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Chronic oxidative stress after irradiation: An unproven hypothesis

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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 for 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 chronic oxidative stress and to identify optimal mitigators of radiation injury.

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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 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 s *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.

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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 h 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 (Fig. 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 h after irradiation [33–37]. The tissues showing this effect were skin [34], kidney [35–37], and lung [33] (Fig. 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

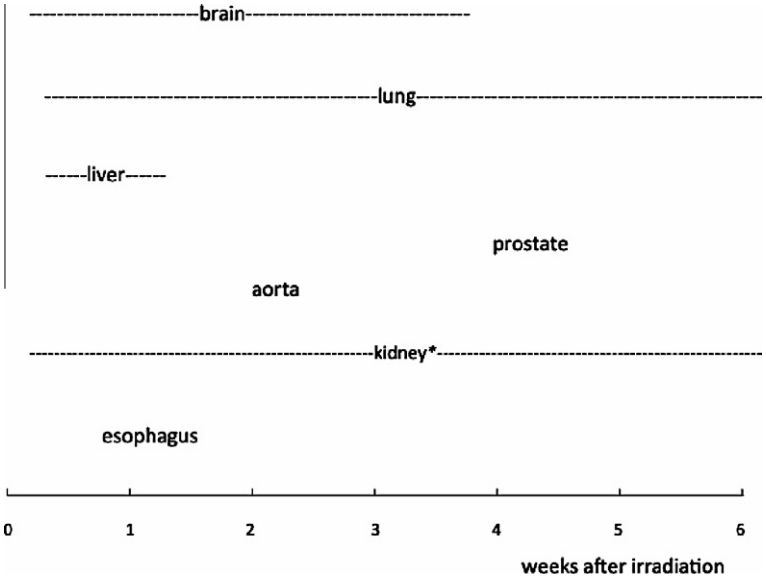

Fig. 1. This shows the evidence for oxidative stress after irradiation, in the organs portrayed and as indicated in the Results. The dotted lines indicate the time intervals at which oxidative stress was found. In the case of the kidney, and as indicated by an asterisk, there is little or no evidence for oxidative stress after irradiation.