Nebivolol effect on oxidative biomarkers in Tamoxifen-Induced Hepatotoxicity in Female White Albino Rats: In Vivo Study

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Abstract:

Liver injury can arise post-exposure to drugs or their metabolites, as well as herbal and dietary supplements. Tamoxifen is a frequently used drug in breast cancer treatment. Unfortunately, Epidemiological studies have identified that Long-term tamoxifen treatment has been associated with the development of hepatotoxicity so it was used in this study to induce liver damage. Oxidative stress was the major implicated mechanism contributing to tamoxifen hepatotoxicity. Nebivolol is a third-generation selective beta1-adrenergic receptor blocker with vasodilator characteristics with significant antioxidant activity.

The presented study was conducted to investigate the possible protective role of nebivolol against rat hepatotoxicity induced by tamoxifen. Rats utilized in this study were randomized into five groups (6 in each group); **Group 1**- (Control) rats received distilled water (5mL/kg body weight orally) for 14 consecutive days. **Group 2**- Rats received distilled water for 12 consecutive days and tamoxifen (75mg/kg b.w., orally) on days 13 and 14 only. **Group 3**- Rats received Nebivolol (5 mg/kg b.w., orally for 14 consecutive days) and tamoxifen (75mg/kg b.w., orally) only on days 13 and 14. **Group 4**- Rats received Nebivolol (8 mg/kg b.w., orally for 14 consecutive days) and tamoxifen (75mg/kg b.w., orally) only on days 13 and 14. **Group 5**- Rats received Nebivolol (10 mg/kg b.w., orally for 14 consecutive days) and tamoxifen (75mg/kg b.w., orally) on days 13 and 14 only. The current study concluded that pre-administration of nebivolol with tamoxifen showed significant upregulation ($P<0.05$) in glutathione peroxidase (GPx) and significant downregulation ($p<0.05$) in malondialdehyde (MDA) each compared to corresponding levels in the tamoxifen-only treated group. In conclusion, this study demonstrated that pre-administration of nebivolol with tamoxifen attenuated its hepatotoxicity considering that nebivolol is a good choice, particularly for patients with hypertension requiring beta blockers and at risk for liver damage.

Keywords: Hepatotoxicity, Tamoxifen, Nebivolol, GPX, MDA.

تأثيرات النيبيفولول على العلامات الحيوية المؤكسدة في السمية الكبدية الناجمة عن عقار تاموكسيفين في الجرذان البيض: دراسة داخل جسم الكائن الحي

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خلاصة

يمكن أن تنشأ إصابة الكبد بعد التعرض للأدوية أو مستقلباتها والمكملات العشبية والغذائية. ان عقار تاموكسيفين هو دواء مشهور يستخدم في علاج سرطان الثدي. اكدت الدراسات الوبائية ان العلاج طويل الأمد بعقار تاموكسيفين بتطور السمية الكبدية. ويعتبر الإجهاد التأكسدي من الآليات الرئيسية المتورطة التي تساهم في السمية الكبدية لعقار تاموكسيفين.ان دواء نيبيفولول هو حاصر انتقائي لمستقبلات بيتا 1 الأدرينالية من الجيل الثالث مع خصائص موسع للأوعية الدموية مع نشاط مضاد للأكسدة كبير. لقد تم تصميم الدراسة للتحقق من الدور الوقائي المحتمل للنيبيفولول ضد السمية الكبدية للفئران التي تم استحداثها مختبريا باستخدام عقار تاموكسيفين. في هده التجربة تم تقسيم الجرذان المستخدمة في هذه الدراسة بصورة عشوائية إلى 5 مجموعات (6 فئران لكل مجموعة). تلقت المجموعة الأولى (مجموعة المقارنة) فئران التجارب الماء المقطر (5 مل / كجم وزن حي عن طريق الفم) لمدة 14 يومًا متتاليًا. في المجموعة الثانية: تلقت الجرذان الماء المقطر (75 مجم / كجم وزن حي، عن طريق الفم) لمدة 12 يوم وعقار تاموكسيفين (75 مجم / كجم وزن حي، عن طريق الفم) في الأيام 13، 14 فقط.وفي المجموعة الثالثة: تلقت الجرذان النيبيفولول (5 مجم / كجم من وزن الجسم، عن طريق الفم لمدة 14 يومًا متتاليًا) وتاموكسيفين (75 مجم / كجم من وزن الجسم، عن طريق الفم) في الأيام 13، 14 فقط. وفي المجموعة الرابعة: تلقت الجرذان نيبيفولول (8 مجم / كجم من وزن الجسم، عن طريق الفم لمدة 14 يومًا متتاليًا) وتاموكسيفين (75 مجم / كجم من وزن الجسم، عن طريق الفم) في الأيام 13، 14 فقط.وفي المجموعة الخامسة: تلقت الجرذان نيبيفولول (10 مجم / كجم من وزن الجسم، عن طريق الفم لمدة 14 يومًا متتاليًا) وتاموكسيفين (75 مجم / كجم من وزن الجسم، عن طريق الفم) في الأيام 13، 14 فقط. أظهر التناول المسبق للنيبيفولول بجرعات مختلفة مع عقار تاموكسيفين ارتفاعا ملحوظا في غلوتاثيون بيروكسيداز ,وتنظيمًا هابطًا ملحوظًا في المالونديالدهيد مقارنةً بالمستويات المقابلة في المجموعة المعالجة بالتاموكسيفين فقط. في الختام، أظهرت هذه الدراسة أن التناول المسبق للنيبيفولول بجرعات مختلفة مع عقار تاموكسيفين أدى إلى تخفيف سميته الكبدية معتبرا أن النيبيفولول خيار جيد خاصة للمرضى الذين يعانون من ارتفاع ضغط الدم والذين يحتاجون إلى حاصرات بيتا والمعرضين لخطر تلف الكبد.

الكلمات المفتاحية: السمية الكبدية، تاموكسيفين، نيبيفولول، GPx, MDA

Introduction

Drug-induced liver injury (DILI) refers to the occurrence of hepatic damage caused by the use of drugs, herbs, or xenobiotics [1]. Tamoxifen, which is Z-1-[4-(2-dimethylaminoethoxy)-phenyl]-1,2diphenyl-1-butene or TAM, is widely regarded as the standard adjuvant therapy for both early and late breast cancer [2]. Tamoxifen's proposed mechanism of action involves the suppression of estradiol binding to the ligand-binding domain of the estrogen receptor alpha (ERα), resulting in conformational alterations that impede the interaction between the estrogen receptor and co-activator proteins [3]. In contrast, the prolonged administration of tamoxifen has been linked to hepatic steatosis and steatohepatitis. The observed pathological findings include multifocal fatty infiltration, fibrosis, cirrhosis, and necrosis [4]. Tamoxifen-induced hepatotoxicities have been found in both human and rat models [5], and it was employed in this work as an inducer of hepatotoxicity. The primary mechanisms involved in developing hepatotoxicity in TAM are oxidative stress, and inflammation [6] including the effect on glutathione peroxidase (GPx) a reducing agent that effectively removes hydrogen peroxide and lipid peroxides [7], and malondialdehyde (MDA) a byproduct resulting from the process of lipid peroxidation induced by free radicals [8]. The adverse effects associated with these treatment adherence measures (TAM) can negatively impact patient adherence, resulting in the termination of treatment and subsequently leading to inferior clinical outcomes [9]. Therefore, it's crucial to investigate novel approaches to enhance TAM's safety profile, particularly for patients with chronic conditions such as hypertension who require beta blockers.

Nebivolol (NEB) is a pharmacological agent belonging to the third generation of selective β1-adrenergic receptor blockers. It has been officially licensed for the treatment of hypertension. It exhibits valuable antioxidant action. Nebivolol exhibits receptor-

independent scavenging of reactive oxygen species by interaction with free radicals [10]. By scavenging reactive oxygen species, nebivolol effectively decreases oxidant stress and enhances nitric oxide availability.

Moreover, previous studies have demonstrated that it effectively suppresses Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase activity in different hypertension models [11]. This presents an additional pathway by which the medication can mitigate the overproduction of O^{+2} and alleviate oxidative stress. Furthermore, it should be noted that nebivolol has vasodilatory characteristics through its direct stimulation of the endothelial nitric oxide synthase (eNOS)-L-arginine- Nitric oxide (NO) pathway [12]. Nitric oxide (NO) is crucial in regulating liver function and the development of liver diseases. The production of nitric oxide (NO) in liver sinusoidal endothelial cells (LSECs) by endothelial nitric oxide synthase (eNOS) at low levels plays an essential role in regulating the vascular tone and blood flow within the intrahepatic sinusoids. NO can limit the activity of hepatic stellate cells (HSCs) and Kupffer cells (kc), the last constitute resident macrophages in the liver and are responsible for the release of inflammatory mediators, growth factors, and reactive oxygen species that trigger acute and chronic hepatocyte injury [13].

This study was aimed to investigate the potential hepatoprotective effects of nebivolol against tamoxifen-induced hepatotoxicity in rats, together with the fundamental mechanisms involved in it.

Materials and Methods

Animals

Thirty female Albino Wister rats, classified as healthy adults, were purchased from the Iraqi Center for Cancer and Medical Genetics Research (ICCMGR) at Mustansiriyah University. Rats weighing 150 to 240 grams and aged 8 and 12 weeks were included in the current study. Animals were subjected to controlled environmental conditions, including a temperature range of 24 ± 2°C and a light cycle of 12 hours of light followed by 12 hours of darkness. During this period, the animals were provided unrestricted access to pellets and water *ad libitum.*

Preparation of drugs used in this study:

Nebivolol hydrochloride, obtained from Sigma-Aldrich in St. Louis, USA, was suspended in distilled water to prepare a suspension with a concentration of 1 mg/mL. The mixture was homogenized using a magnetic stirrer for 10 minutes. Subsequently, the suspension was administered orally via gavage, considering the animals' body weight. Similarly, Tamoxifen citrate (Sigma-Aldrich, St. Louis, USA) was suspended in distilled water to prepare a 10 mg/ml suspension. The mixture was then stirred using a magnetic stirrer for 10 minutes. Subsequently, the suspension was administered orally via gavage, considering the animals' body weight.

Experimental design

Animals were allocated into five equal groups, with six rats assigned to each group.

Group 1 (control): The rats were received an oral administration of distilled water (DW) (5mL/kg b.w.) for 14 days.

In Group 2, the rats were administered distilled water (DW) for 12 days, followed by the oral administration of tamoxifen (TAM) at a dosage of 75mg/kg body weight on days 13 and 14, as previously described [14].

In Group 3 (NEB5), the rats were orally administered nebivolol at a dosage of 5 mg/kg body weight [15] for 14 days. Additionally, tamoxifen was administered orally at 75 mg/kg body weight on days 13 and 14 only.

In Group 4 (NEB8), the rats were administered nebivolol orally at an 8mg/kg body weight (16) dose for 14 days. Additionally, tamoxifen was administered orally at 75mg/kg body weight on days 13 and 14 only.

In Group 5 (NEB10), rats were orally administered nebivolol at 10 mg/kg body weight [17] for 14 days. Additionally, tamoxifen was administered orally at 75 mg/kg body weight on days 13 and 14 only.

Sample collection

At the end of the study, on day 15, rats were subjected to sedation using intramuscular administration of 50 mg/kg ketamine and 5 mg/kg xylazine. After the rat was euthanised, the liver was quickly excised, rinsed in ice-cold buffer phosphate saline pH 7.4 to remove excess thorough blood and weighed before homogenization, then minced tissue and homogenized with the aid of homogenizer after putting the tube in a beaker containing ice. After that, the homogenate was then centrifuged for 20 min at 3000 rpm using a cold centrifuge, and the supernatant was utilized for the estimation of tissue GPX and MDA levels.

A. Liver Tissue Glutathione peroxidase (GPX) assessment.

The competitive enzyme immunoassay approach is used in the GPX ELISA kit, which includes a polyclonal anti-GPX antibody and a GPX-HRP conjugate. In a pre-coated plate, the test sample and buffer are incubated for one hour with the GPX-HRP conjugate. The wells are decanted and cleaned five times after incubation. The wells are then treated with an HRP enzyme substrate. The result of the enzyme-substrate reaction is a blue complex. Finally, a stop solution is added to stop the reaction, which causes the solution to become yellow. Color intensity is measured spectrophotometrically in a microplate reader at 450nm according to manufacturers' instructions (kit catalog number: MBS744364, company: MyBioSource).

B. Liver Tissue Malondialdehyde (MDA) assessment

A monoclonal antibody specific for MDA had been pre-coated on the microtiter plate. Standards and homogenized tissue samples were added to the microtiter plate wells. MDA bound to the antibody pre-coated wells. A standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for MDA was added to each well to "sandwich" the immobilized MDA on the plate. The procedure was employed according to manufacturers' instructions (kit catalog number: MBS268427, company: Mybiosource). After microtiter plate incubation, the wells were thoroughly washed to remove all unbound components. Then, in each well, substrate solutions were added. Over a short incubation period, the enzyme (HRP) and substrate were allowed to react. The hue of the wells containing only MDA and enzyme-conjugated antibodies changed. The color shift was detected spectrophotometrically by the Huma reader at a wavelength of 450 nm after the enzyme-substrate reaction was stopped by adding a sulphuric acid solution.

Statistical analysis

The data was analyzed using SAS (Statistical Analysis System - version 9.1). The statistical analysis employed in this study involved a one-way analysis of variance (ANOVA) followed by a post hoc test known as the Least Significant Differences (LSD) test. This approach was utilized to evaluate and determine any significant variations among the means of the variables under investigation. Post hoc tests play a crucial role in the analysis of variance (ANOVA) methodology. Statistical significance was

determined for data differences at a significance level of $P<0.05$[18].

Results

A. Glutathione peroxidase (GPX) level

The rats subjected to tamoxifen treatment in the induction group demonstrated a statistically significant decrease (P-value < 0.05) in hepatic GPx levels compared to the control group and considerably increased (P-value < 0.05) in NEB8, and NEB10 groups that received pretreatment with nebivolol, compared to the induction group. This finding is supported by the data in Table 1 and Figures 1.

B. Malondialdehyde (MDA) level

The rats subjected to tamoxifen treatment in the induction group were demonstrated a statistically significant increase (P-value < 0.05) in hepatic MDA levels compared to the control group and considerably reduced (P-value < 0.05) in the NEB5, NEB8, and NEB10 groups that were received pretreatment with nebivolol, compared to the induction group. Such findings are supported by the data in Table 1 and Figures 1 and 2.

Table 1. Oxidative stress biomarker changes in all groups.

	GPx (ng/ml)	MDA (ng/ml)
G1:Control	10.58 ± 0.19^{a}	1.53 ± 0.07^{d}
G2;Induction	3.53 ± 0.06^{c}	4.26 ± 0.21^{a}
G3:NEB/5mg	3.28 ± 0.04^{c}	2.78 ± 0.07^{b}
G4:NEB/8mg	6.43 ± 0.50^{b}	2.03 ± 0.20^{c}
G5;NEB/10mg	8.94 ± 1.42^{a}	1.28 ± 0.05^{d}
LSD	1.98	0.41

The results were expressed as Mean ±SD. Results with unidentical superscripts (a, b, c, d) are significantly different ($p<0.05$). **LSD:** a least significant difference. **GPx:** glutathione peroxidase, **MDA:** malondialdehyde, **ng/mL:** nanograms Per milliliter, **Control:** normal control group, rats given distilled water for 14 days; **Induction**: negative control group, rats exposed to Tamoxifen on days13 and 14 only; **NEB/5mg:** Rats treated with nebivolol (5mg/kg/d) orally plus tamoxifen on days13 and 14 only; **Neb/8mg**: Rats treated with nebivolol (8mg/kg/d) orally plus tamoxifen on days13 and 14 only; **NEB/10mg**: Rats treated with nebivolol (10mg/kg/d) orally plus tamoxifen on days13 and 14 only.

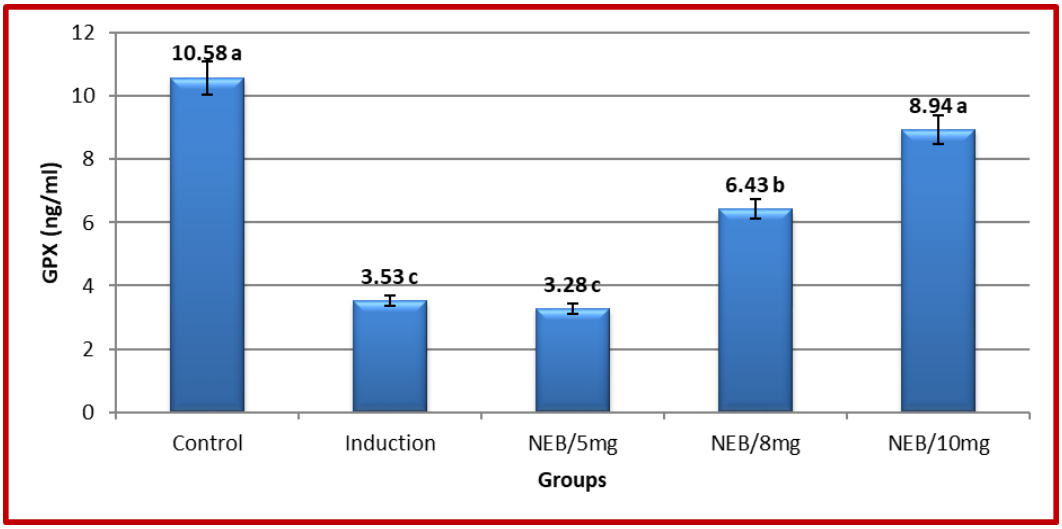

Figure 1. Bar chart showing levels of glutathione peroxidase GPx in different experimental groups. GPx:glutathione peroxidase, **ng/mL:** nanograms Per milliliter, **Control:** normal control group, rats given distilled water for 14 days; **Induction**: negative control group, rats exposed to Tamoxifen on days13 and 14 only; **NEB/5mg:** Rats treated with nebivolol (5mg/kg/d) orally plus tamoxifen on days13 and 14 only; **Neb/8mg** : Rats treated with nebivolol (8mg/kg/d) orally plus tamoxifen on days13 and 14 only; **NEB/10mg**: Rats treated with nebivolol (10mg/kg/d) orally plus tamoxifen on days13 and 14 only, Results with unidentical superscripts (a, b, c, d) are significantly different ($p<0.05$).

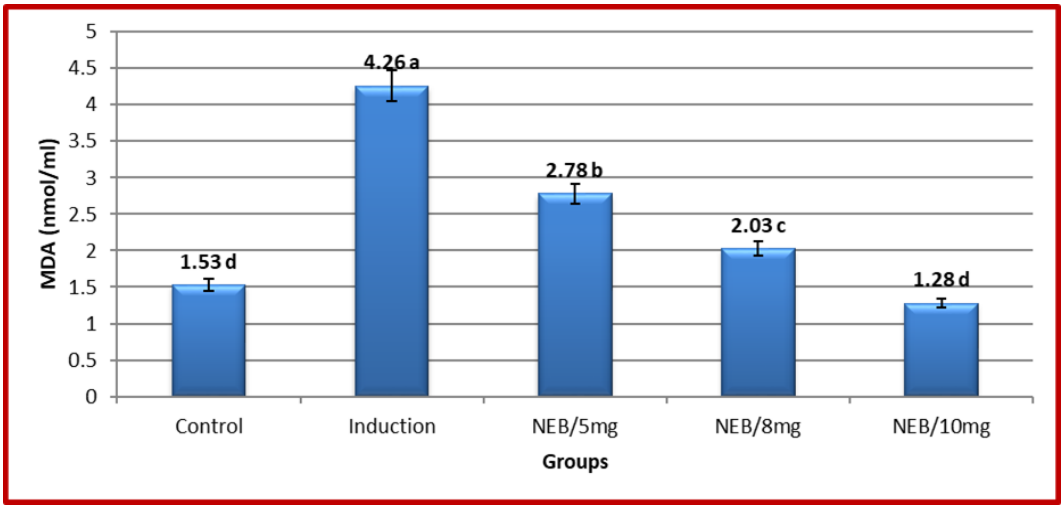

Figure 2. Bar chart showing levels of MDA in different experimental groups.MDA: malondialdehyde, **ng/mL:** nanograms Per milliliter, **Control:** normal control group, rats given distilled water for 14 days; **Induction**: negative control group, rats exposed to Tamoxifen on days13 and 14 only; **NEB/5mg:** Rats treated with nebivolol (5mg/kg/d) orally plus tamoxifen on days13 and 14 only; **Neb/8mg** : Rats treated with nebivolol (8mg/kg/d) orally plus tamoxifen on days13 and 14 only; **NEB/10mg**: Rats treated with nebivolol (10mg/kg/d) orally plus tamoxifen on days13 and 14 only, Results with unidentical superscripts (a, b, c, d) are significantly different ($p<0.05$).

Discussion

Liver injury can occur with exposure to medications or their metabolites, as well as herbal and nutritional supplements (1). Previous researches has primarily concentrated on investigating the potential preventive properties of antioxidant and anti-inflammatory substances against hepatotoxicity (19,20). Tamoxifen has been widely utilised as the preferred pharmaceutical intervention for treating and preventing breast cancer, with an average prescription duration ranging from 3 to 5 years. Unfortunately, epidemiological studies have indicated that 40% of patients acquired hepatotoxicity through steatohepatitis after two years (21). Additionally, in Iraq, a case-control study conducted at a hospital revealed that 86% of patients treated with tamoxifen experienced fatty liver (22).

Oxidative stress is a significant contributing factor in the pathogenesis of various disorders, including hepatic injury. Oxidative stress refers to the condition characterised by an imbalance between the amounts of antioxidants and free radicals. The radicals exhibit an affinity for nucleic acids, proteins, and lipids, thereby interfering with crucial cellular mechanisms such as lipid peroxidation. This interference has the potential to lead to necrosis, fat degeneration, fibrosis, cirrhosis, cell death, and cancer. The severity of these effects is dependent on the dosage and duration of exposure, which can result in increased levels of ALT, AST, and ALP enzymes in the bloodstream(23).

The current study proved that the administration of tamoxifen resulted in an elevation of oxidative stress markers in the liver, as seen by the elevated levels of malondialdehyde (MDA) and considerable reduction in glutathione peroxidase (GPX) levels, when compared to the control group in line with previouse studies(24,25).

The enzyme glutathione peroxidase (GPx) employs a reducing agent to effectively remove hydrogen peroxide and lipid peroxides. This enzymatic activity serves to protect the cell membrane and other organelles from peroxidative damage by modulating the levels of glutathione (GSH). Glutathione peroxidase (GPx) plays a pivotal role in the recycling of glutathione (GSH) and the regulation of GSH renewal(7).

Lipid peroxidation is a prominent contributor to the impairment of cellular membranes in hepatocytes. Malondialdehyde is a byproduct resulting from the process of lipid peroxidation induced by free radicals within the human body. It serves as an essential signal for assessing the presence of oxidative stress and acts as a biomarker specifically associated with oxidative stress(8).

The current study results showed a depletion of GPx in the group treated with 5mg/kg of nebivolol compared to those treated with tamoxifen only, this may be because of other side conditions like not enough waste disposal of the cage leading to stressed aggressive rats. in the bright side, Results indicated a noteworthy rise in antioxidant enzyme activity in the groups treated with nebivolol 5mg/kg and 10mg/kg compared to those treated with tamoxifen only. such finding supported the notion that nebivolol possesses antioxidant properties, which aligns with the previous findings(26) and provided empirical evidence demonstrating that nebivolol show a notable decrease in lipid peroxidation, a prominent factor contributing to the impairment of cellular membranes in hepatocytes. The observed decline in MDA levels serves as proof of this phenomenon. This finding aligns with the previous research(15,17).

The presented study demonstrated that nebivolol exhibited a protective effect against tamoxifen-induced liver injury. The

favorable impact of nebivolol is attributed to its antioxidant and anti-inflammatory effects by cutting the road to inflammation because oxidative stress is the leading cause of inflammation.

Conclusion

The current study concluded that pretreatment of nebivolol can alleviate tamoxifen-induced oxidative damage scavenging free radicals in the liver this appears by upregulation of GPx and downregulation of MDA within the oxidative pathway in liver tissues. As such, this combination needs to be further studied to evaluate other oxido-inflammatory parameters.

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Ethics Statements

The study was approved by the ethics committee of College of Pharmacy/ Mustansiriyah University (acceptance number 8 on 11/10/2023).

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