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Effects of Vitamin D, Infliximab, and Leflunomide on Isolated Rat Striated Muscle with Electrical and Mechanical Stimuli in Statin-Induced Rhabdomyopathy

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ABSTRACT

Objective: The most common adverse effects of statins are muscle disorders. This study investigated the effects of vitamin D, infliximab, and leflunomide on statin-induced rhabdomyopathy by evaluating myofibrillar contractions under electrical and mechanical stimulation.

Materials and Methods: Twenty-four rats were induced rhabdomyopathy using atorvastatin (100 mg/kg/day for three weeks). The rats were then divided into four groups: Group 1 (n=6) received vitamin D (0.2 µg/kg), Group 2 (n=6) received infliximab (7 mg/kg), Group 3 (n=6) received leflunomide (10 mg/kg), and Group 4 (n=6) received saline as a control. *In vitro* organ bath experiments were performed using electrical and mechanical stimulations on the isolated extensor digitorum longus muscle to assess contraction responses. Blood and tissue samples were analyzed for creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), glutathione peroxidase (GPx), and malondialdehyde (MDA).

Results: Group 1 demonstrated significant improvements in contractile responses (Twitch: Group 1 vs. Group 2, p=0.004; Group 1 vs. Group 3, p=0.025; Group 1 vs. Group 4, p<0.001. At 20 Hz: Group 1 vs. Group 2, p=0.018; Group 1 vs. Group 4, p=0.001; Group 3 vs. Group 4, p=0.015. At 80 Hz: Group 1 vs. Group 2, p=0.008; Group 1 vs. Group 3, p=0.0016; Group 1 vs. Group 4, p<0.001. At 100 Hz: Group 1 vs. Group 2, p=0.006; Group 1 vs. Group 3, p=0.015; Group 1 vs. Group 4, p<0.001). At 40–60 Hz and 120–140 Hz, Groups 1, 2, and 3 showed higher responses than Group 4 (p<0.05). Impulse conduction velocity in Group 1 was higher than in Group 4 (p=0.019). Group 1 exhibited significant differences in renal GPx (Group 1 vs.

Group 3, p=0.023) and MDA (Group 1 vs. Group 2, p=0.001; Group 1 vs. Group 3, p=0.003). Additionally, Group 1 showed significant improvements in histopathologic evaluation compared to the control group (p<0.05).

Conclusion: Vitamin D enhanced impulse conduction velocity and contraction response of striated muscle following electrical and mechanical stimulation in rhabdomyopathy. Monitoring vitamin D levels in dyslipidemic patients may serve as a predictive marker for adverse effects of statins. Providing these patients with vitamin D supplementation may improve statin intolerance.

Keywords: Infliximab, statins, leflunomide, skeletal muscle, vitamin D.

INTRODUCTION

Statins are the drugs of choice for lowering and controlling serum cholesterol levels in cardiovascular disorders. In addition to their preventive effects on coronary artery disease, they also exhibit anti-inflammatory and pleiotropic effects.[1] The most common muscle disorders associated with statin use include myalgia, myopathy, myositis, and muscle injury.[2] Muscle toxicity can lead to rhabdomyolysis in 0.1% of patients.[3]

The exact mechanism by which statins induce rhabdomyopathy (RMP), muscle toxicity, or related muscle disorders remains unclear. Current genetic studies focus on CYP3A4, CYP3A5, CYP2D6, and the vitamin D receptor genes to explain the pharmacokinetic distribution and metabolism of statins and their relevance to statin-related muscle symptoms.[4,5]

Vitamin D, a fat-soluble molecule with a four-ring cholesterol structure, regulates gene transcription and protein synthesis in advanced stages by binding to its intracellular receptors.[6] Without adequate exposure to sunlight, vitamin D deficiency can result in rickets and osteomalacia.[7]

Disease-modifying antirheumatic drugs (DMARDs) refer to a group of medications frequently used to reduce or prevent pain, inflammation, and joint damage in rheumatological diseases. The most commonly used conventional DMARDs include methotrexate, sulfasalazine, hydroxychloroquine, and leflunomide.[8] Leflunomide, a pyrimidine synthesis inhibitor, modulates T-cell responses by shifting from T-helper type 1 (Th1) to Th2, resulting in a beneficial effect in inflammatory conditions associated with T cells.[9] Biological DMARDs, also known as targeted biological agents, are produced using recombinant DNA technology. These agents specifically target molecules on immune system cells, including tumor necrosis factor-alpha (TNF-α), which is initially synthesized as a transmembrane precursor protein by activated macrophages and T cells.[10] TNF inhibitors include etanercept, adalimumab, infliximab, certolizumab pegol, and golimumab. Infliximab binds to the variable regions of human constant immunoglobulin G-1 and mouse-derived anti-TNF-α. Although the tumor-lysis activity of TNF-α raises concerns about the potential risk of malignancy with its inhibition, infliximab has proven effective in treating autoimmune rheumatologic and enteric diseases.[11]

KEY MESSAGES

- The most common adverse effects of statins are muscle disorders.
- Vitamin D enhances impulse conduction velocity and contraction response in striated muscle following electrical and mechanical stimulation in rhabdomyopathy.
- Monitoring vitamin D levels in dyslipidemic patients may serve as a predictive marker for adverse effects of statins. Providing vitamin D supplementation to these patients may help improve statin tolerance.

In this study, we analyzed the effects of vitamin D, infliximab, and leflunomide on rat striated muscle myofibrillar contractions using electrical and mechanical stimulation.

MATERIALS AND METHODS

In this study, 24 adult male Wistar rats weighing 250–300 g were used. The rats were housed in hygienic cages for two weeks before the study to acclimate to laboratory conditions and were provided with ad libitum access to food and water. Once suitable conditions were achieved, the study commenced in May 2018. The room temperature was maintained at 22°C, humidity at 60±5%, and a routine 12-hour light/dark cycle was applied. All experiments were conducted in accordance with the National Institutes of Health Guidelines for the Care

and Use of Laboratory Animals. The experimental prothocols were approved by the Kahramanmaraş Sütçü İmam University Laboratory Animal Care Ethics Committee (File No: 2018/07/03, Approval Date: 10.04.2018).

Drugs

Atorvastatin was purchased from Sanovel (İstanbul, Türkiye) and administered via orogastric gavage once daily for 21 days at a dose of 100 mg/kg. Vitamin D was obtained from Koçak Farma (İstanbul, Türkiye) and administered via orogastric gavage once daily for 14 days at a dose of 0.2 µg/kg. Infliximab was obtained from Schering-Plow (Innishannon, County Cork, Ireland) and administered intraperitoneally in 1 mL of 0.9% saline at a dose of 7 mg/kg, given in two doses per day. Leflunomide was obtained from Abdi İbrahim (İstanbul, Türkiye) and administered via orogastric gavage in two doses per day at a concentration of 10 mg/kg.

Inducing Rhabdomyopathy in Rats

After the 21-day RMP induction model, the rats were divided into four groups, and treatment was initiated: Group 1 received vitamin D (0.2 µg/kg) for 14 days; Group 2 received two doses of infliximab (7 mg/kg) on a single day; Group 3 received two doses of leflunomide (10 mg/kg) on a single day; and Group 4 received saline for 14 days. Before the interventional procedure, the rats were anesthetized with ketamine hydrochloride (HCl) (80 mg/kg) and xylazine (10 mg/kg).

The following experimental procedures were performed:

1. *In vitro* isolated organ bath assays were conducted with electrical and mechanical stimulations on the extensor digitorum longus (EDL) muscle to evaluate striated muscle contraction responses.

2. Histopathological evaluation of the EDL muscle was performed to assess statin-induced RMP damage and the effects of treatments on the muscle.

3. Blood sample analysis was conducted to measure serum creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen (BUN).

4. Heart and kidney tissue analysis was performed to evaluate oxidation parameters, including tissue glutathione peroxidase (GPx) and malondialdehyde (MDA) levels.

In Vitro Isolated EDL Muscle Electrical and Mechanical Activity Contractions

In vitro isolated organ bath assays were conducted to measure the contractile properties of the EDL muscle.[12] Care was taken to prevent damage to the vessels and nerve structures in contact. Muscle tendons were attached vertically to the isolated organ bath using silk ligatures. Before starting the experimental prothocol, the left EDL muscle was moistened with Krebs solution, rested for 30 minutes, and perfused with 95% O2–5% CO_2. The EDL muscle was fixed at its proximal end to an isometric force transducer. The muscle length was adjusted to its optimal length (L0), which was determined before the experimental prothocols. To maintain the properties of living tissue in the experimental environment, the bath solution was kept at 37°C using a heater. In accordance with the experimental prothocol, the muscle was directly stimulated using 0.5 ms impulses and supramaximal amplitude stimuli via electrodes placed around the EDL. Force-frequency characteristics were calculated by sequentially stimulating the EDL muscle at the following frequencies: twitch, 10, 20, 40, 60, 80, 100, 120, and 140 Hz. Contractile force was expressed in grams (g). Mechanical activity parameters included maximum contraction tension, total contraction time, peak contraction time, half relaxation time, and maximum tetanic force.

Electromyography (EMG) parameters included conduction velocity, amplitude, electrical activity of the muscle, and compound muscle action potential. These parameters were recorded using a square pulse stimulus under supramaximal stimulation conditions. Contractile forces of the EDL muscle, recorded under isometric conditions, were analyzed using the BIOPAC MP36 Data Collection and Analysis Program.

Histopathologic Evaluations of the EDL Muscle

The right EDL muscle was preserved in 10% formalin for 24 hours and then embedded in paraffin. Tissue processing was performed using an Autotechnicon device (Leica ASP 300, Germany), and 4 µm-thick microsections were prepared using a Leica Microtome RM 2145 (Germany). Muscle samples were examined for RMP-associated changes using an Olympus BX53 polarization microscope (Germany). The severity of myofibrillar damage was semiquantitatively assessed based on nucleus location, focal necrosis, lymphocytic inflammatory cell infiltration, and congestion with extended erythrocytes, as previously described.[13]

Biochemical Assay in Serum and Tissue

Blood samples were collected via cardiac puncture. Serum levels of CK, LDH, AST, ALT, and BUN were measured spectrophotometrically using a Siemens ADVIA 1800 Chemistry Autoanalyzer (Germany).

Supernatants were centrifuged at 10,000 × g for 20 minutes to obtain the post-mitochondrial fraction. GPx activities were determined using cumene hydroperoxide as the substrate[14] and attributed to the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) by glutathione

Table 1. Electrical and mechanical activity contractions of extensor digitorum longus (EDL) muscle

Variable	Group 1 (vitamin D, n=6) Mean±SD	Group 2 (infliximab, n=6) Mean±SD	Group 3 (leflunomide, n=6) Mean±SD	Group 4 (saline, n=6) Mean±SD
Twitch (g)	4.18±0.42	2.78±0.85[a]	3.07±0.70[b]	2.19±0.93[c]
20 Hz (g)	15.98±4.29	10.50±3.51[d]	13.78±3.56[f]	8.09±2.33[e]
40 Hz (g)	20.15±4.22	18.30±7.18[h]	16.06±5.42[i]	8.40±4.79[g]
60 Hz (g)	21.41±2.92	16.84±7.42[m]	15.27±6.61[n]	6.42±2.61[k]
80 Hz (g)	17.96±2.02	10.27±5.70[p]	11.09±4.99[r]	7.12±3.84[s]
100 Hz (g)	14.09±3.89	6.76±3.94[t]	7.77±4.89[u]	3.51±2.71[v]
120 Hz (g)	11.43±5.14	8.26±4.44[z]	9.24±2.91[w]	1.96±0.80[y]
140 Hz (g)	10.87±4.06	8.20±4.00[aa]	7.50±3.13[bb]	1.84±0.98[x]
Conduction velocity (m/sec)	11.77±3.14	7.95±5.73	8.42±3.04	3.42±2.80[cc]

Hz: Hertz; g: Grams; m: Meters; sec: Seconds; EDL: Extensor digitorum longus; SD: Standard deviation. Twitch: aGroup 1 vs. Group 2, p=0.004; bGroup 1 vs. Group 3, p=0.025; cGroup 1 vs. Group 4, p<0.001. 20 Hz impulse: dGroup 1 vs. group 2, p=0.018; eGroup 1 vs. Group 4, p=0.001; fGroup 3 vs. Group 4, p=0.015. 40 Hz impulse: gGroup 1 vs. Group 4, p=0.001; hGroup 2 vs. Group 4, p=0.007; iGroup 3 vs. Group 4, p=0.031. 60 Hz impulse: kGroup 1 vs. Group 4, p<0.001; mGroup 2 vs. Group 4, p=0.003; nGroup 3 vs. Group 4, p=0.011. 80 Hz impulse: pGroup 1 vs. Group 2, p=0.008; rGroup 1 vs. Group 3, p=0.0016; sGroup 1 vs. Group 4, p<0.001. 100 Hz impulse: tGroup 1 vs. Group 2, p=0.006; uGroup 1 vs. Group 3, p=0.015; vGroup 1 vs. Group 4, p<0.001. 120 Hz impulse: yGroup 1 vs. Group 4, p<0.001; zGroup 2 vs. Group 4, p=0.012; wGroup 3 vs. Group 4, p=0.005. 140 Hz impulse: xGroup 1 vs. Group 4, p<0.001; aaGroup 2 vs. Group 4, p=0.005; bbGroup 3 vs. Group 4, p=0.010. Conduction velocity: ccGroup 1 vs. Group 4, p=0.019.

Table 2. Oxidant/antioxidant markers in cardiac and renal tissues; muscle injury parameters in serum

Variable	Group 1 (vitamin D, n=6) Mean±SD/Median (Quartile 1–3)	Group 2 (infliximab, n=6) Mean±SD/Median (Quartile 1–3)	Group 3 (leflunomide, n=6) Mean±SD/Median (Quartile 1–3)	Group 4 (saline, n=6) Mean±SD/Median (Quartile 1–3)
Renal GPx (nmol/g)	319.36±64.99	239.88±29.87	249.24±63.31	272.19±23.30
Renal MDA (nmol/g)	30.87±7.49	57.94±16.09	55.22±16.30	44.75±4.57
Cardiac GPx (nmol/g)	383.5 (310–558)	301.5 (234–364)	393.96 (320–420)	378.28 (349–421)
Cardiac MDA (nmol/g)	40.88±10.56	50.66±13.41	42.85±13.64	45.49±14.37
AST (U/L)	117 (111–132)	137 (105–211)	99 (65–429)	393 (314–416)
ALT (U/L)	58 (50–65)	51 (49–62)	53 (50–80)	171.5 (141–225)
LDH (U/L)	639.33±493.30	863.00±439.36	478.67±235.17	624.75±146.99
BUN (mg/dL)	25.67±2.31	23.83±1.17	22.00±2.00	26.75±3.86
CK (U/L)	689.66±291.37	1222.00±561.51	1467.66±1428.80	1415.25±704.76

GPx: Glutathione peroxidase; MDA: Malondialdehyde; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: Lactate dehydrogenase; BUN: Blood urea nitrogen; CK: Creatine kinase; SD: Standard deviation. Renal tissue GPx (Group 1 vs. Group 3, p=0.023); renal tissue MDA level (Group 1 vs. Group 2, p=0.001; Group 1 vs. Group 3, p=0.003); cardiac GPx and MDA levels among treatment groups (p>0.05); CK, LDH, AST, ALT, and BUN among treatment groups (p>0.05).

reductase. The oxidation of NADPH was monitored spectrophotometrically at 340 nm at 37°C. The results were calculated using the extinction coefficient (6.22 × 103/M cm). Lipid peroxidation levels in heart and kidney tissues were determined using the thiobarbituric acid test.[15] GPx and MDA levels were expressed as nmol/g tissue.

Data Analysis

The data obtained in this study were presented as arithmetic mean and standard deviation or median (quartile 1–3). The Kolmogorov-Smirnov test was used to determine the normal distribution of the samples and the homogeneity of variances. The post-hoc Tukey test and variance analysis test were used to

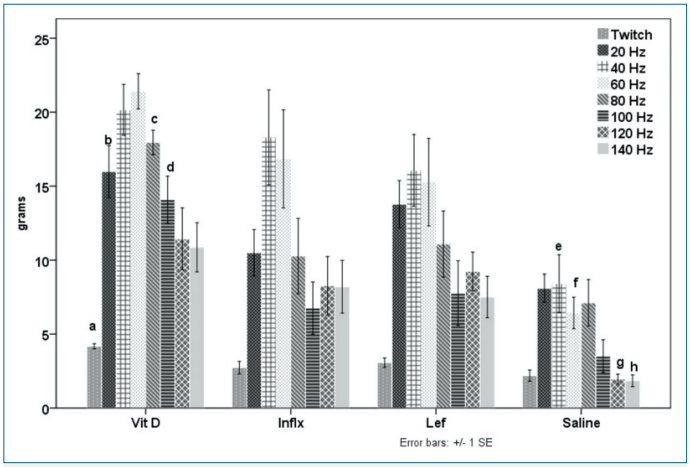

Figure 1. Contractile response following electrical and mechanical stimulation of the extensor digitorum longus (EDL) muscle in Group 1 was significantly higher than in the other groups.

a) Twitch: Group 1 vs. Group 2, p=0.004; Group 1 vs. Group 3, p=0.025; Group 1 vs. Group 4, p<0.001; b) 20 Hz impulse: Group 1 vs. Group 2, p=0.018; Group 1 vs. Group 4, p=0.001; Group 3 vs. Group 4, p=0.015; c) 80 Hz impulse: Group 1 vs. group 2, p=0.008; Group 1 vs. Group 3, p=0.0016; Group 1 vs. Group 4, p<0.001; d) 100 Hz impulse: Group 1 vs. Group 2, p=0.006; Group 1 vs. Group 3, p=0.015; Group 1 vs. group 4, p<0.001. At 40-60 Hz and 120-140 Hz impulses, Groups 1, 2, and 3 exhibited significantly stronger responses than Group 4: e) 40 Hz impulse: Group 1 vs. Group 4, p=0.001; Group 2 vs. Group 4, p=0.007; Group 3 vs. group 4, p=0.031; f) 60 Hz impulse: Group 1 vs. Group 4, p<0.001; Group 2 vs. Group 4, p=0.003; Group 3 vs. Group 4, p=0.011; g) 120 Hz impulse: Group 1 vs. Group 4, p<0.001; Group 2 vs. Group 4, p=0.012; Group 3 vs. group 4, p=0.005; h) 140 Hz impulse: Group 1 vs. Group 4, p<0.001; Group 2 vs. Group 4, p=0.005; Group 3 vs. Group 4, p=0.010.

determine significant differences in normally distributed data across multiple groups. For non-normally distributed data, the Kruskal-Wallis test with post-hoc Dunn test was applied. Categorical variables were analyzed using the Chi-square test or Fisher's exact test, as appropriate. Results with p<0.05 were considered statistically significant. Data were evaluated at a 95% confidence interval, and all analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, Illinois).

RESULTS

We found that the contractile response following electrical and mechanical stimulation of the EDL muscle in Group 1 was significantly higher than in the other groups (Twitch response: Group 1 vs. Group 2, p=0.004; Group 1 vs. Group 3, p=0.025; Group 1 vs. Group 4, p<0.001. 20 Hz impulse: Group 1 vs. Group 2, p=0.018; Group 1 vs. Group 4, p=0.001; Group 3 vs. Group 4, p=0.015. 80 Hz impulse: Group 1 vs. Group 2, p=0.008; Group 1 vs. Group 3, p=0.0016; Group 1 vs. Group 4, p<0.001. 100 Hz impulse: Group 1 vs. Group 2, p=0.006; Group 1 vs. Group 3, p=0.015; Group 1 vs. Group 4, p<0.001).

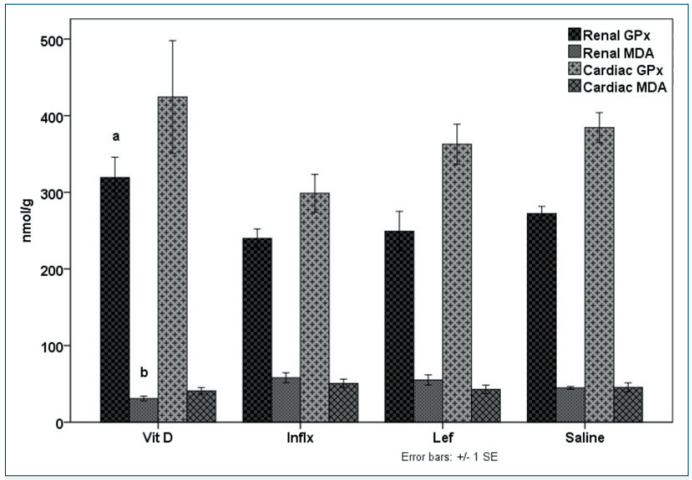

Figure 2. Group 1 showed significantly better outcomes in renal tissue glutathione peroxidase (GPx) level (a) Group 1 vs. Group 3, p=0.023) and renal tissue malondialdehyde (MDA) level (b) Group 1 vs. Group 2, p=0.001; Group 1 vs. Group 3, p=0.003).

As shown in Table 1 and Figure 1, we demonstrated that at 40–60 Hz and 120–140 Hz impulses, Groups 1, 2, and 3 exhibited significantly stronger responses than Group 4 (40 Hz impulse: Group 1 vs. Group 4, p=0.001; Group 2 vs. Group 4, p=0.007; Group 3 vs. group 4, p=0.031. 60 Hz impulse: Group 1 vs. Group 4, p<0.001; Group 2 vs. Group 4, p=0.003; Group 3 vs. Group 4, p=0.011. 120 Hz impulse: Group 1 vs. Group 4, p<0.001; Group 2 vs. Group 4, p=0.012; Group 3 vs. Group 4, p=0.005. 140 Hz impulse: Group 1 vs. group 4, p<0.001; Group 2 vs. Group 4, p=0.005; Group 3 vs. Group 4, p=0.010). Additionally, we found that conduction velocity following electrical and mechanical stimulation of the EDL muscle in Group 1 was significantly higher than in Group 4 (p=0.019).

Serum levels of CK, LDH, AST, ALT, and BUN did not differ among the treatment groups (p>0.05) (Table 2). As shown in Figure 2, Group 1 had significantly higher renal tissue GPx levels (Group 1 vs. Group 3, p=0.023) and lower renal tissue MDA levels (Group 1 vs. Group 2, p=0.001; Group 1 vs. Group 3, p=0.003) compared to Groups 2 and 3. However, cardiac GPx and MDA levels did not differ significantly among the groups.

We observed a significant absence of focal necrosis in Group 1 compared to the control group (p=0.021). However, there were no significant differences in the absence of necrosis in Groups 2 and 3 compared to the control (p=1 and p=0.505, respectively). Additionally, we found a significant presence of mononuclear leukocytes in Group 1 compared to the control group (p=0.015), whereas Groups 2 and 3 did not show significant differences for this parameter (p=0.242 and

p=0.079, respectively). Although congestion was absent in Group 1, this result was not significantly different from the other groups (p=0.558). Similarly, the absence of congestion in Groups 2 and 3 did not show any significant differences when compared to the other groups (p=0.505 and p=1, respectively).

DISCUSSION

In this study, we established a RMP model by administering atorvastatin to twenty-four rats. After 21 days, the rats were treated with vitamin D, infliximab, and leflunomide, respectively. *In vitro* organ bath assays were performed using electrical and mechanical stimulations on isolated EDL muscle to evaluate striated muscle contraction responses. Additionally, histopathological evaluation of the EDL muscle was conducted to assess statin-induced RMP damage and the effects of treatments. Blood and tissue samples were analyzed for biochemical markers of muscle damage and oxidation parameters in distant tissues. We confirmed that atorvastatin induced RMP by the end of the 21st day. Following treatment, we observed significantly better functional, biochemical, and histological outcomes in rats receiving vitamin D compared to the other groups.

The extent of muscle damage may vary depending on the type and dose of statins. It has been reported to be significantly higher for lovastatin, simvastatin, and atorvastatin, particularly as they are substrates of CYP3A4. For this reason, we selected atorvastatin for this study. The drug was prepared at a concentration of 100 mg/kg/day, dissolved in tap water, and administered to the rats via orogastric gavage. We induced the RMP model by administering atorvastatin for 21 days, as previous experimental studies have shown that lower doses (10, 30, and 50 mg/kg) of atorvastatin result only in myopathy symptoms. However, a dose of 100 mg/kg atorvastatin for 21 days has been shown to be sufficient to induce a progressive RMP model in rats,[16] which we also verified through histopathological assays.

In striated muscle, statin-induced myopathy is caused by decreased adenosine triphosphate (ATP) production due to mitochondrial damage, reactive oxygen radicals, cytochrome c release, and calcium influx. Lipid peroxidation in cellular membranes leads to the formation of oxidative epitopes, which act as downstream mediators of oxidative stress in tissues.[17] To assess the potential distant organ effects, we measured GPx and MDA levels in the cardiac and renal tissues. We found that the vitamin D group exhibited significantly better outcomes in renal oxidative parameters compared to the groups receiving infliximab or leflunomide. Increased oxidative stress leads to peroxidation of membrane lipids, which is associated with oxidation-specific epitopes. These epitopes, along with immune responses, can trigger inflammatory reactions, including atherosclerosis. In atherosclerosis, oxidized low-density lipoproteins expose oxidation-favoring epitopes. Previous studies have identified MDA epitopes as major targets of immune responses, as they have been found in damaged tissues.[17] GPx, investigated in the current study, is an antioxidant and scavenger that modulates ferroptosis as an upstream regulator and removes lipid peroxides from cells. Prior research has suggested that GPx functions as a sensor for oxidative stress and cell death. A decrease in GPx levels leads to an increase in oxidative species.[18–20]

In rats, sarcolemmal chloride conductance and muscle chloride channel CIC-1 expression were reported to be reduced during statin-induced skeletal muscle damage. Statins also phosphorylate the CIC-1 channel leading to an increase in protein kinase C, which closes the channel. This process elevated intracellular calcium levels and reduced lactate excretion at rest.[21] These changes result in muscle pain, cramps, myalgia, and elevated serum CK levels. For this reason, we investigated serum CK, LDH, AST, ALT, and BUN levels in this study. CK catalyzes the reversible phosphorylation between creatine and phosphocreatine, balancing ATP and ADP. It is also considered a therapeutic signal for various diseases.[22] We found that serum CK, LDH, AST, ALT, and BUN levels did not differ among the treatment groups. Although the biochemical analysis results showed no significant differences among groups, serum CK, LDH, AST, ALT, and BUN levels tended to be lower in the vitamin D group compared to the other groups. Previous studies have reported cases of rhabdomyolysis with elevated serum transaminases and CK levels following leflunomide administration. However, muscular side effects due to this agent have been reported to occur in only 2–5% of the population.[23,24]

Leflunomide, a DMARD that inhibits the mitochondrial enzyme dihydroorotate dehydrogenase, exhibits therapeutic effects in the treatment of rheumatoid arthritis.[25] It works by inhibiting leukocyte adhesion to vascular endothelial cells, interfering with dendritic cell function, impairing antigen presentation, and blocking the differentiation of osteoclasts mediated by nuclear factor kappa B (NF-κB).[9] Since leflunomide has a half-life of 15 days, we administered the drug orally for one day in two equal doses. One of its side effects is the potential to trigger polymyositis.[26] In our study, although histopathological findings were not statistically significant, necrosis, mononuclear infiltration, and congestion were increased in the leflunomide group. This finding suggests that leflunomide should not be used in cases of pre-existing muscle damage.

TNF-α is an important inflammatory mediator involved in tissue damage during immune responses. TNF-α directly induces

protein degradation and atrophy of myofibrils in muscle cells. This degradation process is linked to the light chain enhancer of the NF-κB protein complex. The NF-κB signaling pathway is activated by proinflammatory cytokines and macrophages, which subsequently lead to cell proliferation. Following NF-κB activation, genes favoring immunomodulation are also triggered, ultimately promoting cell survival or proliferation.[27] Previous experimental studies have demonstrated that polyarthritis formation is associated with an increase in TNF-α expression.[28,29] Consequently, TNF-α inhibitors are commonly used to suppress the inflammatory response of TNF-α. A study reported that infliximab treatment enhances muscle strength by the 14th day following muscle injury and reduces apoptosis and leukocyte infiltration in histopathological assessments.[30] However, there are also clinical reports of patients diagnosed with myositis while undergoing TNF inhibitor therapy.[31,32] In our study, we did not observe significant histological muscle recovery in the group receiving infliximab treatment. Since infliximab has a half-life of 7–12 days, we administered the drug intraperitoneally for one day in two equal doses. The lack of significant histopathological muscle recovery in the infliximab-treated group may be attributed to the short duration of our treatment prothocol. Previous studies utilized a 14-day treatment regimen. Histopathological changes may require more time to become evident, but we observed positive improvements in muscle contraction responses compared to the saline control group. Our results showed that vitamin D, infliximab, leflunomide treatments at 40–60 Hz and 120–140 Hz impulses produced significantly stronger responses than the saline control group. Overall, the contractile response following electrical and mechanical stimulation of the EDL muscle was significantly higher in the vitamin D group compared to the other groups.

There is a well-established link between low serum vitamin D levels (<10–20 ng/mL) and muscle weakness in humans.[33] Recent studies have demonstrated that statin-induced myopathy can be reversibly treated with vitamin D supplementation.[34,35] However, the optimal vitamin D concentration required to improve muscle function has not yet been determined in randomized studies.[36] Similarly, in the vitamin D group, we observed significantly positive histopathological results, including the absence of necrosis and the presence of mononuclear leukocytes. Myofibrillar damage was significantly reduced in muscle histopathology. Our findings align with previous studies linking low vitamin D levels to statin-induced muscle damage.[37,38] In skeletal muscle, cellular proteolysis occurs through the ubiquitin-proteasome pathway. The conjugation of ubiquitin serves as a mechanism that flags proteins for degradation, thereby promoting muscle atrophy.[39]

CONCLUSION

There is a multifactorial relationship between vitamin D and RMP, involving various molecular processes and distant tissue interactions. Our study demonstrated that vitamin D enhanced impulse conduction velocity and contraction response of striated muscle following electrical and mechanical stimulation in rhabdomyopathy. Monitoring vitamin D levels in dyslipidemic patients may serve as a predictive marker for adverse effects of statins. Providing these patients with vitamin D supplementation may help improve statin tolerance.

Ethics Committee Approval: The Kahramanmaraş Sütçü İmam University Clinical Research Ethics Committee granted approval for this study (date: 10.04.2018, number: 2018/07-03).

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