Effect of Fansidar Drug on the Chromosomal Aberrations of Albino Rats

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Abstract: Malaria, a mosquito-borne disease is a serious health challenge to mankind. Human malaria results from infection with *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale or Plasmodium malariae,* but a large majority of the clinical cases and mortalities is caused by *Plasmodium falciparum.* Fansidar[®] is a fixed combination of two antimalarial agents pyrimethamine (25 mg/tablet) and sulfadoxine (500 mg/tablet) that have been used extensively worldwide for the treatment of chloroquine resistant. Folic acid used to avoid or at least to reduce toxic effects of fansidar drug. The present work searched for the cytogenetic effect of fansidar and /or protective effect of the folic acid *in vivo* using albino rats. This was achieved by using cytogenetic studies. The results of the present investigation illustrated that aberrations in chromosomes due to single fansidar dose administration may reduced by folic acid administration.

Keywords: Antimalarial drugs. Fansidar (Pyrimethamine - sulfadoxine), Chromosomal aberrations and Albino Rats.

1.Introduction

Pyrimethamine (Daraprim[®]) is a medication used for protozoal infections. It is commonly used as an antimalarial drug (for both treatment and prevention of malaria) and is also used (combined with sulfadiazine) in the treatment of *Toxoplasma gondii* infections in immunocompromised patients such as HIV-positive individuals. Pyrimethamine is typically given with a sulfonamide and folinic acid. Sulfonamides inhibit dihydropteroate synthetase, an enzyme that participates in folic acid synthesis from para-aminobenzoic acid. Hence sulfonamides work synergistically with pyrimethamine by blocking a different enzyme needed for folic acid synthesis [1].

Leucovorin is a form of folic acid. Folic acid is a naturally occurring substance that is important for the formation of red and white blood cells. Folic acid is present in many foods, such as beans, peas, oranges, spinach. Leucovorin is used to reduce the side effects of large doses of medications, such as methotrexate (Rheumatrex), pyrimethamine (Daraprim), trimethoprim (Trimpex, Proloprim), that may reduce the effects of folic acid in the body [2].

The genotoxic effect of PYR which is a drug used in the therapy of toxoplasmosis and malaria, in bone marrow cells of Swiss albino mice.The result showed no statistically significant change in numerical chromosome abnormalities while, structural chromosome aberrations and micronuclei were increased in a dose- dependent manner by cytogenetic and statistical evaluations[3].

Aberrations due to PYR in the chromosomes were reduced by ascorbic acid and folinic acid significantly depending on the given dose [4].

The interaction of the folic acid antagonist pyrimethamine with the centromeres of chromosomes. The authors found that the modification lead to induction of aneuploidy in exposed cells. Genetic change can be produced by gene mutations, by structural chromosomal changes and by numerical chromosome changes (aneuploidy) [5].

It appears that this drug is able to induce moderate genotoxic effects as revealed by the increases found in sister – chromatid exchanges and micronuclei frequencies in cultures from donors. In addition, cytotoxic/cytostatic effects of fansidar were revealed by a decrease in the proliferative rate index and in the cytokinesis block proliferation index. So they suggested that the use of this drug should be restricted to situation where other antimalarial drugs cannot be used [6].

Furthermore, treatment of human lymphocyte with PYR/SDX does not appear to cause numerical chromosome abnormalities. However, structural chromosome abnormalities increased by the increase in the concentration of PYR/SDX given to the medium. It is observed that the highest increase is in the number of gaps, breaks and rearrangements at highest concentration[7].

2.Material and Methods
Experimental design:

Sixty five (65) adult male albino rats were used for the investigation of the effect of fansidar and / or

the protective effect of folic acid. They were allocated into four main groups as following:

(I)   Control group: In this group 5 rats were kept as control and orally injected by gastric tube with an equivalent volume of distilled. water.

(II)   Fansidar group: twenty (20) rats were orally injected with therapeutic dose of 18.9 mg /rat of fansidar taken as single dose

The animals then subdivided randomly into 4 equal subgroups each subgroup was contained 5 rats as following:

-   1st sub-group: 5 rats were sacrificed after one day of stopping administration.

-   2nd sub-group: 5 rats were sacrificed after three days of stopping administration.

-   3rd sub-group: 5 rats were sacrificed after seven days of stopping administration.

- 4th sub-group: 5 rats were sacrificed after fourteen days of stopping administration.

(III)   Folic acid group:

Twenty (20) rats were orally injected with therapeutic dose of 0.02 mg /rat/day dose of folic acid taken for three successive days. The animals then subdivided randomly into 4 equal subgroups and sacrificed after one, three,seven and fourteen days post treatment with the drug typically as mentioned above in fansidar group (II)

(IV) Folic acid and fansidar group.

Twenty (20) rats were orally injected with therapeutic doses of folic acid 0.02 mg /rat/ day for three successive days followed by 18.9 mg /rat /day for fansidar taken as single dose. The animals then subdivided randomly into 4 equal subgroups and sacrificed after one, three,seven and fourteen days post treatment with the drug typically as mentioned above in fansidar and folic acid groups (II & III).

In the previous subgroups,

The chromosomal aberrations were taken from the bone marrow cells of the animals.

Chemicals and solutions

1.a. Chromosomal Aberrations Assay:

Reagents: reagents used in the present work for the investigation of chromosomal aberrations from bone marrow cells of rats were prepared as follow:

-        Colchicine 0.04% solution: 40 mg of dry colchicine was dissolved in 100 ml of sterile distilled water and stored in freezer.

-        Phosphate Buffer Saline (PBS): 8 gm NaCL, 0.2gm KCL, 1.874 gm Na_2HPO_4 and 0.2 gm KH_2PO_4 were dissolved in 1 litter dist. H_2O.

-        Hypotonic solution: 5.6 gm KCL dissolved in liter distilled water. The solution was pre- warmed to 37°c.

-        Clarke's fixative: methanol- glacial acetic acid, 3/1 V/V was freshly prepared at time of the cultures to be harvested.

-        Giemsa stain (1.5%): it was prepared according to the method described by **(8)**

As follow:

1.1. Stock solution:

one gm of Giemsa powder was dissolved in 66 ml of glycerol heated to 60°c.

-        The mixture was left to stand for 2-3 hours with periodic mixing.

-        The mixture was allowed to cool and then 66 ml of absolute methanol was added.

-        The stock was kept in a dark bottle and filtered before use.

1.2 Giemsa Working Solution:

10 ml stock solution   100   ml distilled water

The working solution was prepared just before use.

1.3   Chromosomal preparation from bone marrow cells:

-        Colchicines solution (0.04%) was injected intraperitonealy to each rat (0.25 ml/ 70 gm body weight) to arrested mitosis at the metaphase stage.

-        Two hours after colchicine treatment, the animals were sacrificed by cervical dislocation and opened at both ends by cutting off the epiphyses. Six ml of phosphate buffer solution were injected from one end of bone femur collecting the cell suspension from the other end in a centrifuge tube, using one tube for each femur.

-        The tubes were centrifuged for 10 minutes at 1000 r.p.m and the supernatant was discarded the cells were resuspended in 6 ml of KCl solution, incubated at 37°C for 20 minutes and centrifuged for 10 minutes at 1000 r.p.m, and the supernatant was discarded.

-        Six ml of freshly prepared fixative 3: 1 methanol: glacial acetic acid were added, then cells mixed gently and left for 20 minutes in freezer.

-        The solution was centrifuged at 1000 r.p.m for 10 minutes the supernatant was discarded and 4ml of fixative were added. This treatment was repeated 2-3 times.

-        Finally, one ml of the fixative was added to give a Condensed cell suspension.

-        The cell suspensions were dropped on clean glass slides, previously put in cold 70% ethanol and keeping in freezer.

-        The slides were allowed to air- dry and then stained with Giemsa (1.5%) working solution for 10-15 minutes in coplen jars and washed by running water.

The metaphase spreads were examined using oil immersion objective lens (X100) of an ordinary light microscope and (X10) eye pieces.

The chosen metaphases printed using suitable enlargements. One or two slides were chosen for each sample and 50 metaphases cell was examined randomly for chromosomal aberrations for each rat.

A search carried out for the chromosomal aberrations, which were either:

Structural chromosomal aberrations in the form of chromatid and chromosomal breaks, chromatid & chromosomal gap, dicentric, ring chromosomes, exchanges figures, deletion, centromeric fusion and acentric fragment.

Numerical chromosomal aberrations in the form of: Aneuploidy (which involves the gain or loss of one or a few chromosomes, i.e. hyperdiploid or hypodiploid), polyploid (which involves complete set of chromosomes, if the chromosomes duplicate normally in interphase) and endoreduplication.

1.4 Statistical Analysis:

Incidence of abnormal metaphases was analyzed for significance by Student's Test [9]

3. Results

The chromosomal aberrations from bone marrow cells of rats were recorded in (Table 1) and shown in photographic plates (Fig.1) & histogramically(Fig.2).

From the comparison between the side effect of fansidar alone and combination of fansidar with folic acid on the chromosomal aberrations from bone marrow cell of male albino rats it was clear that, the percentages of total chromosomal aberrations from bone marrow cell of male albino rats after the administration of single fansidar dose (18.9 mg /rat/day) alone for all time of examination were 60.8%, 69.2, 71.2% and 88.8% after one, three, seven and fourteen days post treatment of the drug respectively.

However, the percentages of total chromosomal aberrations from bone marrow cell of male albino rats after the administration of folic acid 0.02 mg / rat/day for 3 successive days followed by single fansidar dose (18.9 mg /rat/day) at all time of observation were 48.8%, 44.4%, 39.6% and 37.2% after one, three, seven and fourteen days post treatment of the drugs respectively.

From the above results we can concluded that, the combination of folic acid with fansidar drug decreased the side effect of treatment with fansidar alone.

Table (1): Comparison between the frequencies of total chromosomal aberrations from bone marrow cells of male rats administered only single fansidar dose 18.9 mg/ rat /day and other rats that administered folic acid dose 0.02 mg / rat / day for 3 successive days followed by single fansidar dose 18.9 mg/ rat /day**.**

Groups	No. of rats	No. of scored cells 50/rat	Total Chromosomal aberrations		
			No.	%	Mean ±SE
Control group	5	250	59	**23.6**	11.8± 1.28
Fansidar after one day	5	250	152	**60.8**	30.4 ± 1.4***
Fansidar after three day	5	250	173	**69.2**	34.6± 3.54***
Fansidar after seven day	5	250	178	**71.2**	35.6 ± 3.35***
Fansidar after fourteen day	5	250	222	**88.8**	44.4 ± 3.15***
Folic acid +fansidar after one day	5	250	122	**48.8**	24.4±3.55**
Folic acid +fansidar after three day	5	250	111	**44.4**	22.2±3.24*
Folic acid +fansidar after seven day	5	250	99	**39.6**	19.8± 2.63*
Folic acid +fansidar after fourteen day	5	250	93	**37.2**	18.6 ± 2.76

 * Significant at $P<0.05$, ** Significant at $P <0.01$ and *** Significant at $P <0.001$.
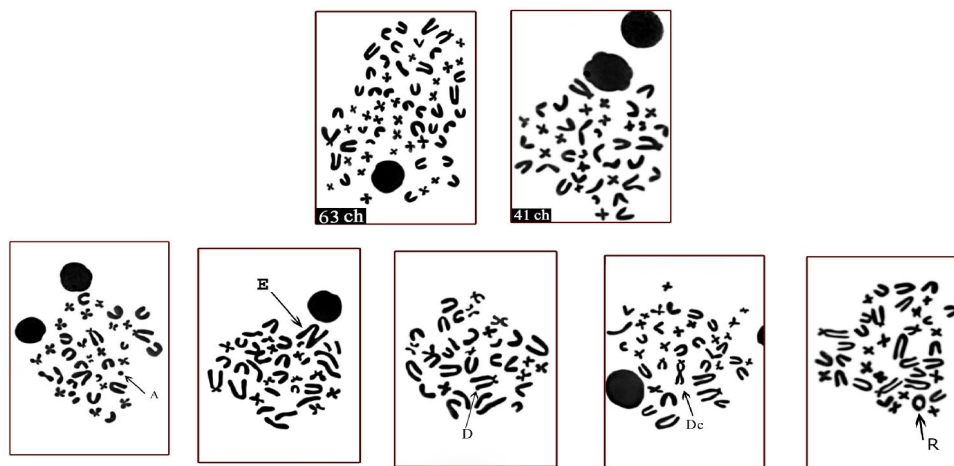

Figure (1): Several metaphases from bone marrow cells of male albino rats administered a single fansidar dose (18.9mg/rat/day) showing: **Up:** Numerical chromosomal aberrations. **Down:** Structural chromosomal aberrations: A centric fragment (A), exchange figure (E), chromosomal deletion (D), dicentric chromosome (Dc) and ring chromosome (R).
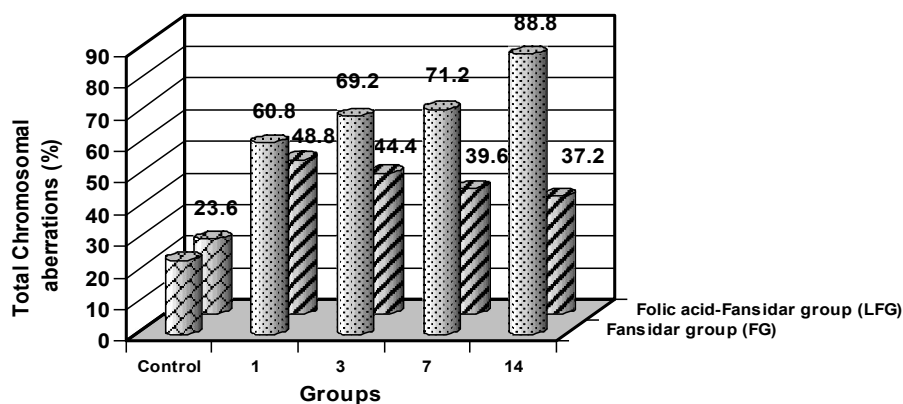
Fig. (2): Comparison between the frequencies of total chromosomal aberrations from bone marrow cells of male rats administered only single fansidar dose 18.9 mg/ rat /day and other rats that administered folic acid dose 0.02 mg / rat / day for 3 successive days followed by single fansidar dose 18.9 mg/ rat /day.

4.Discussion

In the present work, the chromosomal aberrations are dependent on mainly three factors of treatment, the fansidar drug, folic acid drug and combination of fansidar and folic acid drugs.

The aberrant metaphases studied were those for four subgroups of male albino rats administered single safety fansidar dose (18.9 mg/day) and sacrificed after one, three, seven and fourteen days respectively.

The examined chromosomal aberrations, included numerical and structural aberrations, from bone marrow cells of male rats were found to be very highly significant ($p < 0.001$) at all time of the drug treatment. Also the percentage of total aberrations was more increased gradually till the end of treatment at 14 day from stopping treatment. This data pointed to there main activation of single fansidar drugs for long time in the cells.

These data are agreement with some previous data for the mechanism of drugs for infections caused by protozoan parasites as Pyrimethamine belongs to the group of antifolate drugs blocking the enzyme dihydrofolate reductase essential for the synthesis of folic acid, a co-factor required for DNA synthesis. It is used in the treatment of infections caused by protozoan parasites such as *T. gondii* and *P. falciparum* as a single dose [10].

Pyrimethamine (PYR) is a folic acid antagonist used single or multiple dose in acute infestation for the treatment of malaria and toxoplasmosis [11].

The incidence of structural aberrations was increased and this increase was highly significant after 14 days post treatment of the drug. The most aberrations were exchange figures, deletion, acentric fragment and dicentric chromosomes this data agree with data reported by [12] who noted the pyrimethamine / sulfadoxine can lead to structural chromosome defects such as gaps, breaks, a centric fragments 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.

The commonly used antimalarials included chloroquine, sulfadoxine- pyrimethamine and amodiaquine their were increases in sister chromatid exchange in antifolate- treated cells and fragile sites in human chromosomes [13].

Pyrimethamine is an antimalarial agent widely used in clinical therapy. Pyrimethamine was found to produce a significant increase in structural chromosomal aberrations after acute treatment in bone marrow cells of mice.It also induced chromosome abnormalities in spermatogonial cells at the highest dose[14].

On the contrast, the genotoxic effect of PYR which is a drug used in the therapy of toxoplasmosis and malaria, in bone marrow cells of Swiss albino mice. The result showed no statistically significant change in numerical chromosome abnormalities while, structural chromosome aberrations and micronuclei were increased in a dose- dependent manner by cytogenetic and statistical evaluations[15].

Furthermore, treatment of human lymphocyte with PYR/SDX does not appear to cause numerical chromosome abnormalities. However, structural chromosome abnormalities increased by the increase in the concentration of PYR/SDX given to the medium. It is observed that the highest increase is in the number of gaps, breaks and rearrangements at highest concentration[7].

The numerical aberrations were metaphases with more or less number of chromosomes (aneuploidy) and

metaphases with more than two haploid sets of chromosomes (polyploidy).

In the present work the total numerical aberrations were very highly significant at all time of treatment of single fansidar dose drugs, and the aneuploidy that metaphases with less number of chromosomes are more affected than the more diploid number of 42 chromosome.

In our opinion the aneuploidy that metaphases with less number of chromosomes are more affected than the more diploid number of 42 chromosome referred to the mechanism of action of PYR/SDX that interaction with the protein of centromeres and hit specific chromosome lead to anaphase dysfunction of separation chromosomes in nuclear division and induced aneuploidy**.**

Several data are concurrent with our results , the interaction of the folic acid antagonist pyrimethamine with the centromeres of chromosomes and found that the modification lead to induction of aneuploidy in exposed cells. i.e. monosomic cells. The study concluded that the primary mechanism of action of pyrimethamine is the induction of damage to centromeric DNA which results in a failure of attachment of such damaged chromosomes to mitotic spindle. Chromosomes with damaged centromeres fail to correctly attach to the spindle thus producing monosomy[5].

On the contrast PYR did not increase the frequency of numerical or structural chromosome changes in female mouse germ cells[16]. Pyrimethamine does not induce numerical chromosomal aberrations in human lymphocyte cultures [17].

The second main factor is the folic acid treatment

The aberrant metaphases studied were those for four subgroups administered folic acid 0.02 mg / day for 3 successive days and sacrificed after one, three, seven and fourteen days. The examined chromosomal aberrations, included numerical and structural aberrations, from bone marrow cells of male rats were found to be insignificantly at statistical level at all time of drugs treatment except in subgroup 3 where total chromosomal aberrations were significant and numerical aberrations were highly significant on the statistical level. However the percentage of total aberrations gradually decreased and reached its minimum value after 14 days post treatment of the drug. This data are agreement with some data reported by*(18)*.

The three main factor is combination of Fansidar and Folic acid drugs

The aberrant metaphases for rats administered folic acid 0.02 mg/day for 3 successive days followed by a single fansidar dose (18.9 mg/day) and sacrificed after one, three, seven & fourteen days of stopping administration decreased compared to the aberrations

induced when the rats were administered with fansider drug alone at all time of treatment. However the data remain significant at the statistical level.

These data are concurrent with several data as the administration of folic acid or folinic acid supplements during treatment with pyrimethamine may help to prevent adverse effects associated with folate deficiency, which occur as an extension of the mechanism of action of the drug. [19]

The embryonic and maternal genotoxicity of PYR (a potent teratogen and folate antagonist). Pyrimetamine induced DNA damage in all maternal organs (liver, kidney, lung and brain) except spleen and bone marrow. Pyrimethamine also induced DNA damage in maternal and fetal placentas and embryos. Co-treatment of folinic acid calcium salt prevented the PYR- induced DNA damage in all target tissues examined after treatment.The observed embryonic and maternal DNA damage caused by PYR may be related to folate deficiency[20].

Folate deficiency impedes tumor growth rate, but supplementation does not accelerate it in folatereplete animals. Nutritional folate status has an important influence on the efficacy and toxicity of some commonly used cancer chemotherapeutic drugs[21].

Folic acid sometimes used to prevent toxic effects of high doses of antimicrobial dihydrofolatereductase inhibitors such as pyrimethamine. Pyrimethamine can cause birth defects in the fetus. Pyrimethamine also can affect bone marrow function. Potential bone marrow toxicity that may result from an escalating dose schedule of pyrimethamine should respond to the addition of folic acid (leucovorin) to the regimen[22] .

Pyrimethamine dosage used for treatment of toxoplasmosis approaches toxic levels and is associated with adverse effects resulting from folic acid deficiency. Megaloblastic anemia, leukopenia, thrombocytopenia, and pancytopenia reported. When pyrimethamine is used for treatment of toxoplasmosis, give leucovorin (folinic acid) concomitantly. Adverse hematologic effects, including megaloblastic anemia, generally reversible when drug discontinued. Pyrimethamine may be carcinogenic. Chronic granulocytic leukemia and reticulum cell sarcoma reported rarely after long-term use for treatment of toxoplasmosis, increase in lung tumors reported in animal study [23].

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