Oxidative Stress: A Possible Mechanism of Atazanavir/Ritonavir Induced Renal Toxicity

Elias Adikwu[1], Igbans Rejoice Obele[2], Apiakase Williams[2]

ABSTRACT

Introduction: Atazavavir/ritonavir (ATV/r) renal toxicity may be associatedwith oxidative stress because oxidative stress has been implicated in drugs induced renal toxicity. This study therefore evaluated the effect of ATV/r on renal function parameters and kidney oxidative stress markers of male albino rats.

Materials and Methods: Adult male albino rats were orally treated with 150/50 mg/kg of ATV/r for 1-4 weeks and (15/5 - 120/40) mg/kg of ATV/r for 8 weeks. Animals were sacrificed at the end of treatment, blood sample was collected and serum extracted and evaluated forserum creatinine, urea, and uric acid levels. Kidneys were collected weighed and evaluated for glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPX) and malondialdehyde (MDA).

Results: Treatment with ATV/r did not produce any significant (p>0.05) effect on absolute kidney weight when compared to the control. Significant (p<0.05) dose and time-dependent increases in serum creatinine, urea and uric acid levels were observed with ATV/r treatment when compared to the control. Also, significant (p<0.05) dose and time-dependent decreases in kidney SOD,CAT, GST, GSH, GR, and GPXlevels with increases in MDA levels occurred with ATV/r treatment when compared to the control.

Conclusion: Observationin this study shows ATV/r induced renal toxicity is time and dose-dependent and may be dueto oxidative stress.

Keyword: Atazanavir/ritonavir, Kidney, Toxicity, Oxidative Stress, Rats

INTRODUCTION

The complications of therapy with antiretroviral drugs have become more important than the consequences of human immunodeficiency virus (HIV) infection itself. The kidney which is the organ of drug excretion has becomevulnerable to the delivery of antiretroviral drugs and their metabolites due to prolong therapy which may serve as potential toxins. Antiretroviral drugs and their metabolites may accumulate within the tubular epithelial cells especially in the process of reabsorption or secretion exposing renal tubule to high concentration which may lead to crystallization and subsequently kidney toxicity.[1] Atazanavir boosted ritonavir is one of the commonly use antiretroviral drugs in the management of human immunodeficiency virus. In January 2004, it was approved by Japan Ministry of Health, Labour and Welfare as an antiretroviral drug for the management of HIV infection. In Europe,2004atazanavir was also approvedas a once-daily dose of 300 mg boosted with 100 mg of ritonavir. The use of unboosted atazanavir 400 mg daily has also proved effective as a switch strategy and is approved in the USA.[2] According to current guidelines, atazanavir/ritonavir (ATV/r) is one of the first-line antiretroviral drugs with high efficacy, tolerability, favorable lipid profile and once-daily dosing.[3]

Atazanavir may be associated with renal toxicity according to Brewster and Perazella (2004)[4] who first described acute interstitial nephritis associated with atazanavir. Further studies showedthat atazanavir renal toxicity may be marked with granulomatous interstitial nephritis (GIN) characterized by crystalluria, and crystal nephropathy.[5] Epidemiological studies have found that exposure to ATV/r is associated with an increased incidence of renal stones when compared to efavirenz and other protease inhibitors based regimens.[6] Treatment with ATV/r was associated with increases inserum creatinine, urea levels and increases in urine levels of total protein and albumin.[7] Cumulative exposure to ATV/r may cause tubular dysfunction leading to decreaseglomerular function. Mostcumulative exposures to ATV/r were associated with the formation of urolithiasis characterized by acute lumbar or flank pain and gross hematuria.[8,9] Atazanavir is slightly soluble in water at the concentration of 4–5 mg/ml and increasing alkalinity of urine may stimulatecrystallization of atazanavir. Atazanavir crystals are usually 8–20 nm sized, rod like-shaped and mildly birefringent and may be found in few asymptomatic patients receiving atazanavir boosted with ritonavir.[10]

Oxidative stress occurs in cells as a consequence of an imbalance between the prooxidant/antioxidant systems. Ox-

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

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

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idative stress has a critical role in the pathophysiology of renal toxicity and kidney disease. Oxidative stress can cause damage to kidney cellular macromolecules such as nucleic acids, proteins, and lipids. Damage to lipids produces lipid peroxidation products which could lead to a facile propagation of free radical reactions. Also, oxidative kidney damage could lead to downregulation in the activities of kidney antioxidants thereby subjecting the kidney to more oxidative damage.[11] Some antiretroviral drugs have been implicated in oxidative kidney damage marked by mitochondria damage, generation of oxidative radicals, lipid peroxidation and down regulation of the activities of endogenous antioxidants.[12] The use of ATV/rmay be associated with oxidative stress which has not been evaluated. This study therefore evaluated the dose and time-dependent effects of ATV/r on kidney oxidative stress markers of male albino rats. Also, effects on absolute kidney weight, and serum renal function parameters were evaluated.

MATERIALS AND METHODS

Drugs: Atazanavir/ritonavir used for this study was manufactured by Myland laboratories India. Atazanavir/ritonavir powder was suspended in normal saline. All other chemicals used for this study were of analytical grade.

Animals: Fifty adult male albino rats of average weight 310 ±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*.

Drug Administration: This study was divided into three sections; control, time-dependent and dose-dependent studies.

Control: Group A which served as the control group contained 10 animals which were divided into two sub groups A1 (placebo control) and A2 (Solvent control) of 5 animals each. Animals in sub group A1 were treated orally with water while animals in sub group A2 were treated orally with normal saline been the vehicle used for atazanavir/ritonavir in this study.

Time-Dependent Studies: Group C contained 20 animals which were divided into 4 subgroups C1 – C4 of five animals each. Animals in subgroups C1 – C4 were treated orally with 150/50 mg/kg of ATV/r for 1- 4 weeks respectively.

Dose -Dependent Studies: Group D contained 20 animals which were divided into 4 subgroups D1 – D4 of five animals each. Animals in subgroups D1 – D4 weretreated orally with 15/5 – 120/30 mg/kg of ATV/r respectively for 8 weeks.

Collection of Sample: Animals were sacrificed at the end of drug treatment with the aid of diethyl ether. Blood sample was collected through cardiac puncture and transferred into a sterile sample container. Blood sample was centrifuged at 1200 rmp for 15 minutes and serum collected for biochemical analysis. Animals were dissected kidneys were collected and weighed. The collected kidneys were washed in an ice cold 1.15% KCL solution. Kidneys were then homogenized with 0.1M phosphate buffer (pH 7.2). The resulting homogenate was centrifuge at 2500rmp speed for 15 minutes then it was removed from the centrifuge and the supernatant was decanted and stored at -20°C until analysis.

Evaluation of Renal Function Parameters: Serum creatinine urea and uric acid were evaluated as reported by Prabu et al., 2010.[13]

Evaluation of Kidney Oxidative Stress Markers: Glutathione (GSH), Superoxide Dismutase (SOD) Catalase (CAT), Glutathione peroxidase, Glutathione S-transferase, and Glutathione reductase were evaluated as reported by Prabu et al., 2010 while Malondialdehyde (MDA) was evaluated as reported by Ahmed and Hassanein, 2013[14]

STATISTICAL ANALYSIS

Results are expressed as mean +SEM. Results were analyzed using one way analysis of variance (ANOVA) and statistical significance was set at $p<0.05$

RESULTS

Time-dependent Studies

Treatment with ATV/r did not produce any significant ($p>0.05$) time-dependent effects on absolute kidney weight when compared to the control **(Table 1)**. Significant ($p<0.05$) and time-dependent increases in creatinine, urea and uric acidlevels were observed in ATV/r treated animals when compared to the control. Increases in serum creatinine levels were observed to represent41, 55,70 and 107% respectively at week 1-4. Also, time-dependent increases in serum urea levels were calculated to represent 38, 67, 89 and 121 % respectivelyat week 1-4 of treatment **(Table 2)**. Furthermore, significant ($p<0.05$) and time-dependent decreasesin kidney SOD, GSH, CAT, GSH, GPX, and GST levels with increase in MDA level were observed in animals treated with 150/50 mg/kgof ATV/r for 1-4 weeks when compared to the control. Observed increases in kidney MDA levels represent 50, 88, 126 and 267 % respectively at week 1-4. Furthermore, decreases in kidney GPX (31, 53, 72 and 94%) and GST (41, 58, 80 and 96 %) respectively were observed in ATV/r treated animals for 1-4 weeks. Also, Kidney CAT levels were time-dependently decreased to27.1±0.03, 20.0±0.06, 18.1±0.05, and 14.3±0.01 respectively while GSH levels were also decreased to 3.40±0.05, 3.00±0.02, 2.51±0.01 and 2.10 ±0.01respec-

tively after 1-4 weeks of ATV/r treatment **(Table 5).**

Dose- Dependent Studies: Treatment with ATV/r did not produce any significant (p>0.05)dose-dependent effects on kidney weight when compared to the control **(Table 1).**Treatment of animals with (15/5 - 120/40) mg/kg of ATV/r for 8 weeks dose-dependently (p<0.05) increased serum creatinine, urea and uric acid levels when compared to the control. Dose-dependent increases observed in serum creatinine levels represent 28, 53, 87 and 151 % while increases in serum urea levels represent 27, 50, 100 and 169 % respectively at week 8 **(Table 2).** Furthermore, dose-dependent decreases in kidney SOD, GSH, CAT, GSP, GST and GR levels with increase in MDA level were observed in animals treated with (15/5-120/40) of ATV/r for 8 weeks when compared to the control. Observed dose-dependent decreases in kidney GSH levels represent 34, 44,64 and 80% while decreases in GR levels represent35, 49, 69 and 84 % respectively. Furthermore, GPX levels were dose-dependently decreased to 6.33±0.01, 4.63±0.02, 2.97±0.04 and 0.41±0.04 while GST levels were decreased to 5.21.±0.01, 2.70±0.01, 1.20±0.02 and 0.32±0.15 respectively in animals treated with (15/5-120/40) ATV/r for 8 weeks **(Table 4).**

DISCUSSION

This study did not observe any effect on absolute kidney weight in ATV/r treated animals. Serum uric acid, urea and creatinine levels were dose and time-dependently increased in ATV/r treated animals. Also, dose and time-dependent decreases were observed in kidney SOD, GSH, CAT, GSH, GPX, GR and GST levels with increase in MDA level in ATV/r treated animals. Urea and creatinine are metabolic waste products that are freely filtered by the glomeruli of the kidneys. In most clinical and toxicological investigations, their serum concentrations are commonly used as surrogate markers of renal toxicity[15,16], therefore increases observed in these serum parameters in ATV/r treated animals suggest signs of renal toxicity. This observation is consistent with some studies that were not time and dose-dependent.[17,18] Observed increases in these parameters maybe due to ATV/r induced kidney damage because studies have shown that damage to tissues and organs could result in the elevation of serum and tissue concentrations of specific biochemical parameters as a result of their release or secretions from the damaged tissues/organs.[19]

Antioxidants are vital defense network that protect organs from free radicals induced oxidative damage. Super oxide dismutase scavenges superoxide anions while catalase catalyzes the dismutation of superoxide anion radicals to hydrogen peroxide which is degraded into a molecule of oxygen and water. Decreases observed in the levels of these antioxidants suggest signs of oxidative damage and can precipitate accumulation of superoxide anions leading to more kidney damage[20,21] Glutathione scavenges oxidative radicals, glutathione peroxidase reduces hydrogen peroxide and hy-

Parameter	Control	Duration of Treatment with 150/50 mg/kg of ATV/r			
		Week1	Week2	Week 3	Week4
Kidney weight(g)	0.77±0.03	0.73±0.05	0.72±0.07	0.89±0.03	0.85±0.02
Parameter	Control	Dose (mg/kg) administered for 8 Weeks			
		15/5	30/10	60/20	120/40
kidney weight (g)	0.75±0.03	0.70±0.01	0.71±0.03	0.89±0.03	0.83±0.07
ATV/r: Atazanavir. Results are expressed as mean ± SEM.					

Table-1: Effect of treatment with atazavavir/ritonavir on absolute kidney weight of male albino rats

Parameters	Control	Duration of Treatment with 150/50mg/kg of ATV/r			
		Week1	Week2	Week 3	Week4
Urea	20.0±0.03	28.2±1.00*	34.0±0.70*	38.4±1.00*	45.0±0.10*
Creatinine	1.63±0.07	2.30±0.01*	2.53±0.02*	2.78±0.01*	3.39±1.06*
Uric acid	1.52±0.03	2.20±0.03*	2.41±0.05*	2.64 ±0.05*	2.91±0.02*
Creatinine, Urea, Uric acid (mg/dl). Results are expressed as mean ± SEM, the superscript* means significant difference with respect to the control at p<0.05(ANOVA)					

Table-2: Time-dependent effects of treatment with 150/50 mg/kg of atazanavir/ritonavir on serum renal function parameters of male albino rats

Parameters	Control	Duration of Treatment with ATV/r (mg/kg)			
		15/5	30/10	60/20	120/40
Urea	20.0±0.03	25.9±0.10*	30.6±0.02*	40.8±1.00*	54.7±0.02*
Creatinine	1.63±0.07	2.10±0.02*	2.50±0.02*	3.06±0.05*	4.10±0.04*
Uric acid	1.52±0.03	1.98.±0.05*	2.10±0.05*	3.01±0.30*	4.15±0.01*
Creatinine, Urea, Uric acid (mg/dl). Results are expressed as mean ± SEM, the superscript* means significant difference with respect to the control at p<0.05(ANOVA)					

Table-3: Dose-dependent effects of treatment with atazanavir/ritonavir on serum renal function parameters of male albino rats

Parameters	Control	Dose (mg/kg)			
		15/5	30/10	60/20	120/40
MDA	0.53±0.01	0.70±0.02*	0.91±0.04*	1.30±0.07*	2.00±0.01*
GSH	6.25±0.02	4.07±0.07*	3.50±0.01*	2.21±0.05*	1.21±0.03*
SOD	10.3±0.04	7.02±0.05*	5.73±0.06*	3.64±0.01*	1.61±0.02*
CAT	45.7±0.06	35.3±0.01*	26.2±0.02*	17.4±0.05*	10.1±0.03*
GST	7.50±0.02	5.21.±0.01*	2.70±0.01*	1.20±0.02*	0.32±0.15*
GPX	9.21±0.06	6.33±0.01*	4.63±0.02*	2.97±0.04*	0.41±0.04*
GR	0.65±0.07	0.42.±0.13*	0.33±2.10*	0.20±0.05*	0.10±0.04*

MDA: Malondialdehyde, (nmol/mg protein), GSH: Glutathione, CAT: Catalase, SOD: Superoxide dismutase (Unit/mg protein), GST: Glutathione-s-transferase (µmol/min mg protein) GR: Glutathione reductase (nmol/min mg protein), GSP: Glutathione peroxidase (µg/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-4: Dose–dependent effects of treatment with atazanavir/ritonavir on kidney oxidative stress markers of male albino rats

Parameter	Control	Duration of Treatment with 150/50mg/kg of ATV/r			
		Week1	Week2	Week 3	Week4
MDA	0.53±0.01	0.80±0.01*	1.00±0.05*	1.20±0.02*	1.42±0.07*
GSH	6.25±0.02	3.40±0.05*	3.00±0.02*	2.51±0.01*	2.10±0.01*
SOD	10.3±0.04	6.10±0.07*	4.54±0.02*	4.00±0.03*	3.12±0.02*
CAT	45.7±0.06	27.1±0.03*	20.0±0.06*	18.1±0.05*	14.3±0.01*
GST	7.50±0.02	4.40±0.00*	3.10±0.01*	1.50±1.32*	0.30±0.05*
GPX	9.21±0.06	6.30±0.04*	4.30±0.02*	2.50±1.04*	0.55±0.08*
GR	0.65±0.07	0.35±0.03*	0.28±0.07*	0.25±0.25*	0.20±0.04*

MDA: Malondialdehyde, (nmol/mg protein) GSH: Glutathione, CAT: Catalase, SOD: Superoxide dismutase. (Unit/mg protein) GST: Glutathione-s-transferase (µmol/min mg protein) GR: Glutathione reductase (nmol/min mg protein), GSP: Glutathione peroxidase (µg/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: Time-dependent effects of treatment with atazanavir/ritonavir on kidney oxidative markers of male albino rats

droperoxide while glutathione-s- transferase conjugates xenobiotic electrophilic substances with GSH to form the corresponding GSH-S-conjugate. Decreases in glutathione peroxidase and glutathione levels observed in ATV/r treated animals suggest signs of oxidative kidney damage through free radical production.[22,23] Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in cells. It is a known marker of oxidative stress and antioxidant status[24]; hence increase in malondialdehyde level observed in the kidneys of ATV/r treated animals is a sign of lipid peroxidation. Observations in this study maybe due to the ability of ATV/r to accumulate and form crystals in the kidney stimulating renal mitochondria damage leading to the release of oxidative radicals.[25] The release of oxidative radicals might have stimulated damage to lipids, membranes, proteins, and DNA in the kidney[26]

Furthermore, ATV/r combination is an antiretroviral agent and studies have shown that antiretroviral agents induced kidney injury can occur through three pathways. The over-expression or competitive inhibition of transport pumps which could lead to tubular cell toxicity, the activation of the mitogen-activated protein kinase pathway which can affect barrier function in renal epithelial cell cascade and the induction of oxidative stress which could damage mitochondria, disrupting fatty-acid oxidation, and energy production.[27,28] Available studies suggest the involvement of oxidative stress as one of the postulated mechanisms in the pathogenesis of drug induced nephrotoxicity. Oxidative stress can induce dysfunction of cell membrane permeability and loss of functional integrity of the kidney.[29] Oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal function directly by initiating renal vasoconstriction or decreasing the glomerular capillary ultra filtration coefficient; and thus reducing glomerular filtration rate.[30] Considering observations in this study, ATV/r induced renal toxicity may involve oxidative stress as one of the possible mechanisms. Oxidative stress involves the generation of free radicals; hence the ability of ATV/r to generate free radicals in the kidney may be evaluated to further buttress the involvement of oxidative stress in ATV/r induced renal toxicity.

CONCLUSION

In this study, treatment with ATV/r produced dose and time-dependent increases in renal function parameters, kidney malondialdehyde and decreases in antioxidants. This shows ATV/r induced renal toxicity is dose and time-dependent and may be associated with oxidative stress as one of the possible mechanisms. The use of ATV/r is still safe because higher doses were used for this study.

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