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Cytogenetic evaluation of Fansidar on human lymphocyte chromosomes *in vitro*

Author Details

Nuzhat Praveen	Section of Genetics, Department of Zoology, Aligarh Muslim University, Aligarh - 202 002, India
Muheet Alam Saifi	Department of Zoology, College of Science, PO Box-2455, King Saud University- Riyadh - 11451, Kingdom of Saudi Arabia
G.G.H.A. Shadab (*Corresponding author*)	Section of Genetics, Department of Zoology, AMU Aligarh - 202 002, India e-mail: gghas.amu@gmail.com

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Abstract

Fansidar is a fixed combination of two antimalarial agents a diaminopyrimidine (Pyrimethamine) and a sulphonamide (Sulphadoxine) in the ratio 1:20- that have been used extensively worldwide for the treatment of Chloroquine resistant *Plasmodium falciparum* malaria, toxoplasmosis and pneumocystis carinii pneumonia in patients with the acquired immunodeficiency syndrome. This study examined the effect of Fansidar on chromosomes in human lymphocyte culture. Fansidar was added to peripheral blood lymphocyte cultures in vitro at four different concentrations: 5, 15, 25 and 50 µl in the ratio 1:20, 3:60, 5:100 and 10:200 µg ml^{-1}. Result shows that this drug induces moderate increase in the frequency of gaps, breaks and rearrangements. Therefore it can be concluded that Fansidar has moderate clastogenic effect on human chromosomes in vitro.

Key words

Fansidar, Lymphocyte chromosomes, Chromosomal aberration, Clastogenicity, Genotoxicity

Introduction

Fansidar (Pyrimethamine/Sulphadoxine) has been used extensively worldwide for the treatment of chloroquine resistant *Plasmodium falciparum* malaria, toxoplasmosis and pneumocystis carinii pneumonia in patients with the acquired immunodeficiency syndrome. Each tablet of Fansidar contains 500 mg N^1 (5, 6-dimethoxy-4-pyrimidinyl) sulfanilamide (Sulphadoxine) and 25 mg 2, 4-diamino-5- (p-chlorophenyl)-6- ethylpyrimidine (Pyrimethamine). Fansidar (PM/SP) is an antifolic agent (Laurence *et al*., 1997; Sims *et al*., 1998) and it has been reported to affect folate dependent DNA-synthesis (Rosenthal and Goldsmith, 2001). The effective dose of Fansidar is in the ratio 1:20 that is used for the treatment of patients with *Plasmodium falciparum* malaria (Reynolds, 1993; Staedke *et al*., 2001). The two components of Fansidar, Pyrimethamine and Sulphadoxine, competitively inhibit the enzyme dihydrofolate reductase which converts dihydrofolate to tetrahydrofolate (Krishaswamy and Teoh, 1980). The blockade of dihydrofolate reductase enzyme by Pyrimethamine prevents synthesis of folic acid (FA) in the cells (Krishaswamy and Teoh, 1980). The prevention of biosynthesis of folic acid inhibits DNA replication and amino acid synthesis (Laurence and Benett, 1980; Waxman and Herbert, 1969). This inhibition can lead to structural chromosome defects such as gaps, breaks, acentric fragments, *etc*. Also, it can cause the development of fragile sites, which are points on chromosomes that usually appear as non staining chromosomes or chromatid gaps. These regions may be involved in chromosome breakage and recombination events (Glover and Stein, 1988).

It inhibits sequential steps involved in the biosynthesis of tetrahydrofolic acid. This depletes folic acids, an essential cofactor in the biosynthesis of nucleic acid, resulting in interference with protozoal nucleic acid and protein production (Watkins *et al*., 1997; Sims *et al*., 1998). Sulphadoxine is a structural analogue of para-amino-benzoic acid PABA and competitively inhibits the enzyme dihydropteroate synthetase. Pyrimethamine is a competitive inhibitor of the enzyme dihydrofolate reductase.

Taking into account lack of sufficient information about the clastogenicity of Fansidar the cytogenetic activity of this drug was investigated.

Materials and Methods

Chemicals: Fansidar (F. Hoffman-La Roche Ltd.-Switzerland) containing 25 mg Pyrimethamine (PM) and 500 mg Sulphadoxine (SP) was dissolved in 1% dimethyl sulfoxide (DMSO) to prepare stock solution of the drug.

Chromosomal aberrations test: Chromosomal aberrations resulting due to Fansidar were observed using lymphocyte culture as per the method of Moorehead *et al.* (1960). 25 subjects (19 males and 6 females of 20-26 yr age group) were taken, from which blood samples were collected to study chromosomal aberrations. In selecting the subjects it was ascertained that they are non smoking and healthy and that they had not received X-ray irradiation in the last two to three months, that they were not suffering from any viral infection, and that they were not taking medications or any drug. Data for each subject are presented in the Table 1.

Lymphocyte cultures were planted in previously cleaned and sterile chamber of laminar flow. All the necessary apparatus (culture vials, pipettes) were sterilized before use. About 0.4 ml of blood was transferred to a culture vial, already containing 5-7 ml of culture medium (RPMI- 1640) and 0.2-0.3 ml of phytohaemagglutinin (PHA), and the treatment was done by using different Fansidar concentrations ranging from 5, 15, 25 and 50 µl in the ratio 1:20, 3:60, 5:100 and 10:200 µg ml^{-1} in each culture vial under the sterile conditions of laminar flow. The vials were closed tightly and were put at 37°C for 72 hr in water bath. Different concentrations of drug were added to the culture vials at 24 hr under laminar flow hood and then sealed with parafilm and were put again at 37°C in water bath. 0.15 ml of colchicine (2 µg ml^{-1}) was added to each vial 1 hr prior to harvesting to arrest the cells at metaphase stage. After 1 hr of colchicine's action in the medium, the cells were spun down by centrifugation (10 min, 1000 rpm) and the button of cells was saved by discarding the supernatant. Hypotonic treatment (0.075 M KCl) was carried out for 10-20 min at 37°C and the cells were recollected by centrifugation. The cell pellet was suspended in freshly prepared chilled fixative (3: 1, methanol: acetic acid). After giving final washing in the fixative, the cells were resuspended in 0.2 ml of fresh fixative. Slides were prepared by flame drying method. Three drops per slide was generally enough to get a satisfactory cell count. Giemsa stain was used for staining the metaphase chromosomes. Photographs were taken on 100X magnification with an automatic digital camera attached in a Nikon microscope (Eclipse 80i).

Results and Discussion

The result of chromosomal aberration in human lymphocytes culture either exposed or unexposed to Pyrimethamine/Sulphadoxine has been given in the Table 1. For each subject lymphocytes exposed to PM/SP, a concentration dependent increase in clastogenic damage was found. The total percentages of chromosomal aberrations for control were - Gaps 2.25%, Breaks+ Rearrangements 2.25% in subject (A), Gaps 4%, Breaks + Rearrangements 2% in subject (B), Gaps 4%, Breaks + Rearrangements 8% in subject (C), Gaps 2.75%, Breaks + Rearrangements 2.75% in subject (D) and Gaps 2.9%, Breaks + Rearrangements 0% in subject (E). The values of chromosomal aberration at 1:20 µg ml^{-1} of concentration were - Gaps 16.6%, Breaks+ Rearrangements 16.6% in subject (A), Gaps 16%, Breaks + Rearrangements 20% in subject (B), Gaps 24.1%, Breaks + Rearrangements 10.3% in subject (C), Gaps 20%, Breaks + Rearrangements 20% in subject (D) Gaps 17.6%, Breaks + Rearrangements 23.5% and in subject (E). From the above data it is clear that due to the exposure of drug at the concentration of 1:20 µg ml^{-1} mitotic index is decreased and the number of gaps, breaks and rearrangements is increased. At the concentration of 3:60 µg ml^{-1} the chromosomal aberrations were - Gaps 12%, Breaks+ Rearrangements 40% in subject (A), Gaps 30%, Breaks + Rearrangements 20% in subject (B), Gaps 19.2%, Breaks + Rearrangements 38.4% in subject (C), Gaps 21.4%, Breaks + Rearrangements 35.7% in subject (D) and Gaps 40.9%, Breaks + Rearrangements 18.1% in subject (E). The results observed for chromosomal aberration at the concentration of 5:100 µg ml^{-1} were - Gaps 20%, Breaks + Rearrangements 30% in subject (A), Gaps 40%, Breaks + Rearrangements 20% in subject (B), Gaps 24%, Breaks + Rearrangements 36% in subject (C), Gaps 32%, Breaks + Rearrangements 32% in subject (D) and Gaps 30%, Breaks + Rearrangements 32.5% in subject (E) and the results observed for chromosomal aberration at the concentration of 10:200 µg ml^{-1} were - Gaps 26%, Breaks + Rearrangements 17.6% in subject (A), Gaps 30 %, Breaks + Rearrangements 22.5% in subject (B), Gaps 28.5%, Breaks + Rearrangements 28.5% in subject (C), Gaps 16.6%, Breaks + Rearrangements 41.6%, in subject (D) and Gaps 40.9%, Breaks + Rearrangements 18.1% in subject (E).

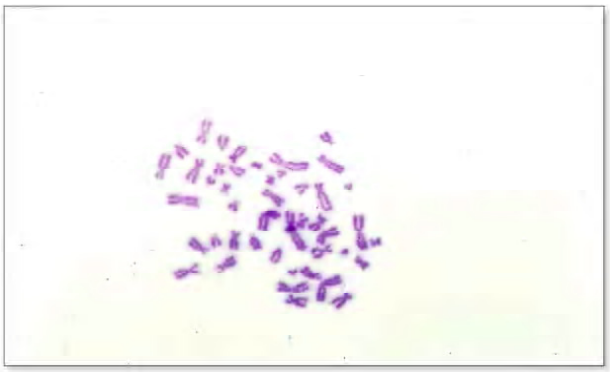

Fig. 3: Pyrimethamine/Sulphadoxine treated metaphase plate showing chromosomal aberration on 100X

From the above data it is clear that there is a significant decrease in metaphase plates at the highest concentration as it seems toxic to the cells. Among the control cultures, the mean frequency of total damaged cells was 6.18%. At 1:20 µg ml^{-1} concentration, the percentage of total damaged cells increased to 36.94%. At 10:200 µg ml^{-1} concentrations, the percentage of total damaged cells was 54.08%.

Fansidar is an antifolic agent (Laurence *et al*., 1997; Sims *et al*., 1998) and it has been reported to affect folate dependent DNA-synthesis (Rosenthal and Goldsmith, 2001). The two components of Fansidar, Sulphadoxine and Pyrimethamine, competitively inhibit two enzymes in the folate synthesis pathway - dihydropteroate synthetase and dihydrofolate reductase respectively, that catalyse the conversion of dihydrofolate to tetrahydrofolate, and this impairs DNA synthesis (Ouellette, 2001; Roper *et al*., 2003). In the

Table - 1: Chromosomal aberration due to Pyrimethamine/Sulphadoxine

Concentration	Subject	Metaphase	Gaps	Breaks and rearrangements	Total	Gap%	Breaks and rearrangements	Total %
Control	A	50	1	1	2	2.25%	2.25%	4.5
	B	50	2	1	3	4	2	6
	C	50	2	4	6	4	8	12
	D	36	1	1	2	2.75	2.75	5.5
	E	34	1	0	1	2.9	0	2.9
1:20 μg ml^{-1}	A	30	5	5	10	16.6	16.6	33.3
	B	25	4	5	9	16	20	36
	C	29	7	3	10	24.1	10.3	34.4
	D	50	10	10	20	20	20	40
	E	34	8	8	14	17.6	23.5	41
3:60 μg ml^{-1}	A	50	6	20	26	12	40	52
	B	20	6	4	10	30	20	50
	C	26	5	10	15	19.2	38.4	57.6
	D	28	6	10	16	21.4	35.7	57.1
	E	22	9	4	13	40.9	18.1	59
5:100 μg ml^{-1}	A	25	5	8	13	20	30	50
	B	10	4	2	6	4	20	60
	C	50	12	18	30	24	36	60
	D	50	16	16	32	32	32	64
	E	40	12	13	25	30	32.5	62.5
10:200 μg ml^{-1}	A	46	12	8	20	26	17.6	43.4
	B	40	12	9	21	30	22.5	52.5
	C	14	4	4	8	28.5	28.5	57.1
	D	12	2	5	7	16.6	41.6	58.3
	E	22	9	4	13	40.9	18.1	59.1

same respect, Fansidar was found to have an inhibitory effect on lymphocyte transformation *in vitro,* whereas this inhibition was attributed to suppression of DNA synthesis (Bygbjerg *et al*., 1986). Thus, DNA synthesis inhibition has been suggested to be an indirect way for inducing genetic damage, the increased genotoxicity usually occurring at a concentration range of toxic doses (Galloway *et al*., 1998). The probable mechanism of antifolate - induced mutation has been attributed to nucleotide precursor pool imbalance of mammalian cells to result in deoxyuridine misincorporation into DNA (Veigl *et al*., 1991). In addition, deoxyuridine misincorporation or pool imbalance-induced replication defects may also underline increases in SCE as seen in antifolate- treated cells and fragile sites in human chromosomes. Folate deficiency is known to induce chromosomal aberrations (CA) and fragile site expressions (Kunz, 1982 and 1988).

The antimicrobial agent pyrimethamine is a folic acid antagonist (Waxman and Herbert, 1969; Webster, 1985) and by inhibition of folate reductase enzyme inhibits replication by preventing the formation of thymidilate which is used in DNA synthesis (Waxman and Herbert, 1969; Dipelema, 1971; Meyers *et al*., 1974; Dyke, 1982). As a result, lesions in the DNA molecule are produced and consequently gaps, breaks and rearrangements are formed (Waxman and Herbert, 1969). Such breakage can lead to SCEs, deletions and translocations under appropriate conditions (Glover and Stein, 1988). In support to this it has also been reported that folic acid deficient medium significantly increases the expressions of spontaneous chromosome breakage (Wekemans *et al.*, 1983) and those inhibitors of DNA synthesis increase chromosome breakage (Moore and Hodgson, 1983).

Treatment of human lymphocyte with PM/SP does not appear to cause numerical anomalies. However, structural chromosome abnormalities increased by the increase in the concentration of PM/SP given to the medium. It is observed that the highest increase is in the number of gaps, breaks and rearrangements at highest concentration.

In conclusion, our study shows that Pyrimethamine/ Sulphadoxine is a clastogenic chemical inducing various structural abnormalities in human chromosomes.

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