RBE of HTO to $^{60}$Co Gamma-Rays for Cell Killing of A Radioresistant

E. coli Harboring Plasmid

OSAMU YAMAMOTO$^1$, TAEKO JO$^2$, MASANORI SUGIYAMA$^3$ AND
TAKUJI ITOH$^4$

$^1$Department of Biology, Faculty of Sciences, Hiroshima University, 1–3, Kagamiyama,
Higashi-Hiroshima 724,
$^2$Department of Radiation Biology, Research Institute for Nuclear Medicine and Biology,
$^3$Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, 1–2–3,
Kasumi, Minami-ku, Hiroshima 734,
$^4$Faculty of Biological Resources, Hiroshima Prefectural University, 562, Nanatsuka-cho, Shobara 727, Japan

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Radioresistant E. coli TG1 harboring pUC 18 plasmid which was Ampicillin-resistant was exposed to
$^{60}$Co gamma-rays or $^3$H beta-rays in saline to determine whether the relative biological effectiveness of $^3$H
beta-rays is higher than one. After exposure to $^{60}$Co gamma-rays at a dose rate of 0.465 Gy/min, the $D_0$ by
colony formation was 145 Gy in the presence of Ampicillin in or absence from the agar medium; whereas,
the $D_0$ was calculated as 118 Gy with and without Ampicillin after exposure to $^3$H beta-rays at a dose rate
of 0.431 Gy/min. The relative biological effectiveness established for $^3$H beta-rays to $^{60}$Co gamma-rays was
1.23. The reason for the higher effectiveness of $^3$H beta-rays as compared to the reference $^{60}$Co gamma-rays
is discussed in terms of nascent 0 production.

INTRODUCTION

The relative biological effectiveness (RBE) of $^3$H beta-rays in comparison to $^{60}$Co
gamma-rays was reported in 1957 to be higher than one for LD$_{50/30}$$^1$, and for splenic and
thymic atrophies$^2$, and Fe uptake$^3$. Later it was reported to be higher than one in
a number of systems such as in the killing of mammalian cells$^3$–$^9$, chromosomal aber-
ration$^{10–12}$ in and transformation$^{13–15}$ of mammalian cells, and the mutation of mam-
malian$^{8,16,17}$, Drosophila$^{18}$, yeast$^{19}$ and bacterial$^{20}$ cells. Lunec and Cramp$^{21}$ reported

山本 隆：広島大学理学部生物学科、広島市鷺山1-3 〒724
城多雄子：広島大学放射線科学研究院環境放射線研究所、広島市南区霞1-2-3 〒734
杉山 勇：広島大学医学部総合養生学科、広島市南区霞1-2-3 〒734
伊藤卓朗：広島県立大学生物資源学部、庄原市七塚町562 〒727
an RBE of 1.21 for $^3$H beta-rays to 7 MeV electrons for bacterial cell killing of radiosensitive *E. coli* B$_{3-1}$. So far, no studies have shown the RBE in a radioresistant bacterium or the RBE for a plasmid with a target size much smaller than the bacterial nucleus. We used *E. coli* TG1 harboring an Ampicillin-resistant plasmid, which is much more radioresistant than the radioresistant strain, *E. coli* H/r3022) to investigate the RBE of $^3$H beta-rays both for the radioresistant *E. coli* TG1 and for the plasmid.

**MATERIALS AND METHODS**

**Cell Culture**

Plasmid pUC 18 (Ampicillin-resistant, Toyobo Co., Japan) was introduced into *E. coli* TG1 (supE hsd$\Delta 5$ thi $\Delta$ (lac-proAB)/F$^+$ [traD36 proAB$^+$ lacI$^q$ lacZM$\Delta 15$])$^{23}$) (a gift from the Pasteur Institute, Paris). This *E. coli* harboring the plasmid was first cultured overnight at 37°C in 50 ml of LB medium (10 g Bactotryptone, 5 g Bacto-Yeast extract, 10 g NaCl in 1 l adjusted to pH 7.0). Two milliliters of this cultured cell suspension was resuspended in 200 ml of LB medium then incubated for 2.5–3.0 h at 37°C. Cells were harvested at OD$^{600}$ 0.8 by centrifugation at 5,000 rpm for 5 min, after which they were washed 3 times with 50 ml saline (0.9 g NaCl/l) by repeated centrifugation. Finally, the cells were resuspended in 10 ml of a double concentration of saline (4.10 $\times$ 10$^{10}$ cells/ml).

**Irradiation**

One-half milliliter of the cell suspension was diluted with an equal volume of pure water to obtain a final concentration of 2.05 $\times$ 10$^{10}$ cells/ml. This suspension was irradiated at 4°C with $^{60}$Co gamma-rays (Shimadzu 10000S) at a dose rate of 0.465 Gy/min. In contrast, HTO with an activity of 1.74 $\times$ 10$^{11}$ Bq/ml (Amersham International plc, U.K.) was diluted with water to obtain an activity of 1.74 $\times$ 10$^{10}$ Bq/ml. The diluted HTO solution was incubated with catalase then distilled in vacuo to remove H$_2$O$_2$, as described elsewhere$^{24}$. One-half milliliter of the H$_2$O$_2$-removed HTO solution was added to 0.5 ml of the cell suspension, the final HTO and cell concentrations being 8.7 $\times$ 10$^9$ Bq/ml and 2.05 $\times$ 10$^{10}$ cells/ml. The dose rate at this HTO concentration was calculated as 0.476 Gy/min (=$\frac{8.7 \times 10^{12}}{60} \times 5.7 \times 10^{-12}$ J/eV $\times$ 1 Gy/J$^{-1}$) in water. The free water in *E. coli*, however, constitutes 73% of the weight of the cell, and the $^3$H beta-particles that originate inside the cell deposit 65% of their dose outside the cell$^{21}$). Therefore, the corrected dose rate for the exposure of the cells was calculated with a factor of 0.905 as 0.431 Gy/min. The HTO-containing cell suspension was left for a specified time at 4°C for the beta-irradiation. The temperature, 4°C, for both the $^{60}$Co gamma- and $^3$H beta-irradiations was controlled by a thermo leader (Taiyo Service Center Co., Model EZL-80).

**Clonogenic Assay**

The irradiated cell suspensions were centrifuged at 5,000 rpm for 5 min, after which
the cell pellets were washed 3 times with 2 ml of saline by repeated centrifugation then resuspended in 1 ml of saline. Difference cell dilutions were plated in 60 mm dishes containing LB medium with 5% agar in the presence or absence of Ampicillin (0.1 mg/ml). The colonies formed were counted after an overnight incubation at 37°C.

RESULTS

After exposure to $^{60}$Co gamma-rays at a dose-rate of 0.465 Gy/min, the $D_0$ was the same, 145 Gy, whether or not Ampicillin was present in the colony dish (Upper in Figure 1). Ampicillin was used to determine the difference in the intrinsic radiation response of *E. coli* and the harbored plasmid. In the absence of Ampicillin in the clonogenic assay, even if the plasmid sustained damage, the observed response would be due to *E. coli* alone. In contrast, Ampicillin would selectively kill those surviving *E. coli* cells in which the harbored plasmid sustained damage. Therefore, the observed radiation response would include not only that of *E. coli* but that of the plasmid. But, as numerous plasmids exist in a single *E. coli* cell, there is the possibility that at least one might survive or repair the damage done, thereby maintaining the Ampicillin resistance of the *E. coli* cell. Therefore, after low dose rate irradiation, when the possibility of repair is high, the fact that there was no marked difference between the presence and absence of Ampicillin is evidence that the observed response was that of *E. coli* in both conditions, the survival rate of the plasmids not being clear. A very radiosensitive bacterium that harbors only one plasmid and a high dose rate at radiation must be used in order to determine the survival rate of the plasmid.

After exposure to HTO ($8.7 \times 10^9$ Bq/ml), the dose rate of $^3$H beta-rays to the cells was calculated as 0.476 Gy/min on the assumption that the irradiated material consisted only of water; this rate became 0.431 Gy/min when a correction factor of 0.905 was used in the calculations as described in the previous section. This dose rate was comparable to the 0.465 Gy/min used for gamma-irradiation. Nor was any difference found between to $D_0$ values in the presence or absence of Ampicillin (both $D_0$ values were 130 Gy before correction and 118 Gy after correction) in $^3$H beta-irradiation (Lower in Fig. 1).

The RBE of $^3$H beta-rays to $^{60}$Co gamma-rays was

$$\frac{D_0 \text{ of } ^{60}\text{Co gamma-rays}}{D_0 \text{ of } ^3\text{H beta-rays}} = \frac{145 \text{ Gy}}{118 \text{ Gy}} = 1.23$$

DISCUSSION

A number of estimations of the RBE of $^3$H beta-rays with reference to gamma-rays or other radiations have given a value higher than one (Table 1). The RBE calculated 1.23 in
Fig. 1. Survival curves for *E. coli* TG1 harboring the pUC 18 plasmid after $^{60}$Co gamma- and $^3$H beta-irradiations. ———: Incubation on agar plates containing LB medium without Ampicillin after irradiation, ———: incubation on agar plates containing LB medium with Ampicillin after irradiation.
our study is in good agreement with past reported values.

An important question is why is RBE of $^3$H beta-rays higher than one?. Iwanami and Oda, and Ito, independently made microdosimetric models to explain the RBE of $^3$H beta-rays. Iwanami and Oda estimated that the somewhat higher magnitude of the inactivation cross section of $^3$H beta-rays than that of the reference radiation is cancelled by the relatively smaller magnitude of the total electron fluence per unit absorbed dose of HTO. They concluded that the RBE of $^3$H beta-rays depends on the ability of the cell to repair DNA damage. The $E.\ coli$ TGI ($D_0=145 \text{ Gy}$ for $^{60}\text{Co}$ gamma-irradiation) we used shows a higher reparability than the $E.\ coli$ B$_k$-1 ($D_0=19.5 \text{ Gy}$ for 7 MeV electron-irradiation) used by Lunec and Cramp. The former’s RBE of 1.23 is almost the same as the 1.21 of the latter. It appears therefore that higher repair capacity of the cells exposed to the reference radiation does not result in a higher RBE.

Ito estimated the RBE as 1.26, based on double-strand breaks of DNA. He proposed that the double-strand breaks were produced by two ionizations (0.74% as dsb/ssb for $^{60}\text{Co}$ gamma-irradiation and 0.88% for $^3$H beta-irradiation), one ionization and one OH reaction.
(0.80% and 1.06%, respectively), and two OH reactions (0.02% for both irradiations). If the double-strand breaks do cause cell death, his hypothesis would be adequate because his given value of 1.26 corresponds to our result of 1.23. But in his hypothesis the respective ratios of the direct to the indirect effect are 2.71:1 (0.74%+0.40%:0.40%+0.02%) and 2.56:1 (0.88%+0.53%:0.53%+0.02%) for $^{60}$Co gamma-irradiation and $^3$H beta-irradiation. Such a major contribution of the direct effects to cell death is inadequate in terms of the $O_2$ and radical-scavenger effects. Indeed, a high OER value has been reported both for double-strand breaks (G values:0.13 in $N_2$ and 0.27 in $O_2$) and for the base release (G values:0.2 in $N_2$ and 0.6 in $O_2$). Roots and Okada reported that the strand break yield could be reduced by a factor of 3.5 in the presence of scavengeers. If the direct effect is really the predominant factor, then the $O_2$ and scavenger effects could not have been observed.

Previously, one of us reported that the OH yield after $^3$H beta-irradiation is higher than that after gamma-irradiation ($\beta/\gamma=1.36$). We now feel that the yield of nascent $O$ should have been included in the reported OH yield as it can act as two OH radicals in the case of $^3$H beta-irradiation. This is based on our observation of excessive epoxide formation from mesityl oxide in the case of $^3$H beta-irradiation but not in the case of $^{60}$Co gamma-irradiation, which indicates that the production of nascent $O$ was much greater for the former irradiation than for the latter.

In fact, the relative effectiveness of mesityl oxide degradation is greater than that of monohydroxide but is much less than that of epoxide (Table 2). This is evidence that the reaction mechanism of $^3$H beta-radioisolation in aqueous systems differs markedly from that of the reference radiation. In the case of chemicals, reactions with the nascent $O$ resembling two OH [$M+O→MO, MO+H_2O→MOH_2$ or MOH+OH] may result in a higher RBE. We also obtained the relative effectiveness higher than one both for single-strand and double-strand breaks of DNA in in vitro system. Such a higher yield of damaged sites produced by interaction both with OH and nascent $O$ in the $^3$H beta-irradiation also may occur in the cells. Therefore, in the case of cell death, the RBE value may become higher than one and be unrelated to the repair capacity. Frankenberg et al. recently reported that for yeast cells the RBE of $Al_X$ X-rays (1.5 keV, 15 keV/μm LET) and $C_X$ X-rays (0.288 keV, 20 keV/μm LET), which have a slightly higher LET than $^3$H beta-rays (5.7 keV, 5 keV/μm LET), were 2.2 and 3.8 for double-strand breaks of DNA and 2.4 and 2.6

<table>
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<tr>
<th>Yield (%)</th>
<th>-Mesityl oxide</th>
<th>Epoxide</th>
<th>Monohydroxide</th>
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<tr>
<td>$^{60}$Co gamma-rays</td>
<td>21.0</td>
<td>4.4</td>
<td>11.7</td>
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<tr>
<td>$^3$HHO beta-rays</td>
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<td>23.2</td>
<td>20.6</td>
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<td>$^3$HHO/$^6$Co</td>
<td>2.5</td>
<td>5.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Table 2. Radiolytic Yields of Mesityl Oxide Degradation, Epoxide Formation and Monohydroxide Formation, and Relative Effectivenesses of $^3$H beta-rays to $^{60}$Co gamma-rays. (Data derived from Fig. 3 in Ref. 24)
Fig. 2. Relation of the 'G value' of the three oxidative species: OH, H2O2 and HO2, with LET. Diff.: Total 'G values' of the oxidative species (G_{OH}+2G_{H2O2}+3G_{HO2}) for irradiation with high LET radiation was subtracted from that for 60Co gamma-irradiation. 'G values' are cited from the tables given by Spinks and Woods\(^{32}\) and Buxton\(^{33}\). ( ) Extrapolated or interpolated. A: 60Co gamma-rays, 0.23 keV/µm; B: 3H beta-rays, 4.7 keV/µm; C: 18 MeV D\(^+\), 12.3 keV/µm; D: 32 MeV He\(^2+\), 61 keV/µm; E: 12 MeV He\(^2+\), 108 keV/µm; F: 210Po alpha-rays, 136 keV/µm; G: 10B(n, α)\(^7\) Li recoil nuclei, 180 keV/µm.
for killing.

Figure 2, based on the tables prepared by Spinks and Woods\textsuperscript{32} and Buxton\textsuperscript{33}, shows the relations of the yields of the three oxidative species \( \text{G}_{\text{OH}}, 2\text{G}_{\text{H}_2\text{O}}, \) and \( 3\text{G}_{\text{HO}_2} \) (1, 2 and 3, are shown as an OH equivalent ratio) in water with LET. The resulting yield of these three oxidative species (\( \text{G}_{\text{OH}}+2\text{G}_{\text{H}_2\text{O}}+3\text{G}_{\text{HO}_2} \)) decreases up to 100 keV/\( \mu \)m, and then increases; the difference in total yield for \( ^{60}\text{Co} \) gamma-irradiation and higher LET particle irradiations increases up to 100 keV/\( \mu \)m then decreases. This difference in the resulting yield of the oxidative species for gamma-rays and other particle radiations is ascribable to some factor other than \( \text{OH}, \text{H}_2\text{O}_2 \) and \( \text{HO}_2 \). We propose that it is nascent \( \text{O} \). Formation of nascent \( \text{O} \) probably induces oxidation of the solute, thereby resembling two \( \text{OH} \) reactions as stated above, although the production of \( \text{O}_2 [\text{O}+\text{O} \rightarrow \text{O}_2] \) also may be involved. Indeed, in the formation of \( \text{O}_2 \) after exposure to high LET radiation, a “G value” of 0.4 has been established in neutral deaerated water for \( ^{222}\text{Rn} \) alpha-rays (134 keV/\( \mu \)m LET)\textsuperscript{34,35}. This value is identical to the different in the resulting value of 0.43 for \( ^{210}\text{Po} \) alpha-rays (136 keV/\( \mu \)m LET) (Fig. 3). Therefore, RBE should vary in different systems because of the competing reactions between \( \text{M}+\text{O} \) and \( \text{O}+\text{O} \). If production of \( \text{O}_2 \) did not occur, the RBE could become greater than the actual value under aerobic conditions. An RBE of more than one would reflect the considerably high contribution of the nascent \( \text{O} \) to the radiolytic reactions caused by exposure to \( ^{3}\text{H} \) beta-rays. In addition, if the irradiation condition was anaerobic, the production of \( \text{O}_2 \) would enhance the RBE because of the \( \text{O}_2 \) effect\textsuperscript{30}.

\section*{REFERENCE}


