

The reno-protective effects of atorvastatin in crush syndrome and rhabdomyolysis: is there a dilemma?

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Background/aim: We aim to determine the effects of low-dose atorvastatin treatment together with crush fluid resuscitation on renal functions and muscle enzyme levels in a rat model of crush syndrome.

Materials and methods: The study involved female Wistar Albino rats weighing 250–300 g that were housed with free access to food and water. The crush model was obtained by compression. Rats were randomly divided into four groups: control (C) group, atorvastatin + crush fluid (ACF) group, crush fluid (CF) group, and hypertonic saline (3%) + mannitol + sodium bicarbonate (SM) group. Blood was obtained at 24, 48, and 72 h, and serum creatinine kinase, myoglobin, urea, creatinine, and lactate dehydrogenase levels were studied.

Results: All parameters were statistically significantly higher in the control group than in the treatment groups at all hours. However, there was no statistically significant difference among treatment groups regarding any of the parameters.

Conclusion: This is the first study determining the role of atorvastatin in the treatment of renal ischemia/reperfusion injury in a crush syndrome and rhabdomyolysis model setting. Larger studies with different atorvastatin doses are required to define the role of this drug in the treatment of renal ischemia/reperfusion injury during crush syndrome.

Key words: Crush syndrome, atorvastatin, rhabdomyolysis

1. Introduction

Ischemia/reperfusion injury (IRI) is the mainstay of organ damage in crush syndrome that develops following prolonged continuous pressure on the limbs (1). Unfortunately, re-establishment of the blood supply after a prolonged limb compression results in local muscle cell necrosis and consequent rhabdomyolysis. Moreover, the metabolites and cytokines leaked from the myocytes of disintegrated muscle tissue may induce systemic inflammatory response and multiple organ dysfunctions (2). The reperfusion injury is associated with the release of oxygen-free radicals and massive accumulation of calcium in ischemic muscles. Recently, oxidative stress, as determined using a free radical elective evaluator, was reported to be markedly increased after ischemia/reperfusion injury (3).

Prevention of reperfusion-induced injury of ischemic muscles is extremely important because it results in salvage of cells (4). Atorvastatin is a HMG-Co

A enzyme inhibitor that is on the market primarily as a lipid-decreasing agent. However, the antioxidant and antiinflammatory properties of statins have been known for years. A dose-dependent reduction and normalization of oxidative stress marker levels in joint inflammation has been reported after atorvastatin administration (5). In a recent meta-analysis of 8 randomized trials, the use of short-term statins was associated with a reduction in the incidence of contrast-induced nephropathy, and was recommended even in patients with low LDL-cholesterol levels (6). On the other hand, myotoxicity is one of the adverse effects of statins and may lead to a large spectrum of muscle diseases, resulting from myalgia or myopathy to rhabdomyolysis in a dose-dependent manner (7).

In this study, we aimed to determine the effects of low-dose atorvastatin treatment together with crush fluid resuscitation on renal functions and muscle enzyme levels in a rat model of crush syndrome.

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2. Materials and methods

2.1. Rats and crush model

Female Wistar Albino rats, weighing 250–300 g, were housed in a room maintained at a temperature of 22 °C and a relative humidity of 55%, with a 12-h light–dark cycle and free access to food and water (*ad libitum*). The study was approved by the Ankara Experimental Animal Laboratory Ethics Committee and all animal experiments were conducted according to guidelines for animal use.

To compress bilateral hind limbs, we prepared a pair of rubber tourniquets that were 2.4 cm in width and 1 mm in thickness. The tourniquets were applied by wrapping 5 turns around a metal cylinder (22-mm outer diameter, 20-mm inner diameter, and 70 mm in length) under a 2-kg weight load, and the end of the band was glued. The rat was held ventral surface up, and its foot was dropped through the metal cylinder. Hind limb compression was induced by pushing the tourniquet high onto the rat's thigh. After 5 h, compression was released by cutting and removing the tourniquet (Figures 1a and 1b) (8).

2.2. Experimental design

The rats were randomly divided into four groups: control (C) group, atorvastatin + crush fluid (ACF) group, crush fluid (CF) group, and hypertonic saline (3%) + mannitol + sodium bicarbonate (SM) group.

All experiments were conducted under general anesthesia (80 mg/kg intraperitoneal ketamine hydrochloride and 5 mg/kg xylazine). Compression was performed in all groups. The control group did not receive any treatment after compression. After the removal of the tourniquets, the rats in the ACF group obtained atorvastatin (ampule 100 mg, Sanofi Aventis, İstanbul, Turkey) 0.2 mg/kg and concomitant crush fluid. The CF group only obtained crush fluid, and the SM group obtained hypertonic saline (3%) + mannitol + sodium bicarbonate together. The tail vein was catheterized for the iv fluid infusions. The infusion rate of the fluids was 1 mL/100 g body weight/hour. The fluid amount was calculated and administered at 2, 4, and 6 h by iv bolus.

Blood was obtained at 24, 48, and 72 h, and serum creatinine kinase (CK), myoglobin, urea, creatinine and lactate dehydrogenase (LDH) levels were studied. At 24 and 48 h, a 1.5–2 cc blood sample was obtained from rat tail veins. At 72 h, the blood was obtained in an intracardiac way under general anesthesia, and the rats were sacrificed this way.

2.3. Statistical analysis

Data are shown as mean \pm standard deviation. Statistical analysis of data was performed using SPSS 21.0. Two-way analysis of variance (ANOVA) was used for comparison of all groups with Tukey posthoc tests. A 95% confidence interval was considered statistically significant ($P < 0.05$).

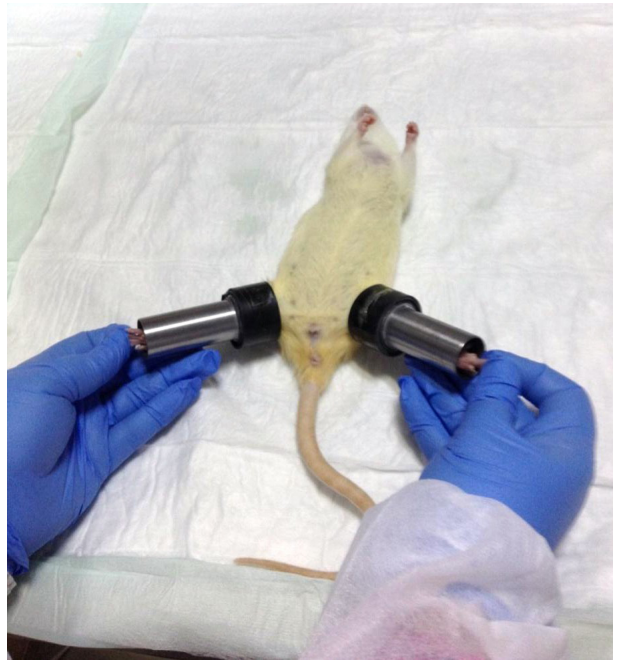


Figure 1a and 1b. Crush syndrome model.

3. Results

The survival rates of the rats remained at 100% throughout the experiment. All rats completed the study.

The mean results of the groups at 24, 48, and 72 h are summarized in Tables 1–3, respectively. All parameters

Table 1. Results at 24 h.

	Control	ACF	CF	SM	P
BUN (mg/dL)	39.75 ± 5.59	17.50 ± 9.53	20.75 ± 12.17	19.87 ± 8.50	0.03
Creatinine (mg/dL)	0.62 ± 0.17	0.37 ± 0.14	0.56 ± 0.32	0.37 ± 0.39	0.007
CK (IU/L)	780.42 ± 126.52	160.87 ± 185.29	197.37 ± 95.39	425.12 ± 330.68	0.008
LDH (IU/L)	1483.14 ± 421.90	429.37 ± 176.56	496.75 ± 180.93	815.75 ± 393.55	0.04

BUN: blood urea nitrogen, CK: creatinine kinase, LDH: lactate dehydrogenase.

Table 2. Results at 48 h.

	Control	ACF	CF	SM	P
BUN (mg/dL)	59.46 ± 11.61	15.12 ± 4.81	17.37 ± 4.49	18.50 ± 5.41	0.001
Creatinine (mg/dL)	1.11 ± 0.42	0.23 ± 0.14	0.27 ± 0.11	0.24 ± 0.12	0.01
CK (IU/L)	1086.25 ± 532.04	152.00 ± 138.72	189.50 ± 128.95	228.62 ± 149.82	0.04
LDH (IU/L)	1014.75 ± 528.25	325.87 ± 162.80	374.25 ± 103.97	422.25 ± 164.26	0.005

BUN: Blood urea nitrogen, CK: creatinine kinase, LDH: lactate dehydrogenase.

Table 3. Results at 72 h.

	Control	ACF	CF	SM	P
BUN (mg/dL)	50.98 ± 7.02	17.66 ± 1.10	20.68 ± 1.34	19.66 ± 2.53	0.03
Creatinine (mg/dL)	0.79 ± 0.34	0.25 ± 0.32	0.27 ± 0.12	0.27 ± 0.07	0.02
CK (IU/L)	562.50 ± 182.05	270.00 ± 171.49	310.00 ± 108.37	441.25 ± 192.44	0.01
LDH (IU/L)	743.00 ± 154.92	190.37 ± 92.99	245.85 ± 106.48	191.37 ± 94.64	0.01

BUN: blood urea nitrogen, CK: creatinine kinase, LDH: lactate dehydrogenase.

were statistically significantly higher in the control group than in the treatment groups at all hours. At 24 h, the BUN value of the control group was significantly higher than those of the ACF, CF, and SM groups ($P = 0.028$). Creatinine values were statistically significantly higher in the control group than in the ACF and SM groups ($P = 0.030$ and $P = 0.032$, respectively). The CK value of the CF group was significantly lower than the value determined in the control group ($P = 0.016$). At 48 h, the BUN values of the control group were significantly higher than the BUN values in the ACF, CF, and SM groups ($P = 0.0001$, $P = 0.0001$, and $P = 0.0001$, respectively). The creatinine values of the control group were significantly higher than those of the ACF, CF, and SM groups ($P = 0.007$, $P = 0.012$, and $P = 0.006$, respectively). CK level detected in the SM group was significantly lower than the value determined in the control group ($P = 0.020$). The LDH level in the control group was

significantly higher than the levels in the ACF, CF, and SM groups ($P = 0.002$, $P = 0.003$, and $P = 0.004$, respectively). At 72 h, BUN values of the control group were significantly higher than the values in the ACF, CF, and SM groups ($P = 0.012$, $P = 0.016$, and $P = 0.018$, respectively). The creatinine values of the control group were significantly higher than those of the ACF, CF, and SM groups ($P = 0.001$, $P = 0.001$, and $P = 0.001$, respectively). CK levels in the SM group were significantly lower than the value determined in the control group ($P = 0.070$). The LDH level in the control group was significantly higher than the levels in the ACF, CF, and SM groups ($P = 0.016$, $P = 0.021$, and $P = 0.030$, respectively). However, there was no significant difference between treatment groups regarding any of the parameters.

The laboratory measured myoglobin levels as normal or higher than normal. In this respect, at 24 h only one

rat in the control group had higher myoglobin levels than normal; at 48 h, one rat in the C group and one rat of the CF group had higher myoglobin levels than normal; at 72 h, 4 rats of the C group, 4 rats of the CF group, 2 rats of the ACF group, and 2 rats of the SM group had higher myoglobin levels than normal. There was no significant difference among groups regarding myoglobin levels at any time.

4. Discussion

In this study, we evaluated the effects of atorvastatin treatment together with crush fluid in a rat crush model. We determined that the addition of atorvastatin to the crush fluid did not result in any significant difference in renal functions or muscle enzyme levels when compared to other treatment (only crush fluid or hypertonic saline treatments) groups. Although the means of all parameters were lower in the atorvastatin group than in the other groups at all hours, the difference was not significant between treatment groups.

Acute limb ischemia results in a sudden onset decline in the perfusion of the limbs that threatens both the limb functions and patients' lives (9).

Muscle cell ischemia and massive cell death result in the release of myoglobin, potassium, and lactic acid. Acute reperfusion of muscle compartments causes sudden intravascular spreading of these toxic metabolites, resulting in acute rhabdomyolysis and renal failure (10).

Data about the renal therapeutic effects of statins in IRI are limited. Sabbatini et al. reported that low-dose treatment with atorvastatin enhances nitric oxide availability, improving renal dynamics and conferring a histologic protection at tubular level after ischemia in an experimental study (11).

Tododrovic et al. investigated the effects of acute pretreatment with a single dose of simvastatin (1 mg/kg, iv; 30 min before ischemia) on renal dysfunction caused by IRI in the rats. They reported that simvastatin significantly improved both parameters of glomerular and tubular dysfunction, and especially improved the histological score compared to rats treated with saline or 10% dimethylsulfoxide only (12).

Haylor et al. reported that 10 mg/kg atorvastatin, administered after clamping the renal hilus but prior to kidney reperfusion, significantly reduced tubular apoptosis, necrosis, and interstitial apoptosis, as well as caspase-3 activity, but did not change serum creatinine and cholesterol levels (13).

Tucci Jr et al. investigated the effects of lovastatin (15 mg/kg per day) on renal ischemia, followed by reperfusion on 31 rats, and reported significantly lower mortality rates as well as serum urea and creatinine levels in the lovastatin group compared to the control cases without any treatment (14).

Very recently, Wu et al. tested the hypothesis that postischemic acute renal failure could be ameliorated with atorvastatin in 24 rats, and reported that atorvastatin treatment reduced the elevation of serum creatinine level by 18%. Moreover, concentrations of advanced oxidation protein products and malondialdehyde were reduced in the atorvastatin group (15). Interestingly, in that study, atorvastatin was injected 30 min before reperfusion, not after reperfusion, and at a dose of 10 mg/kg, which was much higher than the doses used in our study.

In recent years, several different antioxidant treatments have been suggested to diminish IRI in crush syndrome. Murata et al. evaluated the role of nitrite as a therapeutic agent for crush syndrome. They reported that nitrite administration restored nitric oxide bioavailability by enhancing nitrite levels of the muscle, resulting in a reduction of rhabdomyolysis markers such as potassium, lactate dehydrogenase, and creatine phosphokinase in rats (16).

Similarly, Murata et al. studied a rat model to define the therapeutic role of dexamethasone in the laboratory findings, clinical course, and outcome of crush syndrome. They reported that a single injection of high-dose dexamethasone immediately before reperfusion activated endothelial nitric oxide synthase (eNOS) and exhibited antiinflammatory effects by modulating proinflammatory and antiinflammatory mediators, resulting in a complete recovery of the rats from lethal crush syndrome (17).

Recently, Garbaisz et al. studied a mitochondria-specific drug, NIM-811 (N-methyl-4-isoleucine-cyclosporin), which can prevent IRI in a rat model. They reported that muscle mitochondrial viability proved to be significantly higher and renal function parameters significantly better in the treatment group (18).

To the best of our knowledge, this study is the first to determine the role of atorvastatin in the treatment of renal IRI in a crush syndrome and rhabdomyolysis model setting. One of the most common side effects of statins is the statin-associated myopathy that is reported in about 10%–15% of statin users in observational studies (19,20), and this adverse effect was determined to have a dose-response relationship (21). For that reason, a low dose of statin was administered to the rats in this study.

There are certain limitations to this study. Firstly, the number of rats used in the study was not high in order to protect rats from redundant injury. Secondly, the treatment period was brief. The rats were only treated from their tail veins for the first 6 h, which may not be sufficient to determine their direct effects. Lastly, we did not measure the oxidant–antioxidant status of the rats, nor did we evaluate their kidneys histopathologically, which may be important in showing the effects of treatment directly.

In conclusion, we investigated the potential therapeutic role of low-dose atorvastatin treatment, as an antioxidant and antiinflammatory agent, on renal functions of rats with crush syndrome and rhabdomyolysis. Although there was an improvement in the renal functions of rats

following atorvastatin administration, the difference was not significant when compared to other treatment groups. Larger studies with different atorvastatin doses are required to define the role of this drug in the treatment of renal IRI during crush syndrome.

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