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Quinine Induced *Escherichia coli* DNA Base-Pair Substitution Mutation

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Quinine and chloroquine have similar mode of antimalarial activity by forming intercalated complexes between the DNA base pairs of the malarial parasites (Estensen et al. 1969; Hahn and Fean 1969; O'Brien et al. 1966; Strube 1975; Schupbach 1979). Although these two drugs intercalate within the DNA double helix, it is not known if they mediate the same mutagenic effect. Chloroquine was reported to induce frameshift reverse mutation (Schupbach 1979), its interaction with DNA is resistant to 6-M urea solution and has the affinity for attaching to G/C base pairs O'Brien et al. 1966; Schupbach 1979). The molecular interaction of quinine with the DNA base pairs although not properly understood had been reported to occur through the formation of urea-sensitive hydrogen bonds (O'Brien et al. 1966).

This paper presents experiments carried out to determine the mutagenic effect of quinine.

MATERIALS AND METHODS

The bacterial tester strains were the Escherichia coli WP_2 trp; WP_2 trp, uvrA; WP_6 trp, polyAl; WP_{67} trp, uvrA, polyAl (Green and Muriel 1976; Green 1984). These strains are base-pair substitution mutants known to detect base-pair substitution mutagens (Green and Muriel 1976; Bruswick et al. 1980). The Salmonella typhimurium his strains TA97 and TA102 detect frameshift mutagens by reversion mutation from his to his^+ (Levin et al. 1982; Maron and Ames 1983).

The minimum inhibitory concentration (MIC) of quinine hydrochloride against the tester strains was determined on Davis and Mingioli (1950) minimal salts agar with glucose as carbon source using the agar-dilution method following the protocol earlier described (Obaseiki-Ebor 1984). The plates contained varying concentrations of quinine hydrochloride ranging from 10 to 1000 ug/ml respectively containing either tryptophan or histidine (10 ug/ml). The plates were inoculated with about 10^5 - 10^6 colony forming units (cf)/ml of the washed tester strains in normal saline. The MIC was the

lowest concentration of quinine that completely inhibited growth after 24h incubation at 37°C.

The mutagenicity tests were carried out with subinhibitory concentrations of quinine employing the spot test and agar incorporation methods (Brusick et al. 1980) and the modified fluctuation test (Green et al. 1976).

In the spot-test, 10^6 cfu/ml of the test culture were spread on the minimal salts agar plates (unsupplemented) and few crystals (10 mg) of quinine hydrochloride were placed at a central area on the surface of the agar. The plates were incubated at 37°C for 48h and observed for revertant colonies and presence of a zone of inhibition around the central spot of the quinine.

For the plate incorporation procedure, sub-inhibitory concentrations of quinine hydrochloride were each added to tubes containing 2.5 ml of molten agar-agar (Oxoid) 0.7% w/v, held at 45°C. 0.1 ml of 10^{-5} dilution of the test culture (to give about 200 colonies on the plate) was added to each tube and quickly poured onto the surface of the minimal salts agar in the petri-dish containing biotin 5 ug/ml (for the Salm. typhimurium strains). The test plates contained a very low concentration of tryptophan or histidine (67 ng/ml). This low concentration of the amino acid would stimulate the growth of the auxotrophic cells until it is exhausted, thereafter, only trp$^+$ or his$^+$ revertants could continue to grow. The control positive plates contained tryptophan or histidine at a concentration of 10 ug/ml and the control negative plates contained no amino acid. All the plates were incubated at 37°C for 48h and the mean number of colonies growing on each plate in triplicate determination were recorded.

The method of Green et al. (1976) was suitably modified for the fluctuation test. 0.4 ml of a saline washed overnight grown test culture of about 10^8 cfu/ml was inoculated into 400 ml of minimal salts medium containing biotin 5 ug/ml (for the Salm. typhimurium TA97 and TA102 strains), bromocresol purple indicator 12 ug/ml and a trace amount of tryptophan or histidine (67 ng/ml) to stimulate growth without actually allowing the growth of colonies. The 400 ml growth media was aseptically divided into 4 aliquots of 100 ml each. 2 x 100 ml aliquots contained the test concentrations of quinine hydrochloride and another 100 ml aliquot contained tryptophan or histidine (10 ug/ml) as positive control, and the remaining 100 ml aliquot contained neither amino acids nor quinine as the negative control. Each aliquot was aseptically distributed in 2 ml volumes to 50 small tubes and were incubated at 37°C for 72h. The tubes containing his$^+$ or trp$^+$ revertants would be turbid with yellow (turned from purple) colouration.

RESULTS AND DISCUSSION

Quinine hydrochloride inhibited the E. coli and the Salm. typhimurium strains except WP_6 at 200 ug/ml. The E. coli WP_6 strain was inhibited at a relatively lower concentration of 120 ug/ml. The muta-

genecity tests were carried out with the subinhitory concentrations of 25, 50 and 100 ug quinine hydrochloride/ml. There were no revertants in the E. coli and Salm. typhimurium strains except in E. coli WP_6. The E. coli WP_6 strain showed revertant colonies (50 trp^+ colonies after a mean triplicate determination) and a clear zone of inhibition (45 mm) around the central spot of the quinine crystals. Table 1 shows the number of E. coli WP_6 revertants with varying concentrations of quinine hydrochloride in the plate incorporation test.

Table 1. Mutagenic effects of quinine hydrochloride in E. coli trp, polyA1 strain WP_6

Strain	Concentration of quinine (ug/ml)	Agar-incorporation reversion test* (revertant colonies)	Fluctuation test+ (Positive tubes/No. test tubes)
WP_6	0	0	0
	25	52	45/50
	50	75	37/50
	100	26	42/50

* results of mean triplicate determinations
\+ results of duplicate determinations

The decreased number of revertant colonies at the 100 ug/ml of quinine hydrochloride indicated the inhibitory quinine toxicity effects against the cells. The results of the fluctuation test also indicated that quinine was mutagenic in E. coli WP_6 (Table 1).

Quinine was earlier reported to inhibit DNA polymerase activity (O'Brien et al. 1966) and in this study it was found to specifically relatively inhibit the polyA strain more than the other repair deficient strains. This indicated that the repair of DNA damage by quinine was $polyA^+$ dependent. It is plausible that during the DNA damage of the E. coli WP_6 polyA by quinine, the presence of the defective polymerase activity could have prevented the accurate repair of the DNA damage, with the consequent mutation of trp to trp^+. However, there was no quinine induced mutation in strain WP_{67} indicating that the presence of uvrA and polyA genes in the same cell could annul the occurence of mutations induced by quinine.

The results of this study indicate that quinine mediates base-pair substitution mutation. This study is of interest because in parts of tropical Africa, Asia and South America where malaria is endemic, quinine is still therapeutically used against the malarial parasites with multiple resistance to the synthetic antimalarial agents such as chloroquine, primaquine etc (Aug-thaw-batu 1975). Quinine had also been reported effective in combined use with mefloquine for the treatment of chloroquine-resistant falciparum malaria (Hall 1976).

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