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ORIGINAL RESEARCH ARTICLE

Mutagenicity Assay on Ames *Salmonella typhimurium*

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ABSTRACT

Antimalarial drugs are frequently administered to people in tropical regions of the world. It is known that commonly used antimalarial drug primaquine binds strongly to deoxyribonucleic acid (DNA). In the present study the mutagenic effects of antimalarial drug Primaquine was evaluated in the Ames Salmonella assay. PRQ is the most commonly used antimalarial drug at present in different parts of the world. PRQ is in clinical trials as an investigational antiretroviral in humans with HIV-1/AIDS the results of the mutagenicity assay indicate that primaquine is a weak mutagen in *Salmonella* strain TA100. PRQ showed a very weak mutagenic effect in the absence of S9 mix in strain TA100. But this compound didn't show any mutagenic effect on TA98 strain both in presence or absence of S9 mix. This observation suggests that metabolic products of PRQ might not play a significant role in induction of mutation.

Key words: Mutagenic Effects, Ames Salmonella Assay, Primaquine

INTRODUCTION

Today, quinine is still used to treat primaquine resistant *Plasmodium vivax* as well as severe and cerebral stages of malaria, but is not generally used for prophylaxis. Chemically the antimalarial drugs are classified as Aryl amino alcohols (Quinine, quinidine, mefloquine, halofantrine), 4-aminoquinolines (Chloroquine, amodiaquine), Folate synthesis inhibitors (sulphones, sulphonamides, proguanil, chloroproguanil, diaminopyrimidine like pyrimethamine) and 8-aminoquinolines (Primaquine, WR238, 605) etc.Among the above mentioned drugs, primaquine (PRQ) is frequently used for treatment of malaria. PRQ is the most commonly used antimalarial drug at present in different parts of the world. After the malaria parasite *Plasmodium vivax* started to develop widespread resistance to PRQ, new potential utilizations of this cheap and widely available drug have been investigated. The test uses several strains of the bacterium *Salmonella typhimurium* that carry mutations in genes involved in histidine synthesis, so that they require histidine for growth. For mutagenicity assays, *Salmonella* strains TA97a, TA98, TA100 and TA102 are usually used. Each tester strain contains a different type of mutation in histidine operon.

MATERIALS AND METHODS

Animals

Charles river male rats of 150-175 g were used for the preparation of liver homogenate (S9) for bacterial anti mutagenicity assays. They were kept four per cage with husk bedding. Animals were received from the animal house of our institute and were fed balanced rodent pellet diet (Gold Mohar, Lipton Ltd., Chandigarh, India). The environment had a controlled 12h light and 12h dark cycle. Ambient temperature and relative humidity were 22º ± 2ºC and 55% ± 5% respectively.

Chemicals

Primaquine was purchased from the local market. Biotin, histidine, ampicillin trihydrate, sodium azide (SA), 4-nitro-o-phenylenediamine (NPD),were supplied from Sigma Chemical Company ,agar and nutrient broth were supplied from HiMedia laboratories Ltd (India). Magnesium sulfate, magnesium chloride, potassium chloride, citric acid monohydrate, dibasic potassium phosphate, sodium ammonium phosphate, sodium hydroxide, glucose were purchased from E. Merck Limited .

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Preparation of S9 Fraction

Charles river male rats of 150-175 g were fed 0.1% Phenobarbital in their drinking water for seven days. On day 6, no foods were provided for these rats. The next day, they were killed for the rat liver homogenate (S9). All steps of this preparation were performed at 0°C to 4°C with cold and sterile solutions and glassware. The livers (10-15 gm each) were washed in an equal volume of 0.15 M KCl (3 ml/gm of wet liver) and homogenized with a homogenizer. The homogenate was centrifuged for 10 minutes at 9000g and the supernatant that is called as S9 fractions were distributed in 2 ml in small sterile plastic tubes. The S9 mix was prepared by the method of Maron and Ames.

Bacterial Mutagenicity assay:

Standard mutagenicity assay tests were performed on TA98 and TA 100 strains. The test drug PRQ was dissolved in distilled water and its different concentrations (10, 100, 500, 1000 µg/plate) were used in mutagenicity assay. In the assay, 0.1 ml/plate of the different concentrations of PRQ (i.e. 0.1 ml containing 10, 100, 500 and 1000 µg/plate) were used. The plates were inverted within 1h and placed in a dark vented incubator at 37°C for 48 h. Similar experiments were carried out for positive mutagens (4-nitro-o-phenylenediamine for TA98 and SA for TA100) and negative control in absence of S9 mix. Four plates were used for each concentration tested and for both positive and negative controls. After 48 h of incubation, the revertant colonies on the test plates were counted. The presence of the background lawn on all the plates was confirmed. A similar experiment was also carried out using liver homogenate (S9) fractions. Positive mutagens 2-AF was used for TA98 experiments with S9 mix. Due to shortage of time similar experiment with TA100 was not been able to carry out. The spontaneous reversion rates of these two different *Salmonella* strains were checked and were similar as reported earlier.

RESULTS

(**Table 1 & Table 2**) are summaries of the results of the Ames mutagenicity assay in *salmonella typhimurium* strains TA100 and TA98 after PRQ treatment. A significant increase in revertant colonies was observed at the doses 100 µg/plate to 1000 µg/plate in the salmonella strain TA100 when compared to solvent treated control. At 5000µg/plate a significant decrease in the revertant colonies indicate that the PRQ was toxic at this dose. The results of solvent treated control and positive control are at per the values reported by other authors. No significant revertant colonies were observed in any of the concentration tested in case of *salmonella* strain TA98 either with or without S9 activation. So this result shows that the PRQ was not mutagenic in salmonella strain TA98. This overall results indicate that the PRQ is a weakly mutagenic in *salmonella* strain TA100 which detect the base pair substitution mutants. The number of revertant colonies of strain TA100 and TA98 were significantly decreased at 5000µg/plate dose when compared to solvent treated control. This observation suggests that at this dose the drug PRQ shows toxic effects in both the strains.

Table 1: Number of revertants induced by Primaquine in *salmonella* plate incorporation test using TA 100 strain

Chemicals (µg/plate)	Revert ants/plate -S9
Solvent control (100µl distilled water)	145.00 ± 12.215
Primaquine	
10	195.20 ± 19.21
100	241.75 ± 8.20*
500	269.45 ± 19.31*
1000	289.21 ± 10.50*
5000	101.45 ± 9.79
Positive control	
SA (1.5µg/plate)	1239.8 ± 76.31

- S9 = without metabolic activation. All data represented here are Mean ± SD of four plates. Results for each concentration were compared with the solvent treated control by Dunnet's multiple comparison tests.
SA=Sodium azide.
* $p < 0.01$

Table 2: Number of revertants induced by Primaquine in *salmonella* plate incorporation test using TA 98 strain

Chemicals (µg/plate)	Revert ants/plate	
	-S9	+S9
Solvent control (100µl distilled water)	37.70± 12.72	40.19 ± 15.69
Primaquine		
10	41.20 ± 16.40	44.23 ± 17.88
100	49.15 ± 17.60	51.50 ±15.20
500	50.10 ± 08.78	50.35 ± 26.19
1000	54.07 ± 8.21	52.30 ± 13.20
5000	20.44 ± 16.15	14.30 ± 19.08
Positive Control		
NPD (20µg/plate)	1309.15 ± 141.50	
2-AF (10µg/plate)		2032 ± 241.03

- S9 = without metabolic activation; + S9 = with metabolic activation.
All data represented here are Mean ± SD of four plates. Results for each concentration were compared with the solvent treated control by Dunnet's multiple comparison test. NPD=4-nitro-o-phenylenediamine, 2AF=2-aminofluorene.

DISCUSSION

The results of the mutagenicity assay indicate that Primaquine is a weak mutagen in *Salmonella* strain TA100. PRQ showed a very weak mutagenic effect in the absence of S9 mix in strain TA100. But this compound didn't show any mutagenic effect on TA98 strain both in presence or absence of S9 mix. This observation suggests

that metabolic products of PRQ might not play a significant role in induction of mutation.

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