RADIATION RESEARCH **155**, 744–747 (2001)
0033-7587/01 $5.00

SHORT COMMUNICATION

Detection of Elevated RBE in Human Lymphocytes Exposed to Secondary Electrons Released from X-Irradiated Metal Surfaces

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Regulla, D., Panzer, W., Schmid, E., Stephan, G. and Harder, D. Detection of Elevated RBE in Human Lymphocytes Exposed to Secondary Electrons Released from X-Irradiated Metal Surfaces. *Radiat. Res.* 155, 744–747 (2001).

Monolayers of human lymphocytes, attached to a 2-μm Mylar film, were irradiated with 60 kV X rays in the presence and absence of a 150-μm gold film backing the Mylar film. With the gold film present, the absorbed dose imparted to the cells was increased by a factor of 45.4 due to the release of photoelectrons from the gold film. The frequencies of dicentric chromosomes and centric rings as well as of excess acentric fragments were increased in agreement with this dose enhancement, and in addition an RBE of about 1.7 compared to the frequencies observed in the absence of the gold film was found. These radiation effects, which contribute to risk considerations in radiology, are interpreted in terms of the increased dose-mean restricted LET of the photoelectrons backscattered from the metal and slowed down in the Mylar film before they enter the cell layer.

INTRODUCTION

The human body comprises natural interfaces and may comprise artificially generated interfaces between soft tissues and materials of higher atomic number (*Z*), such as bones, teeth, metallic implants and X-ray contrast media. Irradiation of these materials with photons of energies common in X-ray diagnosis releases secondary electrons, particularly by the photoelectric and Auger effects. Some of these electrons are outscattered into the adjacent tissues, contributing to an enhancement of absorbed dose compared to the same irradiation in the absence of the high-*Z* inhomogeneity. We have recently quantified this dose enhancement using a very thin physical detector (resolution ≤100 nm) placed in contact with the surface of a gold film backing the detector (*1*). The dose enhancement was found to exceed a factor of 100, but to remain spatially confined to a very thin layer of matter lining the high-*Z* material surface. Radiobiological experiments using C3H 10T½ mouse embryo fibroblasts 2–3 μm thick, with survival as the end point, revealed a corresponding dose enhancement. Further experimental details are given in refs. (*1–3*).

The present work focuses on the cytogenetic consequences of the interface-related dose enhancement described above, using chromosome aberrations in human T lymphocytes as the biological end point. Our objective was (1) to find further confirmation of the large magnitude of dose enhancement at tissue/gold interfaces, and (2) to investigate the possible changes in relative biological effectiveness (RBE) which may be associated with the low energy of the electrons released from the gold surface, especially with electron track ends.

MATERIALS AND METHODS

For the present experiments, human T lymphocytes supplied by a healthy male donor were isolated from the peripheral blood by density gradient centrifugation. The isolated cells were suspended at a final concentration of 1 million lymphocytes per milliliter in complete medium. Our cell preparation and culture techniques have been reported in detail (*4*); only a brief description is given here. Cell cultures were established in special dishes consisting of a glass ring of 48.3 mm inner diameter and a stretched 2-μm bottom Mylar film (polyethyleneterephthalate) glued to the glass ring. These dishes contained 0.5 ml lymphocyte suspension and 4.5 ml RPMI 1640 medium supplemented with 16% fetal calf serum and antibiotics. During a 3-h incubation time in the presence of 2.5% phytohemagglutinin (PHA), the cells were allowed to settle and attach to the Mylar film. Subsequently, the film was washed with medium to remove unattached cells. The attached lymphocytes were 2–3 μm thick, which was determined almost completely by the cell nuclei.

For irradiation, the dishes were placed so that the Mylar film was in a vertical position and exposed free in air with the cell layer facing the X-ray source, with or without a 150-μm gold film backing the Mylar film. Irradiations were performed with a horizontal X-ray beam in the IAEA/WHO Secondary Standard Dosimetry Laboratory of our Institute of Radiation Protection, using 60 kV X rays filtered with 4 mm aluminum + 0.6 mm copper at a dose rate of about 22 mGy min^{-1}, and using a trans-

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TABLE 1
Chromosome Aberration Yields in Human Lymphocytes after Irradiation with 60 kV X Rays in the Presence or Absence of Secondary Electrons Backscattered from a 150-μm-thick Gold Film

Gold film present	Dose (Gy)		No. of cells scored	Chromosome aberrations per 100 cells	
	D_1	D_2		Dicentrics and centric rings	Excess fragments
No	Controls[a]		92,550	0.050	0.25
No	Controls[b]		9,000	0.022	0.23
No	0.051		2,500	0.36	0.40
No	0.102		1,500	0.80	1.00
No	0.204		3,000	1.07	1.40
No	0.510		2,500	2.88	3.20
No	1.020		700	7.43	8.29
No	1.530		700	13.57	15.29
Yes		0.454	200	5.0	3.5
Yes		1.408	200	8.5	8.0
Yes		2.315	500	21.6	23.6
Yes		3.223	300	32.7	28.3
Yes		4.631	300	70.3	74.0
Yes		6.810	100	90.0	84.0
Yes		9.262	200	119.5	115.5

Notes. From the absorbed doses applied without gold film (D_1), the absorbed doses applied with the gold film present (D_2) are obtained by multiplication by the factor 45.4 when irradiations are performed with the same monitor units. This factor represents the dose enhancement measured with a very thin physical detector (*1–3*) and averaged over 3-μm-thick cell nuclei.

[a] Whole blood samples from 141 individuals (*6*).

[b] Monolayer samples from the present blood donor.

mission ionization chamber for dose monitoring. The source–sample distance in air was 50 cm; the field size in the plane of the cell layer was 10×10 cm^2. Air kerma was measured at the source–sample distance free in air in the absence of the cell samples and was converted to water absorbed dose.

Since the cell monolayer was irradiated without nutrient medium, the irradiation time was limited to about 1 h. Within this time, no influence of the absence of the nutrient medium could be found. It is worth mentioning that the radiation response of the attached lymphocytes has not been found to differ from that of lymphocytes irradiated in whole blood, at least for ^{137}Cs γ rays (*4*).

Immediately after irradiation, the cells were scraped off the Mylar film and were incubated at 37°C with fresh medium containing 2.5% PHA and 2.9 μg/ml BrdU for 48 h, and in the presence of 0.1 μg/ml Colcemid during the final 3 h. Chromosome preparation and Giemsa staining were carried out according to local standard procedures. The culture conditions ensured that the chromosome analysis was performed exclusively in metaphases of the first cell cycle after irradiation. Only complete cell nuclei were analyzed for dicentric chromosomes, centric rings and excess acentric fragments, i.e. all fragments minus one per dicentric chromosome or centric ring.

In the absence of the gold film, the secondary electrons traversing the lymphocytes are released from oxygen and nitrogen in the air between the X-ray source and the cell layer. At a photon energy of 48 keV, representative of the photon spectrum at 60 kV tube potential, about 50% of the absorbed dose administered to the nuclei of the T lymphocytes is due to Compton electrons of maximum initial energy 7.6 keV, and the other 50% due to photoelectrons of initial energy 48 keV. However, with the gold film present, the major fraction of absorbed dose imparted to the cell nuclei is contributed by the L- and M-shell photoelectrons outscattered from the gold surface, while the Auger electrons from gold, due to their maximum energy of only 8 keV, are absorbed in the 2-μm-thick Mylar film (*5*). According to the high-resolution dosimetry with a TSEE dosimeter (*1, 2*), the dose fraction attributed by the photoelectrons decreases according to an approximately exponential function of the thickness of the Mylar film inserted between the cells and the gold surface; at 60 kV tube voltage, the attenuation length in Mylar is 5.2 μm. A fraction of the photoelectrons which penetrate the cell layer are backscattered from air into the cells, and an approximately equivalent backscattering into the sensitive layer of the TSEE dosimeter was achieved in the measurement geometry (*1, 2*). The dosimetric data in refs. (*1, 2*) can be used to calculate an enhancement factor. However, those data were obtained in a mini-phantom providing photon backscattering, while the present samples were irradiated free in air, so an appropriate correction must be used. From these considerations, an enhancement factor of 45.4, averaged over 3-μm-thick cell nuclei, was calculated for the irradiations in the presence of the gold film. The energy absorbed by the lymphocytes was estimated based on the assumption that their composition was the same as that of soft tissue.

RESULTS

As shown in Table 1, the control values for the yield of aberrations determined for lymphocytes from the present donor when they were maintained as a monolayer on Mylar without medium are not significantly different from those obtained in whole blood samples from 141 control individuals (total of 92,550 cells) examined in our laboratory (*6*). The measured yields of dicentric chromosomes and centric rings as well as of excess acentric fragments resulting from exposures with 60 kV X rays in the absence of the gold film at absorbed doses to soft tissue D_1 between 0.051 and 1.53 Gy are given in Table 1. The lower part of the table shows the measured yields of chromosome aberrations resulting from absorbed doses to soft tissue D_2 ranging from 0.454 to 9.262 Gy, with the gold film in place.

TABLE 2
Dose–Yield Coefficients α ± SD, β ± SD and Derived RBE ± SD for Chromosome Aberration Yields after Irradiation with 60 kV X Rays in the Presence or Absence of a 150-μm-thick Gold Film, for Irradiations Performed with the Same Monitor Units

Coefficients	Gold film present	Dicentrics and centric rings	Acentric fragments
α_1 (Gy^{-1})	no	0.047 ± 0.008	0.045 ± 0.013
β_1 (Gy^{-2})	no	0.028 ± 0.010	0.034 ± 0.014
α_2 (Gy^{-1})	yes	0.080 ± 0.011	0.076 ± 0.014
β_2 (Gy^{-2})	yes	0.007 ± 0.002	0.007 ± 0.003
RBE = α_2/α_1		1.70 ± 0.37	1.69 ± 0.57

The dose–yield relationships for exposures without and with the gold film were linear-quadratic in the dose range investigated. The α values, i.e. the initial slopes of linear-quadratic dose–effect relationships fitted to the control-corrected data for yield per cell, were evaluated using a maximum-likelihood procedure and are presented in Table 2, which also contains the β values obtained by fitting linear-quadratic dose–effect relationships to the data for yield per cell. The last line of Table 2 shows the values of the low-dose RBE calculated as the ratio of the α values with and without the gold film. These RBE values of about 1.7 differ significantly from unity.

DISCUSSION

To interpret the observed RBE values for exchange-type chromosome aberrations and acentric fragments, we must consider the spectrum of the photoelectrons scattered out across the entrance plane of the 150-μm-thick gold film, traversing 2 μm of Mylar film in the backward direction and finally entering the 3-μm-thick lymphocyte layer. Monte Carlo calculations[2] have shown that the photoelectrons, which are released from the gold L and M shells and, after their energy losses in gold, pass through the gold/Mylar film interface, have continuous spectra that extend preferentially to the low-energy side of asymmetrical peaks situated at about 30 and 40 keV for the photon spectrum of this experiment. With increasing thickness of the traversed Mylar film, the electron spectra are broadened and the peaks are shifted to lower energies, so that the relative weight of the low-energy end of the spectrum increases.

For interpretation of the observed RBE values of about 1.7, we must consider the microdosimetric differences between the electrons entering the nuclei of the irradiated cells in the presence and absence of the gold film. Systematic evaluations of the yields of dicentric chromosomes and excess acentric fragments in human lymphocytes exposed to electrons, photons, protons and neutrons have shown that the α coefficient for these chromosome aberrations is approximately proportional to $\overline{L}_{500,D}$, the dose-mean restricted linear energy transfer associated with the particle track structure of these radiations (*7*). The restriction referred to here is to consider the LET of the ''track core'' separately and to attribute individual LET values to the secondary electrons that spread out beyond the track core if their initial kinetic energies exceed 500 eV. This separation into components corresponds to the geometry of a DNA target of nanometer dimensions, e.g. the 30-nm chromatin fiber, which, if hit by the track core, is essentially not hit by the secondary electrons of higher energy, since these frequently have ranges that are equal to or exceed the molecular dimensions so that their track ends occur outside the target. For electron radiations at energies below 50 keV, it has been shown[3] that LET, if restricted to collisions with energy transfer not exceeding 500 eV, is equal to the linear density of the total energy deposited within a cylindrical track core of radius 7.5 nm. In the presence of an electron spectrum, the dose weighting of parameter L_{500} has to account for the fact that the product αD determines the yield of aberrations. Therefore, if a linear dependence on L_{500} is assumed to represent the variation of α with radiation quality, L_{500} has to be dose-weighted.

Reasonable estimates of the $\overline{L}_{500,D}$ values that are valid in the presence of the gold film have been obtained using the above-mentioned Monte Carlo simulation of the photoelectron spectra[2] together with the values of restricted LET for electrons of various energies.[3] Thereby, we have estimated an $\overline{L}_{500,D}$ value of 10.9 keV/μm for irradiations in the presence of the gold film. For the irradiation with 60 kV X rays in the absence of the gold film, we use Blohm's $\overline{L}_{500,D}$ values for 150 kV X rays (6.36 keV/μm) and 30 kV X rays (6.82 keV/μm),[3] so that their average of approximately 6.6 keV/μm can be taken as a reasonable estimate at 60 kV.

Although the resulting $\overline{L}_{500,D}$ values of 10.9 keV/μm and 6.6 keV/μm for the two irradiation conditions are estimates and fine corrections are neglected, their quotient of 1.65 can be regarded as a reasonable microdosimetric characteristic of the difference between the secondary electrons originating from the gold film and the secondary electrons released in air and subsequently entering the lymphocytes. Considering the above-mentioned proportionality of the α values with $\overline{L}_{500,D}$ according to ref. (*7*), the observed RBE of about 1.7 is reasonable. The main difference between the exposure conditions in air and near the gold film is that the gold film is a secondary electron source located at a defined distance from the cell layer, so that the cells are preferentially hit by electron track ends with elevated LET, whereas

[2] W. Friedland, H. Pruchova, D. Hantke, H. G. Paretzke, D. Regulla and D. Harder, Monte Carlo analysis of the secondary electron field near a gold/tissue interface exposed to X-rays in the diagnostic energy region. To be published.

[3] R. Blohm, The passage of electrons through radiation sensitive regions of the cell nucleus (in German). Dissertation, Georg-August University, Göttingen, Germany, 1983.

air is a source of secondary electrons extending to the cell surface, so that the cell layer is not particularly exposed to electron track ends.

This interpretation is consistent with the established knowledge that low-energy electrons such as the photoelectrons of carbon K-shell radiation (0.27 keV) and of aluminum K-shell radiation (1.5 keV) have high efficiencies per unit dose in producing chromosome aberrations in human lymphocytes (*8*). RBE values similar to those in the present experiment have been observed, and a similar interpretation has been given for radiation effects in hamster ovary cells exposed to 200 kV X rays near material interfaces of high atomic number (*9*).

The yield of electron track ends per unit dose has long been regarded as a determinant for the RBE of cellular effects, because low-energy electrons have a maximum LET of about 28 keV/μm at an energy of about 0.1 keV (*7*). We therefore conclude that the increased RBE for chromosome aberrations in lymphocytes observed in the field of the photoelectrons backscattered from a gold film and slowed down in a 2-μm-thick Mylar film can be explained by the increase in the electron track-end yield per unit of energy deposition compared to the irradiation of lymphocytes in air.

The data in Table 2 also show that the increase in the α coefficient due to the exposure of the lymphocytes to photoelectrons from gold is accompanied by a decrease in the β coefficient. This phenomenon was first observed in a comparison of the α and β values for various electron and photon radiations and has been interpreted to indicate the presence of two competing reaction pathways (*10*).

CONCLUSION

In conclusion, the present experiments illustrate that chromosome aberrations in attached human lymphocytes represent a detection system with a high spatial resolution of 2–3 μm that is able to detect a localized dose enhancement in a tissue lining the surface of a medium with higher atomic number. The physical dose averaged over the cell nuclei was enhanced by a factor of 45.4. Furthermore, this experiment examining chromosome aberrations in lymphocytes permitted us to detect an increase in the RBE by a factor of about 1.7, as would be expected from the increase in the electron track-end yield per unit of dose. Hence the existing surfaces of higher-*Z* media in humans represent ''radiation amplifiers'' under X irradiation, generating ''hot spots'' of dose and also enhanced RBEs in the adjacent cell or tissue layers. Localized doses up to the therapeutic dose level of several grays for patients undergoing certain diagnostic X-ray examinations may occur. To our knowledge, little information is available in the literature concerning the possible tumor risk that may originate from this interface effect (*11–13*).

Apart from a certain risk to be considered as a problem of radiation protection, there may be potential clinical applications of the dose and RBE enhancement observed in interface-related secondary radiation fields. These effects may serve, for instance, for selective tumor control as a kind of photon activation therapy. Relevant experiments are in progress.

ACKNOWLEDGMENTS

The authors express their appreciation to W. Friedland *et al.* of the GSF-Institute of Radiation Protection for kindly sharing the results of their Monte Carlo calculations prior to publication. Our thanks are also addressed to H. Braselmann of the GSF-Institute of Radiation Biology for valuable discussions of dose–yield fit functions.

Received: March 8, 2000; accepted: November 28, 2000

REFERENCES

1. D. F. Regulla, L. B. Hieber and M. Seidenbusch, Physical and biological interface dose effects in tissue due to X-ray-induced release of secondary radiation from metallic gold surfaces. *Radiat. Res.* **150**, 92–100 (1998).
2. D. Regulla, L. Hieber and M. Seidenbusch, The dose enhancement effect at interfaces between tissue and media of higher atomic number: Physical and biological effects in the range of X-ray diagnosis. *Z. Med. Phys.* **10**, 52–62 (2000). [in German]
3. D. F. Regulla, W. Friedland, L. Hieber, W. Panzer, M. Seidenbusch and E. Schmid, Spatially limited effects of dose and LET enhancement in tissue for diagnostic X-ray qualities. *Radiat. Prot. Dosim.* **90**, 159–163 (2000).
4. E. Schmid, L. Hieber, U. Heinzmann, H. Roos and A. M. Kellerer, Analysis of chromosome aberrations in human peripheral lymphocytes induced by *in vitro* α-particle irradiation. *Radiat. Environ. Biophys.* **35**, 179–184 (1996).
5. ICRU, *Stopping Powers for Electrons and Positrons.* Report 37, International Commission on Radiation Units and Measurements, Bethesda, MD, 1984.
6. M. Bauchinger and E. Schmid, LET dependence of yield ratios of radiation-induced intra- and interchromosomal aberrations in human lymphocytes. *Int. J. Radiat. Biol.* **74**, 17–25 (1998).
7. D. Harder, R. Greinert and D. Frankenberg, Characteristics of electron tracks in matter. In *Radiation Research*, Vol. 2 (M. Moriarty, C. Mothersill, C. Seymour, M. Edington, J. F. Ward and R. J. M. Fry, Eds.), pp. 111–114. Allen Press, Lawrence, KS, 1999.
8. R. P. Virsik, C. Schäfer, D. Harder, D. T. Goodhead, R. Cox and J. Thacker, Chromosome aberrations induced in human lymphocytes by ultrasoft Al_K and C_K X-rays. *Int. J. Radiat. Biol.* **85**, 545–557 (1980).
9. D. L. Zellmer, J. D. Chapman, C. C. Stobbe, F. Xu and I. J. Das, Radiation fields backscattered from material interfaces: I. Biological effectiveness. *Radiat. Res.* **150**, 406–415 (1998).
10. R. P. Virsik, R. Blohm, K-P. Hermann and D. Harder, Fast, short-ranged and slow, distant-ranged interaction processes involved in chromosome aberration formation. In *Seventh Symposium on Microdosimetry* (H. G. Ebert, Ed.), pp. 943–955. Harwood Academic Publishers, London, 1981.
11. S. T. Cochran, A. Khodadoust and A. Norman, Cytogenic effects of contrast material in patients undergoing excretory urography. *Radiology* **136**, 43–46 (1980).
12. F. W. Spiers, Dosage in irradiated soft tissues and bone. *Br. J. Radiol.* **25**, 365–370 (1951).
13. C. L. Wingate, W. Gross and G. Failla, Experimental determination of absorbed dose from X-rays near the interface of soft tissue and other material. *Radiology* **79**, 984–1000 (1962).