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Distribution of Sensitivity to 4-Nitroquinoline 1-oxide among Japanese Lymphoblastoid Cell Lines

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The processes through which the UV-mimic chemical carcinogen, 4-nitroquinoline 1-oxide (4NQO), leads to the DNA lesions are well characterized in E. coli, where the formation of stable 4NQO-purine adducts is critical (Tada and Tada 1971; Tada and Tada 1975; Ikenaga et al. 1975). The $uvrA^+$ gene is responsible for the removal of the adducts in the normal strains of E. coli that possesses the DNA repair capacity (Setlow 1968). UV-sensitive E. coli $uvrA^-$ and $uvrB^-$ strains unable to excise UV-induced DNA adducts. The DNA excision-repair mechanisms similar to those for E. coli occur in normal human cells (Cleaver 1974; Cleaver and Trosko 1970; Regan et al. 1968; Setlow et al. 1969).

Xeroderma pigmentosum (XP) is an example of a rare recessive autosomal skin disorder which is characterized biochemically as a DNA repair-deficient disease. Characteristic clinical symptoms include enhanced sensitivity of the skin to sunlight, cutaneous pigmentary abnormalities and high incidence of cancer in the sun-exposed areas of the skin (Robbins et al. 1974; Mascaro 1976). Skin fibroblast cells isolated from patients with the disease show defective repair of UV light-induced damage to DNA (Cleaver 1968). The 4NQO-induced lethality of cells is mainly due to the some kinds of 4NQO-purine adducts as it is in E. coli.

The fluorescein diacetate (FDA) method (Tsuda and Maeda 1982) was recently used to determine the sensitivity of lymphoblastoid cell lines to 4NQO. Viable cells take up, non-fluorescent chemical, FDA and convert it to, a fluorescent molecule, fluorescein by intracellular esterases (Rotman and Papermaster 1966). DNA damage produced by 4NQO could be evaluated on the basis of the cell lethality by this FDA method. In the present study we describe the distribution of sensitivity to 4NQO among lymphoblastoid cell lines established from Japanese.

MATERIALS AND METHODS

Fluorescein diacetate (FDA), uranine and 4NQO were obtained from

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Wako Pure Chemical Ind. (Osaka, Japan). RPMI-1640 and Eagle's essential minimum medium (MEM) were from Nissui Pharmaceutical Ltd. (Osaka, Japan). Lymphoblastoid cell lines derived from XP patients (XP homozygote; complementation group A, XPL15 and XPL17) and from their parents (XP heterozygote; XPPL17 and XPML17) were a gift from Dr. A. Oikawa, Department of Pharmacology, Research Institute for Tuberculosis and Cancer, Tohoku University (Katsuki and Hinuma 1978).

On the other hand, heparinized peripheral blood (3-10 ml) was collected from apparently healthy 50 adult (both non-smokers and alcohol abstainers) and 8 child volunteers (aged 2 to 48) and was then established by the same method mentioned above (Katsuki and Hinuma 1978). These cells were seeded at a density of 3×10^5/ml (counted with Erma hemocytometer) and cultured in a RPMI-1640 medium supplemented with 20% heat-inactivated fetal bovine serum (FBS), 100 IU/ml penicillin and 100 ug/ml streptomycin at 37°C in an atmosphere of fully humidified air with 5% CO_2. At 24 h of culture the cell suspensions were centrifuged for 5 min at 1,500 rpm and the pellets were washed twice with the above medium minus FBS. The cells were resuspended in the same medium and the cell density was adjusted to 1.5×10^6 cells/ml. The suspension was mixed with 4-NQO solution (dissolved in ethanol and diluted with the RPMI-1640 medium minus FBS) and cultured for the period specified in what follows. Control cultures were received the solvent (the RPMI-1640 minus FBS and plus ethanol) in the same volume and on the same time schedule as the corresponding test cultures. The lymphoblastoid cells were then treated with 4NQO at a dose of 4×10^{-6} M for 48 hr. Control cells were treated with solvent vehicle alone in the same volume and on the same time schedule.

At the end of treatment period, the cell suspensions (about 10 ml) were spun for 5 min at 1,500 rpm and then the cells were washed twice with PBS. The pelleted cells were then resuspended in the riboflavin-free MEM at a concentration of 5×10^5 cells/ml. One ml of the cell suspension was mixed with 50 µl of the FDA substrate solution and incubated at 25°C for 20 min. The absorbance of fluorescein was measured at an excitation wavelength of 490 nm and emission wavelength of 520 nm, using a Hitachi spectrophotofluorometer Model 650-10S. Cellular esterase activity was expressed as the formation of fluorescein equivalent to 1 nmole uranine (standard solution of fluorescein) per min per 10^6 cells.

RESULTS AND DISCUSSION

The 58 lymphoblastoid cell lines established from 58 healthy individuals were each treated with solvent vehicle (used to dissolve the 4NQO) alone and were measured the amounts of fluorescein formation. The lymphoblastoid cell lines hydrolyzed between 20 and 55 pmoles of FDA/min/10^6 cells, exhibiting a normal distribution as shown in Figure 1. Out of 58, 53 cell lines (about 91%) showed the values between 30 and 50 pmoles/min/10^6 cells, 3 lines (about 5%) less than 30

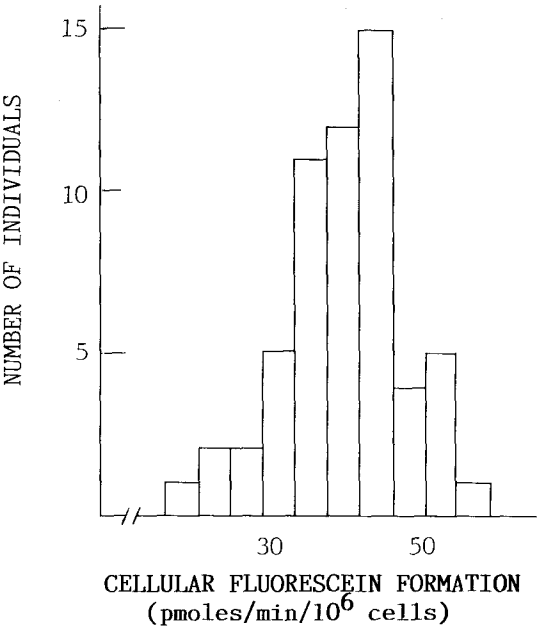

Figure 1. Frequency distribution of fluorescein formation in non-treated lymphoblastoid cell lines from 58 healthy subjects.

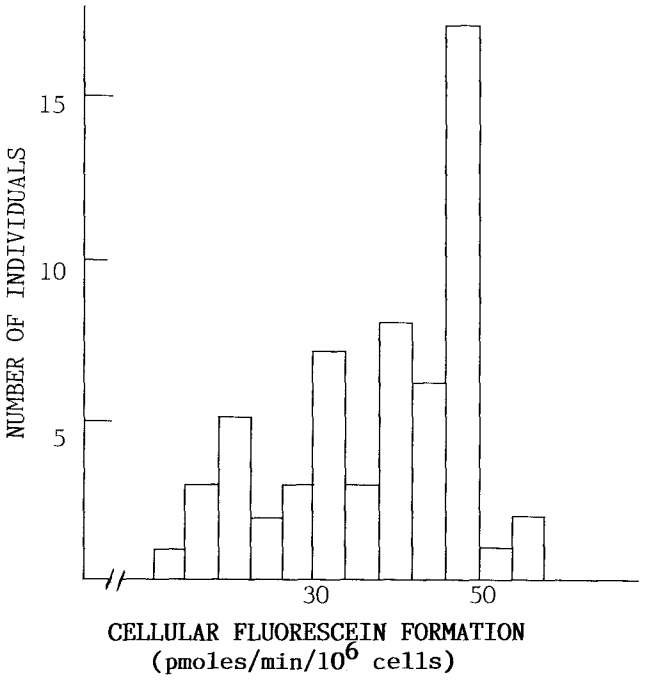

Figure 2. Frequency distribution of fluorescein formation in 4NQO-treated lymphoblastoid cell lines from 58 healthy subjects.

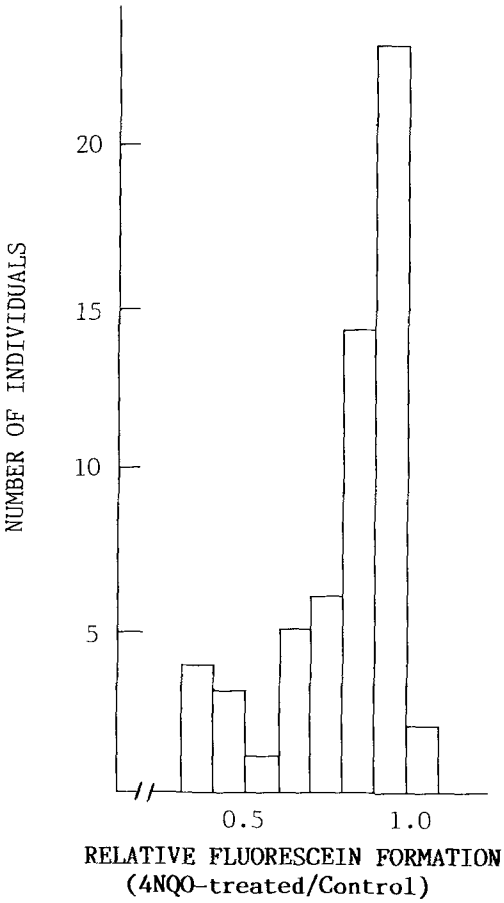

Figure 3. Frequency distribution of relative fluorescein formation (RFF) in lymphoblastoid cell lines from 58 healthy subjects.

pmoles/min/10^6 cells and 2 lines (about 3%) more than 50 pmoles/min/10^6 cells. The mean S.E. of the amount of fluorescein formation of non-treated cells from healthy subjects was 39.61 ± 0.86 (Table 1).

In contrast, the mean ± S.E. of those from XP homozygote and XP heterozygote subjects were 35.12 ± 1.79 and 22.18 ± 2.17, respectively. The cell lines were each treated with 4NQO and the amount of fluorescein formation was measured. The distribution of the values among 4NQO-treated cells gave a skewed pattern as shown in Figure 2. In comparison to the controls the values were relatively lower; the number of cell lines with the amounts of fluorescein between 30 and 50 pmoles/min/10^6 cells decreased to 67% (39 out of 58), and those with the values less than 30 pmoles/min/10^6 cells increased to 33% (19 lines). No cells showed the formation more than 50 pmoles/min/10^6 cells. The mean ± S.E. of the amount of fluorescein formation of 4NQO treated

Table 1. Comparison among fluorescein formation ability of lymphoblastoid cell lines from xeroderma pigmentosum (XP) homozygote, heterozygote and healthy subjects

	Control	4NQO-treated	RFF
Cell line			
XP homozygote (2)[a]	35.12[b]± 1.79[c]	4.75 ± 1.08	0.14 ± 0.04
XP heterozygote (2)	22.18 ± 2.17	10.50 ± 0.41	0.50 ± 0.05
Healthy subjects (58)	39.61 ± 0.86	33.11 ± 1.48	0.86 ± 0.02

a:Figures in parentheses represented number of cell lines used.
b:Expressed in terms of fluorescein formation (pmole/min/10^6 cells) formed.
c:Each value represented mean ± S.E.

cells from healthy subjects was 33.01 ± 1.48. In contrast, the mean ± S.E. of those from XP homozygote and XP heterozygote subjects were 4.75 ± 1.08 and 10.50 ± 0.41 (Table 1), respectively.

The values of relative fluorescein formation (RFF) were calculated from the amount of fluorescein in 4NQO-treated cells normalized by that in the respective non-treated (control) cells. The distribution of the RFF values among the cell lines were shown in Figure 3. The mean ± S.E. of the RFF from healthy subjects was 0.86 ± 0.02. Seventy-six percent cell lines (44/58) showed the RFF value larger than 0.8, while 3 percent cell lines showed it smaller than 0.4 and 11 percent cell lines between 0.4 and 0.8. RFFs were found to be different among healthy individual cell lines we investigated. On the other hand, the mean ± S.E. of RFFs from 2 XP homozygote and 2 XP heterozygote cell lines as references were 0.14 ± 0.04 and 0.50 ± 0.05 (Table 1), respectively.

In the present study the sensitivities to 4NQO of lymphoblastoid cell lines from healthy individuals were compared with each other as well as with XP heterozygote and XP homozygote cell lines as references. The FDA method might mirror, although not directly, the impairment of the DNA excision repair capacity, since the XP cells which are not able to repair DNA when treated with UV or UV-mimic chemicals showed lower RFF values and since 4NQO is known to be a DNA damaging chemical but not a cell damaging agent.

The values of RFF were different among individual cell lines investigated. The cell lines with RFFs more than 0.8 could be regarded as resistant toward 4NQO and 76% (44/58) were found to be as such. Those with the RFF values less than 0.4 may be sensitive to 4NQO and 3% (2/58) of the cell lines were categorized to this class. The remaining 21% (12/58) have RFFs between 0.4 and 0.8. These facts clearly show that the

sensitivity to 4NQO varies from cell line to cell line. This in turn implies that the sensitivity to 4NQO was different among the individuals from which the respective cell lines were derived.

It is worth noting here that the mean ± S.E. of RFF values of the heterozygote and homozygote cell lines for XP were 0.50 ± 0.05 and 0.14 ± 0.04, respectively. Some of the present normal cell lines therefore had the 4NQO sensitivity almost at the same level as XP. It is highly possible that 4NQO susceptible individuals are present in the phenotypically normal populations of Japanese. In Japan the frequency in occurrence of XP patient (XP homozygote) is said to be between one per 40,000 and one per 400,000. If the frequency in occurrence of XP homozygote were one per 100,000 that of XP heterozygote would be about 0.6%, namely one per 163. In contrast, nearly 3% of the 'normal' cell lines we tested had the RFF values that were close to those of XP heterozygote cells. The experimentally detected abnormality was 50 times more frequent than expected if the result was explained only by XP related gene(s). Thus individuals may exist who have hereditary disease(s) or recessive gene(s) other than XP but invoking low resistance toward UV or UV-mimic chemicals.

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