J. Toxicol. Pub. Health
Vol. 15, No. 1, pp. 75-78, 1999

Mutagenicity of Typhoid Vaccine

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(Received October 29, 1998)
(Accepted January 11, 1999)

ABSTRACT : *In order to evaluate the mutagenic potential of Typhoid vaccine, 3 sets of mutagenicity tests were performed. In the reverse mutation test using Salmonella typhimurium TA98, TA100, TA1535 and TA1537, Typhoid vaccine did not increase the number of revertant at the doses of 100, 50, 25, 12.5, 6.25 μg/plate. In chromosome aberration analysis using CHO cells were not found chromosomal aberration in different concentrations with or without metabolic activation at the doses of 0.25 mg/ml, 0.5 mg/ml, 1 mg/ml. In mouse micronucleus test, no significant increase in the occurrence of micronucleated polychromatic erythrocytes was observed in ICR male mice intramuscularly administered with Typhoid vaccine at the doses of 0.1 mg/ml, 0.5 mg/ml, 1 mg/ml. These results indicate that Typhoid vaccine has no mutagenic potential in these in vitro and in vivo systems.*

Key Words : *Reverse mutation, Chromosome aberration, Micronucleus, Typhoid vaccine, Mutagenicity*

I. INTRODUCTION

Typhoid fever is caused by several virulent serovars of *Salmonella typhimurium* and is acquired by ingestion of food or water contaminated by feces of infected humans or animals. In earlier centuries the disease occurred in great epidemics (Lansing M. Prescott, 1993).

Typhoid fever remains a substantial public health problem in developing countries. Each year 33 million people become ill and over 500,000 people die of this infection. Typhoid is rare in industrialised nations, though travellers to endemic countries may occasionally acquire the disease (Eric *et al.*, 1998).

The interest in vaccines to prevent this typhoid disease is long standing. Whether any of the available vaccines would be useful in typhoid prevention in the developing world remains uncertain.

Recently, Korea Green Cross Cooperation developed, for the convenience in practical immunization, the combined vaccine for the typhoid fever. The efficacy of the combined vaccine was confirmed. In this study the elucidate mutagenicity for Typhoid vaccines was performed using the bacterial mutation assays, chromosome aberration analysis *in vitro*, and micronucleus tests *in vivo*.

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II. MATERIALS AND METHODS

1. Materials

Typhoid vaccine were obtained from Green Cross Cooperation. This test material was used after dilution in sterilized saline. sodium azide (SAZ), 9-aminoacridine (9-AA), mitomycin C, 2-aminofluorene (2-AA), $NaHCO_3$ and colcemid were purchased from Sigma Chemical Co. (St. Louis, Mo, USA). S-9 fraction and cofactor were purchased from the Oriental Yeast Co., Ltd.. Japan.

2. Ames mutagenicity test

The *Salmonella* bacterial tester strains used in this study were obtained from the Korea Food and Drug Administration.

After testing for the bactericidal effects, the standard testing doses of Typhoid vaccine (6.25, 12.5, 25, 50 and 100 μg/plate) were selected. These were diluted in Saline before adding to petri dishes. All of the experiments were conducted by

the pre-incubation method as published by Ames *et al.* (1975). Complete test for each testing dose consisted of a nonactivated (without the S-9 mix) and an activated (with the S-9 mix), each with appropriate negative and positive controls.

The plate were incubated at 37°C for 48 hr and then the revertants bacterial colonies on each plate were counted. Dose response tests of the mutagens on the tester strain were carried out to determine the regions of revealing mutagenicity induced by test compound. Statistical analysis was performed by analysis of variance. Significant differences between treatment means were determined by using the Student's test.

3. Micronucleus assay using ICR mouse

The male ICR mice (7 week old) were group-housed (10 mice per cage). Varying doses (0.1, 0.5 and 1 mg/ml) of Typhoid vaccine dissolved in saline were intramuscularly administered as a single treatment. The highest dose used was the half of known intramuscular LD_{50} value. Animals were sacrificed by cervical dislocation 24 hr after the treatment and femora were isolated. Bone marrow cells obtained from the femora were suspended in fetal bovine serum and were stained by Giemsa (Gurr. PH 6.8) stain. These were then examined under a microscope.

The negative control samples were obtained from the saline treated mice and the positive control samples from mice given 2 mg/ml Mitomycin C via a single intramuacular injection.

4. Chromosome aberration assay using cultured chinese hamster ovary cells

The CHO-K_1 cells was obtained from the Veterinary Medicine College of SNU. The medium used in this study was the Ham's F-12 nutrient mixture medium (Gibco BRL) supplemented with 5% FBS (Gibco BRL) and 1176 mg $NaHCO_3$. The cultured cells were maintained in exponential growth by subculture for every two to three days using 0.25% trypsin-EDTA (Sigma Co.) and phosphate buffered saline. The cultures were treated with varying doses of Typhoid vaccine (0.25, 0.5 and 1 mg/ml) which have been dissolved in Saline.

For the aberration assay, three different doses were prepared and separately added to 3-day old cultures (approximately 10^5 cells/60 mm dish). In the absence of metabolic activation, cultures were treated for 24 hr and 48 hr with the test article, while in the presence of metabolic activation, cells were treated S-9 mix for 6 hr and then maintained for 18 hr in the fresh medium. Treatment was followed by addition of medium containing colcemid 0.2 μg/ml for 2 hr, and then harvested by centrifugation after trypsinization. The cells were swollen with hypotonic 0.075 M KCl solution for 15 min at 37°C. After spin down, cell pellets were fixed with ice-cold fixative (methanol : glacial acetic acid=3 : 1 (v/v)) twice, and washed three times in fixative. After centrifugation, the fixative was removed, and cell pellet suspensions were prepared by pipetting gently. A few drop of cell pellet suspension were dropped onto precleaned dry slides, and air dried. Slides were stained with 5% Giemsa buffered solution (PH 6.8). The number of cells with chromosome aberrations was recorded on 200 well-spread metaphases at the magnification of 1,000 with Olympus microscope. The types of aberration were followed by Motoi Ishidate, Jr. (1988) classification. Breaks less than the width of a chromatid were designated as gaps in our criteria, and not included as chromosomal aberration. The incidence of polyploid and endoredupolicated cells was also recorded when these events were observed. Control groups consisted of the negative control (Saline 10%) and the positive control (Mitomycin C 0.03 μg/ml).

III. RESULTS AND DISCUSSION

A reverse mutation test using *Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 did not show significant increase in reverse mutation colony at all doses of Typhoid vaccine (Table 1). But positive control materials showed significant increases in mutation colony compared to control.

In the incidences of micronucleated polychromatic erythrocytes tests using the mice. Typhoid vaccine

Table 1. Reverse mutation test of Typhoid vaccine in *Salmonella typhimurium*

Compound[a]	Dose (μg/plate)	S9 Mix	No. of revertant colonies per plate (Mean±S.D.)			
			TA1535	TA1537	TA98	TA100
Negative (Saline)	0	-	16.7±1.5	3.7±1.5	18.7±6.1	90.7±6.4
Typhoid vaccine	100	-	25.7±12.7	5.3±2.1	19.3±6.5	136.7±12.9
	50	-	19.0±4.0	5.3±3.5	16.0±1.0	128.7±21.9
	25	-	41.0±7.0	8.7±1.5	29.3±11.7	151.3±31.6
	12.5	-	21.3±5.9	5.0±2.7	24.7±3.6	112.0±15.9
	6.25	-	11.7±4.7	5.3±3.1	32.0±3.2	99.3±14.7
2-AF	10	-	NT[b]	NT	64.0±29.1*	NT
	1	-	NT	NT	57.3±22.2*	NT
	0.1	-	NT	NT	58.7±17.2*	NT
SAZ	1	-	310.7±48.0*	NT	NT	534.7±141.2*
	0.1	-	294.7±85.5*	NT	NT	341.3±76.9*
	0.01	-	249.3±70.5*	NT	NT	149.7±22.9
9-AA	10	-	NT	56.0±16.1*	NT	NT
	1	-	NT	13.0±5.3	NT	NT
	0.1	-	NT	11.7±2.5	NT	NT
Negative (Saline)	0	+	18.0±1.0	21.0±9.8	41.0±8.7	103.0±3.6
Typhoid vaccine	100	+	15.3±4.9	16.0±2.6	48.0±10.4	218.0±105.7
	50	+	17.0±5.3	11.7±4.5	36.7±22.0	146.0±38.0
	25	+	11.0±2.6	9.3±0.6	54.7±36.8	162.7±26.6
	12.5	+	12.3±3.1	12.3±3.8	36.0±6.1	100.0±13.1
	6.25	+	15.7±4.7	12.0±4.3	31.7±10.7	120.3±8.7
2-AF	10	+	NT	NT	580.0±262.4*	404.0±90.2*
	1	+	NT	NT	239.3±32.9*	352.0±96.1*
	0.1	+	NT	NT	136.0±17.40	232.7±77.6*
SAZ	1	-	357.3±88.3*	NT	NT	NT
	0.01	-	134.0±27.9*	NT	NT	NT
	0.1	+	120.7±4.2*	NT	NT	NT
9-AA	10	-	NT	88.7±18.2*	NT	NT
	1	-	NT	19.0±3.5	NT	NT
	0.1	-	NT	18.3±3.2	NT	NT

[a]2-AF, 2-aminofluorene,; SAZ, sodium azide; 9-AA, 9-aminoacridine hydrochloride. [b]NT, not tested.
*Significantly different from negative control ($p<0.05$).

Table 2. Micronucleus test of Typhoid vaccine in mice

Compound	Route	Dose (mg/ml)	No. of animals	MNPCE[a] (‰, Mean±SD)	PCE/(PCE+NCE)[b] (%, Mean±SD)
Vehicle[c]	i.m.	0.25 ml	6	1.00±0.63	53.87±8.28
Typhoid vaccine	i.m.	0.1	6	0.83±0.75	51.60±4.26
	i.m.	0.5	6	0.83±0.75	48.33±6.05
	i.m.	1	6	1.5±0.55	49.70±6.05
Mitomycin C	i.m.	2 mg/kg	6	12.2±1.47*	33.23±9.81*

[a]Micronucleated polychromatic erythrocytes/1,000 polychromatic erythrocytes. [b]Polychromatic erythrocytes/500 erythrocytes. [c]Sterile saline.
*Statistically significant from vehicle control ($P<0.05$, Dunnett t-test).

treatment groups incidences of micronucleated polychromatic erythrocytes were not significantly increased, compared with control group (Table 2).

In a chromosome aberration assay using the CHO cell *in vitro*, no significant changes were found in Typhoid vaccine treatment groups compare with control group (Table 3). But Mitomycin C treatment group shows significant increased incidence of chromosome aberration. Dose-dependency was not found in chromosome aberration assay on Typhoid

Table 3. Chromosomal aberration test of Typhoid vaccine on CHO-K_1 cell

Compound	Dose (mg/ml)	S9 mix	Time (hr)[a]	Observed cell	No. of Structural aberration[b]							No. of Aberrant cells[c]		
					ctg	csg	ctb	csb	cte	cse	Frg	TAG(%)	TA(%)	Polyploidy[d] (%)
Negative Control	0	-	6-18	200	2	1	2	1	1	1	0	7 (3.5)	4 (2.0)	1 (0.2)
Vehicle (Saline)	0	-	6-18	200	2	2	2	2	0	0	0	8 (4.0)	4 (2.0)	2 (0.4)
	1	-	6-18	200	2	2	1	1	1	1	0	8 (4.0)	4 (2.0)	2 (0.4)
Typhoid vaccine	0.5	-	6-18	200	2	2	2	1	1	0	0	8 (4.0)	4 (2.0)	2 (0.4)
	0.25	-	6-18	200	2	1	2	1	0	1	0	7 (3.5)	5 (2.5)	1 (0.2)
Cyclophosphamide	0.005	-	6-18	200	6	3	2	2	2	2	1	18*(9.0)	9*(4.5)	4 (0.8)
Negative Control	0	+	6-18	200	2	1	1	2	1	1	0	8 (4.0)	5 (2.5)	2 (0.4)
Vehicle (Saline)	0	+	6-18	200	3	2	1	1	1	1	0	9 (4.5)	4 (2.0)	2 (0.4)
	1	+	6-18	200	2	2	2	1	0	2	0	9 (4.5)	5 (2.5)	2 (0.4)
Typhoid vaccine	0.5	+	6-18	200	2	1	2	2	1	0	0	8 (4.0)	5 (2.5)	1 (0.2)
	0.25	+	6-18	200	1	2	2	1	1	1	0	8 (4.0)	5 (2.5)	1 (0.2)
Cyclophosphamide	0.005	+	6-18	200	16	6	6	5	5	2	1	41*(20.5)	19*(9.5)	4 (0.8)
Negative Control	0	-	24-0	200	2	0	2	1	1	1	0	7 (3.5)	5 (2.5)	1 (0.2)
Vehicle (Saline)	0	-	24-0	200	3	2	0	1	2	0	0	8 (4.0)	3 (1.5)	2 (0.4)
	1	-	24-0	200	3	1	2	1	0	1	0	8 (4.0)	4 (2.0)	2 (0.4)
Typhoid vaccine	0.5	-	24-0	200	2	2	2	1	1	1	0	9 (4.5)	5 (2.5)	1 (0.2)
	0.25	-	24-0	200	3	1	1	2	0	1	0	8 (4.0)	4 (2.0)	1 (0.2)
Mitomycin C (μg/ml)	0.03	-	24-0	200	12	6	6	8	12	6	2	52*(26.0)	34*(17.0)	2 (0.4)
Negative Control	0	-	48-0	200	3	2	1	1	1	1	0	9 (4.5)	4 (2.0)	1 (0.2)
Vehicle (Saline)	0	-	48-0	200	2	2	2	1	1	0	0	8 (4.0)	4 (2.0)	1 (0.2)
	1	-	48-0	200	3	2	2	1	1	0	0	9 (4.5)	4 (2.0)	1 (0.2)
Typhoid vaccine	0.5	-	48-0	200	2	2	2	1	1	1	0	9 (4.5)	5 (2.5)	1 (0.2)
	0.25	-	48-0	200	2	2	2	0	1	1	0	8 (4.0)	4 (2.0)	2 (0.4)
Mitomycin C (μg/ml)	0.03	-	48-0	200	26	10	10	12	12	10	2	82*(41.0)	46*(23.0)	3 (0.6)

[a]Treatment time-Expression time. [b]ctg, chromatid gap; csg, chromosomal gap; ctb, chromatid break; csb, chromosomal break; cte, chromatid exchange; cse: chromosomal exchange; Frg: fragmentation. [c]TAG: total number of aberrant cells including gaps. TA: total number of aberrant cells excluding gaps. [d]Polypoidy cells per 500 mitosis/dose.
*Significantly different from negative control group ($p<0.05$).

vaccine.

Therefore, all above results represent that Typhoid vaccine has no mutagenic effect in these experimental condition.

ACKNOWLEDGEMENT

This work was supported by a 1998 research grant from Veterinary Research Institute, SNU.

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