Published in final edited form as:

Med Hypotheses. 2013 February ; 80(2): 172–175. doi:10.1016/j.mehy.2012.11.022.

Chronic oxidative stress after irradiation: an unproven hypothesis

Samuel R Cohen, B.S.¹ and Eric P Cohen, M.D.²

¹University of Wisconsin-Milwaukee, Zablocki VA Medical Center, Medical College of Wisconsin

²Department of Medicine, Zablocki VA Medical Center, Medical College of Wisconsin

Abstract

Injury and organ failure after irradiation of late-responding tissues is a substantial problem in radiation oncology and a major threat after accidental or belligerent exposures. The mechanisms of injury may include death of clonogens, vascular injury, activation of cytokine networks, and/or chronic oxidative stress. Knowledge of mechanisms may guide optimal use of mitigators. The hypothesis of chronic oxidative stress as a mechanism of late radiation injury has received much attention. We review herein the published evidence for chronic oxidative stress *in vivo*, and for use of antioxidants as mitigators of normal tissue radiation injury. We conclude that there is only indirect evidence for chronic oxidative stress after irradiation, and there are only limited published reports of mitigation by antioxidants. We did not find a differentiation of persistent markers of oxidative stress from an ongoing production of oxygen radicals. It is thus unproven that chronic oxidative stress plays a major role in causing radiation injury and organ failure in late-responding tissues. Further investigation is justified, to identify persistent oxidative stress and to identify optimal mitigators of radiation injury.

Keywords

ionizing radiation; oxidative stress; normal tissue injury; mitigation

INTRODUCTION AND BACKGROUND

The latent period between irradiation and damage of late-responding, normal tissues is poorly understood, despite over a century of research. In 1906, Bergonié and Tribondeau proposed their “law” that ionizing radiation is more damaging to cells having faster turnover (1). This explains the radiation injury of acutely-responding (bone marrow, gastrointestinal mucosa) and cancerous tissues via mitotic cell death or death in apoptosis, but this simple explanation does not appear to hold for late-responding tissues (kidney, lung, brain). Recent studies have shown that late-responding tissue injury can be mitigated by agents started after irradiation (2). That implies that the initial effects of radiation to cause double-stranded

Correspondence to: Eric P Cohen, Department of Medicine, Zablocki VA Medical Center, 5000 W National Ave, Milwaukee, WI, 53295, Tel: 414-384-2000, Fax: 414-383-9333, Eric.Cohen@va.gov.

Current address for Samuel R Cohen: Department of Medicine, Zablocki VA Medical Center, 5000 W National Ave, Milwaukee, WI, 53295, Tel: 414-384-2000, Fax: 414-383-9333, samcohen@mcw.edu

Conflict of interest statement

There are no conflicts of interest for either author.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

DNA breaks are followed by events during the so-called latent period upon which mitigators can intervene. Damage to vascular tissue, cell proliferation, the renin-angiotensin system, chronic oxidative stress, hypoxia, and inflammation have all been proposed as mechanisms for late radiation injury (3–8). Oxidative stress is the presence of excessive reactive oxygen species including superoxide, hydrogen peroxide, and the hydroxyl radical (9). These species are not easily detected, in contrast to biomolecules that they alter. Thus, carbonylated proteins, 8-hydroxy-2-deoxyguanosine, isoprostanes, and for intracellular oxidative stress, the dichlorofluorescein probe are acknowledged biomarkers of oxidative stress. According to the chronic oxidative stress model, toxic concentrations of reactive oxygen species (ROS) and their products persist during the latent period, leading to the injury of late-responding tissues (8,10,11). It is noteworthy, however, that the superoxide derived from the initial radiochemistry may not persist beyond ten seconds after low linear energy transfer (LET) irradiation (12), and it is speculative as to whether the longest-lived ROS in water, hydrogen peroxide, persists for more than 100 seconds *in vivo* (Riley, personal communication). Nonetheless, the theme of chronic oxidative stress has received much attention during the past decade.

HYPOTHESIS AND THEORY

It has been hypothesized that chronic oxidative stress plays a significant role in injury of late-responding tissues after irradiation. This, in turn, has guided attempts to identify both antioxidant mitigators and treatments of late radiation injury. This is important for total or partial body exposures as may arise from radiotherapy or accidental or belligerent events. If chronic oxidative stress (OS) is found during the latent period between irradiation and tissue injury, its antagonism could be an important aspect of mitigation.

EVALUATION OF THE HYPOTHESIS

PubMed searches were done for both evidence of chronic oxidative stress and use of antioxidant mitigators for late-responding tissues. Key words were “radiation chronic oxidative stress” and “mitigation radiation injury”, respectively. Studies that tested prevention of radiation injury, using compounds started before irradiation, were not considered at all. That search was accompanied by a retrospective search of the references from that search and a prospective search using Google Advanced Scholar™ for the publications that cited those references. Criteria for retention for this analysis included use of *in vivo* studies of irradiation of late-responding tissues, use of accepted techniques for measuring oxidative stress during the latent period before the onset of injury, and use of known antioxidants as mitigators. Accepted, direct markers (i.e., those that are biochemical consequences of oxidative stress) included measurement of malondialdehyde, thiobarbituric acid reactive substances, protein carbonyls, 8-hydroxy-2'-deoxyguanosine, the molar ratio of 9,11 linoleic acid to 9,12 linoleic acid, desferrioxamine chelatable iron, and the dichlorofluorescein diacetate and the dihydroethidium indicator method. We did not include secondary methods such as detection of reactive enzymatic activity that could itself be secondary to oxidative stress, but gene expression studies were accepted. No article was found that reported direct evidence of oxidative stress *in vivo* by direct measurement such as with electron paramagnetic resonance (EPR) spectroscopy (9). A further triage was made based on timing, to exclude evidence for oxidative stress that could merely be the persistence of the initial radiochemical changes after irradiation. Only those reports were retained that showed oxidative stress at more than one day after irradiation. This is consistent with recent United States National Institutes of Health (NIH) preference for radiomitigators that are used at times of more than 24 hours after radiation exposure (NIH Request for Applications RFA-AI-12-023). For reports of mitigation by antioxidants, only those reports were retained that tested antioxidant mitigators at times starting more than one

day after irradiation, and that reported the effect of the mitigator in terms of organ function or animal survival.

RESULTS

Evidence of chronic oxidative stress

Forty articles were identified. Twenty-one did not meet our criteria, as stated above, and are reported in the supplement. Nineteen articles reported evidence of chronic oxidative stress by our criteria (13–32). The tissues in which this was shown included brain (13,21,22,25,27), aorta (28), esophagus (32), lung (15–17,19,23,24,29), kidney (20), liver (14,26,30,31), and prostate (18), at times ranging from 1 day to 6 weeks or more after irradiation (figure 1). In each of these reports, evidence of chronic OS appeared to precede evidence of tissue injury. In the case of kidney, there was little or no evidence for chronic oxidative stress.

Mitigation of radiation injury

Fifteen articles were identified. Ten did not meet our criteria, and are also reported in the supplement. Five articles were found that reported mitigation of late-responding, normal tissue radiation injury by antioxidants at more than 24 hours after irradiation (33–37). The tissues showing this effect were skin (34), kidney (35–37), and lung (33) (figure 2).

DISCUSSION

These data show some evidence for chronic oxidative stress after irradiation of late-responding tissues, and also show limited evidence for mitigation of radiation injury in some but not all tissues by use of antioxidant agents that are started at more than one day after irradiation. This may implicate chronic oxidative stress as a mechanism for radiation injury.

Our search used specific key words, and was greatly expanded by a “backward” and a “forward” search. The former was by identification of references of the articles of the first search, and the latter was by identification of articles citing the articles of the first search. This ensures a comprehensive identification of the relevant literature. We excluded reports that reported nitrosative stress only. While there may be overlap between conditions of oxidative stress and those of nitrosative stress, they are not identical, and the presence of the one does not mean that the other is always present. We excluded reports in which there was evidence of injured tissue at the time of testing for OS, for instance, the report of Gencel *et al.* that showed evidence for oxidative stress at a time when irradiated liver showed degenerative changes (38). That is because injury itself can cause OS, in particular via inflammatory leukocytes that produce reactive oxygen species via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase.

It is recognized that the reports for evidence of chronic OS and the reports of mitigation were in different species, including mice, rats, and guinea pigs. Varying doses of x- or gamma irradiation were used in the cited studies, but were similar in that they were sufficient to cause tissue injury. For the reports of mitigation, we used consistent criteria of better organ function or animal survival.

Our cut-off of more than one day after irradiation for evidence of chronic OS is subject to debate. But there is no accepted definite time after irradiation that clearly separates the initial, acute radiochemical changes from those that may be termed chronic OS. The choice of more than one day is very relevant to clinical mitigation of radiation injury, as occurs in radiotherapy and as could occur in a radiation accident or belligerent exposure. For the former exposures, a delay in start of a mitigator is desirable, because its use during

irradiation could attenuate the benefit of irradiation on a cancer. For the latter exposures, it is not likely that a mitigator would be started before one day after irradiation. That is because of delays in recognition of radiation injury and delays in the provision of mitigators to those exposed. This is consistent with the recent United States National Institutes of Health (NIH) preference for radiomitigators that are used at times of more than 24 hours after radiation exposure (NIH Request for Applications RFA-AI-12-023).

CONCLUSIONS REGARDING MECHANISM AND MITIGATORS

The testing for OS in these studies has used chemical markers of OS and may not necessarily indicate active ongoing OS. It remains possible that the presence of indicators of oxidative stress is the persistent expression of the initial radiochemistry, rather than indicators of an ongoing abnormal redox state. *In vivo* measurement of OS will be needed to resolve that question, for instance, using electron paramagnetic resonance (EPR) spectroscopy (9) or Overhauser-enhanced Magnetic Resonance Imaging (OMRI) (39).

For partial body exposures as may result from accidental or belligerent irradiation, starting mitigators in those who may need them will be delayed by tardy recognition, problems in dosimetry, and logistics of obtaining mitigators for use. The present data do support some efficacy of antioxidant radiomitigators that are started at more than one day after irradiation, but the data are limited. The evidence in some tissues for persistent chronic OS is stronger, and supports the ongoing efforts to identify better radiomitigators that may act via reduction of oxidative stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was supported by the National Institute of Allergy and Infectious Diseases at the National Institutes of Health [U19 AI067734].

We thank Mark Dietz, Brian Fish, Meetha Medhora, and John Moulder for useful discussions

References

1. Bergonié J, Tribondeau L. De quelques résultats de la radiothérapie et essai de fixation d'une technique rationnelle. C R Acad Sci. 1906; 143:983–985.
2. Moulder JE, Cohen EP. Future strategies for mitigation and treatment of chronic radiation-induced normal tissue injury. Semin Radiat Oncol. 2007 Apr; 17(2):141–148. [PubMed: 17395044]
3. Rezvani M, Hopewell JW, Robbins ME. Initiation of non-neoplastic late effects: the role of endothelium and connective tissue. Stem Cells. 1995 May; 13(Suppl 1):248–256. [PubMed: 7488953]
4. Moulder JE, Fish BL, Regner KR, Cohen EP. Angiotensin II blockade reduces radiation-induced proliferation in experimental radiation nephropathy. Radiat Res. 2002 Apr; 157(4):393–401. [PubMed: 11893241]
5. Cohen EP, Fish BL, Moulder JE. The renin-angiotensin system in experimental radiation nephropathy. J Lab Clin Med. 2002 Apr; 139(4):251–257. [PubMed: 12024113]
6. Robbins ME, Zhao W, Davis CS, Toyokuni S, Bonsib SM. Radiation-induced kidney injury: a role for chronic oxidative stress? Micron. 2002; 33(2):133–141. [PubMed: 11567882]

7. Vujaskovic Z, Anscher MS, Feng QF, Rabbani ZN, Amin K, Samulski TS, et al. Radiation-induced hypoxia may perpetuate late normal tissue injury. *Int J Radiat Oncol Biol Phys*. 2001 Jul 15; 50(4): 851–855. [PubMed: 11429211]
8. Zhao W, Robbins ME. Inflammation and chronic oxidative stress in radiation-induced late normal tissue injury: therapeutic implications. *Curr Med Chem*. 2009; 16(2):130–143. [PubMed: 19149566]
9. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *Br J Pharmacol*. 2004 May; 142(2): 231–255. [PubMed: 15155533]
10. Robbins ME, Zhao W. Chronic oxidative stress and radiation-induced late normal tissue injury: a review. *Int J Radiat Biol*. 2004 Apr; 80(4):251–259. [PubMed: 15204702]
11. Zhao W, Diz DI, Robbins ME. Oxidative damage pathways in relation to normal tissue injury. *Br J Radiol*. 2007 Sep; 80(1):S23–31. [PubMed: 17704323]
12. Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. *Int J Radiat Biol*. 1994 Jan; 65(1):27–33. [PubMed: 7905906]
13. Ahaskar M, Sharma KV, Singh S, Sisodia R. Post treatment effect of *Grewia asiatica* against radiation induced biochemical changes in brain of Swiss albino mice. *Iran J Radiat Res*. 2007; 5(3):105–112.
14. El-Missiry MA, Fayed TA, El-Sawy MR, El-Sayed AA. Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. *Ecotoxicol Environ Saf*. 2007 Feb; 66(2):278–286. [PubMed: 16793135]
15. Fleckenstein K, Zgonjanin L, Chen L, Rabbani Z, Jackson IL, Thrasher B, et al. Temporal onset of hypoxia and oxidative stress after pulmonary irradiation. *Int J Radiat Oncol Biol Phys*. 2007 May 1; 68(1):196–204. [PubMed: 17448873]
16. Jack CI, Cottier B, Jackson MJ, Cassapi L, Fraser WD, Hind CR. Indicators of free radical activity in patients developing radiation pneumonitis. *Int J Radiat Oncol Biol Phys*. 1996 Jan 1; 34(1):149–154. [PubMed: 12118544]
17. Jackson IL, Zhang X, Hadley C, Rabbani ZN, Zhang Y, Marks S, et al. Temporal expression of hypoxia-regulated genes is associated with early changes in redox status in irradiated lung. *Free Radic Biol Med*. 2012 Jul 15; 53(2):337–346. [PubMed: 22588005]
18. Kimura M, Rabbani ZN, Zodda AR, Yan H, Jackson IL, Polascik TJ, et al. Role of Oxidative Stress in a Rat Model of Radiation-Induced Erectile Dysfunction. *J Sex Med*. 2012 Apr 10.
19. Lee JC, Krochak R, Blouin A, Kanterakis S, Chatterjee S, Arguiri E, et al. Dietary flaxseed prevents radiation-induced oxidative lung damage, inflammation and fibrosis in a mouse model of thoracic radiation injury. *Cancer Biol Ther*. 2009 Jan; 8(1):47–53. [PubMed: 18981722]
20. Lenarczyk M, Cohen EP, Fish BL, Irving AA, Sharma M, Driscoll CD, et al. Chronic oxidative stress as a mechanism for radiation nephropathy. *Radiat Res*. 2009 Feb; 171(2):164–172. [PubMed: 19267541]
21. Limoli CL, Rola R, Giedzinski E, Mantha S, Huang TT, Fike JR. Cell-density-dependent regulation of neural precursor cell function. *Proc Natl Acad Sci U S A*. 2004 Nov 9; 101(45): 16052–16057. [PubMed: 15522966]
22. Lonergan PE, Martin DS, Horrobin DF, Lynch MA. Neuroprotective effect of eicosapentaenoic acid in hippocampus of rats exposed to gamma-irradiation. *J Biol Chem*. 2002 Jun 7; 277(23): 20804–20811. [PubMed: 11912218]
23. Machtay M, Scherpereel A, Santiago J, Lee J, McDonough J, Kinniry P, et al. Systemic polyethylene glycol-modified (PEGylated) superoxide dismutase and catalase mixture attenuates radiation pulmonary fibrosis in the C57/bl6 mouse. *Radiother Oncol*. 2006 Nov; 81(2):196–205. [PubMed: 17069914]
24. Mahmood J, Jelveh S, Calvey V, Zaidi A, Doctrow SR, Hill RP. Mitigation of radiation-induced lung injury by genistein and EUK-207. *Int J Radiat Biol*. 2011 Aug; 87(8):889–901. [PubMed: 21675818]
25. Manda K, Ueno M, Moritake T, Anzai K. Radiation-induced cognitive dysfunction and cerebellar oxidative stress in mice: protective effect of alpha-lipoic acid. *Behav Brain Res*. 2007 Feb 12; 177(1):7–14. [PubMed: 17145083]

26. Meydan D, Gursel B, Bilgici B, Can B, Ozbek N. Protective effect of lycopene against radiation-induced hepatic toxicity in rats. *J Int Med Res.* 2011; 39(4):1239–1252. [PubMed: 21986126]
27. Sisodia R, Kumari S, Verma RK, Bhatia AL. Prophylactic role of melatonin against radiation induced damage in mouse cerebellum with special reference to Purkinje cells. *J Radiol Prot.* 2006 Jun; 26(2):227–234. [PubMed: 16738418]
28. Soucy KG, Lim HK, Attarzadeh DO, Santhanam L, Kim JH, Bhunia AK, et al. Dietary inhibition of xanthine oxidase attenuates radiation-induced endothelial dysfunction in rat aorta. *J Appl Physiol.* 2010 May; 108(5):1250–1258. [PubMed: 20167676]
29. Terasaki Y, Ohsawa I, Terasaki M, Takahashi M, Kunugi S, Dedong K, et al. Hydrogen therapy attenuates irradiation-induced lung damage by reducing oxidative stress. *Am J Physiol Lung Cell Mol Physiol.* 2011 Oct; 301(4):L415–26. [PubMed: 21764987]
30. Ueda T, Toyoshima Y, Moritani T, Ri K, Otsuki N, Kushihashi T, et al. Protective effect of dipyrindamole against lethality and lipid peroxidation in liver and spleen of the ddY mouse after whole-body irradiation. *Int J Radiat Biol.* 1996 Feb; 69(2):199–204. [PubMed: 8609456]
31. Umegaki K, Sugisawa A, Shin SJ, Yamada K, Sano M. Different onsets of oxidative damage to DNA and lipids in bone marrow and liver in rats given total body irradiation. *Free Radic Biol Med.* 2001 Nov 1; 31(9):1066–1074. [PubMed: 11677039]
32. Vujaskovic Z, Thrasher BA, Jackson IL, Brizel MB, Brizel DM. Radioprotective effects of amifostine on acute and chronic esophageal injury in rodents. *Int J Radiat Oncol Biol Phys.* 2007 Oct 1; 69(2):534–540. [PubMed: 17869666]
33. Mahmood J, Jelveh S, Calvey V, Zaidi A, Doctrow SR, Hill RP. Mitigation of lung injury after accidental exposure to radiation. *Radiat Res.* 2011 Dec; 176(6):770–780. [PubMed: 22013884]
34. Jourdan MM, Olasz EB, Moulder JE, Fish BL, Mader M, Schock A, et al. Mitigation of combined radiation and skin wound injury by SOD/catalase mimetic. *Radiation Research meeting abstracts.* 2009
35. Cohen EP, Fish BL, Irving AA, Rajapurkar MM, Shah SV, Moulder JE. Radiation nephropathy is not mitigated by antagonists of oxidative stress. *Radiat Res.* 2009 Aug; 172(2):260–264. [PubMed: 19630531]
36. Rosenthal RA, Fish B, Hill RP, Huffman KD, Lazarova Z, Mahmood J, et al. Salen Mn complexes mitigate radiation injury in normal tissues. *Anticancer Agents Med Chem.* 2011 May 1; 11(4): 359–372. [PubMed: 21453241]
37. Sieber F, Muir SA, Cohen EP, Fish BL, Mader M, Schock AM, et al. Dietary selenium for the mitigation of radiation injury: effects of selenium dose escalation and timing of supplementation. *Radiat Res.* 2011 Sep; 176(3):366–374. [PubMed: 21867430]
38. Gencel O, Naziroglu M, Celik O, Yalman K, Bayram D. Selenium and vitamin E modulates radiation-induced liver toxicity in pregnant and nonpregnant rat: effects of colemanite and hematite shielding. *Biol Trace Elem Res.* 2010 Jun; 135(1–3):253–263. [PubMed: 19763408]
39. Kosem N, Naganuma T, Ichikawa K, Phumala Morales N, Yasukawa K, Hyodo F, et al. Whole-body kinetic image of a redox probe in mice using Overhauser-enhanced MRI. *Free Radic Biol Med.* 2012 Jul 15; 53(2):328–336. [PubMed: 22579576]

Figure 1.

This shows the evidence for oxidative stress after irradiation, in the organs portrayed and as indicated in the Results. The dotted lines indicate the time intervals at which oxidative stress was found. In the case of the kidney, and as indicated by an asterisk, there is little or no evidence for oxidative stress after irradiation.

Figure 2.

This shows the evidence for efficacy of antioxidant mitigators, in the organs portrayed and as indicated in the Results. The time of start of the mitigators is shown by the first letter of the name of the mitigator relative to the x-axis time scale. In the case of deferiprone, and as indicated by an asterisk, there is no evidence that this antioxidant mitigates radiation injury to kidneys.