Effect of EMX on biological function of mouse treatment with anti-cancer drugs

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Summary
Cancer is currently occupies the first place among the causes of death in Japan, and anti-cancer chemotherapy (anti-cancer drug) and radiotherapy have been said to contribute to a curative rate of 7 to 10%. In anti-cancer treatments using chemotherapy and radiotherapy, inhibition of cell division and suppression of protein synthesis damage cancer cells, but these also injure normal cell at the same time. Therefore, adverse side effects such as allergic reactions, anorexia, alpaca, hematological toxicity, immunosuppression, and secondary carcinogenesis are serious problems in patients receiving anti-cancer therapies. In the present study, we examined the effects of EMX, an antioxidant substance extracted from fermentation of microbial effective materials, on the biological functions of mice treated with anti-cancer agents.

Five-week old mice were divided into 4 groups: a 6-MP control group given 6-mercaptopurine (anti-cancer agent) injection; an EMX-drinking group treated with 6-mercaptopurine and given EMX orally; an EMX-injection group treated with 6-mercaptopurine and given EMX by intraperitoneal injection; and an untreated control group. Treatments were conducted for 3 or 8 weeks. After two months, no significant differences in body weight were observed among the treated group.

After 3 weeks of treatment, significantly higher spleen index and bone marrow DNA content were observed in the EMX-drinking and EMX-injection groups compared to the untreated and 6-MP group. Thymus index tended to be higher in the EMX-treated groups compared to the control group, although no significant difference was found. Blood level of 8-hydroxy-2, -deoxyguanosine (8-OhdG), which is an indicator of oxidative DNA damage, were measured by enzyme-linked immunosorbent assay. Compared with the untreated group, the 8-OhdG level was 128.48% in the 6-MP group, 82.00% in the EMX-drinking group, and 54.02% in the EMX-injection group. The frequency of micronuclei in bone marrow, which indicate chromosomal aberration, was examined. The serum level of GOT and GPT, and liver level of lipid peroxide were measured.

The results in this study suggest that EMX given orally or by peritoneal injection suppress anti-cancer agent-induced damages of biological function including those of immune organs, and may prevent adverse side effects of anti-cancer agents.
Materials and Methods

1. Animal: 85-week-old DDY mice were divided into 50 and 30 mice and used in the experiment. Fifty mice were divided into 4 group: a 6-MP25 group injected intra-abdominally with 25mg/kg 6-mercaptopurine once a week; an EMX-drinking group treated with 6-mercaptopurine and given EMX diluted 1/500 in drinking water; an EMX-injection group treated with 6-mercaptopurine and injected intra-abdominally with 0.1 ml of EMX twice a week, and an untreated group. These mice were given treatments for 3 weeks. Thirty mice were similarly divided into 4 group: a 6-PM50 group injected intra-abdominally with 50 mg/kg 6-mercaptopurine once a week; an EMX-drinking group treated with 6-mercaptopurine and given 1/100 EMX in drinking water; an EMX-injection group treated with 6-mercaptopurine and injected intra-abdominally with 0.1ml of EMX twice a week, and an untreated group. These mice were given treatments for 8 weeks. During the treatment period, body weights were measured periodically. At the end of the treatment period, the mice were sacrificed, and thymus and spleen indices were measure.

2. Bone marrow DNA content: Measured according to the method of Liao Fang et al.

3. Micronucleus test: A mouse from each group was sacrificed and the left femur was extracted. Both ends of the femur were excised, and bone marrow samples were prepared according to the method of Hayasi, M et al. Fr Giemsa staining and acridine orange fluorescence staining. One thousand polychromatic erythrocytes (PCE) were counted, and the frequency of PCE containing micronucleus (MNPCE) was noted.

4. 8-hydroxydeoxyguanosine (8-OhdG): Measured using an enzyme-linked immunosorbent assay kit for 8-OHdG obtained from the Japan Control Research Institute.

5. Serum GOT and GPT: Measured by the UV method. 6. Lipid peroxide: Measured according to the method of Uchiyama et al.
Fig. 12. Comparison of the frequency of PCE containing micronucleus (MN-PCE) for different treatment groups 8 weeks after the start of 6-MP administration once a week at the rate of 50 mg/kg.

* Large letters of alphabet indicate group differences with statistical significance.

Fig. 8. Comparison of the treatment effects on thymus index 3 weeks after the start of 6-MP administration, once a week at the rate of 25 mg/kg.

Fig. 9. Comparison of the treatment effects on spleen index 3 weeks after the start of 6-MP administration, once a week at the rate of 25 mg/kg.

* Small letters of alphabet indicate group differences.

Fig. 13. Comparison of GOT content in serum for different treatments, 8 weeks after the start of 6-MP administration, once a week at the rate of 50 mg/kg.

* Small letters of alphabet indicate group differences with statistical significance.

Fig. 14. Comparison of GPT content in serum for different treatments, 8 weeks after the start of 6-MP administration.
Conclusion

1. From the observation in the present study, including body weight changes and hair loss, EMX suppressed the adverse side effects of the anti-cancer agent 6-mercaptopurine.

2. The anti-cancer agent 6-mercaptopurine strongly induced micronuclei indicating chromosomal (48/1000). By administering 1:100 EMX in water and twice a-week intra-abdominal injection of 0.1 ml of EMX, the frequency of micronuclei was reduced by 48.93 and 60.24%, respectively.

3. 8-OHdG is an indicator of oxidative DNA damage. Compared with the untreated group. The 8-OHdG level was 128.84% in the 6-MP25 group, 82.00% in the EMX-drinking group, and 54.02% in the EMX-injection group. EMX suppressed oxidative DNA damage in blood to levels lower than that in the untreated control group.

4. From the results of bone marrow DNA content, thymus index and spleen index, drinking of low-dose EMX or intra-abdominal injection of EMX suppressed the damages of immune organs induced by anti-cancer agent in growing mice, and also enhanced growth of these mice.

5. Most anti-cancer agents are metabolized in the liver, causing liver dysfunction. High dose of 6-MP increased the serum levels of GOT and GPT. The use of EMX mitigated the hepatotoxicity.

Quoted from: 10th Biennial Meeting of the Society for Free Radical Research International Monday 16th - Friday 20th October, 2000
Figure 1 Untreated control Group: no administration of anti-cancer drug. 2 months after the start of experiment.

Figure 2 Control Group. Injection of 6-MP anti-cancer drug, once a week at the rate of 50mg/kg, 2 months after the start of such as anorexia, and depressed growth were observed. Experiment
Figure 3 EMX Drinking Group: Injection of 6-MP anti-cancer drug, once a week at the rate of 50mg/kg treated with EMX drinking water at the rate of 1

Figure 4 EMX Injection Group: Injection of 6-MP anti-cancer drug, once a week at the rate of 50 mg/kg treated with 0.1 ml intraperitoneal injection twice a week, no adverse side effects of 6-MP
Figure 5 Micronucleus appeared inside the Polychromatic Erythrocyte of bone marrow for the groups given 6-MP

Figure 6 Micronucleus appeared inside the Normochromatic erythrocyte of bone marrow for the groups given 6-MP. Adverse side effects