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MUTAGENICITY AND CLASTOGENICITY POTENTIAL OF MEXAFORM*

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ABSTRACT

Mexaform, an anti-amoebic drug, was used in this study. By Rec Assay, it was shown to possess slight DNA damaging capacity. However, by the use of the Ames test, it was not mutagenic before metabolic activation. After metabolic activation, it is transformed to a base-pair mutagen. It also possesses chromosome breaking effects, as measured by the number of micronucleated polychromatic erythrocytes in bone marrow cells of mice. It is therefore, a clastogen. Its clastogenic property, however, was greatly reduced by cysteine.

Introduction

Mexaform, which contains a hylogenated hydroxyquinoline, is used for the treatment of amoebic dysentery. The parent compound, quinoline, was found to be a hepatocarcinogen in rats (1).

Since the drug can be purchased over the counter, many who are involved in self-medication are exposed to it. It is, therefore, imperative to study the effects of this drug on DNA, the genetic substance of the living cell.

Review of Literature

Mexaform is a luminal or contact amoebicide that contains two active components, 7-iodo-5-choro-8-hydroxyquinoline, the major active component and 4,7-phananthroline -5,6-dione (2).

Studies in Japan established a relationship between mexaform and a neurological syndrome described as sub-myelo optic neuropathy (SMON). This was attributed to iodochlorhydroxyquinoline, the major component of mexaform (3).

When fed to rats, iodochlorhydroxyquinoline produced benign and malignant tumors of the liver (4).

Another quinoline derivative, 8-hydroxyquinoline, induced chromatid aberrations in human leukocytes (5).

This study is undertaken to find out if mexaform is genetically toxic.

Materials and Method

Mexaform used in this study was a Ciba-Geigy Ltd. product. Bacillus subtilis mutants for the Rec assay were gifts from Dr. T. Kada, National Institute of Genetics, Mishima, Japan. Salmonella typhimurium strains 1535, 1537, 98, and 100 were obtained from Dr. Bruce N. Ames, Department of Biochemistry, University of California, Berkeley. Salmonella typhimurium G46 was a gift from Dr. Masaaki Moriya, Tokyo, Japan.

Fetal calf serum was obtained from Grand Island Biological Co., New York. Giemsa and May-Grunwald stains were Merck products. The experimental mice came from the Bio-organic colony of the Department of Chemistry, University of the Philippines, Diliman, Q.C.

Rec assay was done based on the method of Kada (6). Cultures of wild and recombinant deficient strains were streaked on broth agar plates from one point. Mexaform suspension was applied to filter paper discs which were placed on that point. Zones of inhibition were measured after 20 hours of incubation at 37°C.

Mutations induced without metabolic activation were studied using the method of Ames (7). Salmonella typhimurium mutants TA 1535, TA 1537, TA 98 and TA 100 were used. In this method, the bottom of a sterile petri dish was covered with minimal bottom agar containing Vogel Bonner E medium. A mixture of 0.1 ml suspension of the test organism with 2 ml of molten top agar premixed with histidine-biotin solution was poured into the hardened bottom agar. After the top agar hardened, a sterile paper disc with a diameter of 8 mm, containing mexaform suspension was placed in the center of the plate. Different concentrations of mexaform were used. The plates were incubated for 48 hours at 37°C after which the revertant colonies visible as small, white opaque dots were counted.

To find out if mexaform is metabolized to mutagens or to non-mutagens, the host-mediated assay of Legator and Gabridge (8), was employed. This is a combination of in-vivo mamalian metabolism and microbial mutation tests. An indicator bacteria, Salmonella typhimurium G 46, is injected into the peritoneal cavity of the experimental mouse and mexaform is administered to the animal orally by gavage. After 3 hours, when the bacteria have come in contact with the metabolites of mexaform, they are withdrawn from the peritoneal cavity and induced mutation frequency is determined.

Chromosome breaking effects of mexaform was determined using the micronucleus test of Schmid (9). It is an in-vivo method for screening chemical systems for cbromosome breaking effects. Mexaform was administered intraperitoneally. A subacute treatment over 30 hours was chosen. Applications were given 30 and 6 hours before the animal was killed. Mice, 7-12 weeks old were used. Micronucleated polychromatic erythrocytes were scored for every thousand erythrocytes.

Table 1. DNA Damaging Capacity of Mexaform as Measured by the Rec Assay Using Bacillus subtilis Strains H17 and M45.*

Test system	*Strain*	*Zone of Inhibition mm*
Mexaform	H17 Rec+	2.18
	M45 Rec–	4.97
Quinoline**	H17 Rec+	22.59
	M45 Rec–	29.21
Control	H17 Rec+	0.00
	M45 Rec–	0.00

*About 0.05 ml of mexaform suspension was used on paper disc.
**Quinoline is used as positive control. (0.02 ml of a solution 1 mg/ml)

Table 2. Mutagenicity Potential of Mexaform Without Metabolic Activation Using Salmonella typhimurium TA 1535

Test System	*Revertants per plate**
Pure mexaform	3.96
90% mexaform in corn oil	6.52
80% mexaform in corn oil	4.88
Ethylmethane sulfonate**	Too numerous to count
Control	7.63

*Average of 25 plates
**Positive control

Results and Discussion

Table 1 shows that mexaform has DNA damaging capacity. This is indicated by the greater inhibition of growth of the Rec strain which does not possess the recombination repair system. The Rec strain was also inhibited to a lesser extent. Although it has the recombination repair system, it could not completely cope up with the stress given by high concentrations of mexaform.

The data depicted in Tables 2 to 5 are the results of the tests using the Ames method to determine whether mexaform interacts with DNA without metabolic activation. The data suggest that mexaform does not react directly with DNA with-

Table 3. Mutagenicity Potential of Mexaform Without Metabolic Activation Using Salmonella typhimurium TA 1537

Test System	*Revertants per plate**
Pure mexaform	8.84
90% mexaform in corn oil	3.60
80% mexaform in corn oil	3.81
9-aminoacridine**	Too numerous to count
Control	9.24

*Average of 25 plates
**Positive control

Table 4. Mutagenicity Potential of Mexaform Without Metabolic Activation Using Salmonella typhimurium TA 100

Test System	*Revertants per plate**
Pure mexaform	98.52
90% mexaform in corn oil	76.27
80% mexaform in corn oil	65.96
Ethylmethane sulfonate**	Too numerous to count
Control	106.17

*Average of 25 plates
**Positive control

out metabolic activation. It is not a direct base-pair mutagen nor a direct frameshift mutagen.

The results of the host-mediated assay are shown in Table 6. The data show that the test organism was reverted to prototrophy by metabolites of mexaform. Thus mexaform is metabolized to a mutagen in the experimental mouse. This has some bearing with the biotransformation iodochlorhydroxyquinoline to its metabolites in the liver and the kidney (10). Urakubo and coworkers identified 5-chloro-8-hydroxyquinoline as a metabolite (11). This was shown to be mutagenic to Salmonella typhimurium TA 98 and TA 100 in the presence of liver enzymes (12). Hollstein and coworkers (13) proposed that quinoline is converted to a 2, 3-epoxide by human and rat liver enzymes. This intermediate actively alkylates DNA.

The effect of mexaform at the chromosomal level in the bone marrow cells can be analyzed from the data shown in Table 7. The data based on the micronucleus test reveal chromosome breaking effects of mexaform. The micronucleus test is based on the principle that mitotic cells with chromatid breaks lag behind when the centric elements migrate towards the spindle poles and that after telo-

Table 5. Mutagenicity Potential of Mexaform Without Metabolic Activation Using Salmonella typhimurium TA 98

Test System	*Revertants per plate**
Pure mexaform	36.48
90% mexaform in corn oil	25.87
80% mexaform in corn oil	22.19
Malathion**	Too numerous to count
Control	39.56

*Average of 25 plates
**Positive control

Table 6. Mutagenicity Potential of Mexaform After Metabolic Activation Based on Host-Mediated Assay Using Salmonella typhimurium G46 as Indicator Organism

Test System	*Revertants/10^8 survivors*
Mexaform	19.87
Dimethylnitrosoamine**	43.10
Control	1.10

**Positive control

phase, a sizable portion of such displaced chromatid is not included in the nuclei of the daughter cells but forms a single or multiple micronuclei in the cytoplasm of these cells. A few hours after their last mitosis, erythroblasts expel their nuclei but the micronuclei remain behind in the cytoplasm. Staining allows to distinguish micronucleated polychromatic erythrocytes.

It is therefore suggested that mexaform is metabolically transformed to a clastogenic metabolite. Thus mexaform can cause damage of the chromosomes. This is in agreement with the finding by others (14) that quinoline and its derivatives can cause gaps and fragmentation of chromosomes.

The effect of cysteine on the mutagenic and clastogenic potential of mexaform is shown in Table 8. The reducing effect of cysteine shows that it is an antimutagen to mexaform. The presence of the sulfhydryl group in cysteine allows it to be very reactive with mexaform metabolites that possess electrophilic sites. An epoxide metabolite can be readily inactivated by cysteine, preventing its tendency to alkylate some bases of DNA.

Summary

Mexaform possesses DNA damaging capacity. Although it is not a direct mutagen, it is metabolized to a base-pair mutagen. Aside from its being mutagenic

Table 7. Clastogenicity Potential of Mexaform on Bone Marrow Cells

	Number of micronucleated polychromatic Erythrocytes per thousand
Mexaform	
4 ml/kg	6.45
6 ml/kg	7.68
8 ml/kg	10.86
Control	2.34

Table 8. Effect of Cysteine on the Clastogenicity Potential of Mexaform

	Number of micronucleated polychromatic Erythrocytes per thousand
Mexaform (8 ml/kg)	10.86
Mexaform + cysteine*	2.77

*214 mg/kg

after metabolic activation, mexaform induces chromosome damage. It is therefore a clastogen. Its chromosome breaking effects, however, are greatly reduced by cysteine.

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