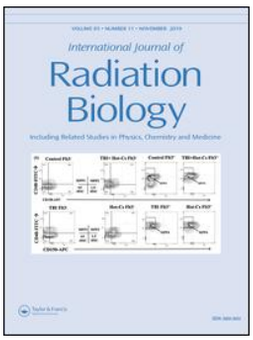

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REVIEW

Expert consultation is vital for adverse outcome pathway development: a case example of cardiovascular effects of ionizing radiation

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ABSTRACT

Background: The circulatory system distributes nutrients, signaling molecules, and immune cells to vital organs and soft tissues. Epidemiological, animal, and in vitro cellular mechanistic studies have highlighted that exposure to ionizing radiation (IR) can induce molecular changes in cellular and subcellular milieus leading to long-term health impacts, particularly on the circulatory system. Although the mechanisms for the pathologies are not fully elucidated, endothelial dysfunction is proven to be a critical event via radiation-induced oxidative stress mediators. To delineate connectivities of events specifically to cardiovascular disease (CVD) initiation and progression, the adverse outcome pathway (AOP) approach was used with consultation from field experts. AOPs are a means to organize information around a disease of interest to a regulatory question. An AOP begins with a molecular initiating event and ends in an adverse outcome via sequential linkages of key event relationships that are supported by evidence in the form of the modified Bradford-Hill criteria. Detailed guidelines on building AOPs are provided by the Organisation for Economic Cooperation and Development (OECD) AOP program. Here, we report on the questions and discussions needed to develop an AOP for CVD resulting from IR exposure. A recent workshop jointly organized by the MELODI (Multidisciplinary European Low Dose Initiative) and the ALLIANCE (European Radioecology Alliance) associations brought together experts from the OECD to present the AOP approach and tools with examples from the toxicology field. As part of this workshop, four working groups were formed to discuss the identification of adverse outcomes relevant to radiation exposures and development of potential AOPs, one of which was focused on IR-induced cardiovascular effects. Each working group comprised subject matter experts and radiation researchers interested in the specific disease area and included an AOP coach.

Conclusion: The CVD working group identified the critical questions of interest for AOP development, including the exposure scenario that would inform the evidence, the mechanisms of toxicity, the initiating event, intermediate key events/relationships, and the type of data currently available. This commentary describes the four-day discussion of the CVD working group, its outcomes, and demonstrates how collaboration and expert consultation is vital to informing AOP construction.

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Cardiovascular disease

Ionizing radiation (IR) can impact the circulatory system, leading to diseases of the brain and heart. Among the diseases of the circulatory system, cardiovascular disease (CVD) is the leading cause of death worldwide, with 1 in 10 people aged 30–70 dying from diseases of the cardiovascular system (CVS) (Ahmad & Anderson 2021; Roger et al. 2012). It encompasses a variety of heart pathology sub-classes including stroke, peripheral artery disease, cardiomyopathy, cardiac hypertrophy, and ischemic heart disease (Figure 1). Reviews on the subject are extensive and many mechanisms have been proposed for IR-induced CVD (Little 2016; EPRI 2020). Historically, the adult heart has been considered radio-resistant due to the low proliferation rate of cardiomyocytes (ICRP 1984). However, new research suggests that

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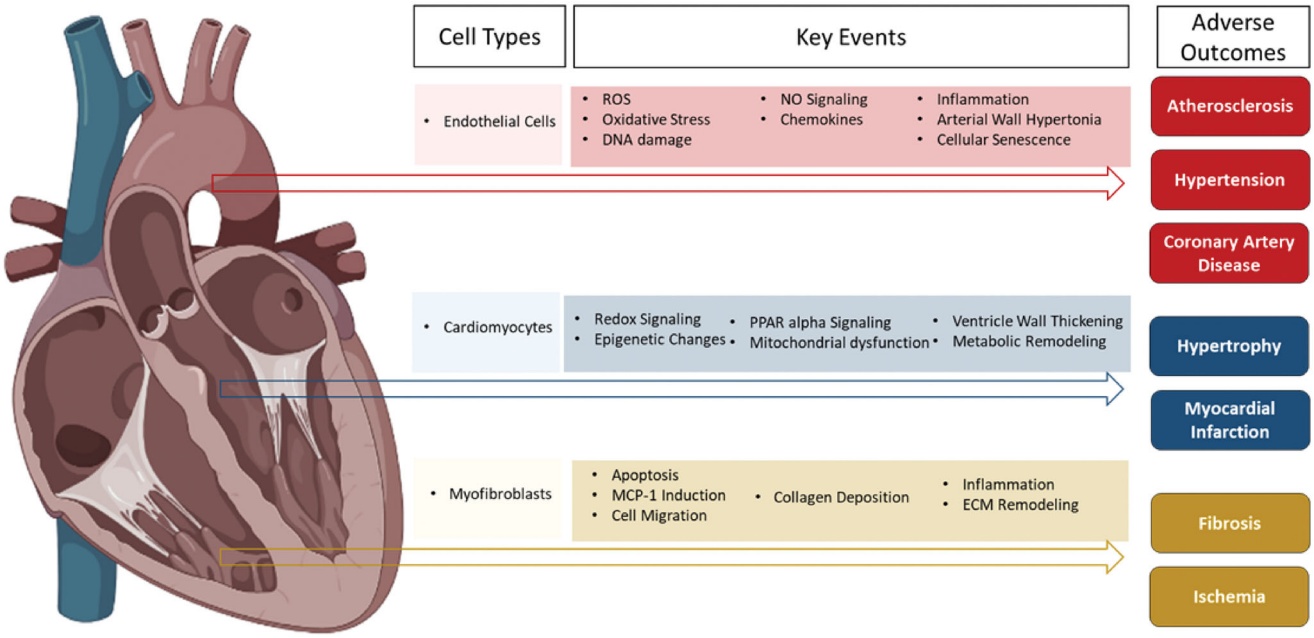

Figure 1. Proposed cell types in the heart, key events and adverse outcomes that may contribute to cardiovasular disease. Not all potential cell types and key events are listed, only those that were discussed in the working group meetings. Also some of the key events listed may be common across the different cell types. Heart schematic was created in BioRender.com. ECM: extracellular matrix; NO: nitric oxide; ROS: reactive oxygen species.

the CVS is relatively sensitive to IR, with subclinical changes detectable as early as a week following an acute exposure and leading to chronic disease over time, depending on dose (ICRP 2012).

Epidemiological reports clearly show that cardiac exposure to moderate (0.5–1 Gy) and high doses (1–5 Gy) of IR increases the risk of CVD (Kreuzer et al. 2015; Little 2016). For example, a significantly increased risk of mortality is observed in patients with left-sided breast cancer who received the heart doses ranging from 3 to 17 Gy (Reinders et al. 1999; Guldner et al. 2006; Darby et al. 2010; Andratschke et al. 2011; Bouillon et al. 2011; Darby et al. 2013). Moreover, it is now proposed that doses of irradiation much lower than previously considered can cause adverse effects on the CVS. Cardiac exposure to low doses of IR has been shown to induce marked structural, cellular, and molecular alterations in the irradiated heart, with mild but significant functional impairment. An increased risk of radiation-associated CVD after chronic and low to moderate dose (0.1-0.5 Gy) exposures has been shown in epidemiological studies on accidentally or occupationally exposed populations (Preston et al. 2003; Azizova et al. 2010; Shimizu et al. 2010; Simonetto et al. 2014; Azizova et al. 2015a, 2015b). Data from the Japanese atomic bomb survivors suggest that the injury to the vascular system may be an important component of the heart risk (Takahashi et al. 2017). Further evidence is provided by animal models that support this notion (Tapio 2016; Baselet et al. 2019; Hamada et al. 2020). Single acute high dose exposure (2, 8 and 16 Gy) in a mouse model suggests that irradiation directly induces stress signals by triggering an inflammatory response that further results in progressive structural damage of the myocardium and microvasculature (Seemann et al. 2012; Baselet et al. 2016). These studies have implicated the vascular endothelium as a target for radiation exposure. Oxidative stress and chronic inflammation associated with radiation exposure may trigger endothelial dysfunction and downstream vascular remodeling (Baselet et al. 2019). However, clear mechanisms have not been delineated, particularly at the low doses. With decades of research in this field, there is a significant need to organize and consolidate the information into a structured framework.

Adverse outcome pathways

The adverse outcome pathway (AOP) approach helps to assemble current knowledge on well-accepted critical events linked to disease progression. The approach is being used in chemical toxicology to understand key events (KEs) causing diseases that are of relevance to the human population (Villeneuve et al. 2014). AOPs begin with a molecular initiating event (MIE) defined as the first interaction of the stressor with a cell. This initiation then leads to downstream KEs that are critical hallmarks along the path to the adverse outcome (AO). A KE is represented at the molecular, cellular, tissue, organ, and individual level and linked by key event relationships (KERs) that are supported by in silico, in vitro, and in vivo experimental data (OECD 2016a).

The Organisation for Economic Cooperation and Development (OECD) launched a program in 2012 to support the AOP approach (Ankley et al. 2010; OECD 2016b). Currently, there are over 500 AOPs in their AOP Wiki (www.aopwiki.org), of which only a few are built using radiation studies, while the remainder are chemical/ecotoxicological-centric. Although there are only a few radiation AOPs, a number of the KEs and KERs in the AOP Wiki are relevant and can be populated with radiation-relevant studies. This facilitates interconnectivity across simple AOPs, allowing them to form networks that more accurately depict

the complexity of a given disease. An example of effective collaboration between the chemical and radiation communities has already been shown through a case study of an AOP developed for lung cancer outcomes relevant to the stressor of radon gas (Chauhan et al. 2019; Chauhan et al. 2021; Stainforth et al. 2021). This AOP contains shared KEs leading to different AOs and is supported by evidence from both chemical and radiation stressors.

Effective building of AOPs is dependent on available information from studies that support each KER. The process of finding evidence to support an entire AOP, or even individual KERs, is a challenge requiring an understanding of the scientific literature and subsequently, organization of those studies to evaluate the weight of the evidence of causality, plausibility, consistency and specificity for each and whole KERs according to the modified Bradford-Hill criteria (Becker et al. 2015). This is best achieved through collaborations and expert consultations. Such an approach can be facilitated through working groups with subject matter experts in the AO of interest and with knowledge spanning biological levels of organization.

MELODI workshop

The Workshop on AOP development for IR effects, organized by the Multidisciplinary European Low Dose Initiative (MELODI) and the European Radioecology Alliance associations, was held virtually on April 12-16, 2021. The main objective was to raise the level of interest for the use of the AOP approach in radiation protection, leveraging experience from the chemical toxicology field. The workshop fostered discussions on the AOP approach among different communities: health and environmental effects, radiation protection and chemical toxicity, scientists and regulators. As part of the workshop, four working groups were formed to focus discussions on AOP development for specific diseases of interest to the radiation field: leukemia, vascular effects, reproduction, and fetal development. Meetings were held over a four-day period to promote the exchange of ideas and information and detail next steps.

Vascular effects is an important area of interest for AOP development. It encompasses many disease pathologies with different underlying mechanistic basis. Within vascular disease it was highlighted that there was available epidemiological evidence to demonstrate an increased risk of CVD following exposure to IR. The decades of available in vitro and in vivo studies on this topic make IR-induced CVD an attractive candidate for AOP development. The findings of these studies facilitate structuring of a framework and provide the evidence needed to support the KERs, including any areas of uncertainty.

Over the course of the workshop, the AOP approach was explored and discussions centered on identifying the dominant mechanistic events associated with CVD progression, including the relevant information sources such as review articles and existing work in the AOP Wiki (https://aopwiki.org/). The working group members were knowledgeable in the field of CVD and from institutions of both regulatory and research-based interests. Daily discussions were guided by a CVD subject matter expert and an AOP coach. Although it was not feasible to develop a full AOP in the short timeframe of this workshop, the ensuing working group discussions described in the following sections provided the opportunity to review, distill, and organize a broad amount of information. A final consensus was reached with a focus on several KEs and distinct disease sub-classes that were organized into a proposed CVD AOP, which is presented in the last section of this paper.

CVD Working group discussions

State of the science

Day 1 of the CVD working group focused on examining the landscape of radiation-induced CVD by discussing the key findings of a recent review by Tapio et al. (2021). The review summarizes available human data across occupational, environmental, and diagnostic studies. Together, these studies in the field of IR show that at moderate doses (0.5–1 Gy), there is suggestive evidence of effects whereas from low doses (<0.1 Gy) evidence is still emerging. Mechanistically, the topic of radiation effects on the CVS is well-studied, and much information is available from data generated by in vitro and in vivo studies. The group deliberated on the need to distinguish low dose from high dose effects and how these would inform AOPs. It was highlighted that the development of an AOP would not be critically affected by the selection of a specific radiation dose. The goal is to compile key evidence, focusing on studies that support the evidence streams of the modified Bradford-Hill criteria for causality (Becker et al. 2015). The working group agreed that the best path forward was to examine the entire body of evidence, irrespective of dose, dose-rate and ionizing radiation types. Particularly, since the evidence in the area of low dose and low dose-rate (<100 mGy, <5 mGy/h) is somewhat still limited and sometimes variable, it would be practical to start structuring findings from high doses (1-5 Gy) where evidence would be rich and more consistent (Tapio et al., 2021). It was also noted that datasets for both acute and chronic radiation exposures could potentially be available. However, there is uncertainty on the extent that this data meets the stringent requirements of the modified Bradford-Hill criteria for incorporation in AOP. It was acknowledged that the potential contribution of dose, dose-rate, and different exposure scenarios (acute, chronic and fractionation) in a quantitative AOP needs to be systematically evaluated. Such a quantitative AOP can provide information for dose-response relationships among the MIEs.

The CVD working group also deliberated on several known KEs following irradiation including protein/DNA damage, cellular senescence, endothelial dysfunction, and specifically epigenetic changes, all based on a recent report (Tapio et al. 2021) (a summary schematized in Figure 1). It was agreed that endothelial dysfunction is an important etiological event following radiation exposure that impacts multiple KEs related to CVD development. Radiation-induced

endothelial dysfunction is characterized by decreased levels of nitric oxide (NO) and perturbation of the NO signaling pathway (Yentrapalli, Azimzadeh, et al. 2013; Yentrapalli, Azimzadeh, et al. 2013; Azimzadeh et al. 2015; Azimzadeh, Subramanian, et al. 2017; Hamada et al. 2020). Deficient NO bioavailability is a known hallmark of endothelial dysfunction, resulting in impaired endothelium-dependent vasodilation (Yu et al. 2011; Farah et al. 2018). However, whether the availability of studies would support these as independent KEs or a combined broad KE would need further investigation. Additionally, both cardiac metabolic and structural remodeling, especially fibrosis, were highlighted as important endpoints (Tapio et al. 2021; Yang et al. 2021). Together, these KEs could be formulated into multiple AOPs that target specific pathologies of vascular disease.

Oxidative stress as MIE

Day 2 focused on the importance of oxidative stress in radiation-induced CVS injury and its potential as the MIE for the AOP. Other MIEs were proposed including deposition of energy, hydrolysis, and ionizing events. The ionizing events would be distinguishable from minuscule thermal effects of deposition of energy. The MIE may also need to be discernable from a chemical stressor, as the origin of damage may be important when building a quantitative AOP, which brings together numerical dose- and time-response data. Participants of the working group came to a consensus that although oxidative stress is central to the process of CVD, 'deposition of energy' may be the more accurate event defining initiation and progression of damage. While some working group members argued that including both deposition of energy and oxidative stress in the AOP would be redundant, the relevance and essentiality of both these events could be delineated in terms of evidence, which underscored the need for each to be independent events in the AOP. Discussions also focused on a review by Higashi et al. (2009) that provided an overview of mechanistic events following oxidative stress in the development of CVD. The review documents that lipoproteins are central targets of oxidative stress, leading to oxidized low-density lipoproteins and NO inactivation. Loss of NO initiates a cascade of events including thrombosis, apoptosis, and altered vasomotion (spontaneous rhythmic low frequency oscillations of the arteries), eventually resulting in cardiovascular complications. This same series of events potentially contributes to similar vascular pathologies following radiation exposure (Higashi et al. 2009).

Space stressors: AOP as an example

Day 3 of the CVD working group discussions focused on existing AOPs – those found in the AOP Wiki as well as in an AOP being developed by Health Canada on health outcomes resulting from space travel. This AOP comprises a network of four AOs (cognitive disorders, CVD, cataracts, and bone loss) from exposure to space environmental stressors. It was noted that, although there is no evidence of cardiovascular effects from the relatively short-term space travel experienced by astronaut crews to date (Cucinotta et al. 2016; Ade et al. 2017; Elgart et al. 2018), the risk would be relevant after longer missions to Mars, where astronauts would be exposed to multiple stressors including galactic cosmic rays (Patel et al. 2020; Simonsen et al. 2020). However, due to the small cohort size, studies on astronauts will likely lack statistical power to detect these effects even at higher levels of exposure (Elgart et al. 2018). The space flight AOP begins with an MIE of deposition of energy leading to oxidative stress, resulting concurrently in the modification of proteins and the activation of pro-inflammatory mediators, leading to altered signaling pathways, endothelial dysfunction, and eventually, to vascular remodeling. Studies supporting this pathway are available and were recently reviewed by Meerman et al. (2021). The review highlights that space radiation can cause endothelial dysfunction in the aortic wall and in the myocardium. Such damage was shown to activate tissue resident immune cells and increase nuclear factor kappaB (NF-kB) activity, causing sustained inflammation and reactive oxygen species production, eventually leading to increased cellular apoptosis and decreased DNA methylation levels, which is an epigenetic hallmark (Tungjai et al. 2013; Koturbash et al. 2016; Meerman et al. 2021). The role of epigenetic changes in radiation-induced CVD was further discussed by the working group. A concern was raised that this was an emerging KE with insufficient evidence to show relevance to disease progression in the form of causation. It was also highlighted that in the space research field, the evidence to support causation of KERs may be lacking; therefore, a stressor-agnostic approach to building AOPs would be of benefit. The evidence for supporting interconnectivities could be made using clinical studies and ground-based animal studies that have identified potential mechanisms to CVD (Boerma et al. 2016). Additionally, there was a discussion on the interconnectivities of the space flight AOP with existing ones in the AOP Wiki (www.aopwiki.org) including AOPs on hypertension and atherosclerosis, which are under development and relevant to exposures involving airborne particulate matter. Discussions also highlighted that there are various components to CVD including fibrosis, atherosclerosis, and metabolic remodeling by virtue of fetal cardiac gene expression leading to myocardial pathology that could be included in the network (Sárközy et al. 2019) (Figure 1). Many biological events were identified for each of these diseases; however, there was uncertainty on how these interconnectivities would be made.

Identifying KEs and data

The last day of the working group session focused on the crosstalk between molecular events and tissue events. It began with discussions across three different cell types in the heart tissue: (1) endothelial cells (endothelium), (2) cardiomyocytes (myocardium), and (3) fibroblasts (extracellular matrix). These may lead to three different AOs (atherosclerosis, myocardium pathology, and fibrosis) contributing to

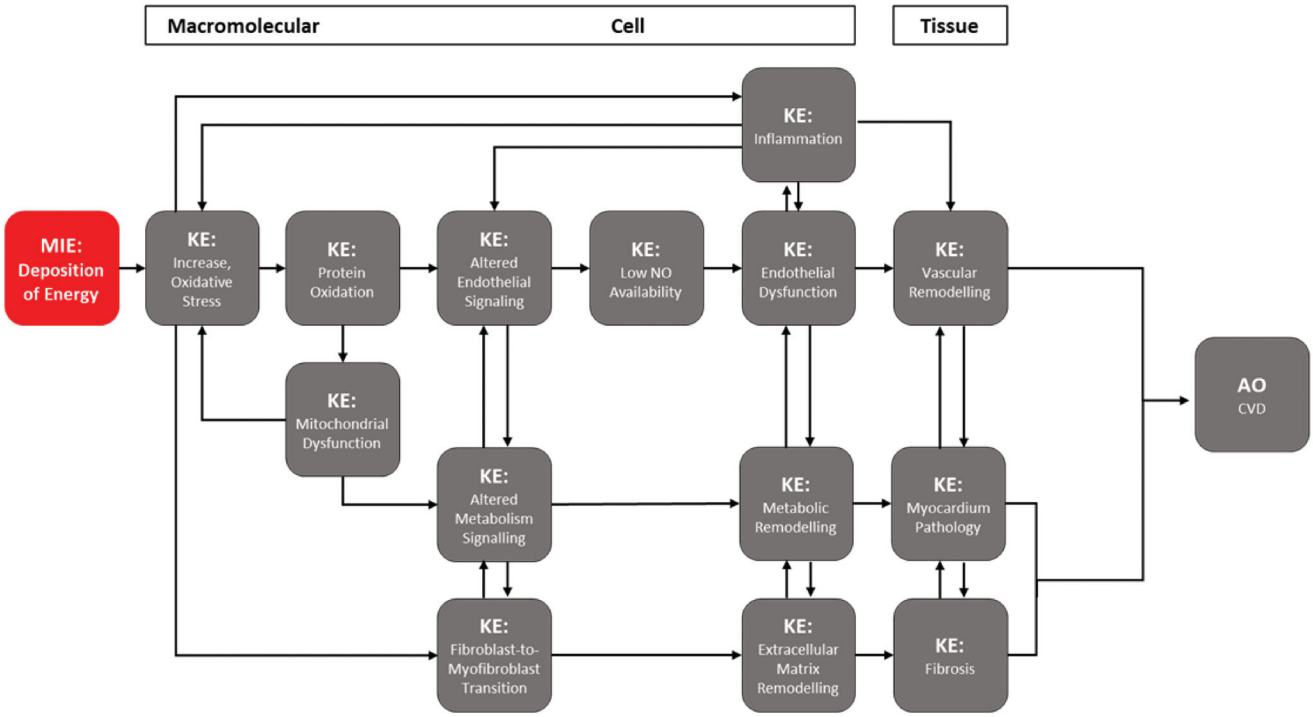

Figure 2. A hypothetical simplified adverse outcome pathway network to cardiovascular disease (CVD) derived from knowledge of working group members and subject matter experts. These connectivities may be modified upon a complete systematic assessment of the literature using OECD AOP guidelines. Not all non-adjacent linkages are shown.

progression of CVD (Figure 2). It was highlighted that there are a sufficient number of studies to support KEs to each of these pathologies at the cellular level (Baselet et al. 2016; Tapio 2016; Azimzadeh & Tapio 2017; Banfill et al. 2021).

In the context of endothelial cells, it has been shown that the effect of radiation exposure on endothelial cells is characterized by impairment of endothelial signaling pathways, increased adhesiveness, accelerated senescence, enhanced release of pro-inflammatory cytokines, and accelerated atherosclerotic process (Yentrapalli, Azimzadeh, et al. 2013; Yentrapalli, Azimzadeh, et al. 2013; Azimzadeh et al. 2015; Sievert et al. 2015; Azimzadeh, Subramanian et al. 2017; Baselet et al. 2017).

Atherosclerosis, defined as a chronic inflammatory disease affecting large and medium arteries and considered to be a major underlying cause of CVD, would be a key outcome to endothelial dysfunction. The pathophysiologic process by which atherosclerosis occurs is complex, driven by inflammation, and the different steps are connected and usually appear simultaneously (Libby et al. 2019; Libby 2021). Radiation has been shown to initiate and accelerate atherosclerosis in atherogenesis-prone animal models such as apolipoprotein E-deficient ($ApoE^{-/-}$) mice following acute, chronic, high and low dose exposure (Hoving et al. 2008; Stewart et al. 2006; Yu et al. 2011; Hoving et al. 2012; Monceau et al. 2013; Mancuso et al. 2015; Ebrahimian et al. 2018). Experimental studies have shown that high doses of IR promote inflammatory reaction and hemorrhages of atherosclerosis plaques (Ebrahimian et al. 2018). However, low and moderate dose studies have shown immunomodulatory response in the context of atherosclerosis and can lead to a decrease in plaque inflammatory profile and lesion size in a disease-prone mouse model (Ebrahimian et al. 2018; Rey et al. 2021). Recently, the pathologic sequence to atherosclerosis has been implemented into a biologically based risk model to describe the incidence of myocardial infarction in the German KORA (Cooperative Health Research in the Augsburg Region) population (Simonetto et al. 2021). The discussions also highlighted the differences between low and high dose irradiation effects on the development of atherosclerotic plaques.

In terms of cardiomyocytes, thoughts within the working group noted that the effect of radiation exposure on cardiomyocytes would be centered on mitochondrial dysfunction, leading to metabolic changes due to the impairment of the peroxisome proliferator-activated receptor (PPAR)-alpha pathway and energy production (Azimzadeh et al. 2013; Barjaktarovic et al. 2013; Subramanian et al. 2017; Azimzadeh, Azizova et al. 2017; Subramanian et al. 2018; Azimzadeh et al. 2020). This energy deposition subsequently affects heart contraction, leading to myocardium injury and pathology. Dose- and time-response effects on cardiomyocytes following radiation exposure are available and would inform the causality linkages in the AOP.

Lastly, the effect of IR on fibroblasts was discussed. Irradiation of fibroblasts can result in activation of transforming growth factor (TGF) beta signaling and onset of fibroblast to myofibroblast differentiation, resulting in extracellular matrix remodeling due to collagen deposition. A series of pathological events that accompany endothelium injury and inflammation leads to chronic tissue dysfunction and fibrosis (Seemann et al. 2012; Monceau et al. 2013; Stewart et al. 2013; Subramanian et al. 2017; Subramanian et al. 2018; Kosmacek & Oberley-Deegan 2020). Together,

the three pathologies of endothelial dysfunction, myocardial injury, and fibrosis would provide a more complete picture of radiation-induced damage on the CVS. The proposed network was identified as a potential path forward to collaborative AOP building. The role of the inflammatory process in IR-induced CVD was discussed and subsequently, it was emphasized that all types of cardiac cell injury are tightly associated with changes in the local and systematic inflammatory events and immunological responses. An increase in the levels of several inflammatory mediators such as interleukin (IL)-1, IL-6, IL-8, and TGF-beta, have been shown following high and moderate irradiation in in vitro or in vivo studies (Baselet et al. 2017; Tapio et al. 2021). In contrast, the studies using low doses identified pathways contributing to the anti-inflammatory effect of irradiation (Lumniczky et al. 2021). These findings indicate that the relation between radiation exposures and alterations in immunological events, including inflammation, is complex and sometimes not proportional to each other. The inflammatory response is strongly affected by the initial inflammatory state of the exposed tissue, the dose and dose-rate and quality of exposure.

Discussions on this day also emphasized the role of AOPs in identifying critical knowledge gaps for future IR-induced CVD research. For example, the establishment of an experimental model dedicated to the investigation of heart responses to low doses of IR is necessary, as current experimental model systems are not ideal. The uncertainties observed in the development of vascular injuries, especially atherosclerosis in available animal models, need to be addressed. Although wild-type mice were shown to be resistant to the development of atherosclerotic lesions after irradiation, the findings obtained from disease mouse models, particularly in $ApoE^{-/-}$ mice after low dose irradiation, remain inconsistent (Monceau et al. 2013; Mitchel et al. 2013; Mancuso et al. 2015). Additionally, understanding epigenetic effects induced by radiation is also a topic of interest with currently limited available data. Genome-wide association studies (GWAS) have identified hundreds of loci associated with coronary artery disease and myocardial infarction. A majority of these variants are located in the non-coding regions of the genome, indicating that epigenetics plays an important role in the disease process. However, functional analyses of these variants are needed to determine which cell types and processes are involved (Örd et al. 2021). There is limited information on epigenetic effects, but research on the topic is underway in context of radiation exposures (Lowe and Raj 2014; Koturbash et al. 2016; Miousse et al. 2019)

It was also highlighted that the study of IR-induced CVD in human cohorts remains challenging. In contrast to early-onset subclinical changes in heart function observed after high dose exposures applied in radiotherapy (Walker et al. 2019; van den Bogaard et al. 2021), there is a long latency time ($\geq$10 years) at low and moderate dose before any measurable clinical complications can be detected (ICRP 2012). Morbidity risk factors such as family history and lifestyle choices, inevitably influence the final disease outcome. Contribution of age is also noteworthy, particularly in the context if an individual has preexisting atherosclerosis and persistent inflammation compared to a young healthy person. This is an important issue that needs to be addressed from the radiation protection viewpoint as there is limited contribution of human data to support AOP development. This latency time and the uncertainty of CVD development after low dose exposure also emphasize the importance of using all available knowledge for AOP development.

Proposed hypothetical AOP

Based on the four-day discussion, it was evident that mapping out a simplified, linear, unidirectional AOP from deposition of energy from radiation exposure to CVD would be challenging. As a result, the proposed AOP evolved from a complex pathway to three distinct sub-classes of diseases for AOP development: vascular remodeling, myocardium pathology, and fibrosis (Figure 2). Deposition of energy is designated as the MIE in this AOP and leads to a KE of increased oxidative stress at the macromolecular level. Oxidative stress would be instrumental in the initiation of further tissue- and organ-level responses resulting in inflammation, an event that contributes to the more severe CVD adverse outcomes.

This proposed AOP is a starting point for the collation of data and appropriate studies meeting the modified Bradford-Hill criteria. Next steps include the compilation of evidence to support the KERs (linkages) in the AOP. That will need to be done systematically and transparently to identify literature that supports the dose-, time- and incidence-concordance as well as biological plausibility. Work is also required to identify the inconsistencies and controversies in the published literature. Following this evidence review and mapping, the AOP could be refined to those KEs that are data-rich and mechanistically well-defined. Eventually, the KEs that lack sufficient data or have only emerging data could be excluded.

Conclusions

The CVD working group discussions led to productive conversations and highlighted important processes and pathways for consideration in AOP development for IR-induced cardiovascular effects. Discussions centered on the scope of building AOPs, the potential KEs, MIE, data composition, and relevant scenarios of exposure. Knowledge gaps in the area of low dose and low dose-rate ($<$100 mGy, $<$5 mGy/h) irradiation were highlighted, emphasizing the need for more studies in the context of the cellular communication where the interplay of cardiac cells contributes to heart injury following exposure (Tapio et al. 2021). Moreover, the retrospective studies on archival autopsies such as collected from nuclear workers can be a better alternative for animal or cellular models to investigate the late and lasting effect of low dose, chronic radiation exposure on the human heart (Rybkina et al. 2014; Azimzadeh, Azizova, et al. 2017; Azimzadeh et al. 2020).

The AOP proposed here allows for structuring and simplification of the available mechanistic information about radiation-induced CVD and can facilitate predictive interpretations, beyond cellular or animal models, at the human population level. Collation of data will provide the linkages from macromolecular events to population relevance using the large body of epidemiological data supporting the relationship between IR and CVD, thus making this AOP directly relevant for regulatory applications. Overall, organization of the literature surrounding this disease within an AOP framework could provide avenues for prioritizing experiments and targets for the development of mitigation strategies to address the population health issues.

The final proposed network is complex, with interconnectivities and communication across different cell types (endothelial cells, cardiomyocytes, and fibroblasts), which results in three distinct pathologies across the cardiac and vascular structures. This shows the difficulty in attempting to simplify the knowledge using the AOP framework where some KEs and KERs are commonly and clearly defined while knowledge gaps exist for others and require further research. The productive discussions in these working group sessions highlighted, once more, the importance of interactions between diverse research communities to identify areas of relevance for AOP development. As screening and identifying relevant studies is critical to AOP construction, it was acknowledged that this resource-intensive, time-consuming process would benefit from collaboration availing expertise across various levels of biological organization. Together, through cross-disciplinary collaborations, a developed AOP in the area of CVD and radiation can effectively identify areas of knowledge gap and guide future research.

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Disclosure statement

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