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Effect of Antitumor Alkylating Agents on Semiconservative and Unscheduled DNA Syntheses in Rat Ascites Hepatoma Cells[1)]

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The effects of several compounds on ultraviolet light-induced unscheduled desoxyribonucleic acid (DNA) synthesis and semiconservative DNA synthesis were examined in rat ascites hepatoma, AH-44 cells. The compounds used did not inhibit unscheduled DNA synthesis as much as or more than semiconservative DNA synthesis. Only improsulfan had a tendency to inhibit unscheduled DNA synthesis to a greater extent than semiconservative DNA synthesis.

Keywords——rat ascites hepatoma cells; unscheduled DNA synthesis; semiconservative DNA synthesis; antitumor alkylating agent; radioactive thymidine; ultraviolet light

The extensive studies have been attempted to investigate unscheduled desoxyribonucleic acid (DNA) synthesis in response to ultraviolet light (UV),[3–6)] X-ray,[7)] alkylating agents[4,7)] and other substances[8–10)] in various eukaryotic cells. Most studies to quantitate unscheduled DNA synthesis have involved either the use of density-gradient analysis with radioactive substrates or autoradiography to resolve unscheduled DNA synthesis from semiconservative DNA synthesis. Since hydroxyurea has been shown to suppress semiconservative DNA synthesis and have little or no effect on unscheduled DNA synthesis,[5,11)] it has been used routinely to reduce semiconservative DNA synthesis and to measure the relatively small amount of unscheduled DNA synthesis.

The substances including co-carcinogens are known to inhibit unscheduled DNA synthesis,[12–14)] but do not inhibit unscheduled DNA synthesis without influencing semiconservative DNA synthesis.[14,15)] In this study, the effects of improsulfan and nitrogen mustard (HN_2) on unscheduled DNA synthesis induced by UV-irradiation and semiconservative DNA synthesis were examined in AH-44 cells.

Materials and Methods

A transplantable rat ascites hepatoma AH-44 was maintained by successive intraperitoneal inoculation in male Donryu rats, 6 weeks old. AH-44 cells harvested from the peritoneal cavity of tumor-bearing rats

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were suspended in ice-cold saline solution and washed several times with saline solution. The washed cells were resuspended in thymidine (TdR) deprived F-12 medium buffered with 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid[16] and supplemented with 20% calf serum.

In the measurement of semiconservative DNA synthesis, the cell suspension was incubated at 37° with 1 μCi/ml [methyl-^{3}H]TdR (specific activity of 45 Ci/mmole, Radiochemical Centre). At the end of the incubation, the tumor cells were washed twice with 5 ml phosphate-buffered saline (PBS) containing 1 mM hydroxyurea and 0.5 mM TdR. The washed cells were collected on glass-fiber filters (GF/C, Whatman) and washed twice with 5 ml of ice-cold 5% trichloroacetic acid (TCA).

In the case of measuring unscheduled DNA synthesis, the tumor cells were preincubated in the medium containing 10 mM hydroxyurea at 37° for 15 min. Ten ml of the preincubated cell suspension were poured into a plastic dish (90 mm in diameter) and exposed to 500 erg/mm^2 of 254 nm UV, derived from Mazda 15 W germicidal lamp. After 1 volume of the irradiated cell suspension was added to the same volume of the medium containing 10 μCi/ml ^{3}H–TdR, the mixture was incubated at 37°, and washed twice with PBS containing 1 mM hydroxyurea and 0.5 mM TdR at the end of the incubation. The washed cells were resuspended in ice-cold 5% TCA for 15 min, collected on the glass-fiber filter and finally washed with 10 ml of ice-cold 5% TCA. The DNA fraction was extracted from the tumor cells by the method of Rowland.[17] The content of DNA was determined by optical density at 260 mμ.

To 10 ml of scintillation fluid (7 g of 2,5-diphenyloxazole, 0.3 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene and 100 g of naphthalene in 1 liter of dioxane), 0.5 ml of the DNA extract was added. The dried filter was transferred to 5 ml of toluene scintillation fluid as described previously.[18] The radioactivity was measured with the Beckman LS-100C scintillation spectrometer.

The compounds used were dissolved in the medium or dimethyl sulfoxide, and successive dilutions were made in the medium so as to make 0.5% dimethyl sulfoxide in a final concentration. All dilutions were made just before use to minimize possible decomposition in the medium.

Results and Discussion

AH-44 is one of rat ascites hepatomas, insensitive to HN_2. It has been found that the resistance of AH-44 cells to NH_2 is related with the repair replication activity.[19,20]

Fig. 1 shows unscheduled DNA synthesis induced by UV-irradiation and semiconservative DNA synthesis in AH-44 cells. Unscheduled DNA synthesis appears to reach almost a plateau within 4 hr after UV-irradiation.

The effects of several compounds on unscheduled and semiconservative DNA syntheses are shown in Table I and Fig. 2. HN_2 and improsulfan inhibited both DNA syntheses. Semiconservative DNA synthesis was preferentially inhibited by NH_2. Improsulfan may inhibit unscheduled DNA synthesis to about the same extent or slightly more than semiconservative DNA synthesis. The other compounds did not inhibit unscheduled DNA synthesis as much as semiconservative DNA synthesis or had no effect on unscheduled DNA synthesis. No inhibition or nonspecific inhibition of unscheduled DNA synthesis by these compounds except alkylating agents was found in cultured mammalian cells and peripheral human leukocytes by other workers.[11,14,21]

In the therapeutic experiment, improsulfan showed an effect on the turmors with resistance to HN_2 and its derivatives.[22] The synergistic action was also observed against Yoshida sarcoma by combinations of improsulfan with HN_2 derivatives.[23,24] The precies reasons for these therapeutic effects of improsulfan are not clear, but these can be explained to some extent as differences in drug distribution and an alkylating velocity after drug administration, and in

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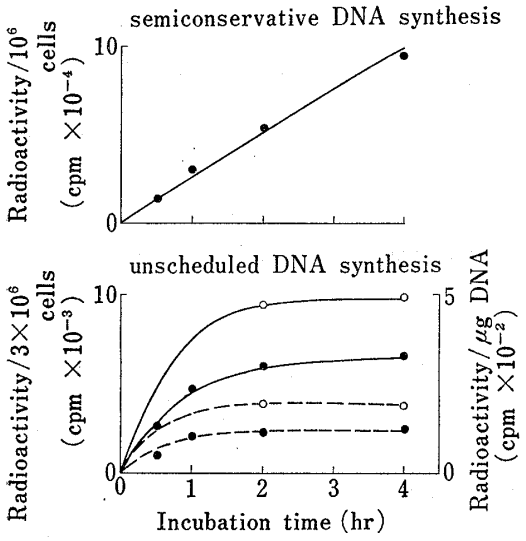

Fig. 1. Semiconservative and Unscheduled DNA Syntheses in AH-44 Cells

The cell suspension (10^6 cells/ml) was incubated at 37° with 1 μCi/ml ^{3}H-TdR for semiconservative DNA synthesis and 5 μCi/ml ^{3}H-TdR for unscheduled DNA synthesis. Each circle represents the radioactivity of ^{3}H-TdR in the tumor cells (●) or in the DNA extract (○). Broken lines show unirradiated controls.

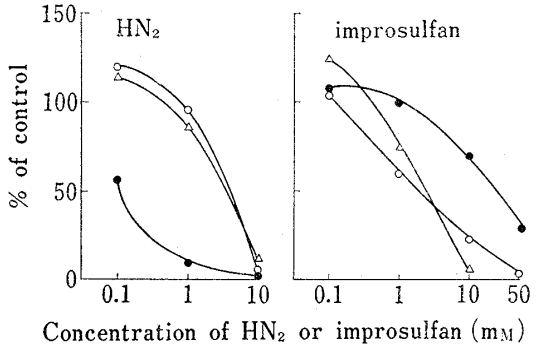

Fig. 2. Effects of HN_2 and Improsulfan on Semiconservative and Unscheduled DNA Syntheses in AH-44 Cells

The experimental condition was the same as described in Table I. Each symbol represents the value obtained by the radioactivity of ^{3}H-TdR in the tumor cells (●, ○) or in the DNA extract (△).

TABLE I. Effects of Compounds on Semiconservative and Unscheduled DNA Syntheses in AH-44 Cells

Compound	Concentration	% of control	
		Semiconservative	Unscheduled
Cytosine arabinoside	0.01 mM	8.6	92.3
5-Aminouracil	10 mM	42.9	93.7
Cycloheximide	0.5 mM	38.1	110.0
Crystal violet	0.1 mM	0	0
Dimethylsulfoxide	10 %	54.6	98.8
Tween 80	1 mg/ml	44.7	89.2

The cell suspension (10^6 cells/ml) was incubated at 37° for 2 hr with a compound and 1 μCi/ml ^{3}H-TdR for semiconservative DNA synthesis or 5 μCi/ml ^{3}H-TdR for unscheduled DNA synthesis. Each value was obtained by the radioactivity of ^{3}H-TdR incorporated into the tumor cells.

drug transport by tumor cells between improsulfan and HN_2 or its derivatives. The relatively specific inhibition of semiconservative DNA synthesis by HN_2, but not by improsulfan, suggests at least the partial difference in mechanism of the cytotoxic action between HN_2 and improsulfan, which is possibly derived from the quantitative difference of alkylation to certain cellular components.