Cytogenetic Test of Captan and Triethylenemelamine in Mouse Bone Marrow

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CYTOGENETIC TEST OF CAPTAN AND TRIETHYLENEMELAMINE\textsuperscript{1}

IN MOUSE BONE MARROW

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\textsuperscript{1}In partial fulfillment of requirements of a BS Degree with Honors in The Honors College of WMU.

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SUMMARY

Following a single non-toxic i.p. injection with 0.5 or 1.0 mg triethylene melamine/kg a significant increase in chromosome aberrations in mouse bone marrow was observed 6, 12, 30, but not 54 h after treatment. A single i.p. injection with 250 mg captan/kg caused up to 68% lethality above controls, but no significant increase in chromosome aberrations after the same time intervals. One metacentric chromosome was observed 6, 30, and 54 h after captan treatment among 300, 300, and 101 metaphase spreads respectively, whereas none appeared in 1495 control spreads.
INTRODUCTION

Captan is an extensively used fungicide known to produce base-pair substitutions in *Escherichia coli* (1, 4, 8, 13), *Salmonella typhimurium* (3, 7, 9, 17, 18), and *Neurospora crassa* (14, 15) and chromatid and chromosome breaks in cell cultures (13). Different studies using rats and mice gave conflicting results with respect to dominant lethal mutations (5, 6, 12). A recent review of captan by Bridges (2) pointed out the need for additional information on the possible genetic effects of captan in vivo.

This study reports results of bone marrow cytogenetic tests with captan in mice. The clastogenic alkylating agent, TEM, was used as positive control.

Abbreviations: TEM, triethylenemelamine.
MATERIALS AND METHODS

Animals and chemicals

Random bred Upjohn strain Swiss albino male mice weighing 25-40 g were used. Captan 50 WP containing 50% technical captan lot # 01007-10, CAS # 133-06-2 was obtained from the Stauffer Chemical Co. and TEM from Pfaltz and Bauer Inc.

Cytogenetic analysis of bone marrow metaphases

All chemicals were injected i.p. into four test groups of animals. Animals of the first group were injected with 1 ml of 0.9% saline. The second group was similarly injected with 250 mg captan/kg and the last two groups with 0.5 and 1 mg TEM/kg. Animals from each treatment group were killed by cervical dislocation at 6, 12, 30, and 54 h following injection. Five h prior to killing, the animals were injected i.p. with 1 mg/kg colchicine in 1 ml 0.9% saline. With a 23 G needle 1.5 ml of 2.2% sodium citrate was injected through each end of the excised femurs. The bone marrow cells were suspended and collected by centrifuging at 1000 rpm for 5 min. The supernatant was discarded leaving about 0.25 ml of fluid in the test tube. The cells were thoroughly suspended and 3 ml of 37°C 0.56% KCl hypotonic solution was added and the suspension incubated in a water bath at 37°C. In the last 5 min of the 30 min hypotonic treatment the cells were collected as above. Three ml fresh fixative (3 parts absolute methanol : 1 part glacial acetic acid) was slowly added with constant agitation to prevent cell clumping. Additional mixing was achieved with 5 aspirations. The fixative was changed three times. During the last resuspension enough fixative was added to give a turbid cell suspension.

Grease free microscope slides previously placed in a freezer were allowed to collect condensation. Three to four drops of cell suspension were placed
on each slide and immediately passed through a flame allowing the methanol to burn completely. Once completely dry the slides were flooded for 4-5 min with fresh Giemsa stain prepared by mixing 5 ml Giemsa stock solution, 6 ml acetone, and 100 ml tap water. Giemsa stock solution contained 3.6 g Giemsa powder (Polysciences Inc.) dissolved in 250 ml glycerol and 250 ml methanol.

One hundred cells per animal were scored from randomized slides when possible. A gap was an achromatic lesion less than the width of a chromatid. Gaps and isochromatid gaps were not scored separately. Fragments were scored as a single chromatid break. Cells with more than 10 breaks were scored as multiple breaks. A typical metaphase spread from control mice is shown in Fig. 1.

Statistical analysis

The significance of differences in the frequencies of chromosome aberrations with captan and TEM in comparison to the control was determined using the method described by Kastenbaum and Bowmen (11).

RESULTS AND DISCUSSION

Toxicity

Shortly after injection of captan toxicity was noted. Signs included edema, decreased body temperature, sluggishness, and bluish skin color. In 6, 12, 30, and 54 h captan treatment lethality increased over control animals by 0, 25, 20, and 68% respectively. TEM treated animals showed no signs of toxicity.
**Time and concentration response**

Six and 12 h following i.p. injection of 0.5 mg TEM/kg chromatid breaks were predominant over chromosome breaks or rearrangements (Fig. 1; Table I). In agreement with the present results, in mice (10) and rat (16) bone marrow, the most TEM-induced chromosome aberrations were detected 30 h after treatment. At this time interval a similar frequency of chromatid and chromosome type aberrations were observed. Predominance of chromatid breaks in metaphases examined at the shortest intervals after treatment (6 and 12 h) indicates that the majority of breaks were induced during the $G_2$ phase. A similar frequency of chromatid versus chromosome type aberrations (breaks, rearrangements, and rings) at 30 h suggests that aberrations in both $G_1$ and $G_2$ cells were induced (Table I).

One mg TEM/kg induced a higher percent of cells with aberrations at 12 and 30 h than 0.5 mg/kg. The increase at 12 h was the greatest for rings and rearrangements suggesting that the increased dose was more likely to induce double-breaks necessary for these aberrations. Fiftyfour h after treatment chromosome damage was not observed with either dose indicating that TEM induced aberrations cause cell lethality and/or are not transmitted (10, 16).

At no time did the incidence of chromosome aberrations in animals treated with captan deviate significantly from the control (Table I). However, one metacentric chromosome (Fig. 1) did appear at 6, 30, and 54 h in 1001 captan treated metaphases, whereas none appeared in 1495 control spreads. Because of these unique chromosome rearrangements it cannot be determined with certainty that 250 mg captan/kg i.p. does not break chromosomes in vivo.
ACKNOWLEDGEMENTS

The authors are grateful to the Stauffer Company for the gift of captan of known production batches. We also appreciate the support to S.F. from the Russel H. Seibert Fund and the valuable suggestions of Drs. L. Beuving, S. Friedman, and M. McCarville.
REFERENCES


EVALUATION OF CONTROL, CAPTAN, AND TEM TREATED MOUSE BONE MARROW METAPHASES SAMPLED AT DIFFERENT TIME INTERVALS.

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<th>Rearrangements</th>
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a p < 0.05; significant
b p < 0.01; highly significant