ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Effects of Hydrochlorothiazide on Tenofovir Disoproxil Fumarate-Induced Nephrotoxicity in Rats

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Abstract

Background: Tenofovir disoproxil fumarate (TDF), a nucleotide reverse transcriptase inhibitor used for the treatment of hepatitis B virus (HBV) and human immunodeficiency virus (HIV) infections, is now one of the most widely used antiretroviral drug. This is largely due to its high antiretroviral activity, relatively good metabolic profile and more importantly good compliance as it has a once-daily dosing. However, tenofovir disoproxil fumarate can induce renal toxicity which can be attributed to the accumulation of such drug in the proximal renal tubular cells, leading to mitochondrial toxicity, and subsequent renal tubular acidosis leading to acute kidney injury. Objective: This study was designed to investigate whether hydrochlorthiazide has renoprotective effects on tenofovir disoproxil fumarate - induced nephrotoxicity in rats. Methods: Twenty eight healthy adult male albino rats weighing 180-200g were utilized in this study. Rats were randomly divided into four groups (7animals each). Group I: Negative control (given distilled water) orally by gavage tube for 5 weeks; Group II: Rats orally received 600 mg/kg/day tenofovir disoproxil fumarate by gavage tube for 5 weeks; Group III: Rats administered hydrochlorothiazide alone at a dose (10 mg/kg/day) by gavage tube for 5 weeks and Group IV: Rats administered hydrochlorothiazide at a dose (10 mg/kg/day) plus tenofovir disoproxil fumarate 600 mg/kg/day by gavage tube for 5 weeks. Results: Administration of hydrochlorothiazide plus tenofovir disoproxil fumarate to rats for 5 weeks produced significant elevation (P<0.05) of MDA content and a significant reduction (P<0.05) in the total antioxidant level in renal homogenate compared to the corresponding levels of negative control animals. Conclusion: treatment with hydrochlorothiazide plus tenofovir disoproxil fumarate in an attempt to prevent the renal toxicity-induced by tenofovir disoproxil fumarate is not effective for inhibiting oxidative stress process.

Introduction

Tenofovir disoproxil fumarate (TDF) is a prodrug of tenofovir (TFV) and it is a nucleoside reverse transcriptase inhibitor (NRTI) which was approved by Food and Drug Administration (FDA) in 2001 for the treatment of hepatitis B virus (HBV) and human immunodeficiency virus (HIV) infections. TDF is considered as an attractive antiviral agent; however, nephrotoxicity is a challenging issue regarding the use of such prodrug in the clinical practice.

Tenofovir's nephrotoxicity is unclear but it may be attributed to the interaction between such drug and the organic anion transporters (hOAT1, and to a lesser extent, OAT3), which are the major transporters in the basolateral membrane of kidney proximal tubules [1]. After oral administration, TDF can be metabolized to TFV, which in turn, can intracellularly be phosphorylated to the active moiety, tenofovir diphosphate (TFV-DP). However, higher circulating plasma levels of TFV have been associated with both renal and bone adverse effects of the prodrug (TFD) [2, 3].The most common renal defects of TDF were reported such as Fanconi syndrome, progressive decline in renal function, nephrogenic diabetes insipidus (NDI) because of distal tubular dysfunction (≤0.1 %) [4], reduced bone mineral density and osteomalasia (0.1 %) [5] and/or acute tubular necrosis [6], and developing chronic kidney disease (CKD) [7].

Numbers of agents have been reported by Yang, Y. *et al* at 2016 that including some clinical drugs may possess renoprotective effects in acute kidney injury (AKI) models [8].

Thiazide diuretics have high to intermediate potency of inhibition of OAT1s and OAT3 [9].

Methods

Drugs

Tenofovir disoproxil fumarate (TDF) tablet (300 mg) was purchased from Cipla, India. Hydrochlorthiazide tablet (25 mg) was purchased from T and D Pharma GmbH, Germany.

Animals

Twenty eight healthy adult male albino rats weighing 180-200g were utilized in this study; they were obtained from and maintained in the Animal House of the College of Pharmacy, Baghdad University, under conditions of controlled temperature. Animals were fed commercial pellets and tap water *ad libitum* throughout the experiment period. The study was approved by the Scientific and the Ethical Committees of the College of Pharmacy/University of Baghdad.

Experimental Protocol

Healthy rats were randomly divided into four groups (7 animals/ group) as follows:

Group I

Rats orally administered distilled water by gavage tube for 5 weeks. This group served as negative control.

Group II

Rats orally administered 600 mg/kg/day of tenofovir disoproxil fumarate by gavage tube for 5 weeks.

Group III

Rats orally administered hydrochlorothiazide alone at a dose of 10 mg/kg/day by gavage tube for 5 weeks.

Group IV

Rats orally administered hydrochlorothiazide at a dose of 10 mg/kg/day plus tenofovir disoproxil fumarate 600 mg/kg/day by gavage tube for 5 weeks.

Twenty-four hour after the end of the treatment duration (i.e. at day 36), each animal was euthanized by diethyl ether. Preparation of renal tissue homogenate involved that renal tissues were minced to small pieces and rinsed in ice-cold phosphate buffer saline (PBS) (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS volume (mL) = 1:9) with a glass homogenizer on ice.

To further break the cells, it was subject to freeze-thaw cycles. Homogenates were then centrifuged for 5 min at 5000×g to get the supernatant, which was used for the determination of malondialdehyde (MDA) content, which was measured according to the method of Buege JA and Aust, SD., 1978 [10]; and total antioxidant capacity (TAOC) level by a method of Apak R, GU ¨ C¸ LU ¨ R *et al*. 2005 [11].

Statistical Analysis

Data were expressed as mean±standard error of the mean (SEM). The statistical significance of the differences among various groups was determined by one-way analysis of variance (ANOVA). Differences were considered statistically significant for $P<0.05$.

Results

Table 1 and Figure 1 summarize the effect of different treatments on malondialdehyde (MDA) content in renal tissue homogenate of rats' groups. Malondialdehyde was significantly elevated ($P<0.05$) in renal tissue homogenate of rats orally administered TDF for 5 weeks compared to negative control group; the mean ± SEM values were 1.54 ± 0.11 µmol/g, and 0.23± 0.01 µmol/g, respectively.

Furthermore, there was a significant elevation ($P<0.05$) in MDA content in both hydrochlorthiazide-treated group, the mean±SEM value was (2.23± 0.18 µmol/g)]; moreover, the group treated with hydrochlorthiazide plus TDF, the mean± SEM value was (3.59± 0.23 µmol/g) compared with control group (0.23± 0.01).

Besides, table 1 and figure 1 showed that there were significant elevation ($P<0.05$) in MDA contents in renal tissue homogenate of rats group administered hydrochlorothiazide (10mg/Kg) alone (group III) or

hydrochlorothiazide (10 mg/kg) plus TDF (600 mg/kg) compared to the corresponding contents in TDF-treated rats.

Table 1: Effect of different treatments on malondialdehyde (MDA) content in renal tissue homogenate of rats' groups

Group / Treatment	MDA content in renal tissue homogenate (µmol/g)
Group I/ negative control (distilled water)	0.23± 0.01
Group II/ TDF (600 mg/kg)	1.54 ± 0.11* A
Group III/ hydrochlorthiazide (10 mg/kg)	2.23± 0.18 * B
Group IV/ hydrochlorthiazide (10 mg/kg) plus TDF (600 mg/kg)	3.59± 0.23 * C

Data expressed as mean± Standard error of mean (SEM).
*: *P*<0.05: Significant difference compared to negative control group.
Values with non-identical capital letters (A, B, and C) are considered significantly different (*P*<0.05).
TDF, tenofovir disoproxil fumarate

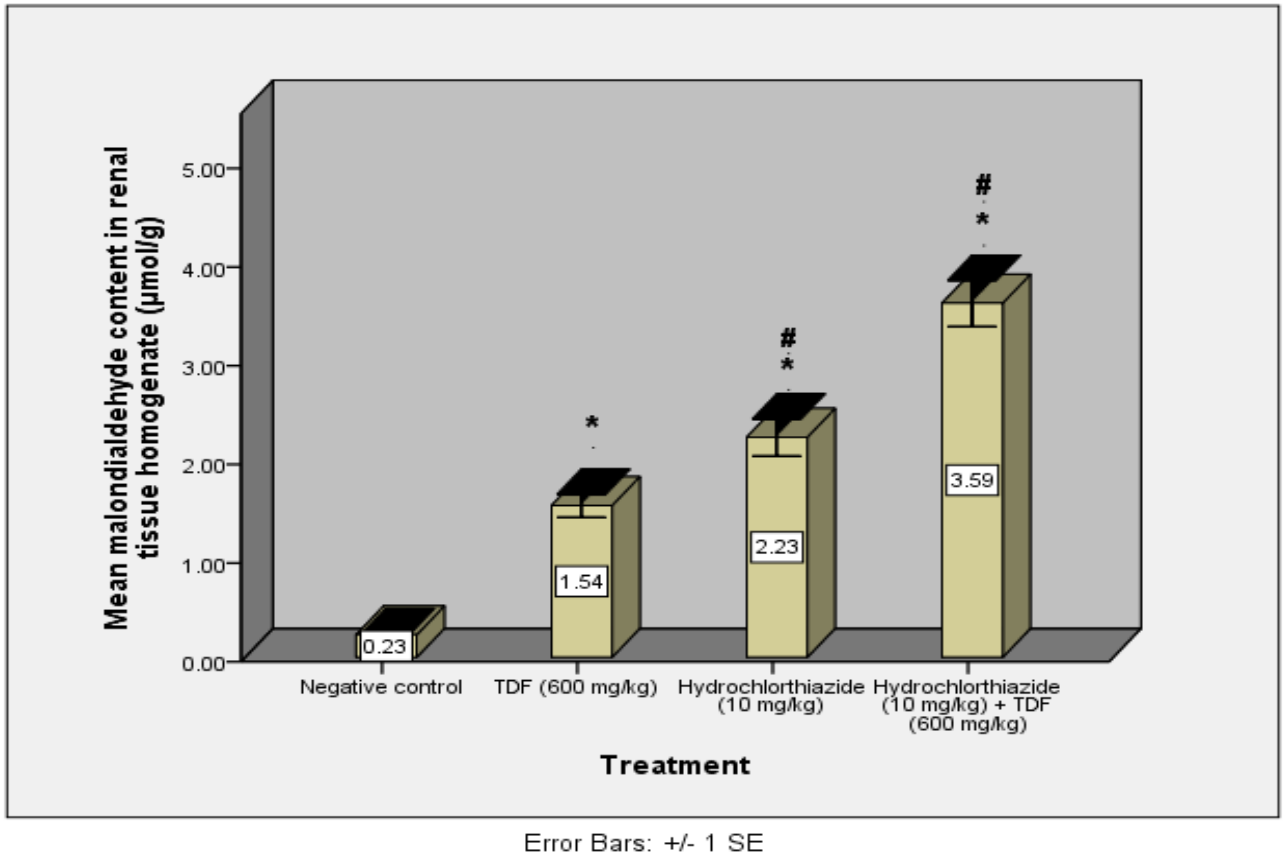

Figure 1: Effect of different treatments on malondialdehyde content in renal tissue homogenate of rats' group
(*):-Indicate a significant difference (*P*<0.05) compared to negative control group
(#):- Indicate a significant difference (*P*<0.05) compared to tenofovir disoproxil fumarate-treated group

Table 2 and figure 2 summarize the effect of different treatments on total antioxidant capacity (TAOC) level in renal tissue homogenate of rats' groups. There was significant reduction (*P*<0.05) in TAOC level in renal tissue homogenate of TDF-treated group (group II) [the mean±SEM value was (5.07 ± 0.19 µmol/g)], hydrochlorothiazide-treated group (group III) [mean±SEM value was (5.18± 0.17 µmol/g)], and in group of rats administered hydrochlorothiazide plus TDF (group IV) [mean±SEM value was (4.24 ±0.14 µmol/g)] compared to the corresponding levels in negative control group [mean±SEM value was (6.65± 0.3)].Furthermore, table 2 and figure 2 showed that there was significant reduction (*P*<0.05) of TAOC level in renal tissue homogenate among rats of group II, III, and IV.

Table 2: Effect of different treatments on total antioxidant capacity (TAOC) level in renal tissue homogenate of rats' groups

Group / Treatment	TAOC level in renal tissue homogenate (µmol/g)
Group I/ negative control (distilled water)	6.65± 0.3
Group II/ TDF (600 mg/kg)	5.07 ± 0.19 *A
Group III/ hydrochlorthiazide (10 mg/kg)	5.18± 0.17* A
Group IV/ hydrochlorthiazide (10 mg/kg) plus TDF (600	4.24 ±0.14* B

mg/kg)	

Data expressed as mean± Standard error of mean (SEM).
*: P<0.05: Significant difference compared to negative control group.
Values with non-identical capital letters (A and B) are considered significantly different (P<0.05).
TDF, tenofovir disoproxil fumarate

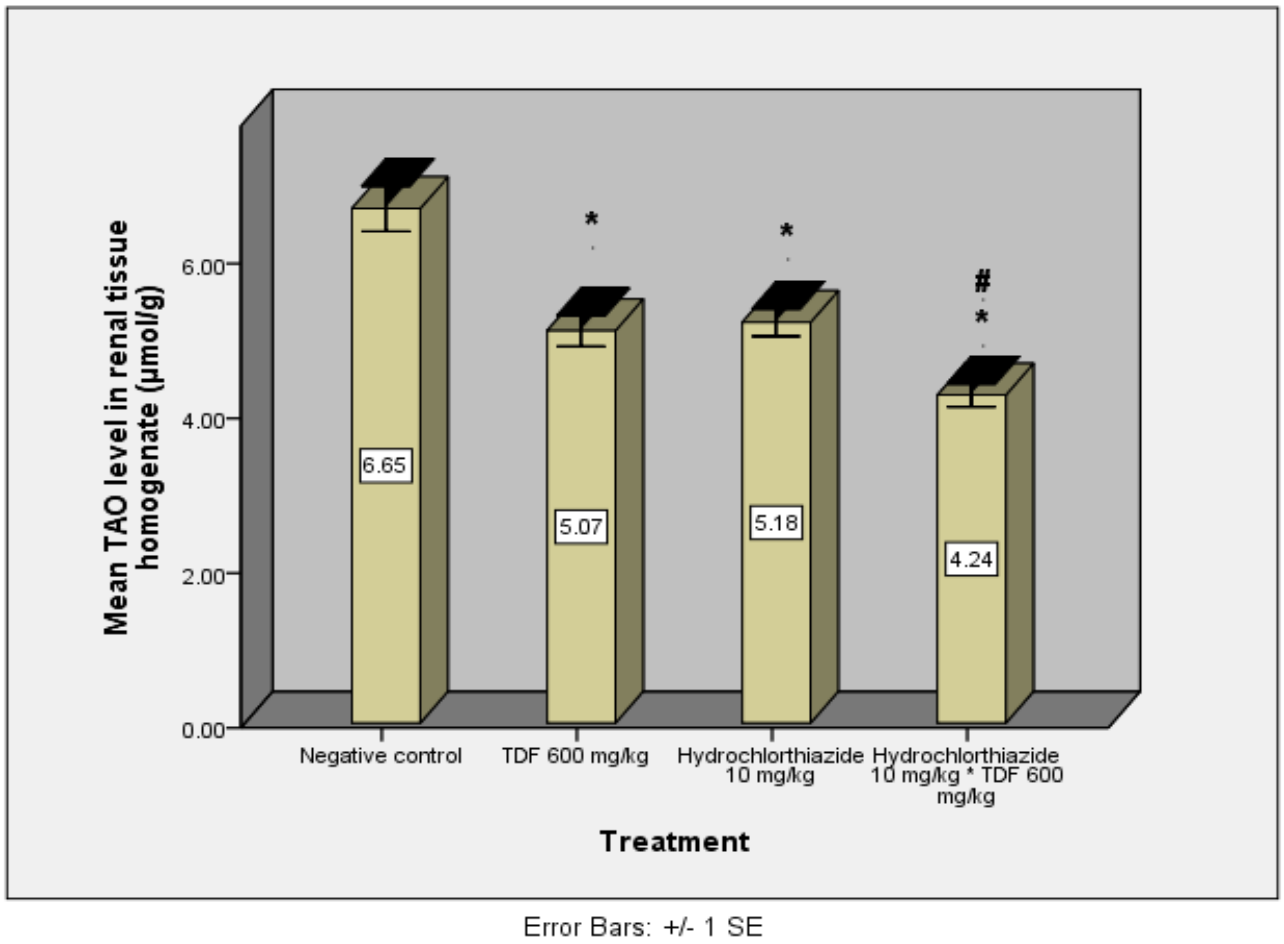

Figure 2: Effect of different treatments on total antioxidant level in renal tissue homogenate of rats' group
(*):-Indicate a significant difference (P<0.05) compared to negative control group.
(#):- Indicate a significant difference (P<0.05) compared to tenofovir disoproxil fumarate-treated group.

Discussion

Tenofovir disoproxil fumarate (TDF) is an orally bioavailable pro-drug of TFV [12]. The renal proximal tubule (PT) is the main target of TFV toxicity [13]. The relationship of counter-balancing transport processes on intracellular accumulation of TFV and toxicity has been *in vitro* illustrated; where, authors showed that overexpression of OAT1 and OAT3 can increase cytotoxicity while co-transfection of multidrug resistance protein type 4 (MRP-4) may cause an incremental reduction in the unwanted effects of TDF [14], favoring intracellular accumulation in renal proximal tubule cells and this may lead to ultra-structural mitochondrial abnormalities and decreased mtDNA levels which could stimulate reactive oxygen species (ROS) production, depletion of antioxidants and antioxidant enzymes [1].

Animal studies have revealed that TFV can cause PT damage in mice [15], rats [16, 17], and non-human primates [18]; furthermore, numerous case reports and case series illustrated that FS or AKI in HIV-infected patients was produced by TFV [19, 20]. Authors reported that injurious oxidative stress (OS) can be one of the causative factors that may lead to the development of proximal tubular toxicity through formation of reactive oxygen species (ROS) [21, 22]. Moreover, long term administration of TDF to rats resulted in tubular damage and glomerular damage. This was accompanied by elevation of ROS and depletion of antioxidant enzymes in the kidney [23].

The inductions of the process of OS by TDF in the kidneys were observed and this may be due to the overproduction of ROS with the depletion of cellular antioxidant system [24]. The sources of ROS may be produced by damaged mitochondria [25, 26]. Malondialdehyde (MDA) is a substance produced during polyunsaturated fatty acid peroxidation [27]. MDA is known to have

toxic influence on cell membrane structure. It can modulate signal transduction as well as modify proteins and DNA [28].

Ramamoorthy, H. *et al* at 2011 showed that the MDA was increased in the kidneys of TDF treated rats as compared with the control, but the increase was not statistically significant [23].

A report in 2012 showed that lipid peroxidation and OS considered as main cause of TDF toxic effect on proximal tubules [29]. The previous findings coincide with the results of the present study; where, there is significant elevation in MDA content of renal tissue homogenate of TDF-treated rats group was observed compared to negative controls.

Moreover, results of the current study showed that there was a significant increase in MDA content in the renal tissue homogenate of rats treated with hydrochlorthiazide (HCTZ) compared to those levels in negative controls, and TDF-treated group. Such results are coincide with those of Ribeiro, M.C *et al.* at 2013 who showed that HCTZ administered to rats caused a significant increase in hepatic and renal lipid peroxidation [30].

Also results of the present study showed that there was significant increase in MDA content in the renal homogenate of rats administered hydrochlorthiazide (10 mg/kg/ day) plus tenofovir disoproxil fumarate (group IV) compared to the corresponding content of negative control (group I), and TDF treated group (group II); this may be due to the aggravation of peroxidation on renal lipid. Furthermore, results of the current study are coincide with the results of others concerning TAOC level; where, there were significant reduction (P<0.05) in TAOC level in renal tissue homogenate of TDF-treated rats group (600 mg/kg/day) compared to the corresponding levels in negative control group. Authors reported that TFV can be taken up into PT cells via basolateral membrane organic anion transporters (OAT1 and OAT3) [31]; and in OAT1 knock-out mice, protection from TFV toxicity was observed [32].

Moreover, it has been reported that thiazide diuretics may have high to intermediate potency of inhibiting OAT1s and OAT3 [33, 34]. Meanwhile, a study by Ribeiro, M.C *et al.* 2013 also showed that treatment with HCTZ alone caused a significant decrease in renal SOD activity [30]. Besides, in the present study treatment of rats with hydrochlorthiazide (10 mg/kg/ day) plus tenofovir disoproxil fumarate (group IV), produced a significant decrease in TAOC level in renal tissue homogenate as compared with both group I and group II rats, this could be due to lots of free radical generation that may overcome the antioxidant capacity of tubular cells with consequent tubular cells damage.

Conclusion

According to the results obtained from this study, it could be concluded that treatment with hydrochlorthiazide plus tenofovir disoproxil fumarate in an attempt to prevent the renal toxicity-induced by tenofovir disoproxil fumarate is not effective for inhibiting oxidative stress process.

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