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Review

The radiation-induced fibroatrophic process: therapeutic perspective via the antioxidant pathway

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Abstract

The radiation-induced fibroatrophic process (RIF) constitutes a late, local and unavoidable sequela to high-dose radiotherapy, traditionally considered irreversible. Today, this process is partly reversible, thanks to recent progress in understanding the physiopathology of the lesions it causes and the results of recent clinical trials using antioxidant therapy. This review includes a synthetic description of the static and dynamic features of the RIF process, as reflected by its clinical, instrumental and histopathological characteristics, and by its cellular and molecular regulation. Schematically, three successive clinical and histopathological phases can be distinguished: a pre-fibrotic aspecific inflammatory phase, a constitutive fibrotic cellular phase, and a matrix densification and remodelling phase, possibly ending in terminal tissular necrosis. The respective roles of the chief actors in the RIF process are defined, as well as their development with time. A fibroblastic stromal hypothesis is suggested revolving around a 'gravitational effect' exerted by the couple ROS (reactive oxygen species)—fibroblasts, and partly mediated by TGF-β1. A variety of strategies have been tested for the management of RIF. In the light of the mechanisms described, a curative procedure has been proposed via the antioxidant pathway. In particular, it was showed that superoxide dismutase and combined pentoxifylline–tocopherol treatment enables the process of established radiation-induced fibroatrophy to be greatly reduced or even reversed, both in clinical practice and animal experiments. The efficacy of combined pentoxifylline–tocopherol treatment in superficial RIF was confirmed in a randomised clinical trial, and then in successful phase II trials especially in uterine fibroatrophy and osteoradionecrosis. It is of critical importance to evaluate these new management approaches in larger clinical trials and to improve the recording of results for better outcome analysis. Mechanistic studies are always necessary to improve understanding of the RIF process and the antifibrotic drug action.

Keywords: Fibrosis; Radiotherapy; Late radiation effects; Antioxidant pathway

1. Introduction

Late radiation-induced fibroatrophy (RIF) is an occasional irreversible damage, which is unavoidable and may last for years after radiotherapy (RT). In the long term, it may adversely affect the functional and aesthetic prognosis, as well as the vital prognosis, of patients free of tumour disease. Although RIF lesions were first described when ionising irradiation began to be used, progress in understanding their physiopathology was made recently, thanks to the recent advances in cellular and molecular biology [49,80,144]. This review will mainly deal with superficial RIF tissue. It includes a synthetic description of

Abbreviations: EC, endothelial cell; ECM, extracellular matrix; IFN, interferon; IL-1, interleukin 1; ORN, osteoradionecrosis; PDGF, platelet-derived growth factor; PTX, pentoxifylline; RIF, radiation-induced fibrosis; RNS, reactive nitrogen species; ROS, reactive oxygen species; RT, radiotherapy; SIPS, stress-induced premature senescence; SOD, superoxide dismutase; SOMA, Subjective, Objective, Medical management and Analytic evaluation of injury; TGF-β1, transforming growth factor β1; TIMP, tissue inhibitors of metalloproteinases; TNFα, tumour necrosis factor alpha; Vit.E, vitamin E (alpha-tocopherol).

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the static and dynamic features of RIF from its clinical and pathological characteristics to its cellular and molecular regulation. The fibrotic process associated with tumours, i.e. local relapses in irradiated volumes, or tumour stroma, are excluded, because they require separate consideration. In the light of the cellular and molecular mechanisms described, we show that this process can be greatly reduced or reversed via the antioxidant pathway, both in clinical practice and animal experiments.

2. Clinical and pathologic features

Late radiation-induced effects can be described in different ways, depending on the methods of assessment chosen, i.e. in terms of clinical description, imagery, or histopathology. However, these descriptions are not always equivalent, because indisputable histopathological lesions may be observed, even when the clinical examination is virtually normal.

2.1. Clinical description and latency

The clinical appearance of late radiation-induced effects is polymorph, and ranges from the absence of detectable anomalies to severe trophic complications [56,77,86]. In theory, tissular damage is confined to the irradiated volume (in-field damage). Most clinical findings of fibrosis are caused by excessively indurated and thinkened tissues, and findings of atrophy by superficial or deep necrosis, fistula, loss of specific tissular function.

(a) *In superficial RIF* with cutaneomuscular damage, it may range from loss of irradiated skin elasticity followed first by mild and then significant induration with surface layer rigidity, to retractile whitish sclerosis or even surface ulceration (Fig. 1(A and B)), either spontaneous or caused by a microtrauma with delayed healing [8,12,13,32, 77,78,140,156–159]. Superficial changes are often associated and include hyperpigmentation, epilation, or skin dryness, telangiectasia. These superficial damages may be combined with underlying fibronecrotic lesions affecting the bones (ribs and sternum (Figs. 2 and 3), or femur head), pleura, lung, heart, or pericardium. They may also induce downstream repercussions by compression and/or local retraction, resulting in painful limitation of joint function, local or regional lymphedema of the face or limbs, distal peripheral plexitis and neuropathy, or vascular stenosis.

(b) *When damage is deep-seated*, fibrotic manifestations differ, depending on the organ concerned and range, for instance, from minor digestive disturbances to subocclusive syndrome, from an irritative cough to respiratory distress, or from cystalgia to haemorrhagic cystitis. An international consensus system categorising the severity of RT complications called the SOMA scale (Subjective, Objective, Medical management, Analytical evaluation of injury) was devised in 1995 [90]. A new scoring system, developed

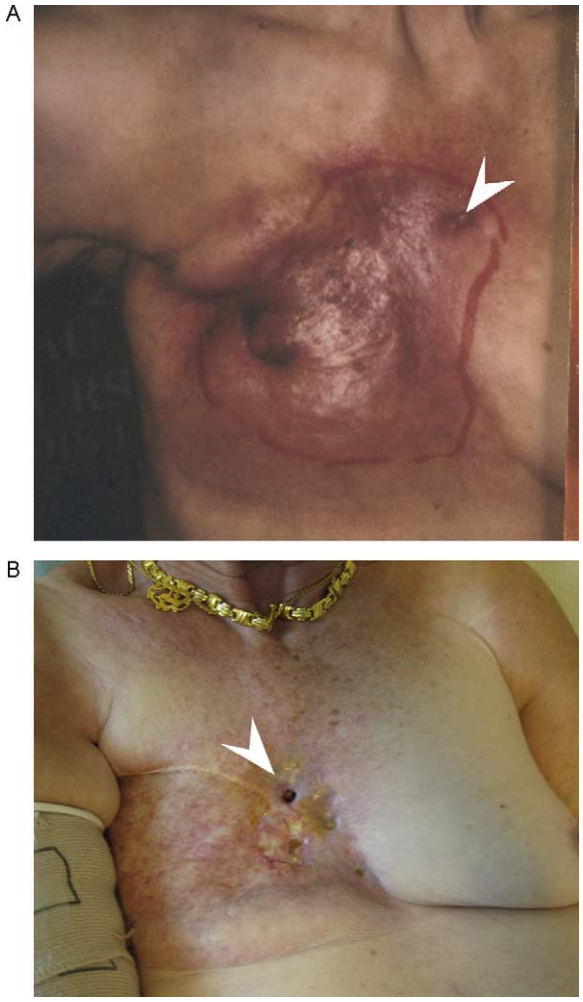

Fig. 1. Clinical photographs of women presenting with a radiation-induced fibroatrophic chest wall, 25 years after radiation therapy: (A) a 62-year-old patient with local and general inflammatory signs; the cutaneous thoracic projection of 16×12 cm fibrosis outlined includes the breast, and a fistulous track is visible in the upper part of the sternum (arrow). (B) A 70-year-old patient with several areas of cutaneous delayed necrosis of the right chest wall (arrow).

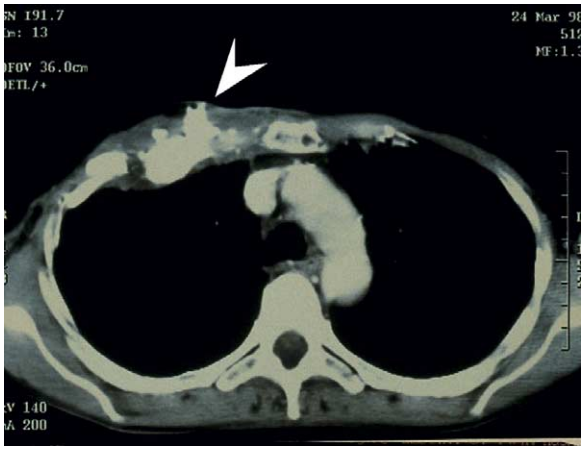

Fig. 2. Chest CT-scan of the woman described in Fig. 1(A): costal lysis is combined with aberrant calcium depositions (arrow).

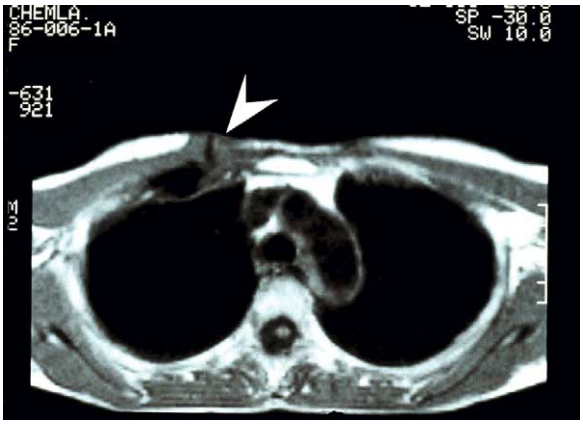

Fig. 3. MRI, 1.5 T, T2-weighted images of the woman described in Fig. 1(A). The right chest wall exhibits a large area of costal and muscular radionecrosis (arrow) and a fistulous track is visible in the right part of the sternum (arrow).

from the earlier scoring system, has recently become available: Common Terminology Criteria for Adverse Events v3.0 (CTCAE, http://ctep.cancer.gov/reporting/ctc.html) [145]. The various anatomic sites irradiated may exhibit different outcomes, because for the same degree of RT injury, the clinical impact varies according to the anatomic site concerned. Variation symptoms from organ to organ were recently reviewed, including the thorax (lung and breast cancers), pelvis (prostate and cervical cancers) and head and neck [145]. The SOMA scale, which is based on severity levels, was designed to provide accurate quantitative descriptions of the lesions caused and their evolution.

The spontaneous clinical development of RIF is characterised by gradual stepwise aggravation over several years or even decades, culminating in an irreversible *sequela*. During this process, it is useful to distinguish schematically [37,38]: (a) an initial pre-fibrotic phase that lasts for the first few months after RT and is often asymptomatic but may be marked by signs of aspecific chronic local inflammation; (b) a constitutive phase of organised fibrotic sequelae during the first few years after RT, in which the local inflammation signs have disappeared, and the tissues have thickened and hardened, with irregular widened capillaries such as telangiectasia; (c) a phase of late fibroatrophy that lasts for 5–30 years after RT, with retractile atrophy and concomitant gradual destruction of the normal tissues included in the irradiated volume.

2.2. Biological and biophysical RIF assessment

Assessment of the severity of tissue fibrosis using clinically based rating scales and quantitative biophysics analysis is not easy. The recording of superficial RIF manifestations can be completed by photograph archiving and measurements such as limb circumference [73] or surface area planimetry [43], and measurement of the serum level of transforming growth factor beta (TGF-β1), which has been reported in generalised fibrosing pathologies and breast and lung cancer patients [112] at risk of developing RIF [99].

Among the methods of imagery, ultrasonography permits the measurement of superficial RIF thickness but its tissue characterisation is poor, and standard X-rays (mammography or chest X-ray) or tomodensitometry can reveal local increases in tissue density (Fig. 2). MRI seems the best method of soft tissue imaging with spontaneous contrast [34,114] that permits effective measurement of RIF volume (Fig. 3) and detects changes in vascularisation before any change occurs in RIF tissue volume [84,85,93].

Other methods of investigation have been proposed in the course of clinical research, such as skin surface microrelief studies by cutaneous profilometry and microtopography [21,95], transcutaneous measurement of partial oxygen pressure [131], and cutaneous laser-Doppler to assess microvascular perfusion [4,44]. Other techniques again allow non-specific assessment of functional organ degradation, such as functional lung, kidney, or liver exploration.

2.3. Interindividual variations in healthy tissue tolerance

The risk, severity, and nature of radiation-induced effects in a patient, depend on several factors.

(a) *Treatment-related factors*. Radiotherapy-related factors include the total dose, the dose per fraction or fraction size, the RT volume and the schedule of treatment [147]. RIF is chiefly observed after an intrinsic RT problem affecting field junctions, during salvage RT of previously treated areas, or when the volume irradiated [82,172], total dose and/or fractionated doses of irradiation are large [19,151,159].

Surgery in an irradiated site is known to increase the risk of RIF in case of post-operative haematoma or chronic infection [117,135,138,148,149]. Chemotherapy especially when concomitant with RT, might intensify certain acute and delayed reactions such as RIF development [100]. Tamoxifen after post-mastectomy RT, significantly increased the risk of lung fibrosis in aged menopausal women [87].

Patient-related factors. The incidence and severity of early and late reactions to RT may vary depending on the physiological status of the patient (e.g. advanced age, obesity) [12,19,118,148,173] and/or co-morbidity factors particularly those involving impaired vascularity such as hypertension or diabetes [28,79,141], or pre-existing collagen vascular diseases [40,66,127,131,161].

There may also be ‘hypersensitive patients’ whose healthy tissues are particularly sensitive to ionising radiations [2,5,6,20,52,81,160,171], as well as very rare congenital diseases characterised by deficient DNA repair mechanisms such as Ataxia Telangiectasia, Xeroderma Pigmentosum, or Cockayne’s syndrome [71].

2.4. Histopathological features

In the clinical practice, the histopathology of RIF development varies from inflammation to sclerosis, depending on the structure of the affected organ or tissue [61,132,133,139]. Although RIF closely resembles the chronic healing of a traumatic wound, it is subject to perturbation by irradiation, because all the cells and extracellular components of the irradiated volume have been affected. Fibrosis is essentially involved in the genesis of late reactions in slowly renewed healthy connective tissue with a non-compartmentalised structure, such as the dermis and subcutaneous tissues [7,8,154], or vasculo-connective parenchymal tissue [49]. Schematically, RIF development can be divided into three histopathological phases, each of which is predominantly cellular, matricial or a mixture of both [37,109].

(a) In the *initial pre-fibrotic phase*, the endothelial cells (EC) play a very important part. Chemokines, released in response to injury, attract leukocytes to the site of injury thereby contributing to the chronic non-specific inflammation that is usual in this phase [49]. This inflammation is characterised by increased vascular permeability with edema formation. The collagen degradation fragments and fibronectin attract the local connective and epithelial tissue cells, and the blood cells. The subsequent destruction of the EC and the associated vascular thrombosis may lead to necrosis of the microvessels and local ischaemia. Loss of this natural EC barrier may result in direct exposure of the connective tissue cells to stimuli which are normally foreign to them, and might, in particular, trigger fibroblastic activation [96].

(b) In the *constitutive organised phase*, the RIF tissue is essentially composed of fibroblasts and extracellular matrix (ECM), although the EC are still active during the secondary neoangiogenesis linked to RIF extension. Constitutive RIF is characterised by a patchwork comprising active RIF areas containing a high density of activated fibroblasts (myofibroblasts) in a disorganised ECM, and pauci-cellular RIF areas containing poorly proliferative senescent fibroblasts (fibrocytes) in a dense sclerotic ECM [96]. However, combined damage to the EC and connective tissue cells, amplified by the action of cytokines, generate the permanent state of RIF.

(c) Lastly, *in the late fibroatrophic phase*, RIF tissue is progressively densified by the successive remodelling of the ECM deposits that occur throughout RIF development, as it was observed by immunohistochemistry in the irradiated skeletal muscle [37,96]. At this late stage of lesions constituted decades after RT, the tissues are friable and develop poorly vascularised and cellularised fibroatrophy, with a few fibroblasts and a dense ECM. However, these healed irradiated areas remain fragile, and may be subjected to surges of late reactivated inflammation after any physicochemical trauma.

3. Pathogenesis: concepts leading to fibroatrophy

In the last decades, various theories concerning the pathogenesis of the RIF have been proposed, mainly deduced from pathological descriptions: these theories are based on vascular or fibroblastic-stromal concepts. Although many cell types and extracellular elements in the irradiated volume are affected by irradiation and often react to it, they do not all have the same impact on the RIF process and its potential regression.

3.1. Vascular concept

The vascular concept was initially based on a theory of gradual ischemia—hypoxia that is still debated as a result in or a consequence of irradiation [49]. For example, in rat lung, severe hypoxia, which developed 6 months after irradiation, was associated with a significant increase in lung fibrosis [167]. In rabbits, however, no change in subcutaneous tissue oxygen pressure (pO_2) was recorded during the delayed fibrotic phase, although light microscopy of affected tissues showed cutaneous fibrosis and blood vessel changes, whereas reduced pO_2 was shown during the acute edema phase of irradiation injury [1].

Similarly, in a series of 112 patients, Marx compared the pO_2 level of irradiated cervical skin versus non-irradiated thoracic skin in the same patient, and considered this parameter as a capillary index for assessing the risk of mandible osteonecrosis [102]. On the other hand, Rudolph reported the absence of ischaemic lesions in irradiated skin, and observed that transcutaneous pO_2 remained normal in a series of 100 patients affected by radiation-induced skin damage [137]. Lastly, ischaemic injury appears to be limited in human tissues, although the capillary network is particularly vulnerable to RT [124].

The more recent concept of vascular damage concerns EC reactions to irradiation, which range from apoptosis to lasting phenotype changes. In particular, the EC dysfunctions reported include the procoagulant, mitogenic, proinflammatory, and profibrogenic effects of locally generated thrombin, an aspect not developed here. However, although these radiation-induced vascular dysfunctions play an important role in generating the initial pre-fibrotic phase of RIF [49,91], we believe that in the constitutive delayed fibrotic phase, this role is more indirect.

3.2. Stromal concept

The fibroblastic stromal concept sheds a different but complementary light on the RIF process, by postulating the existence of a ‘gravitational effect’ that centres on the couple reactive oxygen species (ROS)—fibroblasts, which is partly mediated by TGFβ1, and forms in a vicious circle. This effect is attributed to the existence of a continuous ROS attack and the deregulation of fibroblast proliferation and metabolism, as described in lung fibrosis and liver cirrhosis

after its induction by attacks by alcohol, viruses, or silica [68,120]. This concept is more fully described below.

4. Cellular and molecular features

Like all human pathological fibrogenetic processes, the RIF process may be regulated at several levels [37], including chemotaxis and fibroblast proliferation, metabolism secretion and regulation of ECM components [130].

4.1. Fibroblast differentiation and proliferation

The fibroblast, which is the key cell in connective tissue, exhibits a morphology that varies according to the tissue concerned and to its activity and differentiation stages, which range from the quiescent fibrocyte in mature connective tissue to the active myofibroblast in normal wound healing. The fibroblast is a secretory cell, which produces the components of the ECM and ensures its renewal in a balance between synthesis and degradation. The cytokines and growth factors secreted by the fibroblast, including interferon β (IFNβ), platelet-derived growth factor (PDGF), epidermal growth factor, fibroblast growth factor (FGF), TGF-β1, connective tissue growth factor (CTGF), interleukins (IL), and prostaglandins, also make it a regulatory cell, especially as regards communications with various types of mesenchymatous and epithelial cells [134,146]. The different stimuli of the usual inflammatory reaction and healing process induce the differentiation of the fibroblast phenotype into a myofibroblast one endowed with contractile, secretory and macrophagic properties. These myofibroblasts constitute an intermediate cell type, somewhere between the fibroblast and smooth muscle cell [51,70,122]. During normal wound healing, the myofibroblastic phenotype is a temporary one, as they disappear once the wound has been packed, by a process of phenotypic reversion to fibroblast or smooth muscle cell, or apoptosis.

After irradiation, the *myofibroblasts* appear during the initial inflammatory phase, are then present during fibrogenesis, and persist during the constitutive fibrotic phase. The persistent excess of myofibroblasts corresponds to the histopathological description of hypercellularised fibrosis areas [96] and to the clinical observation of radiation-induced fibrous swellings (Fig. 4). The myofibroblast phenotype is characterised by increased proliferation and altered ECM secretion, combined with overall reduction in ECM metalloproteinases and hyperexpression of anticollagenase enzymes. In this case, the RIF process is characterised by persistent cellular activation whose retroregulation is overwhelmed, because a chronic activation connected with permanent cyokines production [96,101].

At the same time, gradual fibroblast rarefaction combined with incomplete cell replacement might correspond either to the paucicellular areas of fibrosis described in histopathology, or to the clinical processes of atrophy

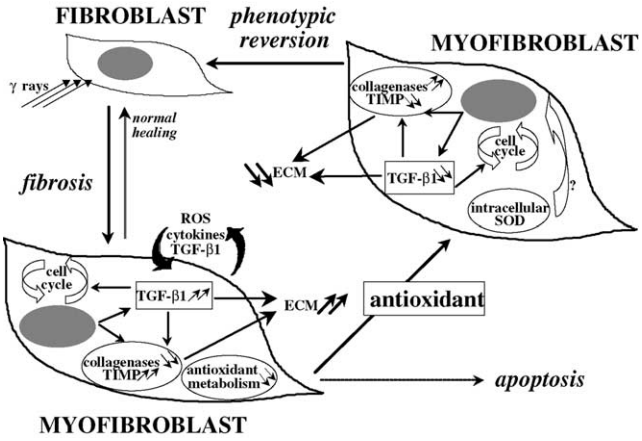

Fig. 4. Main RIF actors and possible cell phenotype reversion after antioxidant treatment.

and secondary radionecrosis. The usual fate of these irradiated fibrocytes is gradual ageing by stress-induced premature senescence (SIPS) [153] with a reduction in ECM secretion, and slowed proliferation due to the loss of ability to divide, as we observed in vitro [42,45]. Cell death then occurs by apoptosis and/or necrosis [22,136].

4.2. Extracellular matrix, cytokines and growth factors

Qualitative and quantitative changes in the ECM have been described in RIF tissue, including increased synthesis of type I and type III collagen [36], fibronectin and hyaluronic acid combined with changes in the collagen I/III ratio, and undersulfatation of sulfated glycosaminoglycans. In addition, the accumulation of ECM in RIF tissues is linked to the deregulation of matricial remodelling enzyme activities including matrix metalloproteinases, type I collagenase, gelatinase and stromelysin, concomitantly with the deregulation of metalloproteinase inhibitors [89]. The homeostasis of collagen turnover is a delicate balance between its molecular synthesis and degradation, depending on the cells secreting degradation enzymes, such as macrophages and fibroblasts, and on the different cytokines which amplify the process [37]. Under these conditions, discrete or major deregulation of this balance at any stage in the metabolic process, such as that encountered in fibrosis, leads to excessive ECM deposition.

The expression and activity of the different cytokines and growth factors may change, according to whether they play a direct or indirect part in the formation and maintenance of the fibrotic process. Depending on the tissue affected and the phase of RIF development, the main factors incriminated are TNFα, PDGF, FGFb, IL1, 4 and 6, TGF-β1 and more recently CTGF [88,165]. Trapped in the ECM in different molecular forms, these factors may later be released locally from matrix receptors, thus allowing persistent local stimulation. Since the immunocytochemical report by Canney and Dean [24], TGF-β1, via the Smad proteins, is today considered to be the main cytokine involved in the RIF

process in vivo [11,49,101], as in other human fibrotic processes such as those involved in atherosclerosis, and kidney, liver and lung fibrosis [17,18,101,134]. On the other hand, based on in vitro studies, it was showed that TGF-β1 could induce fibroblast proliferation via an expansion of the progenitor fibroblast pool, as well as a premature differentiation of progenitor fibroblasts into post-mitotic fibrocytes capable of extracellular matrix components synthesis in far greater quantity than progenitor fibroblasts [22,129]. TGF-β1 has a determinant role in increasing radiosensitivity as described for lung fibroblasts [163,167] or transgenic mice [162]. In the fibrotic process, TGF-β1 is considered responsible for the initiation, development and persistence of fibrosis. During the initial pre-fibrotic phase, the TGF-β1 secreted by the platelets may initiate a cascade of events, including the recruitment and activation of macrophages, which in turn secrete factors that are chemotactic and mitogenic for the fibroblasts. During the constitutive and chronic fibrous phases, circulating TGF-β1 and the TGF-β1 mainly synthesised by the myofibroblasts may contribute to the self-perpetuation of the fibrotic process. However, it is still possible that, in some steps of this long in vivo process, the presence of TGF-β1 may simply act as a marker rather than a cause of the condition, which may also be true of other cytokines [144].

Fibrotic process was recently better understood at the molecular level by a cDNA array hybridisation in human late radiation enteritis. This analysis identified differentially, many genes involved with (a) increased expression of gene coding for proteins involved in the composition and remodelling of ECM, in cell–cell and cell–ECM interactions, in myofibroblast tissue contraction such as rho/HSP27, and (b) alteration in inflammatory response, stress response and antioxidant metabolism [166].

5. Oxidative stress and fibrogenesis

Under physiological conditions, free radicals like reactive oxygen (ROS) or nitrogen species (RNS) perform useful functions such as cell differentiation and proliferation. They are also useful in inflammatory reactions and in signal transduction pathway like intercellular messengers of the growth factor type [3,111]. However, excess ROS/RNS production induced by a wide variety of environmental factors including physical, chemical or infectious agents, and/or deficient ROS/RNS removal by antioxidant defences including intracellular enzymes like glutathion peroxidase, superoxide dismutase (SOD), or low molecular-mass compounds (e.g. vitamin E and vitamin C), may result in pathological stress to tissues and cells [27,35,60].

The involvement of ROS in primary pathological mechanisms is a feature to which radiation is perhaps the major contributor [107,126]. The interaction of radiation with living tissue generates, directly and transiently, ROS originating from the initial seat of inflammation. During secondary exudation, polymorphonuclear cells and macrophages are stimulated by contact with collagen degradation products, thus releasing additional waves of free radicals, and this process can then be self-maintained in a chronic seat of inflammation whose homeostatic balance is upset [152]. In addition, tissue hypoxia when present, may disturb the ROS/RNS balance and cause depletion of tissue NO level by increased ROS level [143].

Biological changes linked to the abnormal presence of ROS have been observed in the extracellular, cellular and membranous compartments [26,64,105,119]. In the extracellular compartment, these radicals affect ECM degradation, leukocyte chemotaxis and phagocytosis, EC surface thrombomodulin, and fibroblast activation [49]. In the cellular compartment, the adaptative reactions to oxidative stress occur via the activation of the genes and proteins characterising the cellular responses to this stress, and trigger a series of processes including DNA repair, cell cycle arrest, and the secretion of growth factors such as TNFα, PDGF and IL1. The processes observed may also include, for example, c-fos induction, membrane and nuclear protein ribosylation by ADP, the activation of phosphorylation of protein kinase C, and induction of the manganese SOD. Lastly, ROS also interfere with biological membranes, via lipid peroxidation processes, thus inducing genetic modulation via transcription factors sensitive to the redox state of cells, such as those of the NF-kB type [125]. In some cases of cytolysis, cell debris may play a chemotactic role.

Under pathological conditions, all these oxidative biological reactions are involved, and the level of damage may rise so much that the stress response mechanisms are transiently overwhelmed [153]. Evidence of such reaction has been described in various diseases in which fibrogenesis occurs in the liver, lung, arteries, and nervous system. Subsequent additional oxidative stress may enhance ROS production, thus helping to extend and densify the fibrotic process. A situation of chronic stress or repeated short stress, resulting from exposure to abnormal ROS concentrations such as those produced by chronic inflammation after RT, can lead to non-lethal effects on cells with a SIPS-like phenotype and changes in the expression of specific proteins termed ‘molecular scars’, or trigger cell death induction by apoptosis and/or necrosis that may eventually contribute to the formation of a necrotic core [153].

6. RIF management

Better physiopathological understanding of the fibroatrophic process has made it possible, in theory, to envisage the regulation of several functions, including collagen metabolism, fibroblastic proliferation, and interactions between cells and the ECM [38], and then to reduce fibrosis. In clinical practice, however, the choice of RIF treatment is based firstly, on the restriction of all

aggravating factors, and secondly, on the stage of disease (prefibrotic, established organised, late fibroatrophy, and necrosis). Care must be taken to avoid confusion as to whether the treatment is designed to prevent RT complications before RIF constitution after RT in a radioprotection perspective, or to manage established RIF injury.

6.1. Restriction of aggravating factors

Removal of the inciting stimulus is clearly the best way to prevent RIF development.

(a) *Stopping co-morbidity related factors* has proved helpful in controlling local RIF progression: firstly, any local trauma such as surgery or biopsy should be avoided, and local infections treated with antibiotics and antiseptic coverage if necessary. Secondly, it is important to stop alcohol abuse and control any imbalance in diabetes, or high blood pressure. It is also useful to avoid fibrogenic chemotherapy like bleomycin.

(b) *Controlling inflammation*. Corticosteroids have been long used for the treatment of radiation injury as anti-inflammatory agents. In all cases, corticosteroids and non-steroidal anti-inflammatory drugs are of interest in the pre-fibrotic phase and in mitigating the acute inflammation associated with fibrosis [38,72,75,104]. However, they have not proven any efficacy in reduction of established RIF. Moreover, several in vitro and in vivo studies have suggested that non-steroidal anti-inflammatory drugs may protect normal tissues from radiation injury [93].

The established RIF treatment has usually been disappointing, although many drugs were available. Some drugs had a favourable effect in experimental in vitro studies on animal or human cells and occasionally in vivo on animals, but proved ineffective, toxic or even dangerous when administered in therapeutic doses to humans [38].

6.2. Usual therapeutic measures

(a) The results of several studies indicate that some drugs might act directly on the fibrotic process itself, and various molecules have been reported to be suitable for clinical use in fibrotic pathology, such as D-penicillamine in scleroderma [142], and colchicine [58,121] and interferons [155,174] in liver cirrhosis and idiopathic interstitial pulmonary fibrosis. However, these drugs have not been precisely evaluated in human RIF [168].

(b) *Inhibitors of angiotensin-converting enzyme* (angiotensin II blocker), such as captopril, have been found effective in its ability to reduce radiation pneumonitis [169] or nephropathy [110] in rats and to protect kidneys, lungs, and the heart from radiation damage [108]. However, their therapeutic effects in human RIF have not been studied [144], except one clinical case of radiation nephropathy in a kidney transplant recipient associated with a stabilisation of the kidney function during 5 years treatment [31].

(c) The authors of two pilot human studies reported that low-dose interferon gamma for 12–36 months could reduce irradiated skin thickness in 6 Tchernobyl survivors accidentally irradiated [116], and in 5 patients after radiotherapy [74].

(d) *Hyperbaric Oxygen* (HBO). Evidence for a benefit of HBO in established fibrosis is not apparent in the literature, although a reduction of related symptoms as pain, erythema, edema, has been described [25,73,123]. In late rectal morbidity (bleeding), resolution of symptoms in several patients, have been reported after HBO [103,115]. As early 1973, HBO was reported to be effective as an adjunctive treatment for osteoradionecrosis. However, the results reported in the literature vary considerably from 15 to 43% recovery after HBO alone, and 18–90% after combination with limited surgery [115,150]. In addition, a recent French randomised trial involving 68 patients failed to demonstrate that HBO alone had any beneficial effect in patients with ORN of the jaw, as only 19% recovered in the HBO group versus 33% in the placebo group [50]. HBO is useful in the prevention of late complications after irradiation, but today no clinical evidence exists to support the hypothesis that HBO could slow or reverse RIF.

6.3. Antioxidant approach

The first effective agent reported in 1983 to reduce RIF was liposomal Cu/Zn SOD in a brief French publication [57]. These two cases, presenting with very severe RIF and necrosis after exposure to high doses of pelvic irradiation, opened the way of subsequent studies with antioxidant drugs.

(a) *Superoxide dismutase*. Bovine liposomal Cu/Zn SOD was successfully used to treat established RIF [9], and enabled the reversibility of human RIF to be demonstrated for the first time in 1994 [39]. This liposomal SOD was administered to patients who had been irradiated for cancer and presented with measurable areas of superficial RIF. After 6 weeks of treatment, mean RIF surface regression was 57%, with complete response in 17% of cases. An other clinical study, using topical SOD, in superficial breast RIF also showed good results [14,23].

A study in irradiated pigs using liposomal SOD treatment also resulted, at 12 weeks, in a major, homogeneous clinical and ultrasonographic response, i.e. 75% of surface regression, confirmed by autopsy [94]. Histopathological analysis of the skeletal muscle around the residual fibrotic block enabled tissue normalisation to be shown for the first time in 1996. This clinical efficacy raised the question of the mechanisms of action of SOD in RIF (Fig. 4).

Thus, in cultured human RIF fibroblasts, reduced TGF-β1 and anti-collagenase TIMP expressions were observed, with an increased endogenous Mn SOD expression [45]. In cultured myofibroblasts from experimental RIF in the pig, a reversion of the myofibroblast phenotype to a normal fibroblast phenotype was observed, with a down regulation of TGF-β1 gene expression [164].

However, although SOD activity seems extremely attractive in RIF patients, this molecule, or mimics, are not at present available for clinical use.

Concerning radioprotection and the antioxidant pathway, Epperly and Greenberger have recently developed SOD gene therapy (plasmid/liposome Mn SOD) in vitro and in vivo, to protect the tissue from irradiated-induced damages, demonstrating to confer cellular resistance to irradiation [59,76].

A different and more recent modulation of the antioxidant pathway was clinically performed using the combination of pentoxifylline and tocopherol (PTX-Vit.E), whose synergy is assumed to be related to that of SOD [43].

(b) *Pentoxifylline*. PTX is a methylxanthine derivative used to treat vascular diseases such as intermittent claudication. In vivo, it has been reported to have an anti-TNFα effect, increase erythrocyte flexibility, vasodilate, and inhibit inflammatory reactions. Many in vitro studies have indicated that PTX has antioxidant properties [83], inhibits human dermal fibroblast proliferation and extracellular matrix production [15,16], and increases collagenase activity [54,55]. However, no clinical or histological change was observed in RIF after 6 months of treatment with PTX alone in experimental pig study [97]. The high concentration of PTX necessary to suppress fibroblast collagen synthesis or to increase collagenase activity, deduced by extrapolating the results of in vitro studies, might be extremely toxic and suggests that administration of PTX alone does not constitute an antifibrotic treatment.

Clinical reports of PTX as sole agent for radiation-induced fibrosis appear to be contradictory. One case report mentioned that PTX relieved pain [170], and in a preliminary report, 1200 mg/d PTX reduced non-measurable RIF in 8 patients, with functional improvement in some of them, although there were three cases of poor tolerance [69]. More recently, 22 patients presenting with radiation-induced fibrosis, treated by 1200 mg/d PTX during 8 weeks showed one-third improvement in functional deficits (active and passive range of motion, muscle strength, limb edema) [113]. However, a rebound effect was described for some patients after the end of treatment (at 16 weeks), suggesting that the duration of treatment was not optimal [113]. Eight weeks course of 1200 mg/d PTX appeared to exert a modest effect in 16 patients with severe trismus by increasing dental gap [29]. Lastly, superficial RIF response was identical in the PTX alone-treated group versus the placebo one, in a recent randomised trial [47].

In contrast, PTX have showed a value in soft-tissue necrosis, reducing the time course of healing in 12 patients [53].

(c) *Tocopherol*. The functions of endogenous tocopherol are to scavenge the ROS generated during oxidative stress, whose production is not limited in vivo by antioxidant enzymes, to protect cell membranes against lipid peroxidation, and to partly inhibit TGF-β1 and procollagen gene expression [27]. Vitamin E may also reduce free radical-induced chromosomal damages, by inhibiting ROS formation and endonuclease activation, that can be triggered by increasing the rate of damaged DNA removal [30].

In a preliminary clinical study in which 700 IU/d of Vitamin E as sole agent was administered to 53 patients, the mean linear regression of superficial breast RIF areas was 20% after 4 months [10]. More recently, superficial RIF response was identical in the Vit.E alone-treated group versus the placebo one, in a randomised trial [47].

Concerning radioprotection, in a recent randomised trial, rinsing the oral cavity in an oil solution containing Vit.E (versus placebo) decreased the incidence of symptomatic oral radiation-induced mucositis in patients with head neck cancer [63].

Thus, PTX or Vit.E alone proved unable to reverse the human RIF. Nevertheless, the fact that they possess all the major properties necessary to make them excellent antifibrotic synergic agents seemed to indicate their joint action might be beneficial.

(d) *Combined pentoxifylline–tocopherol treatment and constituted fibrosis*. A first report in 1998, showed a significant antifibrotic effect, reversing superficial and deep cervico-thoracic RIF, after 18 months of combined PTX-Vit.E treatment [41]. In a phase II study, this combination of 800 mg/d PTX and 1000 IU/d Vit.E was administered to patients with superficial RIF, previously irradiated for head and neck or breast cancer [43]. The mean regressions were 53% for the RIF surface and 53% for the SOMA scale at 6 months, and 66 and 48%, respectively, at 12 months, with a continuous therapeutic effect at 1 year. The first randomised trial on the subject, using this PTX-Vit.E combination, included the same type of patient with superficial established RIF after breast cancer, and confirmed that at 6 months, RIF regression was significantly better than with double placebo [47]. The total duration of PTX-Vit.E treatment is not yet determined, but a treatment shorter than 12 months exposed the patient to a partial rebound effect after initial good response. A long duration of combined PTX-Vit.E treatment, longer than 2–3 years, seemed necessary for severe fibrosis or fibronecrosis with a continuous response on several years (Delanian et al. in preparation, [41,46]).

A similar study was conducted in the pig model, with a RIF surface and volume regression of 70% in the PTX-Vit.E treated pigs, as compared with the PTX alone group or the placebo group of animals. Histopathological analysis made it possible to show tissue normalisation in the PTX-Vit.E group only, combined with a significant reduction of TGF-β1 expression [97].

Moreover, an other team treated several victims of accidental irradiation, and described regression or stabilisation of multiple fibrotic and necrotic lesions after combined PTX-Vit.E treatment [33]. One case with a painful massive RIF mass adherent to the anterior chest wall was successfully treated by 1 year of combined PTX-Vit.E

treatment, with reduced pain and significant mass regression allowing subsequent surgery to remove the residual mobile mass, without any delayed sequelae [33].

This efficacy raised the question of the mechanisms of action of PTX and Vit.E (Fig. 4) and the synergy between them in human and experimental RIF. In vitro studies on different cell types, mainly focused on the changes in proliferation, redox status, apoptosis induction, and gene expression are currently in progress.

(e) *Combined pentoxifylline–tocopherol treatment and late deep fibroatrophy*. The treatment of late deep radiation-induced fibroatrophy or necrosis has had also very disappointing results. Combined PTX-Vit.E activity was explored in the uterus and bone, both specifically concerned by this delayed process.

In women with uterine fibroatrophy, irradiated 25 years previously for cancer in childhood, the effectiveness of 12 months of PTX-Vit.E treatment was shown. These adult patients were hormonoresistant, with failed in vitro fertilisation, and the treatment significantly restored the endometrial mucus, myometrial trophicity, and uterine artery vascularisation [98]. Concomitantly, in a more general study of the treatment of fine hormonoresistant mucuses, it was shown that at 6 months, this trophic uterine improvement by PTX-Vit.E might be a fertility-promoting factor, because two spontaneous full-term pregnancies were observed in three of the previously irradiated patients included in the study [92].

As stated above, superficial RIF may be combined with spontaneous or trauma-induced fibronecrotic lesions of the underlying irradiated tissue, including bones. In patients presenting with moderately severe osteoradionecrosis (ORN), HBO may allow the stabilisation or improvement of symptoms [102]. The usual terminal attitude is radical surgery with exeresis of the necrotic area and flap reconstruction. Acceleration of healing was described using PTX alone in pilot studies of superficial radio-necrotic lesions of head and neck [53,69]. The effectiveness of PTX-Vit.E treatment secondarily boosted by an intermittent oral biphosphonate (clodronate) was recently investigated in this fibronecrotic tissue in18 patients [48]. In this phase II trial, all patients improved, displaying a mean length of exposed bone reduction of 84% at 6 months of treatment, and 89% of them experienced complete and rapid recovery in 5 ± 2.5 months [48]. Clodronate that was of great clinical benefit to the first successfully treated ORN patients [46], was added because it greatly inhibits bone resorption by reducing the number and activity of osteoclasts [128]. At the opposite of the last generation of the biphosphonates, clodronate has a significant direct action on the osteoblastic cells increasing bone formation [67], and reduces fibroblastic proliferation [62], without anti-angiogenic properties [106].

Lastly, one case of skin ulcerated RIF after breast cancer treatment healed after 18 months of combined PTX-Vit.E treatment [65].

7. Conclusion

The radiation-induced fibroatrophic process constitutes a rare and possibly orphan disease, usually considered irreversible. The cellular and molecular mechanisms involved in this process are essentially based on the theory that a vicious circle occurs after the disturbance of several kinds of balance, including fibroblast proliferation and extracellular matrix deposition, amplified by the action of cytokines and growth factors. On the one hand, the use of conformational radiotherapy associated with the reduction of weakening co-morbidity factors can reduce the incidence of RT complications, and on the other, present cellular knowledge of this chronic-active fibrosis has enabled us to influence the ‘smallest’ but chief actors involved in the RIF process, i.e. the reactive oxygen species, by a highly curative treatment mainly involving the antioxidant pathway.

Some recent trials have helped to open up a path through the desert of RIF management, and it is of critical importance to evaluate these new management approaches in larger clinical trials and to improve the recording of results for better outcome analysis. Mechanistic studies are always necessary to improve understanding of the RIF process and its regulation, and of the precise mechanisms of antifibrotic drug action. In future, the efficacy of RIF treatment via the antioxidant pathway should be improved by faster and deeper action by more specific drugs, or by boosting the effects of the PTX-Vit.E combination, which is today available for clinical use.

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