The Cytological Effect of Chemicals on Tumors, XIV. Effect of Ayamycin on the MTK-Sarcoma III

By

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(With 16 Text-figures and 1 Table)

Recently, the inhibitory action of Ayamycin, an antibiotic isolated from cultures of *Streptomyces* No. 0–80 (Tanno 1960, Sato 1960), upon tumor growth has attracted interest in cancer chemotherapy. Matsuura and Katagiri (1961) have reported that Ayamycin–A_2, a fraction of Ayamycin–A complex, is effective upon the growth of Ehrlich ascites carcinoma through intraperitoneal injections. Cytological details of the effect of this interesting agent have, however, remained not wholly explored. The present study was undertaken in hope of obtaining some critical information on the cytological effect of this drug upon an ascites tumor of the rat.

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Material and methods: The tumor used for the present study was the MTK-sarcoma III, an ascites tumor of rats. The rats used for tumor transfer were Wistar and Gifu strains, weighing 100 grams. The transplantability of the MTK-sarcoma III was 100 per cent to rats of Wistar and Gifu strains, and the death of hosts occurred ordinarily within 9 days after tumor transfer. On the 3rd day of transfer, the tumor-bearing rats received intraperitoneally the injection of Ayamycin at the following dose levels: 2.5 mg/kg, 5 mg/kg, 10 mg/kg and 20 mg/kg.

For general cytological studies, smear preparations of the tumor ascites were made at appropriate intervals following the injection of the drug. Smears were fixed either with absolute methanol or with acetic alcohol (3:1), and stained with May-Grünwald and Giemsa (Jacobson and Webb 1952), or with Feulgen reagent. For chromosomal study, the preparations were made according to the water pretreatment squash method with acetic dahlia (Makino 1957).

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Results of observations

Effect on tumor growth: In order to obtain some quantitative data on the effect of Ayamycin on the growth of the tumor, the mitotic rate of tumor cells was examined in the samples taken 1, 2, 3, 6, and 12 hours after the injection of the drug at dose levels of 5 mg/kg and 10 mg/kg, and at each 24-hour-interval for the following 5 days (Fig. 1), on the basis of 2,000 to 5,000 tumor cells.

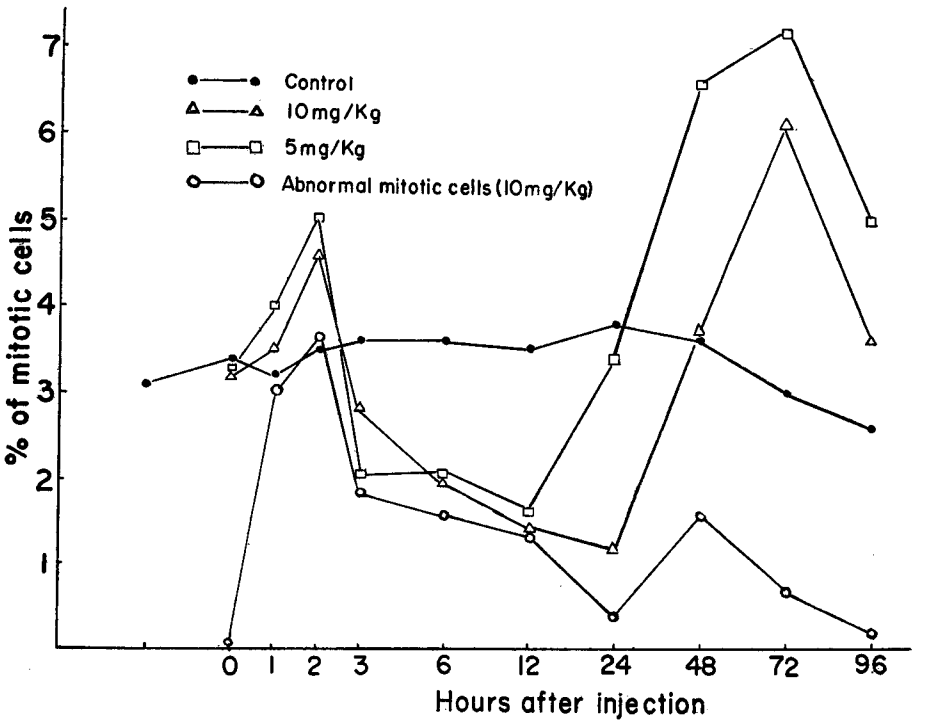


Fig. 1. Curves showing mitotic frequencies of unaffected and affected cells of the MTK-sarcoma III after application of 10 and 5 mg/kg Ayamycin.

The mitotic rate of the treated tumor cells became relatively high in the 2 hours sample, whilst a sudden decrease occurred in the 3 hours sample. The lowest rate was found in the 12 hours sample at the 5mg/kg dose, while it was in the 24 hours sample at the 10 mg/kg dose. In the samples taken from 1 to 3 hours after application, the relative frequency of ana- and telophasic cells decreased in number, with an increase of metaphasic cells (Fig. 3). This is an indication that

cell division had been arrested at metaphase. It was also remarkable that damaged cells increased in number concomitant with a decrease of mitotic cells (Figs. 1 and 2).

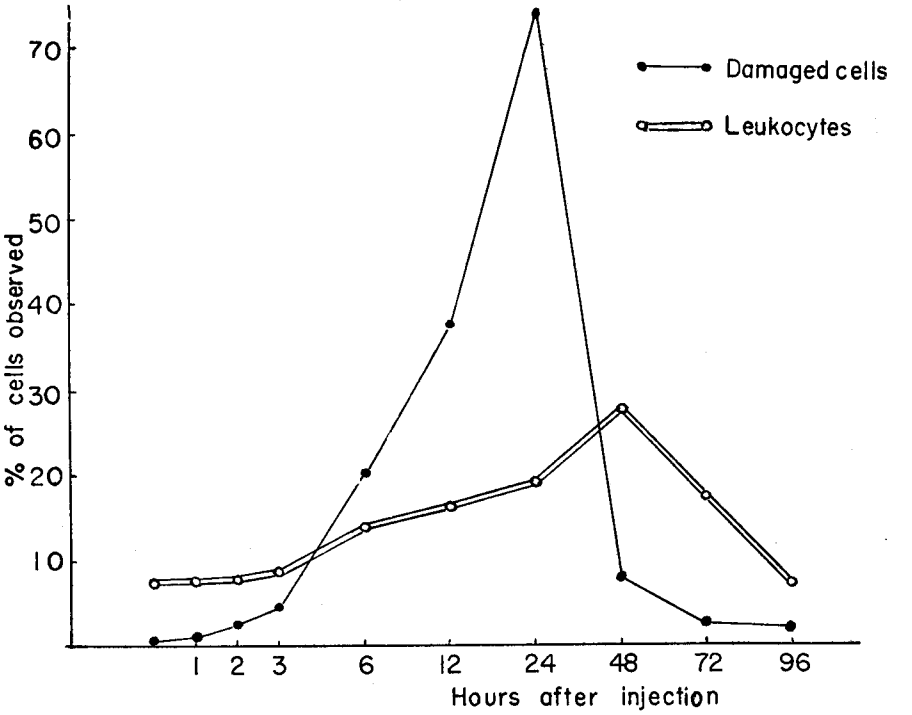


Fig. 2. Curves showing frequencies of damaged cells and leukocytes after application of 10 mg/kg Ayamycin.

Two out of 20 tumor-bearing animals which received the administration of 10 mg/kg Ayamycin showed no sign of tumor growth. The samples taken from those animals on the 7th day after the administration showed no mitotic cells, and on the 9th day they contained no tumor cells in the ascites. Those rats survived more than 150 days. Average survival days of the tumor-bearing rats which received 10 mg/kg or 5 mg/kg Ayamycin are as shown in Table 1.

Daily intraperitoneal injections of 10 mg/kg Ayamycin were given for 5 days to tumor-bearing rats beginning on the 3rd day after the tumor transfer. Only one of those animals survived more than 150 days, and the life span of the remaining tumor-animals showed a prolongation of only 3 days or more (Table 1).

From the above findings, it is evident that the intraperitoneal injection of Ayamycin into tumor-bearing rats resulted in a reliable inhibition of tumor growth,

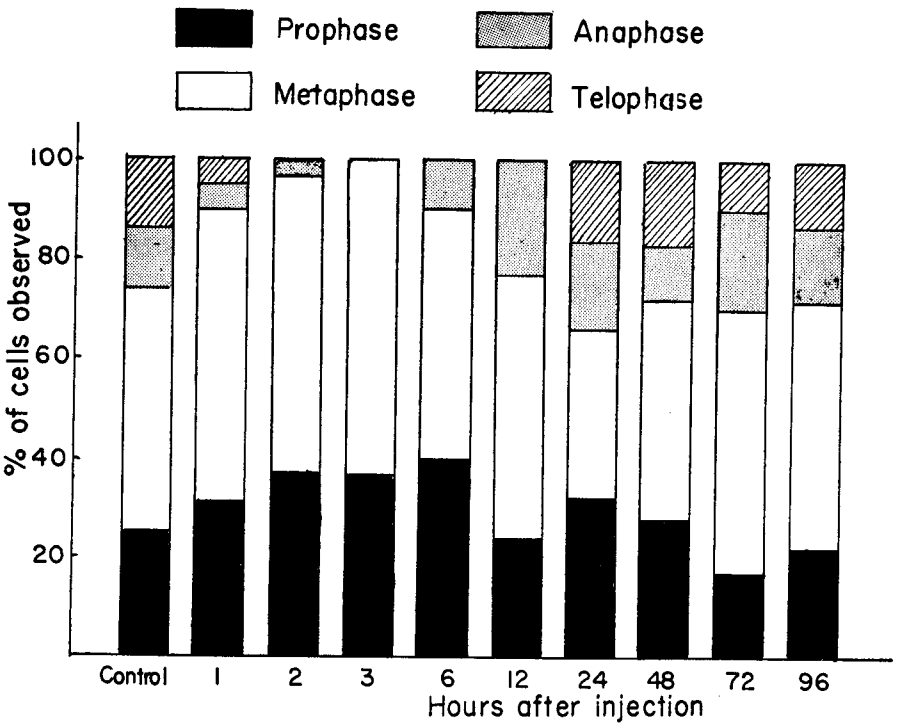


Fig. 3. Histograms showing relative frequencies of mitotic phases after application of 10 mg/kg Ayamycin.

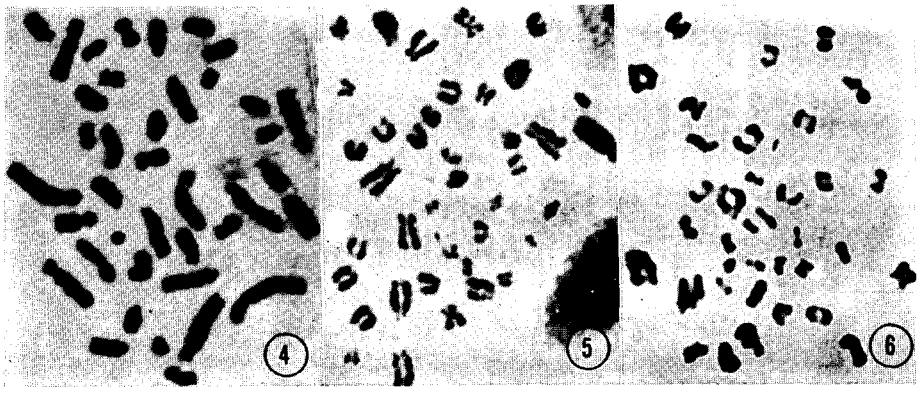
Table 1. Life span of the MTK-sarcoma III rats after the treatment with Ayamycin

Dose of Ayamycin	No. of animals	Survived animals	Average survival days (range)
Control	0/5*	0	8.9(8-10)
2.5 mg/kg	0/4	0	8.0(6- 9)
5 mg/kg	0/8	0	11.1(10-14)
10 mg/kg	2/20	10.0%	10.8(8-13)
20 mg/kg	0/2	0	6.5(6- 7)
10 mg/kg (5 times once daily)	1/9	11.1	12.5(9-18)

* The numerator shows the number of animals survived after the application of Ayamycin, and the denominator indicates the number of animals received injection of the tumor.

particularly at high dosages of the drug.

Cytological effects: Tumor inhibitory action of Ayamycin was found relatively weak at a dose of 5 mg/kg. The following data were based on experiments at a 10 mg/kg dose. Generally, the damage to tumor cells appeared within a rather short time, as early as 30 minutes, after injection of the drug; a remarkable feature was the metaphase block, most tumor cells being blocked at metaphase. The damage was represented by abnormal condensation and irregular scattering of metaphasic chromosomes, aggregation of chromosomes (Figs. 5 and 6), irregular



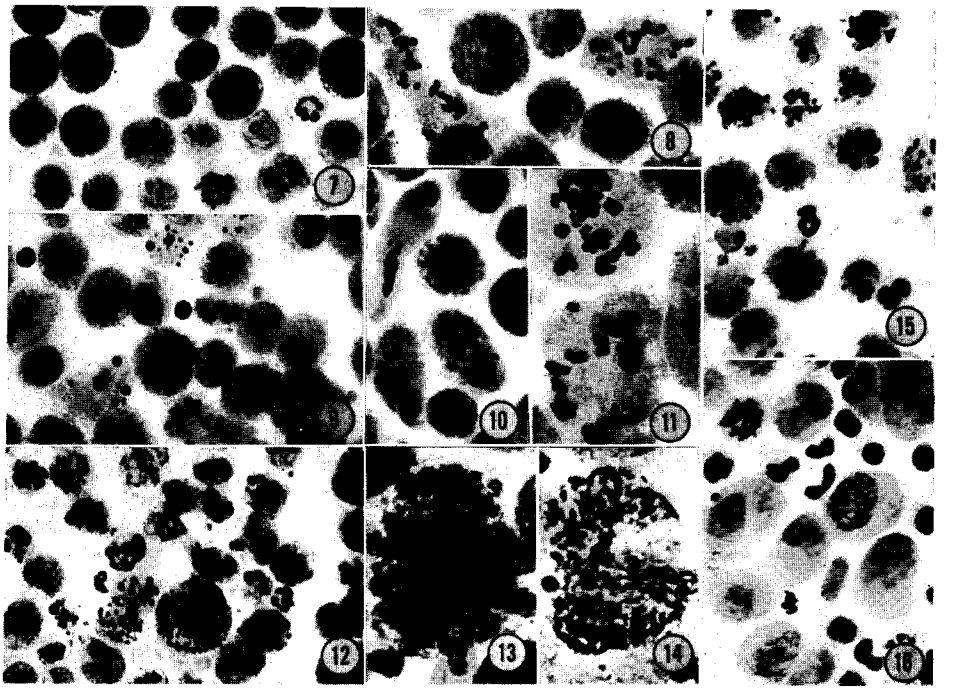
Figs. 4 to 6. Photomicrographs of metaphase chromosomes of treated and untreated cells of MTK-sarcoma III, 1400×. Fig. 4, control. Figs. 5 and 6, Ayamycin-treated (10 mg/kg), 1 and 2 hours after injection.

processes, and atypical amoeboid protrusion of the cytoplasm (Figs. 8–11). These degenerative changes proceeded with the passage of time. In some cases the resting or prophasic cells showed cytoplasmic vacuolization and blebbing of the cytoplasm, following the breakdown of cell body (Figs. 12–14). During a period from 6 to 12 hours after injection, almost all cells at metaphase showed degenerative features evidenced as severe clumping of chromosomes. By 24 hours after injection, the disintegration of tumor cells was very striking, most of the affected tumor cells undergoing degeneration evidenced by pycnotic aggregation and breakdown of chromatin (Figs. 12 and 15).

On the basis of the above findings, the conclusion is possible that Ayamycin exerts influence upon metaphasic cells, producing abnormal condensation or scattering of metaphasic chromosomes, and pycnotic aggregation of chromatin materials.

Abnormal chromosome configurations, such as chromosome translocation, fragmentation or formation of chromosome bridges, were rarely observed in tumor cells treated with Ayamycin.

By 24 hours after injection, the abnormal mitotic cells decreased in number, and by 48 hours there were observed only a few damaged cells in the ascites. In those samples there were present a certain number of tumor cells, which were characterized by a small amount of cytoplasm with a compact nucleus; probably they remained alive undamaged by the drug. Referring to the reports by Makino



Figs. 7 to 16. MTK-sarcoma III cells after the application of 10 mg/kg Ayamycin. Fig. 7; cells on the 3rd day after transfer, just before injection (control), ×300. Figs. 8–11; abnormal condensation, agglutination and scattering of metaphase chromosomes together with deformation of cytoplasm, 1 hour (Fig. 8, ×500, Fig. 11, ×900) and 2 hours after injection of the drug (Figs. 9 and 10, ×500). Fig. 12; pycnosis of chromatin, 12 hours, ×500. Figs. 13–14; cytoplasmic blebbing (Fig. 13, ×800) and pycnosis of chromatin in resting nucleus (Fig. 14, ×1000), 6 hours. Fig. 15; 24 hours, ×500. Fig. 16; regrowth of tumor cells, 48 hours, ×500.

and his co-workers (Makino 1957, 1959), it is most likely that those tumor cells are residual ones having been left alive undamaged by the drug, and that they constitute a primary source of renewed tumor growth.

Discussion

Effects of several antibiotics, such as Sarkomycin, Carzinophilin, Mitomycin and Actinomycin, upon animal and human neoplasms *in vivo* and *in vitro* have been studied cytologically by Reilly *et al.* (1953), Gregory *et al.* (1956), Sasaki (1956), Awa (1959, 1961), Hori and Sasaki (1958, 1959), Kobayashi (1960) and Tonomura (1960), and some others. It was shown that Sarkomycin produced metaphase block, damaging the cells at metaphase, while Carzinophilin and Mitomycin attacked mainly nucleoli of the resting nucleus, and further that Actinomycin injured both resting and mitotic cells.

Recently, inhibitory effects of Ayamycin upon tumor growth have been reported by some authors with particular regard to the prolongation of life of the tumor-bearing animals (Tanno 1960, Matsuura and Katagiri 1961). Matsubara (1960) and Matsumoto (1961) stated that Ayamycin was the most effective purified antibiotic and that it showed severe toxic action against the cytoplasm of HeLa cells *in vitro*.

The present author also found, on the basis of cytological observations, that Ayamycin was pronouncedly effective against the MTK-sarcoma III. The drug affected particularly the tumor cells in process of mitosis; generally the mitotic cells were blocked at metaphase, and then pycnotic disintegration of nuclei with deformation or blebbing of the cytoplasm followed. In the light of the above findings, it is probable that this agent may induce some disturbance of DNA-synthesis. It was found by the present study that the cytological effects of Ayamycin show a general similarity to those of podophyllin and podophyllotoxin, since similar damaging features by the latter two agents were reported by Makino and Tanaka (1953) and Tanaka *et al.* (1955).

It is noticeable that, although the tumor growth was temporarily inhibited by the action of Ayamycin, a renewed growth of the tumor took place in the majority of cases observed. Probably the renewed growth may be due to the proliferation of residual tumor cells which remained alive undamaged by the drug, as shown by Makino and his co-workers (Makino 1957, 1959) in a series of cytological studies on the effects of chemicals upon tumor cells.

Summary

The present study deals with some effects of Ayamycin on the MTK-sarcoma III, with special regard to cell-damage and regrowth of the tumor.

Ayamycin exerts influence upon tumor cells in process of mitosis, especially on metaphasic cells, leading to metaphase block.

The survival days of the tumor-bearing animals treated with Ayamycin at comparatively higher dose levels were prolonged for 2 to 3 days.

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