Potential use of HMG-CoA reductase inhibitors (statins) as radioprotective agents

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HMG-CoA reductase inhibitors (statins) are widely used in the therapy of hypercholesterolemia. Apart from their lipid-lowering activity, they have pleiotropic effects that are attributed to the inhibition of regulatory proteins, including Ras-homologous (Rho) GTPases. Here, we discuss the potential usefulness of statins to prevent normal tissue damage provoked by radiotherapy. Statins reduce the mRNA expression of pro-inflammatory and pro-fibrotic cytokines stimulated by ionizing radiation in vitro and alleviate IR-induced inflammation and fibrosis in vivo. The currently available data indicate that statins accelerate the rapid repair of DNA double-strand breaks and, moreover, mitigate the DNA damage response induced by IR. Furthermore, statins increase the mRNA expression of DNA repair factors in vivo. Thus, although the molecular mechanisms involved are still ambiguous, preclinical data concordantly show a promising radioprotective capacity of statins.

Keywords: HMG-CoA reductase inhibitors/normal tissue damage/DNA damage response/DNA repair/ionizing radiation/Rho GTPases

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Introduction

HMG-CoA reductase is the key enzyme of the mevalonate pathway, which generates isoprene moieties essentially required for the biosynthesis of cholesterol, dolichols and ubiquinones. In addition, isoprene moieties [i.e. farnesyl (C15) and geranylgeranylpyrophosphate (C20)] are required for post-translational modification of regulatory proteins such as Ras and Ras-homologous (=Rho) GTPases. These proteins, which play pivotal roles in signal transduction, require C-terminal prenylation for correct intracellular localization and function. Amongst others, they regulate cellular responses to mitogens,
inflammatory cytokines as well as to oxidative and genotoxic stress. Thereby Rho GTPases eventually affect gene expression, proliferation, cell adhesion, metastasis and cell death.\textsuperscript{1,2} Nowadays, inhibitors of the HMG-CoA reductase (statins) are widely used in the clinic for lipid-lowering purpose. However, beyond their well-known cholesterol-lowering activity, they also possess pleiotropic biological functions independent of their beneficial effects on blood cholesterol levels (Fig. 1). For example, statins interfere with the activity of stress kinases (e.g. c-Jun-N-terminal kinases and p38 kinase) and transcription factors (e.g. NF-\(\kappa\)B and AP1) and control cell cycle progression, angiogenesis and apoptosis. Furthermore, they reduce inflammatory stress responses as reflected for example by a diminished expression of cyclooxygenase-2 (COX-2), endothelial cell adhesion molecules (e.g. ICAM, E-selectin) as well as interleukin-6.

![Fig. 1 Statins—mechanisms of action. Statins inhibit the HMG-CoA reductase thereby causing a depletion of the cellular pool of isoprene precursor molecules. Amongst others, these isoprene moieties are required for C-terminal prenylation of regulatory GTPases, including Ras-homologous (Rho) low-molecular weight GTPases. In consequence of this, statins impact pleiotropic Rho-regulated signal pathways related to cell proliferation and cell survival as well as inflammation and fibrosis. CR, cellular receptor; FPP, farnesylpyrophosphate; GGPP, geranylgeranylpyrophosphate; GDP, guanosine disphosphate; GTP, guanosine triphosphate; GEF, guanine nucleotide exchange factors; GAP, GTPase activating proteins; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IPP, isopentenyl-5-pyrophosphate; Rho, Ras-homologous.](image-url)
It is believed that part of the pleiotropic statin effects rest on the inhibition of Rho signaling. Most important, interference with Rho signaling is believed to be of outstanding relevance for the well-known cardiovascular protection mediated by statins.

**Impact of statins on normal tissue damage provoked by ionizing radiation**

The major target of ionizing radiation (IR) determining its anticancer efficacy is the nuclear DNA. Although IR produces numerous types of DNA lesions, the formation of DNA double-strand breaks (DSBs) is believed to be the most cytotoxic event. DSBs are the main trigger of the DNA damage response (DDR), which leads to a rapid activation of checkpoint mechanisms preferentially arresting cells in G1- or G2/M-phase of the cell cycle. The PI3-kinase-related kinases Ataxia telangiectasia mutated (ATM) kinase, ATM and Rad3-related kinase (ATR) and DNA protein kinase (DNA-PKcs) are key regulators of the DDR and DSB repair and maintain genomic stability. In case DSBs are not properly repaired, the mitochondrial death pathway is activated. However, it should be noted that, apart from damaging the DNA, IR also targets other cellular structures, including membrane receptors. These ‘non-target’ (i.e. DNA damage independent) effects of IR might be especially relevant at low radiation dose. They also lead to complex changes in signaling and trigger genomic instability, cell survival or death functions. In the clinical setting of radiotherapy (RT), it is not possible to exclusively harm the malignant cells. Even under optimized conditions of fractionated RT inside- and/or outside-field exposure damages normal tissue, leading to multiple adverse radiation effects (either related or not to DNA damage induction), which severely impact the life quality of the patients.

A clinically highly relevant adverse effect of RT is acute and chronic inflammation. It is driven by the production and release of pro-inflammatory cytokines from dead or differentiated cells as well as the upregulation of endothelial cell adhesion molecules (e.g. E-selectin and ICAM), which promote inflammatory processes. Cardiovascular complications (e.g. accelerated atherosclerosis, peritonitis and fibrosis) can also result from radiation injury. As a late unwished consequence of reparative or reactive processes of normal tissue, fibrotic tissue remodelling occurs. As a result, the regular tissue architecture is severely and irreversibly damaged, eventually eliciting organ dysfunction. Hence, one goal for improving RT is the attenuation of inflammation and fibrosis.
occurring in the course of RT especially in skin, lung, intestine or the cardiovascular system. Pharmacological strategies for radioprotection of normal tissue are manifold and comprise for example reduction of radiation-stimulated DNA damage induction or inhibition of pro-apoptotic DDR. For example, methylproamine was shown to reduce the level of IR-induced DSBs. Also the inhibition of p53 by pifithrin lowers normal tissue damage. Previously, radioprotective gene therapy through retroviral expression of superoxide dismutase has been suggested. Bearing in mind this report, gene therapeutic approaches aiming to improve the DNA repair capacity of radiosensitive tissues are also conceivable. However, virus-based gene therapy is highly controversial. Hence, alternative approaches are desirable. Apart from strategies aiming to attenuate the genotoxic (i.e. DNA damaging) effects of IR on normal tissue, mitigating of ‘non-target’ radiation effects—in particular of pro-inflammatory and pro-fibrotic radiation responses—is a further important objective. The identification of novel radioprotectants has the disadvantage that their implementation into current radiotherapeutic regimen requires time-consuming phase I and II trials. Hence, in view of an eligible short-term transfer into clinical routine, it would be favourable to have a clinically well-established drug at hand, which turns out to be radioprotective.

A rational strategy to overcome inflammation- and fibrosis-related normal tissue damage is the pharmacological inhibition of the deleterious signaling mechanisms triggered by pro-inflammatory and pro-fibrotic cytokines. Statins have anti-inflammatory properties and inhibit cardiac remodelling as well as lung, hepatic and renal fibrosis. Therefore, they appear to be ideal candidates for protecting normal tissue from the acute and chronic toxicity going along with RT. In line with this, in vitro data showed that statins weaken the IR-induced activation of NF-κB, which is the key transcription factor required for the expression of IL-6 and TNFα. Furthermore, statins counteract the radiation-induced expression of transforming growth factor beta and its downstream effector connective tissue growth factor (CTGF), both of which play pivotal roles in fibrosis. These days, there is a growing number of in vivo studies that substantiate the radioprotective potency of statins. For example, lovastatin was reported to reduce acute and subacute pro-inflammatory and pro-fibrotic responses observed after whole-body irradiation of mice in a tissue-specific manner. Furthermore, simvastatin, pravastatin as well as other HMG-CoA inhibitory drugs, such as γ-tocotrienol, ameliorate radiation-induced intestinal injury. Pravastatin also limits IR-induced vascular dysfunction in the skin and, moreover, has beneficial effects on acute lung injury and fibrosis induced by the radiomimetic agent bleomycin. Oncogenic mutation of Ras and activation of
PI3-kinase/Akt-regulated signaling is known to contribute to radioresistance. Correspondingly, inhibition of prenyltransferases radiosensitizes Ras-mutated cells. Increase in the anti-tumor efficacy of IR in the presence of oncogenic Ras was also reported for statins. Notably, statin use is associated with improved clinical outcomes in patients treated with RT for prostate cancer. Collectively, statins are anticipated to reduce adverse effects of RT on normal tissue while increasing its anti-tumor efficiency in case the malignant cells harbour mutated Ras. Regarding the mechanisms underlying the anti-fibrotic effect of statins, it was shown that the inhibition of Rho/Rho-kinase-regulated expression of CTGF is of particular relevance for the mitigation of IR-induced intestinal injury. Lung and heart toxicity induced by thorax irradiation was also attenuated by inhibitors of the Rho/ROCK pathway. Apparently, Rho-regulated pathways play a key role in radiation-induced fibrosis and, hence, are favoured targets for the development of novel radioprotective drugs in the future.

Do statins interfere with mechanisms of the DDR or DNA repair?

As discussed before, there is substantial pre-clinical evidence of a radioprotective effect of statins. It is likely that part of the beneficial effects of statins are due to the inhibition of Rho/NF-κB- and Rho/ROCK-regulated pro-inflammatory and -fibrotic stress responses, respectively. Bearing in mind that Rho GTPases are localized on the outer membrane, it is tempting to speculate that statins specifically attenuate only the harmful ‘non-target’ (i.e. DNA damage independent) effects of irradiation. In addition, they block the deleterious effects of pro-inflammatory and pro-fibrotic cytokines, which are released from neighbouring dead or living cells (Fig. 2). However, a most important still unanswered question is whether statins solely interfere with such ‘non-target’ effects of IR or whether, in addition, they can also (i) reduce the level of radiation-induced DNA damage, (ii) affect mechanisms of the DDR or (iii) promote DNA repair (Fig. 2). Bearing in mind that the membrane-bound epidermal growth factor receptor promotes the repair of IR-induced DNA damage by stimulating non-homologous end-joining (NHEJ), it is feasible that Rho proteins have a permissive function in the DDR. This hypothesis gains support by the finding that lovastatin blocks IR-induced prototypical DNA damage responses of primary human endothelial cells such as the increase in p53 protein level and ATM/ATR-regulated activation of Chk1. The inhibitory effect of lovastatin on IR-stimulated DDR coincided with a better...
survival of the endothelial cells. It was, however, neither related to the level of initial DSBs nor to the level of residual DSBs as measured 4 h after irradiation by monitoring the level of histone H2AX phosphorylated at Ser139 (γH2AX). Similar observation was made in vivo after whole-body irradiation of mice.23 Recently, it was reported that statins accelerate the repair of DSBs in irradiated vascular smooth muscle cells using a novel Nijmegen breakage syndrome (NBS)-dependent pathway.37 Also in this study, both initial and residual level of DNA damage were not affected by the statin. Since NBS is a rare genetic instability syndrome, the question arises whether statins might impact the integrity of the genomic DNA. This hypothesis is indeed supported by the finding that simvastatin mitigates genomic instability.38 Unfortunately, the question whether this is due to the prevention of genomic damage or the potentiation of repair was not addressed in this study. Collectively, the few data available so far suggest that statins selectively promote the rapid repair of DSBs, indicating that they might interfere with NHEJ rather than with S-phase-dependent homologous

Fig. 2 Statins have pleiotropic effects that might contribute to radioprotection. Bearing in mind that IR can damage both nuclear DNA and membrane structures, it is rational to assume that statins interfere with multiple (i.e. DNA damage dependent and independent) normal tissue responses provoked by irradiation. 1, putative inhibitory effect of statins on Rho-regulated stress responses originating from the activation of various types of membrane receptors; 2, inhibitory effect of statins on cytokine-induced (Rho-regulated) pro-inflammatory and/or pro-fibrotic stress responses; 3, impact of statins on IR-induced mechanisms of the DDR; 4, putative stimulatory effects of statins on (i) the rapid repair of DSBs and (ii) the expression of a subset of DNA repair genes; 5, attenuation of the initial level of radiation-induced DNA damage has not been ascribed to statins so far. CR, cytokine receptors; GCR, G-protein coupled receptors; RTK, receptor tyrosine kinase; --, inhibitory effect; +, stimulatory effect.
recombination. This attractive, yet highly speculative, hypothesis needs careful consideration in forthcoming studies.

Recently, the Rho GTPase Rac1 was found inside the nucleus,\(^{39}\) pointing to novel nuclear functions of Rac1. Besides, the generation of DSBs by use of bacterial cytolethal distending toxin was observed to cause activation of RhoA by stimulation of a Rho-specific guanine exchange factor (Rho) localized inside the nucleus.\(^{40}\) Taking into account these data, it cannot be ruled out that the inhibitory effect of statins on mechanisms of radiation-induced DDR and fast repair kinetics rests on their interference with so far not recognized nuclear functions of Rho proteins. Another question that remains to be answered is whether the observed beneficial effects of statins on radiation-induced normal tissue responses are mainly based on post-translational mechanisms or whether transcriptional mechanisms are involved as well. To elucidate whether statins affect the mRNA expression of DNA repair genes \textit{in vivo}, Balb/c mice were treated with lovastatin (10 mg/kg; per os) for either a short (i.e. on two consecutive days) or extended period of time (i.e. for 3 weeks; three applications/week) and alterations in mRNA expression were analysed in the liver by real-time PCR. To this end a semi-customized PCR array (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) was used, which allows the analysis of the mRNA expression of 96 selected genes, including the housekeeping genes GAPDH and \(\beta\)-actin. The data obtained show that under experimental condition of short-time lovastatin treatment, no major changes in gene expression occur (data not shown). However, upon lovastatin treatment for 3 weeks, complex changes in the mRNA expression of stress responsive genes were observed. Eight genes were found to be significantly downregulated and 23 genes were upregulated (Fig. 3). Amongst others, lovastatin caused increased mRNA expression of a subset of repair-related genes including \textit{fen1}, \textit{ligase 4}, \textit{msh2}, \textit{xpc}, \textit{xrcc1} and \textit{xrcc3}. The data show that lovastatin is able to trigger the expression of a broad spectrum of DNA damage defence mechanisms \textit{in vivo}. Whether or not the statin also impacts IR-induced changes in the expression of repair genes is currently under investigation.

Conclusions and outlook

Based on the currently available pre-clinical \textit{in vitro} and \textit{in vivo} data, HMG-CoA reductase inhibitors (statins) have pleiotropic beneficial effects on radiation-induced normal tissue damage. Besides reducing acute inflammatory responses, statins also protect from delayed radiation-induced fibrotic tissue remodelling and cell death. The radioprotective statin effects seem to be due to their interference with the
function of Rho GTPases, with the inhibition of the Rho/NF-κB- and Rho/ROCK axis likely being of outstanding relevance. A limited number of data indicate that statins, apart from alleviating deleterious non-target (i.e. DNA damage independent) effects of RT, are also radio-protective by promoting DNA repair and/or affecting mechanisms of the DDR. This intriguing aspect remains to be further elucidated in forthcoming studies. Bearing in mind that statins are clinically well established for lipid-lowering purpose, clinical trials assessing their usefulness as radioprotectants in humans are desirable. Provided that adequate clinical data are available, multi-cohort retrospective analyses should be performed to judge the radioprotective potency of statins at the cholesterol-lowering dose.

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