AN EXPERIMENTAL STUDY ON THE ACTION OF CARCINOSTATICS ON LIVER CATALASE ACTIVITY OF MALIGNANT TUMORS

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Carcinostatics, especially 8-azaguanine, have been studied basically and clinically for many years at our Department of Obstetrics and Gynecology.

In 1910, Blumenthal[1] reported the marked decrease of liver catalase activity of human liver carcinoma tissue and the adjacent normal looking liver tissue. This has been confirmed by Brahn,[2] Rosenthal,[3] Appleman[4] and many other investigators. Greenstein[5][6] and his group reported lately the remarkable decrease of liver catalase activity of not only cancer tissue itself but also individuals suffering from cancer. They also reported the recovery of liver catalase activity of individuals by removal or spontaneous healing of tumors and the decrease by retransplantation.

In order to examine the effects of carcinostatics such as nitrogen mustard N-oxide, 8-azaguanine and 6-mercaptopurine on liver catalase activity of normal rats and of ascites hepatoma transplanted rats and to investigate the difference in the action mechanism of each carcinostatic we conducted the experiments as follows.

Nitrogen mustard N-oxide will be abbreviated as NMO, 8-azaguanine as Aza and 6-mercaptopurine as 6-Mer.

MATERIALS AND METHODS

I) Experimental animals

We employed Gifu-hybrid female rats weighing 80 to 120 grams for this experiment. They were placed under the same condition for 2 weeks before the experiments.

II) Method of measuring liver catalase activity

The liver tissue of rats was removed immediately after they were killed. A 1% liver homogenate was prepared with 0.1 g of this liver tissue and 10 ccm of distilled water in a Potter Elvehjem's homogenizer by stirring in ice water for cooling. With 1 ccm of this homogenate, the liver catalase activity was measured by Euler and Josephson's method.[7] The figures are shown by the average for 3 rats except for the normal rats.

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1) Reagents

a) Preparation of substrate: 13.6 g of KH_2PO_4, 17.4 g of K_2HPO_4 and 1 ccm of 30% H_2O_2 were mixed and made by to a volume of 1 000 ccm by adding distilled water. The pH of this solution was 6.8.

b) 0.005 N solution of $KMnO_4$

2) Method of measurement

As shown in Fig. 1., 1 ccm of 1% liver homogenate was added to 50 ccm of the substrate previously cooled in ice water and mixed quickly. The mixture was then placed in 15 ccm of ice cooled 2 N H_2SO_4 in a corn flask to stop fermentation. The remaining H_2O_2 was titrated with 0.005 N $KMnO_4$, and in the same fashion 3, 6, 9 and 12 minutes after adding the homogenate.

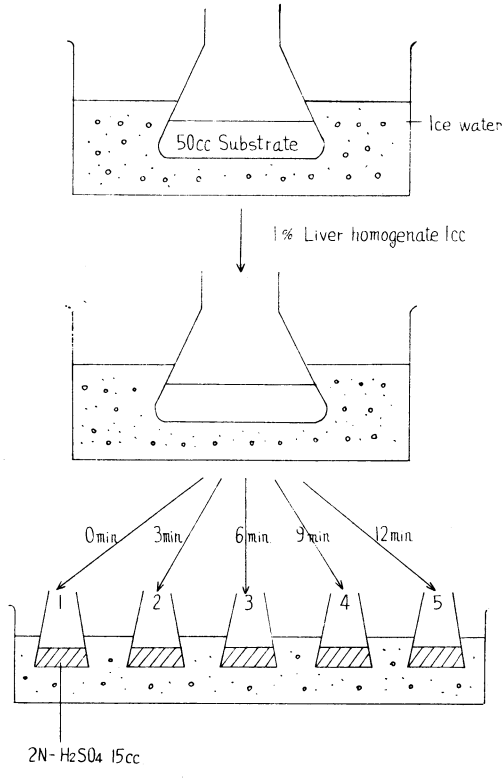

FIG. 1.

3) Method of calculation

The action of liver catalase activity is expressed by the velocity constant of the first order reaction measured under a constant condition.

$$K = \frac{1}{T} \log_{10} \frac{A}{A - X}$$

K: velocity constant, A: initial quantity of $KMnO_4$ required for titration, T: decomposition time $A-X$: quantity of $KMnO_4$ required for titration at T minutes. Therefore, catalase activity is defined as follows

$$\text{Catalase Activity} = \frac{K}{\text{Fresh tissue (g) in 50 ccm of reacting solution}}$$

RESULTS

1) Influence of carcinostatics on liver catalase activity of normal rats (Fig. 2).

Single doses of Aza 30 mg/100 g, NMO. 1 mg/100 g and 6-Mer 5 mg/100 g were injected into the abdominal cavities of 30 rats. The liver catalase activity was measured 24 hours later. The liver catalase activities of the Aza or 6-Mer injected group were almost the same as of normal rats, while the group treated with NMO showed decrease of liver catalase activity.

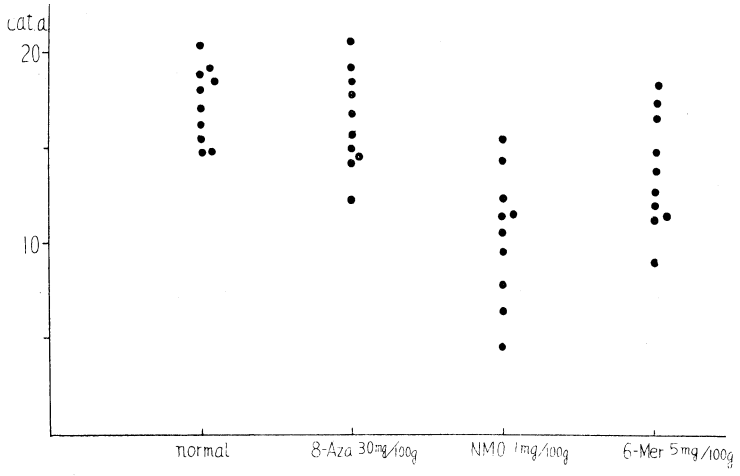

FIG. 2. Influence of anticancerous agents on liver catalase activity of rats.

II) Influence of carcinostatics on liver catalase activity of ascites hepatoma transplanted rats (Fig. 3).

The liver catalase activity of ascites hepatoma transplanted rats, as shown in Fig. 3, decreased markedly up to the 6th day of transplantation but stayed almost the same after the 6th day until death.

When the anticancerous agents Aza 30 mg/100 g, NMO 1 mg/100 g and 6-Mer 5 mg/100 g were injected into the abdominal cavity daily for 6 doses starting from the 4th day of transplantation, it was found that NMO hindered the decrease of liver catalase activity almost completely immediately after the in-

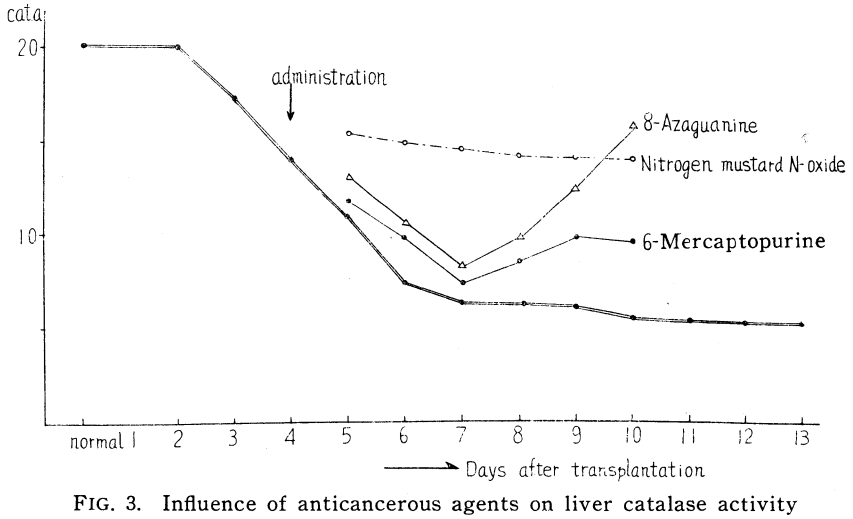

FIG. 3. Influence of anticancerous agents on liver catalase activity of ascites hepatoma transplanted rats.

jection. With Aza it decreased for 3 days after the injection and returned to surpass that of NMO. With 6-Mer the liver catalase activity was a little lower than Aza but it showed the same tendency as Aza.

III) Influence of combination of anticancerous agents and riboflavin on liver catalase activity of rats transplanted with ascites hepatoma (Fig. 4).

Flavin mono-nucleotide was used as riboflavin. This will be abbreviated as F.M.N.

Aza 10 mg/100 g, NMO 0.5 mg/100 g, Carcinophilin 250 u/100 g and Sarkomycin 10 mg/100 g were each injected into the abdominal cavities every day, starting 24 hours after the transplantation of ascites hepatoma. F.M.N. 5 mg/100 g was injected subcutaneously in the right side of the back at the same time. As shown in Fig. 4, in the group treated with the combination of anticancerous substances and F.M.N. greater hindrance in decrease of liver catalase activity was observed than in the groups treated with the anticancerous agents alone. Regarding the combination of the above anticancerous agents and F.M.N., the hindrance in decrease of liver catalase activity was greatest when Aza was used, followed by NMO, Carcinophilin and Sarkomycin in order. Especially, with Aza the temporary decrease in liver catalase activity was not observed and the hindering action was most remarkable.

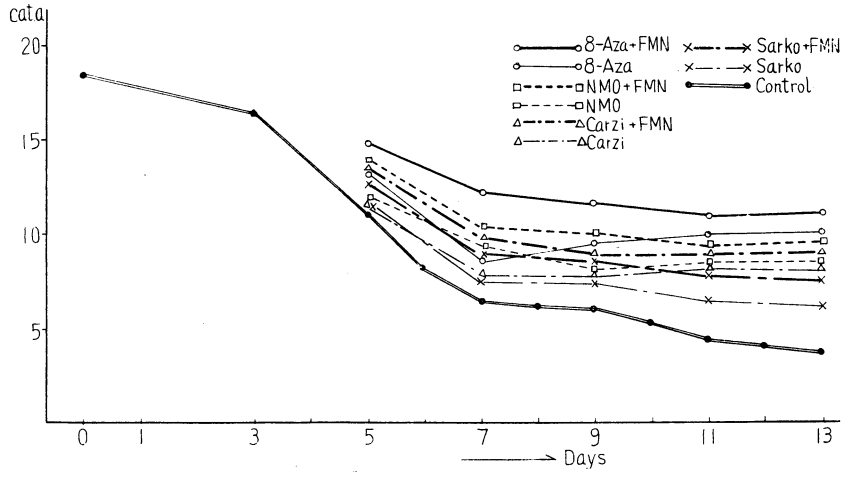

FIG. 4. The liver catalase activity of rats transplanted with hepatoma ascites at the treatments of various anti-cancerous agents alone and various anti-cancerous agents together with FMN.

COMMENT

As Kidder[8] stated, Aza affects only tumor cells and not normal cells as an inhibiting substance of purine metabolism. Therefore, Aza has almost no influence on liver catalase activity, while the decrease in liver catalase activity by NMO is apparently due to the fact that NMO affects both tumor cells and

normal cells as a cytotoxin.

The ascites hepatoma we used was less malignant than Yoshida sarcoma and the animals lived longer. Liver catalase activity markedly decreased until death with Yoshida sarcoma,[9] while in ascites hepatoma it decreased to only 1/3 to 1/4 that of Yoshida sarcoma by around the 6th day and stayed almost the same thereafter.

Regarding the influence of the injection of carcinostatics on liver catalase activity, the activity remained almost unchanged in case of NMO while with Aza it fell up to the 3 day, but at a higher level than that of the non-treated controls rising thereafter to above that of NMO (Fig. 3). This is presumably due to the difference in action mechanism between Aza and NMO. The action of Aza is slower and the affective time is different between the two carcinostatics. With 6-Mer the curve of liver catalase activity resembled that of Aza, only except that the level was slightly lower. The chemical structure of 6-Mer is also similar to Aza. Therefore we cannot expect more from 6-Mer than from Aza.

Regarding the combination of F.M.N. and the various anticancerous substances, marked inhibition of decrease in liver catalase activity was observed with Aza. This is presumably because of the fact that F.M.N. hinders the deamination of liver guanase.[10]

SUMMARY AND CONCLUSIONS

For the chemotherapy of malignant tumors, various carcinostatics were subjected to further investigation according to the result of screening tests. Evaluation of histological and cytological effects and prediction of prognosis were tried experimentally and clinically with these carcinostatics. In order to investigate the actions of the various carcinostatics and in combination with rivoflavin on liver catalase activity, which is believed to have a close relationship biochemically, we made the above experiments and obtained the following results.

1) Influence of carcinostatics on liver catalase activity of normal rats: Liver catalase activity was almost not affected by Aza, decreased markedly by NMO, with 6-Mer in between.

2) Liver catalase activity of rats transplanted with ascites hepatoma decreased gradually until the 6th day, to about 1/3 or 1/4 of the normal level and stayed almost the same thereafter until death.

3) Influence of carcinostatica on liver catalase activity of rats transplanted with ascites hepatoma: NMO hinderd the decrease in liver catalase activity to keep it at the same level. With Aza the liver catalase activity decreased gradually for 3 days and increased again to be above that of NMO. With 6-Mer, the curve of liver catalase activity was slightly lower than with Aza.

4) Combination of carcinostatics and F.M.N. is more effective than carcinostatics alone in hindering the decrease of liver catalase activity. With Aza this action was most marked, followed by NMO, Carcinophilin and Sarkomycin in order.

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