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

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Abstract

Tenofovir disoproxil fumarate, a nucleotide reverse transcriptase inhibitor utilized for the treatment of hepatitis B virus and human immunodeficiency virus infections; and is now one of the most widely used antiretroviral drug. However, tenofovir disoproxil fumarate can induce nephrotoxicity, which may be attributed to the interaction between such drug and the organic anion transporters (hOAT1, and OAT3) with consequent changes in levels of some parameters that may have a role in nephrotoxicity. Thiazide diuretics have high to intermediate potency of inhibition of OAT1s and OAT3; thus, it may possess nephroprotective effects. This study was designed to investigate whether hydrochlorthiazide has nephroprotective effects on tenofovir disoproxil fumarate-induced nephrotoxicity in rats**.**

Twenty eight healthy adult male albino rats weighing 180-200g were utilized in this study for duration of 5weeks (35 days) treatment. Rats were randomly divided into four groups (7animals each). **Group I**: Negative control (orally given distilled water) by gavage tube; **Group II**: Rats orally received 600 mg/kg/day tenofovir disoproxil fumarate by gavage tube; **Group III**: Rats orally administered hydrochlorothiazide alone at a dose (10 mg/kg/day) by gavage tube, and **Group IV**: Rats orally administered hydrochlorothiazide at a dose (10 mg/kg/day) plus tenofovir disoproxil fumarate 600 mg/kg/day by gavage tube. On day 36 of the study, after euthanization of each animal by diethyl ether, 3-5ml of blood samples were collected from each rat by an intra-cardiac puncture, then centrifuged at 3000 rpm for 15 minutes to obtain serum, which was then transferred into suitable plain tubes and preserved at -20 °C; and it was utilized for the estimation of cystatin C and IL-10 level.

Rats administered tenofovir disoproxil fumarate for 5 weeks (group **II**) produced a significant - elevation ($P<0.05$) in serum cystatin C level and – reduction in serum IL-10 levels compared to negative control group (group **I**); similarly, administration of hydrochlorothiazide alone to rats (group **III**) produced a significant -elevation ($P<0.05$) in serum cystatin C level and – reduction in serum IL-10 levels compared to negative control group (group **I**) ; also, rats administered combination of hydrochlorothiazide plus tenofovir disoproxil fumarate to rats for 5 weeks (group **IV**) produced significant elevation ($P<0.05$) in serum level of cystatin C, and a significant reduction ($P<0.05$) in IL-10 serum level in treated rats compared to the corresponding levels of negative control animals (group **I**); beside that in (group **IV**) rats there were significant reduction ($P<0.05$) in serum level of both cystatin C, and IL-10 in treated rats compared to the corresponding levels compared to TDF-treated (group **II**). In conclusion, treatment with hydrochlorthiazide plus tenofovir disoproxil fumarate in an attempt to prevent nephrotoxicity induced by tenofovir disoproxil fumarate is not attained.

Key words: Nephrotoxicity, Tenofovir, Hydrochlorothiazide, Cystatin C, IL-10.

تأثيرات الهيدروكلورثايزايد على السمية الكلوية المستحثة بواسطة التينوفوفير ثنائي البروكسيل فيوماريت في ذكور الجرذان

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الخلاصة

يعد التينوفوفير ثنائي البروكسيل فيوماريت مثبطا لإنزيم النوكليوتيدات العكسي و يستخدم لعلاج فيروس التهاب الكبد B وعدوى فيروس نقص المناعة البشرية وهو الآن واحد من أكثر الأدوية المضادة لفيروسات النسخ العكسي المستخدمة على نطاق واسع. ومع ذلك ، يمكن أن يسبب التينوفوفير ثنائي البروكسيل فيوماريت السمية الكلوية ، والتي يمكن أن تعزى إلى التفاعل بين التينوفوفير ثنائي البروكسيل فيوماريت وناقل الأنيون العضوي (hOAT1 ، OAT3) مع ما يترتب على ذلك من تغييرات في مستويات بعض المعايير التي قد يكون لها دور في السمية الكلوية. مدرات البول الثيازيدية لديها قدرة متوسطة الى عالية من تثبيط OAT1s و OAT3 ؛ وبالتالي ، قد يكون لها تأثيرات واقية للكلى.

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تهدف هذه الدراسة لاستكشاف تأثيرات الهيدروكلورثايزايد على السمية الكلوية المستحثة بواسطة التينوفوفير ثنائي البروكسيل فيوماريت. تم استخدام ٢٨ جرذا بالغا من الذكور السليمة وزنها يتراوح بين ١٨٠-٢٠٠ غم و تم تقسيمها عشوائيا الى أربع مجموعات في كل مجموعة سبعة جرذان حيث تم معاملتها لمدة ٥ اسابيع (٣٥ يوما). المجموعة الأولى – السيطرة السلبية (الماء المقطر) فمويا عبر أنبوب التزقيم يوميا .المجموعة الثانية – اعطيت فمويا٦٠٠ ملغم / كغم من تينوفوفير يوميا عبر أنبوب التزقيم .المجموعة الثالثة - أعطيت هيدروكلوروثيازيد لوحده بجرعة (١٠ ملغم/ كغم/ يوم) عبر أنبوب التزقيم. المجموعة الرابعة -أعطيت فمويا هيدروكلوروثيازيد بجرعة (١٠ملغم / كغم / يوم) بالإضـــافة إلى تينوفوفير ٦٠٠ ملغم / كغم عبر انبوب التزقيم.

في اليوم ٣٦ من الدراسة، تم اجراء القتل الرحيم للحيوانات بواسطة ثنائي الأثير إيثيل، وأخذت عينات الدم عن طريق ثقب داخل القلب وجمعها في أنابيب عادية (غير محتوية على مواد ضد التخثر) للحصول على المصل لقياس مستوى السيستاتين C و الانترليوكين -10.

نتج من اعطاء التينوفوفير ثنائي البروكسيل فيوماريت الى الجرذان (المجموعة الثانية) ارتفاعا معنويا ($P<0.05$) حادا في مستوى السيستاتين C في المصل و-انخفاضا معنويا ($P<0.05$) حادا في مستويات IL-10 في مصل الدم مقارنة بمجموعة السيطرة السالبة (المجموعة الأولى) ، كما نتج ايضا من اعطاء الهيدروكلوروثيازيد لوحده للجرذان (المجموعة الثالثة) ارتفاعا معنويا ($P<0.05$) حادا في مستوى السيستاتين C في المصل وانخفاضا معنويا ($P<0.05$) حادا في مستويات IL-10 في مصل الدم مقارنة بمجموعة السيطرة السالبة (المجموعة الأولى). ايضا في مجموعة الجرذان التي تم اعطاؤها توليفة من هيدروكلوروثيازيد بالإضـافة إلى التينوفوفير ثنائي البروكسيل فيوماريت لمدة ٥ أسـابيع (المجموعة الرابعة) أدت هذه التوليفة إلى ارتفاع كبير ($P<0.05$) في مستوى مصل cystatin C ، وانخفاض كبير ($P<0.05$) في مستوى IL-10 في مصل الدم في الجرذان المعالجة مقارنة بالمستويات المقابلة لحيوانات السيطرة السلبية (المجموعة الأولى). الى جانب ذلك، في مصل جرذان المجموعة الرابعة كان هناك انخفاضـــا معنويا ($P<0.05$) لكل من مســتويات cystatin C و IL-10 مقارنة بالمســتويات المقابلة لحيوانات المجموعة الثانية التي اعطيت التينوفوفير ثنائي البروكسيل فيوماريت.

يمكن الاستنتاج بان المعالجة بالهيدروكلورثيازايد مع التينوفوفير ثنائي البروكسيل فيوماريت في محاولة لتقليل السمية الكلوية المستحثة بواسطة التينوفوفير ثنائي البروكسيل فيوماريت لم تؤد الى تقليل السمية على مستوى العوامل المقاسة في هذه الدراسة.

الكلمات المفتاحية: السمية الكلوية، التينوفوفير، الهيدروكلورثيازايد، السيستاتين C ،الانترليوكين – ١٠ .

Introduction

The kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs [1]. Many drugs and their metabolites can be excreted by the kidney either by glomerular filtration, by tubular secretion, or in some cases by both [2].

Nephrotoxicity may have a wide shade, reflecting to various nephron segments based upon mechanisms of individual drug and heavy metals; moreover, both glomeruli and tubules has been recognized as targets for drug toxicity and may result in acute or chronic functional changes [3,4].

Drugs may exert their nephrotoxic toxic effects by one or more common pathogenic mechanisms. Drugs-induced nephrotoxicity tend to be more common among certain patients and in specific clinical situations. It has been reported that, successful prevention require knowledge of pathogenic mechanisms of renal injury, patient-related risk factors, drug-related risk factors, and pre-emptive measures, coupled with vigilance and early intervention [5, 6].

Tenofovir disoproxil fumarate (TDF) is a bioavailable prodrug of tenofovir, which is a potent nucleotide analog reverse transcriptase inhibitor with activity against human immunodeficiency virus (HIV) and HBV [7]. Such drug is considered as an attractive antiviral agent; where, the International guidelines recommend tenofovir as first-line antiretroviral therapy regimen, and the majority of single-tablet antiretroviral therapy regimens include tenofovir [8]; however, nephrotoxicity is a challenging issue regarding the use of such prodrug in the clinical practice. Tenofovir disoproxil fumarate (TDF) is eliminated by the kidney, largely via glomerular filtration, with 20% to 30% being actively transported into the renal proximal tubule cells [9]. Authors reported that, TDF-associated nephrotoxicity may primarily result in proximal tubular injury; where, severe acute tubular necrosis was seen in 33 (77%) of 43 biopsy-proven cases of TDF nephrotoxicity [10].

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 [7].

It has been shown that after oral administration, TDF can be metabolized to tenofovir (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) [11, 12].

Yang, Y. et al (2016) have been reported numbers of agents that including some clinical drugs may possess renoprotective effects in acute kidney injury (AKI) models [13].

Thiazide diuretics have high to intermediate potency of inhibition of organic anion transporters, OAT1s and OAT3 [14]. Thiazides are sulfonamide-related organic acids that are secreted into the proximal tubule by an organic secretory mechanism; they act to increase the excretion of Na^+ and Cl^- by inhibiting the Na^+/Cl^- symporter in the distal convoluted tubule. Natriuresis may be accompanied by some loss of potassium and bicarbonate. Moreover, thiazides can enhance Ca^{+2} reabsorption in the distal convoluted tubule by increasing Na^+/Ca^{+2} exchanges (which makes thiazides useful in treating the calcium-subtype of kidney stones). Furthermore, authors reported that thiazide diuretics can also reduce the urinary excretion of Ca^{+2} and

therefore can be employed in the treatment of kidney stones and may also be useful for treating osteoporosis [15].The aim of this study is to investigate whether hydrochlorthiazide has nephroprotective effects on tenofovir disoproxil fumarate-induced nephrotoxicity in rats.

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 (TDF) by gavage tube for 5 weeks [16].

Group III- Rats orally administered hydrochlorothiazide alone at a dose of 10 mg/kg/day by gavage tube for 5 weeks [17].

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.

Preparation of serum samples

Twenty-four hour after the end of the treatment duration (i.e. at day 36), each animal was euthanized by diethyl ether. Blood samples were collected (3-5 ml from each rat) by an intra-cardiac puncture, then centrifuged at 3000 rpm for 15 minutes to separate serum, which was then transferred into suitable plane tubes and preserved at -20 °C. The serum of each rat was used for the estimation of cystatin C and IL-10 level.

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 cystatin C level in serum of rats' groups. Cystatin C was significantly elevated ($P<0.05$) in serum of rats that were orally administered TDF for 5 weeks (group **II**) compared to negative control group (group **I**); where, the mean ± SEM values were 0.453± 0.017 ng/ml, and 0.190± 0.02 ng/ml, respectively. Furthermore, there was a significant elevation ($P<0.05$) in cystatin C level in serum of rats in hydrochlorthiazide-treated group (group **III**) compared to negative control group (group **I**), the mean±SEM value was (0.245± 0.015 ng/ml)]; moreover, in group of rats treated with hydrochlorthiazide plus TDF (group **IV**), mean± SEM serum cystatin C levels was significantly elevated compared to the corresponded serum level in negative control rats ($P<0.05$); where, the mean±SEM values were (0.392± 0.01 ng/ml) compared with negative control group (0. 190± 0.02ng/ml).

Moreover, table 1 and figure 1 showed that there were significant elevations ($P<0.05$) in serum cystatin C level among rats in groups [**II** (orally administered TDF (600mg/kg), **III** (orally administered hydrochlorthiazide (10mg/kg), and **IV (**administered hydrochlorthiazide (10 mg/kg) plus TDF (600 mg/kg)].

In addition rats treated with hydrochlorthiazide plus TDF (group **IV**), the mean± SEM serum cystatin C levels was significantly reduced compared to the corresponded serum level in TDF-treated rats (group **II**) ($P<0.05$); where, the mean±SEM values were (0.392± 0.01 ng/ml) compared with TDF-treated group (0.453± 0.17ng/ml).

Table 1. Effect of different treatments on cystatin C level in serum of rats' groups.

Group / Treatment	Mean serum cystatin C level (ng/ml)
Group I/ negative control (distilled water)	0. 190± 0.02
Group II/ TDF (600 mg/kg)	0.453± 0.017* A
GroupIII/ hydrochlorthiazide (10 mg/kg)	0.245± 0.015 * B
GroupIV/ hydrochlorthiazide (10 mg/kg) plus TDF (600 mg/kg)	0.392± 0.01 * 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.

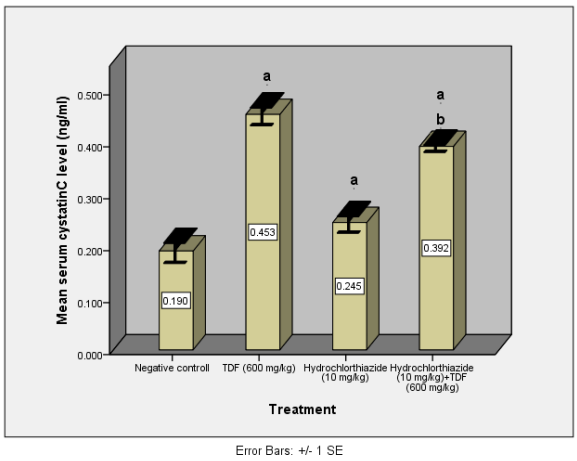

Figure 1. Effect of different treatments on cystatin C level in serum of rats' group.
(**a**):-Indicate a significant difference (P<0.05) compared to negative control group.
(**b**):- Indicate a significant difference (P<0.05) compared to tenofovir disoproxil fumarate-treated group.

Table 2 and figure 2 summarize the effect of different treatments on serum interleukin-10 (IL-10) level of rats' groups. There was a significant reduction (P<0.05) in interleukin-10 level in serum of -TDF-treated group (group **II**) [the mean±SEM value was (20.341± 0.45pg/ml)], -hydrochlorothiazide-treated group (group **III**) [mean±SEM value was (16.99± 0.412 pg/ml)], and -in group of rats administered hydrochlorothiazide plus TDF (group **IV**) [mean±SEM value was (13.655±0.512 pg/ml)] compared to the corresponding levels in negative control group (group **I**) [mean±SEM value was (28.846± 0.56 pg/ml)].
Additionally rats treated with hydrochlorthiazide plus TDF (group **IV**), the mean± SEM serum interleukin-10 levels was significantly reduced compared to the corresponded serum level in TDF-treated rats (group **II**) (P<0.05); where, the mean±SEM values were (13.655±0.512 pg/ml) compared with TDF-treated group (20.341± 0.45 pg/ml).
Furthermore, table 2 and figure 2 showed that there was a significant reduction (P<0.05) of interleukin-10 level in serum among rats of group **II, III,** and **IV**.

Table 2. Effect of different treatments on interleukin-10 level in serum of rats' groups.

Group / Treatment	Mean serum IL-10 level
Group I/ negative control (distilled water)	28.846± 0.56
Group II/ TDF (600 mg/kg)	20.341± 0.45 *A
Group III/ hydrochlorthiazide (10 mg/kg)	16.991± 0.412* A
Group IV/ hydrochlorthiazide (10 mg/kg) plus TDF (600 mg/kg)	13.655±0.512* B

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.

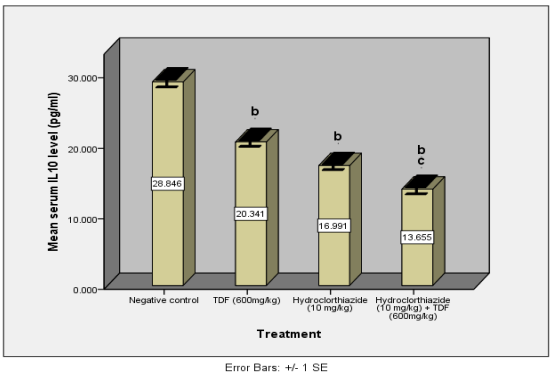

Figure 2. Effect of different treatments on interleukin-10 level in serum of rats' groups
(**b**):-Indicate a significant difference (P<0.05) compared to negative control group.
(**c**):- Indicate a significant difference (P<0.05) compared to tenofovir disoproxil fumarate-treated group.

Discussion

The widespread introduction of highly active antiretroviral therapy (HAART) in the mid-1990s dramatically altered the course of human immunodeficiency virus (HIV) infection, with improvements in survival and reductions in the incidence of AIDS-defining illnesses. Although, antiretroviral therapy has been shown to reduce the incidence of both AIDS-defining and non-AIDS conditions, long-term exposure to HAART may also be associated with significant toxicity [18].

Tenofovir disoproxil fumarate (TDF) is an orally bioavailable pro-drug of TFV [19]. The renal proximal tubule (PT) is the main target of TFV toxicity [20]. Animal studies have revealed that TFV can cause proximal tubular (PT) damage in mice [21], rats [22, 23], and non-human primates [24]; furthermore, numerous case reports and case series illustrated that

Fanconi Syndrome (FS) or acute kidney injury (AKI) in HIV-infected patients was produced by TFV [25, 26].

Moreover, most studies considered creatinine clearance (CrCl) as a marker of renal function for the assessment of TDF-induced nephrotoxicity. However, creatinine clearance (CrCl) was reported to be a weak indicator for evaluation of kidney function for TDF-induced nephrotoxicity [27]; in addition to that, creatinine is derived from skeletal muscle and HIV-infected patients can have abnormal muscle mass, these are important considerations when interpreting studies [28].

Horberg M. et al (2010) showed that TDF-exposed patients had greater development of proximal tubular dysfunction over time, reduced GFR, and had greater risk of medication discontinuation, especially as renal function worsened and serum creatinine were also reported to be significantly elevated among TDF-exposed patients compared with TDF -sparing patients [29]. Thus, in the current study, serum level of cystatin C as a marker for tubular damage was measured instead of serum creatinine.

The results of this study showed that there was significant elevation ($P<0.05$) in serum level of cystatin C in rats orally administered TDF for 5 weeks (group **II**) compared to the corresponding levels in negative control (group **I**) and this coincide with that founded by Horberg M. et al (2010) from the point of the effect.

Cystatin C is a non-glicolized protein with small molecular weight (13.3 kDa), a hundred times bigger than creatinine. It is produced at a constant rate by all the nucleated cells, and is freely filtered by glomeruli and minimally linked to proteins, and is not reabsorbed in the systemic circulation after the filtering [30]. Furthermore, it has shown promise as a replacement for serum creatinine in estimation of glomerular filtration rate (GFR). It has been reported that after glomerular filtration, cystatin C is fully catabolized in the proximal renal tubule and is not returned to blood. Moreover, the concentration of serum cystatin C is not affected by gender, age, race, protein intake, and muscle mass, unlike serum creatinine. When GFR reduced, cystatin C level begin to rise proportionately [31].

Moreover, the results of the present study revealed that there were significant elevations ($P<0.05$) in cystatin C level in serum of rats group administered hydrochlorthiazide (10mg/kg) alone (group **III**) compared to negative control rats (group **I**) or hydrochlorthiazide (10 mg/kg) plus TDF (600 mg/kg) (group **IV**) compared to the corresponding levels in -TDF-treated rats (group **II**) and -negative control (group **I**). Thus, results of this study concerning the effects of hydrochlorthiazide on kidney are coincide with that of Wadei H.M, et al at 2008 who reported that low kidney function and glomerular and tubular injury can be more commonly manifested in thiazide-treated rats [32].

Furthermore, in the current study, it was found that there was statistically a significant ($P<0.05$) reduction in serum IL-10 level of TDF-treated rats (group **II**) compared to the corresponding level in negative controls (group **I**). Moreover, treatment of rats with hydrochlorthiazide (10 mg/kg/day) alone (group **III**), and with hydrochlorthiazide (10 mg/kg/ day) plus TDF (600 mg/kg) (group **IV**) produced significant ($P<0.05$) reduction in serum IL-10 level compared to TDF-treated rats (group **II**) and negative control (group **I**), respectively.

Interleukin-10 (IL-10) is an anti-inflammatory cytokine produced by a number of activated immune cells like monocytes/macrophages, and T helper-1 (Th1) cells [33]. Moreover, it has been reported that such cytokine is a potent inhibitor of inflammation and immune responses to infections and antigens [34, 35]. Furthermore, pretreatment of human peripheral blood mononuclear cells (PBMCs) with TDF caused a reduction in levels of IL-10, and strongly reduced the induction of IL-10 [36].

Moreover Farkhondeh N and Ali D. A. (2012) showed that, clinically relevant concentration of hydrochlorthiazide could elevate the secretion of the proinflammatory cytokine IL-1β by the peripheral blood mononuclear cells (PBMCs), and might result in aggravation of inflammatory processes in vascular wall and worsen the condition in long-term [37]. To our knowledge, the current study is the first that study the effect of hydrochlorthiazide on IL10, thus we could not have a chance to compare the results obtained from this study with others concerning this respect.

Conclusion

It could be concluded that hydrochlorthiazide had no nephron-protective effect against tenofovir disoproxil fumarate- induced renal damage.

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