STUDIES ON AUGER ENHANCEMENT IN BIOLOGICAL SYSTEMS WITH THE USE OF MONOCHROMATIC SYNCHROTRON RADIATION

Kunio Shinohara
Dept. of Radiation Research, Tokyo Metropolitan Institute of Medical Science
Bunkyo-ku, Tokyo 113, Japan

The Auger effect has been demonstrated to be highly toxic to cells (1-4). This highly toxic effect has been characterized with the use of radioisotopes such as ^{125}I (5-9). For clinical application of the Auger effect, Fairchild et al. (10) have proposed the use of 5-iododeoxyuridine and x rays at an energy slightly higher than the K absorption edge of iodine. The enhancement of radiation effects by induction of the Auger effect has been observed in some systems with the use of fluorescence x rays (11-14).

For experiments on induction of the Auger effect by external radiation, synchrotron radiation is quite useful. Since synchrotron radiation became available at the Photon Factory, National Laboratory for High Energy Physics in Japan, facilities have been constructed for using intense monochromatic synchrotron radiation in studies by the Radiation Biology Working Group (led by Dr. Takashi Ito of the University of Tokyo). With these facilities, studies on many aspects of induction of the Auger effect are now in progress. Maezawa et al. are studying bromouracil-incorporating E. coli. Their preliminary results (15) show no increased sensitivity to 13.49 keV compared with 12.40 keV--above and below the K absorption edge of bromine, which is 13.47 keV (9.920 A). In contrast, Ohara et al. (16) observed the highest frequencies of chromosome aberrations in mammalian cells (CHO) irradiated with synchrotron radiation at 0.90 A after 5-bromodeoxyuridine (BrdUrd) incorporation, in experiments on cells with and without BrdUrd and irradiated at 0.90 A or 1.00 A. Studies on DNA (Hieda et al., personal communication) and on the survival of CHO cells (Majima et al., personal communication) are also in progress.

We have also studied Auger enhancement in HeLa cells (17), which is the main subject of this paper. The two parts of the study dealt with the elements bromine and gallium.

HeLa cells were allowed to attach themselves to a membrane filter (18) and were then incubated either with BrdUrd ($5\times10^{-5}M$) and deoxycytidine ($10^{-5}M$) for 18 hr (corresponding to one generation time) or with gallium citrate ($10^{-4}M$) for 24 hr. The cells on the membrane filter were irradiated with monochromatic synchrotron radiation at 0.90 A, 1.00 A, or 1.14 A obtained with a 111-channel cut Si crystal monochromator from the synchrotron radiation produced by the Photon Factory's electron storage ring. The intensity of the monochromatic radiation was monitored by a free-air ionization chamber, and cell survival was determined by colony-forming ability.

The results on the effect of synchrotron radiation on BrdUrd-labeled HeLa cells have been reported (17). Briefly, HeLa cells labeled with BrdUrd were irradiated with monochromatic synchrotron radiation at 0.90 A or 1.00 A. The sensitivity of BrdUrd-labeled cells was higher when they

were irradiated at 0.90 A than at 1.00 A, but cells without BrdUrd showed no difference in sensitivity when irradiated at these two wavelengths (see Fig. 1 of Ref. 17). Table 1 shows the ratio of D_0 (the dose required to reduce the linear portion of the survival curve by 63%) in the presence of bromine to that in its absence (average of three sets of experiments). The increased sensitivity of BrdUrd-labeled HeLa cells at 0.90 A may be attributable to induction of the Auger effect since the K absorption edge of bromine is 0.920 A. The results were in good agreement with the findings of Halpern and Mütz (14) that the sensitivity of BrdUrd-labeled dried bacteria is higher to 14.4-keV than to 12.2-keV monoenergetic x rays, and that this obtains also in wet mammalian cells.

Table 1. The ratio of D_0 in the presence of bromine to that in its absence

Wavelength (A)	$D_0(-Br)/D_0(+Br)$
0.90	1.41 ± 0.03
1.00	1.22 ± 0.01

Another element interesting to study in this wavelength range is gallium. Since gallium has been demonstrated to accumulate selectively in solid tumors (19), it is of interest whether or not it is effective for Auger enhancement of cell killing. Figure 1 shows the growth curve of HeLa cells in the presence of gallium citrate (10^{-4}M). During the test period of 20-60 hr the colony-forming ability was not affected although the growth rate decreased slightly. Figure 2 shows no increase in the sensitivity of gallium-labeled HeLa cells over that of controls to irradiation with monochromatic synchrotron radiation at 1.14 A, slightly shorter than the K absorption edge for gallium (1.196 A).

Since gallium has been shown to concentrate in lysosome (20), the present results, together with findings with the use of ^{67}Ga (21), suggest that the range of Auger enhancement is not great enough for interaction with DNA in nucleus from lysosome in cytoplasm, although the possibility should be considered that the accumulated number of gallium atoms ($\sim 5\times10^7$/cell) may not be enough to produce detectable enhancement effects.

In summary, studies on Auger enhancement in Japan are briefly introduced, and findings are described which show that Auger enhancement in HeLa cells with the use of monochromatic synchrotron radiation takes place in combination with BrdUrd but not with gallium citrate.

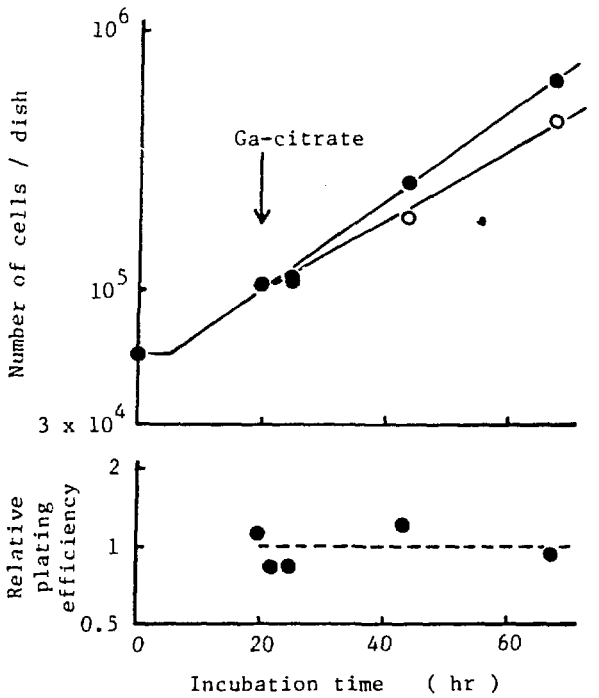

Figure 1. Growth curve of HeLa cells in the presence of gallium citrate (10^{-4} M), added to culture medium at time indicated by arrow. At each point shown, cells were collected by trypsinization, counted, and plated for colony formation. (Upper panel: o, test cells; •, controls.)

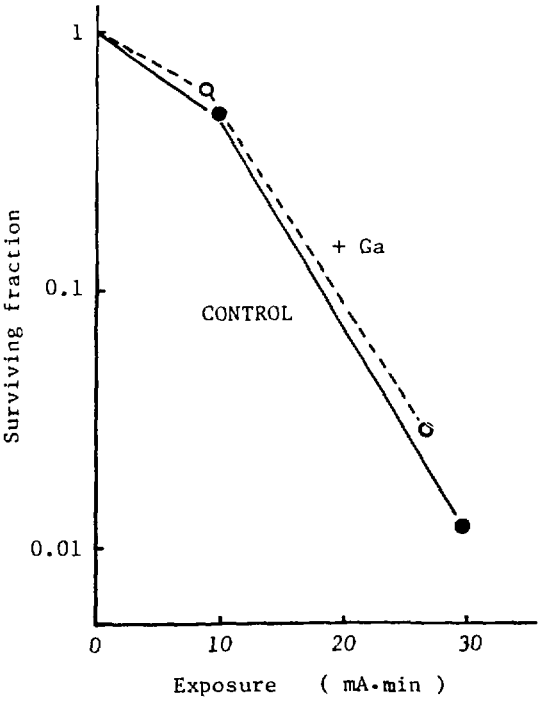

Figure 2. Survival curve of HeLa cells incubated in the presence or absence of gallium citrate (10^{-4} M) for 24 hr and irradiated with monochromatic synchrotron radiation at 1.14 A. The exposure is stored current (mA) x irradiation time (min).

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