Risk Assessment of Co-treatment with Tenofovir and Rifampicin on Kidney Oxidative Stress Markers of Male Albino Rats

Elias Adikwu¹, Igbans Rejoice Obele², Odoko J. Onyedenyifa², Apiaakase Williams Ebipade²

ABSTRACT

Introduction: Rifampicin (RIF) and tenofovir (TDF) may be associated with oxidative kidney damage; hence co-therapy with tenofovir-rifampicin in human immunodeficiency virus and tuberculosis co-infection may be characterized by synergistic oxidative kidney damage. This study, therefore evaluated the toxicological effect of co-treatment with tenofovir-rifampicin on kidney oxidative stress markers of male albino rats.

Materials and Methods: Adult male rats used for this study were divided into five groups A-E of sixteen animals (16) each. Animals in group A (placebo control) were orally treated with water, while animals in group B (solvent control) were orally treated with arachis oil. Animals in groups C-D were orally treated with 80 mg/kg of rifampicin, 32 mg/kg of tenofovir and tenofovir-rifampicin for 1-8 weeks respectively. At the end of drug therapy animals were sacrificed kidney collected and analyzed for superoxide dismutase, catalase, glutathione, glutathione-s-transferase, and malondialdehyde.

Results: Co-treatment with tenofovir-rifampicin insignificantly (p>0.05) decreased superoxide dismutase, catalase, glutathione, and glutathione-s-transferase levels with increase in malondialdehyde level when compared to treatments using individual doses of rifampicin and tenofovir.

Conclusion: This study shows co-therapy with tenofovir-rifampicin may not be associated with synergistic oxidative kidney damage.

Keywords: Rifampicin, Tenofovir, Kidney, Oxidative Stress, Rats

INTRODUCTION

The kidney performs several excretory and regulatory functions including blood pressure control, maintenance of extracellular environment and drug excretion.¹ The kidney is highly vulnerable to injury due to constant exposure to chemical agents which may lead to accumulation in the kidney causing damage to the architecture of the kidney. Drugs are known to precipitate mitochondrial damage in the kidney which could result in the overproduction of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to oxidative and nitrosative damage to the lipids, proteins and DNA in the kidney.² Renal toxicity is a dose limiting toxicological effect of tenofovir, one of the first line antiretroviral drugs. About 3% of patients on tenofovir containing antiretroviral drugs may suffer drugs related renal damage.³ Several human and animal studies have reported tenofovir induced damage to proximal tubules, resulting in Fanconi syndrome, characterized by bicarbonate wasting, phosphaturia, amino aciduria, glycosuria, acidosis, and hypophosphatemia.⁴,⁵ Also, increases in serum creatinine, urea and uric acid levels are frequent features in patients on tenofovir containing antiretroviral regimens.⁶ Tenofovir renal toxicity has been associated with various degrees of histopathological damage characterized by proximal tubular injury, tubular necrosis, tubulointerstitial scarring and mitochondria damage.⁷ Tenofovir renal toxicity was also reported to be associated with reduction in the activities of proximal tubular glutathione, catalase, and superoxide dismutase.⁸ These antioxidants are integral part of kidney defense system; hence depletion will predispose the kidney to oxidative injury.

Rifampicin is used in combination with other anti-tuberculosis drugs in the treatment of tuberculosis. It has bactericidal activity against organisms that are dividing rapidly and against semi-dormant bacterial populations, thus accounting for its sterilizing activity.⁹ Its mechanism of action involves binding to β subunit of bacterial DNA-dependent RNA polymerase in prokaryotic, but not in eukaryotic cells thereby inhibiting RNA synthesis.¹⁰ The use of rifampicin has contributed to decrease in morbidity and mortality rate due to tuberculosis, but its use has been associated with renal toxicity characterized by acute renal failure.¹¹ Rifampin renal toxicity may be associated with increases in serum levels of creatinine, urea and uric acid. Also, histopathological changes characterized by tubulointerstitial nephritis, tubular necrosis, papillary necrosis and acute cortical necrosis are common features of rifampicin induced kidney damage.¹² Rifampicin induced kidney damage has been characterized by depletion

¹Faculty of Basic Medical Sciences, Department of Pharmacology, University of Port Harcourt, Choba, Rivers State, ²Lecturer, Department of Community Health Sciences, College of Health Technology, Otuogidi, Bayelsa State, Nigeria

Corresponding author: Adikwu Elias, Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Choba, Rivers State, Nigeria.

of kidney antioxidants (glutathione peroxidase, catalase, superoxide dismutase) and increase in malondialdehyde level which may increase the vulnerability of kidney to oxidative injury.\(^{13}\)

Rifampicin containing anti-tuberculosis regimes and tenofovir containing antiretroviral regimes are used as co-therapy for the management of human immunodeficiency virus and tuberculosis co-infection.\(^{14}\) Because rifampicin and tenofovir are individually associated with kidney damage characterized by decrease in kidney antioxidants. Co-therapy with these drugs may synergistically deplete kidney antioxidants which may be of grave consequence. This study, therefore evaluated the toxicological effect of co-treatment with tenofovir- rifampicin on kidney oxidative stress markers of male albino rats.

**MATERIALS AND METHODS**

**Drug**

Rifampicin used for this study was manufactured by Mangalcare Pharmaceuticals, India while pure sample of tenofovir disoproxilfumarate was purchased from Shijiazhuang Aop-china Import & Export Trading Co., Ltd. Shijiazhuang, China. All other chemicals used for this study were of analytical grade.

**Animals**

Eighty (80) adult male albino rats of average weight 330 ±5 g were used for this study. The animals were obtained from the animal house of the Department of Pharmacology and Toxicology, Madonna University, Elele, Rivers State. The animals were allowed to acclimatize for 14 days and had free access to food and water ad libitum.

**Dose Selection and Drug Preparation**

The doses of rifampicin and tenofovir disoproxil fumarate used for this study were 80mg/kg and 32mg/kg respectively and were higher than the clinically recommended doses.\(^{15,16}\) Rifampicin and tenofovir disoproxil fumarate powder were suspended in arachis oil\(^{17,18}\).

**Grouping of Animals and Drug Administration**

Animals were divided into five groups A – E of 16 animals per group. Animals in each group were further sub divided into four groups of four animals each. Animals in group A (placebo control) were orally treated with water while animals in group B (solvent control) were orally treated with arachis oil. Animals in groups C-E were orally treated with 80 mg/kg of RIF, 32 mg/kg of TDF and TDF- RIF combination for 1-8 weeks respectively.

**Collection of Sample for Analysis**

At 1, 2, 4 and 8 weeks, after overnight fast, animals were dissected under diethyl ether anesthesia; kidney collected and washed in an ice cold 1.15% KCL solution. Kidney was then homogenized with 0.1M phosphate buffer (pH 7.2). The resulting homogenate was centrifuge at 2500rpm speed for 15minutes then it was removed from the centrifuge and the supernatant was decanted and stored at -20°C until analysis.

**Evaluation of Kidney Oxidative Stress Markers**

Kidney malondialdehyde, superoxide dismutase, glutathione, catalase, and glutathione- S- transferase levels were evaluated using methods reported by Ahmed and Hassainein, 2013\(^{19}\).

**STATISTICAL ANALYSIS**

Results are presented as mean± SEM. Statistical significance and differences from control and test values were evaluated using one way Analysis of Variance (ANOVA). Statistical probability of \(p<0.05\) was considered to be significant.

**RESULTS**

Treatment with TDF for 1-8 weeks produced time-dependent decreases in kidney GSH levels with significant \((p<0.05)\) decreases observed at weeks 6 and 8 when compared to the control. Also, treatment with rifampicin produced time-dependent decreases in GSH levels with significant \((p<0.05)\) decrease observed at week 8 with respect to the control. But effects produced by individual doses of these agents on GSH levels were insignificant \((p>0.05)\) when compared to effects produced by their combined doses (Table 1). Also, kidney SOD levels were time-dependently decreased in animals treated with individual doses of TDF and RIF with significant \((p<0.05)\) difference from the control observed at weeks 6 and 8. These observed decreases were insignificant \((p>0.05)\) when compared to decreases produced by combined doses of TDF and RIF (Table 2). The following kidney CAT values 40.8±0.20, 35.1±0.22, 25.2±0.20 and 20.5±0.24 U/mgprotein were obtained in TDF treated animals for 1-8 weeks respectively .These values represent time-dependent decreases in kidney CAT levels with significant \((p<0.05)\) difference from the control observed at weeks 6 and 8. Furthermore, co-treatment with TDF-RIF produced time-dependent decreases in kidney CAT levels but, these decreases were insignificant \((p>0.05)\) when compared to treatments using individual doses of TDF and RIF (Table 3). This study also noticed time-dependent decreases in GST levels in animals treated with individual doses of TDF and RIF. These dose-dependent decreases were observed to be significant \((p<0.05)\) at weeks 6 and 8 in TDF treated animals while significant \((p<0.05)\) difference was observed at week 8 in RIF treated animals when compared to the control. But, these dose-dependent decreases were insignificant \((p>0.05)\) when compared to decreases produced by their combined doses (Table 4). Furthermore, kidney MDA levels were time-dependently increased in animals treated with individual doses of TDF and RIF. Increases were observed to be significant \((p<0.05)\) at weeks 6 and 8 in TDF treated animals while sig-
significant ($p<0.05$) increase was observed at week 8 in RIF treated animals when compared to the control. Increases in MDA levels were insignificant ($p>0.05$) in animals treated with a combination of TDF-RIF when compared to increases observed in animals treated with individual doses of TDF and RIF (Table 5).

**DISCUSSIONS**

Kidney has cellular antioxidants that protect it from chemicals induced damage. These antioxidants are usually depleted in drugs induced oxidative kidney injury and additive or synergistic deletion of antioxidants can occur with concurrent use of drugs. This study, therefore evaluated the toxicological effect of co-treatment with tenofovir-rifampicin on kidney glutathione, super oxide dismutase, catalase, glutathione-s-transferase and malondialdehyde levels. In this present study, time-dependent increases in malondialdehyde levels with decreases in superoxide dismutase, glutathione, catalase and glutathione -s- transferase levels were significant ($p<0.05$). An increase was observed at week 8 in RIF treated animals when compared to the control. Increases in MDA levels were insignificant ($p>0.05$) in animals treated with a combination of TDF-RIF when compared to increases observed in animals treated with individual doses of TDF and RIF (Table 5).

**Table 1:** Effect of treatment with tenofovir and rifampicin on kidney glutathione of male albino rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>WK1</th>
<th>WK2</th>
<th>WK4</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>5.21±0.05</td>
<td>5.29±0.01</td>
<td>5.20±0.02</td>
<td>5.24±0.05</td>
</tr>
<tr>
<td>TDF 32mg/kg</td>
<td>5.17±0.04</td>
<td>4.32±0.03</td>
<td>3.02±0.04*</td>
<td>2.26±0.07*</td>
</tr>
<tr>
<td>RIF 80mg/kg</td>
<td>5.10±0.06</td>
<td>4.91±0.05</td>
<td>4.32±0.02</td>
<td>3.10±0.03*</td>
</tr>
<tr>
<td>TDF/RIF</td>
<td>4.07±0.03</td>
<td>4.23±0.03</td>
<td>3.01±0.08*</td>
<td>2.02±0.08*</td>
</tr>
</tbody>
</table>

TDF: Tenofovir. RIF: Rifampicin. GSH: Glutathione (μ/mg protein). Results are expressed as mean ± SEM, the superscript (*) means significant difference with respect to the control at $p<0.05$ (ANOVA).

**Table 2:** Effect of treatment with tenofovir, and rifampicin on kidney superoxide dismutase of male albino rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>WK1</th>
<th>WK2</th>
<th>WK4</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>6.50±0.01</td>
<td>6.47±0.01</td>
<td>6.49±0.05</td>
<td>6.51±0.06</td>
</tr>
<tr>
<td>TDF 32mg/kg</td>
<td>6.32±0.05</td>
<td>5.21±0.03</td>
<td>3.82±0.08*</td>
<td>3.47±0.02*</td>
</tr>
<tr>
<td>RIF 80mg/kg</td>
<td>6.35±0.05</td>
<td>6.09±0.01</td>
<td>5.43±0.01</td>
<td>3.87±0.03*</td>
</tr>
<tr>
<td>RIF/TDF</td>
<td>5.60±0.01</td>
<td>5.10±0.06</td>
<td>3.61±0.08*</td>
<td>3.13±0.05*</td>
</tr>
</tbody>
</table>

TDF: Tenofovir, RIF: Rifampicin. SOD: Superoxide Dismutase (u/mg protein). Results are expressed as mean ± SEM, the superscript * means significant difference with respect to the control at $p<0.05$ (ANOVA).

**Table 3:** Effect of treatment with tenofovir and rifampicin on kidney catalase of male albino rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>WK1</th>
<th>WK2</th>
<th>WK4</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>43.0±0.12</td>
<td>42.9±0.26</td>
<td>43.1±0.13</td>
<td>42.7±0.15</td>
</tr>
<tr>
<td>TDF 32mg/kg</td>
<td>40.8±0.20</td>
<td>35.1±0.22</td>
<td>25.2±0.20*</td>
<td>20.5±0.24*</td>
</tr>
<tr>
<td>RIF 80mg/kg</td>
<td>42.3±0.03</td>
<td>38.75±0.15</td>
<td>35.5±0.31</td>
<td>24.7±1.21*</td>
</tr>
<tr>
<td>RIF/TDF</td>
<td>38.2±0.05</td>
<td>34.0±0.22*</td>
<td>23.1±0.18*</td>
<td>18.3±0.42*</td>
</tr>
</tbody>
</table>

TDF: Tenofovir, RIF: Rifampicin. CAT: Catalase (u/mg protein). Results are expressed as mean ± SEM, the superscript * means significant difference with respect to the control at $p<0.05$ (ANOVA).

**Table 4:** Effect of treatment with tenofovir, and rifampicin on kidney glutathione-s-transferase of male albino rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>WK1</th>
<th>WK2</th>
<th>WK4</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>8.70±0.07</td>
<td>8.77±0.09</td>
<td>8.69±0.05</td>
<td>8.71±0.01</td>
</tr>
<tr>
<td>TDF 32mg/kg</td>
<td>7.90±0.03</td>
<td>7.30±0.04</td>
<td>5.10±0.01*</td>
<td>4.73±0.07*</td>
</tr>
<tr>
<td>RIF 80mg/kg</td>
<td>8.13±0.07</td>
<td>7.42±0.08</td>
<td>6.97±0.07</td>
<td>5.12±0.02*</td>
</tr>
<tr>
<td>RIF/TDF</td>
<td>7.90±0.06</td>
<td>7.20±0.05</td>
<td>5.01±0.08*</td>
<td>4.60±0.07*</td>
</tr>
</tbody>
</table>

TDF: Tenofovir, RIF: Rifampicin. GST: Glutathione-S-Transferase (μmol/min mg protein). Results are expressed as mean ± SEM, the superscript * means significant difference with respect to the control at $p<0.05$ (ANOVA).

**Table 5:** Effect of treatment with tenofovir and rifampicin on kidney malondialdehyde of male albino rats

<table>
<thead>
<tr>
<th>Dose</th>
<th>WK1</th>
<th>WK2</th>
<th>WK4</th>
<th>WK8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (A)</td>
<td>1.52±0.01</td>
<td>1.60±0.01</td>
<td>1.53±0.04</td>
<td>1.49±0.05</td>
</tr>
<tr>
<td>TDF 32mg/kg</td>
<td>1.71±0.02</td>
<td>2.03±0.01</td>
<td>3.21±0.07*</td>
<td>4.10±0.04*</td>
</tr>
<tr>
<td>RIF 80mg/kg</td>
<td>1.62±0.03</td>
<td>1.85±0.05</td>
<td>2.13±0.01*</td>
<td>3.21±0.04*</td>
</tr>
<tr>
<td>TDF/RIF</td>
<td>1.80±0.02</td>
<td>1.90±0.06</td>
<td>3.5±0.03*</td>
<td>4.4±0.02*</td>
</tr>
</tbody>
</table>

TDF: Tenofovir. RIF: Rifampicin. MDA: malondialdehyde (nmole/mg protein). Results are expressed as mean ± SEM, the superscript * means significant difference with respect to the control at $p<0.05$ (ANOVA).
observed in animals treated with individual doses of tenofovir and rifampicin. These effects on oxidative stress markers were not synergistic in animals co-treated with tenofovir and rifampicin. Considering observations in this study co-treatment with tenofovir-rifampicin in human immunodeficiency virus and tuberculosis co-infection may not be associated with synergistic oxidative kidney damage.

In this study, observed time-dependent decreases in superoxide dismutase, glutathione, catalase, glutathione peroxidase and glutathione transferase with increase in malondialdehyde level in tenofovir treated animals is a sign of oxidative kidney damage. This is consistent with the work of Adaramoye and colleagues who reported increase in malondialdehyde level and decreases in antioxidants in animals treated with 50mg/kg/day of tenofovir for 4 weeks. Also, time-dependent increases in malondialdehyde levels with decreases in superoxide dismutase, catalase, glutathione, and glutathione transferase levels observed in rifampicin treated animals suggest signs of oxidative kidney damage. Malondialdehyde is the major oxidative product of the per-oxidation of polyunsaturated fatty acids by oxidative radicals. This makes increase in malondialdehyde level observed in this study a sign of lipid per oxidation induced by these agents. Glutathione whose active and oxidized form is glutathione disulfide detoxifies xenobiotic and scavenges oxidative radicals; decrease in glutathione level observed in this study could stimulate accumulation of oxidative radicals leading to oxidative kidney damage. Catalase is a cytosolic enzyme that protects biological system against oxidative radicals, and catalyzes the dismutation of superoxide anion radicals to hydrogen peroxide which is degraded into a molecule of oxygen and water. Decrease in its level could stimulate accumulation of superoxide anion radicals leading to kidney damage. Super oxide dismutase is a vital scavenger of superoxide anions, decrease in its level could instigate superoxide accumulation which may inactivate several mitochondrial enzymes, and stimulate pro-inflammatory processes. Inactivation of superoxide dismutase could also result in the accumulation of toxic superoxide anion which could further react with nitric oxide to form peroxynitrite. Peroxynitrite is a potent nitrosating agent that can cause direct damage to proteins, lipids, and DNA. Glutathione-s-transferase catalyzes the Conjugation of xenobiotic electrophilic substance with GSH to form the corresponding GSH-S-conjugate. Glutathione reductase utilizes NADPH and maintains GSH in a reduced form. Decreases in glutathione-s-transferase and glutathione reductase observed in this study will further expose the kidney to oxidative injury. Observed decreases in antioxidants in the kidney of animals treated with these agents may be due to oxidative stress through the generation of free radical. This observation is supported by the fact that oxidative stress is one of the mechanisms reported to be associated with tenofovir and rifampicin induced renal toxicity. In addition, studies suggest that rifampicin induced kidney damage is either a type II or type III hypersensitivity reaction induced by rifampicin antigens in which anti-rifampin antibodies form immune complexes are deposited in renal vessels, glomerular endothelium, and interstitial area.

CONCLUSION

Observation in this study shows co-therapy with tenofovir-rifampicin in the management of tuberculosis and human immunodeficiency may not be associated with synergistic oxidative kidney damage.

ACKNOWLEDGEMENT

We appreciate the technical assistance of Mr Charles Okeibuno and MrEze Ihekwumere of the Faculty of Pharmacy Madonna University, Elele, Rivers State.

REFERENCES

11. Managing Drug Interactions in the Treatment of
HIV-Related Tuberculosis National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention Division of Tuberculosis elimination

Source of Support: Nil; Conflict of Interest: None
Submitted: 25-11-2015; Published online: 10-12-2015