represents the cornerstone of the prevailing modelling conceptualisation of 'the volume effect'. Withers et al. [180] proposed the concept that normal tissues are composed of functional sub-units which are the largest unit of cells capable of being regenerated from a single surviving clonogen. Using an electrical circuit analogy, Withers pointed out that tissues are organised in one of two ways from a functional perspective: (1) 'in parallel' (e.g. organs such as kidney, lung, liver, etc) or 'in series' (e.g.organs such as spinal cord, gut, etc). This organisation determines the functional outcome of irradiating a portion of that organ and has implications for the way in which irradiated organ volume data, such as now widely available from the dose–volume histograms produced by modern treatment planning computers, can be modelled to produce normal tissue complication probability (NTCP) estimates. Although in many instances the models derived on this basis, such as the critical volume model of Niermerko and Goitein [111], produce realistic estimates of NTCP, there are specific situations in which the models do not provide accurate predictions. For tissues organised 'in series' such as gut and spinal cord, careful experimental work in the last 15 years indicates that the tolerance of short lengths of these organs is considerably higher than predicted by the critical element model presumably due to the reparative proliferation and migration of 'tissue rescuing' stem cells outside of the irradiation portals. In reviewing the data Travis [151] noted that a refinement of the model, the threshold probability model, would provide a satisfactory fit. In the same review Travis concurred with Hopewell and Trott [70] and Hill et al. [67] that the satisfactory modelling of injury to tissues organised 'in parallel' such as lung were challenged by out-of-field injurious effects that might be mediated by cytokines, and by regional variations in sensitivity within the irradiated volume.

3.3. Future prospects

It is reasonable to expect that in the next 10 years many of the mechanisms of injury that have been described imperfectly in this review will be thoroughly understood. With this understanding will come the evolution of effective clinical strategies. No doubt many will build upon the concepts alluded to in the last few paragraphs, but further gains may

be achievable by addressing the interaction between pre-existing pathological processes and the mechanisms that contribute to injury that are induced by radiation.

At present it is difficult to predict how much the successful interruption of the processes that contribute to injury (besides cell killing) will impact on the overall burden of delayed injury to the therapeutically irradiated community. Prediction will be especially difficult if successful intervention leads to an expansion in the indications for radiotherapy.

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