Changes of Cell Cycle Affecting Cidal Effect of Triethylene Thiophosphoramide on Cultured Human Bladder Carcinoma Cells

TETSURO KATO, KIYOSHI ISHIKAWA, RYOSUKE NEMOTO and ROBERT J. IRWIN, Jr.*

Department of Urology, Akita University School of Medicine, Akita and *Division of Urology, University of Mississippi Medical Center, Mississippi, USA

KATO, T., ISHIKAWA, K., NEMOTO, R. and IRWIN, R. J., Jr. Changes of Cell Cycle Affecting Cidal Effect of Triethylene Thiophosphoramide on Cultured Human Bladder Carcinoma Cells. Tohoku J. exp. Med., 1978, 125 (2), 163-167 — The cell cycle of the human bladder carcinoma cell line (MGH-U1 cells) was prolonged immediately after low dose of triethylene thiophosphoramide (Thio Tepa). Although the elongated cell cycle time seemed to return to the control level 48 hr following the treatment, there still remained postsynthetic accumulation and postmitotic diminution in the cell population and the cells were insensitive to high dose of the agent. The present results suggest that Thio Tepa is less effective for G2 cells, but more effective for G1 or S cells.

An established cell line, MGH-U1, has been studied on the cell kinetics following the treatment with triethylene thiophosphoramide (Thio Tepa), a potent alkylating agent for the bladder carcinoma. The cell cycle was elongated immediately after the drug treatment with a low dose, the main effect being seen in S phase, and recovered nearly to the control level 48 hr after the treatment. Although analysis of the cell cycle parameters disclosed slight differences between the treated and untreated cells, repeated exposures at 12 or 48 hr interval to the low concentration of the drug did not lead to enhancement nor decrement of the drug effect on the cells (Kato and Irwin 1977). In view of the above findings, experiments were made to study if the cidal effect of the drug would be affected by pretreatment with the low dose.

MATERIALS AND METHODS

Cell line. MGH-U1 cells, which were established from human urinary bladder carcinoma (Kato et al. 1977), were cultured with McCoy’s 5A modified medium supplemented with 10% fetal calf serum in a CO2 incubator. Dispersing the monolayered cell sheets by trypsin-EDTA, 5 × 10⁴ cells were inoculated in a Petri dish (35 mm in diameter, Falcon). For the autoradiographic study, the same concentration of cell suspension was planted

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on round coverslips placed in the Petri dish.

Autoradiography. The cells grown on the coverslips were pulse-labeled for 30 min with 0.1 μCi/ml 3H-TdR and sampled every 2 hr through 36 hr. The cells were fixed with acetic ethanol (1:3) and extracted with 3% perchloric acid. The dipping method was carried out using Kodak NTB-2 emulsion, followed by 2 weeks exposure and Giemsa staining. Two hundred mitoses were counted on each sample and scored as labeled when 5 or more grains on the nucleus were observed. The FLM (fraction of labeled mitoses) curve was traced as a function of time and analyzed by Mendelsohn-Takahashi's asymmetry method (Mendelsohn and Takahashi 1971; Kato et al. 1977).

Drug treatment. The cells were exposed to Thio Tepa at concentrations of 10, 50 and 100 μg/ml for 2 hr on day 2 of culture. In the second phase of the experiment, the cells on day 4 of culture were exposed to 100 μg Thio-Tepa/ml for 2 hr with or without the pretreatment of 10 μg Thio Tepa/ml for 2 hr 12 or 48 hr before. Viable cell numbers were calculated by trypan blue exclusion method as a function of time.

RESULTS AND DISCUSSION

The low dose of Thio Tepa (10 μg/ml for 2 hr) brought temporarily a static effect on the cell proliferation, and the high dose (100 μg/ml for 2 hr) produced a cidal effect. The FLM curves of the cells treated with the low dose of the agent on day 2 showed a prolongation of the cell cycle time by approximately 30% of the control, and seemed to go back to the control level on day 4, that is, 48 hr after the treatment. The cell cycle parameters analyzed are presented in Tables 1 and 2. These findings were in good agreement with the previous results (Kato and Irwin 1977). As the cell cycle time (Tc) of the treated cells on day 3 was estimated to be about 35 hr, the cells must remain in the first generation for 35 hr following the drug treatment and pass into the second generation thereafter. The second generation cells 48 hr after the treatment were characterized as being relatively prolonged in TG2+0.7M and reduced in TG1+0.3M as compared with the first generation cells as well as the untreated control (Tables 1 and 2). From the above findings, it is suggested that the second generation cells consist of a larger part of postsynthetic cells and a smaller part of postmitotic cells than the others.

<table>
<thead>
<tr>
<th>TABLE 1. Cell cycle parameters of MGH-U1 cells immediately following the treatment with 10 μg/ml of Thio Tepa for 2 hr on day 2 of culture</th>
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<td>TC</td>
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Ratios of the parameters to TC are presented in the parentheses.

<table>
<thead>
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<th>TABLE 2. Cell cycle parameters of MGH-U1 cells 48 hr following the treatment with 10 μg/ml of Thio Tepa for 2 hr</th>
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<td>TC</td>
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Ratios of the parameters to TC are presented in the parentheses.
Fig. 1. Macroscopic findings of MGH-U1 cells 18 days after the treatment with 100 µg/ml of Thio Tepa for 2 hr on day 4. Group I (left), Group II (middle) and Group III (right). Viable colonies are seen in Group I alone. Further explanation, see text.

Three experimental groups were designed: 1) Group I (the second generation cells); The cells were exposed to 10 µg/ml of Thio Tepa for 2 hr on day 2, refed with fresh medium for 48 hr and exposed again to 100 µg/ml of Thio Tepa for 2 hr on day 4. 2) Group II (the first generation cells); the cells were exposed to 10 µg/ml of Thio Tepa for 2 hr on day 3, refed for 12 hr and exposed to 100 µg/ml of Thio Tepa for 2 hr. 3) Group III (the treated control cells); the cells were exposed to 100 µg/ml of Thio Tepa for 2 hr on day 4 without the drug pretreatment. Following the drug treatment on day 4, all of the experimental groups were refed with fresh medium every 4 days and the viable cell numbers were counted through 4 weeks. The cells in all experimental groups showed an exponential cell death and there remained no detectable cells by hemocytometer within 10 days. However, a few microcolonies appeared in Group I about two weeks after the last treatment and began to grow again. No visible colonies could be observed in the remaining two groups (Figs. 1 and 2). Triplicated experiments led to the same results.

Recent advances in investigation of the cell cycle have disclosed that alkylating agents keep some effects on the cell cycle, suggesting a cell cycle phase specificity of the agents. In general, alkylating agents induce a prolongation of DNA synthetic (S) phase (Kato and Irwin 1977; Wheeler et al. 1970a, b). In addition, G2 (postsynthetic) cells have been reported to be insensitive to nitrogen mustard (Wheeler et al. 1970), nitrosourea (Harrod and Cortner 1968) and sulfur mustard (Walker and Thatcher 1968). On the other hand, G1 and S (postmitotic) cells have been demonstrated to be considerably sensitive to nitrogen mustard as well as
alkyl alkanesulfonates (Mauro and Madoc-Jones 1970). In the present study, the high dose of Thio Tepa produced a cidal effect on both the untreated control cells and the first generation cells with the drug pretreatment. With the same manipulation, however, the re-growth of the cells were demonstrated in the second generation cells which were characterized by accumulation of postsynthetic cells and diminution of postmitotic cells. These results would indicate that Thio Tepa is more effective on $G_1$ or $S$ cells and less effective on $G_2$ cells.

References


5) Mendelsohn, M.L. & Takahashi, M. (1971) A critical evaluation of the fraction of...

