

Investigation of lipid peroxidation as probable mechanism of rifampicin toxicity *in vivo*

Awodele Olufunsho and Alade Akintonwa

Department of Pharmacology, College of Medicine, University of Lagos, Lagos-NIGERIA

KEY WORDS

Rifampicin
Toxication
Free radicals
Malondialdehyde
Superoxide dismutase

ABSTRACT

Background: Xenobiotics may exert their toxic effects on tissues directly or after they have been metabolized. Increased reactivity of xenobiotics owing to their conversion to electrophiles, free radicals, nucleophiles and redox-active reactants may also contribute to toxicity. **Purpose:** The present study attempts to investigate the possible "lipid peroxidation/free radical generation" mechanism behind rifampicin toxicity. **Methods:** Measurement of antioxidant enzymatic activity and MDA level was done according to standard procedures. **Results:** The results showed a low, non-significant ($p > 0.05$) increase in the malondialdehyde (MDA) levels of the testes, serum, brain and liver of Rif treated group compared with the control. The superoxide dismutase (SOD) results in Rif treated group also showed a low, non-significant ($p > 0.05$) decreased levels in the testes, serum, brain and liver when compared with the control. However, there was a significant ($p \leq 0.05$) decrease in the level of catalase in the testes of Rif treated rats compared with the control. **Conclusion:** The low, non-significant ($p > 0.05$) increase in the Malondialdehyde (MDA) levels of the testes, serum, brain and liver suggest that Rif toxicity may also be through other mechanisms such as direct toxic effects on cells, cellular dysfunction, conversion of Rif to electrophiles, nucleophiles and redox-active reactants; other than only via lipid peroxidation. It may be concluded that just as a lot of attention is directed towards targeting drug toxicity arising due to free radical generation by the use of antioxidants, similarly other mechanisms leading to drug toxicity should also be targeted.

Corresponding Author:

Awodele Olufunsho Ph.D
Tel : +2348023624044
E.mail : awodeleo@yahoo.com

doi : 10.5214/ans.0972.7531.12190204

Introduction

Xenobiotics may exert their toxic effect on tissues directly or after they have been metabolized.¹ Increased reactivity of xenobiotics owing to their conversion to electrophiles, free radicals, nucleophiles and redox-active reactants may also contribute to toxicity.¹ The studies of Peters *et al*² and Armstrong *et al*³ have also shown toxicities to occur through cellular dysfunction which may include eregulations of gene expression, transcription and signal transduction. However, there are ways by which damaged tissues can be repaired. The repair may be via molecular mechanisms (repair of proteins, repair of lipids, and repair of DNA) or cellular mechanisms (Apoptosis, regeneration of tissues, replacement of extracellular matrix, side reactions to tissue injury). The scientific review on antioxidants done by Lamson and Brignall⁴ showed the preventive potentials of dietary and endogenous antioxidants against cellular damage by reacting with and eliminating oxidizing free radicals.

Our earlier research findings had shown Rifampicin which is a crucial component in treatment regimens for tuberculosis⁵ to demonstrate some systemic toxicities such as hepatotoxicity, meningeal congestion and sperm quality damage.⁶ It further showed the positive modulatory effect of vitamins E and/or C against the Rifampicin induced systemic toxicities, but, the level of lipid peroxidation was determined neither in the serum nor the tissue.

Therefore, this present research is geared to investigate whether the Rifampicin toxicity was induced by lipid peroxidation/free radical generations.

Methods

Drugs

Rifampicin Capsules (300 mg per capsule), Ascorbic acid {Vitamin C Tablets (100 mg per tablet)} and Alpha Tocopherol {Vitamin E Tablets (100 mg per tablet)} were obtained from

the outpatient Pharmacy Department of the Lagos University Teaching Hospital, Lagos-Nigeria

Animals

Rats were obtained from animal laboratory centre of College of Medicine, University of Lagos, Nigeria. The animals were authenticated in Zoology Department, Faculty of Science, University of Lagos, Nigeria. They were made to acclimatize for two weeks before the commencement of the experiment. The animals were fed on Pfizer animal feed cubes ad water libitum.

Sub-Acute toxicity test

Twenty male albino rats (150-250g) were used for the Sub-Acute toxicity experiment. The animals were equally divided into two treatment groups as follows:

Group 1 (Control group): 0.25ml of distilled water was orally administered for 28 days

Group 2 (Positive control): 9 mg/kg/day of Rifampicin was orally administered for 28 days.

After the 28 days administration, rats were sacrificed by cervical dislocation and the blood was obtained by cardiac puncture. The testes, liver and brain were also skillfully isolated. All the organs isolated and the blood was used to determine the oxidative stress parameters (Lipid peroxidation (MDA), Superoxide dismutase (SOD), Catalase, Peroxidase).

The investigation conforms to *The Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996)⁷ for studies involving experimental animals and approval from ethical committee was obtained.

Measurement of antioxidant enzymes and MDA levels

Measurement of antioxidant enzymes activity and MDA level was done according to standard procedures: catalase^{7,8,9}; SOD⁹; Peroxidase^{10,11} and MDA.^{9,12}

Statistical analysis

Results are presented as mean \pm S.E.M. Statistical significance between the control group and the test group was analyzed by means of student *t*-test. *P* values less than 0.05 were considered significant.

Results

A low, non-significant ($p > 0.05$) increase in the malondialdehyde (MDA) level of both the testes (63.10 ± 4.99) and serum (65.86 ± 4.33) of the Rif treated group compared with the control (60.97 ± 3.51 ; 59.92 ± 5.29) respectively were obtained (Table 2). The Superoxide dismutase results in Rif treated group also showed a low, non-significant ($p > 0.05$) decrease in both the testes and serum compared with the control (Table 2). However, the catalase results of the testes showed a significantly ($p \leq 0.05$) decreased (1.11 ± 0.01 ; 1.04 ± 0.06) in the Rif treated group compared with the control group.

The results on Table 3 also showed a low, non-significant ($p > 0.05$) increase in the malondialdehyde (MDA) level of both the liver (45.13 ± 1.62) and brain (46.03 ± 1.60) of the Rif treated group compared with the control (44.01 ± 2.67 ; 45.11 ± 2.62) respectively. The Catalase and Superoxide dismutase results in Rif treated group showed a low, non-significant ($p > 0.05$) decrease in both the liver and brain compared with the control.

There are no statistically significant ($p > 0.05$) differences between the weight of animals in the control and Rif treated groups over the 28 days of administration (Table 1).

Discussion

To the best of our knowledge, the present study is the first investigation that examined the possible mechanism of Rif toxicity via lipid peroxidation. Our earlier study had proposed that the protective effect of vitamins C and E against the systemic toxicity of Rif may be due to the antioxidant properties of vitamins C and E, which can scavenge the possible free radicals generated by Rif that could cause cell damage.⁶ The study of Ebuehi *et al*¹² had also shown that presence of free radicals may be one of the plausible explanations for elevated MDA levels; also catalytic actions of anti-oxidants enzymes are important for the effective removal of oxygen radicals. The aforementioned facts are consistent with the report of Jahangir *et al*¹³ that showed that enhanced catalase activity may be a protective mechanism against cadmium toxicity. The results obtained from the present study although it showed a low, non-significant ($p > 0.05$) increase in the Malondialdehyde (MDA) level of the testes (63.10 ± 4.99); serum (65.86 ± 4.33); liver (45.13 ± 1.62) and brain (46.03 ± 1.60) of Rif treated group compared with the control (60.97 ± 3.51 ; 59.92 ± 5.29 ; 44.01 ± 2.67 ; 45.11 ± 2.62) respectively, it is an indication that Rif induced free radical generation. It further showed a low, non-significant ($p > 0.05$) decrease in the superoxide dismutase levels (testes, serum, liver and brain) of the Rif treated rats and a significant ($p \leq 0.05$) decreased level of the catalase in the testes of the Rif treated rats compared with the control group. This suggests a reduced level of endogenous antioxidants and subsequent toxicity on cells. These findings may strengthen the studies of Ebuehi *et*

Table 1: Body weight of rats (kg) treated with Rifampicin (Rif)

	Week 1	Week 2	Week 3	Week 4
Group 1 (Control)	180 \pm 6.14	183 \pm 5.23	181 \pm 4.23	182 \pm 4.25
Group 2 (Rif Treated)	185 \pm 4.52	186 \pm 7.34	185 \pm 7.32	186 \pm 5.45

*No statistical significance ($p > 0.05$) difference in between groups over 28 days

Table 2: Determination of Lipid peroxidation and antioxidant parameters in Testes and Serum of Rif treated rats

	TESTES			SERUM		
	Control	RIF Treated	Pvalue	Control	RIF Treated	Pvalue
MDA	60.97 \pm 3.51	63.10 \pm 4.99	0.51	59.92 \pm 5.29	65.86 \pm 4.33	0.13
CAT	1.11 \pm 0.01	1.04 \pm 0.06	0.04	2.46 \pm 0.301	2.45 \pm 0.15	0.98
SOD	4.77 \pm 0.16	4.68 \pm 0.13	0.38	5.88 \pm 0.35	5.84 \pm 0.24	0.88

MDA Malondialdehyde (units/g proteins), SOD Superoxide dismutase (units/g proteins), CAT Catalase(units/g proteins)

Table 3: Determination of Lipid peroxidation and antioxidant parameters in Liver and Brain of Rif treated rats

	LIVER			BRAIN		
	Control	RIF Treated	Pvalue	Control	RIF Treated	Pvalue
MDA	44.01 \pm 2.67	45.13 \pm 1.62	0.51	45.11 \pm 2.62	46.03 \pm 1.60	0.41
CAT	4.53 \pm 1.04	3.80 \pm 0.40	0.24	0.92 \pm 0.03	0.90 \pm 0.04	0.23
SOD	4.33 \pm 0.05	4.33 \pm 0.02	0.61	9.36 \pm 0.29	9.01 \pm 0.43	0.23

MDA Malondialdehyde (units/g proteins), SOD Superoxide dismutase (units/g proteins), CAT Catalase(units/g proteins)

*al*¹² and Jahangir *et al*¹³ to propose that Rif toxicity may be via free radical generation. However, the low, non-significant ($p > 0.05$) increase in the Malondialdehyde (MDA) levels in the testes, serum, brain and liver indicates that Rif toxicity may also be through other toxication mechanisms such as direct toxicity of cells, cellular dysfunction, conversion of Rif to electrophiles, nucleophiles and redox-active reactants,¹ other than only via lipid peroxidation. However, the non-significance levels of MDA and SOD in the tissues may also be due to short period of Rif administration. The slight increase in the level of MDA of Rif treated group may therefore justify our earlier findings⁶ that showed vitamin C to demonstrate a questionable hepatoprotection due to the fact that other toxication mechanisms may be involved in the hepatotoxicity induced by rifampicin.

Conclusion

It may be concluded that as much as great attention is directed to scavenging free radicals using antioxidants to prevent drugs toxication, it is also more important to direct strategies on modulating drug toxication via other mechanisms such as increased reactivity via conversion of xenobiotics to electrophiles, nucleophiles and redox-active reactants.

This article complies with International Committee of Medical Journal editor's uniform requirements for manuscript.

Competing interests: None, Source of Funding: Self Funded

Received Date : 29 November 2011; Revised Date: 23 February 2012

Accepted Date : 29 March 2012

References:

1. Gregus Z and Klaassen CD. Mechanism of Toxicity. IN: Casarett and Doull's Toxicology-The basic Science of Poisons, 6th edition, McGraw Hill Medical Publishing Division, New York, 2001; 35-83.
2. Peters JM, Aoyama T, Cattley RC. Role of Peroxisome proliferator activated receptor alpha in altered cell cycle regulation in mouse liver. *Carcinogenesis* 1998; 19: 1989-1994.
3. Armstrong RB, Kim IU, Grippo JF *et al*. Retinoids for the future: Investigational approaches for the identification of new compounds. *J. Am Acad Dermatol* 1992; 27: S38-S42.
4. Lamson DW and Brignall MS. Antioxidants in Cancer Therapy; Their Actions and Interactions with Oncologic Therapies. *Alternative Medicine Review* 1999; 4(5): 304-329.
5. Rekha VV, Santha T, Jawahar MS. Rifampicin induced renal toxicity during retreatment of patients with pulmonary tuberculosis. *J Assoc Physician India*. 2005; 53: 811-813.
6. Awodele O, Akintonwa A, Osunkalu V *et al*. Modulatory Activity of Antioxidants against the Toxicity of Rifampicin *In vivo*. *Rev. Inst. Med. Trop. S. Paulo*; 2010; 52(1): 43-46.
7. Beers RF, Seizer IW. A spectrophotometric method for measuring breakdown of hydrogen peroxide by catalase. *J. Biol. Chem* 1952; 195: 133.
8. Beuge JA, Aust SD. The thiobarbituric acid assay. *Methods Enzymol* 1978; 52: 306.
9. Soon, Y.Y., Tan, B.K.H. Evaluation of the hypoglycemic and antioxidant activities of *Morinda officinalis* in streptozocin-induced diabetic rats. *Singapore Medical Journal* 2002; 43: 077-085.
10. Nicholas MA. A spectrophotometric assay for iodide oxidation by thyroid peroxidase. *Analytical Biochemistry* 1962; 4: 311.
11. Habbu PV, Shastry RA, Mahadevan KM *et al*. Hepatoprotective and antioxidant effects of *Argyrea speciosa* in rats. *African Journal of Traditional, Complementary and Alternative Medicine* 2008; 5: 158-164.
12. Ebuehi OAT, Ajuluchukwu AE, Afolabi OT *et al*. Catalase activity, lipid peroxidation, Cholesterol and Triglyceride levels in Alloxan induced Diabetes Mellitus in Female and Male rats. *Nig. Qt. J. Hosp. Med* 2009; 19: 15-19.
13. Jahangir T, Khan TH, Prasad L *et al*. *Pluchea lanceolata* attenuates cadmium chloride induced oxidative stress and genotoxicity in swiss albino mice. *Journal of Pharmacy and Pharmacology* 2005; 57: 1199-1204.