Cancer promoters and germ cell toxicity of dimethylnitrosamine. Effects of phenobarbital and saccharin.

Dalisay Chionglo Sy & Lumina Eugenio

Department of Biochemistry
Faculty of Medicine & Surgery
University of Santo Tomas, Manila

Studies in the past have shown the enhancing actions of phenobarbital and saccharin on somatic cell toxicity of dimethylnitrosamine. This paper deals with the effects of the two substances on the genotoxicity of DMN. Nine to 10 weeks old Strong A male mice were treated with DMN at zero time, followed by phenobarbital and saccharin after one hour. One week after treatment, animals were mated with virgin females and subsequently impregnated mice were observed for dead implants and dominant lethals. Average total live embryo for animals treated with DMN and phenobarbital or saccharin did not show significant difference from controls. However, animals that received DMN and saccharin exhibited marked increase in percent dead implants compared to controls. Those that received DMN and phenobarbital showed appreciable increase in the percent dead implants only at the phenobarbital dose of 120 mg per kg Body Weight.

Key words: promotion, germ cell toxicity, phenobarbital, saccharin

Previous studies in our laboratory have shown the enhancing action of phenobarbital and saccharin on micronuclei production in Strong A mice treated with dimethylnitrosamine (DMN) and mitomycin C respectively (1, 2). That this effect of the cancer promoters holds true not only in somatic cells but possibly in germ cells will be most significant. A genotoxic defect brought about by DMN and enhanced by phenobarbital and saccharin will have many implications, since both substances have their uses in medicine: phenobarbital as an anti-epileptic drug and saccharin, as a sugar substitute, although its use as such has since been regulated.

Dimethylnitrosamine, a known mutagen has been shown to be formed from secondary amines found in tobacco leaves (3). It is metabolized by liver enzymes, leading to release of carbocations which are active alkylating agents of DNA. It is therefore interesting to note the possibility that individuals receiving or using phenobarbital or saccharin and who are at the same time exposed to DMN through smoking or some other ways may manifest the genotoxic effects these compounds can induce. This study hopes to add to the growing information on the topic.

MATERIALS AND METHODS

Animals. Strong A male mice, 9-10 weeks old were utilized in the experiment. Virgin females, 11-15 weeks old were mated with the treated males in a 1:1 ratio. Animals were maintained on pigeon pellets (containing 18% protein) throughout the experiment. Water was given ad libitum.

Chemicals. DMN and saccharin were obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. Phenobarbital injections were purchased from local drugstores and used to prepare the working standards, using triple distilled water as diluent.

Treatment of Animals. Animals were divided into experimental and control groups. In the experimental group, male mice were injected i.p. with DMN at zero time, followed by phenobarbital or saccharin (as the case may be) after 1 hour. In both cases, DMN concentration of 10 mg/kg BW was used as determined in earlier studies (1). Animals belonging to the control groups were injected separately, i.p. with phenobarbital, saccharin and water. One week after the treatment, both control and experimental animals were mated with virgin females. Mating was established by the presence of vaginal plug. Progressive increase in the weights

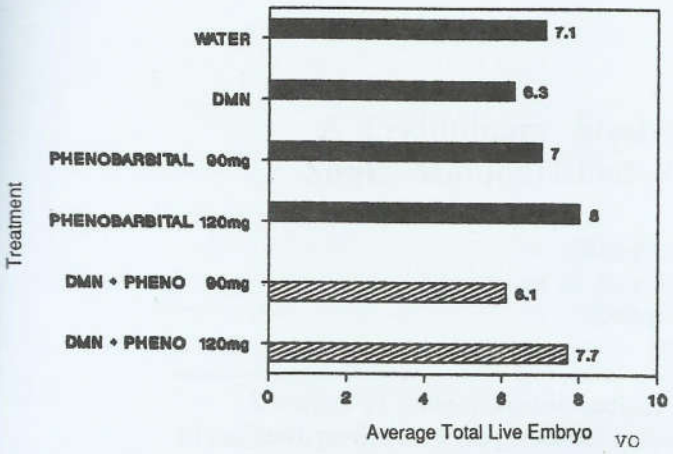

Fig. 1. Effect of phenobarbital on the genotoxicity of DMN

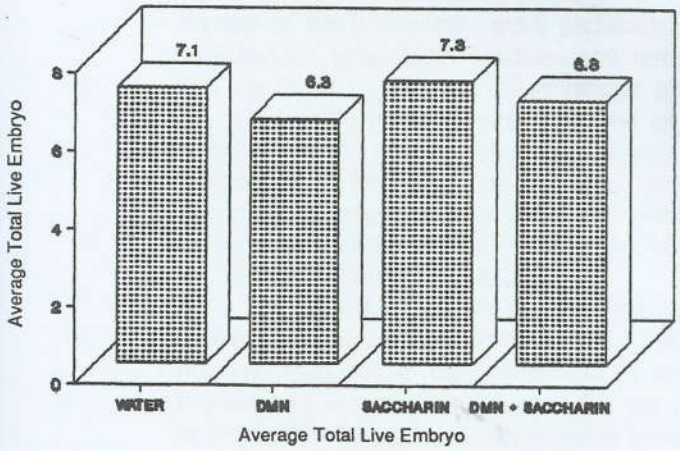

Fig. 2. Effect of saccharin on the genotoxicity of DMN

of female mice was also noted. Nineteen to twenty days after the vaginal plugs were seen, the pregnant mice were killed by cervical dislocation and litters collected. Number of dead implants, resorptions, still-births and total live embryos were noted.

RESULTS AND DISCUSSION

The effects of phenobarbital and saccharin on germ cell toxicity of DMN are compared. Average total live embryo and percent dead implants were calculated as follows;

$$\text{Average Total Live Embryo} = \frac{\text{Total Live Implants}}{\text{Total Implants}}$$

$$\text{Percent Dead Implants} = \frac{\text{Number Dead Implants}}{\text{Total Implants}} \times 100$$

Figures 1 and 2 show that the average number of total live embryo in females mated with phenobarbital-treated and saccharin-treated male mice did not indicate any appreciable effect on the genotoxicity of DMN. However, percent dead implants, as evident in Figures 3 and 4 was increased in similarly-treated animals. In the DMN and saccharin experiment, DMN and water controls gave values of 2% and 0.9% as against experimental animals that gave a value of 6.8%. On the other hand, phenobarbital controls gave percent dead implants of 9.3% for animals that received 90 mg/kg BW phenobarbital. Experimental animals gave values of 7.6% and 13.3% respectively for 60 mg and 120 mg phenobarbital concentrations. This represents a 75% increase in dead implants when phenobarbital is given at a dose of 120 mg/kg BW. Since results obtained represent the effects of the two substances on the first two weeks of spermatogenesis, it is possible that percent total live embryo pattern is not as sensitive as the percent dead implants at this period. Further experimentation involving the latter stages of spermatogenesis may give a different picture.

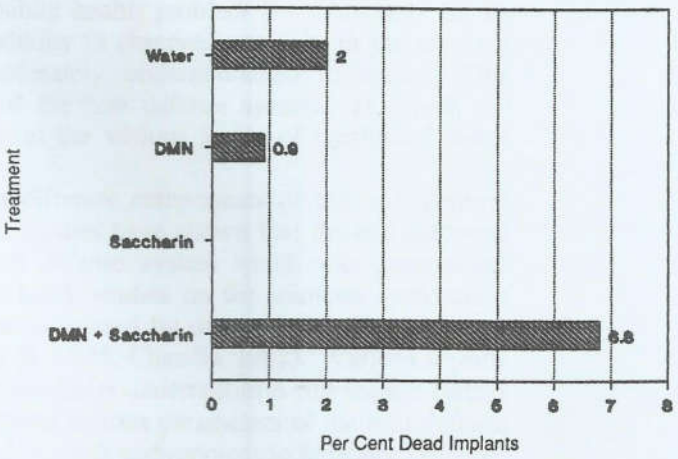

Fig. 3. Enhancing action of saccharin on the dominant lethality of DMN

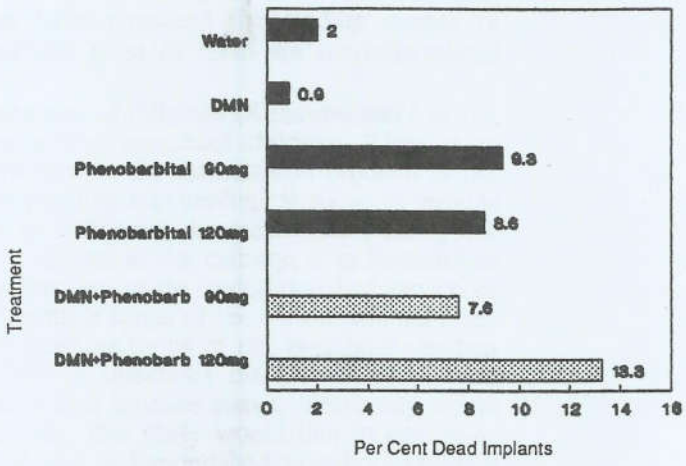

Fig. 4. Enhancing action of phenobarbital on dominant lethality of DMN

LITERATURE CITED

1. Sy, Dalisay Chionglo and Clara Lim Sylianco. Effect of the cancer promoter saccharin on the mutagenicity of mitomycin C. Acta Manilana Vol. 37. p. 21-25, 1988

2. Sy, Dalisay Chionglo. 1989. Cancer promoters and their effect on the mutagenicity of mutacarcinogens. *Environmental and Molecular Mutagenesis*. vol. 14. Supplement 15. p. 568. Alan Liss, N.Y.

3. Sylianco, CYL and Sylianco-Wu, Lorraine. 1990. *Modern Biochemistry*, Aurum Technical Books, Quezon City, p. 57.