Experimental Studies on Chemotherapy of Malignant Growth Employing Yoshida Sarcoma Animals (XVI) Determination of Minimum Effective Dose (MED) of Nitromin and Other Compounds by Means of Cytochemical Detection of the PAS Positive Substances

HARUO SATO, FUMIO NOUCHI, TOMIZO YOSHIDA, MORIZO ISHIDATE
(Department of Pathology, Fukushima Medical College, Fukushima, Sasaki Medical Institute, and Iatrochemical Institute of Pharmacological Research Foundation, Tokyo)

In a series of cytochemical studies of the effects of Nitromin and other compounds on the Yoshida sarcoma cells, it was clearly demonstrated that increase of PAS positive granules (which were of glycogen in nature) was evidently correlated with the doses of Nitromin administered. It has already been reported by Hirono, Belkin, and many others that the Yoshida sarcoma cells possessed an ability to produce glycogen granules in a particular stage of a single transplant generation. And increase of glycogen was also revealed in the Yoshida sarcoma cells following administration of several compounds.

In this series of experiments, experimental schedules were designed as follows: 1) The compounds tested were administered into the tumor bearing animals (3 rats were used in each experiment.) intraperitoneally 5 days after transplantation of the Yoshida sarcoma. Around this time, the ascites represents a well-known feature of “nearly pure culture”. In addition, this is the time when the initial increase of the tumor cells with glycogen begins to decrease in number. 2) Three different doses were administered of each compound, i.e.; Maximum tolerated dose (MTD), minimum effective dose (MED), and half dose or one-tenth of MED which failed to give any effect in the previous observations. 3) Tumor ascites was drawn 3, 6, 9, 12, 24 hours after administration, and thereafter once a day until the host died. 4) Dried smears of the tumor cells were fixed in alcohol, and stained with Lillie’s Periodic Acid Schiff technique, together with the Giemsa method as usual. 5) Number of the PAS Positive tumor cells was examined by counting 2,000 cells observed in each preparation.

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The results in the features of glycogen so far observed in the present experiments are summarized here, although the details will be published elsewhere.

(1) In case of Nitromin administration, there were revealed two phases of increase of the tumor cells which possessed glycogen granules in their cytoplasm. The first increase of glycogen cells observed within 24 hours after administration, stopped once before a marked increase of those was revealed again 24 to 72 hours after. It was noted that the glycogen cells had increased not only in their number, but increase of glycogen in each cell was also remarkable in its quantity. The appearance of glycogen was correlated with the procedures through which a characteristic effect of Nitromin was revealed in the cytological features of the Yoshida sarcoma cells, as described in the previous papers.

(2) These characteristic changes failed to develop in the cases when a smaller amount than the minimum effective dose of Nitromin (17/gr.) was administered.

(3) This phenomenon in glycogen was of importance, correlating with the curative effect of Nitromin in the tumor bearing rats. Thus, for example, the animals in which glycogen disappeared in the tumor cells 72 to 96 hours after administration of Nitromin (17 to 107/gr.), died of tumor, accompanying a marked regrowth of the tumor cells in the ascites. On the other hand, the animals in which glycogen was kept to appear in the tumor cells, survived, representing a marked degeneration and disappearance of the tumor cells in the ascites. These were the usual procedures in the rats administered with the maximum tolerated dose of Nitromin (407/gr.).

(4) The effect on glycogen of several compounds was examined, including nitrogen mustard, benzyl-Nitromin, T.E.M., colchicine, 6-mercaptopurine, Sarcomycin and Carzinophilin. Nitrogen mustard and the derivatives represented the similar results to those obtained with Nitromin, although there were observed differences in the time and the period when glycogen appeared and increased after administration of the substances. Colchicine and Carzinophilin represented a particular effect on glycogen increase to some extent. In general, the same correlation between the dosages administered and glycogen effect could be revealed in each substance, except 6 MP.

Considering the results obtained, it is suggested that the increase of glycogen in the tumor cells of Yoshida sarcoma could be an useful criterion not only for determination of the MED of Nitromin and other compounds, but also for, cytological examination in a screening test for ant-cancer substances.

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