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ORIGINAL ARTICLE

Concurrent vitamin D supplementation and exercise training improve cardiac fibrosis via TGF-β/Smad signaling in myocardial infarction model of rats

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Abstract

Although the role of vitamin D in various types of disorders such as cancer and diabetes has been well recognized, its relation to cardiovascular diseases still remains equivocal. The present study aims to investigate the interactive effects of aerobic-resistance training (ART) and vitamin D_3 (VD_3) on both cardiac fibrosis and heart functions considering TGF-β1/Smad2, 3 (transforming growth factor-β1/mothers against decapentaplegic homolog 2/3) signaling in the myocardial infarction (MI) model of rats. Fifty-six male Wistar rats were divided into 2 groups of sham ($n = 8$), and MI ($n = 48$). Then, MI rats were divided into six groups of VD_3, ART, VD_3+ART, Veh, Veh+ART, and sedentary MI. The animals received the related treatments for 8 weeks, and then their functional exercise capacity (FEC) and strength gain (SG) were estimated through exercise tests. Ejection fraction (EF%) and fractional shortening (FS%) and serum level of VD_3 were measured by echocardiography and ELISA, respectively. Cardiac expressions of TGF-β1, Smad2/3, and collagen I/III were assessed by western blotting and fibrosis by Masson's trichrome staining. The highest EF, parallel with the lowest expression of cardiac TGF-β1, Smad2/3, collagen I, and collagen III were observed in MI + VD_3 ($P = 0.021$), MI + ART ($P = 0.001$), and MI + VD_3 + ART ($P < 0.001$). Furthermore, similar to FS, the highest FEC and SG were related to the groups of MI + VD_3 + ART and MI + ART compared to the MI group. In conclusion, our data indicate that concurrent vitamin D supplementation and ART, compared with monotherapy, successfully improve cardiac function and alleviate myocardial fibrosis via downregulating TGF-β1, Smad2/3 signaling, and also regulating collagen I and III expressions.

Keywords Cardiac fibrosis · Exercise training · TGF-β1 · Smad2/3 · Vitamin D_3

Key points
- Concurrent aerobic-resistance training and VD_3 supplementation are more efficient than exercise training alone in alleviating cardiac fibrosis
- Concurrent aerobic-resistance training and VD_3 supplementation reduce cardiac fibrosis through downregulating TGF-β/Smad signaling
- Concurrent aerobic-resistance training and VD_3 supplementation are better than monotherapy in improving EF and FS

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Introduction

Cardiac fibrosis is a clinical manifestation of several cardiovascular diseases and is characterized by an increase in collagen and expression of several factors, particularly transforming growth factor-β (TGF-β) [5].

TGF-β belongs to the large family of growth factors and is markedly expressed in the myocardia that is following a heart failure [26], and it also activates fibroblasts to induce collagen deposition via its canonical pathway [19]. This pathway involves the phosphorylation of Smad2/3 (mothers against decapentaplegic homolog 2/3) and Smad4, which together promote the expression of genes associating with fibrosis [19]. Consequently, sustained expression of TGF-β results in myocardial stiffness, diastolic dysfunction, and heart failure [13].

Finding a suitable strategy to combat MI progression toward heart failure has great importance for both health care organizations and the public. There is growing evidence that

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exercise training is capable of reducing mortality after MI [10, 36]. For example, doing exercises improves ejection fraction (EF), fractional shortening (FS) [10], and also reduces the progression of fibrosis following MI [22, 36]. However, the questions regarding the type of exercise training programs that are more suitable and efficient for MI and also the mechanisms by which exercise training might exert post-MI cardiac rehabilitation have remained unanswered yet [14].

On the other hand, there are contradictory results regarding the effects of exercise training programs and cardiac rehabilitation in the related literature. Here, we assumed that vitamin D_3 (VD_3) insufficiency might neutralize the beneficial effects of exercise training programs [12]. Epidemiological studies have shown a relationship between low plasma levels of VD_3 and a predisposition to cardiovascular events [32, 35], while the administration of an active form of VD_3 prevents the progression of cardiac hypertrophy and heart failure [3]. Considering the anti-inflammatory and anti-oxidative effects of VD_3 [26], we hypothesized that 8 weeks of VD_3 supplementation together with a special protocol of combined aerobic-resistance training (ART) may be a useful strategy to improve heart function and prevent the progression of fibrosis.

Therefore, the present study was designed to evaluate the effects of concurrent aerobic-resistance training and VD_3 supplementation on myocardial fibrosis and cardiac function considering the TGF-β-Smad signaling pathway in animal model of myocardial infarction.

Materials and methods

Animals and treatment protocol

Fifty-six male Wistar rats (230–250 g) were used in this study. All animals were kept in a standard animal caring room with a 12:12-h light-dark cycle (lights on 7:00 h) and a temperature of 22 + 2 °C. The rats were given free access to standard rat food including crude protein (23%), crude fat (3.5%) crude fiber (4–5%), ash (8%), calcium (0.95–1%), phosphorus (0.65%), and moisture (10%). All studies and experimental protocols were approved by the Ethics committee of Guilan University of medical sciences (IR.GUMS.REC.1398.046) adhered to the EU animal experiments ethical guidelines (2010/63/EU). The general characteristics of the experimental groups are shown in Table 1.

Myocardial infarction

Myocardial infarction was induced by subcutaneous injection of isoproterenol (ISO) (Sigma-Aldrich, 150 mg/kg) on two consecutive days [21]. Three weeks after the second injection of ISO [6], animals underwent echocardiography under anesthesia with intraperitoneal (IP) injection of ketamine 75 mg/kg and xylazine 10 mg/kg, using rodent echocardiography equipment in the Faculty of Veterinary Medicine, University of Tehran, and the ejection fraction cutoff was considered less than 65%. The experimental design is shown in Fig. 1.

Experimental groups

The rats demonstrating heart failure 3 weeks after the injection of ISO were divided into seven groups of myocardial infarction (MI), sham (normal saline), MI + VD_3 (vitamin D_3), MI + Veh (sesame oil), MI + ART (aerobic-resistance training), MI + VD_3 + ART, and MI + Veh + ART ($n = 8$ for each group).

VD_3 supplementation

Vitamin D_3 was purchased from Caspian Tamin Pharmaceutical Company and injected subcutaneously at a dose of 10,000 (IU/kg), once a week for 8 weeks [2]. VD_3 was aliquoted in sesame oil, based on the manufacturer brochure (Barij Essence Pharmaceutical Company).

Measurement of functional exercise capacity and strength gain

Functional exercise capacity (FEC) and strength gain (SG) were measured by placing the rats on a seven-lane treadmill (Sports Sciences Research Center company, Tehran, Iran) and also a rodent ladder with 54 vertical steps, three and 8 weeks after ISO injection.

At the beginning of the training programs, animals were adapted to the situation by running on a treadmill at 5 m/min with no incline and also by climbing the entire length of the ladder without any load.

To measure the FEC, the rats performed running on a graded treadmill for 3 min, while the speed and the incline degrees were increasing. Exhaustion was defined as a state in which a rat could not tolerate a situation containing either running for more than 50% of the expected time (90 s) or staying on the shock grid for 10 consecutive seconds [9].

Also, to measure the SG, the maximal load test (MLT) was performed. For this purpose, the rat was forced to carry a load equal to 75% of its body weight. Progressively, the load was increased to an amount which was 15% more than its previous weight on the subsequent climbs until the animal failed to climb to the next step of the ladder after three attempts [7].

Exercise protocol

The exercise training was performed at 2–4 p.m. First, a pilot study was conducted on six rats to estimate MLT and FEC.

Then, animals belonging to the groups of MI + ART (aerobic-resistance training), MI + VD_3 + ART, and MI + Veh + ART, performed aerobic exercise training protocol of 3 days/week. The training session included running at the speed of 10 m/min and 5° incline for 10 min, then it was gradually increased to the speed of 16 m/min for a total duration of 50 min (workload equal to 30 to 70% of the maximum speed obtained from EFC), and remained unchanged till the end of the experiment [4].

Resistance training consisted of climbing on a rodent ladder while carrying 40–60% of the initial maximal load test (MLT), for 2 days/week. The exercise protocol utilized in our study consisted of 15–20 min of climbing, followed by carrying loads with different intensity plus 1-min intervals [7].

Echocardiography

Before (3 weeks after the second injection of ISO) and also after the end of treatments, electrocardiography(ECG) and echocardiography were performed on anesthetized rats using an echocardiographic system equipped with a 6–12 MHz linear transducer (GE Voluson 730 Pro, Kretztechnik Company Australia). M-mode images were obtained at the level of the papillary muscles. Left ventricular dimensions during systole and diastole, left ventricular end-systolic dimension (LVESD, cm), and left ventricular end-diastolic dimension (LVEDD, cm) were measured according to the guidelines by the American Society of Echocardiography [18]. The following formulas were used to obtain the ejection fraction (EF) and the fractional shortening (FS) of the left ventricle [33]:

$$\mathrm{EF}\ (\%) = [(\mathrm{LVDd3} - \mathrm{LVDs3})/\mathrm{LVDd3}] \times 100$$

$$\mathrm{FS}\ (\%) = (\mathrm{LVDd} - \mathrm{LVDs})/\mathrm{LVDd}\Big] \times 100$$

Biochemical and histological assessments

Blood samples were drawn under deep anesthesia with ketamine 75 mg/kg and xylazine 10 mg/kg from vena cava and then were centrifuged. Serums were stored at − 20 °C for further biochemical assays. Serum 25OHD level was measured using ELISA kit (Monobind, USA) by ELISA Reader (USA-AWARENESS STAT FAX 4200) according to the kit instructions.

Furthermore, immediately after the blood sampling, the hearts were removed from the chests and were cut into three transverse parallel segments. One segment was transferred to a 10% formalin solution for Masson's trichrome staining to evaluate the extent of myocardial fibrosis [33]. To reach this purpose, paraffin blocks prepared from the heart tissues were sliced with a thickness of 5 μm. Masson's trichrome staining was conducted using Image J software, version 1.48, (US National Institutes of Health, Bethesda, MD, USA). The amount of fibrotic area was compared with the control group.

Western blotting

The left ventricle stored at liquid nitrogen was homogenized, and total proteins were extracted using RIPA lysis buffer, and then separated by SDS page. The membrane was incubated with the following diluted primary antibodies against TGF-β1, COL1A1, smad2/3, and COL3A1 (Santa Cruz Biotechnology, INC, USA). Then, the membrane was washed and incubated with an anti-rabbit secondary antibody (Santa Cruz Biotechnology, INC, USA). β-Actin was also used as an internal control. The protein bands were subsequently detected with enhanced chemiluminescence and sections were exposed to an X-ray film. Finally, western blot bands were analyzed with Image J software, version 1.48, (US National Institutes of Health, Bethesda, MD, USA) [36].

Statistical analysis

The normality of data were estimated by Shapiro-Wilk, then one-way ANOVA with Tukey's post hoc test and ANCOVA with Bonferroni post hoc test were used to compare between-group differences. The level of significance was $P < 0.05$ in all statistical evaluations. Data were analyzed in SPSS version 16 and expressed as mean ± SD.

Results

Descriptive characteristics

The characteristics of the animals are shown in Table 1. Despite no significant difference in the final body weight, heart weight and left ventricle weight to body weight ratio were significantly changed (Table 1). As shown in the Table 1, these ratios in the MI group were significantly higher than Sham ($P < 0.01$), while in MI + ART+VD_3, these ratios were less than the MI group ($P < 0.05$).

It should be noted that one rat from each group of MI animals died (overall five rats) and we had to repeat the experiment.

Cardiac function and echocardiography

According to the echocardiography data, 8 weeks after the treatment, significant between-group differences were observed in the EF and FS ($P < 0.001$, Fig. 2a and b), in a way that EF was significantly less in the MI group compared with the sham ($P < 0.001$), while, in the groups of ART ($P = 0.001$), MI + VD_3 ($P = 0.021$), and MI + VD_3 + ART improved significantly ($P < 0.001$, Fig. 3a).

Table 1 Cardiac characteristics and VD_3 level after 8 weeks of the treatment protocol

Group	Sham	MI	MI + Veh	MI + VD_3	MI + VD_3+ ART	MI + Veh + ART	MI + ART	Significance (one-way ANOVA)
FBW (g)	337 ± 21	333 ± 19	334 ± 12	339 ± 16	341 ± 18	347 ± 17	344 ± 16	NS
HW (g)	1.20 ± 0.04	1.39 ± 0.07 ***	1.38 ± 0.07***	1.26 ± 0.06 $^{\$}$	1.24 ± 0.06 $^{\#\#}$	1.28 ± 0.7	1.27 ± 0.06 $^{\#}$	$P < 0.001$
LV (g)	0.84 ± 0.05	0.97 ± 0.03 ***	0.98 ± 0.05***	0.88 ± 0.05 $^{\$}$	0.88 ± 0.04 $^{\#\#}$	0.90 ± 0.05	0.89 ± 0.04 $^{\#}$	$P < 0.001$
HW/BW (g/g) $\times 10^{-3}$	3.59 ± 0.27	4.19 ± 0.46 **	4.17 ± 0.22*	3.73 ± 0.15	3.64 ± 0.28 $^{\#}$	3.72 ± 0.35	3.72 ± 0.33	$P < 0.01$
LV/BW (g/g) $\times 10^{-3}$	2.52 ± 0.26	2.93 ± 0.25 **	2.92 ± 0.17**	2.60 ± 0.14	2.58 ± 0.21$^{\#}$	2.60 ± 0.23	2.60 ± 0.20	P < 0.01
Serum VD_3 level (ng/ml)	33 ± 2.9	30 ± 3.2	31 ± 2.8	61 ± 4.3 †††	64 ± 2 †††	30 ± 3.1	32 ± 2.6	P < 0.001

Values are presented as mean ± SD. Data were analyzed by one-way ANOVA and post hoc tests by Tukey

FBW (final body weight), *HW* (heart weight), *LV* (left ventricular), *Sham* (normal saline injection), *MI* (myocardial infarction), *ART* (aerobic-resistance training), *Veh* (vehicle, sesame oil), *VD_3* (vitamin D_3 injection, 10,000 IU/kg/week)

* Significant increase vs sham group; $P < 0.05$

** Significant increase vs sham group; $P < 0.01$

*** Significant increase vs sham group; $P < 0.001$

$^{\#}$ Significant decrease vs MI group; $P < 0.05$

$^{\#\#}$ Significant decrease vs MI group; $P < 0.01$

$^{\$}$ (Significant decrease vs MI + veh; $P < 0.05$

††† Significant increase vs other groups

In addition, FS was significantly greater in MI + ART ($P = 0.005$), MI + VD_3 ($P = 0.037$), and MI + VD_3 + ART ($P < 0.001$) compared with the MI group (Fig. 3b). The greatest EF and FS amounts were related to the MI + VD_3 + ART in comparison with the other groups.

Functional exercise capacity and strength gain

Eight weeks after ISO injection, FEC and SG were significantly higher in the MI + VD_3 + ART, MI + ART ($P < 0.001$), and MI + VD_3 groups ($P < 0.05$) compared with MI, and MI + Veh groups. The exact P values are shown in Table 2.

Serum level of VD_3

The serum level of VD_3 did not show any significant difference between MI and sham groups ($P = 0.074$). While it was significantly higher in the groups receiving VD_3 (MI + VD_3 and MI + VD_3 + ART) compared with the other groups ($P < 0.001$) (Table 1).

Pathological assessment

The results of Masson's trichrome staining showed significant between-groups differences (Fig. 4a). Tukey post hoc tests displayed a significant reduction in ISO-induced cardiac

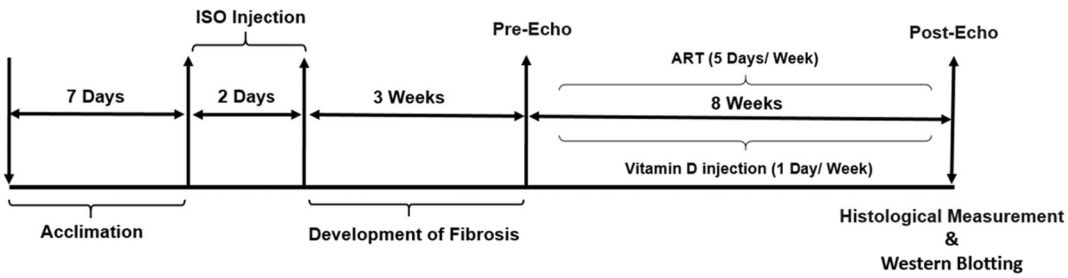

Fig. 1 Experiment design. One week after quarantine and acclimation to the new environment, MI groups ($n = 48$) received a subcutaneous injection of isoproterenol (ISO) (150 mg/kg in two consecutive days) and sham ($n = 8$) received the same volume of saline. Then, in the third week, echocardiography was performed to confirm MI. VD_3 (10,000 IU/kg/week) and sesame oil as the vehicle of VD_3 (at the same volume) was injected, and aerobic-resistance training was performed for 8 weeks (1 day was devoted to vitamin D injection, 5 days of ART, and 1 day of rest). Echocardiography was repeated at the end of the study and cardiac tissues were collected for histological and molecular analyses. ISO (isoproterenol), Echo (echocardiography), ART (aerobic-resistance training), VD_3 (vitamin D_3)

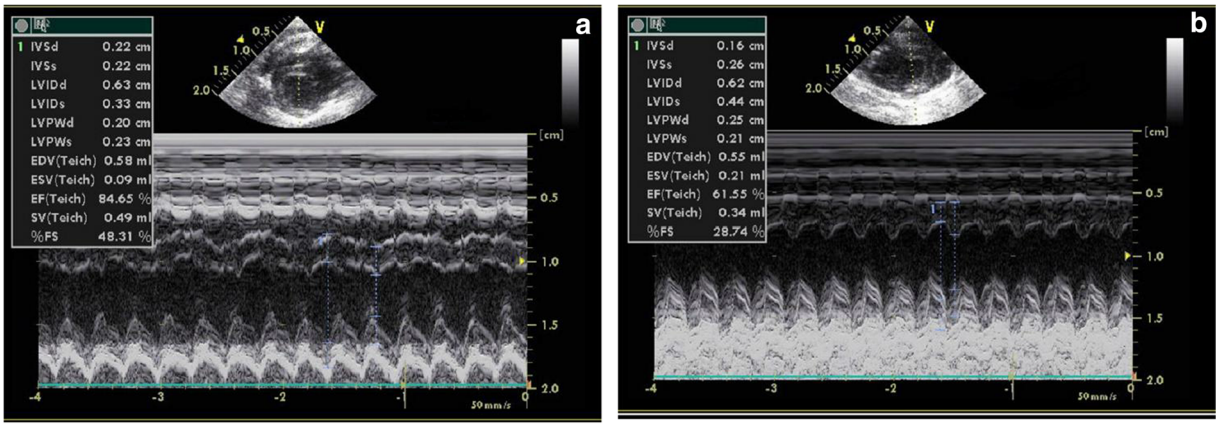

Fig. 2 Echocardiographic evaluation. **a** and **b** related to the sham and MI group, respectively. Ejection fraction cutoff was considered less than 65%

fibrotic area in the MI + VD_3 ($P = 0.010$), MI + ART ($P = 0.004$), and MI + VD_3 + ART groups ($P < 0.001$) compared with the MI group. No significant difference was found between MI + VD_3, MI + ART, and MI + VD_3 + ART groups (Fig. 4b).

Western blotting

One-way ANOWA and Tukey post hoc test revealed a significant change in the expression of TGF-β1 ($P < 0.001$), displaying that it was about 97% greater in the MI group compared with the sham. However, the lowest TGF-β1 expression was related to the MI + VD_3 + ART group (Fig. 5a). Also, Smad2/3 expression was approximately 199% greater in the MI group compared with the sham (Fig. 5b). Additionally, the expressions of collagen I and collagen III in the MI group were about 84% and 195% higher compared with the sham group (Fig. 5c and d). As mentioned, collagen I showed lower expression in the groups of MI + VD_3 + ART, MI + ART, and MI + VD_3 compared with the MI group (Fig. 5b, c, and d).

Discussion

The findings of this study showed that myocardial infarction leads to a significant increase in the expression of TGF-β1, Smad2/3, collagen I, and III together with an enhancement in cardiac fibrosis and cardiomyopathy. In addition, VD_3 supplementation together with ART successfully prevented the progression of cardiac fibrosis, improved ejection fraction, fractional shortening, and alleviated cardiac pathological

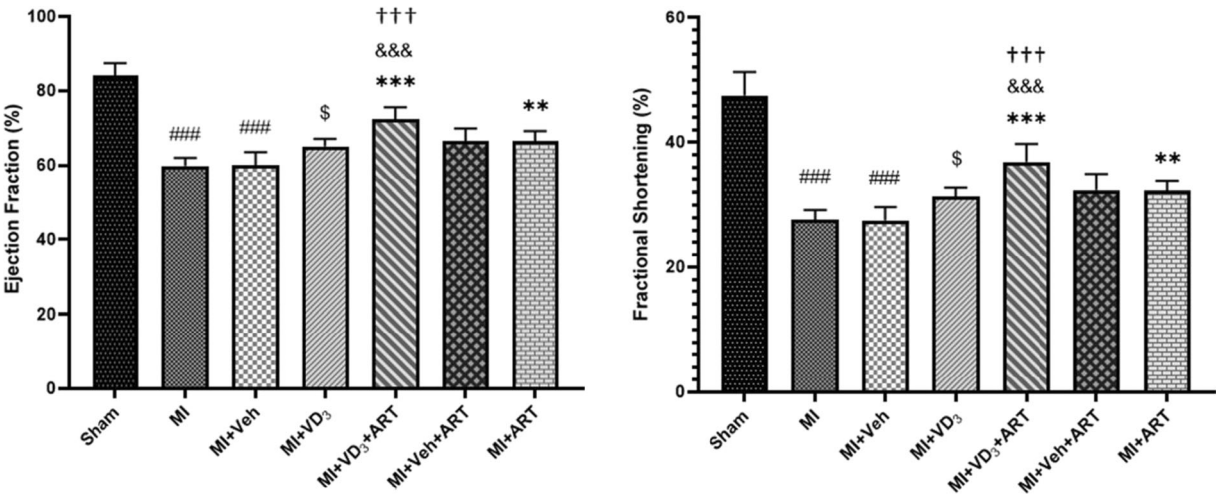

Fig. 3 Effects of aerobic-resistance training and vitamin D_3 injection on heart function. Ejection fraction (**a**) and fractional shortening (**b**) in different groups after 8 weeks of ART and VD_3 injection. Sham (normal saline injection), MI (myocardial infarction), ART (aerobic-resistance training), VD_3 (vitamin D_3 injection, 10,000 IU/kg/week), Veh (vehicle), TGF-β1 (transforming growth factor- beta1), Smad (suppressor of mothers against decapentaplegic). Values are presented as mean ± SD ($N = 8$) and analyzed by one-way ANOVA and post hoc tests by Tukey. ** (significant increase vs MI group; $P < 0.01$). *** (significant increase vs MI group; $P < 0.001$). ### (significant decrease vs sham group; $P < 0.001$). $ (significant increase vs MI + Veh group; $P < 0.05$). &&& (significant increase vs MI + VD3 group; $P < 0.001$). ††† (significant increase vs MI + Veh + ART group; $P < 0.001$)

Table 2 Change in functional exercise capacity (FEC) and strength gain (SG) 8 weeks after ISO

Group	Sham	MI	MI + Veh	MI + VD_3	MI + VD_3 + ART	MI + Veh + ART	MI + ART
FEC (min)	33.3 ± 2.8	16.6 ± 2.9 ###	16.8 ± 2.9 ###	24.7 ± 6.4 $	33.6 ± 6.3 ***, ††	28.6 ± 4.4 ***	29.2 ± 4.9 ***
SG (gram)	340 ± 17	263 ± 24 ###	260 ± 27 ###	302 ± 21 #, $	371 ± 23 ***, †††	341 ± 27 ***	339 ± 15 ***

Values are presented as mean ± SD and analyzed by ANCOVA and post hoc tests by Bonferroni. Significant between-group differences were observed. F (6, 48) = 17.51; $P < 0.001$ and F)6, 48) = 5.10; $P < 0.001$, respectively

FEC (functional exercise capacity), *SG* (strength gain), *Sham* (normal saline injection), *MI* (myocardial infarction), *ART* (aerobic-resistance training), *Veh* (vehicle, sesame oil), *VD_3* (vitamin D_3 injection, 10,000 IU/kg/week)

*** Significant increase vs MI group; $P < 0.001$

Significant decrease vs Sham group; $P < 0.05$

Significant decrease vs Sham group; $P < 0.001$

$ Significant increase vs MI + Veh group; $P < 0.05$

†† (Significant increase vs MI + VD_3 group; $P < 0.01$

††† Significant increase vs MI + VD_3 group; $P < 0.001$

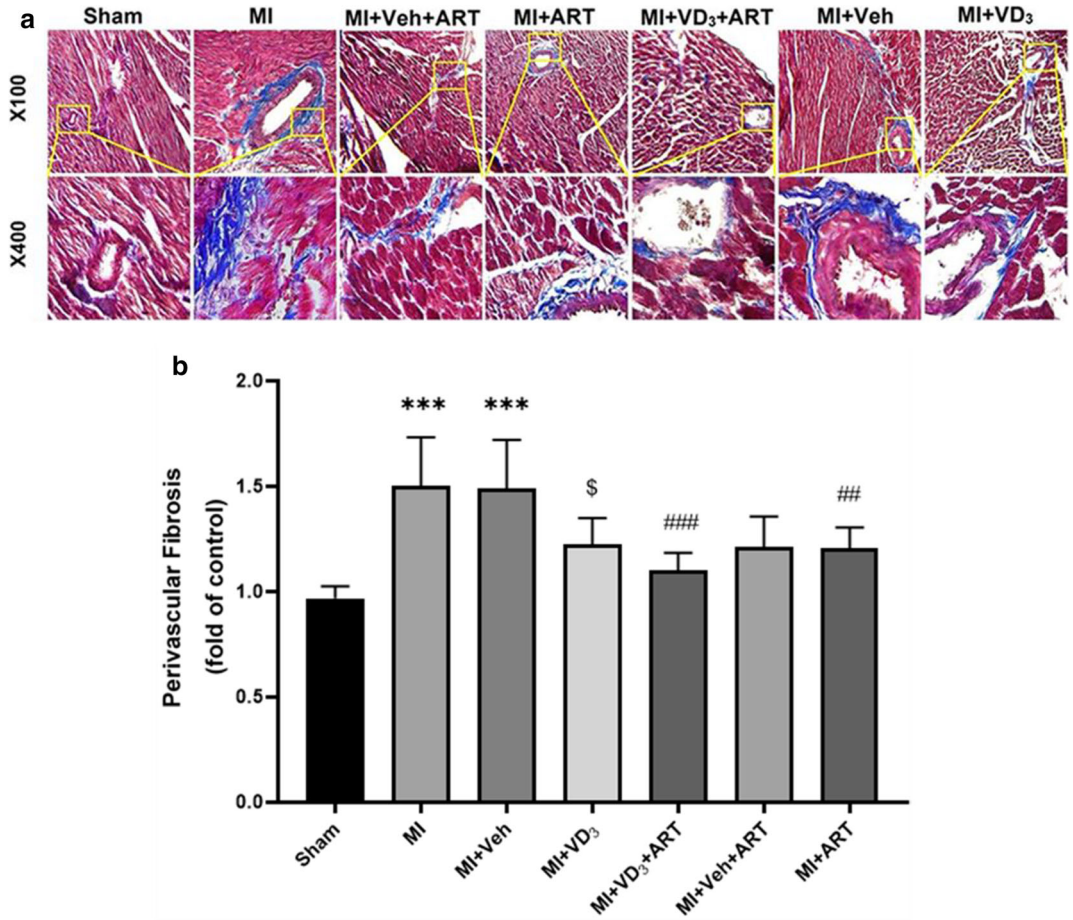

Fig. 4 Effects of ART and VD_3 on heart fibrosis after MI (magnified scales × 100, × 400, respectively). **a** Masson's trichrome staining. **b** The extent of stained perivascular fibrotic areas in different groups. Fibrotic areas were measured with ImageJ software, version 1.48, (US National Institutes of Health, Bethesda, MD, USA). Sham (normal saline injection), MI (myocardial infarction), ART (aerobic-resistance training), Veh (vehicle, sesame oil), VD_3 (vitamin D_3 injection, 10,000 IU/kg/week). Values are presented as mean ± SD ($N = 8$) and analyzed by one-way ANOVA and post hoc tests by Tukey. *** (significant increase vs sham group; $P < 0.001$). ## (significant decrease vs MI group; $P < 0.01$). ### (significant decrease vs MI group; $P < 0.001$). $ (significant decrease vs MI + Veh; $P < 0.05$)

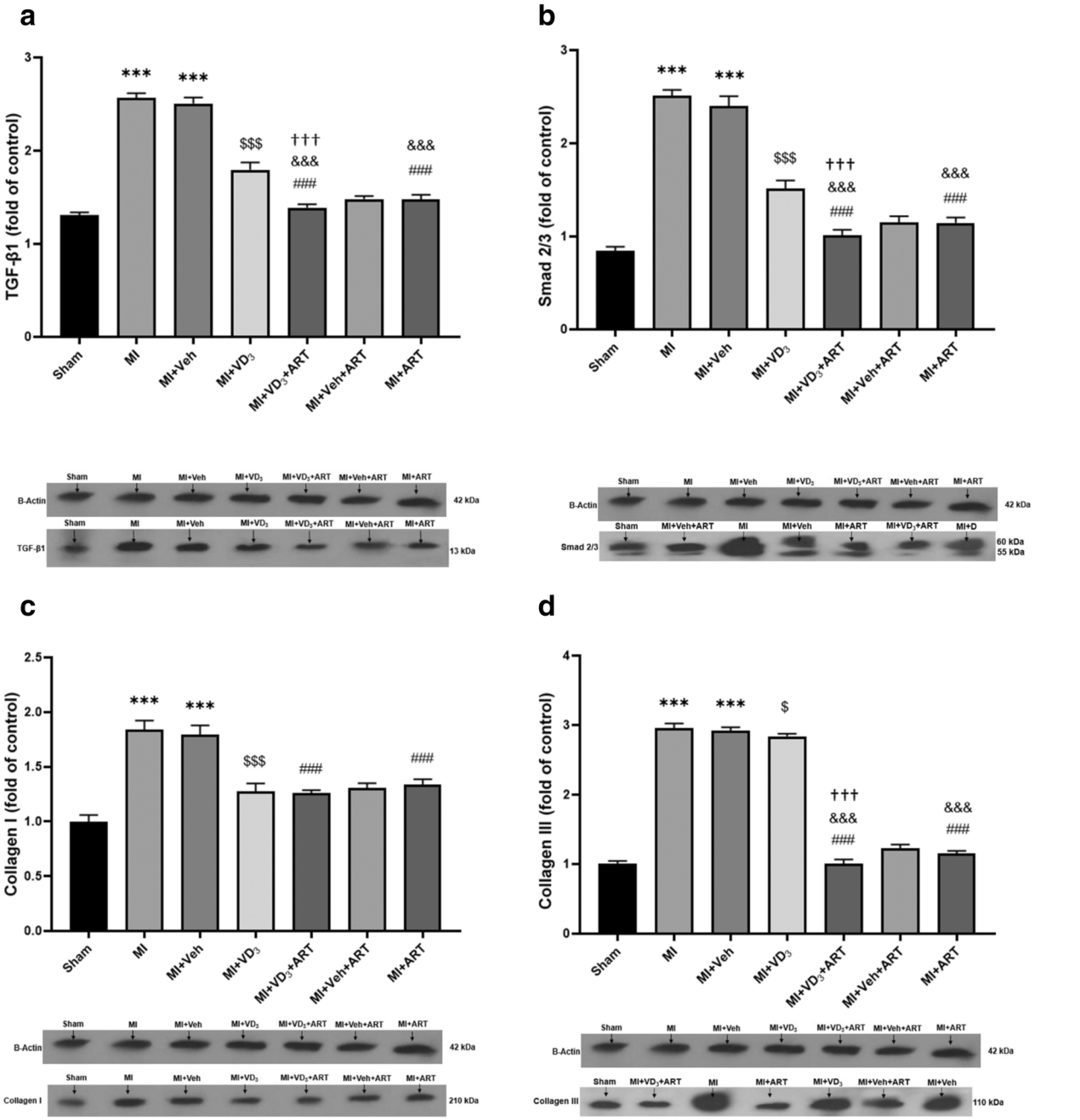

Fig. 5 Western blotting results. **a** The expression of TGF-β1, **b** Smad2/3, **c** collagen I, and **d** collagen III was significantly increased in the MI group when compared with the sham group. On the other hand, their expression except collagen III was significantly decreased compared with the MI group. Sham (normal saline injection), MI (myocardial infarction), ART (aerobic-resistance training), Veh (vehicle, sesame oil), VD_3 (vitamin D_3 injection, 10,000 IU/kg/week), TGF-β1 (transforming growth factor- beta1), Smad (suppressor of mothers against decapentaplegic). Values are presented as mean ± SD ($N = 8$) and analyzed by one-way ANOVA and post hoc tests by Tukey. ** (significant increase vs sham group; $P < 0.01$). *** (significant increase vs sham group; $P < 0.001$). ### (significant decrease vs MI group; $P < 0.001$). $ (significant decrease vs MI + Veh group; $P < 0.05$). $$$ (significant decrease vs MI + Veh group; $P < 0.001$). &&& (significant decrease vs MI + VD_3 group; $P < 0.001$). ††† (significant decrease vs MI + Veh + ART group; $P < 0.001$)

hypertrophy compared with monotherapy. Moreover, our findings suggest that the cardioprotective effects of co-treatment of VD_3 and ART may be mediated by TGF-β1/Smad signaling.

Here, we used isoproterenol, a β-adrenergic agonist to induce MI in rats. Isoproterenol has been known to exert oxidative stress, mitochondrial dysfunction, and apoptosis [25]. Cardiac oxidative stress is associated with increased collagen

production and TGF-β-Smad expression, which both are potential factors resulting in cardiac fibrosis [26]. TGF-β1 has been known to phosphorylate Smad2/3 to bind with Smad4. Then, these complexes translocate to the nucleus [19] and express the target genes such as collagen [34]. Also, TGF-β increases reactive oxygen species (ROS) [17] and then induces epithelial-mesenchymal transition (EMT), which is a process that occurs during tissue fibrosis [15].

Although VD_3 and ART per se were able to reduce the expression of TGF-β1, Smad2/3, collagen I, and collagen III, the simultaneous treatment caused more reduction in the levels of these proteins (Fig. 5a, b, c, and d). Consistent with these data, histological findings confirmed the greatest reduction in the size of the fibrotic area in the group receiving a combination of ART with VD_3 (26.67%), while ART per se induced only 20% and VD_3 18.26% reduction in the fibrotic area (Fig. 4a and b). This finding suggests a synergistic cardioprotective effect of VD_3 supplementation, and ART to alleviate cardiac fibrosis probably mediating by TGF-β1/Smad2/3 signaling.

Vitamin D_3 administration has been known to reduce ROS formation through suppression of NADPH oxidase [16] and also downregulation of the renin-angiotensin-aldosterone system [37]. The Renin-angiotensin-aldosterone system is involved in the pathogenesis of cardiac fibrosis via upregulating fibroblastic TGF-β1, suppressing matrix metalloproteinase-1, a key enzyme causing interstitial collagen degradation and inflammation [26]. Besides, VD_3 exerts anti-inflammatory effects via a reduction in TNF-α concentration [29]. In line with our findings, Lai and colleagues (2016) reported that paricalcitol attenuates the expression of endothelial cell transition markers, in rats with cardiomyopathy [18]. Therefore, considering both anti-inflammatory properties [23] and the antioxidant role of VD_3 [29], TGF-β1-Smad signaling reduces collagen accumulation and finally alleviates myocardial fibrosis [36].

On the other hand, in our study, ART per se prevented the progression of fibrosis by 20% parallel with the suppression of TGF-β1-Smad signaling. These findings are in line with the previous studies showing that exercise training reduces plasma concentrations of angiotensin II [1], cardiac TGF-β1 expression, collagen deposition, and fibrosis [26].

Moreover, regular moderate exercise potentiates the immune system and suppresses inflammation [31] due to antioxidant properties [30]. In line with our findings, Ma and colleagues (2015) reported that swimming attenuates cardiac fibrosis by inhibiting the NADPH oxidase–ROS via AMPK activation [22].

Another part of our results showed that fibrosis leads to a significant elevation in the ratio of HW/BW and LV/BW heart, but the reduction in EF and FS. This confirms that the workload of cardiomyocytes in the fibrotic heart and their gradual loss lead to cardiac hypertrophy and pathological dysfunction, as it is evident by collagen III and EF data [28]. According to our results, the combination of VD_3 and ART more efficiently changed EF, ES, and the ratio of HW/BW and LV/BW (Table 1 and Fig. 3, respectively). It seems that there is a convergence of molecular signaling pathways between two treatments of ART and VD_3, and they might synergistically prevent the progression of fibrosis, in addition to histological remodeling in post-MI status. Mechanistically, VD_3 as a steroid hormone binds to its nuclear receptor (VDR), and increases the expression of peroxisome proliferator-activated receptors of gamma, and then reduces triglyceride levels and lipid deposition [12]. Additionally, VD_3 reduces cardiac hypertrophy modulating several protein kinases [24] and mediators such as atrial natriuretic peptide [27] and extracellular signal-regulated kinase 1/2 (ERK1/2) [11]. On the other hand, ART not only improves the heart function by regulating genes responsible for apoptosis and angiogenesis but also changes the myocardial contractility by an increase in cardiac β-adrenergic receptor signaling [8] such as ERK1/2 and P38 mitogen-activated protein kinases [20].

To our knowledge, this is the first study examining the effect of concurrent aerobic-resistance training and VD_3 supplementation on cardiomyopathy. Our findings suggest that simultaneous and chronic treatment of ART and VD_3 successfully alleviates cardiac fibrosis and improves both functional exercise capacity and strength gain via downregulating TGF-β1 signaling (Table 2). One limitation of the present study is evaluating only one of the several signaling pathways. Other important molecular signaling cascades remain to be elucidated in future studies.

Regarding the clinical importance of this study, our findings suggest that the combination of aerobic exercise and resistance training together with VD_3 supplementation for 8 weeks might be a suitable candidate therapy for cardiac rehabilitation in post-MI.

Compliance with ethical standards

Animals used in these experiments were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the research protocol was approved by the ethics committee of Guilan University of Medical Sciences (IR.GUMS.REC.1398.046).

Conflict of interest The authors declare that they have no conflicts of interest.

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