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Original Article

A combination of dexmedetomidine and thymoquinone is better able to prevent ischemia reperfusion injuries in the liver: an experimental study in rat model

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Abstract: Ischemia and reperfusion (IR) injuryin liver may lead to metabolic and structural damage. The aim of this study was to investigate whether the more effective protective effects of Dexmedetomidine and Thymoquinone combination against liver IR injury via analyzing Total Antioxidant Capacity (TAC), Total Oxidative Status (TOS), Oxidative Stress Index (OSI), histopathological changes. Fifty female Wistar-Albino rats, aged 4 to 8 weeks and average weight 180-240 g were randomly assigned into five groups of 10 rats per group as follows, sham group, control group, Dex group, TQ group and combined (Dex+TQ) group. In Sham group, no procedure and drug were given. In control group, salin, in Dex group, 25 μg/kg dexmedetomidine, in TQ group, 20 mg/kg thymoquinone, and in Combined group, 25 μg/kg dexmedetomidine and 20 mg/kg thymoquinone were given intraperitoneally. TAC activity levels were statistically significantly higher in sham group as compared to the other groups. The closest TAC activity levels to sham group were seen in combined group. The lowest TAC activity levels and highest TOS and OSI activity levels were found in control group as compared to the other groups. The lowest TOS and OSI activity levels were found in sham group and these levels in combined group were close to sham group. The lowest total histopathological damage score was seen in sham group and these scores in combined group were close to sham group. Total histopathological damage score was statistically higher in control group as compared the other groups. Dex and TQ combination has more protective effects on liver IR injury in rat model.

Keywords: Dexmedetomidine, thymoquinone, ischemia, reperfusion, injury, liver

Introduction

Ischemia and reperfusion (IR) injuries in the liver can lead to metabolic and structural damage [1]. Liver IR injury induces a systemic response and releases harmful substances that may affect remote organs such as the lungs and kidneys [1]. Blunt or penetrating trauma, surgery, sepsis, and liver transplantation can all result in liver IR injury, which is a significant negative side-effect of surgical procedures, and limits liver transplantation. Although liver ischemia can cause severe cell damage, the composition of damaging agents can be changed by reperfusion. Many chemical substances interact within the liver, and can result in the production of reactive oxygen species (ROS), which are responsible for reperfusion damage. Therefore, damage is sustained during reperfusion [1].

To prevent IR injury, new treatment strategies using various pharmacological and on-pharmacological antioxidant-like drugs have been suggested. However, these drugs are not often used in clinical practice. Dexmedetomidine (Dex) is a highly selective and potent α2-adrenergic agonist, and is used as a sedative agent in anaesthetic practice [2]. In experimental animal IR models, Dex has been shown to prevent liver IR injury due to its antioxidant properties [3, 4]. Thymoquinone (TQ), the active component of Nigella sativa seeds, is protective effect against IR injury to the liver and vari-

ous other organs [5]. Several experimental animal studies have shown that the use of these drugs to prevent liver IR injury may be possible [1, 6]. However, to the best of our knowledge, no previous studies have examined the combined use of Dex and TQ.

The aim of this study was to investigate whether combining Dex and TQ was more effective at preventing liver IR than either drug alone, by analysing total antioxidant capacity (TAC), total oxidative status (TOS), oxidative stress index (OSI), and histopathological changes.

Material and methods

This study was performed after obtaining approval from the local Ethics Committee. All experiments in this study were performed in accordance with the guidelines for Animal Research from the National Institutes of Health.

Fifty female Wistar-Albino rats, aged 4 to 8 weeks, with an average weight of180-240 g, were randomly assigned into five groups of ten as follows: sham group, control group, Dex group, TQ group, and combined (Dex+TQ) group. The animals were housed in 21±2°C temperatures with 60-65% humidity in controlled rooms with a 12 h light/dark cycle and free access to food and water. Rats were deprived of food, but not water, for 8 h before surgery. TQ (purity ≥98.5%), was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in dimethyl sulphoxide following the manufacturer's instructions. All rats were anaesthetised with an intraperitoneal injection (0.2 mL/100 g) of ketamine hydrochloride. After the abdomen was shaved and disinfected, a midline incision was made, and blood and tissue samples were taken. All the structures (hepatic artery, portal vein, and bile duct) in the portal triad of the left and median liver lobes were uncovered, and blood flow was occluded for 45 min via an atraumatic microvascular clamp. Forty five minutes later, the ischemic liver was reperfused by opening the clamp, and reperfusion continued for 60 min. In the sham group, the rats were anaesthetised, but no additional procedures were performed. The control, Dex, TQ, and combined groups were given saline, 25 μg/kg dexmedetomidine, 20 mg/kg thymoquinone, and both 25 μg/kg dexmedetomidine and 20 mg/kg thymoquinone, respectively, intraperitoneally for 5 minutes before opening the clamp. The doses were chosen based on previous studies [1, 6].

Blood and tissue samples were taken at the end of the reperfusion period in all groups. All rats were sacrificed after blood sampling, and liver tissues were obtained. Serum was obtained by centrifuging the blood, and was rapidly transferred to plastic, Eppendorf-covered tubes for biochemical analysis, and stored at -80°C. Tissue samples taken for histopathological evaluation were put into plastic containers in 10% formaldehyde. A portion of the liver was stored at -80°C for future analysis. Histopathological evaluation was performed by a blinded expert (S.K.).

The TAC and TOS of supernatant fractions were determined using a novel automated measurement method developed by Erel et al. [7, 8]. The TAC results are expressed as nmol Trolox Equiv/mg protein. The TOS results are expressed in terms of nmol H_2O_2 Equiv/mg protein. The percent ratio of TOS to TAC was accepted as OSI. The OSI values were calculated according to the following formula: OSI (arbitrary unit) = TOS/(TAC *10).

Liver samples from each animal were obtained for histopathological evaluation. Tissue samples were placed in formalin and embedded in wax according to standard protocols. The blocks were then sectioned at 5 nm, and stained with hematoxylin and eosin. A magnification of ×200 was used (Olympus BX51 TF, Olympus, San Diego, CA, USA) for evaluation. Histopathological evaluation was performed to determine the severity of ischemia using a predetermined scoring system defined by Akbulut et al. [9]. Based on the scoring system, the severity of liver damage was evaluated as: (1) sinusoidal dilatation; (2) inflammatory cell infiltration; (3) congestion; and (4) hydropic degeneration (cytoplasmic vacuolisation/swelling of hepatocyte), with features scored as 0 (normal), 1 (mild), 2 (moderate), or 3 (severe). The maximum score of 12 indicated the most severe liver injury. The parameters of each sample were collected and used to determine histopathological damage scores.

Statistical analysis

Statistical analyses were performed using SPSS for Windows software (ver. 16.0; SPSS

Table 1. Oxidative, antioxidative parameters and histopathological evaluation in all groups

	Sham (n=10)	Control (n=10)	Dex (n=10)	TQ (n=10)	Combined (n=10)	*P*
TAC	0.33±0.71[a]	0.14±0.02	0.13±0.03	0.13±0.01	0.14±0.03	<0.001
TOS	18.68±3.41	36.97±6.26[b]	27.46±5.90	27.51±3.73	26.41±5.84	<0.05
OSI	5.92±1.89	26.29±5.40[b]	21.29±7.11	21.31±2.97	19.97±1.03	<0.05
Total histopathologicalscore	2.40±1.07	5.20±0.78[b]	4.40±0.84	3.20±1.03	2.60±1.42	<0.05

TAC = Total Antioxidant Capacity; TOS = Total Oxidant Status; OSI = Oxidative Stress Index. *P<0.05* was considered as statistically significant. [a]*P<0.001* (for all comparisons) compared with control, Dex, TQ, and Combined groups. [b]*P<0.05* (for all comparisons) compared with sham, Dex, TQ, and Combined groups.

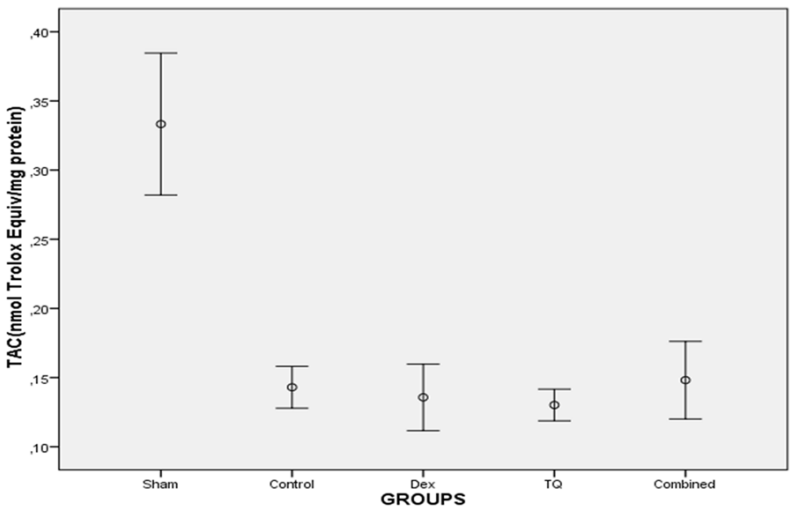

Figure 1. TAC levels for sham, control, Dex, TQ and combined groups. *P<0.001 (for all comparisons) compared with control, Dex, TQ, and combined groups.

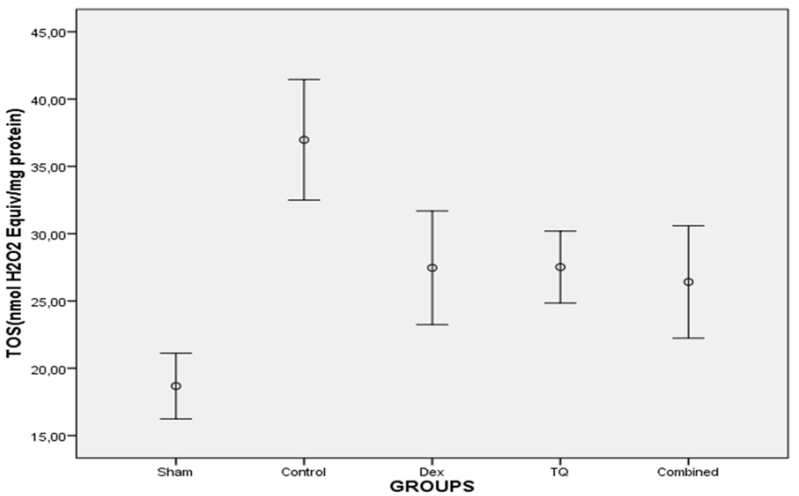

Figure 2. TOS levels in sham, control, Dex, TQ and combined groups. **P<0.05 (forallcomparisons) compared with sham, Dex, TQ, and combined groups.

Inc., Chicago, IL, USA). All data are expressed as means ± SD. The distribution of continuous variables was assessed with a one-sample Kolmogorov-Smirnov test, and all variables were determined to be abnormally distributed. Therefore, nonparametric independent group comparisons were made. The Kruskal-Wallis test was used for multiple comparisons and a Mann-Whitney U test was used to compare values among groups. A two-sided *P* value of <0.05 was considered statistically significant.

Results

All animals survived the experiment. Treatment with Dex or TQ alone, and with Dex and TQ in combination, did not produce any adverse side effects at the tested doses.

The oxidative stress parameters, including TAC, TOS and OSI, are shown in **Table 1** and **Figures 1**-**3**. TAC activity levels were significantly higher in the sham-group compared to the other groups (P<0.001), but the combined group had the next highest TAC value. The lowest TAC values were found in the control group. TOS and OSI activity levels were statistically significantly higher in the control group compared to the other groups (P<0.05). In addition, the lowest TOS and OSI activity levels were found in the sham group,

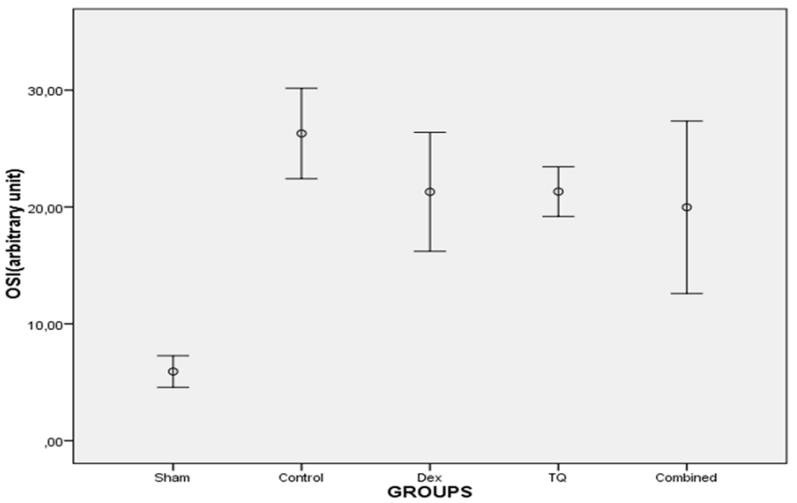

Figure 3. OSI levels in sham, control, Dex, TQ and combined groups. *P<0.05 (for all comparisons) compared with sham, Dex, TQ, and combined groups.

and the levels in the combined group were close to those found in the sham group.The histopathological damage scores from each group are shown in **Table 1**. The histopathological images of the rats' livers are shown in **Figure 4**. The lowest damage score was seen in the sham group, but the combined group had the lowest scores among the other groups. The control group had the highest amount of liver damage compared to the other groups (P<0.05).

Discussion

In this study, the protective effects of a Dex and TQ combination treatment against liver IR injury were evaluated by analysing TAC, TOS, OSI, and histopathological changes. The main findings of this study are as follows. First, ischemia for 45 minutes followed by 60 minutes of reperfusion caused significant damage to the liver.Second, in the sham group, TAC activity levels were the highest, and TOS and OSI activity levels, and histopathological damage scores, were the lowest compared to the other groups. In addition, the group receiving combined treatment had the least liver damage among the surgery groups, suggesting that the TQ and Dex combination was effective at preventing IR. Third, the lowest TAC activity levels, and the highest TOS levels, OSI levels, and histopathological damage scores, were seen in the control group.

Liver IR injury is a complex process accompanied by alterations in intracellular signalling, mediators, cells, and pathophysiological alterations [10]. Damage in the early stages of IR injury is mediated by oxygen free radicals [11]. A massive and abrupt release of oxygen free radicals after reperfusion, followed by endothelial dysfunction or neutrophil infiltration, triggers oxidative damage [11]. An excessive production of oxygen free radicals has been reported in ischemic reperfused livers, which lead to tissue damage, an unavoidable process that occurs during liver transplantation and surgical procedures in which atraumatic microvascular clamping is used [12]. In this study, ischemia for 45 minutes followed by 60 minutes of reperfusion resulted in liver tissue damage, which was likely caused by oxidative damage induced by endothelial dysfunction and neutrophil infiltration.

Ischemia and reperfusion injury usually includes the production of free oxygen radicals [13]. Defence mechanisms of the organism against these radicals are assessed by the measurement of oxidant and antioxidant levels. Although measuring oxidants or antioxidant components alone can give information about oxidative stress, measuring oxidants along with antioxidants is more useful [6]. Therefore, TAC, TOS and OSI activity levels were measured in this study. The results confirm that liver IR injury occurs through oxidative stress, and modulates the production of toxic cytokines leading to inflammation and leukocyte infiltration.

Various pharmacological and non-pharmacological antioxidant-like drugs have been used to reduce liver IR injury in animal models [1, 6]. However, few have been used in clinical practice. Therefore, anaesthetic or sedative agents used in anaesthesia have become an important issue for the reduction of IR injury in recent years [2, 14]. Dex, a highly selective and potent α2-adrenergic agonist used as a sedative agent in anaesthetic practice[2], showed potential biochemical and histopathological benefits, and anti-inflammatory effects, by preventing IR-related cellular damage in experimental rat models [15-18]. Previous studies have reported

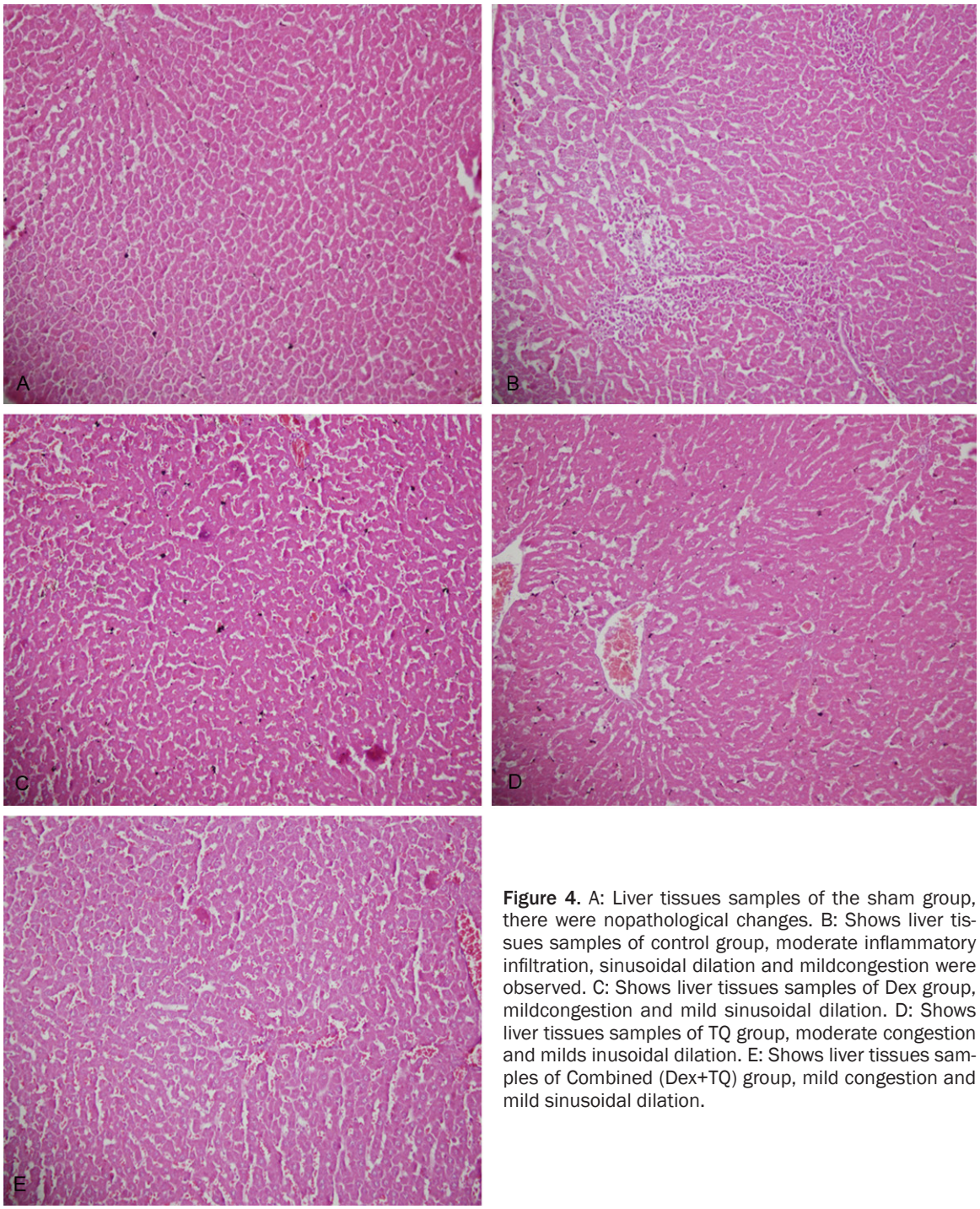

Figure 4. A: Liver tissues samples of the sham group, there were nopathological changes. B: Shows liver tissues samples of control group, moderate inflammatory infiltration, sinusoidal dilation and mildcongestion were observed. C: Shows liver tissues samples of Dex group, mildcongestion and mild sinusoidal dilation. D: Shows liver tissues samples of TQ group, moderate congestion and milds inusoidal dilation. E: Shows liver tissues samples of Combined (Dex+TQ) group, mild congestion and mild sinusoidal dilation.

that the anti-ischemic effect of Dex may be associated with inhibition of ischemia-induced excess noradrenalin secreted via presynaptic alpha adrenoreceptors [19]. Dex can prevent the potentially destructive effects of free-oxygen radicals by preventing the effects of noradrenalin on these presynaptic alpha adreno receptors [19]. Tüfek et al. [1] showed that Dex had protective effects on the liver and remote organs against hepatic IR injury in rats. Several studies have demonstrated that Dex minimises IR injury in the central nervous system and has neuroprotective effects [18, 20].

TQ is the active constituent of Nigella sativa seeds. It may reduce oxidative stress through a

direct antioxidant effect, but also induces endogenous antioxidant enzymes [21] Mansour et al. [22] showed that TQ inhibited both the cyclooxygenase (COX) and 5-lipoxygenase (5-LO) pathways of arachidonate metabolism in rat peritoneal leukocytes [22]. The inhibition of both COX and 5-LO pathways is a key factor mediating the anti-inflammatory effects of TQ [23]. Yildiz et al. [6] reported that TQ relieves the deleterious effects of IR injury on the liver. Previous studies have shown that TQ attenuates lipid peroxidation and increases antioxidant enzyme activity, and that it has strong antioxidant potential due to its ability to scavenge oxygen free radicals [24, 25].

As mentioned above, both Dex and TQ have been identified as potent antioxidants [15-18] and anti-inflammatory compounds [1, 6, 26]. In this study, the results from the combined group were more positive than those for the Dex or TQ group. In the combined group, TAC activity levels were increased, and TOS levels, OSI activity levels, and histological damage scores were decreased. Moreover, our study showed that the Dex and TQ combination was more effective in terms of antioxidant activity and prevents damage by free radicals. These results were likely due to synergistic interaction between the antioxidant and anti-inflammatory effects of the two agents.

In this study, in both the Dex and TQ groups, TAC activity levels were increased-and TOS levels, OSI activity levels, and histological damage scores were decreased-compared to the control group. However, the results in the Dex and TQ groups were still higher than in the sham group. Therefore, it appears that combining Dex and TQ is more effective at preventing liver IR than either drug alone.

This study had several limitations. Firstly, we only evaluated the early effects of Dex and TQ. Extension of survival time post-surgery may have allowed for a longer term analysis. Secondly, ketamine, which was the main anaesthetic in our study, may have influenced the results by mitigating IR injury and affecting our biochemical and histopathological results. However, the effect of ketamine on IR injury is limited and dose-dependent [16, 27]. Third, this is an experimental study, and the animal model used may not accurately reflect the situation in humans.

The results of this study show that the Dex and TQ combination is more protective against liver IR injury compared to Dex or TQ used alone in a rat model. This study is the first to report the protective effects of Dex and TQ combination against liver IR injury. In addition, the combination of both agents may be useful in the treatment of liver IR injury. We expect that further studies on the long-term effects of Dex and TQ combinations will increase the value of our findings. Future studies will be required to verify the effectiveness of these agents. The clinical significance of these preliminary findings should be further investigated in human subjects.

Disclosure of conflict of interest

None.

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