COMMENTARY

Small molecule derived from a natural product that mitigates radiation injury

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Exposure to ionizing radiation is unavoidable for the many cancer patients that require radiotherapy as a component of their treatment. Radiation exposure also occurs from rare events, such as nuclear power plant malfunctions, terrorist attacks with "dirty bombs," or detonation of nuclear weapons. The Chernobyl and Fukushima Daiichi disasters have demonstrated that the first responders to such emergencies have particularly high risks of radiation exposure. The types of ailments that victims suffer following total body exposures are dose-dependent (1). With total body exposures of <2 Gy, lymphocyte counts fall by about 50% in the first 24 h, nadir at 10–15 d, and take 3–6 mo to recover. The hematopoietic syndrome is a more extreme version of this that occurs following exposure to 2.5–5 Gy, during which bone marrow depletion can be fatal without bone marrow transplantation. Following whole-body exposures of 5–12 Gy, the victims who survive hematopoietic crisis via bone marrow transplantation subsequently face fatal intestinal injury. All survivors of these different situations are at risk for developing radiation-induced mutations and associated carcinogenesis. Several academic and governmental groups have organized to develop medical countermeasures in preparation for nuclear disasters. Compounds, termed radioprotectors, are capable of ameliorating radiation effects if delivered before or during the radiation exposure. A more elusive class of agents, termed radiation mitigators, are effective when administered hours or days after the radiation exposure occurs. The distinction between radioprotectors and mitigators is important, because the mobilization of countermeasures is logistically difficult in the midst of an unexpected disaster, so treatment is likely to begin at a time well after the radiation exposure occurs. Both classes of drugs were recently reviewed (2). It is noteworthy that these drugs could be valuable in clinical oncology, if they can protect normal tissues from radiotherapy injury while not simultaneously protecting tumor cells. Currently, the only Food and Drug Administration-approved radioprotector is Amifostine, and its only approved radiation-related indication is resected head and neck carcinomas.

DIM Mitigates Radiation Injury

In PNAS, Fan et al. have presented a major advance in the study of radioprotectors and mitigators (3). The authors demonstrate that a small molecule 3,3′-diindolylmethane (DIM) protects rodents from death after potentially lethal doses of total body irradiation (TBI).

The ability of DIM to mitigate radiation in mice offers strong proof in principle for this small molecule.

Importantly, this treatment was effective when delivered up to 24 h after TBI, thereby demonstrating evidence for true mitigation activity. DIM is a bioactive metabolite of indole-3-carbinol, which is a naturally occurring phytochemical in cruciferous vegetables. This compound is orally bioavailable and stable in the acidic gastric contents. DIM is also active when delivered by intraperitoneal or subcutaneous injection. This versatility in administration routes is relevant and important to the goals of radiation countermeasure programs, because irradiated victims are likely to have impaired intestinal absorption following bowel exposure. The physiochemical properties of DIM satisfy all of Lipinski's rules of drug-likeness (4), suggesting that it may not require medicinal chemical optimization to be an effective drug candidate.

The intracellular binding target of DIM that confers its activity is unknown. However, the mechanistic studies presented by Fan et al. (3) convincingly demonstrate that DIM-treated cells more rapidly rejoin radiation-induced DNA double-stranded DNA breaks (DSBs). Specifically, DIM increases both cellular survival and DSB rejoining in non-tumorigenic epithelial cell lines, and both of these effects require the presence of intact ataxiatelangiectasia mutated (ATM) activity. Furthermore, DIM-treated rodent tissues exhibit rapid ATM activation, as well as the phosphorylation of multiple ATM substrates. This activation of ATM signaling appears to result from the inhibition of protein phosphatase 2A, a negative upstream regulator of ATM.

DIM Effects on DNA Repair

Fan et al. (3) consider the stimulation of ATM-dependent DNA damage response to be the primary mechanism by which DIM mitigates radiation damage in cells. However, challenging questions arise when one considers the fast kinetics of canonical ATM-mediated DNA repair, together with the very long time period (≤24 h) after exposure during which DIM can mitigate damage. The rejoining of radiation-induced DSBs is known to follow a biphasic kinetic pattern, composed of initial rapid phase (10–20 min) followed by a slow phase (several hours). However, even for the 15% of DSBs religated in the slow phase, the large majority (>95%) appear to be religated by 24 h (5). Therefore, it is difficult to attribute all DIM activities to ATM-mediated DSB rejoining. This discrepancy might be explained by recent studies that have investigated DSB repair by the nonhomologous end joining (NHEJ) and homologous recombination (HR) pathways. Helleday and colleagues showed that the cell's choice of whether to use HR or NHEJ is not always a binary decision (6). Even though most radiation-induced DSBs are rapidly rejoined by NHEJ, at least a fraction of the lesions subsequently undergo secondary replication-associated DNA breakage peaking at 7–9 h after radiation exposure. These secondary DSBs are thought to be repaired by HR many hours after the exposure. These data underscore the concept that HR and NHEJ probably have overlapping and complementary roles, such that some DSBs invoke repair by more than one single

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pathway. Interestingly, DIM treatment leads to phosphorylation of two key HR regulators, BRCA1 (breast cancer 1, early onset) and CHEK1 (checkpoint kinase 1). Additionally, BRCA1 activity was shown to be essential for DIM-mediated mitigation. Taken together, these findings suggest that DIM may allow cells to tolerate radiation by promoting HR repair, which occurs well after the initial DSBs are religated.

DIM Exerts DNA Repair-Independent Effects

ATM activation is clearly required for DIM to mitigate radiation injury; however, the degree to which the downstream stimulation of ATM-mediated DSB rejoining confers cellular survival is unclear. DIM was shown to potentiate radiation-induced stimulation of NF-κB activity and to reduce radiation-induced apoptosis. These results are consistent with known downstream effects following ATM activation, which include NF-κB activation and repression of apoptotic death (7). Interestingly, an NF-κB inhibitor essentially eliminated DIM-induced radiation mitigation, indicating that the mitigation activity of DIM depends strongly on this NF-κB activation. This finding raises the possibility that DIM protects mice from radiation, at least in part, by simply blocking apoptotic cell death. If that were the case, DIM's impact would be reminiscent of the p53 inhibitor pifithrin, which prevents TBI-induced death in rodents by reducing apoptotic death (8). This mode of protection is expected to be highly mutagenic because it permits cells to survive with unrepaired DNA damage. A more appealing mechanism for DIM protection might instead involve a combination of both apoptotic repression and DNA repair stimulation. For example, the NF-κB pathway might cooperate with DNA damage response by repressing apoptotic death, thereby providing more time for cells to complete DNA repair before replication and division. This mechanism seems likely because both NF-κB and BRCA1 are required for DIM-mediated mitigation in cell-based experiments.

Cautionary Thoughts and Conclusions

Potential limitations of DIM should be considered in the oncology setting, where DIM could potentially be used to protect normal organs from radiotherapy. This concept for DIM use in this context is supported by the xenograft tumor experiments in Fan et al. (3), which suggest that DIM does not protect tumor cells from therapeutic radiotherapy. However, this lack of observed tumor protection may be because of the tumor type selected for the experiment. MDA-MB-231 breast cancer xenograft tumors exhibit constitutively phosphorylated ATM and defective downstream ATM signaling. Therefore, one would not predict observing DIM-induced cell protection in this peculiar biological background, even if DIM is capable of activating ATM in more typical tumor types. Additionally, the experiment design (e.g., radiation delivery schedule and DIM administration schedule) was quite different between the TIB and tumor experiments, and these differences may explain the apparent lack of tumor protection. Furthermore, a range of different tumor types would need to be examined before reaching this conclusion. Therefore, further preclinical testing is warranted before concluding that DIM will not undermine tumor cure rates with radiotherapy.

Fan et al. (3) should be congratulated for this very interesting study. The ability of DIM to mitigate radiation in mice offers strong proof in principle for this small molecule. Like all good research, however, this study generates both questions and answers regarding DIM's mechanism of action, as well as the underlying biology of radiation tolerance. If their observations are confirmed, these investigators will have opened the door to additional targets that can be exploited to modulate radiation effects in cells.

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