Tetracycline, Amoxicillin and Metronidazole Elicit Oxidative Stress-Induced Testicular Toxicity in Male Rats

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ABSTRACT

Antibiotics (Tetracycline, Amoxicillin and Metronidazole) are common antimicrobial agents used in the treatment and prevention of microbial infections. Little is known on the adverse effects of antibiotics on the testis. We designed the current study to investigate the effects of selected antibiotics (Tetracycline, Amoxicillin and Metronidazole) on markers of oxidative stress (Malondialdehyde; MDA and Glutathione) and testes in male Wistar rats. Adult male Wistar rats weighing 190g-250g were randomly allotted to control (Group A), group B (12mg/100g b.w. of tetracycline), group C (400mg/100g b.w. of metronidazole) and group D (4mg/g b.w. of amoxicillin). The treatments lasted for 2 weeks. Biochemical assays showed significant increase ($p<0.05$) and reduction ($p<0.05$) in MDA and glutathione levels respectively in all the experimental groups. Also, abnormal changes were observed in the histology of testes when compared with control. The study demonstrates that antibiotics (Tetracycline, Amoxicillin and Metronidazole) increased the level of oxidative stress in male Wistar rats and accompanied with testicular toxicity.

Keywords: Antibiotics, Glutathione, Malondialdehyde, Oxidative Stress, Testis

INTRODUCTION

Antibiotics are antimicrobial compounds that kill, or inhibit the growth of microorganisms[1]. Tetracycline is a class of broad-spectrum bacteriostatic antimicrobial drug effective against many gram-postive and gram-negative bacteria, such as strains of streptococci, bacilli, chlamydiae, rickettsiae and spirochetes etc. They are used for the treatment of urogenital tract infections (UTIs) and bronchitis. Tetracycline is polypeptide antibiotics, biosynthesized in a fashion similar to that of fatty acids, erythromycin and a host of other antibiotics. This antimicrobial agent is produced naturally by *Streptomyces aureofaciens* [2], and binds reversibly to the 30S subunit of the bacterial ribosome, blocking the binding of aminoacyl-tRNA to the acceptor site on the mRNA-ribosome complex. This prevents bacterial protein translation[3]. Tetracycline has proved to be an extremely essential type of drug because of their wide range of antimicrobial activity[4].

Amoxicillin is a penicillin antibiotic that fights bacteria. It is shown to be active against stains of gram-positive and gram-negative bacteria in both human and animals. Amoxicillin has a bactericidal action and acts against gram-positive and gram-negative microorganisms by inhibiting the biosynthesis and repair of the bacterial mucopeptide wall. It is semi-synthetic penicillin, which is susceptible to the action of the β-lactamases[5].

Also, metronidazole which is commonly known as flagyl is an antimicrobial compound with wide spectrum of activity against protozoal and anaerobic bacterial infections. Metronidazole has been clinically effective for the treatment of genital tract infections in both men and women[6]. Organisms, such as flagellated protozoa, are more resistant to metronidazole and chemicals that kill these organisms might be toxic to flagellated sperm cell walls[6]. Metronidazole's derivatives such ornidazole have been shown to reversibly suppress fertility in male rats[7].

Prolonged use of antibiotics have been reported to cause detrimental side effects such as ototoxicity, nephrotoxicity, and tendinopathy in patients, possibly through the production of toxic reactive oxygen species (ROS) in bacteria[8]. However it is not yet clear whether selected bactericidal antibiotics like tetracycline, amoxicillin and metronidazole would elicit oxidative stress that is accompanied with testicular toxicity. We therefore hypothesized that treatment with tetracycline, amoxicillin and metronidazole would elicit oxidative stress-induced testicular toxicity in male rats.

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MATERIALS AND METHODS

Animals and Grouping

Twenty matured male Wistar rats with 190-250g *b.w.* were purchased from the animal house, Department of Biochemistry, Faculty of Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria. The rats were housed in wire mesh cages and maintained in a well ventilated room at 25±2 ^{0}C, on a 12-h light/12-h dark cycle. Rats had unrestricted access to standard rat chow and tap water. After acclimatization for a period of two weeks, the rats were randomly allotted to: Group A (vehicle-treated) which served as control, group B (Tetracycline-treated), group C (Metronidazole-treated) and group D (Amoxicillin-treated) with n=5 in each group. We conducted our study with adherent to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the protocol was approved by the University of Ilorin Ethical Review Board. We also made adequate effort to minimize both the number of animals used and suffering.

Protocol

Group A received distilled water 2ml/100g *b.w.* as vehicle (p.o.), group B received tetracycline 12mg/100g *b.w.*, group C received metronidazole 400mg/100g *b.w.* and group D received amoxicillin 4mg/100g *b.w.* orally[9,10]. The selected antibiotics were purchased from Momrota Pharmacy, Ilorin, Kwara state, Nigeria, and the animals were treated daily for two weeks. The initial and final body weights were measured using animal weighing balance (Olympia SCL66110 model, Kent Scientific Corporation, Torrington, CT06790, USA) and body weight gain was calculated.

Sample preparation

After the period of treatment, the rats were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*). Blood was collected through cardiac puncture into sample bottle and was centrifuged at 3000 rpm for 15min. Serum was stored frozen until it was needed for biochemical assay. Testes were excised, blotted and weighed immediately. The testes were fixed in 10% buffered formol saline for histological examination.

Biochemical assays

Serum levels of oxidative stress markers (Malondialdehyde; MDA and Glutathione) were measured by standardized enzymatic colorimetric methods using kit purchased from Randox Laboratory Ltd. (Co. Antrim, UK).

Histological examination

For histological examination, testes were fixed in 10% buffered formol saline overnight, dehydrated, and embedded in paraffin. The paraffin-embedded samples were sectioned at 5-µm thickness, and hematoxylin and eosin (H&E) staining technique was used as previously described[9,10,11]. The slides were examined using light microscopy.

Statistical analysis

All data were expressed as means ± SEM. SPSS statistical software was used to perform statistical analysis. The mean values of variables were compared using one-way analysis of variance (ANOVA), and the significance of pair wise comparison of mean values among the groups were identified with Bonferroni's test . We accepted $p<0.05$ as statistically significant difference.

RESULTS

Effect of tetracycline, amoxicillin and metronidazole on body weight in male rats

Figure 1 depicts body weight gain and metronidazole and amoxicillin-treated groups showed a significant decrease in body weight gain where as the body weight gain of tetracycline-treated group remains unchanged when compared with control rats.

Effect of tetracycline, amoxicillin and metronidazole on oxidative stress markers (MDA and glutathione) in male rats

Figure 2 and 3 depict oxidative markers. There are comparable significant increase in MDA level and significant decrease in glutathione level of all the experimental groups when compared with control rats.

Effect of tetracycline, amoxicillin and metronidazole on the testicular tissue in male rats

Histological changes in the testes have been reported to have an impact on sperm quality, reproductive function and used as an estimation of testicular toxicities[9]. The current histological study shows deleterious lumen, leydig cells, basement membrane, spermatid and spermatogonium in tetracycline-treated group.

Metronidazole-treated group also shows moderate luminal vacuolation and distorted leydig cells, basement membrane, spermatid and spermatogonium, while amoxicillin-treated group shows severe luminal vacuolation and distorted leydig cells, basement membrane, spermatid and spermatogonium (Figure 4).

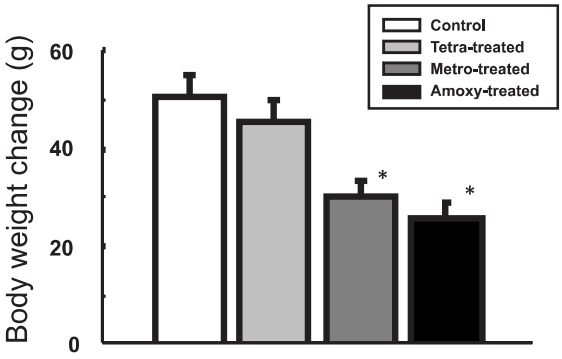

Fig.1. Effect of tetracycline, amoxicillin and metronidazole on body weight gain in male rats. Data are expressed as mean±S.E.M. for 5 rats. (*p<0.05 vs control).

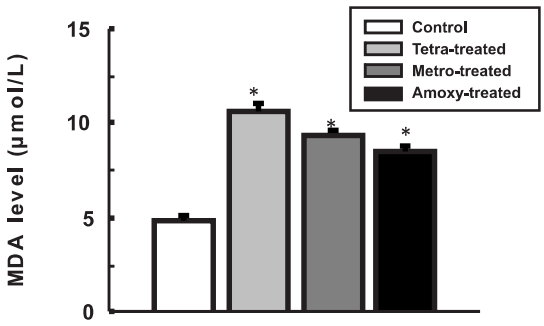

Fig.2. Effect of tetracycline, amoxicillin and metronidazole on MDA level in male rats. Data are expressed as mean±S.E.M. for 5 rats. (*p<0.05 vs control).

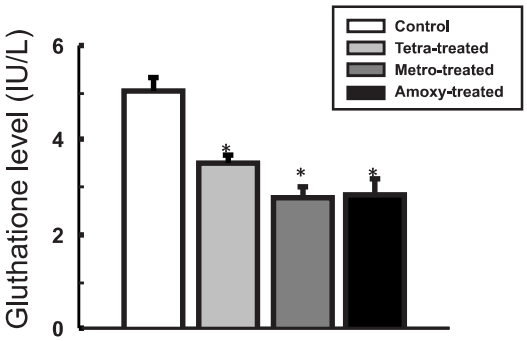

Fig.3. Effect of tetracycline, amoxicillin and metronidazole on glutathione level in male rats. Data are expressed as mean±S.E.M. for 5 rats. (*p<0.05 vs control).

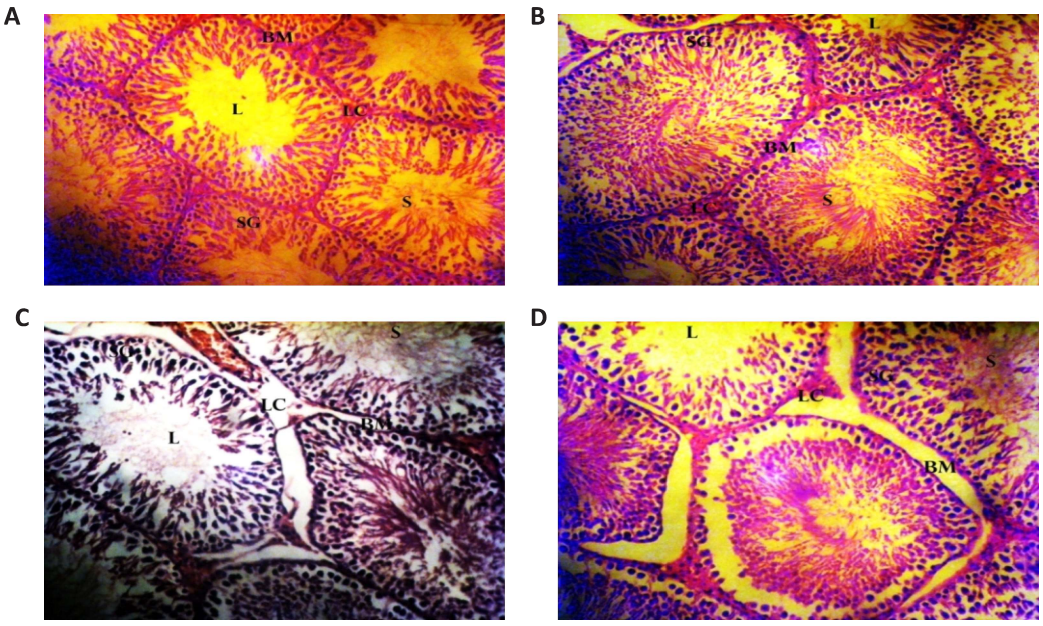

Fig.4. Effect of tetracycline, amoxicillin and metronidazole on histology of testicular tissue in male rats. Control rats show normal lumen (L), leydig cell (LC), basement membrane (BM), spermatid (S) and spermatogonium (SG) (a), tetra-treated rats show deleterious L, LC, BM, S and SG (b), metro-treated rats show luminal vacuolation, distorted LC, BM, S and SG (c) and amoxy-treated rats show luminal vacuolation, deleterious LC, BM, S, SG (d) (H&E paraffin stain; x400, longitudinal section).

DISCUSSION

The current investigation demonstrates that treatment with amoxicillin and metronidazole led to a comparable significant decrease in body weight gain but treatment with tetracycline did not alter the body weight gain when compared with control. In addition, comparable increased oxidative stress (MDA and glutathione) and histopathological changes were observed in all the experimental groups.

The findings that amoxicillin and metronidazole led to significant decrease in body weight gain were consistent with previous studies[12,13]. However, the non-significant effect of tetracycline treatment on body weight gain suggests that bactericidal antibiotics might or not alter the body weight pattern. Also, the present findings that rats treated with tetracycline, amoxicillin and metronidazole exhibited comparable elevated MDA and reduced glutathione serum levels suggest increased oxidative stress, which is inconsonance with earlier report that bactericidal antibiotics could induce oxidative stress[8,14]. Oxidative stress occurs when the formation of Reactive Oxygen Species (ROS) exceeds the level which the natural body antioxidant defense mechanism can cope with; causing damage to macromolecules such as DNA, proteins and lipids[15]. High Malondialdehyde level is a marker of lipid peroxidation which is a fallout of oxidative damage[13, 16]. Moreover, the sperm plasma membrane contains lipids in the form of polyunsaturated fatty acids, which are vulnerable to attack by ROS. ROS, in the presence of polyunsaturated fatty acids, triggers a chain of chemical reactions called lipid peroxidation[17, 18]. Glutathione is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidant in their reduced forms[19]. Glutathione serves as protective mechanism whereby potentially toxic electrophilic metabolites are "mopped up" as glutathione conjugates. The level of glutathione is a measure of the cellular redox status[18,20]. The alteration of serum glutathione levels in this present study suggests that the treatment altered the cellular redox status of the rats. This agreed with previous studies that a reduction in glutathione is a proxy measure for ROS production because it is an intracellular scavenger of ROS and a key component of the enzymatic antioxidant system[14,21,22].

The findings of the present study show for the first time that treatment with tetracycline, amoxicillin and metronidazole did not only elicit oxidative stress but led to testicular toxicity in male rats (Figure 4). Comparable testicular distortion was evident in the histology of the experimental groups as shown in figure 4. The treatments with tetracycline, amoxicillin and metronidazole cause deleterious lumen, diminished leydig cells, distorted basement membrane, abnormal spermatid and spermatogonium in the testes. This is inconsonance with previous studies that testicular tissues are vulnerable to oxidative stress[23,24], although, the testes contain an elaborate array of antioxidant enzymes and free radical scavengers that ensure the twin spermatogenic and steroidogenic functions of this organ are not impacted by oxidative stress[25,26]. However, the results of the current study indicate that tetracycline, amoxicillin and metronidazole treatments impaired the testicular antioxidant enzyme to elicit oxidative stress accompanied with testicular toxicity in rat model.

CONCLUSION

Our study suggests that treatment with tetracycline, amoxicillin and metronidazole at the current dosage elicit oxidative stress and accompanied with comparable testicular toxicity in male rats.

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