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A Systematic Review of Epidemiological Associations between Low and Moderate Doses of Ionizing Radiation and Late Cardiovascular Effects, and Their Possible Mechanisms

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The link between high doses of ionizing radiation and damage to the heart and coronary arteries is established. In this paper, we systematically review the epidemiological evidence for associations between low and moderate doses (<5 Gy) of ionizing radiation and late-occurring cardiovascular disease. Risks per unit dose in epidemiological studies vary over at least two orders of magnitude, possibly a result of confounding factors. An examination of possible biological mechanisms indicates that the most likely causative effect of radiation exposure is damage to endothelial cells and subsequent induction of an inflammatory response, although it seems unlikely that this would extend to low-dose and low-dose-rate exposure. However, a role for somatic mutation has been proposed that would indicate a stochastic effect. In the absence of a convincing mechanistic explanation of epidemiological evidence that is less than persuasive at present, a cause-and-effect interpretation of the reported statistical associations cannot be reliably inferred, although neither can it be reliably excluded. Further epidemiological and biological evidence will allow a firmer conclusion to be drawn. © 2008 by Radiation Research Society

INTRODUCTION

Risks associated with exposure to ionizing radiation have been known for almost as long as ionizing radiation itself. Within a year of the discovery of X rays by Röntgen, skin burns had been reported (*1*), and within 7 years a case of skin cancer was observed (*2*), in all cases associated with high-dose X-ray exposure.

It has generally been assumed that ionizing radiation risks at moderate to low doses and dose rates are dominated by cancer risks in the directly exposed individuals. The mechanisms by which low doses of ionizing radiation cause cancer are reasonably well understood, being fundamentally driven by mutational damage to DNA (*3*), although a role for non-DNA targeted effects cannot be ruled out (*4*). At high radiation doses, such as would be received by patients treated with radiotherapy, a variety of other (so-called deterministic or tissue reaction) effects are observed, resulting from inactivation of large numbers of cells and associated functional impairment of the affected tissue. Among such effects are direct damage to the structures of the heart—including marked diffuse fibrotic damage, especially of the pericardium and myocardium, pericardial adhesions, microvascular damage and stenosis of the valves—and to the coronary arteries; these sorts of damage occur both in patients receiving radiotherapy and in experimental animals (*5*). With the exception of pericarditis, which occurs on time scales of months, most of these end points occur 10 or more years after irradiation (*5*). Heart and coronary arterial doses associated with radiotherapy can be very large for certain groups treated for malignant disease; e.g., in treatment of Hodgkin's disease, some parts of the heart receive >40 Gy (*6*).

However, there is emerging evidence of an excess risk of cardiovascular disease at much lower radiation doses and occurring over much longer intervals after radiation exposure in the Japanese atomic bomb survivor Life Span Study (LSS) cohort (*7–9*) and in a few other groups (*6, 10, 11*), although not in others (*12*). In this paper, we review the evidence for a causal interpretation of these epidemiological associations between low- and moderate-dose radiation exposure and cardiovascular disease. In contrast to a recent review (*6*), we concentrate attention on possible biological

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mechanisms for the effects observed in epidemiological studies, and we are concerned more with quantitative estimates of cardiovascular risk, in terms of excess risk per unit dose. Another recent review (*13*) also considered mechanisms, but it was very largely concerned with experimental data on effects at the high doses relevant to radiotherapy. In this paper, we review epidemiological and experimental studies in which the mean heart or brain doses were generally in the 0–5-Gy dose range. The epidemiological part of the paper is necessary to motivate the biological part—the epidemiological evidence from low- and moderate-dose studies is the justification for examining in depth possible biological mechanisms.

METHODS

A search of the epidemiological literature in the Medline database was conducted on 22 August 2007 using the terms ''radiation'' + ''heart'' + ''disease''. Only peer-reviewed papers in English in which there was reliable ascertainment of cardiovascular morbidity or mortality were considered; abstracts and letters were not included. In general, studies were excluded if there was not reliable estimation of organ (heart or brain) dose; in occupational studies, dose is generally assumed to be administered uniformly, so that whole-body dose (or committed effective dose) should approximate that to the heart. A total of 3541 references relating to articles published in or after 1990 resulted. This was supplemented by searches of the appropriate tables in the most recent UNSCEAR report (*3*), as well as a recent systematic review (*6*). We employed the most recent follow-up of each cohort. There is some degree of overlap between some of these cohorts, as we detail below, but each cohort that we present contributes some underlying study population or period of follow-up not subsumed in other cohorts. We do not present results for any cohort where the extra follow-up amounts to a year or less compared with another study that otherwise properly contains it; therefore, we omit from further consideration the U.S. nuclear worker study (*10*), which contains only 1 more year (1997) of follow-up than the IARC 15-country study (*12*) that otherwise subsumes it.

The basis of all estimations of risk is the value of the excess relative risk (ERR) coefficient (ERR Sv^{-1}). Wherever possible, this was taken directly from the relevant study. For certain studies (*14–19*), this was estimated from tabulations of deaths by dose group in the various papers, together with zero-dose estimated deaths. To make such estimations, a simple linear relative risk model was fitted by maximum likelihood, assuming Poissonian errors (*20*), in which it was assumed that the expected number of deaths in dose group d with average organ dose D (in Sv) is given by

$$E_d \cdot \lambda \cdot [1 + \alpha \cdot D], \tag{1}$$

where E_d is the expected number of deaths in dose group d and λ is the multiplier of the expected number of deaths at zero dose. The parameter α is the excess relative risk per Sv, and central (maximum likelihood) estimates and 95% profile likelihood confidence intervals (CI) (*20*) are given in Table 1. Model fits were performed using the EPICURE package (*21*).

For the peptic ulcer study (*22*) in which the most useful information given is estimates of the (adjusted) relative risk, RR_i (and associated CI) in each dose group i, estimates of α and associated CI are obtained by least squares, i.e., by minimizing the sum of squares:

$$\sum_i w_i [RR_i - 1 - \alpha D_i]^2, \tag{2}$$

where w_i is the weight attached to dose group i, given by

$$w_i = 1/[CI_{ui} - CI_{li}]^2. \tag{3}$$

Likewise, for the Mayak worker study (*23*), in which the most useful information given is estimates of the (age-standardized) mortality rate, MR_i and associated standard deviation (SD_i)] in each dose group i, estimates of α and associated CI are obtained by least squares, i.e., by minimizing the sum of squares:

$$\sum_i w_i \{MR_i - \lambda[1 + \alpha D_i]\}^2, \tag{4}$$

where w_i is the weight attached to dose group i, given by

$$w_i = 1/SD_i^2. \tag{5}$$

REVIEW OF THE EPIDEMIOLOGICAL DATA

General Epidemiology of Cardiovascular Disease

Cardiovascular disease is the leading cause of death in the developed world. Extensive epidemiological research has identified specific risk factors, which include male sex, family history of heart disease, cigarette smoking, diabetes, high blood pressure, obesity, increased total and low density lipoprotein (LDL) cholesterol, and decreased high density lipoprotein cholesterol plasma levels (*24–26*). In addition, markers of socioeconomic status (SES) and lifestyle factors (*27*), infections (*28–30*), and inflammatory and hemostatic variables (*28, 31*) have emerged as potential risk factors for this disease independently of the above factors, although the clinical value of inflammatory and hemostatic biomarkers remains uncertain (*32, 33*). Similarly, genetic polymorphism studies, which have the potential to identify at-risk individuals, are still in their infancy and have yet to provide consistent results (*34*). Age is also a risk factor, and there are well-known secular trends and geographical variations in cardiovascular disease (*35*), although careful study design, stratifying on these risk factors or otherwise, can minimize their potential confounding effect.

Studies that provide an estimate of average radiation dose to the heart and for which quantitative risk assessment is possible are summarized in Table 1.

Findings in the Japanese Atomic Bomb Survivors

Excess radiation-associated mortality due to heart disease and stroke has been observed in the atomic bomb survivor Life Span Study (LSS) cohort (Table 1) (*8*). However, the shape of the dose response was very uncertain, and there is no direct evidence of excess risk under 0.5 Sv (*8*). Statistically significant radiation-associated increases in mortality among the survivors were also found for digestive diseases and respiratory diseases (*8*). In the latest follow-up of the Adult Health Study (AHS) (a subcohort of the LSS subject to biennial assessments of morbidity), Yamada *et al.* (*9*) observed statistically significant radiation-associated excess risks for incidence of hypertension and myocardial infarction (Table 1). The study of Yamada *et al.* (*9*) was the only epidemiological study considered to assess morbidity rather than mortality.

Low- and Moderate-Dose (<5 Gy) Therapeutically Exposed Groups

All the studies considered in this section are of patients treated for benign disease. In contrast to the high doses typical of treatment for malignant disease, doses are generally much lower (typically <5 Gy) in most groups treated for benign disease (*6*). There was a significant (two-sided $P = 0.01$) increasing trend of coronary heart disease mortality with radiation dose in a U.S. cohort of persons treated for peptic ulcer (half with X radiation, half without), although there was no such significant trend for other cardiovascular mortality (*22*). Doses in this study are among the highest considered here and arguably are sufficiently high that this study should be considered outside the scope of the review. In this study the mean heart dose was 1.3 Gy (range 0.0–7.6 Gy) (*22*) (Table 1). A total of 382 of the 3043 patients in this study received average cardiac doses of 3.1–7.6 Gy, with a mean dose to the part of the heart in the beam of 14.4–35.6 Gy (mean 18.4 Gy). Excluding this highest dose group from our regressions has little effect on the central estimates, although confidence intervals become appreciably wider (Table 1). Radiation-associated excess mortality from cardiovascular disease has not been seen in a study of UK ankylosing spondylitis patients (*36*), in which the mean heart dose was 2.49 Gy (range 0–17.28 Gy) and the mean brain dose 0.14 Gy (range 0–4.80 Gy) (*37*) (Table 1).

Diagnostically Exposed Groups

No excess circulatory disease mortality has been observed in a cohort of Massachusetts tuberculosis patients receiving multiple fluoroscopic chest X rays (*14*), although the lung (and probably heart) dose in this group was fairly low, an average of 0.84 Gy (Table 1).

Occupationally Exposed Groups

There are increasing trends for certain cardiovascular disease mortality end points (all circulatory disease, cerebrovascular disease, other circulatory diseases) and decreasing trends for certain other end points (ischemic heart disease, heart failure, deep vein thrombosis and pulmonary embolism) in the IARC 15-country study of radiation workers (*12*) (Table 1), although none are statistically significant (one-sided $P \geq 0.20$). Radiation-associated excess ischemic heart disease and stroke mortality has been observed in excess in a group of Chernobyl recovery workers, although there was no excess mortality due to hypertensive heart disease and other heart disease (*11*) (Table 1). There is a very strong and highly statistically significant increasing trend of circulatory disease mortality with dose in a Canadian cohort of nuclear workers and various other occupationally exposed groups (dentists, radiographers, etc.) (*38*) (Table 1). However, general increases of the same sort of order were seen for a number of other diseases in this study, which implies that there may be bias. In a cohort of workers employed at the UK Atomic Weapons Establishment there was a marginally significant (two-sided $P = 0.0455$) increasing trend of circulatory disease mortality with cumulative film badge dose (*15*) (Table 1). Likewise, generally nonsignificant trends with dose have been seen for a variety of cardiovascular end points in various other UK, U.S. and other workforces (*16, 17, 19, 23, 39, 40*) (Table 1); it should be noted that parts of some of these studies (e.g., *15–17, 19, 38–40*) overlap with the IARC study (*12*). There were no statistically significant trends of circulatory disease mortality with cumulative radon or external γ-ray dose or dose from other radionuclides in a cohort of male German uranium miners (*41*) (Table 1). There was also no trend with any measure of dose for ischemic heart disease; mortality from acute myocardial infarction exhibited a significant (two-sided $P < 0.05$) increasing trend with radon dose, although the authors were inclined to treat this as spurious. Heart doses both from radon and external radiation were low; the average γ-ray dose was 0.041 Sv, with only 124 workers receiving doses of 0.5 Sv or more. Despite the large number of deaths (5417) from circulatory disease, therefore, the statistical power of this study was low. There is no significant trend of coronary heart disease mortality with radon dose in a cohort of Canadian fluorspar miners (*42*).

Environmentally Exposed Groups

There was a decreasing trend in heart disease mortality with dose for males and females in the study of Talbott *et al.* (*18*) of persons exposed as a result of the accident at the Three Mile Island nuclear power station. For females the decreasing trend was significant. The contrast with the conclusions reported by Talbott *et al.* (*18*) should be noted: We base our conclusions on trends of SMR with dose, whereas Talbott *et al.* interpreted elevations in SMR at various dose levels as evidence for significant excess risk. As with all studies of environmental exposure, exposure assessment in this study is problematic, although an attempt has been made to assess individual residence and migration patterns. An additional complication in relation to assessing cardiovascular end points is that stress would be expected to be associated with proximity to the plant and therefore with dose; this confounding would be expected to potentially positively bias the ERR estimate. Given the very small estimated doses, and the possibility of bias, little weight should be attached to these results; this study should be regarded as minimally informative.

Summary of Epidemiological Studies

The variation in magnitudes of trends of cardiovascular disease with dose, which span at least two orders of magnitude (see Table 1), and the possibility of confounding and other sources of bias mean that one cannot be sure that these statistical associations observed with radiation are causal in nature. The well-known independent risk factors

TABLE 1
Excess Relative Risks (per Sv) of Cardiovascular Disease in Published Epidemiological Data Sets with Estimated Average Radiation Dose to the Heart and for which Quantitative Risk Assessment is Possible

Data	Reference	Average heart/brain dose (range) (Sv)	Numbers in cohort (person years follow–up)	End point (mortality unless otherwise indicated)	Excess relative risk Sv^{-1} (and 95% CI)
Japanese atomic bomb survivors					
Mortality	*8*	0.1 (0–4)[a]	86,572 (1,697,861)	Heart disease, 1968–1997 (ICD9 390–429)	0.17 (0.08, 0.26)[ab]
				Stroke, 1968–1997 (ICD9 430–438)	0.12 (0.02, 0.22)[ab]
Morbidity	*9*	0.1 (0–4)[c]	10,339 (n.a.)	Hypertension incidence, 1958–1998 (linear model) (ICD9 401)	0.05 (−0.01, 0.10)[c]
				Hypertension incidence, 1958–1998 (pure quadratic model) (ICD9 401)	0.03 (0.01, 0.06)[cd]
				Hypertensive heart disease incidence, 1958–1998 (ICD9 402, 404)	0.01 (−0.09, 0.09)[c]
				Ischemic heart disease incidence, 1958–1998 (ICD9 410–414)	0.05 (−0.05, 0.16)[c]
				Myocardial infarction incidence, 1964–1998 (ICD9 410)	0.12 (−0.16, 0.60)
				Myocardial infarction incidence, age at exposure <40,1968–1998, pure quadratic model (ICD9 410)	0.17 (0.03, 0.56)[cd]
				Stroke incidence, 1958–1998 (ICD9 430, 431, 433, 434, 436)	0.07 (−0.08, 0.24)[c]
Low–dose radiotherapy and medical diagnostic studies					
Peptic ulcer study	*22*	1.3 (0.0–7.6)	3719 (92,979)	Coronary heart disease (ICD8 410–414)	0.11 (0.01, 0.22)[e]
				Coronary heart disease (ICD8 410–414), excluding highest dose group (3.1–7.6 Gy)	0.10 (−0.12, 0.33)[e]
				Other heart disease (ICD8 400–404, 420–429)	−0.11 (−0.40, 0.17)[e]
				Other heart disease (ICD8 400–404, 420–429), excluding highest dose group (3.1–7.6 Gy)	−0.16 (−0.49, 0.17)[e]
Ankylosing spondylitis	*36*, *37*	0.14 (0.0–4.80)[f] 2.49 (0.0–17.28)[h]	14,106 (183,749)	Stroke (ICD7 430–434)	−2.43 (−4.29, 0.71)[fg]
				Other circulatory disease (ICD7 400–429, 435–468)	−0.01 (−0.12, 0.13)[gh]
TB fluoroscopy	*14*	0.84[i] (n.a.)	13,385 (331,006)	All circulatory disease (ICD8 390–458)	−0.11 (−0.20, −0.01)[i]
Occupational studies					
IARC 15-country nuclear worker study	*12*	0.0207 (0.0–>0.5)	275,312 (4,067,861)	Circulatory disease (ICD10 I00–I99, J60–J69,O88.2, R00–R02, R57)	0.09 (−0.43, 0.70)
				Ischemic heart disease (ICD10 I20–I25)	−0.01 (−0.59, 0.69)
				Heart failure (ICD10 I50)	−0.03 (<0, 4.91)
				Deep vein thrombosis and pulmonary embolism (ICD10 I26, I60–I69, I80, I82)	−0.95 (−1.00, 9.09)[j]
				Cerebrovascular disease (ICD10 O88.2)	0.88 (−0.67, 3.16)
				All other circulatory disease (ICD10 R00–R02, R57, I00–I99 excluding I20–26, I50, I60–69, I80, I82)	0.29 (<0, 2.40)
Chernobyl emergency workers	*11*	0.109 (0–>0.5)	61,017 (n.a.)	Hypertension (ICD10 I10–I15)	0.26 (−0.04, 0.56)
				Ischemic heart disease (ICD10 I20–I25)	0.41 (0.05, 0.78)
				Other heart disease (ICD10 I30–I52)	−0.26 (−0.81, 0.28)

TABLE 1
Continued

Data	Reference	Average heart/brain dose (range) (Sv)	Numbers in cohort (person years follow–up)	End point (mortality unless otherwise indicated)	Excess relative risk Sv^{-1} (and 95% CI)
				Cerebrovascular disease (ICD10 I60–I69)	0.45 (0.11, 0.80)
Canadian nuclear and other workers	*38*	0.063 (0.0–>0.4)	206,620 (n.a.)	Circulatory disease (males) (ICD9 390–459)	2.3 (0.9, 3.7)[b]
				Circulatory disease (females) (ICD9 390–459)	12.1 (−0.4, 24.6)[b]
UK Atomic Weapons Establishment workers	*15*	0.015 (<0.01–>0.1)	22,543 (n.a.)	Circulatory disease (ICD9 390–459)	2.51 (0.01, 5.56)[k]
UK Springfields workers	*16*	0.0228 (0–0.7693)	19,454 (479,146)	Ischemic heart disease (ICD9 410–414)	−0.51 (<−1.67, 0.97)[l]
				Cerebrovascular disease (ICD9 430–438)	1.03 (−0.89, 4.09)[l]
				All circulatory disease (ICD9 390–459)	−0.16 (−1.07, 0.99)[l]
UK Capenhurst workers	*17*	0.00985 (0–>0.4)	12,540 (334,473)	Ischemic heart disease (ICD9 410–414)	−1.67 (<−1.67, 1.00)[l]
				Cerebrovascular disease (ICD9 430–438)	2.82 (<−1.67, 32.77)[l]
				All circulatory disease (ICD9 390–459)	−1.67 (<−1.67, 1.34)[l]
UK Atomic Energy Authority workers	*19*	0.01888 (0–>0.1)	51,367 (1,371,153)	Ischemic heart disease (ICD9 410–414)	−0.66 (−1.46, 0.23)[m]
Mayak workers	*23*	n.a. (0–>1)	9373 (n.a.)	All cardiovascular disease (ICD9 390–405, 410–438, 440–459)	0.00 (−0.06, 0.06)[n]
U.S. Oak Ridge workers	*39*	n.a. (0–>0.1)	14,095 (425,486)	Ischemic heart disease (ICD8 410–414)	−2.86 (−6.90, 1.18)
UK Chapelcross workers	*40*	0.0836 (0–0.3393)	2628 (63,967)	Ischemic heart disease (ICD9 410–414.9)	0.51 (−0.81, 2.54)
				Cerebrovascular disease (ICD9 430–438)	−0.96 (<−2.95, 2.34)
				All circulatory disease (ICD9 390–459)	0.37 (−0.74, 1.95)
German uranium miner study	*41*	0.041 (0–>0.3)	59,001 (1,801,626)	All circulatory disease (ICD10 I00–I99)	−0.26 (−0.6, 0.05)[o]
				Heart disease (ICD10 I00–I52)	−0.35 (−0.7, 0.009)[o]
				Cerebrovascular disease (ICD10 I60–I69)	0.09 (−0.6, 0.8)[o]
Environmental studies					
Three Mile Island study	*18*	0.0001 (0–>0.00016)	32,135 (561,063)	Heart disease (white males)	−274 (−874, 438)[p]
				Heart disease (white females)	−951 (−1433, −390)[p]

[a] Analysis based on colon dose.
[b] 90% CI.
[c] Analysis based on stomach dose, derived from Table 3 of ref. (*19*) with smoking and drinking in stratification.
[d] Derived from excess relative risk at 1 Sv.
[e] Based on model (2) fitted to data in Table 3 of ref. (*22*).
[f] Based on brain dose.
[g] Based on ERR and 95% CI given in ref. (*6*), combined with the median organ dose estimate of ref. (*37*).
[h] Based on heart dose.
[i] Based on lung dose.
[j] Estimate derived from log–linear model, evaluated at 1 Sv.
[k] Estimate derived by Poisson regression applied to aggregate data given in Table 2 of ref. (*15*), assuming average external whole–body doses of 0.005, 0.015, 0.035, 0.075 and 0.15 Sv applied to dose categories <10 mSv, 10–19 mSv, 20–49 mSv, 50–99 mSv and ≥100 mSv, respectively.
[l] Estimate for ref. (*16*) [and ref. (*17*)] derived by Poisson regression applied to aggregate data given in Table 6 of ref. (*16*) [respectively ref. (*17*)], assuming average external whole-body doses of 0.005, 0.015, 0.035, 0.075, 0.15, 0.3, 0.6 Sv applied to dose categories <10 mSv, 10–20 mSv, 20–50 mSv, 50–100 mSv, 100–200 mSv, 200–400 mSv and ≥400 mSv, respectively.
[m] Estimate derived by Poisson regression applied to aggregate data given in Table 4 of ref. (*19*), assuming average external whole-body doses of 0.005, 0.015, 0.035, 0.075 and 0.15 Sv applied to dose categories <10 mSv, 10–20 mSv, 20–50 mSv, 50–100 mSv and ≥100 mSv, respectively.
[n] Estimate derived by fitting model (4) by weighted least squares, applied to aggregate data given in Table 3 of ref. (*23*), assuming average external whole-body doses of 0, 0.5 and 1.5 Sv applied to the dose categories control, 0–1 Gy and >1 Gy, respectively.
[o] Risk estimates in relation to cumulative whole-body external γ-ray dose.
[p] Estimate derived by Poisson regression applied to aggregate data given in Tables 5 and 6 of ref. (*18*), assuming average external whole-body doses of 0.000015, 0.000055, 0.00012 and 0.00024 Sv applied to dose categories <0.03 mSv, 0.03–0.08 mSv, 0.08–0.16 mSv and ≥0.16 mSv, respectively.

for cardiovascular disease, such as cigarette smoking, diabetes, obesity, high blood pressure, and high levels of blood LDL were not available or were not adjusted for in analyses of most of these study groups. This is likely to be particularly problematic in cohorts in which there was no adjustment for socioeconomic status (SES) in the analysis [all except refs. (*10, 12*)]; many of these risk factors, in particular obesity, shift work and cigarette smoking, are correlated with SES, and SES may well be associated with occupational radiation exposure.

Among those treated with radiotherapy for malignant conditions, patients treated for breast cancer show promise for risk estimation because of the substantial and variable heart doses. Although cardiac doses are steadily decreasing over time in these patients, they still remain high for the most part (*43*). For example, a survey of 32 patients in about 2000 using three different radiotherapy plans for treatment of internal mammary lymph nodes assessed mean cardiac dose to be around 4–8 Gy for tumors of the left breast and 2–4 Gy for tumors of the right breast, with parts of the heart receiving more than 20 Gy in some patients from each group (*44*). However, certain other radiotherapy regimens give substantially lower doses (*45*). For example, a reconstruction of doses given in the period ~1950–2000 to about 40 patients by left-sided radiotherapy of the supraclavicular fossa, or of the posterior axilla, estimated mean cardiac doses of about 0.3–0.8 Gy, with maximal doses of 0.7–1.4 Gy; right-sided radiotherapy delivers no cardiac dose (*45*). To the best of our knowledge, there is only a single study concentrating on cardiovascular disease subsequent to these lower-dose radiotherapy procedures, although dosimetry is not given (*46*).

The issue of publication bias is a problem for this review as for any other such review and in particular the previous such survey (*6*)]. However, since radiation-induced cardiovascular disease has been an issue even in the LSS data for at least 15 years (*7, 47*), arguably this should not greatly affect the findings of this review, concentrating as it does on results published since 1990.

Radiation dosimetry is another issue that must be considered, particularly in relation to the radiotherapy studies. Related to this is the question of what the target tissue might be. There are indications that the immune system may be adversely affected in the Japanese atomic bomb survivors, as suggested by variations of T-cell and B-cell population numbers with radiation dose (*48*). Taken together with the known involvement of the immune system in cardiovascular disease (discussed below), this implies that whole-body dose (or possibly bone marrow dose) might be the most relevant dose. Of relevance are the diverse measures of dose used. In all the studies considered here, the predominant dose is from penetrating low-LET radiation. In the Japanese atomic bomb survivors, the dose used is DS86 colon (*8*) or stomach (*9*) equivalent dose, with a neutron relative biological effectiveness (RBE) of 10. Since there are only minor differences between organ doses for a particular survivor, even between the superficial and deep-lying organs, the error introduced by use of the colon or stomach as a surrogate for the heart or brain is probably small. In the peptic ulcer (*22*) and ankylosing spondylitis studies (*36, 37*), the dose used is that to the relevant tissue (heart or brain). In the TB fluoroscopy study (*14*), lung dose is used as a surrogate; heart dose would be expected to be similar to lung dose for the penetrating X rays used here. In the various occupational studies (e.g., *10, 12, 15–17, 19, 38–40*), the dose used is generally external film badge dose, with appropriate weighting factors for neutron radiation. For the German uranium miner study (*41*), the doses from γ radiation (and radon daughters, which are not considered here) were estimated with a job-exposure matrix. Dose from internal emitters generally was not taken into account in these studies [the significant exception being the Canadian nuclear workers (*38*), in which tritium doses were estimated], but for most of these cohorts heart or brain doses from this source would not be expected to be significant with respect to external γ-ray doses. Likewise, the neutron dose in most studies is generally small relative to the low-LET component, so that unless the RBE was much greater than 10 this should not affect dose estimates greatly. The heterogeneity in dose estimation criteria introduced by the dosimetry is therefore expected to be relatively minor, and it probably would not contribute materially to the inconsistency in trends of dose–response relationships.

POSSIBLE RADIATION ETIOLOGY AT LOW AND MODERATE RADIATION DOSES (<5 Gy)

Inflammation is believed to participate in virtually all stages of atherosclerotic disease, including its inception. Epidemiological evidence for the role of inflammation in causing cardiovascular disease has come from findings that elevated levels of systemic inflammation, reflected in the increased levels of the pro-inflammatory cytokine interleukin 6 (IL6), C-reactive protein (CRP), and a variety of cell adhesion molecules such as intercellular cell adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1) and endothelial leukocyte adhesion molecule 1 (ELAM1; E-selectin), are associated with elevated risk of cardiovascular disease in a number of prospectively examined cohorts (*49–53*). While the inflammatory process is recognized as an integral part of the atherosclerotic process (*54*), it does not explain the observation that the proliferation of smooth muscle cells during atherosclerotic plaque development is monoclonal (*55*). Support for the hypothesis of a monoclonal origin for atherosclerosis and hence an affinity with cancer development comes from studies showing changes in expression of proto-oncogenes and tumor suppressor genes and increases in frequencies of chromosome aberrations and other markers of DNA damage in atherosclerotic lesions (*56*). In addition, DNA damage, mitochondrial mutations and chromosome aberrations have been observed at increased frequencies in patients with car-

diac disease, indicating a general increase in somatic mutation (*57–61*). However, chronic inflammation is often associated with increased formation of reactive oxygen species (*62*), and it has recently been suggested that it is these, rather than the direct action of exogenous agents, that may contribute to DNA damage in atherosclerosis (*60*).

When examining possible mechanisms for the association between ionizing radiation and circulatory disease, it is important to recognize the manner in which radiation-induced cellular and molecular responses can influence the pathogenic process. At high doses (>10 Gy), there is abundant evidence from animal studies and radiotherapy patients of direct damage to the structures of the heart resulting in early acute cardiovascular effects (e.g., acute and chronic pericarditis, accelerated atherosclerosis, conduction abnormalities, valvular changes, pericardial or myocardial fibrosis) (*5*). Such effects are predominantly the consequence of microvascular injury, resulting from excessive cell killing and the associated response to cellular damage and leading to myocardial ischemia. Survivors of high-dose exposures may also suffer long-term tissue damage, such as late pericarditis or fibrous scars, and impairment of cellular functions, making them more susceptible to cardiovascular effects. While studies of cellular and molecular responses after high doses may give some indications as to how low-dose radiation can influence the development of cardiac disease, it is important to evaluate these in relation to dose, dose rate and dose response. We consider this evidence in turn for experimental *in vitro*, for experimental *in vivo*, and for human studies.

Experimental In Vitro Studies

Adhesion of leukocytes to the vascular endothelium is an essential step in the inflammatory process and is mediated through the release by endothelial cells (ECs) of selectins and adhesion molecules such as E-selectin and ICAM1. E-selectin was up-regulated in a time-dependent fashion by doses of as little as 0.5 Gy in human vein endothelial cells (HUVECs) through transcriptional regulation (*63*). This expression was independent of cytokines, and it is related to increased binding of nuclear proteins from irradiated EC to the NFκB binding site of the E-selectin promoter: knockout of this binding site eliminated the X-ray up-regulation of E-selectin (*63*). Both E-selectin and ICAM1 were up-regulated in human ECs with X-ray doses of between 1 and 5 Gy, although levels of other adhesion molecules (VCAM, P-selectin) were unaffected (*64, 65*). Induction of E-selectin and ICAM1 occurred immediately and was independent of radiation-induced cytokine [IL1, tumor necrosis factor α (TNF-α, TNFA)] production (*64*). However, while E-selectin could be induced by a dose as low as 0.5 Gy and the effect was transitory (levels had returned to baseline by 20 h), ICAM1 induction required a dose of 5 Gy, and expression still persisted at 48 h (*64*). Quarmby *et al.* (*65*) confirmed the up-regulation of ICAM1 but at the lower dose of 2.5 Gy and also observed increased expression of platelet endothelial cell adhesion molecule 1 (PECAM1/CD31) at doses ≥5 Gy. In human pulmonary microvascular endothelial (HMVEC-L) cells, ICAM1 expression was increased within 24 h after X irradiation with 2 Gy (*66*). In cultured human epithelial skin cells exposed to 5 Gy X rays, there was up-regulation of ICAM1, VCAM1 and E-selectin cell surface expression within 24 h, although the levels of CD31/PECAM1 were unchanged (*67*). However, exposure of cells of a transformed human bone marrow EC line (TrHBMEC) to 2.5 Gy of ^{60}Co γ rays resulted in increased expression of ICAM1 but no change in VCAM1, E-selectin and PECAM1 (*68*). This study also reported up-regulation of certain cytokines, in particular IL6, IL8, IL11, IL1α, G-CSF and GM-CSF, but no change in levels of other pro-inflammatory cytokines, such as TNF-α or LIF. Somewhat at odds with this, Woloschak *et al.* (*69*) demonstrated up-regulation of the pro-inflammatory cytokine IL1 in Syrian hamster embryo cells *in vitro* after exposure to 0.75 Gy X rays, 0.9 Gy γ rays or 0.21 Gy fission neutrons.

In contrast to the above, doses in the range 0.3–0.7 Gy are associated with reduced adhesion of human peripheral blood mononuclear cells (PBMCs) to ECs, this being most likely associated with the shedding of L-selectin from the surface of peripheral T cells (*70*). Further studies in the same dose range with the same cell type have shown that this is accompanied by a decrease in expression of E-selectin and PBMC adhesion to ECs and by an increase in levels of the pro-inflammatory cytokines TGF-β and IL6 (*71*). A study using WHT/Ht mouse peritoneal macrophage cells indicated that 2 Gy X rays down-regulated IL1β and IL6, whereas 0.1 Gy increased IL6 expression but had no influence on IL1β expression (*72*). Thus, in contrast to high-dose radiation, acute doses in the range 0.1–1 Gy may result in down-regulation of the adhesion of leukocytes to the endothelium and thus may have an anti-inflammatory effect. One mechanism influencing the response to radiation at different doses may be the nitric oxide (NO) pathway in stimulated macrophages, since NO is known to play a central role in inflammation. When macrophages were stimulated with lipopolysaccharide (LPS) and interferon gamma (IFN-γ), NO production was suppressed at X-ray doses up to 1.25 Gy but returned to normal and increased at higher doses (*73*). Since levels of TNF-α were unaffected, it was concluded that radiation was having a direct effect on the inducible nitric oxide synthase (iNOS) pathway through post-transcriptional or post-translational regulation of iNOS (*73*).

Experimental In Vivo (Animal) Studies

Generally, animal studies confirm that acute doses of around 2 Gy and above are associated with increased expression of a variety of cell adhesion molecules in endothelial tissue (*13, 74*). Thoracic irradiation of C3H mice

with 2 Gy X rays resulted in increased expression of E-selectin, P-selectin (another pro-inflammatory factor promoting leukocyte rolling) and ICAM1, but expression differed in different tissues (*74*). Thus ICAM1 was expressed primarily in the endothelium of the microvasculature, E-selectin primarily in the endothelium of the larger blood vessels, and P-selectin primarily in the endothelium of Weibel-Palade bodies of the endothelium and never in the microvasculature. This selective expression may explain some of the inconsistencies between *in vitro* studies. The increased permeability of ECs can also lead to increased accumulation of lipids and initiation of atherogenic changes in the presence of hypercholesterolemia in C57BL/6 mice after doses of 2 Gy X rays or more (*75*).

As observed *in vitro*, acute doses in the range 0.1–1 Gy can result in a down-regulation of the adhesion of leukocytes to ECs and thus have an anti-inflammatory effect. For example, whole-body irradiation of rats with 0.1, 0.3 and 0.6 Gy 6 MeV photons had no effect on ICAM1 expression but was found to inhibit leukocyte adhesion after challenge with LPS (*76*). Hildebrandt *et al.* (*73*) extended their *in vitro* work by examining radiation effects on the NO pathway *in vivo* using the BALB/c mouse chronic granulomatous air pouch system and were able to confirm that low-dose radiation modulates the production of NO. Such results provide mechanistic support for the clinical efficacy of low-dose radiation therapy for the treatment of inflammatory conditions (*77*) and for the suggestion that different radiobiological mechanisms are involved at the higher and lower ends of the dose spectrum (*73*).

Human Studies

Elevated levels of the pro-inflammatory cytokines IL6, CRP, TNF-α and INF-γ, but also increased levels of the (generally) anti-inflammatory cytokine IL10, have been observed in the Japanese atomic bomb survivors (*78, 79*). There was also a dose-related elevation in the erythrocyte sedimentation rate and in the levels of IgG, IgA and total immunoglobulins, all markers of systemic inflammation, in this cohort (*79*). Given the possible role of infections in cardiovascular disease (*28, 80*), it is of interest that certain T-cell and B-cell population numbers are known to vary with radiation dose among the Japanese atomic bomb survivors (*48*). The atomic bomb survivors also demonstrate dose-dependent decreases in levels of $CD4^+$ helper T cells (*78*); decreased levels of helper T cells have also been found in blood samples from Japanese atomic bomb survivors with myocardial infarction (*81*).

The up-regulation of a range of cell adhesion molecules and the observation of cytokine markers of the inflammatory response in the atomic bomb survivors reflect the action of acute radiation doses generally of >0.5 Gy. The presence of such markers may be indicative of radiation-induced killing of ECs. However, it is not clear what responses would be induced by lower doses and lower dose rates. When considering the role of the inflammatory response in the etiology of radiation-induced cardiovascular disease, it is therefore also necessary to distinguish between acute and chronic exposures. Occupational doses will be received in daily increments of less than 0.5 mSv for the most part. Thus the killing of one or a few cells in a system that is continually undergoing regeneration is unlikely to be significant and may well not induce the types of response discussed above. Of relevance here is a study of workers employed at the Sellafield nuclear facility that examined a group with cumulative exposures >0.2 Sv (mean 0.33 Sv) and an otherwise similar group with exposure <0.028 Sv (mean 0.014 Sv) and found no differences in levels of $CD4^+$, $CD8^+$ and $CD3^+$/HLA-DR^+ cells or in the $CD4^+$:$CD8^+$ ratio, indicating that such fractionated exposures were not affecting these markers of immune response (*82*).

Of more relevance to low and chronic doses is the suggestion, discussed above, that somatic mutation has a role to play in the etiology of cardiovascular disease (*57–59*). At very low doses and low dose rates, cell death resulting from genetic damage may be of little significance, but genetic changes resulting in a mutated viable cell could have more serious long-term consequences. Thus any increase in the somatic mutation rate (such as might be caused by even low-dose-rate radiation) would have an influence on the disease process. In this respect, atherogenesis could be viewed as a stochastic effect with a finite probability of occurrence even at very low doses and low dose rates (*83*).

Another indirect mechanism for the induction of hypertension and cardiovascular disease has been suggested (*83*) by the known elevation in parathyroid hormone with increasing radiation dose in the atomic bomb survivors (*84*). Parathyroid hormone is known to play a role in regulation of blood pressure, with increasing levels of the hormone resulting in increases in blood pressure. This is consistent with the known associations of blood pressure with radiation dose in the atomic bomb survivors (*85*).

In reviewing the possible mechanisms whereby radiation exposure could influence the induction and/or progression of cardiovascular disease, it is apparent that any effect will be dependent on dose and dose rate and may also be affected by other pre-existing predisposing factors. As indicated above, a clear distinction should be drawn between the mechanisms at high (radiotherapy) doses, reviewed elsewhere (*13*), and those at the relatively low doses (<5 Gy) considered here. The inflammatory response depends on initial cell killing and tissue damage and will certainly play a role at acute doses above 0.5 Gy; at low doses this may be countered by an anti-inflammatory response that could lead to reduced progression of the atherosclerotic process. However, if somatic mutation has a role in this process, then any dose of radiation, however small, will have a finite probability of inducing the appropriate genetic lesion. Whether this is an initiating event or a step in the progression of the process remains unclear. Further work

examining specific markers of cardiovascular disease in populations exposed to chronic low-dose-rate radiation should contribute to the elucidation of the role, if any, of occupational and environmental radiation exposure in cardiovascular disease.

CONCLUSIONS

There is no doubt that the high radiation doses to the heart and coronary arteries received during certain radiotherapy procedures induce tissue damage that results in an increased risk of circulatory diseases; the underlying biological mechanism is the high level of cell killing experienced (*13*). The central question is whether moderate and low doses can elevate the risk of these diseases, as indicated by the findings of some epidemiological studies, presumably through a mechanism different from that for high-dose effects. The epidemiological evidence for an effect of moderate and low doses is suggestive rather than persuasive, and, in the absence of a firm biological mechanism, caution is required in the interpretation of the statistical associations. On the other hand, a causal explanation cannot be reliably excluded, and further research is required to better understand the nature of the epidemiological associations.

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