アセタミドのエーリッヒ腹水癌の分裂速度におよぼす影響

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EFFECT OF ACETAMIDE ON THE MITOTIC RATE IN EHRLICH ASCITES TUMOR

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In *Drosophila melanogaster*, the phenotypic expression of the mutant Bar eye can be changed by various chemicals. Of these, acetamide and lactamide are known to be particularly effective in increasing facet number (Kaji 1954, 1955, 1960; DeMarinis, 1966a, 1966b); whereas mitomycin-C and nitromine were found to have a strong inhibitory effect on the facet-formation.

Many reports indicated that tritiated acetamide incorporated into nuclei of facet-forming cells, which are the precursor cells of the imaginal ommatidia as indicated by autoradiographic studies elsewhere (Hirose and Kaji 1968, 1969; Hirose 1968; Ushioda 1976). These results would also suggest that the acid amides may be closely associated with the metabolism of DNA synthesis in the eye discs.

The present studies were undertaken to investigate whether or not the acid amides act specifically on the facet-formation of the mutant Bar eye. If the acid amides are not specific to *Drosophila*, a similar action might be expected to occur in organisms other than *Drosophila*. On such an assumption, Ehrlich ascites tumor cells which are known to multiply rapidly were used for that experimentation.

This paper deal with an examination of the effect of acetamide, mitomycin-C and nitromine upon the duration of mitotic division in the tumor cells.

MATERIALS AND METHODS

Ehrlich ascites tumor cells.

Ehrlich ascites tumor cells (EAT cells) employed in experiments were mononuclear cells. The cell line, Ehrlich/Takeda (G796) was obtained from the laboratories of Dr. Koichi Ootsu, Takeda Chemical Industries, Ltd. ICR strain were used as host mice. The cell line was maintained by weekely transfer of ascitic fluid. Each ICR mouse was inoculated with 0.1 m*l* of ascitic fluid (approximately 2×10^6 cells). The cells used for all experiments were obtained freshly from the peritoneal cavity.

Microscopy.

The fresh ascitic fluid removed from the peritoneal cavity by the glass capillary tube was mixed with the same amount of distilled water on the slide and smeared. After air dried, specimens were fixed in 100 % methyl alcohol and stained with acetic dahlia. Approximately 1000 cells were counted from each sample. Among the ascitic

fluid, leukocytes, erythrocytes and macrophages are also observed in addition to the tumor cells.

RESULTS

1. *Mitotic rate after inoculation.*

The rate of multiplication of the tumor cells after inoculation was investigated. Changes in the mitotic rate of EAT cells in the peritoneal cavity of 12 mice at 1 to 25 days after inoculation are shown in Figure 1.

The mitotic rate varied at different time after inoculation of EAT cells into mouse peritoneal cavity (Fig. 1). It was obvious that the mitotic rate is at the peak on 5th day after inoculation, and thereafter it gradually decreased. The mitotic indices were in average, 16.5 % for the 1-day, 29.7 % for the 5-days, and 9.4 % for the 20-days after inoculation. After 20-days, mitotic rate was entirely unreliable because many cells were dead or dying.

2. *Effect of acetamide on the rate of multiplication.*

The effect of acetamide to the multiplication of the EAT cells were examined. 0.5 m*l* of 10 % acetamide dissolved in 1 % sodium chloride, was injected into the peritoneal cavities of 6 mice with tumor populations aged two or four days.

Samples from each mouse were taken at an interval of 24 hours after injection of acetamide. The results are shown in Figure 2.

Mitotic rate is markedly increased after injection of acetamide, reached the peak on the third day and showed an abrupt fall thereafter (Fig. 2). It is also clear as

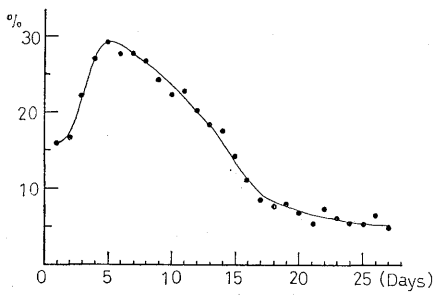

Fig. 1. Per cent of the mitotic rate after inoculation of EAT cells.
Abscissa: days after inoculations; Ordinate: % of mitotic indices. The mitotic rate is at the peak on 5th day after inoculation and thereafter it gradually decreased.

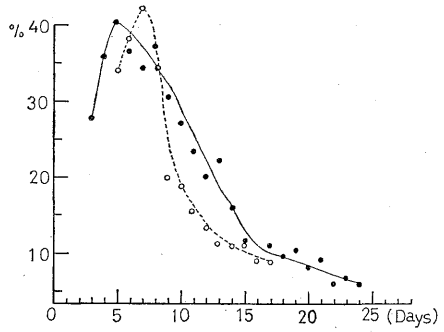

Fig. 2. Change of mitotic rate after injection of acetamide.
Abscissa: days after inoculation; Ordinate: % of mitotic indices. 0.5 ml of 10% acetamide solution was injected into the peritoneal cavities of mice with tumor population aged 2 (●) or 4 (○) days. The mitotic rate is markedly increased after injection of acetamide reached the peak on the third day.

figures 1 and 2 indicated that the mitotic rate was 42.3 % for the acetamide injected mice as compared with 29.7 % for the untreated ones.

3. *Effect of mitomycin-C on the rate of multiplication.*

The concentration of mitomycin-C dissolved in saline solution was 250 μg/m*l*. For comparison, a mixture of 0.5 m*l* of 10 % acetamide plus 0.5 m*l* of 0.25 % mitomycin-C were also used. Six mice were examined in each series. The chemicals were injected intraperitoneally, two or four days after tumor cell inoculation. Samples of the tumor cells were taken every 24 hours after the injection. The results obtained from the effect of mitomycin-C and acetamide-mitomycin-C mixture are shown in Figures 3 and 4.

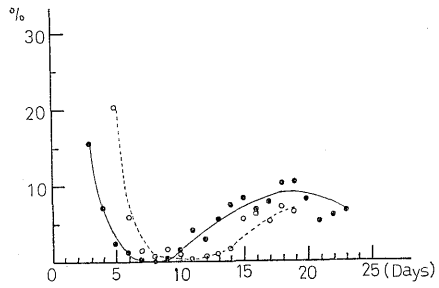

Fig. 3. Change of mitotic rate after injection of mitomycin-C.
Abscissa: days after inoculation; Ordinate: % of mitotic indices. 0.5 ml of 0.25% mitomycin-C solution was injected interperitoneally, 2 (●) or 4 (○) days after tumor cell inoculation. The mitomycin-C has an intensive inhibitory effect on the multiplication of EAT cells.

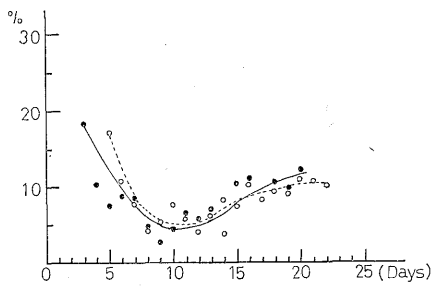

Fig. 4. Change of mitotic rate after injection of acetamide-mitomycin-C mixture.
Abscissa: days after inoculation; Ordinate: % of mitotic indices. A mixture of 0.5 ml of 10% acetamide plus 0.5 ml of 0.25% mitomycin-C was injected into mice 2 (●) or 4 (○) days after tumor cell inoculation. The inhibitory effect expressed in mice injected with a mixture of acetamide-mitomycin-C was not as distinct as that with only mitomycin-C.

Obviously, the mitomycin-C has an intensive inhibitory effect on the multiplication of EAT cells. The mitotic rate decreased markedly, which was especially significant in mice 4 to 12 days after mitomycin-C injection. In an extreme case there was no detectable mitosis 6 days after injection. However, after an elapse of 7 to 8 days, mitosis resumed and mitotic index reached almost 10 %. In contrast, the inhibitory effect expressed in mice injected with a mixture of acetamide and mitomycin-C was not as distinct as that with only mitomycin-C. The minimum mitotic rate was 5 % in the acetamide-mitomycin-C mixture.

4. *Effect of nitromine on the rate of multiplication.*

0.5 m*l* of 0.5 % nitromine was injected into mice 2 or 6 days after tumor cell inoculation. For comparison, a mixture of acetamide and nitromine were also used.

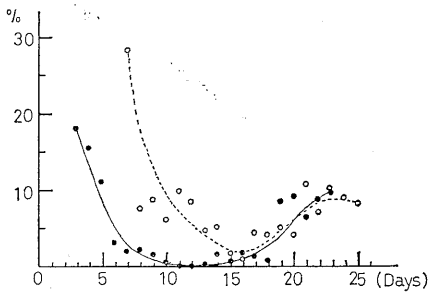

Fig. 5. Change of mitotic rate after injection of nitromine.

Abscissa: days after inoculation; Ordinate: % of mitotic indices. 0.5 ml of 0.5% nitromine was injected into mice 2 (●) or 6 (○) days after tumor cell inoculation. The nitromine also had a strong inhibitory effect on the multiplication of the tumor cells.

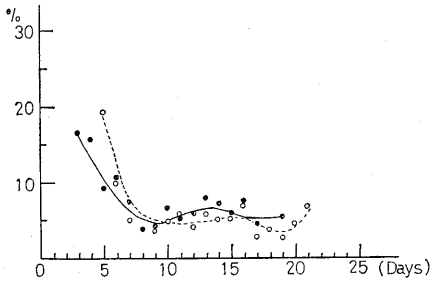

Fig. 6. Change of mitotic rate after injection of acetamide-nitromine mixture.

Abscissa: days after inoculation; Ordinate: % of mitotic indices. A mixture of 0.5 ml of 10% acetamide plus 0.5 ml of 0.5% nitromine was injected into mice 2 (●) or 4 (○) days after tumor inoculation. The acetamide-nitromine mixture relieves the inhibitory effect produced by nitromine.

Each tests were made with six mice. Samples of EAT cells were taken at an interval of 24 hours after chemical injections. Changes in percentages of mitotic EAT cells are illustrated in Figures 5 and 6. Apparently, nitromine also had a strong inhibitory effect on the multiplication of the tumor cells. The mode of inhibition of nitromine to EAT cells resembled that of mitomycin-C, and those from mice 6 to 11 days after injection were markedly inhibited. However, 11 to 12 days later, multiplication of EAT cells resumed and slowly increased to reaching the mitotic rate at about 10 %.

On the contrary, the acetamide-nitromine mixture relieves the inhibitory effect produced by nitromine. Thus, acetamide and nitromine or mitomycin-C showed mutual reverse effect on the multiplication of EAT cells.

DISCUSSION

In our previous work of the Bar eye in *Drosophila*, it was found that acid amides markedly accelerated the facet-formation. When these agents were administered, the facet number increased to as many as those of the wild full-type eye (Kaji 1954, 1955, 1960; DeMarinis 1966a, 1966b). In contrast to this, mitomycin-C or nitromine which is an inhibitor of DNA synthesis had a strong inhibitory action on the facet-formation in both wild-type and mutant Bar eyes (Kaji and Hirose 1968). As pointed out in our previous reports dealing with autoradiographic studies, the ^{3}H-acetamide was mainly incorporated into nuclei in the facet-forming cells of the Bar eye disc (Hirose and Kaji 1968, 1969). Most likely the acid amides may play a rule in the metabolism of DNA synthesis of the eye disc cells.

In *Drosophila*, it was found that acetamide also had an effect on the homoeotic

mutant, *loboid-ophthalmoptera* (Ouweneel 1969). In this respect, acetamide was found to be so effective in increasing the number of facets that the eye size of the mutant may reach that of the wild type; in proportion to its concentration it moreover strongly increases the penetrance of the wing-like outgrowths. Except for the Bar strain, the mutant *ld-opht* was the only other example in *Drosophila* in which acetamide effect has been experimented. These experiments have indicated that the acid amides exert on the morphogenesis of the mutant eye discs. However, no such effect by acid amides was observed in the facet-formation of Oregon-R wild type (Kaji and Hirose 1968) and mutant *bar*-3 or *eyeless* strain (unpublished). In the wild type flies, mitomycin-C or nitromine had an intensive inhibitory effect on the eye formation. In an extreme case, some of these antibiotics-treated flies had almost become eyeless; whereas if they were exposed to the lactamide-mitomycin-C or nitromine mixture, no difference in the facet-formation between treated and untreated ones was observed.

Under these circumstances it seems natural to assume that, the acid amides act specifically on the cell multiplication, i.e., increasing the facet-forming cell of eye disc during morphogenesis. However, it is not clear whether or not the acid amides is specific to the eye development of *Drosophila* only. If it is not, a similar action might be exerted on other organisms to specifically promote cell multiplication.

For probing this, the Ehrlich ascites tumor cells were chosen for experimentation. As expected, multiplication of EAT cells was also affected by acetamide. Mitotic rate increased to a maximum rate of 42.3 % after acetamide injection to the ascitic fluid; whereas it was 29.7 % in the untreated mice (Figs. 1 and 2). In contrast, mitomycin-C or nitromine injected mice showed marked decrease in the mitotic rate of the EAT cells (Figs. 3 and 5). Nitrogen mustard is known to prolong survival time of tumor-bearing animals (El-Merzabani and Sakurai 1965, 1967; Ootsu and Matsumoto 1970). Similary, mitomycin-C is also known to have an anticancer activity, because it is an antimitotic and DNA synthesis blocking agent (Matsumoto and Ootsu, 1969).

At any rate, the mitotic rate of EAT cells in mitomycin-C or nitromine-acetamide mixture did not show any marked effect (Figs. 4 and 6). It seems natural to assume that acetamide has relaxation effect on the inhibitory action of those antibiotics. Thus these two groups of chemicals showed counter effects on the multiplication of EAT cells. The mode of the effect of acetamide and antibiotics on the multiplication of EAT cells resemble that of the facet-formation in *Drosophila*.

Consequently, these experiments showed that the acid amides not only act as specific agents to the eye formation in *Drosophila*, but also cause a considerable increase in the mitotic rate of EAT cells in mouse.

SUMMARY

The effect of acetamide and certain antibiotics on the multiplication of the Ehrlich ascites tumor cells in mice was examined. The acetamide had a marked effect on increasing the mitotic rate of the EAT cells. However, mitomycin-C or nitromine had a strong inhibitory effect on the multiplication of the cells. In contrast the rate of cell multiplication in acetamide-mitomycin-C or nitromine mixture showed no effect what so

ever. The acetamide appeared to counteract either mitomycin-C or nitromine on the metabolic process of the multiplication of EAT cell.

The mode of the action on EAT cells by these two groups of chemicals was similar to that on the facet-formation in *Drosophila*. The acid amides not only are specific to the eye development in *Drosophila*, but also considerably increase the mitotic rate of EAT cells in the mouse.

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