Review Article

Alpha-2-macroglobulin as a radioprotective agent: a review

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Abstract: Radiation is an important modality in cancer treatment, and eighty percent of cancer patients need radiotherapy at some point during their clinical management. However, radiation-induced damage to normal tissues restricts the therapeutic doses of radiation that can be delivered to tumours and thereby limits the effectiveness of the treatment. The use of radioprotectors represents an obvious strategy to obtain better tumour control using a higher dose in radiotherapy. However, most of the synthetic radioprotective compounds studied have shown inadequate clinical efficacy owing to their inherent toxicity and high cost. Hence, the development of radioprotective agents with lower toxicity and an extended window of protection has attracted a great deal of attention, and the identification of alternative agents that are less toxic and highly effective is an absolute necessity. Recent studies have shown that alpha-2-macroglobulin (α_2M) possesses radioprotective effects. α_2M is a tetrameric, disulfide-rich plasma glycoprotein that functions as a non-selective inhibitor of different types of non-specific proteases and as a carrier of cytokines, growth factors, and hormones. α_2M induces protein factors whose interplay underlies radioprotection, which supports the idea that α_2M is the central effector of natural radioprotection in the rat. Pretreatment with α_2M has also induced a significant reduction of irradiation-induced DNA damage and the complete restoration of liver and body weight. Mihailović *et al*. concluded that the radioprotection provided by α_2M was in part mediated through cytoprotection of new blood cells produced in the bone marrow; these authors also indicated that an important aspect of the radioprotective effect of amifostine was the result of the induction of the endogenous cytoprotective capability of α_2M. The radioprotective effects of α_2M are possibly due to antioxidant, anti-fibrosis, and anti-inflammatory functions, as well as the maintenance of homeostasis, and enhancement of the DNA repair and cell recovery processes. This review is the first to summarise the observations and elucidate the possible mechanisms responsible for the beneficial effects of α_2M. The lacunae in the existing knowledge and directions for future research are also addressed.

Keywords: Alpha-2-macroglobulin (α_2M); ionising radiation; radioprotection; radiation-induced fibrosis; mechanism

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Introduction

Radiotherapy is the most common modality in cancer treatment. An estimated eighty percent of cancer patients need radiotherapy at some time or other, either for curative or palliative purposes (1). Radiotherapy is frequently used to obtain local or regional control of malignancies either alone or in combination with other modalities such as chemotherapy or surgery (2). However, exposure of normal tissue to radiation may lead to radiation-induced damage

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that can result in the inability to deliver the intended therapy, a range of symptoms, and a decrease in quality of life. Irradiation of noncancerous "normal" tissues during the course of therapeutic radiation can result in a range of side effects including self-limited acute toxicities, mild chronic symptoms, or severe organ dysfunction. Thus, radiation-induced damage to the normal tissues restricts the therapeutic doses of radiation that can be delivered to tumours and thereby limits the effectiveness of the treatment. To achieve better cancer control and possible cure, a judicious balance between the total dose of radiotherapy delivered and the threshold limit of the surrounding normal critical tissues is required. To obtain optimal results, the normal tissues should be protected against radiation injury. Hence, the role of radioprotective compounds is of great importance in clinical radiotherapy (3).

Radioprotectors are synthetic compounds or natural products that are immediately administered before irradiation to reduce injuries caused by ionising radiation. Over the past 60 years, as a result of the great clinical need for effective radioprotectant agents, many have set out to find more effective, less toxic drugs. Initial attempts focused on synthetic thiol compounds. These agents are highly effective at reducing lethality induced by irradiation. Of this class, amifostine is the only radioprotector that has been clinically approved by the Food and Drug Administration (FDA) for mitigating side effects (xerostomia) in patients undergoing radiotherapy (4,5). This drug offers good protection, but is relatively toxic (nausea, vomiting and hypotension being some of the most common adverse effects) (6,7). In view of this, there is still an urgent need to identify novel, nontoxic, effective, and convenient compounds to protect humans from damaging effects of ionising radiation. Aside from synthetic compounds, some safe and effective naturally occurring products that produce a nonspecific response in the body that increases the power of resistance, function as antioxidants and immunostimulants and restore homeostasis-stimulated radioresistance have also been included in the category of radioprotectors (1). Studies in the recent past have shown that alpha-2-macroglobulin (α_2M) possesses radioprotective effects. This review focuses on the observations and elucidates the possible mechanisms responsible for the radioprotective effects of α_2M.

Radiation injury and radioprotection

The deleterious effects of ionising radiation are mediated through direct deposition of energy to biological molecules and indirectly through generation of highly reactive free radicals (1). Exposure to ionising radiation induces the production of reactive oxygen species (ROS), which include superoxide, hydroxyl radicals, singlet oxygen, and hydrogen peroxide. Free radicals react with DNA, RNA, proteins, and membranes, resulting in cell dysfunction and death. Radiation sickness, also referred to as the acute radiation syndrome (ARS), is a serious illness that occurs when the entire body (or most of it) receives a high dose of radiation over a short period of time (8). Because ionising radiation causes cell dysfunction and mortality, extensive research is devoted to the development of effective radioprotective compounds (9) that would diminish radiation injury in living organisms (1).

Radiation-induced fibrosis is a new theory that accounts for the damage to normal tissues, including bone, after radiotherapy (10). The establishment of radiation-induced fibrosis involves free radical production immediately after the irradiation, resulting in complex multistep activation processes that persist for months and years. This pathology is characterised by the deposition of newly formed extracellular matrix components resulting from an altered balance between connective tissue production and degradation, and by the accumulation of specific fibroblastic cells called myofibroblasts that exhibit contractile properties (11,12). Myofibroblasts are defined as transiently activated fibroblasts exhibiting features intermediate between those of smooth muscle cells and fibroblasts, including the expression of α-smooth muscle actin (α-SM actin) (13,14) and a depleted antioxidant system (15). These cells were introduced in 2004 when recent advances in cellular and molecular biology explained the progression of microscopically observed ORN (*Figure 1*) (16) .

The theory of radiation-induced fibrosis suggests that the key event in the progression of ORN is the activation and dysregulation of fibroblastic activity that leads to atrophic tissue within a previously irradiated area. After radiotherapy the endothelial cells are injured, both from direct damage by radiation and from indirect damage by radiation-generated ROS or free radicals. Injured endothelial cells produce chemotactic cytokines that trigger an acute inflammatory response and then generate a further release of ROS from polymorphs and other phagocytes (17). The destruction of endothelial cells, coupled with vascular thrombosis, lead to necrosis of micro-vessels, local ischaemia, and tissue loss. Loss of the natural endothelial cell barrier allows seepage of various cytokines that cause fibroblasts to become

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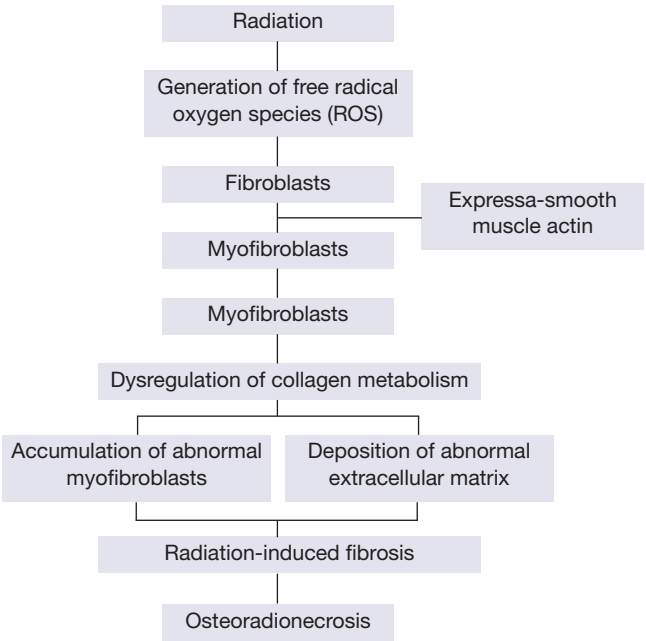

Figure 1 Radiation-induced fibrosis theory.

myofibroblasts. The ROS-mediated release of cytokines such as tumour necrosis factor β (TNF-β), platelet-derived growth factor, fibroblast growth factor α, interleukins 1, 4, and 6, transforming growth factor α1 (TGF-α1), and connective tissue growth factor, result in unregulated fibroblastic activation and the myofibroblast phenotype persists (17).

Because exposure to irradiation in radiotherapy or accidental exposure to radiation can produce significant unwanted side effects, it is important to ameliorate such effects by the use of radioprotective drugs (18). The radioprotectors can elicit their action by various mechanisms, such as: (I) suppressing the formation of reactive species; (II) detoxification of radiation induced species; (III) target stabilisation; and (IV) enhancing the repair and recovery processes (19). The ideal radioprotective agent should fulfil several criteria including significant protection against the effects of radiation, a general protective effect on the majority of organs, an acceptable route of administration, an acceptable toxicity profile and protective time-window effect, an acceptable stability profile and compatibility with the wide range of other drugs (20). Unfortunately, to date, there is no radioprotector that fulfils all of these criteria. A search for alternative agents that are less toxic and highly effective is of great importance.

Alpha-2-macroglobulin (α_2M) as a radioprotective agent

Human α_2M is a 720-kDa glycoprotein in which five reactive sites have been characterised in each of its four identical subunits (180 kDa) (21). α_2M is the largest major non-immunoglobulin protein in plasma. The α_2M molecule is synthesised by numerous cell lineages, including lung fibroblasts, monocytes-macrophages, hepatocytes, astrocytes and adrenocortical cells (22-24).

In cultured bovine adrenocortical cells, synthesis of α_2M may be selectively and significantly increased by TGF-β (25). Interleukin-6 (IL-6) induces α_2M synthesis by human neuronal cells, a mechanism of possible significance in some diseases of the central nervous system (26). A significant increase in α_2M plasma levels is consistently observed during embryogenesis, pregnancy, and childhood, all representing periods of life characterised by growth, development, and differentiation (23,27-29). α_2M is a tetrameric, disulfide-rich plasma glycoprotein with several functions (22). Some of its functions, such as inhibition of different types of nonspecific proteases and transport of cytokines, growth factors, and hormones and a pronounced immune-suppressive activity (30,31), are well established.

α_2M is a pan-proteinase inhibitor capable of inhibiting a large variety of proteinases. After proteolytic cleavage of the "bait" region, the proteinase is entrapped and loses most of its activity, at least toward high molecular weight substrates (22). The concomitant interruption of the thiolester triggers further biological functions of the inhibitor such as binding cytokines, growth factors and hormones (32,33), as well as clearance by the α_2M-R/LRP present on the surface of different cells (34-36). The α_2MR/LRP is a 600-kDa glycoprotein that undergoes proteolysis in the trans-Golgi and is expressed as a non-covalently associated heterodimer of 515 and 85 kDa, respectively (30). The α_2M-R/LRP is expressed in hepatocytes where it plays an important role in the regulation of proteolytic activity in tissue and peri-cellular space and contributes mainly to the clearance of α_2M-proteinase complexes from circulation (37).

Recent studies have shown that α_2M possesses radioprotective effects. Evidence implicates the α_2-macroglobulin fraction (19S) (containing α_2M) in the recovery of mice from radiation damage. The α_2-macroglobulin fraction has been shown to enhance the regeneration of haematopoietic cells (38) and lymphopoietic cells (39) in X-irradiated mice. The haematopoietic system is among the most radiosensitive tissues in the body.

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α_2M most likely plays a role in maintaining hemodynamic equilibrium because the primary cause of death after repeated scalding is circulatory shock provoked by a decrease in plasma volume of more than 50% (40). This is supported by the finding that α_2M inhibits the activity of prostaglandin E2, which increases the permeability of blood vessels (41) and acts as a vasodilator (42). Sevaljević *et al*. reported that the administration of the rat acute-phase (AP) protein a2-macroglobulin (α_2M) that results in a 15-fold increase in its concentration from the normal basal level, 30 min either before the infliction of a lethal scalding (43) or exposure to total body irradiation with 6.7 Gy (LD50/30) X-rays (44,45) enabled 100% survival in experimental animals. Indeed, pretreatment with α_2M before whole-body irradiation allowed for the restoration of body weight, leukocyte count, the complete recovery of liver mass and fully protected liver morphology during a four week follow-up period covering the duration of the ARS. Otherwise, pretreatment with α_2M also induced a significant reduction of irradiation-induced DNA damage and the complete restoration of liver and body weight. Mihailović *et al*. conclude that the radioprotection provided by α_2M was in part mediated through cytoprotection of new blood cells produced in the bone marrow and they also indicate that an important aspect of the radioprotective effect of amifostine is the result of induction of the endogenous cytoprotective capability of α_2M (45,46). Mihailović *et al*. [2009] compare the cytoprotective effects of α_2M and amifostine on rat liver. At 2 weeks after irradiation, Comet assays revealed a 15-fold increase in DNA damage in unprotected rats, while in α_2M- and amifostine-treated rats we observed 3- and 4-fold rise in damage, respectively (45).

Bogojević *et al*. [2011] reported that compared to untreated rats, pretreatment with α_2M and amifostine led to similar increases in levels of the inflammatory cytokine IL-6 and the redox-sensitive transcription factor NFκB, promoting upregulation of MnSOD, the major component of the cell's antioxidant axis, and subsequent increases in Mn/CuZnSOD and catalase enzymatic activities. The results show that α_2M induces protein factors whose interplay underlies radioprotection and support the idea that α_2M is the central effector of natural radioprotection in the rat (47).

Mechanisms responsible for the radioprotection of alpha-2-macroglobulin (α_2M)

The mechanisms responsible for the radioprotective effects of α_2M can be summarised in five aspects as follows: antioxidant, anti-fibrosis, anti-inflammatory, maintaining homeostasis and enhancement of the DNA repair and cell recovery processes. We will elucidate the mechanisms responsible for the radioprotective effects of α_2M in detail point by point below.

Alpha-2-macroglobulin (α_2M) functions as an antioxidant

Ionising radiation causes immediate direct damage to macromolecules and also generates ROS in irradiated tissues and cells (48). The antioxidant system in the liver includes enzymatic and non-enzymatic components that control the flux of ROS (48). The first line of defence against increased levels of ROS is provided by the activities of the major antioxidant enzymes such as superoxide dismutase (SODs) that inactivates the superoxide anion by catalysing its dismutation into oxygen and hydrogen peroxide, and catalase that inactivates the peroxide (49). The enhancement of endogenous intracellular antioxidant enzyme levels can be very important in the adaptive, inherent radiation resistance of cells. An example is manganese superoxide dismutase (MnSOD), which was identified as the most effective endogenous antioxidant enzyme in protecting against radiation-induced cell toxicity (50). Bogojević *et al*. [2011] reported that pretreatment with α_2M led to increased levels of the inflammatory cytokine IL-6 and the redox-sensitive transcription factor NFκB (p65), promoting upregulation of MnSOD, the major component of the cell's antioxidant axis, and subsequent increases in Mn/CuZnSOD and catalase enzymatic activities (47). As α_2-macroglobulin is a carrier protein for IL-6, the powerful inducer of its synthesis, these two proteins can modulate each other's activity. It does not inhibit IL-6 activity or its binding to homologous receptor. In addition, IL-6 bound to α_2M is resistant to proteinases, whereas free IL-6 is easily degraded (51). NFκB is predominantly comprised of p65/p50 heterodimers that are sequestered in the cytoplasm by association with members of the IκB protein family, which bind NFκB and thereby mask its nuclear localisation signal. Upon stimulation by inflammatory cytokines or DNA damage, IκB molecules are degraded, promoting the nuclear translocation of NFκB and binding to target genes. Only p65 and c-Rel activate transcription (52). Pretreatment with α_2M led to increased levels of NFκB (p65), increased nuclear transcription signals, and activation target genes (e.g., MnSOD gene) resulting in increased protein expression. SOD enzymes are naturally occurring intracellular enzymes, which scavenge O_2^- by catalysing its conversion to hydrogen peroxide and

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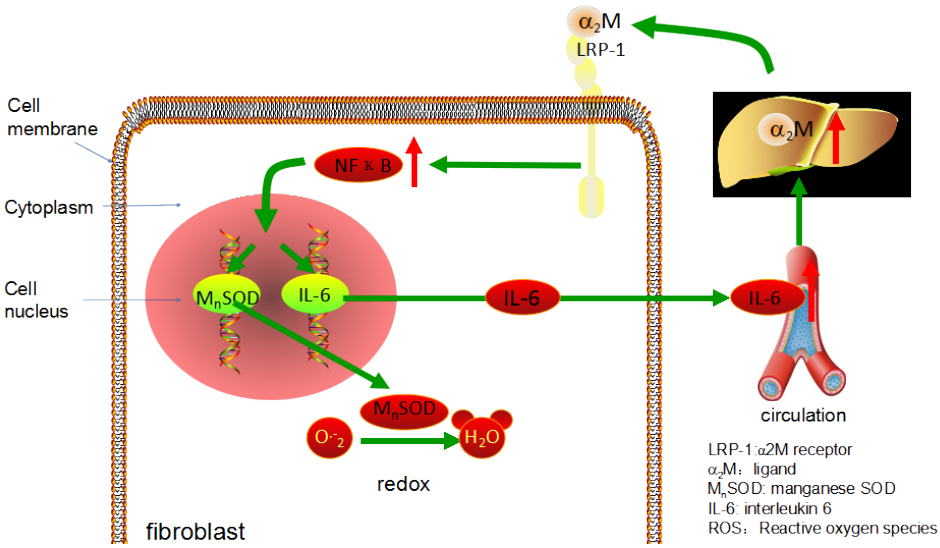

Figure 2 The potential mechanism of antioxidant effect of α_2M on radiation injury.

oxygen. It has become clear that these enzymes provide an essential defence against the superoxide radical. The copper-, zinc- and manganese-containing SODs (Cu, Zn, Mn and SOD) are the most common type of SOD (53,54). Mn/CuZnSOD and catalase are naturally occurring antioxidant enzymes and α_2M lead to subsequent increases in Mn/CuZnSOD and catalase enzymatic activities and then the ability of free radical scavenging increases.

Combined with radiation-induced fibrosis theory, the antioxidant effect of α_2M on radiation injury may be as follows (*Figure 2*). α_2M binds LRP-1 on the fibroblast membrane and sends the signal to NFκB and promotes the nuclear translocation of NFκB and binds to target genes (MnSOD and IL-6 genes), and then the expression of MnSOD and IL-6 increases. MnSOD scavenges O_2^- by catalysing its conversion to hydrogen peroxide and oxygen. IL-6 enters the blood circulation and reaches the liver to induce the synthesis of α_2M. Then, α_2M binds LRP-1 on the fibroblast membrane again.

Anti-fibrosis

The theory of radiation-induced fibrosis suggests that the key event in the progression of its pathophysiology is the activation and dysregulation of fibroblastic activity that leads to atrophic tissue within a previously irradiated area. Activated fibroblasts, namely myofibroblasts, exhibit features intermediate between those of smooth muscle cells and fibroblasts, including the expression of a-smooth muscle actin (a-sm actin) (13,14), and a depleted antioxidant system (15).

To impede the pathological process of radiation-induced fibrosis, we can block the activation of fibroblasts to myofibroblasts to prevent fibrosis. The ROS-mediated release of cytokines such as TNF-β, platelet-derived growth factor (PDGF), fibroblast growth factor α (FGF-α), interleukins 1, 4, and 6 (IL-1, 4, 6), TGF-α1, and connective tissue growth factor result in unregulated fibroblastic activation and the myofibroblast phenotype persists (17). α_2M binds different acute inflammatory mediators (TNF-α, IL-1β, IL-2, IL-6) and growth factors (β-nerve growth factor, platelet-derived growth factor BB, transforming growth factor β1 (TGF-β1), transforming growth factor β2 (TGF-β2)), extremely potent hormone-like polypeptides that play essential roles in the regulation of cellular functions (22,30,55). One aspect of the physiological role of α_2M-cytokine/α_2M-growth factor binding is the downregulation of their activities because bound polypeptides lose their ability to regulate cell functions, whereas the abilities of others become enhanced (56). Thus, α_2M binds cytokines such as TGF-β, PDGF, FGF-α, IL-1, -4, -6, downregulates their activities, and then block the process of activating fibroblasts to become myofibroblasts.

Anti-inflammatory ability

Under conditions of chronic oxidative stress, as would be encountered in irradiated tissue, the generation of ROS

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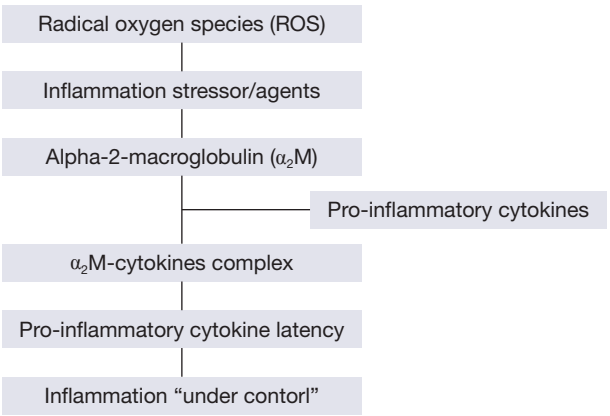

Figure 3 The binding of α_2M with pro-inflammatory cytokines.

triggers an inflammatory response through the activation of cytokines and other inflammatory mediators (57). Indeed, it has been shown that the decreased T-cell response is the consequence of IL-2 degradation and inactivation by α_2M-bound proteinases (58). Other cytokines, such as pro-inflammatory cytokines (IL-1, IL-6 and TNF-α), bind α_2M without being degraded (58). The formation of the α_2M–pro-inflammatory cytokine complex has been suggested as protection from the immediate toxic effect of cytokines, protection from extracellular proteolysis and the loss of cytokines through the kidney and, finally, as a mechanism to target cytokines to α_2M-receptor-bearing cells (58). These phenomena occur as indirect or alternative inhibitory roles of α_2M against increased MMPs, which are in turn temporarily produced in response to exogenous signals by pro-inflammatory cytokines (59).

The binding of α_2M with pro-inflammatory cytokines occurs to induce latency of cytokines themselves (58). As a consequence, inflammation is "under control" (*Figure 3*) (55). In addition, the possible overexpression of these cytokines may also be avoided by the α_2M-cytokine complex through α_2MR/LRP (60). Proinflammatory cytokines (IL-1, IL-6 and TNF-α) increase during inflammation (61), and IL-6 regulates the α_2M gene expression (62). Thus, the interrelationship between α_2M and the immune system is evident with a role of protection against toxic effects by an over production of pro-inflammatory cytokines during inflammation.

Maintaining homeostasis

The pathology of radiation-induced fibrosis is characterised by the deposition of newly formed extracellular matrix components resulting from an altered balance between connective tissue production and degradation and by the accumulation of specific fibroblastic cells called myofibroblasts exhibiting contractile properties (11,12).

In addition to anti-fibrosis, maintaining homeostasis is also of great importance.

The significant release of proteinases by activated neutrophils is one important aspect of the potentially negative side effects of inflammatory processes. Thus, as a result of direct damage by radiation and activated defensive mechanisms, the ensuing large-scale cell destruction that occurs is connected to a burst of proteinase activity. Ionising radiation also stimulates proteolysis of proteins by proteases (63). One aspect of the radioprotective efficacy of α_2M could be attributed to its unique ability to inhibit all classes of proteinases (22,30,64).

Indeed, several studies have demonstrated the importance of α_2M in maintaining the proteinase-proteinase inhibitor equilibrium during inflammation (30). α_2-macroglobulin has been generally viewed as an inflammatory fluid proteinase scavenger. α_2M is able to inactivate an enormous variety of proteinases (including serine-, cysteine-, aspartic-, and metalloproteinases). α_2M has a 35 amino acid "bait" region in its structure. Proteinases binding and cleaving the bait region become bound to α_2M. The proteinase-α_2M complex is recognised by macrophage receptors and cleared from the system (30).

Irradiation induces primary oxidative damage of biomolecules, including lipids, proteins, and DNA (1). Damage to cellular proteins by oxygen free-radicals formed as a result of action of exogenous factors (radiation, oxidants etc.) underlies the pathogenesis of many diseases (65). Thus, oxidation of amino acid residues in the active centre of enzymes could induce considerable alterations of their catalytic properties and, as a consequence, impair cellular regulatory processes. Ionising radiation also stimulates proteolysis by proteases (63). Interaction between α_2M and proteases in the plasma and extracellular fluids involve a unique trapping mechanism by which proteases are incorporated covalently into the α_2M molecule, diminishing their proteolytic effect during irradiation.

The application of α_2M before whole-body irradiation enables 100% survival in experimental rats and allows for the complete restoration of body weight and leukocyte count by the end of a four-week follow-up period covering the duration of acute radiation injury (46). These results and the findings presented here suggest that α_2M, a typical rat

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AP plasma protein, and functions as a natural radioprotector by stimulating processes that restore homeostasis (44). In view of the important role of α_2M in the restoration of homeostasis during the AP response (66,67), we concluded that in the rat α_2M behaved as an adaptogen with a radioprotective function.

We also reported that pretreatment with α_2M prevented the usual decrease in plasma volume observed after scalding (43). α_2M most likely plays a role in maintaining hemodynamic equilibrium because the primary cause of death after repeated scalding is circulatory shock provoked by a decrease in plasma volume of more than 50% (40). This is supported by the finding that α_2M inhibits the activity of prostaglandin E2, which increases the permeability of blood vessels (41) and acts as a vasodilator (42). Trocha and Catravas showed that on the third day after irradiation of amifostine-treated rats, prostaglandin concentrations reached a near physiological level (68). This finding, together with the similar degree of radioprotection observed after either the α_2M or amifostine pretreatments described here, indicates that α_2M affects vascular permeability and promotes normovolemia that in turn protects the organism from the harmful effects of radiation exposure (46).

Enhancement of the DNA repair and cell recovery processes

Evidence has been provided which implicates the α_2-macroglobulin fraction (19S) (containing α_2M) in the recovery of mice from radiation damage. The α_2-macroglobulin fraction has been shown to enhance the regeneration of haematopoietic cells (38), and lymphopoietic cells (39) in X-irradiated mice. Cytological studies of the haematopoietic recovery indicated that the injection of α-globulin fraction into irradiated mice enhanced the recovery, both in time and magnitude, of the granuloid, lymphocyte and lymphocyte-like elements of the bone marrow, as well as of leucocytes in the peripheral blood. The bone marrow, together with lymphoid tissue, the gastrointestinal epithelium, gonads and embryonic tissues, is highly radiosensitive. In the present study, rats from the unprotected irradiated group displayed the highest mortality rate within two weeks of X-irradiation. Up to the end of the 2nd week after irradiation, animals from this group exhibited decreased leukocyte counts, whereas pre-treatment with α_2M-pretreatment provided full survival and restoration of leukocyte numbers by the end of the follow-up period (69). In untreated irradiated animals, the number of leukocytes decreased rapidly after irradiation exposure whereas in the α_2M-pretreated animals during the followed up period it increased.

Cytokines induce the growth and differentiation of many cells of connective tissue and immune, central nervous, vascular and endocrine systems (70). The multiplicity of important functions that are induced by these cytokines indicates that these agents play essential roles in defensive reactions against infections, in the recovery from injury, and in mediating the radioprotective effect of immunomodulators.

α_2M binds different acute inflammatory mediators of which the most important are the cytokines (TNF-α, IL-1β, IL-2, IL-6) and growth factors (β-NGF, PDGF-BB, TGF-β1, TGF-β2) that play essential roles in the regulation of cellular functions that bring about tissue repair and remodelling (55) and participate in mechanism responsible for radioprotection (71,72). Accordingly, α_2M has emerged as a dynamic control point for cytokine/growth factor release and their subsequent functioning. It is plausible that the pre-emptive establishment of very high concentrations of α_2M in the circulation in α_2M-pretreated and pregnant rats provided optimal conditions for highly selective cytokine delivery to target sites of action where repair of irradiation-induced damage was executed (56). Moreover, α_2M exerts an antiapoptotic effect (73).

Conclusions

This article reviews different features that make α_2M a potentially useful radioprotector such as: the ability to stimulate the activity of antioxidant enzymes; anti-fibrosis effects, namely preventing fibroblasts from becoming myofibroblasts; anti-inflammatory effects, reduction of inflammatory response; maintaining homeostasis and hemodynamic equilibrium; and enhancement of the DNA repair and cell recovery processes.

According to the theory of radiation-induced fibrosis (10) and the possible mechanism of the radioprotective effects induced by α_2M, we hypothesised that α_2M may interact with cytokines and antioxidant enzymes to play a role in radioprotection. The mechanisms are summarised as follows (*Figure 4*). α_2M binds LRP-1 on the fibroblast membrane and activates to NFκB to promote the nuclear translocation of NFκB and transcription of target genes (MnSOD and IL-6 genes), and subsequent increased expression of MnSOD and IL-6 increases. MnSOD scavenges O_2^- by catalysing its conversion to hydrogen peroxide and oxygen. IL-6 enters the blood circulation and reaches the liver to

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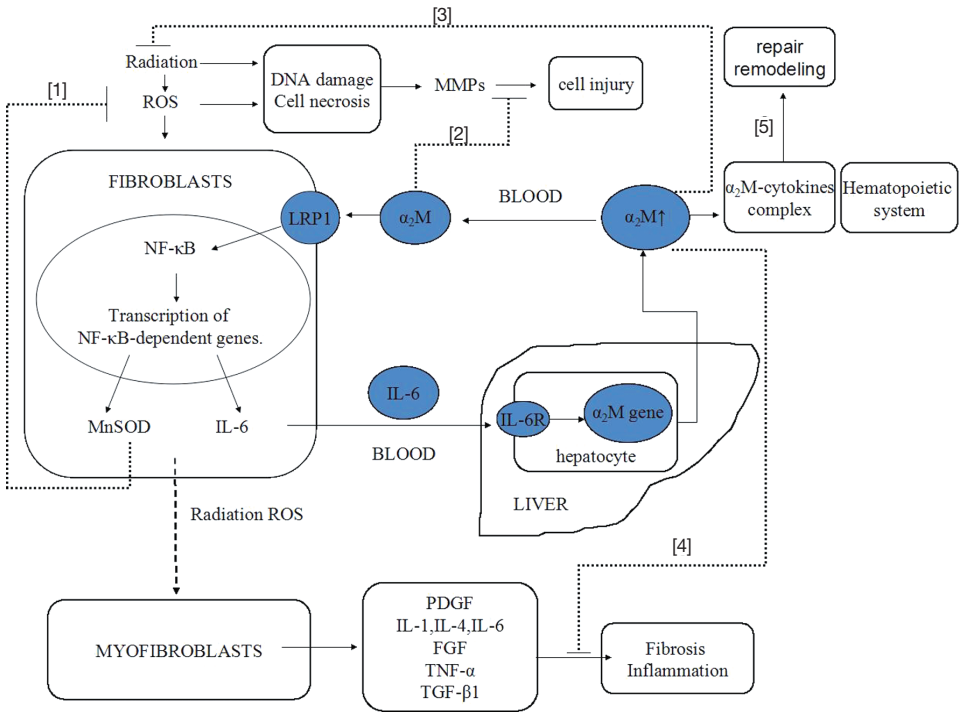

Figure 4 Potential mechanisms responsible for radioprotection of α_2M. [1], α_2M binds LRP-1 on the fibroblast membrane to activate NFκB and promote the nuclear translocation and binding of NFκB to target genes (MnSOD genes), resulting in the increased expression of MnSOD. MnSOD then scavenges ROS (O_2^-) by catalysing its conversion to hydrogen peroxide and oxygen; [2], α_2M functions as an inflammatory fluid proteinase scavenger and maintains homeostasis; [3], α_2M provides natural radioresistance and avoid radiation injury; [4], α_2M binds cytokines and growth factors and inhibits the processes of fibrosis and inflammation; [5], α_2M binds cytokines to develop α_2M-cytokines complex. The α_2M-cytokines complex plays essential roles in the regulation of cellular functions that bring about tissue repair and remodelling. Moreover, α_2M plays a role in repair and remodelling by promoting hematopoietic system cells production.

induce the synthesis of α_2M. Then, α_2M again binds LRP-1 on the fibroblast membrane. The higher level of α_2M plays an important role in radioprotective effects through the mechanisms of anti-fibrosis, anti-inflammatory, antioxidant, homeostasis, and repair and remodelling.

As α_2M exists naturally in all mammals, it has a lower toxicity and provides an extended window of protection. Therefore, we propose that in the future, α_2M improve the therapeutic ratio in radiation oncology. There is continued interest and need for the identification and development of non-toxic and effective radioprotective compounds. Such compounds could potentially protect humans from the genetic damage, mutagenicity, immune system alterations, and teratogenic effects of toxic agents that act through the generation of free radicals. In hundreds of investigations, α_2M appears to provide the desired effect. The optimum dose of α_2M for human radioprotection, however, is yet to be determined.

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