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Novel Dosimeter for Low-Dose Radiation Using *Escherichia coli* PQ37

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Abstract The measurement of radiation response using simple and informative techniques would be of great value in studying the genetic risk following occupational, therapeutic, or accidental exposure to radiation. When patients receive radiation therapy, many suffer from side effects. Since each patient receives a different dose due to different physical conditions, it is important to measure the exact dose of radiation received by each patient to lessen the side effects. Even though several biological dosimetric systems have already been developed, there is no ideal system that can satisfy all the criteria for an ideal dosimetric system, especially for low-dose radiation as used in radiation therapy. In this study, an SOS Chromotest of *E. coli* PQ37 was evaluated as a novel dosimeter for low-dose gamma-rays. *E. coli* PQ37 was originally developed to screen chemical mutagens using the SOS Chromotest-a colorimetric assay, based on the induction of β-galactosidase due to DNA damage. The survival fraction of *E. coli* PQ37 decreased dose-dependently with an increasing dose of cobalt-60 gamma-rays. Also, a good linear correlation was found between the biological damage revealed by the β-galactosidase expression and the doses of gamma-rays. The expression of β-galactosidase activity that responded to low-dose radiation under 1 Gy was $Y=0.404+(0.089\pm0.13)D+(-0.018\pm0.16)D^2$ (Y, absorbance at 420 nm; D, Dose of irradiation) as calculated using Graph Pad In Plot and Excel. When a rabbit was fed with capsules containing an agar block embedded with *E. coli* PQ37 and irradiated with low-dose radiation, the *E. coli* PQ37 showed a linear response to the radiation doses. Accordingly, the results confirm that *E. coli* PQ37 can be used as a sensitive biological dosimeter for cobalt-60 gamma-rays. To the best of our knowledge, this is the first time that a bacterium has been used as a biological dosimeter, especially for low-dose radiation.

Key words: Cobalt-60 gamma-rays, dosimeter, *E. coli* PQ37, SOS Chromotest

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As the use of atomic energy increases, the possibility of humans being exposed to radiation also increases. It has been previously reported that patients under radiation therapy suffer from side effects such as anorexia, nausea, vomiting, and diarrhea. In particular, abdominal radiotherapy causes bacterial overgrowth resulting in intestinal pseudo-obstruction, which induces the absence of intestinal migrating motor complexes [7]. Futhermore, abdominal radiotherapy makes the recipient susceptible to systemic infection from the various endogenous and exogenous pathogens [2]. Therefore, it is important to measure the actual degree of exposure to radiation and cease radiation treatment before a patient suffers from side effect [5].

Several biological dosimetric systems have been developed, such as the electron spin resonance (ESR) technique [11, 12], hair cortical cell count (HCCC) [15], chromosomal aberration in skin fibroblast and hair [9, 20, 22], and micronucleus assay [1, 10, 14, 21]. However, these methods can only be applied to high-dose radiation and not to the low-dose radiation, and they are complicated to perform [4, 19]. Furthermore, since each patient receives a different dose of radiation because of different sensitivities to radiation, and radiation therapy uses only low-dose of radiation, the actual radiation dose received is very difficult to measure exactly. Over the last 20 years, the main focus has been on the development of a genotoxicity screening test using microorganisms or their properties as an indicator system. The SOS Chromotest is one example [8]. The SOS Chromotest is a simple bacterial colorimetric assay for screening genotoxicity, and *Escherichia coli* PQ37 was developed specifically for this assay [6, 13]. *E. coli* PQ37 carries a *sfiA::lacZ* fusion and has a deletion for the normal *lac* region (Fig. 1), therefore, any β-galactosidase activity is strictly dependent on *sfiA* expression induced by DNA damage [17]. So far *E. coli* PQ37 has only been tested with high-dose radiation [18]. Accordingly, based on the hypothesis that, albeit small, low-dose radiation can induce the β-galactosidase activity due to its damaging effect on DNA, *E. coli* PQ37 was

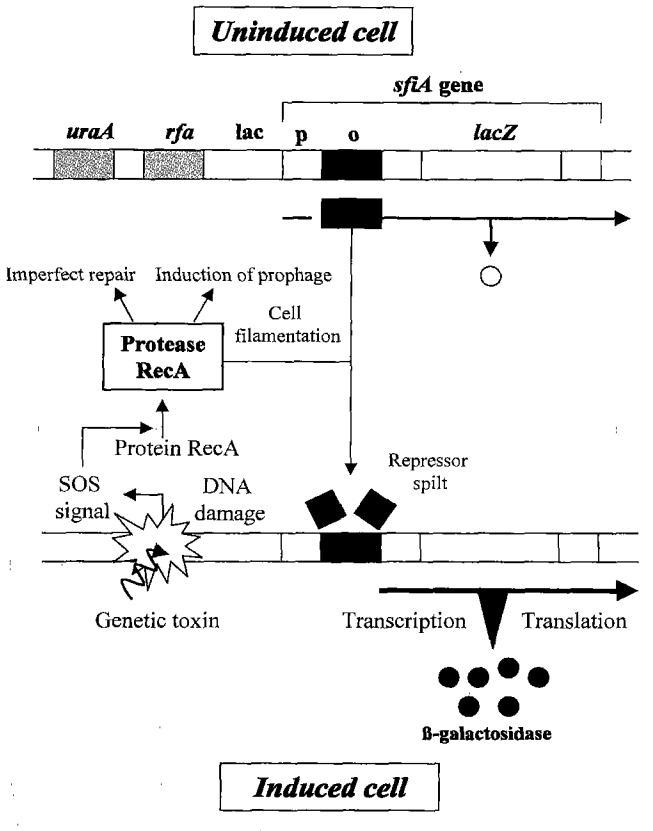

Fig. 1. The principle of the SOS Chromotest.
Upper part: uninduced cell. LexA repressor prevents expression of the *sfiA::lacZ* fusion. Lower part: the induced cell. DNA damage has triggered the activation of the expression of the *sfiA::lacZ* fusion [17].

tested as a novel biological dosimeter for low-dose radiation.

E. coli PQ37 includes the genotype *F⁻ thr leu his-4 pyrD thi galE galK or galT lac ΔU169 srl300::Tn10 rpoB rpsL uvrA rfa trp::Muc⁺ sfiA::Mud* (Ap. *lac) cts* [16] and was cultured in LB (Luria-Bertani) containing 20 μg/ml ampicillin. The media were purchased from Difco (Detroit, MI, U.S.A) and the other reagents were from Sigma Co. (St. Louis, MO, U.S.A.). The β-galactosidase assay was performed according to the method as described previously, with a minor modification [17]. Bacterial cells in the log phase (A_{600}=0.7) were collected by centrifugation and then washed with phosphate buffered saline (PBS, pH 7.4). Fresh LB was added to the cell pellet making 1×10^9 CFU/ml and an aliquot (1 ml) was transferred to a new tube. The bacterial cells were irradiated with cobalt-60 gamma rays (96 cGy/min, Theratron-780 telethrapy unit), incubated at 37°C for 4 h, and diluted tenfold with fresh LB. An aliquot of the bacterial suspension (0.3 ml) was mixed with 2.7 ml of a buffer (0.1 M Na_2HPO_4, 40 mM $NaH_2PO_4 \cdot H_2O$, 10 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$, 0.1% SDS, 40 mM β-mercaptoethanol, pH 7.0) and the reaction was started by the addition of 0.6 ml of 4 mg/ml ONPG in a 0.1 M sodium phosphate buffer (pH 7.4). After 1 h at 37°C, the reaction was stopped by the addition of 2 ml of 1 M Na_2CO_3. The color developed was assayed at 420 nm using a UV spectrophotometer. The mean and equation for the relationship between the absorbance and the radiation dose were calculated using Graph Pad In Plot and Excel.

Bacterial cells in the log phase (A_{600}=0.7) were washed with PBS and dispersed in fresh LB making 2×10^9 CFU/ml. The bacterial suspension was mixed with an equal volume of 1.4% low-melting agarose and the mixture was solidified in a sterile petridish. The immobilized bacteria in a petridish was irradiated with cobalt-60 gamma rays and gel blocks were punched out with the end of a sterile pasteur pipette. The gel blocks were immersed in 2 mg/ml X-gal and incubated at 37°C. After 6 h, each block was dissolved in 0.5 ml DMSO and the color developed was assayed at 634 nm using a spectrophotometer. Bacterial cells in the log phase were collected and washed with PBS. The bacterial cells were mixed with the same volume of 1.4% low-melting agarose, and 2.5 μl of the mixture was injected into a piece of tygon tubing (inner diameter, 0.1 cm; length, 0.8 cm) and solidified at room temperature after each end was sealed with teflon sealing tape. After the capsules were irradiated, blocks were extracted from each piece of tubing with a pipette tip and the β-galactosidase was assayed, as described above. A rubber tube (inner diameter, 0.36 cm) was intubated through the esophagus of a New Zealand white rabbit (male, 6 months old, 2.7 kg) and capsules containing the immobilized bacterial cells were inserted into the tube while flushing with water. The rabbit was irradiated while the capsules were inside the intubation tube. After irradiation, the capsules were collected by retracting the intubation tube and incubated at 37°C. After 9 h, which is the average time for food to stay in the intestines, the agarose blocks were extracted from the capsules and the β-galactosidase activity was assayed as described above.

Methods to estimate the actual dose of radiation by biological indicators have rapidly progressed during recent years [24]. To be used as a biological dosimeter, a dosimetric system should meet with the following criteria [11]: (1) It should show a good dose-dependence over a relevant range of doses; (2) The effect chosen for the estimation of the dose should be primarily specific for ionizing radiation; (3) The results should be available shortly after radiation exposure, within a few days in the case of an accident; (4) The effect should be permanent, otherwise, the time-dependence of the decay of the effect must be known. The time available for measurements should be of reasonable duration; (5) Different radiation qualities should be covered by the method. In particular, exposure due to internal emitters should be measurable; (6) The biological material that shows the effect must be readily accessible without extensive invasive methods; (7) Evaluation

should be either easy and rapid or transferable to machines. Among the known dosimetric systems, the induction of chromosome aberration in human peripheral blood lymphocytes [4, 9, 14, 20] and a micronucleus assay [1, 21] are the two most useful assays. These are useful for accidental whole-body irradiation and can also detect low-dose radiation (0.05 Gy). However, these dosimetric systems have some limitations. A dose distribution over the entire body is highly uneven, and lymphocytes from non-irradiated and irradiated regions are mixed in many situations of exposure to ionizing radiation, thereby resulting in an underestimation of the absorbed dose [19].

For partial-body irradiation, the determination of the hair diameter and chromosome abberations in skin fibroblasts and hair [3, 22] would seem to be advantageous, since better information on the dose distribution can be obtained. A decrease in the number of dividing cells in a hair bulb results in a narrowing of the hair, which can be determined 1–2 weeks post-irradiation: Hairs can be plucked, stained, and observed with a microscope in only a few minutes. However, the method cannot be applied to abdominal irradiation. In the case of *E. coli* PQ37, a good linear correlation curve was found between SOS induction and doses, especially with low-dose irradiation under 1 Gy (Fig. 2). β-galactosidase activity increased dependently on the dose of gamma-radiation under 1 Gy, and the relationship between the absorbance and the dose was calculated using Graph Pad In Plot and Excel as follows:

$$Y=0.404+(0.089\pm0.13)D+(-0.018\pm0.16)D^2$$

Y and D represent the absorbance at 420 nm and the dose of irradiation, respectively. The radiation dose was calculated from Y using the following equation when a, b, and c were 0.404, 0.089±0.13, and 0.018±0.16, respectively.

$$D=\frac{-b+\sqrt{b^2-4a(a-Y)}}{2c}$$

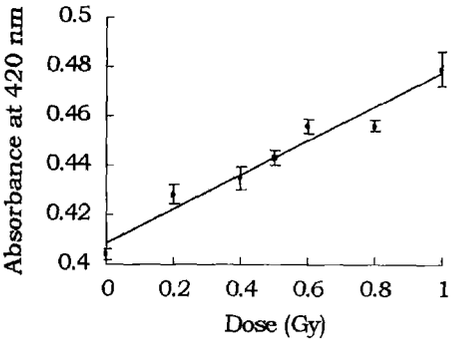

Fig. 2. β-galactosidase assay of *E. coli* PQ37 in broth.
Bacterial cells in LB were irradiated with various doses of cobalt-60 gamma-rays and the β-galactosidase activity was assayed as described in the text. The reactions were performed in triplicate and the mean was plotted.

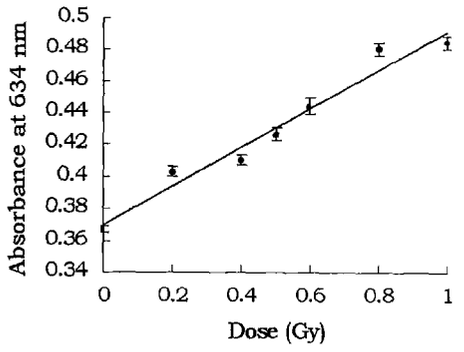

Fig. 3. β-galactosidase assay of immobilized *E. coli* PQ37.
E. coli PQ37 immobilized in a low melting agarose gel was irradiated with various radiation doses of cobalt-60 gamma-rays and the β-galactosidase activity was assayed as described in the text. Each reaction was performed in triplicate.

Since it is impossible to collect specific bacterial cells from the intestine, the bacterial cells were immobilized in low-melting agarose. Also, as the solidified agarose is easily dissolved in DMSO, the color developed in an agar block could be easily measured using a spectrophotometer. To avoid the disturbance from the cell debris and agarose in the sample, β-galactosidase activity of the immobilized cells was assayed with X-gal instead of ONPG. As expected from the above results, the immobilized cells in the low-melting agarose also exhibited a dose-dependent increase (Fig. 3). Furthermore, the capsules were made of tygon tubing to avoid any melting or dispersal of the bacterial cells inside the intestines. When the immobilized bacterial cells were collected from the capsules after irradiation, their β-galactosidase activity was found to be increased as the dose of irradiation increased (Fig. 4). Also, the activity of the β-galactosidase in the capsules inside the rabbit increased as the irradiation dose increased (Fig. 5). All of these results strongly support *E. coli* PQ37 as a biological dosimeter for low-dose radiation.

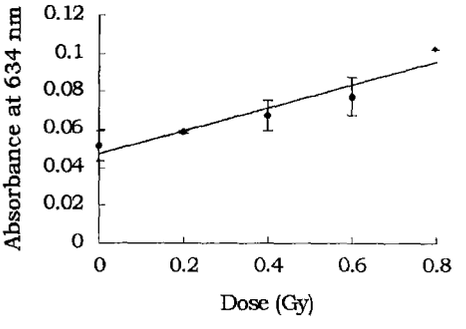

Fig. 4. β-galactosidase assay of immobilized *E. coli* PQ37 in a capsule.
Capsules containing immobilized *E. coli* PQ37 were irradiated with various doses of cobalt-60 gamma-rays and the β-galactosidase activity was assayed as described in the text. Each reaction was performed in triplicate.

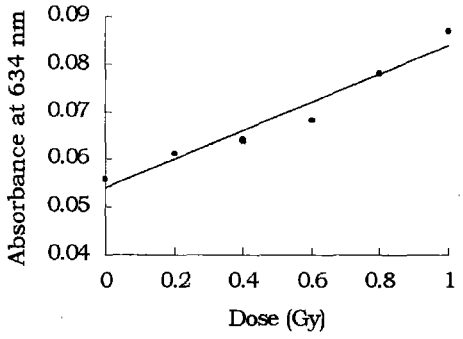

Fig. 5. Animal experiment.
After the capsules were intubated into a rabbit, the rabbit was irradiated with various doses of cobalt-60 gamma-rays and the activity of β-galactosidase was assayed as described in the text.

One concern was whether β-galactosidase activity would continue to be expressed even after a long period in the intestine. Since the stability of bacterial cells can be improved by entrapment [23], *E. coli* PQ37 entrapped in agarose continuously exhibited β-galactosidase activity even after 15 h at 37°C. This suggests that capsules could be ingested before radiation therapy and then subsequently collected without special equipment. Compared to other methods, this new dosimetric system using *E. coli* PQ37 satisfies several criteria, including a good dose-dependence, specificity for ionizing radiation, availability shortly after radiation exposure which takes less than one day to measure the actual dose in the intestine, and ability to detect the partial body exposure, especially in the case of abdominal irradiation. Before this system is used in a patient, however, several requirements should be met. The capsules should withstand the passage through the intestines and be easily collected after radiation therapy without making patients uncomfortable. To shorten the assay time, a different assay system using fluorescence instead of β-galactosidase needs to be developed.

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