

Protective Effect of Resveratrol on Kidney and Liver Histopathology Induced by NMDA Receptor Antagonist Mk-801 in Mice ^[1]

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Abstract

TMK-801 is an antagonist of N-methyl-D-aspartate (NMDA) receptors. Sub-chronic administration of 1 mg / kg dose of MK-801 resulted in schizophrenia-like symptoms and degeneration in the brain of mice. In this study, it was investigated whether this dose could cause histopathological changes in kidney and liver tissues and resveratrol had a protective role against these changes. For this aim, 24 male mice were obtained and divided equally into 4 groups. The animals in the control group were intraperitoneally (i.p.) given 10 mL/kg saline solution, the animals in the experiment groups were i.p. given 1 mg/kg MK-801 alone, 40 mg/kg resveratrol alone and MK-801 by 1 mg/kg for 14 days. The kidney and the liver of the sacrificed mice were collected and routine histology procedure was applied. Glomerular shrinkage was observed in the kidneys after the measurements. Besides this finding, histopathological changes were observed in both kidney and liver tissue and resveratrol inhibited most of the harmful effects. In conclusion, MK-801 caused histopathological changes in both kidney and liver tissues, and resveratrol had a significantly protective role on this injury.

Keywords: Histopathology, Kidney, Liver, Mice, MK-801, Resveratrol

Farelerde NMDA Reseptör Antagonisti Mk-801 İle Böbrek ve Karaciğerde Oluşan Histopatolojik Değişikliklere Karşı Resveratrolün Koruyucu Etkisi

Öz

MK-801 N-methyl-D-aspartate (NMDA) reseptör antagonisti bir kimyasal ajandır. Bu kimyasalın sub-kronik 1 mg/kg dozunda uygulanması farelerde şizofreni benzeri semptomlar görülmesine ve beyinde dejenerasyona yol açmaktadır. Resveratrolünse hem böbrek hem de karaciğer üzerinde koruyucu etkisi vardır. Sunulan bu çalışmada hem bu dozun karaciğer ve böbrek gibi organlara histopatolojik bir zararının olup olmadığı hem de resveratrolün koruyucu etkisinin olup olmadığı araştırılmıştır. Bu amaçla 24 erkek fare alınmış ve 4 eşit gruba bölünmüştür. Kontrol grubuna intraperitoneal (i.p.) olarak %0.9'luk (10 mL/kg) fizyolojik tuzlu su verilmiştir. Deney gruplarındaki hayvanlara 14 gün boyunca MK-801 tek başına 1 mg / kg, resveratrol tek başına 40 mg/kg ve MK-801 ile birlikte i.p. verildi. Farelerin sakrifikasyonundan sonra karaciğer ve böbrek dokuları çıkartılmış, histolojik işlemlerden sonra hematoksilen-eozinle boyanmıştır. Ölçümler sonucunda glomeruluslarda istatistiksel olarak küçülme ile birlikte karaciğer ve böbrek dokusunda histopatolojik değişikliklere rastlanmıştır. Resveratrolün bu zararlı değişikliklere karşı oldukça fazla koruyucu etkisinin olduğu gözlenmiştir. Sonuç olarak MK-801'in kronik dozunun karaciğer ve böbrek üzerine zararlı etkileri olduğu ve resveratrolün bu zararlı etkilere karşı önemli miktarda bir koruyucu özelliğinin olduğu gözlemlenmiştir.

Anahtar sözcükler: Histopatoloji, Böbrek, Karaciğer, Fare, MK-801, Resveratrol

INTRODUCTION

N-methyl-D-aspartate (NMDA) receptors are an important factor in the synapse mechanism of the glutamatergic

system ^[1,2]. Hypofunction of these receptors may cause cognitive and motor problems ^[3-8]. The chemical agent MK-801 is a blocking and neurotoxic antagonist of this receptor ^[6-8]. Blocking of this receptor by the 1 mg/kg



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sub-chronic dose of MK-801 causes neurodegeneration, demyelination, and schizophrenia-like symptoms in mice [7,8]. In addition, transporters and receptors of the glutamatergic system are found in many internal organs such as the testis, intestine, heart, lung, liver and kidney [9]. In the kidney, the NMDA receptor is expressed in the cortex, medulla and particularly in the renal proximal tubule [10,11].

Recently, low dose of MK-801 has been applied to kidney diseases as a therapeutic agent [12-14]. Administration of MK-801 at a dose of 0.5 mg/kg for 28 days relieved renal ischemia and reperfusion-induced glomerular and tubular functional problems [12,13]. In addition, NMDA receptor antagonist MK-801 improved the effects of gentamicin-induced renal injury [14].

However, there are some controversial points on the administration of the MK-801. MK-801 constricts the vessels of the kidney, reduces the rate of single nephron glomerular filtration, and produces abundant podocyte cytoskeleton [15-18]. In addition, MK-801 increased the detrimental effects of dexamethasone on the kidney, and the individual administration of MK-801 caused morphological changes on the kidney [19].

There is also limited information about the effect of MK-801 on the liver. Previous studies have reported that acute administration of NMDA antagonists may prevent from acute liver failure (ALF) and damage [20,21].

Resveratrol, a protective antioxidant, is a natural phytoalexin and is found especially in peanuts, grapes and red wine [22]. It is an NMDA receptor expression regulator and can be used as anti-oxidant, anti-inflammatory, anti-cancer, antiviral, anti-ageing, anti-diabetic, and cardio-protectant [23,24]. In addition, it is also quite effective on some renal diseases such as diabetic nephropathy, drug-induced renal injury, ischemia-reperfusion, sepsis-induced, and obstructed and aging kidney [24]. In addition, resveratrol has a curative effect on the liver diseases [25].

This study investigated the possible deleterious effect of sub-chronic administration of 1 mg/kg MK-801 on the kidney and liver of mice and the possible protective role of resveratrol.

MATERIAL and METHODS

Animals

This study was performed on 24 male Balb/c mice (obtained from the Kobay Firm) under the permission of the Ethical Committee of Experimental Animals, Afyon Kocatepe University, AKUHADYEK-132-16. All the mice were housed at the Experimental Animal Research Centre of Afyonkarahisar, in the 22±2°C room temperature and a 12/12 h light/dark cycle and fed as *ad libitum*.

Groups and Dosages

The 24 mice were divided equally into 4 groups. The animals were grouped as control (i.p. 10 mL/kg saline solution), MK-801 (1 mg/kg), resveratrol (40 mg/kg) and resveratrol+MK-801 (40 mg/kg + 1 mg/kg) (n=6 in each group). The saline, MK-801 and resveratrol doses in this study were chosen from previous studies [8,26]. All injections were performed for 14 days. In the third group, resveratrol was injected in the morning and MK-801 was injected in the afternoon.

Histological Tissue Processing

After the injections, all mice were sacrificed by decapitation and the kidney and liver tissues were collected into 10% formalin solution. After one week tissues were embedded into the paraffin and then trimmed into 5 µm thickness, consecutively. All the slides were stained with hematoxylin-eosin.

Tissue Evaluation

The mean shrinkage of the nephron was evaluated on a computer attached Olympus microscope by M-Shot software. The liver and kidney semiquantitative scoring was done according to a previous research [27].

Statistical Analysis

The data about the nephron and its compartments were estimated as means and standard deviations, and analysed using one-way analysis of variance (ANOVA) followed by Duncan posthoc test on the SPSS 16.0 software computer programme. A difference in the mean values of P<0.05 was considered to be significant.

RESULTS

Effect of Resveratrol on the Kidney

In the group, to which MK-801 was administered, a significant shrinkage (P<0.001) of the glomerular tuft was observed compared to control group (Table 1). Administration of resveratrol significantly protected the mean glomerulus diameter of MK-801 treated mice (P<0.001). Moreover, compared to all the other groups, resveratrol did not change mean nephron diameter and bowman-space (P>0.05; Table 1).

Effect of Resveratrol on Histopathological Examination

The histopathological changes in the kidney and liver of the animals in the experiment groups are shown in Fig. 1 and Fig. 2, respectively. In the control group (Fig. 1a, Fig. 2a) and in the group supplied with resveratrol (Fig. 1b, Fig. 2b), histopathological observations in the kidney, and liver tissues were normal. In the MK-801 group, degenerative and necrobiotic changes in tubular epithelial cells as well as expansion and vacuolar degeneration of

Bowman's capsule were observed (Fig. 1c). The double-nuclei hepatocytes in the periportal area and multifocal coagulation necrosis; severe hyperemia in the vessels; kuppfer cell activation; increase in the number of the bile ducts and focal mononuclear cell infiltration were observed in the liver tissue of the mice (Fig. 2c). In the MK-801 + resveratrol group, slight histopathological changes were observed respectively in kidney and liver tissues (Fig. 1d, Fig. 2d). Moreover, the quantitative assessment of the histopathological changes in the kidney and liver of mice from different groups is summarised in Table 2 like this; -: no lesion; +: mild; ++: moderate; +++: severe. Our results demonstrated that resveratrol reduced the degenerative effects of MK-801 and protected the kidney and liver tissues.

DISCUSSION

The animal schizophrenia-model caused by MK-801 is used to observe the effects of this illness on the brain. Sub-chronic dose of 1 mg/kg MK-801 for 14 days was reported to cause schizophrenia-like symptoms in mice [7,8]. However, there was insufficient information about the

effects of this dose on the liver and kidney. Therefore, this study focused on the effect of this dose on both the kidney and liver tissues. The effect of this dose was evaluated by histopathological inspections on the liver and kidney.

According to the literature, the MK-801 has protective effects on renal and liver degeneration [12-14,20,21]. However, in these studies, the protective dose was relatively lower than the sub-chronic dose. In addition, some previous studies have shown that 1 mg/kg MK-801 has adverse effects on the internal organs [28]. Previous studies have reported that administration of MK-801 to the kidney results in constriction of the renal vessels and reduces glomerular filtration rate and production of podocytes [15-18]. In this study, similar to these reports, the application of MK-801 to the kidney showed some histological changes. MK-801 at a dose of 0.3 mg/kg for 8 days caused a slight narrowing of the urinary cavity in the nephrons and the use of MK-801 with dexamethasone increased the severity of hyperemia and dilatation of these cavities [19]. However, in this study, application of MK-801 caused an expansion in the nephro-glomerular space, contraction of glomerulus diameter,

Table 1. Measurement of the nephron and its compartments

Groups	The Mean Nephron Diameter (µm)	The Mean Glomerulus Diameter (µm)	The Bowman-space (µm)
Control	145±8	116±6 ^a	27±2
MK-801	135±5	98±8 ^c	39±12
RES+MK-801	134±15	104±8 ^{bc}	31±10
RES	140±5	111±4 ^{ab}	32±7
P value	0.175	<0.001	0.084

Values are the mean ± S.D, n=6; ^{a,b,c} The mean glomerulus diameter with different letters in the same column shows statistically significant differences (P<0.05)

Table 2. The semiquantitative scoring of the liver and kidney

Tissue	Histopathological Findings	Control	Resveratrol	MK-801	MK-801 + Resveratrol
Liver	Double-nuclei hepatocytes in the periportal area and multifocal coagulation necrosis	- (7/7)	-(7/7)	+ (5/7) ++ (1/7) +++ (1/7)	- (7/7)
	Kuppfer cell activation	- (7/7)	-(7/7)	- (4/7) + (1/7) ++ (2/7)	- (6/7) + (1/7)
	Hiperemia in the vessels	- (7/7)	-(7/7)	+ (5/7) ++ (2/7)	- (7/7)
	Increase in the number of the bile ducts and focal mononuclear cell infiltration	- (7/7)	-(7/7)	+ (4/7) ++ (3/7)	- (7/7)
Kidney	Vacuolar degeneration in the glomerulus	- (7/7)	-(7/7)	- (1/7) + (4/7) ++ (2/7)	- (7/7)
	Expansion in the bowman-space	- (7/7)	-(7/7)	+ (2/7) ++ (5/7)	- (7/7)
	Degenerative and necrobiotic changes in the tubular epithelial cells	- (7/7)	-(7/7)	+ (4/7) ++ (2/7) +++ (1/7)	- (6/7) + (1/7)

The findings were evaluated and scored as follows: -: no lesion; +: mild; ++: moderate; +++: severe

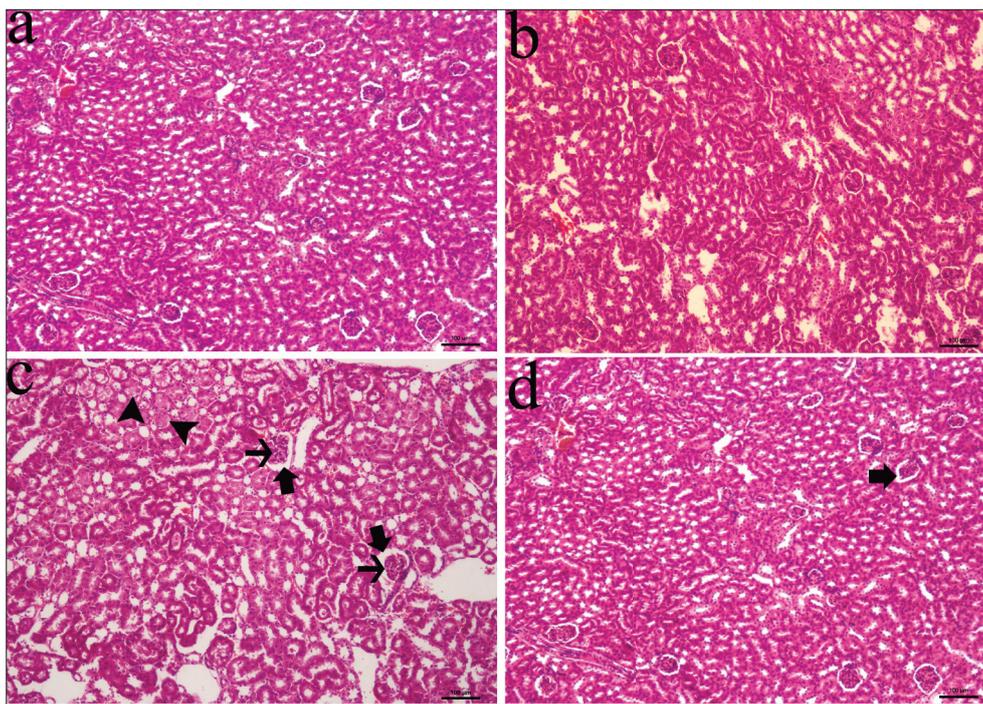


Fig 1. The effect of resveratrol on Mk-801 induced damage in kidney of mice. **a.** Control group, **b.** Resveratrol group, **c.** MK-801 group; *thick arrow*: Expansion in the bowman-space, *thin arrow*: Vacuolar degeneration in the glomerulus, *arrowhead*: Degenerative and necrobiotic changes in the tubular epithelial cells, **d.** MK-801 + Resveratrol group, *thick arrow*: Expansion in the nephro-glomerular space

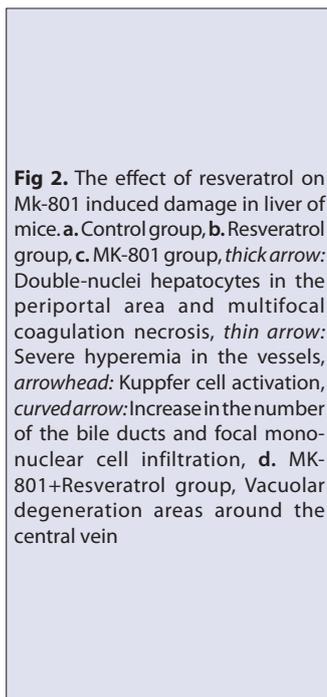
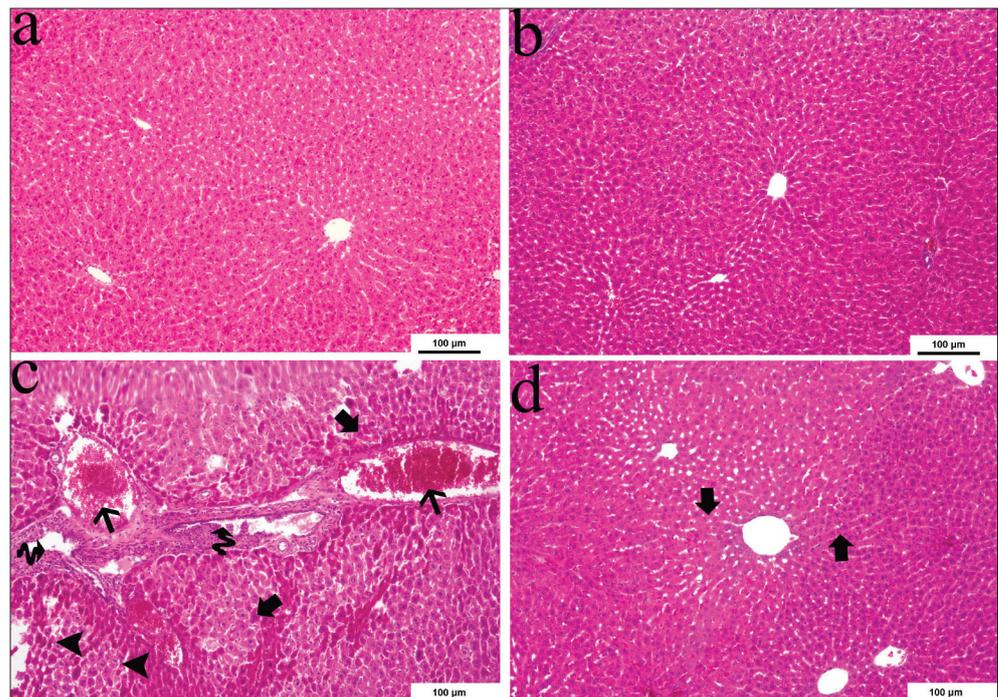


Fig 2. The effect of resveratrol on Mk-801 induced damage in liver of mice. **a.** Control group, **b.** Resveratrol group, **c.** MK-801 group, *thick arrow*: Double-nuclei hepatocytes in the periportal area and multifocal coagulation necrosis, *thin arrow*: Severe hyperemia in the vessels, *arrowhead*: Kuppfer cell activation, *curved arrow*: Increase in the number of the bile ducts and focal mononuclear cell infiltration, **d.** MK-801+Resveratrol group, *thick arrow*: Vacuolar degeneration areas around the central vein



vacuolar degeneration in glomerulus, and degenerative and necrobiotic changes in tubular epithelial cells. The administration of resveratrol has protected the many of these harmful effects. In one study, xenobiotics applied to the body caused histopathological deterioration in kidney and liver tissues and resveratrol protected against these disorders^[29]. Beside the histopathological findings, a dose-dependent increase in cell death and apoptosis were also observed in the renal culture cells exposed to the MK-801. Thus, over excitation or blockade of the renal NMDA receptor leads to cell death^[11].

There is insufficient information about the effect of sub-chronic administration of MK-801 on liver tissue. Recently, it has been reported that MK-801 has a positive effect on ammonia-induced activation of ALF^[21]. ALF leads to activation of NMDA receptors in the brain, and neuronal damage ultimately results in death. Blocking NMDA receptors with MK-801 protects or delays death from ALF. But, in the clinics the routine or chronic administration of MK-801 has secondary effects and this is the biggest handicap for the treatment^[21]. Additionally in the present study, double-nuclei hepatocytes in the periportal area,

multifocal coagulation necrosis, kuppfer cell activation, hyperemia in the vessels, increase in the number of the bile ducts and focal mononuclear cell infiltration in the liver and the protective effect of the resveratrol against these impairments were observed.

As a result, administration of resveratrol against MK-801 significantly protected kidney and liver tissues. Particularly, epithelial cells are NMDA receptor expression sites and resveratrol is thought to be important in protecting this site from necrosis.

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