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COMMENTARY

Is There Reliable Experimental Evidence for a Low-Dose RBE of about 4 for Mammography X Rays Relative to 200 kV X Rays?

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Frankenberg *et al.* (*Radiat. Res.* 157, 99–105, 2002) recently reported, on the basis of observations of neoplastic transformation in human hybrid CGL1 cells, a low-dose relative biological effectiveness (RBE_M) of 4.3 for mammography X rays (29 kV) relative to 200 kV X rays. With reference to data in the literature, they inferred a factor of about 8 relative to ^{60}Co γ rays and concluded that this result is relevant to risk estimation. However, the conclusions do not appear to be valid. The data from the transformation study exhibit uncertainties in the statistical analysis that preclude any generalization of the inferred RBE_M. The data selected or inferred from the literature are likewise insufficient to support the stated RBEs. Our own uniform data set for the yields of dicentrics was obtained for widely varying photon energies with blood samples from the same donor, and it avoids interindividual variations in sensitivity as well as the differences in methodology that are associated with interlaboratory comparisons. Our data provide RBE_M values for 29 kV X rays of 1.64 ± 0.27 relative to 220 kV X rays and 4.75 ± 1.67 and 6.12 ± 2.51 relative to ^{60}Co γ rays.

INTRODUCTION

In a recent paper, Frankenberg *et al.* (*1*) reported, on the basis of their measurements of neoplastic transformation in a human hybrid cell line (CGL1), a low-dose relative biological effectiveness (RBE_M) of 4.3 for mammography X rays (29 kV) relative to 200 kV X rays. With reference to data in the literature, they then inferred an RBE of about 8 relative to ^{60}Co γ rays and concluded that the risk of mammary tumors due to mammography X rays has been substantially underestimated. Their conclusions have had some impact on the debate on mammography screening. It is therefore desirable to examine the evidence that has been brought forward.

An examination of the experimental data presented by Frankenberg *et al.* (*1*) raises questions related to their statistical evaluation. Their consistency with radiation physics and microdosimetry has been examined critically (*2*), and their validity will need to be judged in the context of current experiments with monoenergetic photons. The subsequent discussion is not aimed primarily at these issues, but it assesses critically the presentation and interpretation of experimental data from other laboratories that Frankenberg *et al.* (*1*) offered in support of the presumed high RBE of mammography X rays.

CRITICAL EVALUATION OF THE EXPERIMENTAL EVIDENCE

An enhanced RBE of 29 kV X rays relative to 200 kV X rays was derived by Frankenberg *et al.* (*1*) from their observation of the rates of neoplastic transformation in CGL1 cells (HeLa × human skin fibroblast hybrid cells) within the dose range of 1–5 Gy. Based on a linear-quadratic fit to their experimental data, they reported a low-dose RBE for the 29 kV X rays relative to 200 kVp of about 4, and they concluded that this value applied more generally. In view of their analysis, it is difficult to accept this claim.

Frankenberg *et al.* (*1*) give linear-quadratic fits to the dose dependences for cell transformation, but the reported standard errors of the coefficients are far too large to permit meaningful conclusions. Thus it will be necessary to have appropriate data and analysis that provide RBEs with acceptable uncertainties. Until this is done, the authors' case for the high RBE for radiation used in mammography must rest primarily on the published data that they compiled in their paper and summarized in their Table 3 and Fig. 3. However, this compilation of evidence from other laboratories appears to contain major errors and misinterpretations that need to be corrected.

There are nine items listed in their Table 3. Statistical uncertainties are stated for only two of these items, which

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makes the assessment difficult or impossible. However, in the majority of the cases, there are faults in the assessment that appear to weaken or invalidate the conclusions regardless of the statistical uncertainties that are involved.

Animal Studies

Items 1 and 2 are derived from animal studies. Item 1 gives a value of 2.5 for the RBE of what is termed 600 kVp X rays relative to ^{60}Co γ rays. This is based on a single dose point obtained at one dose of X rays from the electron accelerator Thalie at Valduc, which produces the dose by ultrashort pulses (70-ns) with a broad high-energy photon spectrum. From the original reference (*3*) it is seen that the statistical uncertainty of the RBE is large. If the RBE is indeed substantially larger than unity, this is more likely to be a dose-rate effect than a matter of the rather similar photon energies. Item 2 is apparently derived from studies of tumors of rodents irradiated with fission neutrons and photons, but the description is too unspecific to permit an assessment.

Neoplastic Cell Transformation

Item 3 gives a low-dose RBE equal to 2 for 300 kVp X rays relative to ^{60}Co γ rays. This refers to the study of neoplastic transformation of hamster embryo cells by Borek *et al.* (*4*). It should be noted that this RBE is seen only at a γ-ray dose of 0.03 Gy. In the dose range of the experiments of Frankenberg *et al.* (*1*), i.e. for doses ≥1 Gy, the transformation frequencies are similar for X rays and γ rays. There is thus little justification for multiplying, as in entry 5 of Table 3, the RBE of 4.3 that was obtained at high doses by 2 to obtain an RBE of 8.6 of 29 kV X rays relative to ^{60}Co γ rays.

Item 4 gives an RBE ≈4 for 50 kV X rays relative to ^{60}Co γ rays for oncogenic transformation in mouse C3H 10T½ cells. This is presented as a result of two comparisons against the same common reference, namely fission neutrons. In reality, the 50 kV X rays were tested in one laboratory relative to a broad spectrum of neutrons fission from the JANUS reactor at the Division of Biological and Medical Research, Argonne National Laboratory (*5*), while the 250 kV X rays were compared in another laboratory to monoenergetic neutrons generated at an accelerator facility (*6*). As emphasized in the original publication (*6*), the two neutron fields cannot readily be compared, and the intercomparison of the two experiments is therefore of doubtful validity.

In item 5, Frankenberg *et al.* (*1*) refer to their own transformation data. As stated, these data will need to be assessed on the basis of a corrected statistical analysis and the resulting standard error or confidence range of the RBE.

Chromosome Aberrations in Human Lymphocytes

1. Reciprocal translocations

Item 6 refers to seven studies of reciprocal translocations in human lymphocytes and gives, without further comment, an RBE of 2.3 ± 1.2 for 180–220 kVp X rays.

During the last three decades, several efforts were made to investigate the biological effectiveness of different types of low-LET radiation for the *in vitro* induction of asymmetrical exchanges, such as dicentrics, in human lymphocytes. Major examples are the studies at the cytogenetics laboratories of the NRPB (Didcot, UK) (*7*) and the GSF (*8*). During the last decade, fluorescence *in situ* hybridization (FISH) with chromosome-specific composite DNA probes (chromosome painting) emerged as a useful tool for quantifying symmetrical exchanges such as reciprocal translocations.

None of the experiments using the FISH technique have been performed with more than a single photon energy. This is a serious limitation, because the calibration curves for FISH in different laboratories (including our own laboratory at the GSF) are based on different forms of the FISH technique and on different methods for scoring the chromosome aberrations. Aberrations in the painted chromosomes are variously taken to include two-way translocations, one-way translocations, insertions and, where recognizable, inversions. Depending on the different aberration categories, either the frequencies of complete translocations (reciprocal translocations) or those of total translocations have been used for establishing dose–effect curves. Furthermore, because the application of FISH techniques has made it possible to detect significant frequencies of complex aberrations at doses ≥2 Gy (*9*), complex chromosome exchanges were in part reduced to simple aberration types and were included. Loucas and Cornforth (*10*) used combinatorial multi-fluor FISH (mFISH) to demonstrate that, for a γ-ray dose of 4 Gy, roughly half of the human lymphocytes contained at least one complex exchange that required from 3 to 11 initial chromosome breaks.

There is a further difficulty that is related to the scoring of translocations. The first study in our own series of publications (*11*) on the induction of symmetrical translocations by various radiation qualities was performed without centromere-specific markers, which may have caused a mis-scoring of a substantial fraction of dicentrics as translocations. Such possible mis-scoring has led to considerable confusion in FISH cytogenetics (*12*). An example is the enhanced frequency of reciprocal translocations relative to dicentrics that was reported by Nakano *et al.* (*13*). When the same cells were subsequently evaluated using a conventional staining method, the ratio of 50:50 was restored, in line with theoretical considerations.

Although FISH is regarded as the most objective method of detecting translocations, there are further problems in interpreting and comparing FISH data from laboratories. For example, Lucas (*14*) suggested the use of the ratio of complete to incomplete translocations (S ratio) as a cytogenetic signature for ionizing radiation. Nakano *et al.* (*15*), however, found that the S ratio can vary considerably among laboratories. Consequently, they stated that caution will be required when comparing results from different lab-

TABLE 1
Linear Dose–Effect α Coefficient and Maximum Low-Dose RBE (RBE_M) of 29 kV X Rays Relative to 220 kV X Rays and ^{60}Co γ Rays for Data on Dicentrics Obtained in Human Lymphocytes from the Same Blood Donor

Radiation quality	Mean energy (keV)	Dose range (Gy)	Cells scored	Linear α coefficient ± SEM × 10^{-2} Gy^{-1}	RBE_M ± SEM of 29 kV X rays relative to 220 kV X rays or ^{60}Co γ rays	Reference
29 kV X rays	17.4	0.12–2.19	6100	6.55 ± 0.97	—	(*27*)
220 kV X rays[a]	96	0.05–4.0	15100	4.00 ± 0.30	1.64 ± 0.27	(*22*)
220 kV X rays[b]	135	0.05–4.0	9500	2.20 ± 0.40	2.98 ± 0.70	(*22*)
^{60}Co γ rays[c]	1250	0.05–4.0	14700	1.07 ± 0.41	6.12 ± 2.51	(*25*)
^{60}Co γ rays[d]	1250	0.25–4.0	6800	1.38 ± 0.44	4.75 ± 1.67	(*26*)

[a] 220 kV X rays, filtered by 4.05 mm aluminum + 0.5 mm copper.
[b] 220 kV X rays, filtered by 2.0 mm aluminum + 3.35 mm copper.
[c] γ rays; phantom was 7 cm wide, 11.5 cm high and 2.3 cm thick.
[d] γ rays; phantom was 30 cm long, 30 cm wide and 30 cm high, irradiation at a depth of 5 cm.

oratories. All these difficulties preclude simple comparisons of translocation yields from different laboratories to assess the relative biological effectiveness of different types of radiation. Item 6 in Table 3 of Frankenberg *et al.* (*1*) therefore has little informative value.

2. Dicentrics in human lymphocytes

A more reliable estimation of the RBE for radiation qualities is possible for dicentrics in human lymphocytes, particularly at low doses.

Item 7 presents values for the RBE_M of 220–250 kVp X rays relative to ^{60}Co γ rays of about 2–3. While it is striking that none of the more recent references are given, the statement itself is correct.

A serious problem arises again with item 8, which is highly misleading, because it presents a low-dose RBE of 8.6 relative to ^{60}Co γ rays from experiments by Sasaki *et al.* (*16*) as if it were an RBE for normally filtered 50 kVp X rays, such as that in item 4. In reality, Sasaki *et al.* (*16*) employed, with careful dosimetry, 50 kVp X rays with nothing but inherent filtration. Such X rays have an extremely low mean photon energy and are not representative of the mammography X rays, which have a mean photon energy of around 17.4 keV. As a matter of fact, Sasaki *et al.* (*16*) presented data for monochromatic X rays of 13.8 and 14.6 keV, which are much closer to mammography X rays. These data provide an RBE_M of only 2.7 relative to ^{60}Co γ rays, but they are not cited by Frankenberg *et al.* (*1*).

Finally, item 9 in Table 3 refers to the data of Virsik *et al.* (*17*) for dicentrics in human lymphocytes that are stated to provide an RBE equal to 3 at low doses for 30 kV relative to 150 kV X rays. However, in the experiment with 150 kV X rays, only a single dose below 1.94 Gy, namely 153 cells exposed to 0.65 Gy was analyzed, and the α coefficient of the linear-quadratic dose–effect relationship for the 150 kV X rays was actually negative. More recent studies by the same authors (*18, 19*) using 150 kV X rays provided an α coefficient that is significantly different from zero. However, in the latter studies, there was no evidence for an enhanced RBE_M of 30 kV X rays relative to 150 kV X rays. An estimation of the RBE_M would require accounting for the fact that the work with 150 kV X rays was performed in part under cell cycle control, but there appears to be very little justification for simply omitting references to these data.

In summary, it must be concluded that Frankenberg *et al.* (*1*) give references and interpretations that are neither representative nor reliable, while they fail to present the few results, such as the data of Sasaki *et al.* (*16*), that are most pertinent. To reduce some of the resulting misperception, the subsequent section will deal with some current data from our laboratory that are relevant to the issue.

DATA FOR DICENTRICS FROM OUR LABORATORY

Studies of dicentrics in human lymphocytes are well suited to explore the low-dose RBE of different low-LET radiations. In our laboratory at the GSF, there have been systematic investigations using widely varying photon energies, and a large, uniform set of the α components of the linear-quadratic fits has been obtained with high precision by using blood from the same donor under constant conditions of cell cycle control (*20–27*). As stated earlier with regard to the total set of these α coefficients (*8*), they appear to be quite reliable in view of the excellent agreement between data from NRPB and GSF for similar types of radiation. There is thus good reason to use the existing data set together with new data for 29 kV X rays (*27*) for estimating the RBE_M of 29 kV X rays relative to higher-energy X rays or to ^{60}Co γ rays.

Table 1 presents the data from the experiments with 29 kV X rays (from a molybdenum target), 220 kV X rays filtered with two filters, and ^{60}Co γ rays. The study of Frankenberg *et al.* (*1*) included irradiation with 200 kVp X rays

with a 0.6-mm copper filter. The corresponding comparison in our data is between the mammography X rays ($\alpha = 0.0655 \pm 0.0097$ Gy^{-1}) and 220 kV X rays (*22*) weakly filtered with 0.5 mm copper ($\alpha = 0.040 \pm 0.003$ Gy^{-1}), which results in an RBE_M of 1.64 ± 0.27. A higher RBE_M of 2.98 ± 0.70 of 29 kV X rays results when the 29 kV X rays are compared with 220 kV X rays with hard filtration (3.35 mm copper; $\alpha = 0.022 \pm 0.004$ Gy^{-1}) (*22*). The reference α coefficient for ^{60}Co γ rays changed from 0.0107 ± 0.0041 in earlier experiments (*25*) to 0.0138 ± 0.0044 in the most recent work (*26*). These somewhat different values are compatible within their statistical uncertainty, but if the difference is real, it may be due to differences in the phantom sizes that were used for the γ irradiation (*26*). Depending on whether the earlier or the most recent reference α coefficient for ^{60}Co γ rays is used, the RBE_M of 29 kV X rays is inferred to be 6.12 ± 2.51 or 4.75 ± 1.67. In summary, the data on dicentrics from our laboratory do not support the high RBE_M values reported by Frankenberg *et al.* (*1*).

CONCLUSION

Our critical evaluation does not support the selection of data from the literature that Frankenberg *et al.* (*1*) made to document a low-dose RBE of 4 or more for 29 kV X rays relative to weakly filtered 200 kV X rays. Their own data exhibit uncertainties in the α coefficients that preclude any generalization of the inferred RBE_M. The data they selected from the literature are likewise insufficient to back up the proposed low-dose RBE of 8 of 29 kV X rays relative to ^{60}Co γ rays.

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