The Cytological Effect of Chemicals on Ascites Sarcomas, VII. Effects of Myleran upon the Yoshida Sarcoma[1)2)]

By

Tatsuya Tanaka

(Zoological Institute, Hokkaido University)

(With 5 Text-figures)

In the course of the study with the bifunctional esters of bis-hydroxy-ethylarylamines, Timmis and Haddow (1950) have reported inhibitory effect on various transplantable tumors produced by di-sulfonic esters. In continuation of this research they observed that butane compound exerted a remarkable mitotic inhibition against the Walker rat carcinoma 256 (Haddow and Timmis 1953). This compound (1,4-dimethanesulfoxybutane, so called GT-41 or Myleran), unlike X-irradiation and mustard, was found to produce a considerable decrease in number of neutrophils with very little effect on the other types of blood cells in rats and human, as a result of the depressive influence on the myeloid series. Therefore this drug has been used to treat chronic myelogenus leukemia with some beneficial results (Galton 1953, Petrikis et al. 1954, Ramioul 1955). In view of its practical importance in chemotherapy, it seemed to be of value to study the influence of myleran on tumor cells in order to learn of any possible activity causing cytological damage.

The results reported here deal with the effect of myleran with special regard to the morphological process in causing regression of malignant growth and to its action on cell division.

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Material and methods

The tumor employed in this study was the Yoshida sarcoma, a transplantable rat ascites tumor. Intraperitoneal injections of myleran were made in tumor-bearing rats at various intervals after transplantation of the tumor. Myleran was dissolved with 20 percent propylene glycol in physiological saline solution. In preliminary experiments, one or five doses of 2 to 50mg per Kg of body weight were given. When repeated doses

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were required, injections were given twice daily. Rats weighing 100 to 150gm were used for the experiment.

For observation smear preparations were made from a droplet of the neoplastic exudate obtained by abdominal puncture at specific intervals following the administration of myleran. The slides were colored with acetic-dahlia, and Feulgen, or after Giemsa's method. Observations of treated tumor cells with the phase-microscope were also undertaken for comparison.

Results

1) *Damage to tumor cells by myleran*: A vast amount of experimentation has been done to determine the relationship between the dosage of myleran and degree of tumor inhibition. The maximum tolerated dosage level was judged on the basis of elongation of survival time and loss of body weight of animals under examination. The range of concentration of the compound used was 2, 5, 10, 30 and 50 mg per Kg of body weight, at the twice daily dosage. The degree of inhibition was directly proportional to the dosage of the compound. At higher dosage level within the range from 10 to 50 gm, however, the compound caused immediate loss in body weight which continued until death of the animals. There is no considerable spread in the survival time of treated animals due probably to the toxic effect of the compound, in spite of a remarkable inhibitory effect upon the growth of the tumor. The dosage of 5 mg/Kg in each injection revealed marked inhibition of tumor growth, but it still resulted in a slight decrease in body weight.

After several tests, the intraperitoneal injection of 2 mg/kg was found to result in a remarkable inhibition of tumor growth without decreasing body weight or exerting a marked effect on the body tissue. In all seven rats used for this treatment, the ascites tumor decreased and the tumor cells became scarcely detectable in the abdominal fiuid by the end of eight days' treatment. Two of them died 9 and 14 days after tumor transfer. Autopsy revealed no sign of malignant development, but showed fairly intensive liver degeneration and spleen atrophy. The other four rats, however, showed very healthy condition after 8 days' treatment; they were sacrified at 25 and 30 days after cessation of the treatment. Macro and micro-examinations failed to find any sign of tumor growth. The remaining one rat began to show tumor cells again in the abdominal fluid at about 10 days after cessation of the treatment and died 6 days later. Development of the ascites tumor and of solid abdominal tumors were found at autopsy. To summarize, it may be stated that the twice daily dosage of 2 mg/Kg of myleran was the tolerated concentration which brought about a remarkable inhibition, by predominant selection of the tumor cells.

2) *Effect of myleran on tumor growth*: To study closely the effect of the compound, a single dose of 2 mg/Kg was administrated 4 days after transplantation and the ascitic fluid was sampled at short intervals thereafter. Ordinary

cells occurring in the abdominal fluid did not show marked changes except that there were some monocytes provided with cytoplasmic vacuolization and deformed nuclei. They showed an increase in number of eosinophils, some exhibiting clumping of chromatin threads. In contrast to the above feature, the tumor cells showed significant changes. Generally, tumor damage activity of myleran was found to be slower during the first 3 to 5 hours as compared with that of other antitumor compounds. The first change observed was temporary cell elongation, presumably due partly to hydration. The change was obvious after 1 hour in the probable effect on the cytoplasm first. By the time the movement of cytoplasmic granules was slowed down, fine sting-like processes appeared, and finally the production of amoeboid protrusion and blister-like blebs followed.

The morphological changes in the nuclei of tumor cells were significant. Swollen, poorly stained nuclei were observable within 3 hours after the injection. The nuclei appear provided with fine granules. The chromatin threads were thin but in part deeply stained in some nuclei. With the passage of time, the granules became larger and fewer in number, and tended togather in a part of the nuclear vesicle, leaving a clear homogeneous non-basophilic nuclear space. The changes reached a maximum after about 20 hours. One or two days after the myleran injection some tumor cells had many deformed nuclei showing deeply stained segments on the chromocenter which gradually condensed into aggregates lining the nuclear membrane. Extremely high doses of X-irradiation have been noted to cause similar pycnotic changes (Koller 1952, Swanson 1954).

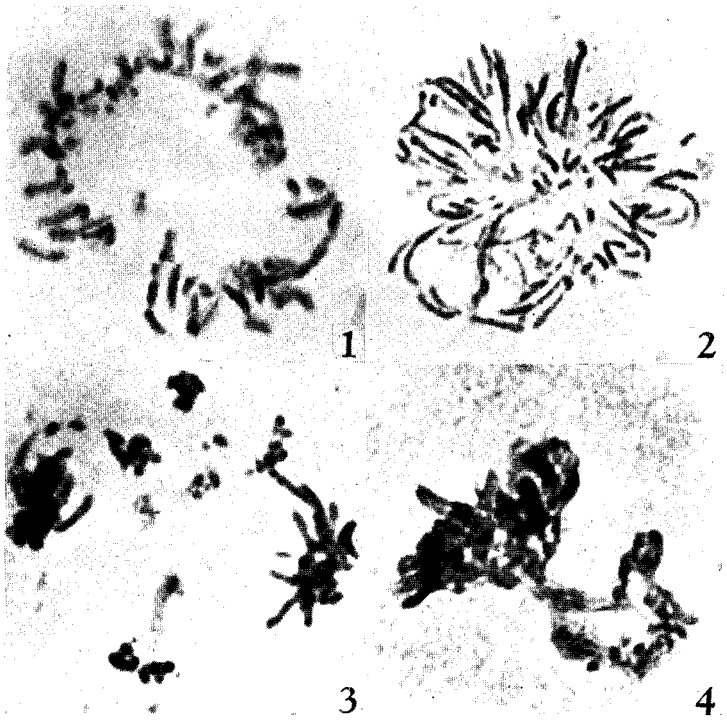
Two or more days after the first injection, the tumor cells at interphase showed advanced changes. The aggregates further condensed forming spherical pycnotic bodies as the boundary of the nucleus became no longer defined. The process of so-called karyorrhexis was thus demonstrated. Eventually, these changes were followed by cytolysis.

Gardella and Lichter (1955) have emphasized the enlargement of malignant tumor cells after X-irradiation due to continued cytoplasmic as well as nuclear growth in the absence of mitosis. Such a change was not observed in the present experiments.

As the tumor cells decreased, the monocytes, eosinophils, neutrophils, mast-cells and lymphocytes became of very frequent occurrence. These cells also showed more or less similar injuries. Especially the changes in the monocytes were marked. Changes in the peritoneal cells caused by antitumor compounds were similar to the results of Makino and Tanaka (1953).

3) *Cytological effects of myleran upon tumor cells*: The mitotic figures were morphologically analyzed. The typical mitotic characters rapidly decreased in number as the 2 mg/Kg 8 days' treatment progressed. Reciprocally, the non-typical characters increased; they are stickiness, bridge formation, micronuclei formation and lagging of chromosomes, condensation and disintegration. Finally, tumor cells either in mitosis or in interphase were scarcely found.

Six to eight hours after a single dose of 2 mg/Kg myleran, some tumor cells at prophase exhibited pycnotic chromosome elements. Further advanced changes were observed in comparison with those of the control; there were observable many chromosome arms containing interstitial Feulgen-negative gaps through each strand was markedly elongating (Figs. 1–2). Duncan and Woods



Figs. 1-4. Effect of myleran (2 mg/Kg) upon mitosis of tumor cells, 8 hours after injection. ×1700. 1, metaphase. 2, early anaphase. Figures illustrate clearly the interstitial Feulgen-negative gaps in each chromosome arms. 3, late-telophase showing irregularly condensed laggards or fragments. 4, telophasic ring after failure of anaphasic separaton.

(1953) have reported a similar effect to occur subsequent to treatment with 5-aminouracil; probably the effect may be due to the delay of mitosis but not to its stopping, since a little delay of mitosis resulted in the appearance of partial pycnotic knotting or irreular segments. Subsequently, stickiness of chromosomes became pronounced at late anaphase.

The most frequent and significant event caused by myleran was the disturb-

ance in chromosome movement. The metaphasic chromosomes which started to split, were disorderly scattered (Fig 3). Also this drug caused a pronounced metaphasic delay with metaphase index rising progressively. Analytical data presented in Figure 5 illustrate this feature clearly. Referring to the above find-

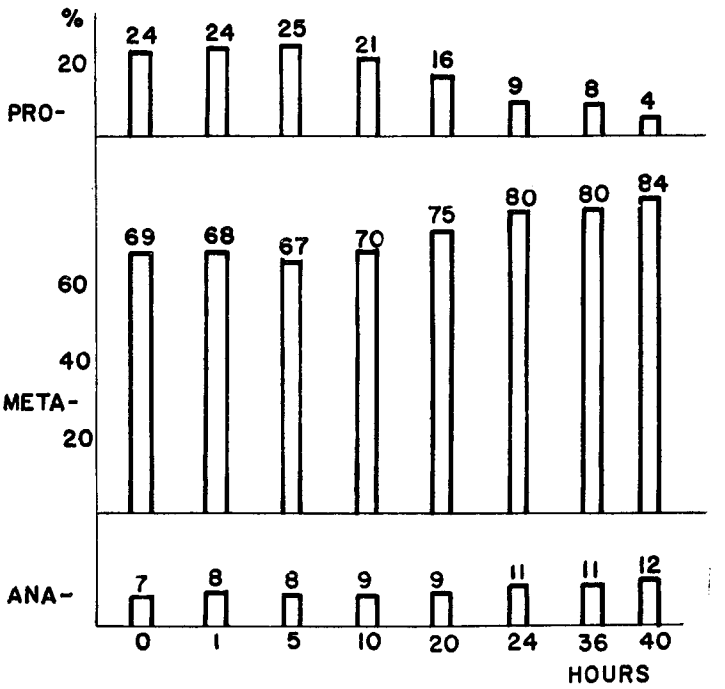


Fig. 5. Relative frequencies of mitotic phase after administration of myleran (2mg/Kg).

ings, it may be deduced that myleran has a potentiality for spindle inactivation. At the present moment, it is difficult to state the difference in spindle inactivation between myleran and colchicine which are similar spindle poisons. The chromosome bridge, telophasic ring-formation, and di- as well as acentric fragmentation appeared at ana- and telophase (Fig. 4). Similar mitotic aberrations have been caused by nitrogen mustard, TEM and X-irradiation. The presence of small supernumerary nuclei called micronuclei in the resting cell is an another remarkable indication of the damage.

Conclusion

Myleran at a tolerated dose of 2 mg/Kg caused an initial inhibition of tumor

growth without decreasing body weight. The results obtained agree with the findings of Haddow and Timmis (1954) and Gellhorn *et al.* (1954).

From the effect upon mitosis produced by myleran, it may be tentatively concluded that myleran causes remarkable damage to mitotic tumor cells, and that the effect of myleran and radiomimetic compounds on tumor cells may be basically related. The mitotic changes induced by myleran may probably be due to an interference in the nucleic acid metabolism at the interphase nucleus. Skipper, Bonnett and Wheeler (1952) have found that nitrogen mustard, one of the radiomimetic compounds, forms insoluble reaction products in connection with nucleic acid bases; they have isolated a guanine-nitrogen mustard reaction product after interaction of nitrogen mustard with desoxyribonucleic acid *in vitro*. Further investigation is needed to determine whether myleran reacts in the same way.

Summary

It was found that the twice daily dose of 2 mg/Kg of myleran was the tolerated concentration which would cause a remarkable inhibitory effect acting selectively on tumor cells of the Yoshida sarcoma. The tumor cells treated with such concentration underwent cytoplasmic vacuolization and karyorrhexis. Mitotic aberrations were considerable. The most significant mitotic abnormalities were the morphological change of chromosomes and the spindle inactivation. This compound evidently reacts effectively in connection with nucleophilic center, especially in cells at mitosis or eligible for mitosis. Prolonged administration of the tolerated dosage induced a remarkable elongation of life span of the tumor-bearing animals or striking tumor regression.

Literature cited

Duncan, R. T. and Woods, O. S. 1953. Chromosoma 6 : 45-60.

Galton, D. A. G. 1953. Lancet 264 : 208-213.

Gardella, J. A. and Lichter, E. J. 1955. Cancer Res. 15 : 529-531.

Gellhorn, A., Peterson, E.R., Kells, A., Hirschberg, E. and Murray. M. R. 1955. Ann. New York Acad. Sci. 60 : 273-282.

Haddow, A. and Timmis, G. M. 1953. Lancet 264 : 207-208.

Koller, P. C. 1952. Heredity 6 : 5-22.

Makino, S. and Tanaka, T. 1953. Jour. Nat. Cancer Inst. 13 : 1185-1200.

Petrikis, N. L., Birman, H. R., Kelly, K.H., White, L. P. and Shimkin, M.B. 1954. Cancer 7 : 383-390.

Ramioul, H. 1955. Acta Unio internat. Cancer 11 : 170-178.

Skipper, H. E., Bennett. L. L. and Wheeler, G. 1952. Second National Cancer Conference, Cincinnati, Ohio.

Swanson, C. P. and Johnston, A. H. 1954. Am. Nat. 88 : 425-430.