BULL. PHIL. BIOCHEM. SOC. 2(1):52-59

# MUTAGENIC AND CLASTOGENIC POTENTIAL OF SOME ANTIHYPERTENSIVE DRUGS

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## ABSTRACT

Some antihypertensive drugs containing reserpine, hydrochlorthiazide, bendroflumenthiazide, mefrusside and inositol isonicotinate were studied to determine their tendency to affect the structure of DNA.

The DNA damaging interactions of reserpine was suggested by its inhibitory effect on the growth of recombination repair deficient strain of *Bacillus subtilis*. Without metabolic activation, reserpine induced frameshift mutations in *Salmonella typhimurium* mutants. In the experimental mouse, reserpine was metabolized to a base-pair mutagen. Its tendency to induce frameshift mutations was still observed even after metabolism in the mammalian system. In addition, reserpine exhibited clastogenic or chromosome breaking effects.

All these properties were not observed when reserpine was in combination with bendroflumethiazide, mefrusside and inositol isonicotinate. On the other hand, its mutagenic and clastogenic effects were enhanced when it was combined with hydralazine hydrochloride and hydrochlorthiazide.

## INTRODUCTION

Essential hypertension or high blood pressure affects millions of people worldwide. The elucidation of chemical and physical changes associated with the onset of hypertension enabled pharmaceutical researchers to synthesize drugs to treat hypertension.

A number of drugs have been characterized by their mode of lowering blood pressure (1). There are antihypertensive drugs that depress the activity of the sympathetic nervous system at postganglionic sites. There are those that act directly on vascular smooth muscles. Some are ganglionic blocking agents while others are diuretics.

Reserpine is a drug that lowers blood pressure by depressing sympathetic nervous system activity. Hydralazine hydrchloride reduces blood pressure by acting directly on arteriolar smooth muscles. Diuretics such as hydrochlorthiazide and bendroflumethiazide counteract sodium retention and enhance activity of other agents.

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Since antihypertensive drugs are used for long periods, lifelong use in some cases, it is of great interest to determine the effect of these systems on DNA, the cellular genetic material.

While the literature is rich in studies on the mechanism of blood pressure lowering of antihypertensive drugs, studies on the effects on the genetic substance of the cell are not many. Reserpine (2) was long associated as a catecholamine depleting drug as a consequence of its interference with catecholamine synthesis. Hydralazine hydrochloride has been shown to be very effective in relaxing vascular tone (3). Effects of diuretics such as bendroflumethiazide, hydrochlorthiazide and mefrusside are related to negative sodium balance or to direct effects on vascular smooth muscles.(1). Studies on long term administration of reserpine and increased risk of breast cancer (4) suggest probable interactions with DNA of somatic cells.

## MATERIALS AND METHODS

**Materials:**

The antihypertensive drugs used in this study were obtained from local drug companies. These are given in Table 1 and their structures are indicated in Table 2.

*Bacillus subtilis* mutants used for Rec assay were gifts from Dr. T. Kada, National Institute of Genetics, Mishima, Japan.

**Table 1. Constituents of Reserpine-containing drugs.**

| Drugs | | Constituents per Tablet |
|---|---|---|
| Drug 1 | R | Reserpine (0.1 mg.) |
| Drug II | R + B | Reserpine (0.1 mg)<br>Bendroflumethiazide (2.5 mg.) |
| Drug III | R + B + K | Reserpine (0.1 mg)<br>Bendroflumethiazide (2.5 mg.)<br>Potassium Chloride (573 mg ) |
| Drug IV | R + M + I | Reserpine (0.15 mg)<br>Mefrusside (15 mg)<br>Inositol Isonicotinate (150 mg) |
| Drug V | R + HN + HT | Reserpine (0.1 mg)<br>Hydralazine hydrochloride (25 mg)<br>Hydrochlorthiazide (15 mg) |

**Table 2. Structures of the different constituents of the antihypertensivedrugs.**

| Drug Constituent | Structure |
|---|---|
| Reserpine | |
| Bendroflumethiazide | |
| Mefrusside | |
| Hydralazine Hydrochloride | |
| Hydrochlorthiazide | |
| Inositol Isonicotinate | |

*Salmonella typhimurium* mutants used in direct mutagenicity studies and as indicators in the host-mediated assay, were gifts from Dr. B.N. Ames, Department of Biochemistry, University of California, Berkeley.

Fetal calf serum was a product of Grand Island Biological supply.

Giemsa and May-Grunwald stains were Merck products.

Experimental mice were obtained from Alabang Stock Farm, Alabang, Metro Manila.

**Methods:**

Rec assay was done based on the method of Kada (5). Cultures of wild and recombinant deficient strains were streaked on broth agar plates from one point. Test drugs were 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 (6). *Salmonella typhimurium* mutants TA 1535, TA 1537 and TA 98 were used. TA 1535 can be reverted to the wild type by base-pair mutagens while TA 1537 and TA 98 can be reverted to the wild type by frameshift mutagens.

To find out if the drugs are metabolized to mutagens or to non-mutagens, the host-mediated assay of Legator and Gabridge (7) was employed. This is a combination of in vivo mammalian metabolism and microbial mutation tests. An indicator bacteria is injected into peritoneal cavity of the experimental mouse and the test compounds administered to the animal by stomach tube. After 4 hours when the bacteria have come in contact with the metabolites of the drug in the peritoneal cavity, they are withdrawn from the peritoneal cavity and induced mutation frequency is determined.

Clastogenic effects of the drugs were studied using the method of Schmid (8). It is an in vivo method for screening chemical systems for chromosome breaking effects. The test substances were administered intraperitoneally. 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 one thousand polychromatic erythrocytes.

## RESULTS AND DISCUSSION

Reserpine, a constituent of a number of antihypertensive drug preparations was shown to have DNA damaging capacity (Table 3). This property was enhanced when reserpine was in combination with hydralazine hydrochloride and hydrochlorthiazide. This DNA damaging capacity was indicated by growth inhibition zones of a strain of *Bacillus subtilis* (Rec-) which lacks

the recombination repair mechanism. The structure of reserpine suggests that its DNA damaging interactions can be a consequence of intercalation as well as H-bond interactions with DNA.

Without metabolic activation, reserpine induced frameshift mutations in *Salmonella typhimurium* (Table 4). This tendency to induce frameshifts

**Table 3. DNA damaging capacity of Reserpine-containing drugs.**

| Drugs | | Zone of Inhibition (mm) | |
|---|---|---|---|
| | | H 17 (Rec ) | M 45 (Rec-) |
| Drug I | R | 0 | 0.15 |
| Drug II | R + B | 0 | 0 |
| Drug III | R + B + K | 0 | 0 |
| Drug IV | R + M + I | 0 | 0 |
| Drug V | R + HN + HT | 0 | 0.52 |

Rec += *Bacillus subtilis* wild strain that has the mechanism for recombination repair

Rec- = *Bacillus subtilis* strain, a mutant strain, that lacks the mechanism for recombination repair

**Table 4. Mutagenic potential of Reserpine-containing drugs without metabolic activation**

| Drugs | | No. of Revertant Colonies per plate | |
|---|---|---|---|
| | | TA 1535 | TA 98 |
| Control | no drug | 8.98 | 9.86 |
| Drug I | R | 9.01 | 17.15 |
| Drug II | R + B | 7.65 | 9.01 |
| Drug III | R + B + K | 8.22 | 9.60 |
| Drug IV | R + M + I | 8.14 | 9.21 |
| Drug V | R + HN + HT | 30.65 | 21.34 |

Table 5. Mutagenicity potential upon metabolic activation of Reserpine-containing drugs

| Drugs | Dosage | Relative Mutation Frequency TA 1535 | TA 1537 |
|---|---|---|---|
| R | 1 mg/kg | 2.400 | 1.220 |
| R + B | 1 mg/kg | 0.880 | 0.113 |
| R + B + K | 1 mg/kg | 1.013 | 0.602 |
| R + M + I | 1 mg/kg | 1.032 | 0.678 |
| R + HN + HT | 1 mg/kg | 48.130 | 2.367 |

Table 6. Clastogenicity potential of reserpine-containing drugs

| Drug | Dosage | Number of micronucleated polychromatic erythrocyte per thousand |
|---|---|---|
| R | 2 mg/kg | 14.27 |
| R + B | 2 mg/kg | 6.08 |
| R + B + K | 2 mg/kg | 8.02 |
| R + M + I | 2 mg/kg | 8.10 |
| R + HN + HT | 2 mg/kg | 16.98 |
| Control | ....... | 8.04 |

is indicated by its effect on TA 98 which contains an R plasmid making it more sensitive to frameshift mutagens than TA 1537. It did not induce reversions in TA 1535 indicating that without metabolic activation, reserpine does not induce base-pair mutations. Although reserpine is a big molecule, it can readily penetrate the cell membranes of these test mutants because they lack the lipopolysaccharide barrier on their cell walls (6). They are very sensitive to systems that can alter the structure of DNA because these mutants do not possess the excision repair mechanism.

Upon metabolic activation, reserpine induced not only frameshift mutations but also base-pair mutations (Table 5). These effects are also reflected at the chromosomal level as shown in Table 6. Reserpine caused

appreciable formation of micronucleated polychromatic erythrocytes in the bone marrow of the experimental mouse. Substances that affect the chromatin material can cause some fragments to lag behind during mitosis and some chromatin fragments will be left behind as micronuclei.

These effects of reserpine (DNA damaging capacity mutagenicity without metabolic activation, enhanced mutagenicity after metabolic activation and clastogenicity) were not observed when reserpine was in combination with bendroflumethiazide, mefrusside and inositol isonicotinate (Tables 3, 4, 5 and 6). However, these effects were enhanced when reserpine was in combination with hydralazine hydrochloride and hydrochlormethiazide.

It is possible that interactions of reserpine with bendroflumethiazide and reserpine with mefrusside and inositol isonicotinate lessened the tendency of reserpine to interact with DNA. With hydralazine hydrochloride, a derivative of hydrazine, DNA damaging interactions can be enhanced especially after metabolism. Hydrazine derivatives can be readily metabolized to alkylating agents (9).

Reserpine alone did not induce base-pair mutations without metabolic activation. But in combination with hydralazine hydrochloride and hydrochlorthiazide, base-pair mutations were induced prior to metabolism. This could be a consequence of the last two components, not to reserpine.

## SUMMARY

Reserpine possesses DNA damaging capacity. Without metabolic activation, it is a frameshift mutagen. Upon metabolic activation, it can induce both frameshift and base-pair mutations. It is also clastogenic. In combination with bendroflumethiazide and also with mefrusside and inositol isonicotinate, the mutagenic and clastogenic effects of reserpine were lost. However, these properties of reserpine were enhanced when it was in combination with hydralazine hydrochloride and hydrochlorthiazide.

## ACKNOWLEDGEMENT

Financial support from the National Research Council of the Philippines is gratefully acknowledged.

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