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Opinion Article

The Need for Increased Efforts to Understand the Early Effects of Ionizing Radiation on Biological Tissues: Unresolved Issues Regarding Oxygen Sensitizing and Radioprotection?

Jim Peterson

Department of Environmental & Occupational Health, University of Pittsburgh School of Public Health 130 DeSoto St, Pittsburgh PA 15219, USA

Correspondence

Jim Peterson

Department of Environmental & Occupational Health, University of Pittsburgh School of Public Health, 130 DeSoto St, Pittsburgh PA 15219 ,USA

E-mail: jimmyp@pitt.edu

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An important lesson to be taken from nuclear accidents, such as the 1986 Chernobyl [1,2] and 2011 Fukushima-Daiichi [3] disasters, is that perhaps while less than a hundred operators and emergency responders are likely to receive significant doses of ionizing radiation, potentially millions of individuals could subsequently be exposed through fallout and/or contamination of groundwater. The 1987 incident at Goiânia [4], where an improperly secured ^{137}Cs-containing source was stolen by scrap dealers, further informs us that while ~250 people were measurably impacted, over 100,000 individuals reported for testing believing they had been exposed. Certainly, from a public health perspective, these observations point clearly to a need for better radioprotectors; that is, prophylactic agents that can be safely given to human subjects before predictable exposures to ionizing radiation have occurred, both to effectively counteract any injurious effects and to allay possible fears that might otherwise lead to social disruption. Of course, emergency response issues are not the only cause for concern here; other important applications being protection of healthy tissue in patients undergoing radiotherapy [5], shielding of workers in occupations where they may occasionally encounter elevated exposures [6,7] and, perhaps, in our longer-term future, allowing our descendants to tolerate the doses of ionizing radiation they will receive during space flights [8].

Almost two decades ago, a report initiated by the U.S. Homeland Security Council in conjunction with the Office of Science and Technology Policy identified "radioprotectors for use prior to exposure" as the nation's number one research need with respect to radiological countermeasures [9]. The driving concern at the time was a fear that terrorist organizations might attack civilian populations with a nuclear device, like a dirty bomb, but less nefarious nuclear accidents present similar problems in terms of human exposures to ionizing radiation. A funding program, the Centers for Medical Countermeasures to Radiation (CMCR) was established to address the problem. Within Five years, however, the National Institutes of Health seems to have decided to ignore the opinion of its own experts when the decision was made to limit CMCR awards exclusively to the study of countermeasures effective if administered at least 24 hours after exposure to the radiation dose; that is, the focus was to be on radiomitigation (therapies to be administered after the dose), essentially to the detriment of radioprotector projects. Not surprisingly, recent progress has stalled; remarkably few newly synthesized, or re-purposed, compounds have emerged demonstrating any practical capability as viable radioprotectors. At this time, nuclear energy appears likely to remain an unavoidable component of our attempts to ameliorate climate change by reducing our dependence on fossil fuels. This entails building more nuclear power stations, particularly those of newer and smaller design; but inevitably, this in turn means there will likely be more accidents. Therefore, it is in our best interest to plan appropriate response strategies, which should include the development of improved radioprotectors.

Radiobiology

For reference and clarity, the ionizing radiation doses under consideration here are in the intermediate range of ~0.5 Sv up to approaching 20 Sv total-body irradiation (TBI); or at least an order of magnitude more than doses received during medical imaging procedures and acceptable annual exposures of nuclear industry workers, but less than the kind of exposures in humans that would prove rapidly lethal due to inflammation within the cranium associated with the cerebrovascular syndrome. Thus, the discussion is focused on single doses that, while lethal from the middle

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of the range upwards, offer some hope of proving survivable with appropriate prophylaxis.

Nowadays and in the recent past, most researchers working in radiobiology have some kind of association with radiotherapy. My own experimental efforts in the Field have largely been in collaboration with colleagues whose primary appointments are in radiation oncology. I think this has resulted in a focus on matters of radiological cell death mechanisms (especially cancer-cell types) and cycles of inflammation, followed by tissue injury and regeneration. These are important biomedical issues, but substantial sections of the signaling pathways involved are not necessarily unique to the radiation injury response and they are a long way downstream of the primary biological effects of ionizing radiation, which are probably more the purview of biochemists, or biophysicists. Lack of consensual understanding regarding the early effects – during and within minutes of the dose, well before there has been commitment apparent to any particular mode of cell death, or inflammation of tissues – remains a significant hurdle to the rationale design and development of better radioprotectors. Consideration of the oxygen sensitizing effect will serve to illustrate the argument.

Oxygen sensitizing

It has been recognized since the earliest days of radiobiology that the damaging effects of ionizing radiation are exacerbated by the presence of oxygen and, conversely, lowering the prevailing oxygen level ameliorates the extent of the injury. Indeed, in the absence of physical controls like separation distance, or effective shielding, there may be nothing more radioprotective than anaerobiosis. Obviously, this can provide a useful experimental variable in experimental work with some cultured cell types, but is of no practical value as a means of ameliorating radiation injury in any air-breathing organism. On the other hand, if the specific cytotoxic product(s) generated as a consequence of the interaction between oxygen and ionizing radiation is(are) identified, it follows that these could be targeted as a strategy for discovering and developing radioprotectors. It is extremely likely that this pre-requisite has already been satisfied. The mitochondrially-targeted manganese superoxide dismutase (MnSOD, SOD2) has been shown to significantly ameliorate the damaging effects of x-ray irradiation at all stages during and after the dose has been received [10,11] clearly implicating superoxide (O_2•–) and/or its secondary products as the primary oxygen-derived species responsible for the sensitizing effect of oxygen. In fact, upregulation of SOD2 activity through transfection procedures is an excellent approach to radioprotection, but it is only applicable to a small number of individuals, neither practical nor cost effective when considering populations. Nevertheless, this was a seminal piece of work having important mechanistic consequences that continue to be underappreciated. The cytosolic copper-zinc enzyme (Cu/ZnSOD, SOD1) does not ameliorate the effects of irradiation unless it is manipulated to include the mitochondrial-targeting sequence from SOD2 in its primary structure [12]. These observations categorically argue that the double-strand beaks of nuclear DNA, originally proposed as the primary lesions following irradiation (the "classical model") and still often described as such, cannot be causative in the mechanism of radiation induced damage in the intermediate dose range. If they were, SOD1 activity (not mitochondrially targeted) would necessarily be radioprotective rather than SOD2 activity. Following doses above 20 Sv, it is certainly possible to detect fragmented nuclear DNA in electron micrographs of tissue samples, but this corresponds to a dosing regimen where the pathological effects are more essentially physical in nature rather than resulting from the initiation of cellular biochemical processes. Currently, the key mitochondrial sites damaged during and immediately following irradiation appear to remain at best controversial, but likely unidentified.

Oxygen-derived cytotoxicity

Elevated superoxide production is clearly cytotoxic, but the superoxide radical, itself, is only a poor electrophile and weak reductant, incapable through direct reaction of leading to the kind of consequences associated with "oxidative stress" secondary to any stressor. To resolve this paradox, the hypothesis that elevated hydrogen peroxide (H_2O_2) formed from superoxide leading to the generation of the considerably more reactive/toxic hydroxyl radicals (OH•) gained popularity, but the reactions suggested to be involved do not proceed to any significant extent under *in vivo* conditions. Other reasons for the relative unimportance of hydroxyl radicals in any kind of biological signaling process where there are specific targets have been documented [13,14]. An alternative and tenable explanation of the cytotoxicity of superoxide is the extremely rapid (diffusion-limited) uncatalyzed reaction between superoxide and nitric oxide (NO•) forming the peroxynitrite anion (ONO_2–). Peroxynitrite and/or its conjugate acid (ONO_2H, pKa = 6.8) are clearly cytotoxic and able to generate the biomarkers normally associated with oxidative stress in addition to some others [15,16]. In the several cases examined by my own research group, it has consistently been found that peroxynitrite is the key oxygen-derived reactive species associated with the early biological response to ionizing radiation [17,18]. It is correct that the hydrogen peroxide-dependent peroxidase activity of cytochrome c is implicated in the oxidation of cardiolipin during radiologic apoptosis [19], but this is almost certainly a long way downstream of the early events that should be blocked to afford optimal radioprotection and does not impact other radiologic mechanisms of cell death, such as cell-cycle arrest [18].

Many, perhaps most, authors gloss over matters of the small reactive species generated during and immediately following ionizing radiation exposures. For example, superoxide, hydrogen peroxide, hydroxyl and other small alkyl radicals are collectively referred to as reactive oxygen species (ROS), or partially-reduced oxygen species (PROS) and, sometimes reactive nitrogen species (RNS) to take into account peroxynitrite and other possibilities. The continued use of these catch-all phrases is less than helpful in my view, not just betraying a lack of insight, but also reinforcing a certain reluctance to think critically about these particular details that one might reasonably suspect could prove crucial to the task at hand of designing better radioprotectors.

Concluding remarks

Overall, the radiobiological response is a multifaceted problem that continues to at least draw the attention of researchers from many diverse backgrounds. In recent decades, however, the Field has become increasingly dominated by projects focused on biomedical questions, especially regarding the treatment of cancers, almost to the exclusion of other related areas of interest. The associated cell biology is highly complicated and, once thoroughly immersed in these research problems, the individuals involved and the irradiation facilities they manage are often not routinely available for collaborative ventures in more peripheral areas that may lie just outside their

sphere of expertise/comfort zone. The case for more effort in the development of radioprotectors is straightforward and compelling. While specific molecular targets have not been identified, mitochondria have been established as the critical organelles damaged by intermediate doses of ionizing radiation. Peroxynitrite formed secondary to superoxide generated in mitochondria has been shown to be a key damaging oxidant following irradiation. Thus, reductants able to undergo facile reaction with peroxynitrite that can also partition into mitochondria might be at least one good starting point for seeking new radioprotectors. This challenge seems much less formidable than trying to delineate and understand the signaling networks associated with the cancer cells and their responses to radiotherapy, so it is not completely out of the question that existing research groups could be engaged in both. However, the two kinds of study require somewhat different motivations and skill sets, so it is unlikely that new radioprotectors are going to start emerging without specific investment of resources directed toward this end.

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