

Clastogenic Potential of Certain Vaccines on Bone Marrow Cells of Swiss Mice

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ABSTRACT Although most of the vaccines are prepared out of the heat killed or attenuated virus and bacteria, study on their genotoxicity/clastogenicity is meagre. Therefore, the clastogenic potential of certain widely used vaccines namely, measles, rubella and rabies (rabipur), has been assessed at 24 h post-treatment from mouse bone marrow cells after a low dose, single intraperitoneal treatment. Mitotic metaphase spreads were scanned from the slides prepared following the colchicine- sodium citrate hypotonic- methanol, glacial acetic acid- flame drying- Giemsa technique. All the three treated doses of measles vaccine (500, 1000, 2000 CCID₅₀ per 100 g b.w. of mice) induced significant chromosomal aberrations in male mice ($P \leq 0.05$ or $P \leq 0.01$), but failed to induce significant aberrations in female mice. Interestingly, the intermediate dose showed the optimum effects. All the three different doses of rubella vaccine (100, 200, or 400 CCID₅₀ per 100 g b.w. of mice) induced statistically significant ($P \leq 0.01$) chromosomal aberrations in both female and male mice. The induced chromosomal aberrations ($P \leq 0.01$) by all the three different treated doses of rabies vaccine (0.25, 0.5, or 1.0 I.U. per 100 g b.w. of mice) were dose related but nonlinear. The results were compared with the scantily available earlier data on the clastogenicity of vaccines and the live viruses in patients. The possible substance present in the vaccines causing chromosomal aberrations has been discussed.

INTRODUCTION

Humans and the livestock are very often vaccinated for the successful prevention and control of different dreaded diseases. However, most of the vaccines are prepared out of the heat killed or attenuated virus and bacteria. While the live bacteria, virus, their culture filtrate and toxins were found genotoxic/clastogenic (Hamper and Ellison 1961; Aula 1965; Cooper et al. 1968; Manna and Chakrabarti 1970; Manna and Das 1974; Manna 1992; Ghosh 1982; Ghosh and Ghosh 1983; Nichols 1970; Nichols et al. 1964, 1965, 1978; Bhatnagar et al. 1984), study on the effects of the vaccines on the hereditary material of the inoculated organisms is very

meagre, although it is directly concerned with human health. Paucity of such studies might have been due to the landmark success of vaccinology over the years. Nevertheless, sporadic but still very important studies have been undertaken earlier showing the induced genotoxicity of certain vaccines on laboratory animals and in inoculated organisms (Kucerova et al. 1980; Lambert et al. 1979; Castillo et al. 1983; Nayak and Das 1986, 1987; Cherkezia et al. 1980; Mikhailova and Gorschunova 1975; Mikhailova et al. 1976; Sharma and Sahai 1981; Volgareva et al. 1991). On the other hand, the contrary results i.e., the nongenotoxic and anticlastogenic effects of tularemia live vaccine (TLV), reported by Nersessian et al. (1986, 1991) are interesting.

A cursory survey of the concerned literature indicates that most of the earlier studies on the genotoxicity/clastogenicity of vaccines on laboratory animals were made after chronic exposures, that to with unusually high doses. Besides, most of such studies have been undertaken following crude techniques and with improper time interval between treatment and observation. Therefore, the results of such studies are having little extrapolative value to humans. Whereas, following a suitable experimental protocol, a low dose single exposure for one cell cycle duration in mammalian *in vivo* is adequate for both qualitative and quantitative estimations of their cytogenetic effects. Therefore, the present study has been undertaken to assess the clastogenic potential of three widely used vaccines, namely measles, rubella and rabipur, on bone marrow cells of Swiss mice after a single intraperitoneal exposure for one cell cycle duration.

MATERIALS AND METHODS

Test Animals: Swiss albino mice, *Mus musculus*, were procured from a live animal

supply farm M/s Ghosh Enterprises, Kolkata and were acclimatized to the animal house of the department for one month before employed them in the experiment. The animal house was maintained to $25 \pm 2^\circ \text{C}$ and a 12 h light and dark cycle. The mice were fed daily with standard balanced diet and tap water *ad libitum*, and were kept in hygienic condition.

Test Chemicals: Cyclophosphamide in Endoxan-Asta, manufactured by Khandelwal Laboratories Ltd., Mumbai, a widely prescribed cancer chemotherapeutic drug for the treatment of various types of cancers and a widely used chemical mutagen for cytogenetic toxicity tests, was selected as the positive control chemical. A single dose of cyclophosphamide 40 mg/kg b.w. of mice was used as the positive control. Sodium chloride 0.9% was used as the negative / vehicle control.

Sii Measles vaccine Live l.p. (lyophilized), manufactured by Serum Institute of India Ltd., Pune, containing live attenuated measles virus (Edmonston-Zagreb strain) propagated on human diploid cells, was tested for its cytogenetic toxicity. Each available dose of 0.5 ml of vaccine contains not less than 1000 CCID₅₀ of measles virus on reconstitution with the diluent provided.

Sii Rubella vaccine Live B. P. (R-Vac) lyophilized, manufactured by Serum Institute of India Ltd., Pune, containing live attenuated rubella virus (Wistar RA 27/3 strain) propagated on human diploid cells was used as one of the test vaccine. Each prescribed dose of 0.5 ml of the vaccine contains not less than 1000 CCID₅₀ of rubella virus on reconstitution with the diluent provided.

Rabipur, the purified chick embryo cell rabies vaccine for human use, manufactured by Chiron Behring Vaccines Pvt Ltd., Ankleswar, India was the other vaccine tested. Each immunizing dose (1ml) of the vaccine in a vial of lyophilized powder contains inactivated rabies virus (Flury LEP strain) of the potency $\geq 2.5 \text{ I.U.}$, polygeline, salts and carbohydrates. The virus are propagated in primary chick fibroblast cell cultures and inactivated by β -propiolactone. The vaccine is purified from antibiotics (Neomycin, Chlorotetracycline and Amphotericin-B) used during virus propagation.

Experimental Protocol: Sixty-six healthy mice (33 females and 33 males), of 15-20 g body

weight each, were selected and were randomly divided into 11 groups, each with 3 females and 3 males. Different groups were caged separately. One group of six mice (3 females and 3 males) was injected intraperitoneally with 0.9% sodium chloride at the rate of 1 ml per 100 g b.w., which served as the negative/ vehicle control group. Another group of six mice was injected intraperitoneally with cyclophosphamide (in Endoxan) at the rate of 4 mg / 100 g b.w. and served as the positive control group.

Three groups of mice were inoculated with measles vaccine diluted in such a way that they received the live attenuated measles virus at the rate of 2000, 1000, or 500 CCID₅₀ per 100 g b.w. of mice.

Three groups of mice were inoculated with rubella vaccine by diluting the reconstituted 0.5 ml dose for humans in such a way that the three groups received 400, 200, or 100 CCID₅₀ of live attenuated rubella virus per 100 g b.w. of mice.

The rest three groups of mice were inoculated with rabies vaccine after diluting the human immunizing dose of 1 ml, with the inactivated virus bearing the potency 2.5 I.U., in such a way that they received 1, 0.5, or 0.25 I.U. per 100 g b.w. Dilution of different chemicals was made in such a way that the volume of each inoculation of the vaccines, and the positive and negative control dose were maintained to 1 ml/ 100 g b.w. of mice.

Procedure: At 24 h post-treatment the mice were treated intraperitoneally with 0.02% colchicine at the rate of 1 ml/ 100 g b.w. After 2 h the colchicinated mice were sacrificed by cerebral dislocation and slides were prepared following the colchicine- sodium citrate hypotonic-methanol, glacial acetic acid- flame drying-Giemsa technique of Choudhury et al. (1995). About 150 well-spread metaphases from each animal were scanned. The metaphases with aberrations, like chromatid gap, chromatid break, chromosome gap, chromosome break, fragment, minute etc. were recorded separately for each animal.

From the data generated for each group of mice, the percentage of aberrant metaphases, aberrations (excluding gaps) per hundred metaphases were calculated. The chromatid and chromosome gaps were recorded separately and were taken into consideration for the calculation of aberrant metaphases. Statistical evaluations

for the tests of significance of the differences in between the data generated for the groups of mice treated with various doses of vaccines and positive control were compared with that of the vehicle/ negative control group of mice. 'P' values were recorded from the tables of Kastenbaum and Bowman (1970), which were prepared specifically for mutation studies.

RESULTS

At 24 h post-treatment the vehicle control group of mice showed 2.58 and 3.02 percent aberrant metaphases with 0.64 and 0.33 aberrations per hundred metaphases (excluding gaps) in the female and male mice respectively (Table 1). The positive control group of mice showed 63.39 and 51.28 percent aberrant metaphases with 110.34 and 81.94 aberrations per hundred metaphases (excluding gaps) in the female and male mice, respectively, which are statistically significant at 1% level ($p \leq 0.01$) when compared to that of the respective vehicle control group of mice (Table 1).

The female mice inoculated with the measles vaccine at the rate of 500, 1000, and 2000 CCID₅₀ per 100 g b. w. exhibited 6.88, 23.77 and 12.88 percent aberrant metaphases, and 3.11, 6.22 and 4.44 aberrations per hundred metaphases, respectively, which are not significant statistically. Whereas, their male counterparts exhibited 20.22, 18.00 and 19.33 percent aberrant metaphases and 8.66, 12.44 and 12.01 aberrations excluding gaps, respectively, which are statistically significant ($p \leq 0.05$ or $p \leq 0.01$) when compared to that of the vehicle control group (Table 1). The induced aberrations were mostly chromatid breaks and fragments. Besides, a large number of chromatid gaps were also recorded.

The female mice, which received rubella vaccine at the rate of 100, 200 and 400 CCID₅₀ per 100 g b.w., showed 21.11, 21.11 and 25.11 percent aberrant metaphases and 17.77, 12.0 and 18.22 aberrations per hundred metaphases respectively. Their male counterparts showed 14.88, 24.0 and 28.22 percent aberrant metaphases and 11.11, 13.55 and 15.33 aberrations per hundred metaphases respectively. The aberrations per hundred metaphases induced by all the three different doses of rubella vaccine in both the sexes of mice are statistically highly significant ($p \leq 0.01$) (Table 2). In addition to a

large number of chromatid gaps, the induced aberrations were mostly chromatid breaks.

All the three doses of rabies vaccine of 0.25, 0.5 and 1.0 I.U. induced 34.44, 62.00 and 73.33 percent aberrant metaphases with 17.33, 34.88 and 40.88 aberrations per hundred metaphases in the female mice respectively. In the male mice the induced percentage of aberrant metaphases were 43.77, 46.44 and 58.22 with 27.33, 28.88 and 31.33 aberrations per hundred metaphases respectively. The induced aberrations per hundred metaphases by all the three different doses of rabies vaccine in both female and male mice are statistically highly significant ($p \leq 0.01$) (Table 3). The aberrations were mostly random chromatid breaks, apart from a large number of chromatid gaps.

DISCUSSION

Cyclophosphamide 4 mg/ 100 g b.w. induced very high percentage of aberrant metaphases and highly significant number of aberrations (excluding gaps) ($p \leq 0.01$) per hundred metaphases in both male and female mice. Apart from the induction of a large number of chromatid gaps, the induced aberrations were mostly random chromatid breaks and fragments, which are in complete agreement with the earlier reports (Datta and Schliermacher 1969; Rohrborn and Basler 1977; Machemer and Lorke 1978; IARC 1981).

All the tested doses of measles vaccine induced significant number of chromosomal aberrations ($p \leq 0.05$ or $p \leq 0.01$) only in the male mice, but failed to induce significant aberrations in the female mice. However, the induced chromatid gaps were more in the female mice. This shows that the chromosomes of male mice are comparatively more susceptible to aberration on exposure to measles vaccine than that of the female mice. Sex hormones might have some role in bringing about such disparity. Interestingly, the intermediate dose (1000 CCID₅₀/ 100 g b.w.) induced the highest percent of aberrant metaphases, highest number of aberrations excluding gaps and the highest number of gaps, even more than that of the highest tested dose (2000 CCID₅₀) (Table 1). This indicates that measles vaccine 1000 CCID₅₀/ 100 g b.w. is the optimal dose in inducing chromosomal aberration in mouse bone marrow cells. The induced clastogenicity of measles vaccine in the

Table 1: Chromosomal aberrations in bone marrow cells of mice induced by Measles Vaccine and Cyclophosphamide (in endoxan) at 24 h post-treatment intraperitoneally.

Chemical	Dose per 100 g b.w. of mice	Number of mice treated/ Gender	Number of Metaphases of Examined	Number of Aberrant Meta-phases	Types and number of chromosomal aberrations						Total number of aberrations (excluding gaps)	Percentage of aberrant meta-phases	Aberrations (excluding gaps) per hundred metaphases
					Chromatid type		Chromosome type	Fragment/Minute					
					Gap	Break		Gap	Break				
0.9% NaCl (Vehicle control) Measles Vaccine	1 ml	3f	465	12	10	-	2	-	3/-	3	2.58	0.64	
		3m	596	18	15	1	2	-	1/-	2	3.02	0.33	
	500	3f	450	31	16	13	1	-	1/-	14	6.88	3.11	
		3m	450	91	50	21	5	3	10/2	39	20.22	8.66*	
	1000	3f	450	107	81	21	-	1	3/1	28	23.77	6.22	
		3m	450	81	30	35	2	2	7/5	56	18.00	12.44**	
Cyclophosphamide (Positive control)	2000	3f	450	58	38	15	1	-	3/1	20	12.88	4.44	
		3m	450	87	32	39	3	1	11/1	54	19.33	12.01*	
	CCID ₅₀	3f	377	239	56	75	15	20	205/48	416	63.39	110.34**	
		3m	427	219	87	104	22	6	158/37	348	51.28	81.49**	
	4.0 mg	3f	450	87	32	39	3	1	11/1	54	19.33	12.01*	
		3m	450	87	32	39	3	1	11/1	54	19.33	12.01*	

* $P \leq 0.05$, ** $P \leq 0.01$, f = female mice, m = male mice

present study is in agreement with the earlier reports (Chun et al. 1966; Mikhailova and Gorschunova 1969, 1975; Gorschunova et al. 1975; Knuutila et al. 1978; Michailova et al. 1976). But, no such earlier report is available showing sexual discrimination in the clastogenicity of measles vaccine. The earlier reports on chromosomal aberrations from measles patients (Nichols et al. 1962, 1964, 1965; Gripenberg 1965; Aula 1965; Grover et al. 1967; Csonka et al. 1975; Tolani et al. 1979) corroborate the clastogenic potential of measles vaccine. Contrarily, Tanzer et al. (1963) and Harnden (1964) failed to find any increase in chromosomal aberration in patients with measles. Tanzer et al. (1963) failed to notice even a single chromatid break out of the 644 metaphase spreads examined from the preparation of short-term leucocyte cultures from 24 patients with measles, except for a few chromatid gaps and chromosome constrictions. Absence of chromosomal aberration in their study might have been due to some technical/procedural shortcomings.

The clastogenicity of all the three different tested doses of rubella vaccine in mouse bone marrow, recorded here, is in agreement with the earlier reports on the induction of chromosomal breaks in human embryonic cell cultures (Plotkin et al. 1965), chromosomal abnormalities in congenital rubella (Nusbacher et al. 1967) and in women receiving drugs during the period of viremia after rubella vaccination (Knuutila 1976). Plotkin et al. (1965) and Rawls and Melnick (1966) also explained chromosome damage, resulting in cell death, as the cause of inhibition of cell multiplication after rubella infection. On the other hand, Mellman et al. (1965) analyzed the chromosomes in cultured leucocytes of six newborn infants with congenital rubella and could not find any chromosomal abnormality. Neu et al. (1964) and Valenti (1965) also could not notice any chromosomal abnormality in therapeutically aborted fetuses of mother with rubella in the first trimester of pregnancy. Coincidentally, all the earlier studies showing negative clastogenic effects of rubella virus have been made at very early stages i.e., on infants and on aborted fetuses. Thus, the clastogenicity of rubella might be related to the age of the infected persons. At early period of life, as there is a rapid lymphoid turnover; the affected lymphocytes might not be surviving during the

subsequent cell divisions, resulting in the non-availability of chromosomal aberrations at the time of observation. Thus, for clastogenicity tests in rapidly proliferating tissue, the timing between treatment and observation after one cell cycle is important.

The induced significant clastogenic action of all the three different tested doses of rabies vaccine of the present investigation is in agreement with the earlier reports of Cherkezia et al. (1980) and Nayak and Das (1987) on chromosomal aberration and micronuclei induction in mice. However, both the earlier studies have been done after chronic exposures of daily inoculation for 24 and 7 days, respectively, that to with very high doses. But in the present study a single inoculation of very low dose (0.25, 0.5, or 1.0 I.U./ 100 g b.w.) also induced significant number of chromosomal aberrations. May be because of high dose and chronic exposure, Cherkezia et al. (1980) reported pulverization of chromosomes, whereas we failed to record any such pulverization. The dose dependent increase, although nonlinear, in the induction of aberrant metaphases, chromosomal aberrations and the chromatid gaps by rabies vaccine in the present study gets support from the earlier reports of Stich and Yohn (1970) on the dose related chromosome damage by the virus. Contrarily, Marquez-Monter et al. (1967) reported the absence of any chromosomal anomaly in the lymphocytes of rabies patients. Although the reason for such contradictory result is not explainable, rabies vaccine possesses the potentiality of affecting the chromosomes.

The exact mechanism of viral mutagenesis is not known. Viruses may attack chromosomes directly. Certain viruses cause activation of lysosomal enzymes including DNase and cathepsin. Light induced selective activation of lysosomal enzymes produces increased chromosomal breakage, either directly or by destroying the proteins associated with the chromosomes (Allison and Panton 1965). This mechanism has not yet been proved, but may be true for certain viral pathogens. The exact mechanism of the clastogenic action of vaccines is obscure. But all the vaccines contain attenuated viruses. Thus, there is remote possibility of multiplication or transcription of the viral genome. But some viral components like virion might be

Table 2: Chromosomal aberrations in bone marrow cells of mice induced by Rubella Vaccine and Cyclophosphamide (in endoxan) at 24 h post-treatment intraperitoneally.

Chemical	Dose per 100 g b.w. of mice	Number of mice treated/ Gender	Number of Metaphases Examined	Number of Aberrant Meta- phases	Types and number of chromosomal aberrations						Total number of aberrations (excluding gaps)	Percentage of aberrant metaphases	Aberrations (excluding gaps) per hundred metaphases
					Chromatid type		Chromosome type						
					Gap	Break	Gap	Break	Fragment	Minute			
.9% NaCl (Vehicle control) Rubella Vaccine	1 ml	3f	465	12	10	-	2	-	3/-	3	2.58	0.64	
		3m	596	18	15	1	2	-	1/-	2	3.02	0.33	
	100	3f	450	95	21	52	3	5	10/4	80	21.11	17.77 **	
	CCID ₅₀	3m	450	67	23	31	-	1	7/5	50	14.88	11.11 **	
	200	3f	450	95	43	49	-	1	1/1	54	21.11	12.00 **	
Cyclophosphamide (Positive control)	CCID ₅₀	3m	450	108	46	54	1	-	7/-	61	24.00	13.55 **	
	400	3f	450	113	35	69	2	4	1/2	82	25.11	18.22 **	
	CCID ₅₀	3m	450	127	60	59	2	1	2/3	69	28.22	15.33 **	
	4.0 mg	3f	377	239	56	75	15	20	205/48	416	63.39	110.34 **	
		3m	427	219	87	104	22	6	158/37	348	51.28	81.49 **	

** P ≤ 0.01, f = female mice, m = male mice

Table 3: Chromosomal aberrations in bone marrow cells of mice induced by Rabies Vaccine (Rabipur) and Cyclophosphamide (in endoxan) at 24 h post-treatment intraperitoneally.

Chemical	Dose per 100 g b.w. of mice	Number of mice treated/ Gender	Number of Metaphases of Examined	Number of Meta- phases	Types and number of chromosomal aberrations						Total number of abe- rrations (exclu- ding gaps)	Perce- ntage of aberrant- meta- phases	Aberra- tions (exclu- ding gaps) per hundred metap- hases
					Chromatid type		Chromosome type		Frag- ment/ Minute				
					Gap	Break	Gap	Break					
0.9% NaCl (Vehicle control) Rabies Vaccine	1 ml	3f	465	12	10	-	2	-	3/-	3	2.58	0.64	
		3m	596	18	15	1	2	-	1/-	2	3.02	0.33	
	0.25 I.U.	3f	450	155	77	73	1	-	3/1	78	34.44	17.33 **	
		3m	450	197	77	109	1	-	8/3	123	43.77	27.33 **	
	0.5 I.U.	3f	450	279	127	146	-	3	1/2	157	62.00	34.88 **	
Cyclophosphamide (Positive control)		3m	450	209	86	115	-	3	1/4	130	46.44	28.88 **	
	1.0 I.U.	3f	450	330	149	172	2	3	2/2	184	73.33	40.88 **	
		3m	450	262	127	119	1	4	8/3	141	58.22	31.33 **	
	4.0 mg	3f	377	239	56	75	15	20	205/48	416	63.39	110.34 **	
		3m	427	219	87	104	22	6	158/37	348	51.28	81.49 **	

** P ≤ 0.01, f = female mice, m = male mice

responsible for the genotoxic effects. Toxin of *Clostridium perfringens* and culture filtrate of *Staphylococcus aureus*, *Salmonella typhi* and *Vibrio cholerae* are reportedly inducing chromosomal damage (Manna and Das 1974; Manna 1992; Hazra and Manna 1981). Thus, the antigenic substances present in the vaccines might be responsible for causing the genotoxic/clastogenic effects. Further study is essential to unveil the exact mechanism of the clastogenic action of different vaccines on the hereditary materials of the inoculated organisms.

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