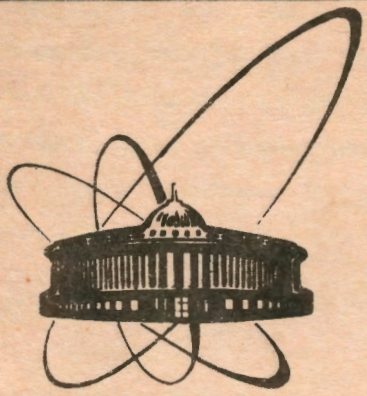

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S.G.Basha, E.A.Krasavin, S.Kozubek

THE EFFECT OF THE ANOXIC RADIOSENSITIZING AGENT TAN ON INDUCTION OF REVERTANTS BY γ-RAYS AND HELIUM IONS IN SALMONELLA TESTER STRAINS

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It is well known that organic nitroxyl-free radicals are able to sensitize different anoxic cells to the lethal action of ionizing radiation. The mechanism of sensitization by N-oxyls consists in interaction of these compounds with radiation-induced DNA-radicals (Johansen 1974; Nakken and Brustad, 1974). The interaction of N-oxyls (for example, TAN) with DNA-radicals leads to formation of hard repair damages (Hohman et al., 1976) and probably can be seen in the peculiarities of radiation-induced mutagenesis.

In previous publications we have shown that the important role in mutagenic action of ionizing radiation with different linear energy transfer (LET) is played by the so-called "complex" DNA injuries, which are not reparable by the *pol A*-dependent repair system in *E.coli* cells (Kozubek et al., 1989a; Tokarova et al.,1989; Bonev et al.,1990). The formation of such damages is increased with growing of LET. We have also shown that induction of direct mutation (Tokarova et al., 1989), the SOS response, which has been measured by means of the SOS-chromotest (Kozubeket al., 1989a) and λ-prophage induction (Bonev et al., 1990) in *E.coli* cells grow with increasing LET. On the other hand, the increasing of the SOS-response in TAN sensitized *E.coli* cells

under anoxia was shown after γ-irradiation (Komova et al., 1990). These data can indicate that binding of a sensitizer to DNA-radicals leads to formation of SOS-inducing and probably of premutational DNA damages. Owing to these results we have investigated the effect of the radiosensitizing agent TAN on the induction of revertants by ionizing radiation with various LET in different *Salmonella* tester strains.

MATERIALS AND METHODS

Bacterial strains

Tester strains *TA98*, *TA100* and *TA102* were provided by Dr. B. Ames, Department of Biochemistry, University of California. The overnight cultures as well as the plates for mutagenicity assay were prepared according to Maron and Ames (1983).

2,2,6,6-tetramethyl-4-piperidine-1-oxyl (TAN) was synthesized in the Institute of Chemical Physics of the Academy of Sciences of the USSR. TAN was dissolved in phosphate buffer and added to the bacterial suspension to give the final concentration 10^{-3} M immediately before degassing.

Irradiation by γ-rays and helium ions

A ^{137}Cs γ-ray source giving a dose rate of about 23 Gy/min was used. Irradiation by helium ions was performed at the accelerator of heavy ions U-200, Joint Institute for Nuclear Research. The energy of helium ions was 5,2 MeV/nucleon. The initial average LET of particles was 38 keV/μm and the dose rate was 90 Gy/min. In experiments with γ-rays the cell suspension was irradiated in glass tubes or in special glass plates. Because of the low penetration of helium ions with energy 5,2 MeV/nucleon, bacteria were irradiated only in glass plates 300 μm

deep and 10 mm in diameter. In this case 0.01 ml of the cell suspension was placed on sterile glass plates and covered by a mylar film 15 μm thick.

Hypoxia was obtained by flowing nitrogen for at least 30 min before irradiation. In the experiments with helium ions the hipoxic cell suspension was placed on plates in the nitrogen atmosphere of a special box. The plates were set into a round magazine, and transported by remote control to the beam exit window. The LET and the dose were determined by semiconductor detectors and by an ionization chamber, respectively (Cherevatenko, 1986). All irradiation procedures were done at 0°C. The number of surviving cells and revertant colonies were counted under slight magnification after 48 h of incubation at 37°C. The experiments were repeated 3-5 times.

Processing of experimental data

The surviving fraction (S) was calculated as the ratio $S = N_s/N_o$, where N_s is the number of surviving cells after irradiation and N_o is the number of nonirradiated bacteria. The mutation rate was measured as the ratio of mutant colonies (N_m) to the number of surviving cells (N). Dose dependence $N_m/N(D)$ was fitted to the following function:

$$N_m/N = k\, D^{\chi}, \qquad (1)$$

where D is the radiation dose. The parameters k and χ were determined for each $N_m/N(D)$ dependence by an optimizing procedure (Tokarova et al., 1989). The oxygen enhancement ratio (OER) and the dose modifying factor (DMF) with respect to mutagenesis were determined as the ratio of the doses inducing the same number of mutations under normal conditions and under anoxia (or anoxia with TAN). The standard deviations of OER and DMF were determined by the same procedure as the deviations of the parameters in Eq.1.

RESULTS

The cell survival as a function of the dose of γ-radiation and helium ions under different conditions is presented in Fig. 1. The survival curves have an exponential shape for all 3 strains and for both types of radiation. The radiosensitivity (D_o^{-1}) and OER for each strain were determined. The data are summarized in Table 1. For *TA98* and *TA100* strains the values D_o^{-1} after γ-irradiation are the same; however, for *TA102* the radiosensitivity is less. The OER after γ-irradiation is approximately equal to 2 for all 3 strains. The value of OER after helium ion irradiation is significantly less and equals 1.3.

The data from Table 1 show, that the cell radiosensitivity after irradiation in oxygen is not modified by TAN but the sensitization effect appears after γ-irradiation under anoxia. The value of DMF for all 3 strains approximately equals 2. The influence of TAN on cell survival after irradiation by helium ions was not revealed.

The mutation rate as a function of the dose of γ-radiation and helium ions under various conditions is shown in Fig. 2. A power dose-response relation was observed for all 3 strains. Mutation rates after γ-irradiation in oxygen and under anoxia were measured in these strains. For *TA98* and *TA100* the modification effect of anoxia on induction of revertants was not revealed; however, for *TA102* the value of OER is equal to 1.6 (Table 2). There is no effect of anoxia on the mutation rate in *TA100* after irradiation by helium ions and after γ-irradiation.

The influence of TAN on the mutation rate after irradiation under anoxia is distinctly observed for all 3 strains (Fig.2, Table 2). Among

TABLE 1

SENSITIVITIES OF DIFFERENT SALMONELLA TESTER STRAINS AFTER IRRADIATION BY γ-RAYS AND HELIUM IONS UNDER VARIOUS CONDITIONS

Type of radiation	Strains	D_o^{-1}, 10^{-2} Gy^{-1}		OER[a]	D_o^{-1}, 10^{-2} Gy^{-1}		DMF[b]
		Oxygen	Anoxia		Oxygen + TAN	Anoxia + TAN	
γ-rays	*TA98*	2.0±0.1	0.9±0.1	2.2±0.2	2.0±0.1	2.0±0.1	2.2±0.2
γ-rays	*TA100*	2.0±0.1	1.0±0.1	2.0±0.2	2.0±0.1	1.9±0.1	1.9±0.1
γ-reas	*TA102*	1.6±0.1	0.7±0.1	2.2±0.2	1.6±0.1	1.2±0.1	1.7±0.2
$^4_2$He	*TA100*	2.7±0.2	2.1±0.2	1.3±0.1	2.6±0.2	2.0±0.2	1.0±0.1

[a]Oxygen enhancement ratios were calculated as the ratio of D_o^{-1} under oxygen conditions and under anoxia.

[b]Dose-modifying factors were calculated as the ratio of D_o^{-1} under anoxia + TAN and under anoxia.

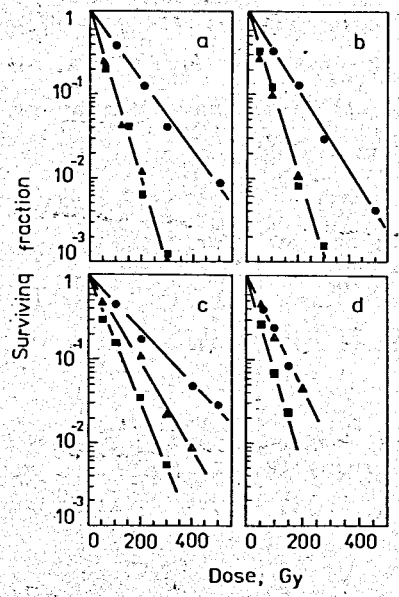

Fig.1. Survival curves of Salmonella typhimurium TA98 (a), TA100 (b,d) and TA102 (c) strains after irradiation by γ-rays (a,b,c) and helium ions (d) under the following conditions: ■, oxygen; ●, anoxia; ▲, anoxia + TAN. Ordinate: surviving fraction. Abscissa: dose of radiation, Gy.

TABLE 2

MUTAGENESIS IN DIFFERENT SALMONELLA TESTER STRAINS AFTER IRRADIATION BY γ-RAYS AND HELIUM IONS UNDER VARIOUS CONDITIONS

Type of radiation	Strains	Anoxia		Anoxia + TAN	
		χ^a	OER[b]	χ^a	DMF[c]
γ-rays	*TA98*	1.7±0.3	1.1±0.2	1.8±0.4	3.4±0.3
γ-rays	*TA100*	1.6±0.4	1.0±0.1	1.5±0.3	5.2±0.4
γ-rays	*TA102*	2.1±0.5	1.6±0.2	2.2±0.5	2.1±0.2
$^4_2$He	*TA100*	1.7±0.4	1.0±0.1	1.5±0.1	3.1±0.3

[a]The values of parameters χ were calculated according to Eq.1.

[b]OER and [c]DMF were calculated as the ratio of the doses inducing the same number of mutations under oxygen conditions and under anoxia (OER) or under anoxia+TAN (DMF).

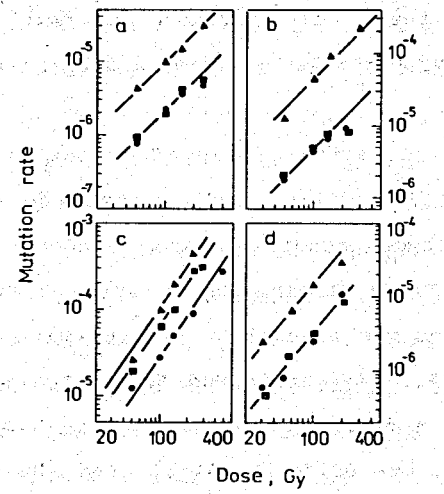

Fig.2. Dependencies of mutation rate of TA98 (a), TA100 (b,d) and TA102 (c) strains on the dose of γ-radiation (a,b,c) and helium ions (d) under the following conditions: ■, oxygen; ●, anoxia; ▲, anoxia + TAN. Ordinate: mutation rate. Abscissa: dose of radiation, Gy.

those strains, the sensitizing effect of TAN on mutagenesis after γ-irradiation was the most strongly marked in the *TA100* strain. The DMF for this strain equals 5.2±0.4. However, the sensitizing effect of TAN after irradiation by helium ions decreases and equals 3.1±0.3.

DISCUSSION

We have investigated the effect of the anoxic radiosensitizing agent TAN on cell survival and mutagenesis in 3 *Salmonella* tester strains after irradiation by γ-rays and helium ions. The results of our experiments show that the influence of anoxia on cell survival and mutagenesis after irradiation is markedly different. The radiosensitivities of all strains under anoxia have the same values, and OER equals approximately 2. The modification effect of anoxia on cell survival after helium ion irradiation is significantly less than after γ-irradiation. The decreasing of oxygen effect in this case can be explained as a result of induction of more oxygen-independent DNA-radicals by heavy particles (Kozubek and Krasavin, 1983; Krasavin, 1989).

In contrast to the lethal assay the mutation rate in *TA98* and *TA100* strains after irradiation under anoxia does not differ from mutagenesis under oxygen conditions and a moderate oxygen effect is found only in the *TA102* strain. The absence of anoxia influence on induction of mutations was also shown earlier with different mutation assays (Anderson, 1951; Bleichrodt and Roos-Verheij, 1979; Sargentini and Smith, 1989). A moderate effect of anoxia was demonstrated on $trp^- \rightarrow Trp^+$ reversion by Bridges (1963) and Deering (1963). The difference in influence of anoxia on mutagenesis in *Salmonella* tester

strains can be explained by considering the nature of premutational DNA injuries in bacteria.

As known, the *TA98* and *TA100* strains determine the reversions after frameshift mutation and after substitution, respectively (Maron and Ames, 1983). The *hisG46* mutation in *TA100* substitutes -CCC- for -CTC- in the wild type cells. Therefore, *TA100* detects mutagens that cause base-pair substitutions of G-C pairs and produce C→T transitions. Among the pyrimidine radiolysis products that are not affected by oxygen there are only 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol) and trans-5,6-dihydroxy-5,6-dihydrouracil (uracil glycol) (Teoule, 1987). Thymine glycols, as shown (Laspia and Wallace, 1988), are not probably mutagenic lesions. On the other hand, different cytosine damages are connected with appearance of C→T transitions (Ayaki et al., 1987). Therefore, the absence of the oxygen effect on γ-radiation mutagenesis in *TA100* can be explained by the induction of mutagenic uracil glycols.

A moderate effect of anoxia in *TA102* can be connected with thymine radiolysis products which are produced in DNA only in the presence of oxygen (for example, hydroperoxides of thymine, 5-hydroxymethyluracil) (Teoule, 1987). This thymine-derived base lesions are probably associated with production of T→C transitions that are determined by *TA102*. On a level with these reasons one should bear in mind that the $N_m/N(D)$ dependence for *TA102* is quadratic. The quadratic dose-response relation is explained by a 2-hit mechanism. The first hit is required to produce a premutational DNA lesion in the target (for example, the thymine damage in TAA nonsense codon of *TA102*). The second hit is required to produce DNA damages triggering the SOS response and activating the error-prone repair. This hit fixes the first lesion (Tokarova et al., 1989). The SOS-inducing damages in the case of

ionizing radiation can be DNA injuries of a "complex" type (Kozubek et al., 1989a), which are not affected by oxygen (Amirtayev et al., 1985). These reasons can explain the moderate effect of anoxia in *TA102*.

Our results show that mutagenesis in 3 strains after irradiation under anoxia is enhanced by TAN. Among these tester strains, *TA100* was more sensitive. A moderate effect of TAN is revealed in *TA102*. The sharply increasing effectiveness of γ-radiation to produce mutations can be explained in this case by 2 factors. Firstly, TAN under anoxia interacts with radiation-induced DNA-radicals and produces TAN-DNA adducts (Johansen, 1974; Hohman et al., 1976). It is shown that N-oxyls interact through covalent binding mainly with thymine and cytosine radicals (Nakken and Brustad, 1974). These adducts probably cannot be repaired by *polA*-dependent excision DNA repair and they restores only by slow repair which is controlled by *recA-lexA* genes. This supports the data on TAN interference in cell DNA synthesis (Hohman et al., 1976). Secondly, TAN increases the SOS response in *E.coli* cells after γ-irradiation under anoxia (Komova et al., 1990). It can be suggested that modification of the mutagenic radiation-induced pyrimidine-radicals and activation of the error-prone repair by TAN may further increase the mutagenesis. A higher sensitizing effect of TAN on mutation induction after γ-irradiation in *TA100* is probably associated with interaction under anoxia of cytosine-radicals (uracil glycols) and TAN leading to production of C→T transitions.

The moderate effect of TAN on mutagenesis in *TA102* is explained by considering the properties of the target leading to T→C transitions. Since first of all the N-oxyls interact with thymine-radicals, TAN will interact under anoxia with oxygen-independent thymine radiolysis products (thymine glycols), which do not seem to be mutagenic. However,

various radiolytic products of thymine, which produce base substitutions at AT and GC sites (the hydroperoxides of thymine and their degradation products) are formed only in presence of oxygen. Therefore, the moderate effect of TAN on mutagenesis in *TA102* depends on the type of molecular injury leading to reversion in this strain. A high radiosensitizing effect of TAN in *TA98* is probably associated with the base damages, which leads to the reversion of the sequenced *hisD3052* (-1 frameshift) mutation (Maron and Ames, 1983) and with the activation of error-prone repair.

A comparative consideration the effect of TAN on mutagenesis in *TA100* after irradiation by γ-rays and helium ions shows that the DMF value after helium ion irradiation is smaller. This probably reflects the increased production of DNA damages with growing LET weakly modifyed by TAN. Such injuries, which are more efficiently produced by high-LET radiation, are multiply damaged single-strand breaks (Kozubek et al., 1989b; Chatterjee and Holley, 1990) and they themselves, without TAN binding, are hard repair injuries. These damages induces the SOS-response in *E.coli* (Kozubek et al., 1989a; Bonev et al., 1990) and some types of complex single strand breaks should be the cause of mutation events (Tokarova et al., 1989).

In conclusion, our results support the earlier hypothesis on the possible important role of "complex" DNA damages in mutagenesis induced by high-LET radiation. Such injuries is result from substantial energy deposition in the DNA molecule and are not repaired by the excision *pol A*-dependent repair system in *E.coli*. The possible candidates for "complex" *pol A*-independent DNA damages are suggested by Bridges and Mottershead (1972). Our data on TAN modification of mutagenesis induced radiation with different LET probably indicate the

important role of the similar injuries in radiation-induced mutagenesis.

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