Effect of Ascorbic Acid on Saccharin-enhanced Mutagenicity of Mitomycin C

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Effectiveness of ascorbic acid in inhibiting the enhancing effect of saccharin on the mutagenicity of mitomycin C was demonstrated. Eight to nine weeks old Strong A male mice were injected i.p. with mitomycin C at a concentration of 3 mg/Kg BW. This was followed 3h after by i.p. injection of saccharin, 2.5 g/Kg BW. Two groups of experimental animals were utilized: one, receiving ascorbic acid lh after saccharin injection and the second group, lh before administration of saccharin. Both groups were sacrificed 30h after mitomycin C treatment and bone marrow cells isolated following Schmid's procedure. Cells were smeared, stained with May Grunwald Giemsa and scored for micronuclei. Animals that received ascorbic acid prior to saccharin treatment showed 48% reduction in the number of micronuclei. In contrast, animals that received the vitamin after saccharin injection showed 3.87% reduction. Results suggest the effectivity of ascorbic acid in diminishing the effect of mitomycin C when the vitamin is given before but not after saccharin administration.

Key words: Mitomycin C, mutagenicity, ascorbic acid, saccharin

For years, man has been in constant search for agents that will protect him against the effects of carcinogens. More interest was generated along this field with the discovery that human carcinogens are so ubiquitous that they are found even in man's diet (1, 14). One such protective agent is ascorbic acid or vitamin C, which has been widely known to possess anti-carcinogenic activity (3, 4, 5, 8, 12, 15). Studies have shown that it prevented formation of carcinogenic nitrosamines, N-nitroso compounds (2, 10, 11) and aromatric amines (9). This paper shows the effect of ascorbic acid on saccharin-induced mutagenicity of mitomycin C. Influence of administration time on the effectivity of vitamin C was also demonstrated.

MATERIALS AND METHODS

Animals and Chemicals

Strong A male mice, 8-9 weeks of age with average weights of 17g were used.

Mitomycin C in 2 mg vial was obtained from Kyowa Hakko Co. Ltd. Solutions for i.p. injections were prepared by dissolving the dry powder in sterile distilled water. Animals received concentrations of 3 mg/Kg BW.

Pure crystalline L-ascorbic acid (C-Vit Wander Phils. Zuellig Pharma) 500 mg per 2 mL potency was mixed with sterile distilled water and given at a dose of 3g per Kg BW.

Fetal calf serum was obtained from the Hong Kong dealer of Gibco (Grand Island Biochemical Co., New York).

Saccharin was purchased from Aldrich Chemical Co., Milwaukee, Wisconsin. Crystals were dissolved in sterile distilled water and injected i.p. at a concentration of 2.5g per Kg BW.

Treatment of animals

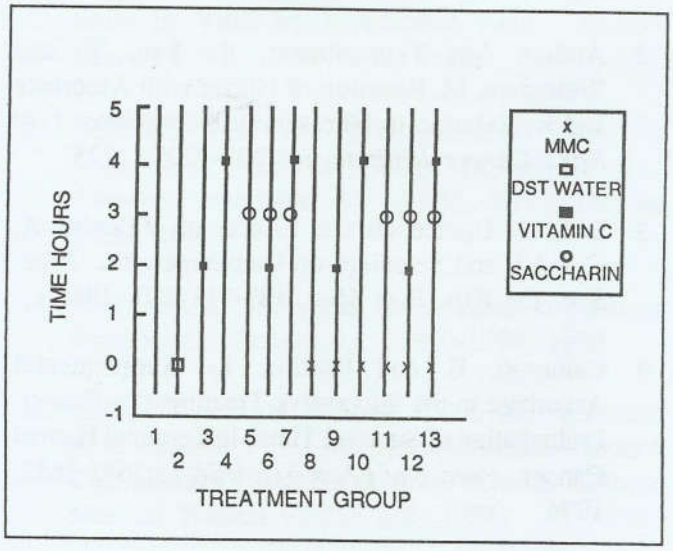
Treatment combinations utilized in the experiment are shown. Treatment 1 is Control A, wherein animals did not receive any injections. Treatment 2 is Control B where only sterile distilled water was injected. Treatment 3 received vitamin C on the second hour while Treatment 4 received it on the 4th hour. For treatment 5, only saccharin was given to the animals. In all cases, saccharin was constantly

given on the 3rd hour. In treatment 6, saccharin was given with vitamin C on the 2nd hour. In treatment 7, animals also received saccharin with vitamin C, the latter given on the 4th hour. Animals with treatment 8 received mitomycin C which was constantly given at zero time. Animals under treatment 9 received mitomycin C combined with vitamin C on the 2nd hour while those in treatment 10 received the same with vitamin C given on the 4th hour. For treatment 11, mitomycin C was combined with saccharin, injections given to the animals at zero hour and 3h respectively. In treatments 12 and 13, mice received mitomycin C and saccharin combined with vitamin C. Animals in treatment 12 received vitamin C on the second hour while those in treatment 13 received vitamin C on the 4th hour.

Isolation of Bone Marrow Cells

Procedure followed was the same as that from previous experiments (6) which was adopted from Schmid (13). Mice were sacrificed by cervical dislocation 30h after injection of mitomycin C. Both femora were cut and cleansed of adhering muscles. Bone marrow contents were flushed out using 0.6 ml fetal calf serum. Solutions were centrifuged at 5000 rpm. Smears were prepared and stained with May Grunwald Giemsa. Scoring for micronuclei was done under oil immersion, 100 x 15 magnification.

Treatment of Animals



Statistical Analysis

Data were analyzed statistically using the Pooled Analysis of Variance for a three factor factorial in complete random design. Treatment means were compared using Duncan's Multiple Range Test. The Student's Test was used to compare trials made.

RESULTS AND DISCUSSION

Figure 1 shows the mean number of micronuclei in mitomycin C-treated animals and control groups. Animals that received separate injections of vitamin C and water (Control B) showed very little difference in the total number of micronuclei as compared to animals that received no injection at all (Control A). Mitomycin C-treated animals showed a mean number of 13.62 micronuclei.

The enhancing action of saccharin on mitomycin C is shown on Figure 2. Animals that received both mitomycin C and saccharin gave an average of 20.91 micronuclei, as compared to those receiving mitomycin C alone with a value of 13.62. Controls, including that for saccharin, gave values of 5.08 and lower. This is in agreement with observations that saccharin acts on the promotion stage of carcinogenesis rather than at the initiation stage. Alone, saccharin does not seem to exert any mutagenic effect. But when given after the administration of a mutagen, in this case, mitomycin C, saccharin increased the mutagenicity as shown by increased number of micronuclei compared to that produced by mitomycin C alone.

Figure 3 shows the effect of vitamin C on the action of mitomycin C. The vitamin causes decrease in the number of micronuclei under 2 conditions: when injected 2h and then 4h after treatment with mitomycin C. However, it can be noted that the time of vitamin injection made a difference in its effectivity. When injected 2h after giving mitomycin C, average number of micronuclei was 8.83, compared to the value of 11.71 obtained when vitamin C was given 4h after mitomycin C administration. In the absence of vitamin C, mean number of micronuclei of 13.62 was obtained.

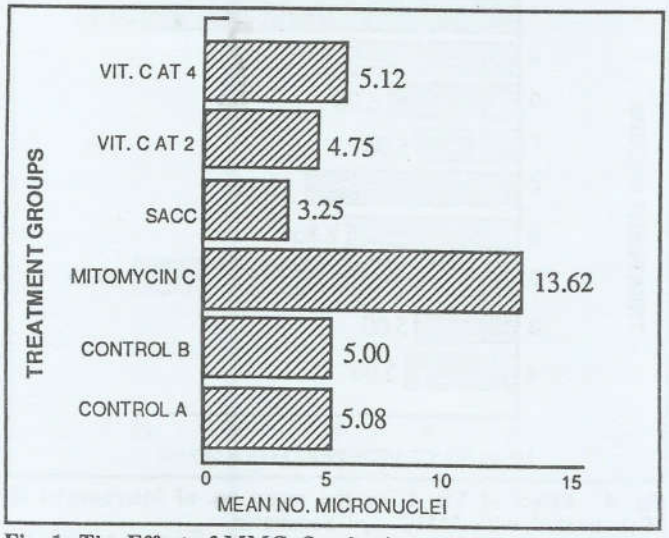


Fig. 1 The Effect of MMC, Saccharin and Vit. C on the mean no. of Micronuclei in Mice.
Control B - Mice were injected with water
Control A - Mice did not receive anything

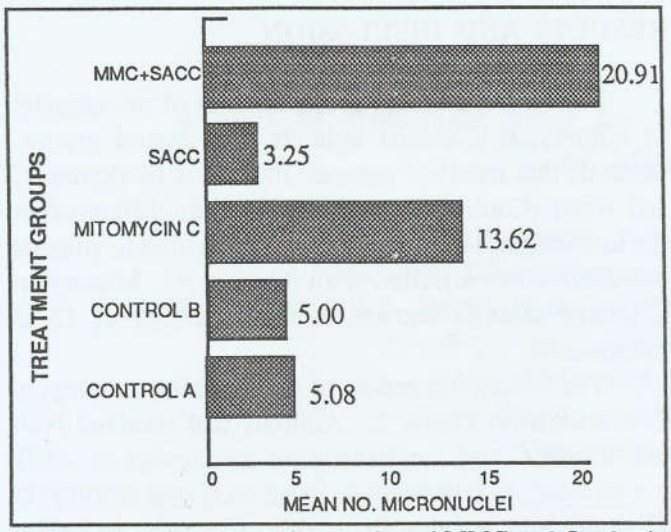


Fig. 2 The effect of combined action of MMC and Saccharin on the mean no. of Micronuclei in Mice
Control B - Mice were injected with water
Control A - Mice did not receive anything

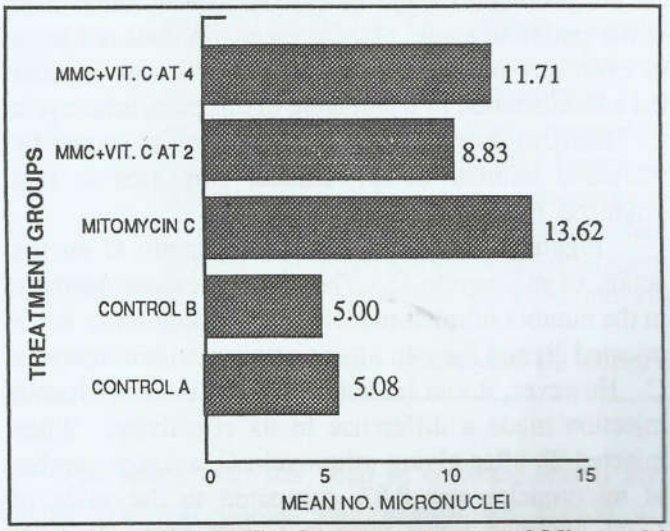


Fig. 3 The effect of Vit. C on the mean no. of Micronuclei in Mice treated with MMC alone.

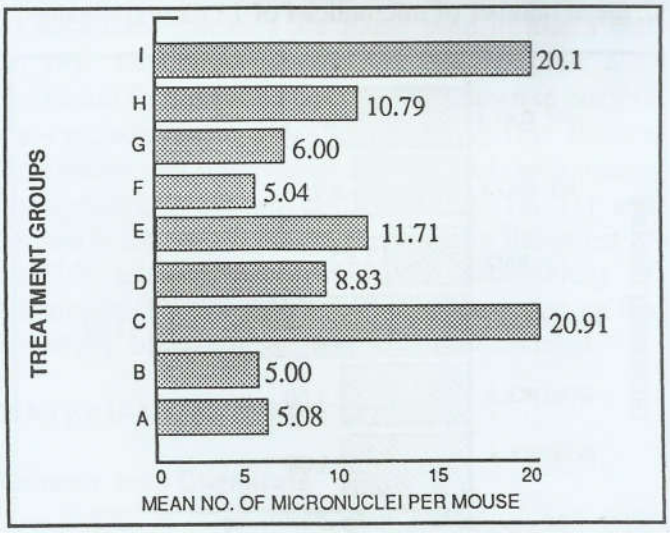


Fig. 4. Effect of Vit. C on the mean no. of Micronuclei in Mice treated with MMC and Saccharin.
Legend: A Control A, B Control B, C MMC + Saccharin, D, MMC + Vit. C at 2, E, MMC + Vit. C at 4, F, Saccharin + Vit. C at 2, G, Saccharin + Vit. C at 4, H, MMC + Vit. C at 2 + Sacch at 3, I, MMC + Sacch at 3 + Vit. C at 4

This effect of vitamin C is also seen when mutagenic activity of mitomycin C is enhanced by the administration of saccharin. It is evident in Figure 4 that when ascorbic acid was given one hour before the administration of saccharin (treatment H), the average number of micronuclei decreased to a value of 10.79. In the same way when ascorbic acid was given one hour after saccharin administration, Figure 4 (treatment I) shows the mean number of micronuclei to be 20.1. In contrast, mitomycin C and saccharin gave a value of 20.91 micronuclei. As the study indicates in treatment G, very little decrease in the average number of micronuclei was obtained representing only 3.87% lowering. The one hour treatment (H) showed 48.39% decrease in the number of micronuclei. This suggests the effectivity of vitamin C in cancer promotion (or mutagenicity) when it is given at an earlier period, when the damage has not been done. When promotion has started and progressed (as after saccharin has been given) the effect of vitamin C is minimal. The anti-mutagenic (anti-carcinogenic) effect of vitamin C can be said to be better when given before a promoter has had its effect. Statistical methods used, Pooled Analysis of Variance for a three factor factorial, as well as Duncan's Multiple Range Test for treatment means gave significant levels of 5%.

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