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ORIGINAL ARTICLE

Morphological effects of chronic efavirenz administration on the kidney of adult Wistar rats

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Abstract Efavirenz is commonly used as part of the highly active antiretroviral therapy (HAART) for the treatment of human immunodeficiency virus (HIV) type 1. The effects of chronic administration of efavirenz on the kidney of adult Wistar rats were investigated. The rats were divided into two groups, those given efavirenz (treatment) and those given water (control), both for 30 days. The animals were sacrificed by cervical dislocation on Day 31 of the experiment and kidneys were dissected, weighed and quickly fixed in 10% formalin for histological analysis. The findings indicate that there was a significant ($p < 0.05$) decrease in weight and increase in relative weight of treated right and left kidneys compared to the control group. Kidneys in the treated group showed disruption of the cytoarchitecture of the renal cortical structure, diffuse glomerulonephritis with some cell congestion, and dilation of Bowman's space compared to the control group. Chronic efavirenz administration may therefore have an adverse effect on the kidney function of adult Wistar rats. Further studies to corroborate these observations should be carried out.

Introduction

Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used as part of highly active antiretroviral therapy (HAART) for the treatment of human immunodeficiency virus (HIV) type 1.[1] Efavirenz is effective in many combination regimes for the treatment of HIV infection, both in previously untreated and in treated individuals. It has been combined successfully with nucleosides such as lamivudine or emtricitabine plus abacavir, didanosine, stavidine, tenofovir or zidovudine to achieve virologic suppression in a high percentage of recipients.[2,3] Most antiviral agents do not efficiently penetrate the blood–brain barrier (BBB) or are actively transported out of the central nervous system

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(CNS).[4] Even after antiviral treatment that successfully controls the virus in the treatment compartments, the CNS may suffer continuing damage induced by HIV infection.[5] Efavirenz may be taken once a day without regard to meals and it can penetrate the CNS and spinal fluid.[6,7]

Adverse effects on the CNS have been associated with efavirenz.[8] The most common CNS effects include confusion, insomnia, abnormal vivid dreams, dizziness and headache. Efavirenz has emerged as the cornerstone of HAART regimens. The side effect profile of the drug is generally regarded as satisfactory. However, there are conflicting study results in the literature and conflicting evidence from patients and physicians regarding neuropsychiatric problems associated with efavirenz.[9] Lipodystrophy, moderate or severs pain, abnormal vision, arthralgia. asthenia, dyspnea, gynecomastia, myalgia, myopathy and tinnitus have all been reported on efavirenz use.[1]

The kidney is a paired organ located in the posterior abdominal wall. Its functions include removal of waste metabolic products from the blood and regulation of water and electrolyte balance in the body. In humans, the majority of drugs are eliminated via a combination of hepatic metabolism and renal excretion.[10] Since the kidney is involved in the excretion of many toxic metabolic waste products, particularly nitrogenous compounds, it would be worthwhile to examine the effects of efavirenz administration on the kidney. The aim of this study was to investigate the morphological effects of chronic efavirenz administration on the kidney of adult Wistar rats.

Materials and methods

Animals

Twenty adult Wistar rats of both sexes with an average weight of 200 g were randomly assigned to two groups: a control group ($n = 10$) and a treatment group ($n = 10$). The rats were obtained from and maintained in the Animal Holding of the Department of Anatomy, School of Basic Medical Sciences, University of Benin (Benin City, Edo State, Nigeria). They were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited (Ewu, Edo State, Nigeria) and had *ad libitum* access to water. Efavirenz was obtained from the President Emergency Plan for AIDS Relief (PEPFAR) Unit, University of Benin Teaching Hospital (Benin City, Edo State, Nigeria).

Drug administration and sample preparation

Rats in the treatment group received the recommended efavirenz dosage of 600 mg/70 kg body weight dissolved in distilled water via orogastric tube administration for 30 days. The control rats received an equal volume of distilled water via the same route for the same period. The rats were sacrificed by cervical dislocation on Day 31 of the experiment. The abdomen was quickly opened to expose the kidneys, which were quickly dissected out, dried, weighed on a Mettler Toledo balance and fixed in 10% formalin for histological analysis. Laboratory chemicals were obtained from May and Baker (Dagenham, UK).

Histological analysis

Tissues were dehydrated in an ascending ethanol gradient, cleared in xylene and embedded in paraffin wax. Serial sections of 7 μm in thickness were obtained using a rotatory microtome. Deparaffinized sections were stained with hematoxylin and eosin.[11] Photomicrographs of the sections were taken using a photographic microscope (Olympus, Tokyo, Japan) in the Department of Anatomy, School of Basic Medical Sciences, University of Benin.

Statistical analysis

Mean values for control and treatment group kidneys were recorded and compared statistically using the paired-sample *t* test and symmetric measured test of the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA).

Results

For the right kidney, the mean weight was 0.58 g and 0.50 g and the relative weight was 0.23% and 0.25% in the control and treatment groups, respectively. For the left kidney, the mean weight was 0.56 g and 0.48 g and the relative weight was 0.22% and 0.25% in the control and treatment groups, respectively. Thus, there was a significant ($p < 0.05$) decrease in weight and an increase in relative weight of right and left kidneys in the treatment group compared to the control group (Table 1, Fig. 1). This is as a result of the programmed cell death that occurs when kidney cells die on activation of an internal suicide program. This in turn results in morphologic cellular shrinkage that leads to a significant decrease in kidney weight.

The photomicrograph of a kidney from the control group shows normal histological features. A detailed cortical parenchyma is evident and renal corpuscles appeared as dense rounded structures, with the glomerulus surrounded by a narrow Bowman's space (Fig. 2). Kidneys in the treatment group showed a level of distortion and disruption of the cytoarchitecture of the renal cortical structure, diffuse glomerulonephritis, and some cell congestion and dilated Bowman's space compared to the control group (Fig. 3).

Table 1 Mean ± SEM kidney weight and relative weight for the animals.

	Control ($n = 10$)	Treatment ($n = 10$)
Body weight (g)	*260 ± 30.19	*197 ± 15.85
Right kidney weight (g)	*0.58 ± 0.14	*0.50 ± 0.18
Relative right kidney weight (%)	*0.23 ± 0.07	*0.25 ± 0.07
Left kidney weight (g)	*0.56 ± 0.19	*0.48 ± 0.06
Relative left kidney weight (%)	*0.22 ± 0.09	*0.25 ± 0.01

*Significant ($p < 0.05$).

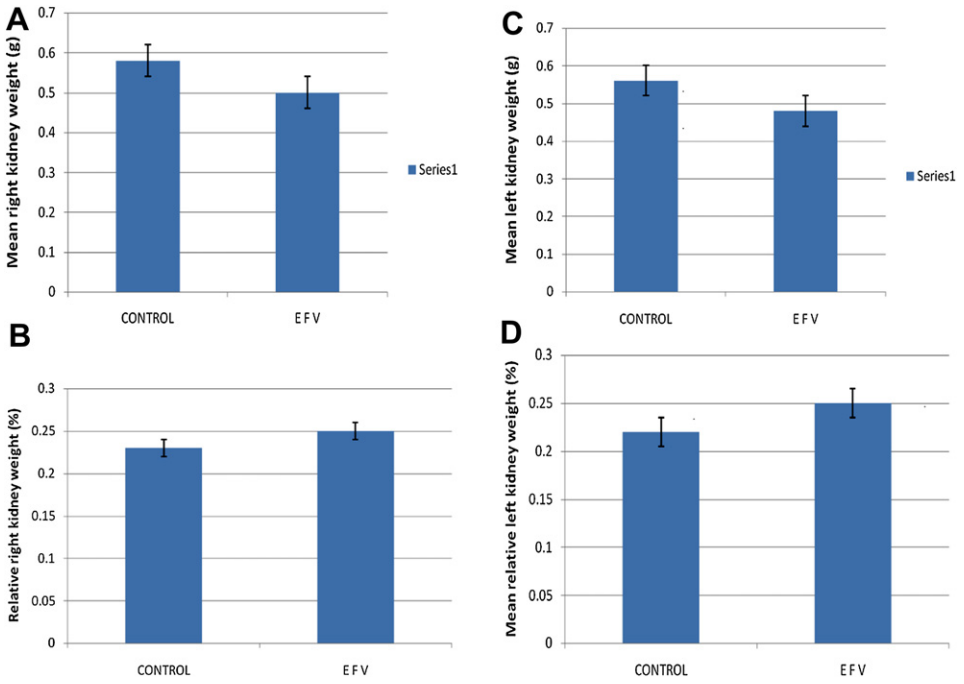

Figure 1 Bar charts showing mean ± SEM for (A) right kidney weight, (B) relative right kidney weight, (C) left kidney weight and (D) relative left kidney weight of the animals.

Discussion

There was a significant decrease in kidney weight and increase in relative weight in the treatment group compared to the control group. Kidneys in the treatment group showed a level of distortion and disruption of the cytoarchitecture of the renal cortical structure, diffuse glomerulonephritis and some cell congestion compared to the control group.

The results obtained are probably due to the effects of chronic efavirenz administration on the kidney. Thus, chronic efavirenz administration may not be as harmless as generally believed. The distortion and disruption of kidney cytoarchitecture observed here may have been associated with functional changes that could be detrimental to the health status of the animals. The significant decrease in kidney weight and increase in relative weight compared to the control group might be due to a cytotoxic effect of efavirenz on the kidney. As tissue swells or shrinks, as observed in this study, the activity of cellular transporters is up- or downregulated, as reported for hyponatramia and hypernatremia. [12]Ischemia or pharmacologic disruption of cellular transporters can cause parenchymal swelling in any organ. Pharmacologic disruption of kidney weight caused by efavirenz is the cardinal feature of the results of this study. There are many different causes of cell swelling or shrinkage, including drug poisoning, water intoxication, hypoxia and acute hyponatremia.[12] Under such conditions,

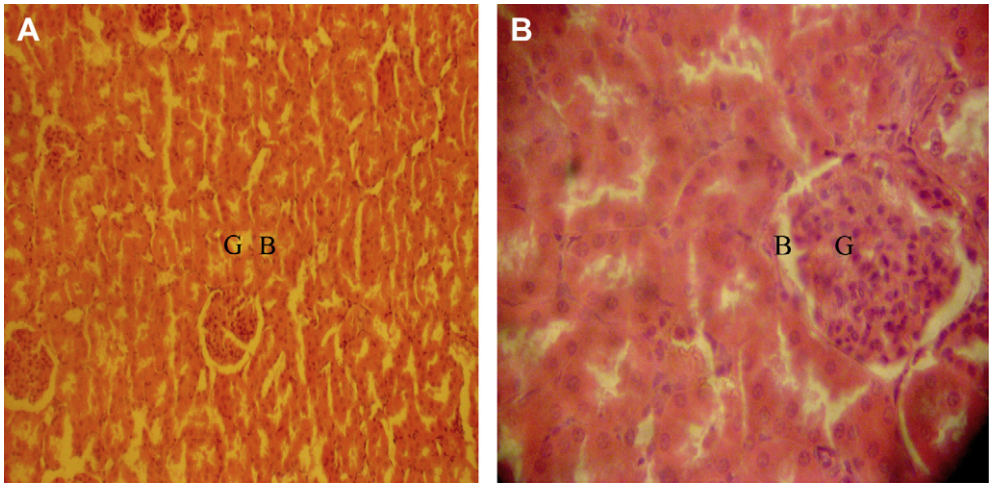

Figure 2 Photomicrographs of a control kidney section at magnification of (A) 100× and (B) 400×. B = Bowman's space; G = glomerulus.

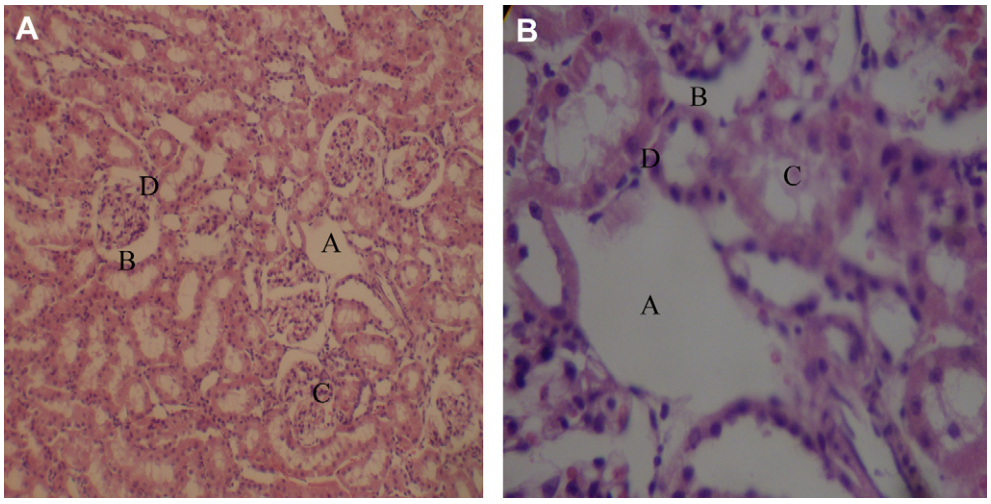

Figure 3 Microanatomy (hematoxylin and eosin staining) of a treated kidney section at magnification of (A) 100× and (B) 400×. A = diffuse glomerulonephritis; B = dilated Bowman's space; C = distortion and disruption of the glomerulus; D = cell congestion.

there is a net shift of water from the extracellular space to the cell interior.[12] The significant decrease in weight and significant increase in relative weight of the kidney observed in this study usually involve intracellular swelling or shrinkage of the endothelia.[12]

The obvious signs of diffuse glomerulonephritis and dilated Bowman's space observed in this study may have been due to cytotoxic effects of efavirenz on the kidney. These findings implicate efavirenz as a possible precipitant of kidney disease by causing congestion of kidney cells. Pathological or accidental cell death is regarded as necrotic and could result from extrinsic insults to the cell such as osmotic, thermal, toxic and traumatic effects.[13] Cellular necrosis involves disruption of membranes, as well as structural and functional integrity. It is not induced by stimuli intrinsic to cells, as in programmed cell death, but by an abrupt environmental perturbation and departure from normal physiological conditions.[14] The rate of progression of cellular necrosis depends on the severity of the environmental insult. The greater the severity of the insult, the more rapid is progression of neuronal injury.[15] This principle holds true for toxicological insult to the brain and other organs.[14] It may be inferred from the present study that prolonged efavirenz administration resulted in increased toxic effects on the kidney. The results are in accordance with work carried out by Enaibe et al[16] who reported that camphor administration resulted in mild edema with glomerulonephritis, glomerular lobulation, tubular necrosis and congestion of blood cells in rabbit kidney. It has been reported that damiana (*Turnera diffusa*) administration to mature Wistar rats resulted in distortion of the renal cortical structure, reduced the size and number of renal corpuscles, and led to a degree of cellular necrosis in the kidney.[17] In the present study, efavirenz may have acted as a toxin to the cells of the kidney, causing distortion and disruption, cell congestion, dilated Bowman's space and glomerulonephritis in renal cortical structures. The results are in line with a study that reported that chronic consumption of soda drinks resulted in varying degrees of distortion and disruption of the cytoarchitecture of the renal cortical structure, diffuse glomerulonephritis with some congestion and tubular necrosis in adult Wistar rats compared to controls.[18]

Conclusions

Our study revealed that chronic efavirenz administration resulted in distortion and disruption of kidney cytoarchitecture compared to control kidneys. Cell congestion and glomerulonephritis were also observed in treated kidneys. These results indicate that kidney function may be adversely affected by chronic efavirenz treatment. Further studies to corroborate these findings should be carried out.

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