Emerging issues in radiogenic cataracts and cardiovascular disease

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In 2011, the International Commission on Radiological Protection issued a statement on tissue reactions (formerly termed non-stochastic or deterministic effects) to recommend lowering the threshold for cataracts and the occupational equivalent dose limit for the crystalline lens of the eye. Furthermore, this statement was the first to list circulatory disease (cardiovascular and cerebrovascular disease) as a health hazard of radiation exposure and to assign its threshold for the heart and brain. These changes have stimulated various discussions and may have impacts on some radiation workers, such as those in the medical sector. This paper considers emerging issues associated with cataracts and cardiovascular disease. For cataracts, topics dealt with herein include (i) the progressive nature, stochastic nature, target cells and trigger events of lens opacification, (ii) roles of lens protein denaturation, oxidative stress, calcium ions, tumor suppressors and DNA repair factors in cataractogenesis, (iii) dose rate effect, radiation weighting factor, and classification systems for cataracts, and (iv) estimation of the lens dose in clinical settings. Topics for cardiovascular disease include experimental animal models, relevant surrogate markers, latency period, target tissues, and roles of inflammation and cellular senescence. Future research needs are also discussed.

Keywords: cataract; cardiovascular disease; threshold; radiation protection

INTRODUCTION

Over the past three plus decades, the International Commission on Radiological Protection (ICRP) has classified radiation effects into tissue reactions (previously called non-stochastic or deterministic effects) and stochastic effects [1]. By definition, tissue reactions result from injury to populations of cells, and are characterized by a threshold below which no effect would occur. Typical examples are cataracts and non-cancer skin changes, the severity of which increases with dose [2]. In contrast, injury to a single cell or small number of cells is supposed to cause stochastic effects, and its probability but not severity is regarded as a linear-non-threshold (LNT) function of dose. Stochastic effects comprise cancer and heritable effects due to somatic cell mutations and germ cell mutations, respectively [2]. In the context of radiation protection (RP), equivalent dose limits aim to prevent tissue reactions, whereas effective dose limits aim to reduce the risks of stochastic effects to the extent reasonably achievable [2].

Human radiation cataracts appeared in the literature as far back as 1903 [3], but atomic-bomb (A-bomb) and cyclotron cataracts observed in the late 1940s created a surge of interest...
in RP of the ocular lens [4, 5]. The ICRP listed cataracts as a radiation health hazard in 1950 [6] and recommended the first dose limit for the lens in 1954 [7]. The belief that cataracts result from a tissue reaction with a threshold dates back to 1969 [8], and the occupational dose limit recommended for the past few decades was calculated as a chronic dose threshold for vision-impairing cataract (VIC) divided by a working lifetime of 50 years. The dose limit for the lens has undergone revisions since 1954. The latest revision took place in 2011 when the ICRP Seoul Statement on tissue reactions lowered the chronic threshold for VIC from >8 Gy to 0.5 Gy and the occupational equivalent dose limit for the lens from 150 mSv/year to 20 mSv/year (100 mSv in defined 5 years with no single year exceeding 50 mSv) [9], leading to a resurgence of interest in RP of the lens. Besides, the Seoul Statement [9] was the first to recommend the threshold for cardiovascular disease (CVD) and cerebrovascular disease of 0.5 Gy to the heart and brain, respectively, although no dose limits have been recommended for such circulatory disease. ICRP Publication 118 (ICRP-118) [9] (report on tissue reactions), issued in 2012 as the scientific basis of the Seoul Statement, was the first set of guidelines to list circulatory disease as a radiation health hazard. These changes have raised various potential issues and may affect the medical sector, especially those who provide and receive interventional procedures (i.e. interventionists and patients) that deliver significant dose to the lens and heart [10]. These new thresholds hence request more attention to optimization and more efforts to monitor or estimate dose to the individuals concerned, but the dose evaluation system has yet to be established. In addition, the boundary between tissue reactions and stochastic effects is becoming fuzzy [11]. ICRP-118 deduced an acute threshold of 0.5 Gy for VIC from two papers on A-bomb data [12, 13]. Likewise, the threshold of 0.5 Gy for circulatory disease was supported by an excess relative risk (ERR)/Gy of ~0.1 that assumed an LNT dose response, where the A-bomb data [14] played central roles. These three papers indicated no threshold [12–14]. Then, ICRP-118 assigned the same threshold of 0.5 Gy for both cataract and circulatory disease irrespective of the rate of dose delivery (i.e. acute, fractionated/protracted and chronic exposures; n.b. the lack of dose rate effect deductively reflects stochastic events). Overall, ICRP-118 judged cataracts and circulatory disease as tissue reactions, but also considered that these effects may be of a stochastic nature with no threshold. These thresholds have been deduced exclusively from epidemiological data, but their validity should be verified biologically; as ICRP-60 [15] mentioned, a certain effect cannot justifiably be classified as a tissue reaction or a stochastic effect without knowledge of the mechanisms leading to the observable defect. In these examples, the mechanism of cataractogenesis remains incompletely understood, and much less is known about circulatory disease, highlighting the necessity for discussion on future research strategies to decipher the mechanistic underpinnings.

This paper summarizes our unique interdisciplinary discussion among researchers from the research fields of radiation, cataract and CVD. Current knowledge and underlying issues are overviewed, followed by discussion on perspectives for future studies.

**RADIOGENIC CATARACTS**

**Key papers used to deduce the new threshold**

ICRP-118 (an update of ICRP-41 [16]) aimed to review and evaluate the literature on tissue reactions from RP viewpoints. As regards cataracts, nearly 70 papers of pertinent epidemiological or other human studies were cited, three papers of which were used to judge the nominal threshold. The acute threshold of 0.5 Gy was deduced from two papers on A-bomb cataracts assessed at 55–57 years after exposure: the first paper [12] provided thresholds of 0.6 Gy for cortical cataracts and 0.7 Gy for posterior subcapsular (PSC) opacities (structures of the lens are schematized in Supplementary Fig. 1); and the second paper [13] provided a threshold of 0.1 Gy for cataract surgery prevalence. It should be noted, however, that its lower 90% or 95% confidence interval (CI) included zero dose. The threshold for protracted/fractionated exposures was considered not to be greater than the acute threshold. This judgment was made using the third paper [17] on cataracts in Chernobyl liquidators that were assessed at 12–14 years after exposure and provided the threshold for cortical or PSC cataracts of 0.34–0.5 Gy with the lower 95% CI of >0.1 Gy. For chronic exposures spread over several to many years, it was considered difficult to judge thresholds due to epidemiological uncertainties, and the same incidence of VIC with >20 years follow-up was assumed regardless of the acute or chronic nature of the exposure over a working life. Consequently, for practical purposes, the threshold of 0.5 Gy was judged as the dose to which 1% of individuals exposed develop VIC >20 years after exposure, independently of dose rates.

**Does a minor opacity progress into VIC?**

Since 1984, and before ICRP-118, the ICRP had recommended four different thresholds (i.e. for detectable minor opacity and VIC, each produced by a single brief exposure or highly fractionated/protracted exposures). Conversely, ICRP-118 recommended a single threshold value, assuming that minor opacities progress to VICs. Historically, ICRP-14 [8] described that minor opacities that do not interfere with vision often do not progress in severity and may regress or disappear spontaneously with time. ICRP-41 (an update of ICRP-14 in the tissue reaction context) mentioned that (i) lens opacities develop within months, progress rapidly and eventually cloud the lens completely at high dose; (ii) opacities take years to develop, remain microscopic in size and cause no significant visual impairment; and (iii) whether the lesion remains stationary or progressive depends on dose.
These descriptions may stem from the observations of Merriam and Focht [18] that the higher the dose, the shorter the time of appearance of the lens changes and the higher the incidence of progressive opacities, and that fractionation delays the onset of cataracts. Moreover, early A-bomb data showed little progression in lenticular lesions: for instance, 36% were unchanged and 19% regressed in 1951 (6 years after exposure) [19, 20], and ~60% were unchanged and ~30% regressed in 1966 (21 years after exposure) [21]. Collectively, these findings suggest that the latency period shortens with increasing dose, but that minor opacities may not necessarily progress to VICs.

Are there any dose rate effects?
ICRP-118 assumed the lack of a dose rate effect for cataracts without epidemiological evidence. For cancers, epidemiological data for residents in high natural background radiation areas [22, 23] can be compared with those of acutely exposed individuals (e.g. A-bomb survivors). Such human studies are essential for cataracts, but it is tempting to discuss whether there is a dose rate effect. In this regard, an attractive hypothesis has been proposed whereby if radiation damage does not accumulate (because of elimination of damaged tissue stem or progenitor cells through tissue turnover or cell death), the irradiated tissue does not undergo radiation carcinogenesis [24]. Namely, dose rate and tissue turnover rate are postulated to determine the dose rate effect, where irradiation may stimulate tissue stem cell turnover depending on the tissue type [25]. In this context, the lens represents a unique tissue derived from a single cell type. Throughout life, the lens continues to grow and all cells stay inside the lens. Tissue turnover cannot thus be expected in the lens, and hence the dose rate effect may not exist for cataractogenesis. Taken together, to be stationary or not to be is the question. Reanalysis of the afore-cited human data of Merriam and Focht [18] reveals that there is little dose rate effect for progressive cataracts, unlike stationary cataracts (Fig. 1). The lack of a dose rate effect indicates accumulation of radiation damage within the tissue, and production of initial radiation damage to DNA is a stochastic event, implying that generation of a progressive cataract is a stochastic process.

Is cataractogenesis of a stochastic nature?
Besides the assumed lack of a dose rate effect, the lower CI of the threshold doses in the two papers used to deduce the acute threshold included 0 Gy, indicating the lack of a threshold. The following experimental findings support the hypothetical stochastic nature of cataractogenesis. First, the number of lens opacities is an LNT function of dose at 0–3 Gy of X-rays and 0–0.38 Gy of neutrons [28]. Second, mice heterozygous or nullizygous for DNA repair genes ATM, RAD9 and/or BRCA1 develop radiation cataracts earlier than wild-type counterparts [29–31]. ATM-disrupted mice of Elson et al. [32] used as the 129SvEv strain crossed with the Black Swiss strain manifested an accelerated phenotype of spontaneous and radiation cataractogenesis [29], but this was not the case for the ATM-disrupted

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**Fig. 1.** Changes in estimates of the minimum cataractogenic dose for a 50-year exposure estimated by a Strandqvist plot. The minimum cataractogenic dose to produce human cataracts in each of three exposure conditions (a single exposure, exposure over 3 weeks to 3 months, and exposure over 3 months to 9 years) was taken from Merriam and Focht [18], and plotted on a log–log scale as a function of exposure time (i.e. a Strandqvist plot [26]). Equation of the fitted line, its correlation coefficient square \( r^2 \), and the estimated minimum cataractogenic dose in röntgen (r) for a 50-year exposure \( (D_{50y}) \) estimated from the fitting equation are shown in each panel (n.b. 1 r roughly corresponds to 1 cGy). The time of a single exposure was treated as 4 h according to Merriam et al. [27], and 50 years were treated as 18.262.5 days. (A) The minimum dose to produce a cataract in each exposure condition, irrespective of whether the cataract is stationary or progressive. (B) The minimum dose to produce a stationary cataract in each exposure condition. (C) The minimum dose to produce a progressive cataract in each exposure condition. Note that Merriam and Focht [18] used the term ‘cataract’ to mean all clinically recognizable opacities (i.e. both minor opacities and VICs), ‘stationary cataract’ to mean the opacity that remains stationary at any stage or progresses slowly over a considerable period and then remains stationary (i.e. a cataract that does not progress to a VIC), and ‘progressive cataract’ to mean the opacity that continues to progress to a VIC and becomes non-specific.
mice of Barlow et al. [33] used as the C3H strain [34] (data not shown), suggestive of strain sensitivity or individual susceptibility [35]. ATM gene polymorphism also appears to affect cataract surgery risk among A-bomb survivors [36]. Intriguingly, whilst the human lens does not develop tumors, cataractogenesis possibly involves diverse tumor suppressor or DNA repair genes (e.g. p53, p16\textsuperscript{Ink4a}, p19\textsuperscript{Arf}, p27\textsuperscript{Kip1}, NBS1, XRCC1, HSF4 and WRN) [37–41] in addition to ATM, RAD9 and BRCA1. Notwithstanding, ICRP-118 concluded that cataracts are a tissue reaction with a threshold, albeit small, because there is no direct evidence that a single damaged progenitor lens epithelial cell (LEC) produces a cataract. In this regard, LECs in the germinative zone (GZ) around the equatorial region have been considered to be the relevant cells at risk, because cataracts are produced after localized irradiation of the equatorial region [42] but not after irradiation when the GZ is shielded, nor after irradiation with LEC divisions inhibited [43–45]. Moreover, recent studies highlight that flow-cytometrically identified putative murine stem cells localize around the GZ [46], and that a lens progenitor-like cell differentiated from human embryonic stem cells forms a lentoid body [47]. It would hence be worth testing whether an irradiated, single lens stem cell generates a cloudy lentoid body, though the difference in physical conditions between in vivo exposure and in vitro exposure needs to be recognized.

**Implications of findings obtained from analysis of the effects of oxidative stress and ultraviolet exposure**

ICRP-14 stated that there is no evidence that lens opacities depend quantitatively on cell killing, but ICRP-58 [48] considered that cell killing is the sole mechanism behind all deterministic effects. ICRP-92 [49] was the first to propose that whereas abnormal differentiation underlies radiation cataracts, cell killing underlies all other deterministic effects. It may be tempting to speculate on the mechanisms of cataractogenesis from the findings obtained in pertinent fields of research. The following two subsections overview the current knowledge on the response of the lens and crystallin proteins to oxidative stress, ultraviolet (UV) light and ionizing radiation.

**Role of antioxidants in lens opacification**

Oxidative stress-induced cataracts may result either from diminished antioxidants due to aging or from increased reactive oxygen species (ROS) following exposure to various external insults such as ionizing radiation, UV light and chemicals [50–52]. UV exposure generates ROS in the lens [53], which in turn causes degradation, cross-linking and aggregation of lens proteins, and also DNA damage. UV-modulated ROS are thus important for cataractogenesis [53, 54].

The lens has evolved an impressive repertoire of antioxidant defense systems (Fig. 2). Of these, the peroxiredoxin (Prdx) family is actually a superfamily of selenium-independent peroxides and comprises at least six members (Prdx1–6) [55–58]. Prdxs use redox-active cysteines (Cys) to reduce peroxides, and fall into two categories: the 1-Cys Prdx (Prdx6) is different from 2-Cys Prdxs due to the lack of a C-terminal Cys residue required for intersubunit disulfide bond formation in the 2-Cys Prdxs [58]. The lens expresses all six Prdxs, among which Prdx6 expression is most pronounced [55, 59]. Prdx6 protects cells from damage to membranes, DNA and proteins produced by ROS-driven...
oxidative stress and/or lipid peroxidation [55–60]. Patients aged >70 years express a lower level of Prdx6 mRNA than younger patients at the time of cataract surgery [61]. Nuclear and cortical cataracts were more severe in patients aged ≤70 years, whose LECs express a lower level of Prdx6 mRNA. The progression of age-related cataracts should thus involve increased oxidative stress resulting from the depletion of antioxidants such as Prdx6 in the lens. Importantly, introduction of the tagged Prdx6 into rodent LECs reduces cell death and DNA damage, and delays opacification following hydrogen peroxide treatment [60, 62]. Depletion of Prdx6 in mouse LECs increases ROS levels and cell killing following UVB exposure, implying that reduced Prdx6 expression underlies cellular damage and impaired homeostasis against UVB exposure in the lens, leading to cataractogenesis [62]. UV exposure may promote the progression of radiation cataracts [63], and free radical scavengers delay radiation cataracts [64, 65]. An antioxidant (e.g. by the addition or induction of Prdx6 controlling ROS in the lens) may be useful for delaying radiation cataracts.

Modification of lens proteins
The mechanism of cataract development is incompletely understood, but thought to involve the abnormal lens protein aggregation that lets incoming light scatter instead of focusing on the retina. Human lens proteins mainly consist of α-, β- and γ-crystallins, and their overall structure, stability and short-range interactions are thought to contribute to lens transparency. α-crystallin is a large molecule of ~800 kDa comprised of two subunits, αA and αB. Each subunit is a polypeptide of ~20 kDa, and α-crystallin is an aggregate of ~40–50 subunit molecules. The β/γ-crystallin superfamily comprises oligomeric β-crystallin and monomeric γ-crystallin [66]. αA- or αB-crystallins are members of the small heat-shock protein family and function as molecular chaperones to protect β- and γ-crystallins from aggregation [66]. Lens crystallins account for ~90% of the total water-soluble proteins in a highly concentrated form and constitute the refractive medium. Lenses constantly receive UV light and oxidative stress, and therefore damaged proteins accumulate in water-insoluble fractions due to the absence of turnover of the lens proteins. Water insoluble proteins increase in aged and cataractous lenses [66]. Furthermore, lens crystallins undergo various post-translational modifications, such as isomerization and inversion of aspartyl (Asp) residues (i.e. l, β, γ, and η formation), deamidation of asparagine or glutamine residues, disulfide bonding of cysteines, oxidation of methionine or tryptophan, backbone cleavage, phosphorylation and glycation during the aging process [67–69]. These modifications may reduce crystallin solubility, alter lens transparency and lead to cataract development. Indeed, post-translational modifications are strongly related to aggregation and loss of solubility of crystallin. Of these, η-Asp formation was proposed to underlie the change in the higher order structure and the loss of function of crystallins, considering that η-Asp formed in the protein should make the configuration of the Asp residue opposite. In addition, the β-linkage produced with Asp formation may affect the crystallin quaternary structure because the main chain of the protein becomes elongated. The Asp isomers may therefore cause insolubilization, abnormal aggregation and induction of partial unfolding of proteins, leading to a disease state. In fact, γ-ray and UV irradiation causes the η-Asp formation in α-crystallin, which results in abnormal aggregation and reduction of its chaperone activity [70–72]. Mechanisms for spontaneous formation of η-Asp residues have been clarified [67], and Fig. 3 explains the possible steps for the simultaneous formation of β- and η-Asp residues in the protein. The η-Asp formation results from the racemization of specific Asp residues in the lens crystallins: a newly developed detection method could reveal that Asp residues 58, 76, 84 and 151 of αA-crystallin, and Asp 62 and 96 of αB-crystallin are highly converted to l, β, γ- and η-α-isomers in age-related cataracts [73]. Detection of such isomers may be useful for molecular epidemiological survey of cataracts induced by radiation or other causes.

Radiation weighting factor
The relative biological effectiveness (RBE) varies with the linear energy transfer (LET) of radiation such that high-LET radiation (e.g. neutrons and energetic heavy ions) is more genotoxic and cytotoxic than low-LET radiation (e.g. X-rays and γ-rays) [74, 75]. The lens was the only tissue for which ICRP recommended a special radiation weighting factor (wR) in 1964–1977 [76, 77] because it was believed that the lens is specifically vulnerable to neutrons. ICRP-92 could not recommend the wR or RBE values for cataracts due to lack of human data and concern as to how to apply the experimental data, and ICRP-118 did not deal with tissue reactions following high-LET irradiation. Whilst the wR and RBE of ≤20 have been commonly used, much higher RBE values have been reported: for example, the 95% CI of neutron RBE for A-bomb cataracts was 12–228 at 70 mGy [78]; the 80% CI of neutron RBE for rat cataracts was 250–500 at 2 mGy [79]; and the heavy ion RBE for rat cataracts was 50–200 at 10 mGy [80]. The wR relating to low-dose stochastic effects (chromosome aberrations) should not be used for cataracts and other tissue reactions without justification, awaiting further studies considering human exposure to high-LET radiation (e.g. accelerator or nuclear workers, cancer patients and space travelers).

The necessity to standardize cataract evaluation systems for epidemiological studies
ICRP-103 [2] defined the threshold as the dose that causes a particular effect in 1% of exposed individuals. With the approach of ICRP-99 [81], the population size required to detect a 1% risk with statistical significance was estimated to
be ~46,000 people, given the background cataract incidence of 75% [82]. Nevertheless, the population size for each cataract-related epidemiological study has not exceeded 10,000. A pooled analysis should help solve this issue, but has yet to be conducted because of varying endpoints and scoring systems in different studies. For instance, let us consider the three papers used in ICRP-118 to judge the threshold. One paper [13] looked at cataract surgery as a surrogate for VIC, whereas two papers [12, 17] graded lenticular changes using the Lens Opacity Classification System (LOCS) II or the Merriam–Focht method. Opacities and cataracts classified by these two different methods cannot be directly compared, but approximation algorithms have been proposed to convert from the LOCS III to either the Oxford system [83] or the Wisconsin system [84] (and vice versa). Such conversion may be useful for the pooled analysis of epidemiological data obtained with different grading systems, though approximation processes may cause some artifacts. Taken together, the boundary between opacities and cataracts is ophthalmologically vague, and various diagnostic modalities have been used (e.g. slit-lamp biomicroscopy, retroillumination or Scheimpflug imaging). Endpoints, diagnostic approaches and classification systems should therefore be standardized for future epidemiological studies.

Fig. 3. Possible reaction pathways for spontaneous isomerization of aspartic acid (Asp) and deamidation of asparagine (Asn) residues in protein. The simultaneous formation of β- and d-Asp residues in the protein could be explained in the following four steps. (i) When the carbonyl group of the side chain of the L-α-aspartyl residue/L-α-Asn is attacked by the nitrogen of the amino acid residue following the Asp residue, L-succinimide is formed by intramolecular cyclization. (ii) L-succinimide may be converted to d-succinimide through an intermediate [I] that has the prochiral α-carbon in the plane of the ring. (iii) Protonation of the intermediate [I] may proceed from the upper or lower side of the plane in an ordinary peptide or protein. (iv) d- and L-succinimide are hydrolyzed at either side of their two carbonyl groups, yielding both β- and α-Asp residues, respectively. Thus, four isomers, L-α-Asp, L-β-Asp, D-α-Asp and D-β-Asp, are simultaneously formed in the protein.

The dose limit: preventing cataracts versus reducing cancer risks
Cataracts are not life threatening, and most of them are surgically curable. Notwithstanding, cataracts limit occupational performance and interfere with daily life activities. Cataracts should hence be prevented (c.f. its risk should be reduced if stochastic). By definition, the new equivalent dose limit for the lens of 20 mSv/year aims to prevent VICs at a morbidity of 1%/0.5 Sv (and a mortality of 0%/0.5 Sv). For comparison, the effective dose limit of 20 mSv/year aims to reduce cancer risks to a mortality of 2.06%/0.5 Sv (and a morbidity of 5.84%/0.5 Sv) (n.b. excluding the contribution of heritable effects) [85]. For instance, exposure of the Japanese population to 0.5 Sv is estimated to increase VICs morbidity from 75 to 76%, cancer morbidity from 48 to 54% and cancer mortality from 20 to 22% [85]. Thus, these dose limits deal with cataract morbidity more strictly than cancer mortality, necessitating the development of a common scale to compare diverse effects regardless of the life-threatening and stochastic nature.

Potential issues arising from implementation of the new dose limit
The new dose limit for the lens was not 10 mSv/year (0.5 Sv divided by a working life of 50 years) but 20 mSv/year [9].
This was because a higher limit would not be protective given the substantially lower threshold, and because alignment with the effective dose limit of 20 mSv/year facilitates regulatory implementation [86]. In this scenario, the lifetime occupational exposure at the rate of the dose limit (i.e. 0.1 Sv/5 years × 50 years = 1 Sv) doubles the nominal threshold. Accordingly, optimization of protection was also recommended [86].

The new dose limit may affect some medical (e.g. interventionists) or nuclear (glove box users fabricating mixed oxide fuel) workers. Supplementary Table 1 provides the estimated lens dose of ~260,000 workers (~40% of all registered radiation workers) in Japan, showing that >0.5% of medical workers exceeded 20 mSv/year in the last two years. Supplementary Table 2 lists the recently reported lens doses of interventionists and support staff, indicating a mean dose per procedure of 3.9–141 µSv (ranging from 1–1083 µSv) and a mean annual dose of 19–500 mSv (ranging from 1–4674 mSv). Of the three basic elements of RP, shielding with protective tools would be the most realistic to reduce the lens dose of these workers, because it would be difficult to shorten the time (e.g. by increasing workers or decreasing patients) or to increase the distance from the radiation source.

**Estimation of the lens dose in clinical settings**

The lens dose of medical workers and patients has seldom been evaluated, given that the occupational dose limit has rarely been exceeded. However, lens dose management will become more important considering the new threshold. The dose should be known at each time when a worker or a patient undergoes repeated examinations or interventional procedures. For this, it would be practical if the patient dose could be estimated using irradiation parameters. The Medical Internal Radiation Dose (MIRD)-based mathematical phantom is generally used for Monte Carlo simulations of dose, but this does not include the lens. By contrast, the MIRD-type phantom presented in Fig. 4 includes the lens in addition to other major organs used for effective dose calculation. With this mathematical phantom and the use of irradiation parameters, the absorbed dose to any organ can be calculated. The difference between the simulated dose and the dose experimentally determined using a physical phantom was mostly within ±20%, depending on the organ irradiated (e.g. ±7% for a cranial computed tomography scan), and this can serve as a practical patient dose evaluation approach.

Regarding the occupational dose to interventionists and their support staff, information on the spatial dose distribution in an X-ray examination room is important in order to prepare the work plan and to accurately perform the safety assessment of radiation medical examination. Such information is generally obtained by measuring the dose at many points with radiation survey meters etc., but it would be impractical to measure dose distributions every time and also be difficult to know how irradiation conditions and direction of radiation are affecting dose distributions. The measurement data can be used in dose estimation for a certain model case, but may sometimes result in poor accuracy and underestimation of the dose. In this regard, the lens dose of medical workers involved in interventional procedures can be evaluated with the Monte Carlo method by estimating spatial dose distributions (Fig. 5) [87]. The difference between the simulated dose and the experimentally determined dose is within ±10% at a source-to-patient distance of 3 m. Besides, this approach can calculate spatial dose distributions using recorded irradiation patterns (e.g. an irradiation angle), and is thus useful for evaluating the absorbed dose to any organs of workers in a range of standing positions and to conduct planning for adequate protection.

In summary, Monte Carlo simulations will be useful for estimating patient dose and spatial dose distribution in the X-ray room for work planning and retrospective dose reconstruction. Future related issues include how to manage and record cumulative patient dose.

**What else do we need to know about cataracts?**

Various fundamental questions remain unanswered about cataractogenesis, necessitating a greater mechanistic understanding. Several examples of such questions, in addition to those already mentioned, are as follows.
What are the target cells for PSC cataractogenesis?

PSC cataracts are most common radiogenic cataracts (c.f. least common of senile cataracts); in contrast, cortical cataracts, which are also radiation-related, almost always track behind PSC cataracts in time. PSC cataracts have been believed to result from either abnormal proliferation and migration of LECs, or aberrant fiber cell terminal differentiation [88, 89]. LECs in the GZ appear to be the relevant cells at risk, as discussed above, and aberrant and excessive LEC proliferation around the equator seems to most plausibly account for radiogenic PSC cataractogenesis [44, 45, 90, 91]. However, the target cells are not known, and the question of whether a cataract arises from a single cell or a group of cells remains unanswered, as does the question of whether cataractogenesis is attributable to damage to stem, progenitor cells and/or functional cells, warranting further studies. Findings on non-radiogenic PSC cataracts (e.g. those caused by steroids) should also help improve mechanistic understanding. What determines the anatomical location of cataract development remains a fascinating question.

What triggers lens opacification?

LECs divide in the GZ, move posteriorly, and terminally differentiate into elongated fiber cells that have no organelles. Newly formed fiber cells surround existing fiber cells concentrically. Proper fiber cell terminal differentiation and maintenance of the highly ordered arrangements of crystallins and fiber cells are responsible for lens transparency. Abnormal LEC behaviors would surely be one of the upstream events in radiation cataractogenesis, because the
inhibition of LEC divisions prevents radiogenic misalignment of fiber cells and lens opacification [44, 45]. Furthermore, the tumor suppressor protein p53 has been proposed as protecting the lens against spontaneous PSC cataractogenesis [37], so it would be interesting to test if topical enhancement of p53 function in the lens alleviates radiation carcinogenesis, and if ‘super p53’ mice [92] manifest fewer radiation cataracts than their wild-type counterparts.

Irradiation disturbs fiberogenesis via the posterior displacement of the nuclei of abnormally elongating fibers, which disorganizes the cytoarchitecture of the lens bow [93]. Disruption of NBS1 or of HSF4 promotes spontaneous cataractogenesis and makes fiber cell denucleation incomplete [39, 94], but their roles in radiation cataractogenesis are unknown. Future studies should clarify these issues.

Whether protein aggregation is an early trigger event for radiation cataractogenesis or the result of the cataractogenesis remains unknown. Whole body exposure of a mouse to ≥0.5 Gy of γ-rays changes crystallins [95], though its impact on lens opacification is unclear. Accumulation of β-amyloid (Aβ) in the human lens has recently been proposed as promoting lens protein aggregation and opacification [96]. Aβ accumulates in the opaque rat lens epithelium, but this was not the case in its cortex or nucleus [97]. Cerebral Aβ plaque accumulation occurs in a mouse model of Alzheimer’s disease exposed to 0.1 Gy of high-LET iron ions [98], but not in wild-type mice exposed to 0.1 Gy of X-rays [99]. Dose and radiation quality dependence of Aβ accumulation and its impact on the lens would thus be interesting.

The earliest lenticular changes following irradiation include the formation of ‘vacuoles’ in the equatorial regions, which then appear in the central portion of the posterior cortex and PSC regions [100, 101]. These vacuoles appear to be transient in nature [102] and greatly differ in diameter (e.g. 10 µm–1.3 mm [103]), but the composition of the intravacuolar fluid with lower refractive index than the surrounding lens material is currently uncharacterized and requires elucidation.

**Do calcium ions play roles?**

The development of senile nuclear or cortical cataracts involves a lens ionic imbalance, such that whereas the intracellular levels of Ca2+ and Na+ in the lens escalate with age, those of Mg2+ and K+ decline. Induction of inducible nitric oxide synthase (iNOS) appears to precede the elevation of the lens Ca2+ levels, which in turn causes opacification via enhanced lens protein degradation and nitration [104, 105]. Increased serum Ca2+ levels are associated with A-bomb PSC cataracts but not with cortical cataracts [31, 106]. Changes in and roles of lens Ca2+ levels are unknown, but this evaluation may be warranted because radiation is an iNOS inducer [107].

**What determines the latency period?**

Human radiation cataracts take from a few months to more than half a century to appear, and dose cannot simply explain such difference [18, 108]; thus further studies are required to address whether different mechanisms work for early- and late-occurring cataracts, or for cataracts, which are induced by low- and high-dose irradiation, and how individual differences in radiosensitivity affect cataracts in the latency period.

**RADIgenic CVD**

**Current knowledge and unresolved issues concerning CVD**

ICRP-118 used the ERR/Gy and baseline disease risk to explain the threshold. The ICRP recognized uncertainties in the underlying scientific evidence, but assumed the same ERR/Gy independent of dose rate for RP purposes. This has raised precautionary consideration of the inclusion of circulatory disease in the RP system for chronic or fractionated low-dose exposure. The most important biological issues to be solved for scientific substantiation will be whether small lesions caused by low-dose radiation persist and accumulate long enough to show symptoms, and whether low-dose-rate radiation causes disease in proportion to total dose.

Epidemiological studies provide information for identification of radiation-associated effects. Studies on high-dose exposure (e.g. medical exposure) [9] and the meta-analysis of moderate- to low-dose exposure [109] have suggested an associated increase in ischemic heart disease (IHD). Acceleration of atherosclerosis is thus assumed as a possible mechanism for radiation-associated CVD.

ICRP-118 discussed the possible mechanism of radiation-associated CVD being mainly based on an inflammation theory [110]. Inflammation is a known key mechanism in atherosclerosis [111], and radiation induces inflammatory responses. An enhanced pro-inflammatory response has been observed at >2 Gy [112] and at 0.1–0.5 Gy [113, 114], but anti-inflammatory effects have also been observed at <0.5 Gy [115, 116]. Acute exposure of mice to 0.1 or 0.3 Gy inhibits leukocyte recruitment against lipopolysaccharide challenge [115]. Fractionated low-dose exposures decrease diabetes-induced inflammatory gene expression in the heart, whereas a slight but significant increase is observed in non-diabetic control mice [116]. These results suggest a non-linear dose response for radiogenic inflammation, implying different etiological mechanisms at a low dose. However, these findings were obtained in animal models under highly inflammatory conditions, and suitable experimental models are necessary to study radiation effects on the circulatory system under physiological conditions. Taken together, whereas these experimentally observed inflammatory responses are transient in nature, CVD has a long latency period. Thus long-lasting and cumulative effects need to be analyzed.
Vascular endothelial cell senescence has recently been recognized as an important surrogate marker for CVD [117]. Chronic low-dose-rate irradiation also appears to accelerate vascular endothelial cell senescence in vitro [118]. Senescence may thus be a good marker (Fig. 6), and more detail is provided below. The roles of inflammation and of endoplasmic reticulum (ER) stress in CVD and metabolic disease are also discussed.

**Role of cellular senescence in CVD**

Vascular cells have a finite lifespan in vitro and eventually enter a state of irreversible growth arrest called cellular senescence. Flattening and enlargement of vascular cells are known morphological characteristics of senescence [119]. The expression of negative cell cycle regulators (e.g. p53 and p16) increases with cell division, thereby promoting growth arrest [120]. Primary cultured cells undergoing senescence in vitro show increased expression of senescence-associated β-galactosidase (SA β-gal) activity, which is correlated with the aging of cells and thus regarded as a biomarker for cellular senescence [121]. Vascular cells obtained from human atherosclerotic plaques have impaired in vitro growth and develop senescence earlier than those from normal vessels [122]. Both vascular endothelial cells and vascular smooth muscle cells (VSMCs) exhibit the morphological features of cellular senescence [123]. These findings suggest vascular cell senescence in vivo, which has been actually confirmed. For instance, repeated endothelial denudations markedly enhance the accumulation of SA β-gal-positive vascular cells in damaged rabbit carotid arteries [124]. Moreover, SA β-gal-positive vascular cells exist in atherosclerotic plaques obtained from the coronary arteries of IHD patients [125]. These SA β-gal-positive cells are predominantly localized on the luminal surface of the atherosclerotic plaques and are identified as endothelial cells, while such cells are not observed in the internal mammary arteries of the same patients where atherosclerotic changes are minimal. In advanced plaques, however, SA β-gal-positive VSMCs are detected in the intima but not in the media [126]. This may be due to extensive cell replication in the lesions, as is observed in arteries subjected to double denudation [124]. The finding that SA β-gal-positive cells in human atheroma exhibit increased expression of p53 and p16 further serves as evidence in favor of in vivo senescence. These cells also show various functional abnormalities, such as decreased expression of endothelial NOS (eNOS) and increased expression of pro-inflammatory molecules [126, 127]. Cellular senescence may thus contribute to the pathogenesis of human vascular aging.

Telomerase adds telomers to chromosome ends [128]. Most somatic cells, including vascular cells, show progressive telomere shortening due to low telomerase activity, in contrast to the high telomerase activity and the maintained telomere length observed in some stem cells. Critically short telomeres resemble damaged DNA and trigger cellular senescence via a p53-dependent pathway [129]. Such telomere shortening occurs in human blood vessels and may be related to atherogenesis. For instance, the telomere length of endothelial cells from the abdominal aorta and iliac arteries is inversely correlated with age [130]. Interestingly, telomere shortening occurs faster in iliac artery endothelial cells than in internal mammary artery endothelial cells [130]. A high level of hemodynamic stress may thus enhance endothelial cell turnover in the iliac arteries compared with that in vessels subjected to less stress. Telomeres are shorter in coronary artery endothelial cells from patients with coronary heart disease than in cells from healthy subjects [131]. Shorter endothelial telomere length has been found in patients with a longer history of CVD risk factors [132], suggesting that these factors override the effect of chronological aging on endothelial cell turnover by accelerating stress-induced damage. Identification of the factors that accelerate endothelial telomere attrition could provide a novel strategy with which to treat human atherosclerosis.

**Role of inflammation and ER stress in CVD and metabolic disease**

Over the past decade, ample evidence has demonstrated an integration of metabolic and immune response pathways. Metabolic and immune systems share common regulatory mechanisms, and ‘metaflammation’ (defined as metabolic-driven, low-grade, chronic inflammation) has been observed in metabolic diseases including obesity, insulin resistance, type 2 diabetes and CVD [133]. Excess metabolic nutrients trigger metaflammation and activate signaling pathways involved in classical and canonical inflammation, such as c-Jun N-terminal kinase (JNK), inhibitor of nuclear factor κB kinase (IKK), and double-stranded RNA-dependent protein kinase (PKR) [133].

Inflammatory cytokines, bioactive lipids and metabolic stress pathways act in both metabolic and immune responses, leading to metaflammation in metabolically critical organs and tissues. Both extracellular mediators (e.g. inflammatory cytokines and lipids) and intracellular stress (e.g. ER stress...
and ROS) initiate metaflammatory pathways. Fatty-acid-binding proteins also play significant roles in the development of metabolically triggered inflammation [134, 135]. All of these mediators activate a number of inflammatory signaling pathways including JNK, IKK and PKR [133, 136].

The ER is an important organelle that serves as a protein quality control device and coordinates synthesis, folding and trafficking of proteins [137]. Pathological conditions including increased protein synthesis, hypoxia, and irradiation interfere with the ER folding capacity and lead to ER stress. ER stress activates complex signaling pathways called the unfolded protein response (UPR). The UPR activates inflammation and has been implicated in the etiology of several chronic inflammatory diseases [137]. The UPR involves the integration of adaptive responses through three ER membrane sensors: PKR-like ER kinase (PERK), inositol-requiring enzyme-1 (IRE1) and activating transcription factor 6 (ATF6). These sensors activate adaptive responses such as the inhibition of protein translation, transcriptional production of protein-folding chaperones, and ER-associated degradation [137]. The UPR also induces apoptosis if the stress is too severe and prolonged.

Regarding ER stress-related inflammation, IRE1 interacts with tumor necrosis factor receptor-associated factor 2 and activates JNK and IKK, which in turn induce the expression of inflammatory cytokines [138, 139]. PERK activation decreases the translation of inhibitor of NF-κB (IκB), thereby augmenting NF-κB transcriptional activity and the expression of its pro-inflammatory target genes [140]. ER stress also activates PKR, a member of eIF2α kinase family [136].

Disruption of ER function is associated with the development of obesity, insulin resistance and type 2 diabetes [141]. JNK activation mediated by IRE1 and PKR suppresses insulin receptor signaling during ER stress [136, 141]. PKR inhibitors reduce adipose tissue inflammation and improve insulin sensitivity in obese and diabetic mice [142]. Enhancement of the ER folding capacity in obese mice through transgenic strategies or the use of chemical chaperones (e.g. sodium 4-phenylbutyrate and tauroursodeoxycholic acid) relieves ER stress in liver and adipose tissue and restores glucose homeostasis [143, 144].

Prolonged activation of ER stress pathways contributes to macrophage death and subsequent plaque necrosis in advanced atheroma [145]. Expression of the UPR effector C/EBP-homologous protein (CHOP) that triggers apoptosis is markedly increased in plaques with vulnerable morphology in the human coronary artery [146]. Moreover, deletion of CHOP in mouse models of advanced atherosclerosis suppresses macrophage death and plaque necrosis [147].

What more should we know about CVD?

Possible mechanistic models have been proposed for radiogenic CVD [111], but a consensus has yet to be reached, especially for low-dose and/or low-dose-rate exposures. The absence of established experimental model systems is an underlying problem. The following four issues are discussed here: (i) experimental model animals; (ii) relevant surrogate markers; (iii) time-span and mode of exposures; and (iv) target tissues and endpoints.

Mice lacking apolipoprotein E have been used in the field of radiation biology because of their known predisposition to atherosclerosis, but this type of experimental model with spontaneous pathological conditions may not be suitable for the purposes of extrapolation to human risk estimation. Even though low-dose irradiation of such genetically predisposed mice may cause suppressive effects (i.e. hormesis), these effects should not be regarded as beneficial effects. This is because the radiation-induced suppression of spontaneous pathological conditions may not necessarily reflect the changes that would be induced by irradiation of normal mice.

Given the myriad of differences between mice and humans (e.g. in lipid metabolism, cell cycle checkpoints, immune system and lifespan), mice may not be a good model system for human atherosclerosis. We expect that porcine models of accelerated coronary atherosclerosis [148] will be important for radiation research, but the use of rodent animals is more practical because many irradiation facilities are designed for rodents (especially mice for exposure to low-dose-rate radiation). Identification of radiation-related markers in mice relevant to human CVD is thus a priority.

Surrogate markers need to work similarly in humans and in animal models. Vascular cell senescence has been recognized as a surrogate marker for the risk of vascular dysfunction, but causality needs to be established. For ER stress, there are no surrogate markers that can be measured in humans. It is difficult to show causal associations between inflammatory changes and atherosclerosis or CVD. Considering the long latency of CVD, the time-lag between irradiation and manifestations is important, and radiation-related endpoints observable for long periods after irradiation will need to be identified as surrogate markers. Dose rate is a related issue. Although the ICRP have suggested the same threshold dose for acute and chronic exposures, it is questionable to assume that they involve the same mechanism, as mentioned in ICRP-118.

If CVD is to be regarded as a tissue reaction caused by injury in populations of cells, the dose rate that affects all functional units should be used for testing. It would be of the order of 1 mGy/h based on the unit dose of a single photon track [149] and the rate of DNA repair [150], or of the order of 10 mGy/week based on the stem cell turnover rate in tissues (e.g. [151]).

It remains unknown whether CVD induced by acute or chronic radiation exposure is the same as age-related CVD. Acute radiation accelerates cellular senescence without shortening telomeres [152], suggesting a possible difference between radiogenic and age-related CVD. Low-dose-rate irradiation of cultured human umbilical vein endothelial cells induces senescence with a potential dose-rate threshold...
of <2 mGy/h [153]. Given that long-lasting stress stimulates stem cell turnover and induces cellular senescence, chronic exposure experiments will be important.

The target tissue of radiation effects is also an issue. For instance, chronic exposure to 20 mGy/day increases adiposity without an increase in feed consumption [154], but it is unclear which tissue (adipose tissue or other organs such as endocrine systems) is critical for inducing such effects. Both epidemiological studies [155] and biological studies [156] have raised the possibility that radiogenic renal dysfunction contributes to the acceleration of CVD. Thus we need to consider the possibility that radiation effects on other tissues could modify or amplify the effects and affect circulatory systems (Fig. 6).

Epidemiological studies provide information for the estimation of human risks, but are compromised by several issues. First, epidemiological associations between radiation and circulatory disease collectively consist of many different endpoints, but analysis (e.g. dose response) of each specific disease should provide intrinsically important information. Second, risk transfer between populations is not easy, because the baseline disease rate affects ERR.

Because no single relevant markers for radiogenic CVD are available for animal experiments, markers should be identified that can explain human risks and which are observable for long periods (even after low-dose or low-dose-rate exposures) and consistent with epidemiological results. From the viewpoints of RP and the characteristics of CVD, it would be important to explore the effects after long-term low-dose-rate exposure. Molecular mechanistic markers are needed to validate the generality of the expected cellular/animal findings for extrapolation to humans, but pathological pre-symptomatic changes that contribute to the process of CVD development would also be useful to evaluate causal associations between the observed effects and disease.

CONCLUSIONS

Here we have discussed the emerging issues in radiation-induced cataracts and CVD, and new thresholds surely led to an increased interest in its manifestations and mechanisms. The similarities and dissimilarities observed in early- and late-occurring cataracts, and in CVD induced by acute high dose and chronic low doses of radiation are not well understood. The possible stochastic nature of cataracts and of CVD renders the boundary between tissue reactions and stochastic effects vague and may call for a new concept for the classification of radiation effects [11]. Because the new thresholds indicated by the guidelines are as low as 0.5 Gy, and also because the dose for patients is clearly higher than that for medical workers, ‘therapeutic reference level’ may need to be considered in addition to ‘diagnostic reference level’. The target tissues for the prevention of circulatory disease remain uncertain, and may include the heart, brain, kidney and/or the entire vasculature. In short, more studies on radiogenic non-cancer effects are evidently necessary.

SUPPLEMENTARY DATA

Supplementary data is available at the Journal of Radiation Research online.

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