Protective effect of vitamin C and or vitamin E on micronuclei induction by rifampicin in mice

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Abstract: Rifampicin which is a known antituberculous agent has been reported to induce both chromosomal breakage and numerical chromosomal abnormalities. This study was carried out to determine the mutagenicity of rifampicin and more importantly to investigate the protective roles of antioxidants-vitamin C and E individually and in-combination therapy against rifampicin mutagenicity using micronucleus assay. Therapeutic concentrations of rifampicin alone (9mg/kg), rifampicin plus vitamin E (5mg/kg), rifampicin plus vitamin C (8mg/kg) and rifampicin plus vitamin C plus vitamin E were administered orally for 28 consecutive days using 6 mice in each group. The negative and positive control mice received same volume of distilled water and cyclophosphamide (40 mg/kg) intraperitoneally 6 hours before sacrifice, respectively. The results showed rifampicin alone treated group to demonstrate significant (P<0.05) increase in the proportion of micronucleated polychromatic erythrocyte (MPCE) to polychromatic erythrocyte (PCE) compared with the negative control group while a significant decrease (P≤0.05) in the proportion of MPCE to PCE was demonstrated in the rifampicin plus vitamin E; rifampicin plus vitamin C plus E and rifampicin plus vitamin C groups compared with cyclophosphamide treated group and rifampicin treated group. These findings suggest that rifampicin has damaging effects on the deoxyribonucleic acid (DNA). However, co-administration of rifampicin and antioxidants (vitamin C and E) has protective effect on the damaging potentials of rifampicin.

Key words: rifampicin, vitamin, deoxyribonucleic acid, mutagenicity, micronucleus, mice

Introduction

Rifampicin which is a known antituberculous agent has been reported to induce both chromosomal breakage and numerical chromosomal abnormalities (Sabry, 2007). A critical review of previous literatures has clearly shown an increase in hepatoma in female mice following one year's administration of rifampicin (Rate *et al.*, 1979). The remarkable observation of a 3-fold increase in the mutagenic activation of 2-amino-3-methylimidazole (4, 5-f) quinoline in cynomolgus monkeys treated with rifampicin is also evident of the mutagenic potentials of rifampicin (Sadriel & Snyderwine, 1995). In the same sequence with the earlier studies (Sadriel & Snyderwine, 1995; Jaynet & Suresh, 1998) showed rifampicin to produce thrombocytopenia and also induced nucleated cells in bone marrow. Apart from the genotoxic and cytotoxic effect of rifampicin, our recent study has shown rifampicin to be hepatotoxic and have the potential to cause sperm quality damage and congestion of the meninges (Awodele *et al*., 2010).

The mechanistic studies (Curtis *et al.*, 1988) have shown that micronucleus formation may be due to free radical generation from an agent leading to lipid peroxidation of membrane causing the breakages of the deoxyribonucleic acid (DNA) and covalently binding between the product of lipid peroxidation and DNA. However, it has also been

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documented by Pacher *et al.* (2007) that free radicals play an important role in a number of biological processes, some of which are necessary for life, such as the intracellular killing of bacteria by neutrophil granulocytes and also in certain cell signalling processes.

Antioxidants (vitamin A, C, E, carotenoids) are known to be reducing agents and they are molecules capable of slowing or preventing the oxidation of other molecules. They terminate the oxidative chain reactions by removing free radicals and prevent the oxidation of unsaturated fats which is the cause of rancidity (German, 1999). Thus, lot of previous scientific reports have clearly documented the anti-genotoxic and antimutagenic potentials of antioxidants (Vitamin C, E, A, carotenoids). The work of Khan & Sinha (1993) demonstrated that vitamin C (ascorbic acid) when administered concurrently with a pesticide could significantly decrease the frequency of pesticide-induced clastogenic and mitotic disruptive changes. Vitamin C also appears to efficiently protect the bone marrow cells when given together with rifampicin (Aly & Donya, 2002). The antigenotoxic effect of ascorbic acid against megesterol acetate- induced genotoxicity in mice has also been reported (Dique *et al.,* 2005). Furthermore, these studies have shown the antigenotoxic effect of apigenin against mitomycin C induced genotoxic damage in mice bone marrow cells, antigenotoxic effect of grape seed procyanidin extract in Fao cells submitted to oxidative stress and the antigenotoxic effect of ascorbic acid on mutagenic dose of three alkylating agents respectively (Ddique *et al.,* 2009; Liopiz *et al.,* 2004; Kaya, 2003). The antioxidant-vitamin E (alpha tocopherol) has also been documented to be protective against chromosomal aberration and mutation induced by Sodium Chromate in Chinese hamster V79 cells (Sugiyama *et al.*, 1991).

The mechanism of action of antioxidants are multidimensional, it may be due to their ability to scavenge free radicals which are often produced by the activities of some drugs, competition with the nucleophilic sites on DNA for an electrophilic mutagens, inhibition of promutagen bioactivation by blocking oxidation processes and reaction with the electrophilic metabolites of a promutagens (Goncharowa & Kuzhir, 1989; Nikolic *et al.,* 2006).

Therefore, this study was carried out to further determine the mutagenic effect of rifampicin and also investigate the protective roles of antioxidants-vitamin C and E against rifampicin induced micronuclei in mice. The results obtained from this study may proffer a strategy for the rational use of rifampicin in the management of tuberculosis so as to reduce possible genetic adverse effect.

Materials and Methods

Animals and drugs

Four to six weeks old pathogen free mice weighing between 15-25g, obtained from animal breeding units, University of Lagos, Lagos, Nigeria, were used for this study. The animals were acclimatized for two weeks before the commencement of the experiment. Rifampicin (MOPSON Pharmaceutical Ltd, Lagos-Nigeria), Cyclophosphamide (OLPHARM Nigeria Ltd, Oregun Ikeja, Lagos), vitamin C and vitamin E tablets (KUNIMED Pharmachem. Lagos, Nigeria). The drugs were all obtained from the outpatient Pharmacy Department of the Lagos University Teaching Hospital Lagos, Nigeria. Vitamin E (Evitol^{R}) which is fat soluble was granulated into fine powder before reconstitution and was vigorously shaken before each oral administration.

Experimental procedure

Therapeutic concentrations of drugs were administered using the following treatment groups of 6 mice per treatment group: Group 1: Rifampicin alone (9mg/kg); Group 2: Rifampicin plus vitamin E (5mg/kg vitamin E); Group 3: Rifampicin plus vitamin C (8mg/kg vitamin C); and Group 4: Rifampicin plus vitamin C plus vitamin E. The drugs were administered orally for 28 consecutive days following the method of Brusick (1980) and Alimba *et al.* (2006). The negative and positive control mice received same volume of distilled water and cyclophosphamide (40 mg/kg) intraperitoneally 6 hours before sacrifice respectively.

The animals were sacrificed by cervical dislocation and bone marrow cells were prepared from the femoral bone marrow and whole blood was obtained from orbital sinus of the eyes by the conventional method (Brusick, 1980). Foetal calf serum was used to flush bone marrow cells from both femurs into empendof tubes and cells were centrifuged at 2000 rpm for 10 min. Pelleted bone marrow cells were then resuspended in 0.8 ml of foetal calf serum and allowed to mix well by shaking. Smears were made out of this mixture and from the whole blood.

The slides were fixed with methanol for 10 minutes and left overnight to dry. The fixed slides were stained with 0.4% raw May Grunwald for three minutes; 1:1 ratio of 50% May Grunwald and 50% distilled water for six minutes and 5% Giemsa stain for 20 minutes. All slides were examined under oil immersion at high magnification for micronucleated polychromatic erythrocyte (MPCE) and polychromatic erythrocyte (PCE). The ratios of MPCE to PCE were transformed as arcsine square root {Y=sqrt(y)}. Results are presented as mean ± S.E.M and statistical significance between the groups was analyzed by means of student t-test and ANOVA. *P*- values less than 0.05 were considered significant.

Results

The results showed cyclophosphamide, rifampicin alone, rifampicin plus vitamin C and rifampicin plus vitamin E treated groups to significantly ($P<0.05$) increase the proportion of MPCE to PCE compared with the distilled water treated group (Table 1).

Table 1: Proportion of mironucleated polychromatophilic erythrocyte to polychromatophilic erythrocyte in blood smear of treated mice

Treatment Group	Mean ± SE	P≤ 0.05
Negative control	0.5690±0.26	Abcd
Cyclophosphamide	3.571±0.22	Efg
Rifampicin alone	6.194±0.25	hij
Rifampicin+Vitamin C	3.774±0.23	kl
Rifampicin+Vitamin E	2.035±0.49	
Rifampicin + Vit E + C	1.146 ±0.37	

Keys:- a: comparison between negative and positive control; b: comparison between negative control and rifampicin alone; c: comparison between negative control and rifampicin+vit.C; d: comparison between negative control and rifampicin+vit E; e: comparison between positive control and rifampicin; f: comparison between positive control and rifampicin+vit E; g: comparison between positive control and rifampicin+vit C+vit E; h: comparison between rifampicin and rifampicin+vit C; i: comparison between rifampicin and rifampicin + vit E; j: comparison between rifampicin and rifampicin + vit E+ vit C; k: comparison between rifampicin +vit C and rifampicin+vit E; l: comparison between rifampicin + vit C and rifampicin + vit E + vit C.

Rifampicin alone treated group also showed a significant ($P<0.05$) increase in the proportion of MPCE to PCE compared with the cyclophosphamide treated group, rifampicin plus

vitamin C treated group, rifampicin plus vitamin E treated group and rifampicin plus vitamin C plus vitamin E treated group. While a significant (P<0.05) decrease in the proportion of MPCE to PCE was demonstrated between rifampicin plus vitamin C, rifampicin plus vitamin E, rifampicin plus vitamin C plus E and cyclophosphamide treated group. The results further showed a significant decrease (P<0.05) in the proportion of MPCE to PCE between rifampicin plus vitamin C plus vitamin E treated group and rifampicin plus vitamin C treated group. There is no significant (P<0.05) difference between the negative control group and the rifampicin plus vitamin C plus vitamin E treated group.

Table 2: Proportion of mironucleated polychromatophilic to polychromatophilic erythrocyte in bone marrow of treated mice

Treatment Group	Mean±SE	P≤ 0.05
Negative control	1.024±0.35	Abcde
Cyclophosphamide	11.26±1.01	fg
Rifampicin alone	17.02±0.91	hij
Rifampicin+Vitamin C	9.876±0.24	
Rifampicin+Vitamin E	9.240±0.16	
Rifampicin + Vit E + C	7.869 ±0.29	

Keys:- a: comparison between negative and positive control; b: comparison between negative control and rifampicin alone; c: comparison between negative control and rifampicin+vit.C; d: comparison between negative control and rifampicin+vit E; e: comparison between negative control and rifampicin+vit C+vit E; f: comparison between positive control and rifampicin alone; g: comparison between positive control and rifampicin+vit C+vit E; h: comparison between rifampicin and rifampicin+vit C; i: comparison between rifampicin and rifampicin + vit E; j: comparison between rifampicin and rifampicin + vit E+ vit C.

The results showed cyclophosphamide, rifampicin alone, rifampicin plus vitamin C, rifampicin plus vitamin E plus vitamin C treated groups to significantly (P<0.05) increase the proportion of micronucleated polychromatic erythrocyte (MPCE) to polychromatic erythrocyte (PCE) compared with the distilled water treated group (Table 2). Rifampicin alone treated group also showed a significant (P<0.05) increase in the proportion of MPCE to PCE compared with the cyclophosphamide treated group. More so, cyclophosphamide treated group showed a significant (P<0.05) increase in the proportion of MPCE to PCE compared to rifampicin plus vitamin C plus vitamin E group. The results further showed rifampicin plus vitamin C, rifampicin plus vitamin E and rifampicin plus vitamin C plus vitamin E to significantly decrease (P<0.05) the proportion of MPCE to PCE compared with rifampicin alone treated group.

Discussion

The micronucleus assay is devised primarily for evaluating the ability of test agents to induce structural and /or numerical chromosomal damage (Miller *et al*., 1991). Both kinds of damages are associated with the appearance and/or progression of tumours and with adverse reproductive and developmental outcomes. An increase in the proportion of micronucleated polychromatophilic erythrocyte (MPCE) to polychromatophilic erythrocyte (PCE) in test agent treated animals is an indication of induced chromosome damage (Gopala & Makoto, 2000). These PCE's can be found in bone marrow, blood and spleen and they can be identified by the method of Schmid (Miller *et al*., 1991).

The results obtained from the blood smear of the treated mice in this present study indicate that cyclophosphamide which is a known mutagen and rifampicin significantly

increase the proportion of MPCE to PCE compared with control group. However, rifampicin alone treated group significantly increase proportion of MPCE to PCE compared with cyclophosphamide (a non specific cell cycle phase dependent antineoplastic agent) treated group.

This observation may be as a result of the duration of administration of rifampicin (28 days) which is more than the cyclophosphamide which was only given 6 hrs before sacrificing the animals. Thus, it may be preferable to administer cyclophosphamide 18 hours prior to sacrificing the animals in this kind of study. Studies have already shown that micronucleus formation may be due to free radical generation from an agent leading to lipid peroxidation of membrane causing the breakages of the DNA and covalently binding between the product of lipid peroxidation and DNA (Curtis *et al.*, 1988).

The results obtained from this study corroborate the above findings because co-administrations of rifampicin plus vitamin C, rifampicin plus vitamin E and rifampicin plus vitamin C and E significantly decrease the proportion of MPCE to PCE compared with rifampicin alone treated group. More so, the co-administration of rifampicin plus vitamin C plus E produced no significant difference in the proportion of MPCE to PCE compared with the distilled water treated group. This may indicate absolute protection against the DNA damaging effect of rifampicin.

The results obtained from the bone marrow of treated rats produced relatively similar results as obtained with the blood smear however, the rifampicin plus vitamin C group and rifampicin plus vitamin E group did not produce a significant difference in their protection against the damaging effect of rifampicin on DNA as to the blood smear results that showed rifampicin plus vitamin E group to demonstrate significant protection against the damaging effect of rifampicin on DNA than rifampicin plus vitamin C group.

These findings suggest that rifampicin has damaging effects on the DNA and this damage may be induced by free radicals generated by this drug. However, co-administration of rifampicin and antioxidants (vitamin C and vitamin E) has protective effect on the damaging potentials of rifampicin on the DNA. The co-administration of vitamins C and E plus rifampicin and vitamin E plus rifampicin produced better and more significant protection against the damaging effect of rifampicin on the DNA than co-administration of rifampicin plus vitamin C. More so, co-administration of vitamin C and E plus rifampicin produced the best protection against the damaging effect of rifampicin on the DNA than the individual (vitamin C or E) administration. The differences in the efficacy of combination pattern of vitamin E and vitamin C on the protection against the rifampicin induced DNA damage may initiate further study on the rate of free radical scavenging potentials of these antioxidants. It may then be recommended that the clinician always incorporate antioxidants in the regimen of patients with tuberculosis so as to reduce the possible adverse effect on the DNA.

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