Repair Mechanism in Sendai Virus Carrying HeLa Cells after Damage by 4-Hydroxyaminoquinoline 1-Oxide

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SUMMARY

4-Nitroquinoline 1-oxide (4NQO), a potent carcinogen, inactivates both HeLa cells and cells carrying Sendai virus [HeLa(HVJ)], but the latter cells are relatively resistant, whereas the former are quite sensitive to this compound. However, in both types of cells, the activity of diaphorase, the enzyme presumed to convert 4NQO to 4-hydroxyaminoquinoline 1-oxide is similar, and both cell types display the same relative resistance to 4-hydroxyaminoquinoline 1-oxide as they do to 4NQO and to UV light. Moreover, when herpes simplex virus was treated with 4-hydroxyaminoquinoline 1-oxide and assayed simultaneously on both types of cells at any given exposure period, the number of plaque-forming survivors on cells carrying Sendai virus was much higher than on HeLa cells. These results suggest that cells carrying Sendai virus have more effective repair activity both for cellular DNA and for foreign DNA than have HeLa cells.

INTRODUCTION

4NQO, a potent carcinogen, inactivates bacteria and induces prophase (8). We isolated many 4NQO-sensitive mutants of Salmonella typhimurium, all extremely sensitive to UV light; these were subdivided into 2 groups: host cell reactivation-deficient mutants lacking repair activity for UV-damaged superinfecting phage and recombination-deficient mutants (8). Furthermore, UV-sensitive Salmonella mutants were also sensitive to 4NQO (8). These findings suggest that 4NQO, or its metabolic intermediates, react with the bacterial genome, resulting in damage of DNA, and that the damaged bacterial DNA is repairable by the repair mechanism of UV damage. Thus, the supposedly dark repair mechanism may be a common repair mechanism, not only for UV damage, but also for chemical damage (8). Similar findings have been extended to human cells (9).

We recently found that 4NQO at a concentration of about 0.2 μM inactivated HeLa cells and its carrier culture HeLa(HVJ) cells carrying parainfluenza virus type 1 Sendai virus. HeLa(HVJ) cells were relatively resistant to 4NQO as well as to UV light, whereas HeLa cells were quite sensitive to both 4NQO and UV. In addition, UV-exposed herpes simplex virus was more efficiently repaired in HeLa(HVJ) cells than in HeLa cells (9).

Since 4NQO does not react with DNA (7, 8), it must be converted in vivo to an active metabolic intermediate to be reactive with the genome. Sugimura et al. (6) reported that 4NQO is converted to 4HAQO in animals and proposed that 4HAQO reacts with DNA. Diamond (1) suggested that the carcinogen sensitivities of various cell types depend on the activity levels of activating enzymes for proximal carcinogens in the cells. Therefore, it was desirable to examine the sensitivity of HeLa and HeLa(HVJ) cells toward 4HAQO. In this communication we report the effect of 4HAQO on HeLa and HeLa(HVJ) cells and examine further the concept that sensitivity depends on the reparability of 4HAQO-damaged cellular and foreign DNA.

MATERIALS AND METHODS

Mammalian Cell Cultures. Sendai virus is a parainfluenza virus type 1, previously designated hemagglutinating virus of Japan (HVJ) (5). Infection of HeLa cells with HVJ causes a cytopathic effect, but the virus does not multiply. HeLa cells surviving after HVJ infection have been established as a carrier culture, HeLa(HVJ) (4), capable of releasing noninfectious hemagglutinin. Cell cultures were grown in Eagle's basal medium containing double quantities of amino acids and vitamins, supplemented with 10% calf serum as growth medium. For cell inactivation experiments, cell cultures were trypsinized, inoculated in 60-mm diameter plastic Petri dishes at a density of 1 × 10⁴ cells/plate, and incubated at 37° in a 5% CO₂ incubator. After 24 hr, incubation cells attached to Petri dishes were washed twice with prewarmed Dulbecco's PBS and then exposed to 4HAQO.

Preparation of the Stock of Herpes Simplex Virus. Monolayers of HeLa cells were infected with herpes virus at a multiplicity of 2. The culture fluid was harvested on the 3rd day after infection and centrifuged at 8,000 × g for 10 min to remove cell debris. Then the supernatant was centrifuged at 36,000 × g for 3 hr, and the pellet was suspended in M/15 PBS, pH 7.0, containing 0.1 M NaCl and 1 mM MgSO₄. A dilute sample (10⁸ plaque-forming units/ml in the same buffer) of this virus stock was used for 4HAQO treatment.

Chemicals. The carcinogens used were 4NQO and its reduction product, 4HAQO, kindly supplied by Dr. Kawazoe, National Cancer Center Research Institute, Tokyo, Japan.
Dimethyl sulfoxide, purchased from Fisher Scientific Co., Pittsburgh, Pa., was used as a medium for saponification of the carcinogens. NADH and dichlorophenol indophenol were purchased from Sigma Chemical Co., St. Louis, Mo.

Treatment of Cells with 4HAQO. Cell cultures grown on Petri dishes were incubated in Dulbecco's PBS medium containing various concentrations of 4HAQO for appropriate periods at 37° in a CO2 incubator. The 4HAQO-treated cells were washed twice with Eagle's medium and incubated further with Eagle's growth medium. The number of colonies formed after incubation for 7 days served as the measure of cell inactivation.

Assay for Diaphorase Activity. Diaphorase activity of the cell cultures was determined by the method described by Sugimura et al. (6) in a reaction medium of the following composition: 0.05 M phosphate buffer, pH 7.0; 40 μM dichlorophenolindophenol, 100 μM NADH, and an appropriate amount of enzyme. Samples for diaphorase activity were prepared as follows. The cell cultures were washed well with Dulbecco's PBS; a 10% cell suspension was sonically disrupted for 2 min and centrifuged for 10 min at 500 X g; the supernatants of these samples were further centrifuged for 1 hr at 29,000 X g and used for assay of diaphorase activity. Protein concentrations of all enzyme preparations from the 2 different cultures were determined by the method of Lowry et al. (3) and adjusted to the same amount for the enzyme assay.

Treatment of Herpes Simplex virus with 4HAQO. All manipulations were carried out at room temperature (25°). One volume of 2 mM of 4HAQO in Dulbecco's PBS (pH 7.0) with 5% dimethyl sulfoxide was added to the same volume of herpes simplex virus suspension, and, periodically, 0.05-ml samples were withdrawn by pipet and diluted to 10^-2 or further in Eagle's basal medium to stop the reaction of any residual reagent. Samples were diluted further, and 0.5-ml aliquots of each diluted sample were inoculated onto monolayers of HeLa and HeLa(HVJ) cells grown in Petri dishes. After absorption for 3 hr, cells were overlaid with 1% Noble agar containing Medium 199 supplemented with 2% calf serum and incubated in a CO2 incubator at 37°. On the 4th day after infection, a secondary overlay agar containing 1:30,000 neutral red was applied. The number of plaques following 24 hr incubation at 37° served as the measure of cell inactivation.

RESULTS

Diaphorase Activity.

Studies from our laboratory with a microbial system (2) confirmed the previous study of Sugimura et al. (6) suggesting that 4HAQO was the intermediate reactive toward DNA and also indicated that NADH diaphorase was the enzyme responsible for its formation from 4NQO.

Diamond (1) reported that cell sensitivity to carcinogens depends on amounts of proximal carcinogen synthesized and suggested that the activating enzyme levels in cells are responsible for the cell sensitivities. Thus, it became of interest to study the diaphorase activity for conversion from 4NQO to 4HAQO in HeLa and HeLa(HVJ) cells.

As shown in Chart 1, both cell types demonstrated diaphorase activities practically to the same extent. In addition, at 3 different protein concentrations, no difference in the diaphorase activity of these cells was observed. These results indicate that these cell cultures should produce the same amounts of 4HAQO. Therefore, the difference in sensitivities of these cell cultures toward 4NQO must be due to factors other than diaphorase activity.

4HAQO Sensitivities of HeLa Cells and Its Carrier Culture HeLa(HVJ) Cells

Inactivation Kinetics of HeLa and HeLa(HVJ) cells. When HeLa and HeLa(HVJ) cells were treated with 4HAQO at a concentration of 10 μM for various periods at 37°, rapid inactivation of colony-forming ability was observed. As shown in Chart 2, the number of colonies of HeLa(HVJ) cells was consistently higher than that of HeLa cells at any given exposure period. Incubation periods required for 10- to 100-fold inactivation of HeLa cells caused only 30 to 60% inactivation of HeLa(HVJ) cells. This result is similar to the UV- and 4NQO-sensitivities of these cells (9). Since all 4NQO-sensitive bacterial mutants are far more sensitive to UV than the wild-type bacteria and 4NQO-damaged bacterial genome is reparable by the bacterial dark repair mechanism (8), HeLa(HVJ) cells may also carry more repair activity for damaged genomes than HeLa cells.

Concentration Effect of 4HAQO on Inactivation by 4HAQO of HeLa and HeLa(HVJ) Cells. Relative sensitivities of the mammalian cell cultures described in the preceding section were further examined with the use of various concentrations of 4HAQO for a given incubation period at 37°. When HeLa and HeLa(HVJ) cells were incubated with various concentrations of 4HAQO for 1 hr at 37°, it was observed that colony-forming ability decreased as 4HAQO concentration increased and HeLa(HVJ) cells were more resistant to 4HAQO than HeLa cells. As shown in Chart 3,

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concentrations required for 10- to 100-fold inactivation of HeLa cells caused only 35 to 60% inactivation of HeLa(HVJ) cells.

Inactivation Kinetics Curve of Herpes Simplex Virus by 4HAQO and Repair by Host Cells.

We previously reported that HeLa(HVJ) cells carry more repair activity for UV-damaged herpes simplex virus when present as foreign DNA than similarly infected HeLa cells (9). It was of interest to examine reparability of 4HAQO-damaged herpes simplex virus in these 2 types of cells.

When herpes simplex virus was treated with 1 mM of 4HAQO and assayed simultaneously on HeLa and HeLa(HVJ) cells, survival of herpes simplex virus in HeLa(HVJ) cells observed by plaque formation was significantly higher than in HeLa cells, as shown in Chart 4. Incubation periods required for 10- to 100-fold inactivation of herpes simplex virus assayed on HeLa cells produced 3- to 10-fold inactivation on HeLa(HVJ) cells. Moreover, in a treatment of the virus with a higher concentration, 2 mM 4HAQO, a similar result was obtained, although inactivation of the virus on both the cell types was rapid.

DISCUSSION

From the data presented in this paper and from our previous studies (8), it would appear that the repair enzyme system of mammalian cells can repair both UV- and 4HAQO-damaged genomes. This situation is analogous to our finding that the bacterial repair mechanism for UV-damaged bacterial genomes can also repair 4NQO- and 4HAQO-damaged bacterial genomes (8). However, 4HAQO-damaged phage genomes were not repaired by bacterial excision activity (8) but by bacterial DNA polymerase I (T. Kato and N. Yamamoto, unpublished data). This may be explained by a study of Sugimura et al. (7), that in vitro treatment of DNA with 4HAQO results in single-strand breaks in DNA molecules.

Since 4HAQO-damaged herpes simplex virus was repaired more efficiently by HeLa(HVJ) cells than by HeLa cells, the differences in repair activity should be due to repair enzymes other than excision enzyme activity. Our preliminary experiment showed that DNA polymerase I activity in HeLa(HVJ) cells is twice as high as in HeLa cells (T. Satoh and N. Yamamoto, unpublished data). Because HeLa(HVJ) carrier culture carries a remarkably higher repair activity for both UV- and 4HAQO-damaged cellular and viral DNA than HeLa itself, this mammalian system should be useful to study the interaction of chemicals and proximal carcinogens with genes.
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REFERENCES


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