Original Article

Cardioprotective Effect of Vitamin D2 and Rosuvastatin on Isoproterenol-induced Myocardial Infarction in Albino Rat

Rami Alfar[1]*, Wael Khattab[2], Mahmoud Metwally Emara[3], Mohamed Ali Mahmoud Elmoghazy[4,5], Mohammad O. Zarad[6], Albayoumi AB Fouda[7], Ahmed Mohammed Hassan Mostafa[8], Ahmed Abdrabo Elshaday Elsisi[9], Taher Said Abd Elkareem[10], Shimaa Fouad[11], Ahmed G. Bakry[12], Ahmed Elsayed[13], Ramadan Hassan Ibrahim Thabet[14], Hassan Saad Hassan Elwakeel[15], Mai Ali Mohammad Etewa[16], Ahmad Mohamed Mohamed Awad[17], Ehab Kamal Ali[18], Alaa M AboSeif[19], Noha O. Shawky[20]

[1]Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan. Email: raalfar@just.edu.jo

[2]Department of Medical Physiology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. Email: waelkhattab025@gmail.com

[3]Department of Pharmacology, Faculty of Medicine, Al-Azhar university, Egypt; Alghad College for Applied Medical sciences, Riyadh, Saudi Arabia. Email: memara2005@gmail.com

[4]Department of Oral Surgery and Diagnostic Sciences, Faculty of Dentistry, Applied Science Private University, Al-Arab Street, Amman, Jordan.

[5]Department of Physiology, Damietta Faculty of Medicine, Al-Azhar University, Damietta, Egypt. Email: drmohamedali122@gmail.com

[6]Department Medical Physiology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. Email: mohammadzarad560.el@azhar.edu.eg

[7]Department of Medical Physiology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. Email: fouda82@yahoo.com

[8]Department of Intensive Care Medicine, National Heart, Egypt. Email: ahm_hassan79@yahoo.com

[9]Department of Pharmacology, Faculty of Medicine, Al-Azhar University, Assiut, Egypt. Email: zezo20082001@yahoo.com

[10]Department of Cardiology, Ischemic Center of Cardiology, Al-Azhar University, Egypt; Consultant Cardiology, Nizwa Hospital, Oman. Email: tahersalman2017@gmail.com

[11]Department of Medical Physiology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. Email: drshimaafouad88@gmail.com

[12]Department of Cardiology Division of Internal Medicine, Faculty of Medicine, South Valley University, Qena, Egypt. Email: ahmedgbakry@gmail.com

[13]Department of Microbiology, Sharurah Armed Forces Hospital, Saudi Arabia. Email: elamgad99@hotmail.com

[14]Department of Pharmacology, Faculty of Medicine, Al-Azhar University, Assiut, Egypt. Email: ramadan332@yahoo.com

[15]Department of Anatomy and Embryology, Faculty of Medicine, Al-Azhar University, Damitta, Egypt. Email: hasan.alwakil@domazhermedicine.edu.eg

[16]Department of Medical Physiology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. Email: mai.ali.md1@gmail.com

[17]Department of Medical Biochemistry, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. Email: ahmadawad84@yahoo.com

[18]Department of Anatomy and Embryology, Faculty of Medicine, Al-Azhar University, New Damietta, Egypt. Email: ehab0512@gmsil.com

[19]Department of Pharmacology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. Email: alaaclinic@yahoo.com

[20]Department of Medical Physiology, Helwan University, Cairo, Egypt. Email: noha.osama@med.helwan.edu.eg

Abstract

Background: Myocardial infarction (MI), a predominant cause of worldwide mortality, arises from permanent myocardial necrosis resulting from ischemia. Vitamin D2 and rosuvastatin exhibit antioxidant and anti-inflammatory characteristics; nevertheless, their cardioprotective effectiveness in myocardial infarction remains ambiguous and need additional investigation. **Objectives**: is to assess the distinct cardio-protective effects of vitamin D2 and rosuvastatin in alleviating isoproterenol (ISO)-induced myocardial injury in albino rats through the analysis of cardiac enzyme levels, oxidative stress indicators, and histological alterations. **Methods:** The research employed adult male albino rats as an animal model to examine the effects of isoprenaline (ISO) on myocardial infarction. The rats were categorized into four groups: normal control rats, rats administered ISO subcutaneously twice at 24-hour intervals, rats treated with rosuvastatin daily for 10 days, and rats receiving daily pretreatment with vitamin D for 30 days. The study entailed isoprenaline injection for two days, succeeded by oral administration of rosuvastatin for ten days and daily oral intake of vitamin D for thirty days. Blood samples were obtained 12 hours post-second ISO injection and analyzed for lipid peroxidation, superoxide dismutase, and reduced glutathione levels. The findings indicated that ISO therapy markedly increased cardiac enzymes and oxidative stress indicators, while diminishing antioxidant levels. Vitamin D2 and rosuvastatin separately mitigated these effects, although did not fully normalize the values. Histopathology corroborated the chemistry

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Address for Correspondence: Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan
Email: raalfar@just.edu.jo

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results. **Conclusion:** Vitamin D2 and rosuvastatin are recognized for their protective effects against ISO-induced myocardial injury, evidenced by enhanced biochemical markers and histological observations. Nonetheless, neither medication completely mitigated the harm, underscoring the necessity for additional research into their synergistic potential, dosage refinement, and practical implementation. The trial employed pretreatment with rosuvastatin and vitamin D2, without post-treatment treatments, and had limited biochemical and histological assessments. The study's unique temporal focus and homogenized tissue masked specific localized effects. Further investigation is necessary to see if the combination with vitamin D2 offers further benefits, and any modifications to medication should be conducted under professional supervision.

Keywords: Vitamin D2, Rosuvastatin, Oxidative Stress, Cardiac Enzymes, Isoproterenol-induced Myocardial Infarction.

Introduction

Myocardial infarction (MI) is a leading cause of morbidity and mortality worldwide. It occurs when myocardial ischemia causes irreversible myocardial necrosis.[1] The identification of targeted drugs for pre-beneficial changes in acute MI could lead to significant discoveries. Isoproterenol (ISO) induced MI is widely used to determine the therapeutic efficiency of standardized compounds.[2,3] Multiple biochemical indicators, including LDH, creatine kinase, Aspartate Aminotransferase (AST), and Alanine Aminotransferase (ALT), are utilized to predict energy and oxidative stress-related alterations in isoproterenol-induced myocardial infarction models for the identification of the drug constituent.[4,5]

Vitamin D is one of the most important human hormones, and it has several roles in the body related to bone growth, calcium and phosphorus homeostasis, and skin cell growth differentiation.[6] Recently, it has been proposed that there is an association between low levels of serum concentration of vitamin D and an increased risk of developing adverse cardiovascular events.[7]

The mechanism of cardiovascular disorders includes activation of the Renin-Angiotensin system, where the activation can overproduce reactive oxygen species.[8] These Reactive Oxygen Species (ROS) can promote lipid peroxidation, which would lead to myocardial uptake of peroxidative lipids and finally result in infarction and necrosis of cardiac muscle cells.[9,10]

In addition, the expression of an inflammatory mediator, tumor necrosis factor-alpha, and its intracellular cascades lead to the activation of protein kinases and transcription factors that can increase cell survival and apoptosis signaling.[11,12]

The cardioprotective ability of vitamin D2 was evident through the lowering of the levels of heart injury markers such as serum C-reactive protein, cardiac troponin I, and creatine kinase.[13] In addition, vitamin D2 treatment could weaken the level of lipid peroxidation and intracellular free calcium ions in ischemic-hypoxia cells in a dose-dependent manner.[14]

MI is a disease that causes myocardial cell death, leading to a decrease in heart function.[1,15] The disease is caused by various factors, including insufficient coronary blood flow to the myocardium.[16,17]

Isoproterenol (ISO) is a synthetic catecholamine that binds to α and β adrenergic receptors and induces MI in rats.[18,19] Acute administration of high ISO levels lead to a sudden increase in the amount of oxygen required and produces free radicals, subsequently causing myocardial necrosis. In the rat ISO-induced model of MI, the heart undergoes several pathophysiological changes during acute ischemia and reperfusion.[20,21]

The imbalance between the production and elimination of free radicals' results in extensive lipid peroxidation in the cellular and subcellular compartments.[1]

Vitamin D2 and rosuvastatin treatment has been shown to have a combined cardioprotective effect on isoproterenol-induced myocardial infarction in albino rats.[22] This study was designed to investigate the combined cardioprotective effect of vitamin D2 and rosuvastatin on isoproterenol-induced myocardial infarction in albino rats.

Objectives

This research aimed to evaluate the individual and combined cardioprotective effects of vitamin D2 and rosuvastatin in an ISO-induced MI model in albino rats.

Materials and Methods

Experimental Animals

This study utilized adult male albino rats as the animal model. Rats were obtained from the animal house at the Faculty of Medicine, Assiut University, Assiut, Egypt, and were sustained on a balanced diet with unrestricted access to water in clean containers. They were irate for two weeks while adjusting the laboratory conditions prior to the commencement of the experiment. Fifty-six age-matched male albino rats, with initial body weights between 150 and 200 grams, were utilized.

The rats were categorized into four groups, each consisting of 14 rats. Eight animals were utilized for biochemical assessments, while six animals were employed for histopathological analysis.

Group I: Normal control rats.
Group II: Rats were subcutaneously administered ISO (100 mg/kg) twice at 24-hour intervals, serving as the control for groups III and IV.
Group III: Rats were administered rosuvastatin (15 mg/kg) once daily for 10 days, followed by subcutaneous injections of ISO at 24-hour intervals for 2 days.
Group IV: Rats received a daily pretreatment of vitamin D (100 mg/kg) for 30 days, followed by subcutaneous injections of ISO at 24-hour intervals for 2 days.

Vit. D and Rosuvastatin were obtained from Oxford laboratory - Mumbai – India. In addition; Isoprenaline hydrochloride was obtained from Sigma Aldrich chemical –USA

Study Procedure

- **Induction of Myocardial Infarction**

Over the course of two days, rats were given isoprenaline at a dose of 100 milligrams per kilogram (mg/kg) subcutaneously in saline at intervals of twenty-four hours. According to Kumaran and Prince[23], the presence of elevated serum levels of creatine kinase (CK), troponin-I, aspartate transaminase (AST), and lactate dehydrogenase (LDH) in rats served as evidence that ISO-induced myocardial infarction was subsequently confirmed.

Drug Administration

- Rosuvastatin was administered orally at a single dose of 15 milligrams per kilogram of body weight each day for a period of ten days according to Priscilla and Prince[24]. Rusovastatin was dissolved in saline and then given to rats through an intragastric tube.
- Vitamin D was administered orally at a dose of 100 milligrams per kilogram of body weight every day for a period of thirty days. It was suspended in distilled water and then administered through a stomach tube.[25]

Collection of Blood Samples

At the end of the experimental period, after 12 h of second ISO injection.

All the rats were sedated with ether and euthanized via cervical decapitation. Blood was collected in a dry, clean, graduated glass centrifuge tube. The sample was promptly centrifuged at 5000 r.p.m for 10 minutes to separate the serum for biochemical analysis. Hearts were removed, weighed, and homogenized in a tris HCl buffer (10 mM, pH 7.4) at a concentration of 10% (w/v). The homogenates underwent centrifugation at 10,000 rpm for 20 minutes. The transparent supernatant was utilized for the assessment of lipid peroxidation, superoxide dismutase, and reduced glutathione.

Biochemical Measurements

- **Estimation of Serum aspartate transaminase (AST) and Serum Alanine Aminotransferase (ALT)**

The serum activities of AST and ALT were measured using an enzymatic colorimetric method provided by Vitro Scient Egypt.

- **Estimation of Serum Lactate Dehydrogenase (LDH)**

Serum LDH levels were measured using the enzymatic-colorimetric method provided by Chemelax Industria, Barcelona, Spain.

- **Estimation of Serum Creatine Kinase**

Serum creatine kinase levels quantified using the enzymatic-colorimetric approach by Chema Diagnostica, Italy.

- **E-Estimation of Serum Troponin-I (CTnI)**:

The serum level of cardiac troponin I (CTnI) was measured using an immune-enzymometric assay by Monobind Inc. - United States.

- **Estimation of the Level of Reduced Glutathione (GSH), Superoxide Dismutase (SOD), and Malondialdehyde (MDA)**

The concentration of glutathione (GSH) in the heart was assessed using a colorimetric approach obtained from Biodiagnostics. Egypt.

Histopathological Examination

Following decapitation, the hearts were swiftly excised, rinsed with saline, and subsequently fixed in 10% neutral buffered formalin. They were then embedded in paraffin, sectioned to a thickness of 5 µm, stained with Hematoxylin and Eosin (H&E), and examined under a light microscope for histopathological evaluation.[25]

Statistical Analysis

Statistical analysis was conducted with the software application SPSS. The quantitative results were displayed as mean ± standard error (S.E.). Statistical analysis of group differences was conducted using one-way analysis of variance (ANOVA), followed by the Tukey-Kramer test for mean comparisons. A P-value of less than 0.05 was established as the threshold for statistical significance.

Ethical Approval

The study was approved by research ethics committee at Faculty of Medicine, Al-Azhar University. Under number (._0000016)

Results

Rats treated with ISO demonstrated a significant ($p<0.01$) increase in serum aspartate aminotransferase (AST) activity compared to normal control rats. The prior treatment of vitamin D (100 mg/kg) daily for 30 days and rosuvastatin (15 mg/kg) daily for 10 days considerably ($p<0.01$) diminished the enzyme activity compared to rats only induced by ISO; however, a considerable ($p<0.01$) increase persists relative to normal rats. Rats treated with ISO demonstrated a significant ($p<0.01$) increase in blood alanine aminotransferase (ALT) activity compared to normal control rats. The prior treatment of vitamin D (100 mg/kg) daily for 30 days and rosuvastatin (15 mg/kg) daily for 10 days considerably ($p<0.01$) diminished the enzyme activity compared to rats only induced by ISO; however, a considerable ($p<0.01$) increase persists relative to normal rats as demonstrated in Table 1.

The research demonstrated that isoproterenol (Isoprenaline) successfully produced myocardial infarction in a rat model, resulting in cardiac tissue destruction and the subsequent release of enzymes into the bloodstream. Vitamin D2 and rosuvastatin exhibited cardioprotective properties by decreasing AST and ALT levels, which serve as indicators of myocardial injury. Nonetheless, neither intervention fully restored the harm inflicted by isoproterenol.

Table 1: Effect of Pretreatment with Vitamin D and Rosuvastatin on the Activity of AST and ALT in Serum of ISO-induced Myocardial Infarcted Rats.

Groups	AST (IU/l)	ALT (IU/l)
Normal control	34.62±4.27	26.48±2.62
Isoprenaline	60.37±3.50 *	50.31±2.28 *
Vit D+ Isoprenaline	50.50±8.82 * #	40.04±4.19 * #
Rosuvastatin+ Isoprenaline	47.03±6.03 * #	34.36±2.36 * #

Each value represents the mean ± SE (standard error) of 8 animals
*Significant result as compared to control group
#Significant result as compared to isoprenaline group

The research demonstrated that isoproterenol (Isoprenaline) successfully produced myocardial infarction in a rat model, resulting in cardiac tissue destruction and the subsequent release of enzymes into the bloodstream. Vitamin D2 and rosuvastatin exhibited cardioprotective properties by decreasing AST and ALT levels, which serve as indicators of myocardial injury. Nonetheless, neither intervention fully restored the harm inflicted by isoproterenol.

The findings clearly demonstrate that isoproterenol effectively produced myocardial infarction in the rat model. Both vitamin D2 and rosuvastatin exhibited cardioprotective properties by diminishing the levels of AST and ALT, which serve as indicators of myocardial injury. Nonetheless, neither intervention fully restored the harm inflicted by isoproterenol. Additional statistical analysis is required to ascertain whether Rosuvastatin provides significantly more protection than Vitamin D. This study demonstrates that both Vitamin D2 and Rosuvastatin possess potential therapeutic efficacy in reducing myocardial infarction.

Rats administered ISO exhibited a substantial ($p<0.01$) elevation in serum lactate dehydrogenase (LDH) activity relative to normal control rats. The previous administration of vitamin D (100 mg/kg) daily for 30 days and rosuvastatin (15 mg/kg) daily for 10 days significantly ($p<0.01$) reduced the activity of this enzyme compared to rats only induced by ISO; however, a notable ($p<0.01$) increase remains in comparison to normal rats. Rats administered ISO exhibited a substantial ($p<0.01$) elevation in serum creatine kinase (CK) activity relative to normal control rats. Previous administration of vitamin D (100 mg/kg) daily for 30 days and rosuvastatin (15 mg/kg) daily for 10 days considerably ($p<0.01$) reduced the activity of this enzyme in ISO-induced rats; however, a considerable ($p<0.01$) increase remains when compared to normal rats. Rats administered ISO exhibited a significant ($p<0.01$) increase in serum levels of cTnI compared to normal control rats. Previous administration of vitamin D (100 mg/kg) daily for 30 days and rosuvastatin (15 mg/kg) daily for 10 days to ISO-induced rats significantly ($p<0.01$) reduced cTnI levels compared to rats induced solely with ISO; however, a significant ($p<0.01$) increase remained when compared to normal rats, as illustrated in Table 2.

Table 2: Effect of Pretreatment with Vitamin D and Rosuvastatin on the Activity of LDH, CK, and cTnI in Serum of ISO-induced Myocardial Infarcted Rats.

Groups	LDH (IU/l)	CK (IU/l)	cTnI (ng/ml)
Normal control	81.98±14.63	166.76±20.56	0.116± 0.127
Isoprenaline	161.88±12.20*	280.47±26.57*	2.15±0.515*
Vit D + Isoprenaline	107.27±14.02* #	199.06±26.96* #	1.19±0.049* #
Rosuvastatin + Isoprenaline	91.13±15.26* #	193.60±14.88* #	0.92±0.148* #

Each value represents the mean ± SE (standard error) of 8 animals
*Significant result as compared to control group #significant result as compared to isoprenaline group

The research illustrates that isoproterenol proficiently induces cardiac injury in a rat model. Both vitamin D2 and rosuvastatin demonstrated substantial cardioprotective benefits, as indicated by the decrease in LDH, CK, and cTnI levels. These markers are indicative of cardiac muscle injury; thus, a decrease in these markers is highly significant. The findings offer compelling evidence that isoproterenol successfully produced myocardial injury in the rat model. Both vitamin D2 and rosuvastatin had notable cardioprotective effects, as indicated by the decrease in LDH, CK, and cTnI levels. Rosuvastatin seems to demonstrate a marginally superior protective effect compared to vitamin D2, although statistical analysis is required for confirmation. These data indicate the prospective therapeutic efficacy of both vitamin D2 and rosuvastatin in alleviating myocardial infarction. The incomplete normalization indicates that although the therapies provided protection, they failed to fully restore the harm. The findings offer compelling evidence that isoproterenol successfully produced myocardial injury in the rat model.

Rats administered ISO exhibited a significant ($p<0.01$) reduction in cardiac levels of GSH compared to normal control rats. Pretreatment with vitamin D (100 mg/kg) daily for 30 days and rosuvastatin (15 mg/kg) daily for 10 days in ISO-induced rats considerably ($p<0.01$) elevated GSH levels compared to rats induced solely with ISO; nevertheless, a considerable ($p<0.01$) reduction remained when compared to normal rats. Rats administered ISO exhibited a significant ($p<0.01$) reduction in SOD activity in the heart relative to normal control rats. Pretreatment with vitamin D (100 mg/kg) daily for 30 days and rosuvastatin (15 mg/kg) daily for 10 days in ISO-induced rats significantly ($p<0.01$) enhanced SOD activity compared to rats induced solely with ISO; nevertheless, a considerable ($p<0.01$) reduction persisted in contrast to normal rats. Rats administered ISO exhibited a

substantial ($p<0.01$) elevation in myocardial malondialdehyde (MDA) levels relative to normal control rats. Pretreatment with vitamin D (100 mg/kg) daily for 30 days and rosuvastatin (15 mg/kg) daily for 10 days considerably ($p<0.01$) reduced the level of MDA compared to rats generated just by ISO; however, there remained a considerable ($p<0.01$) elevation when compared to normal rats, as illustrated in Table 3.

Table 3: Effect of Pretreatment with Vitamin D and Rosuvastatin on the Levels of GSH, SOD, and MDA in the Heart of ISO-induced Myocardial Infarcted Rats

Groups	GSH (mmol/g tissue)	SOD(U/g tissue)	MDA (nmol/g tissue)
Control	11.42±1.83	17.22±2.12	1.19±0.19
Isoprenaline	3.40±0.39*	6.23±0.65*	2.47±0.33 *
Vit D + Isoprenaline	8.17±1.67*#	11.13±0.78*#	1.50±0.58*#
Rosuvastatin + Isoprenaline	9.83±1.62* #	11.65±0.94*#	1.66±0.56*#

Each value represents the mean ± SE (standard error) of 8 animals
*Significant result as compared to control group #significant result as compared to isoprenaline group

The research demonstrated that isoproterenol caused considerable oxidative stress in the cardiac tissue of rats, resulting in diminished levels of GSH and SOD, alongside an elevation in MDA. Vitamin D2 and rosuvastatin exhibited notable antioxidant and cardioprotective properties, reinstating GSH and SOD levels while diminishing MDA levels. Rosuvastatin exhibited elevated levels of GSH and SOD relative to the vitamin D2 group, indicating it may offer a marginally superior level of antioxidant protection.

The findings indicated that although both treatments enhanced antioxidant status and decreased lipid peroxidation, they failed to fully restore GSH, SOD, and MDA levels to normal control values. This indicates that although the treatments provided protection, they did not fully rectify the oxidative damage. The findings indicate that isoproterenol caused considerable oxidative stress in the cardiac tissue of rats, while both vitamin D2 and rosuvastatin exhibited notable antioxidant and cardioprotective properties. Rosuvastatin seemed to provide a somewhat enhanced level of antioxidant protection; however, additional statistical analysis is required. The results endorse the prospective therapeutic efficacy of vitamin D2 and rosuvastatin in alleviating oxidative stress and myocardial injury during myocardial infarction. Oxidative stress constitutes a substantial component of the damage induced by ISO.

Histopathological Examination of the Hearts of the Normal Rats

Analysis of H&E-stained slices from the hearts of normal rats revealed typical striation of the myocardium. (Figure 1).

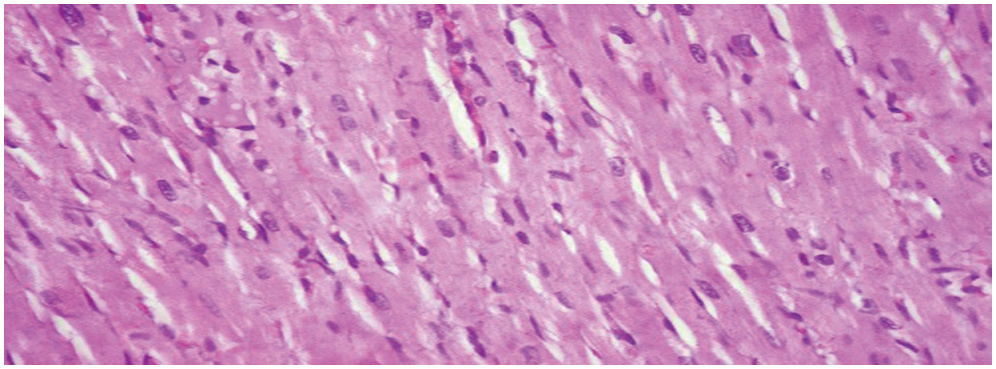
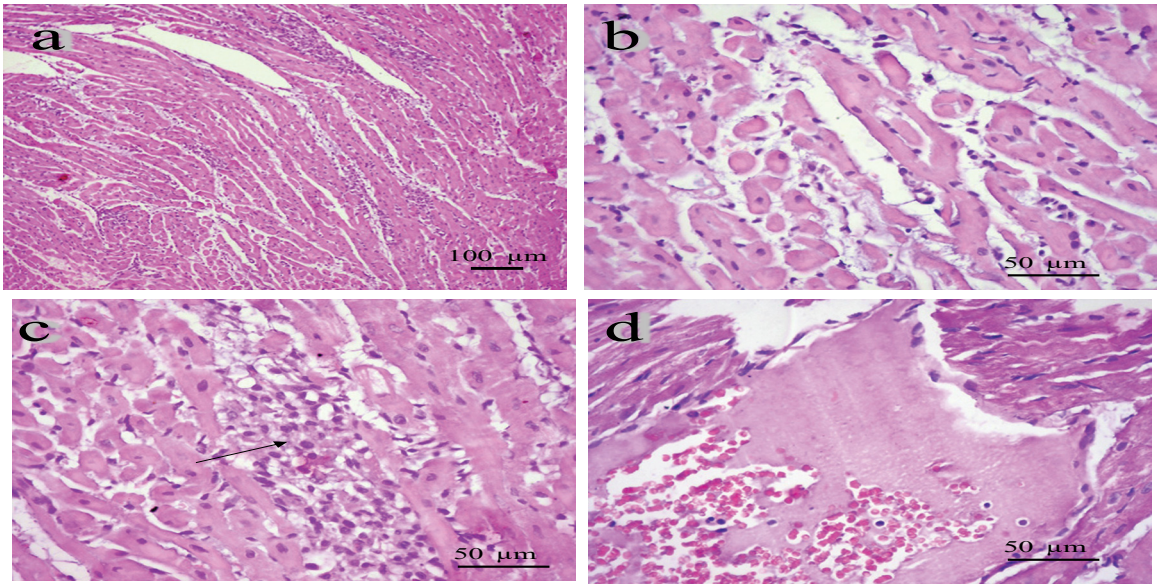


Figure 1: Histopathological Examination of the Hearts of the Normal Rats.

The histological analysis of cardiac tissue from normal rats demonstrates the characteristic architecture of cardiac muscle. Cardiomyocytes, the contractile cells of the heart, are elongated, spindle-shaped cells organized in parallel rows. The nuclei are dark-staining oval structures located within the cells. Striations, consisting of alternating bright and dark bands perpendicular to the longitudinal axis, are distinctive of heart muscle owing to the organization of contractile proteins (actin and myosin). Intercalated discs, specialized cell connections oriented perpendicularly to muscle fibers, facilitate the coordinated contraction of cardiac muscle. Nonetheless, the intercalated discs are challenging to identify owing to staining and resolution issues.

Histopathological Examination of the Hearts of Isoprenaline Treated Rats

Analysis of H&E-stained sections from the hearts of ISO-treated rats revealed histopathological alterations characterized by isolated areas of coagulative necrosis in the myocardium (Figure 2a). The myofibers exhibit karyopyknosis, hypereosinophilia, and loss of striation (Figure 2b), along with macrophage infiltration in the interstitium (Figure 2c). Thrombosis of blood vessels is observable (Figure 2d).



Figur 2: Histopathological Examination of the Hearts of ISO Treated Rats. (a) Localized Region of Coagulative Necrosis in the Myocardium. Pyknosis and Eosinophilia of the Myocytes, Accompanied by Lack of Striation. Infiltration of Macrophages in the Interstitium (arrow). (d) Thrombosis of Blood Vessels.

Isoproterenol-induced myocardial injury is a form of cell death generally resulting from ischemia, characterized by insufficient blood flow. The damage is confined to a specific area of the heart, indicating that it is localized rather than widespread. The myocyte nucleus undergoes shrinkage and exhibits intense staining, signifying irreversible cellular injury. Myocytes exhibit a more pronounced pink staining with eosin dye because to the enhanced binding of denatured proteins to the dye. Healthy cardiac myocytes exhibit a characteristic striated morphology resulting from the systematic organization of contractile proteins, which are degraded during necrosis, resulting in the disappearance of striations. Macrophages, immune cells that phagocytize and eliminate dead cells and debris, are located in the interstitium to eradicate necrotic tissue. Thrombosis of blood vessels transpires when a thrombus forms within the heart's vasculature, hence diminishing blood supply to the myocardium and intensifying the damage induced by ISO.

Histopathological Examination of the Hearts of the Rats Treated with Isoprenaline+ Rosuvastatin

Analysis of H&E-stained slices from the hearts of rats administered isoprenaline and rosuvastatin demonstrated solely vascular congestion alongside intact myofibers (Figure 3).

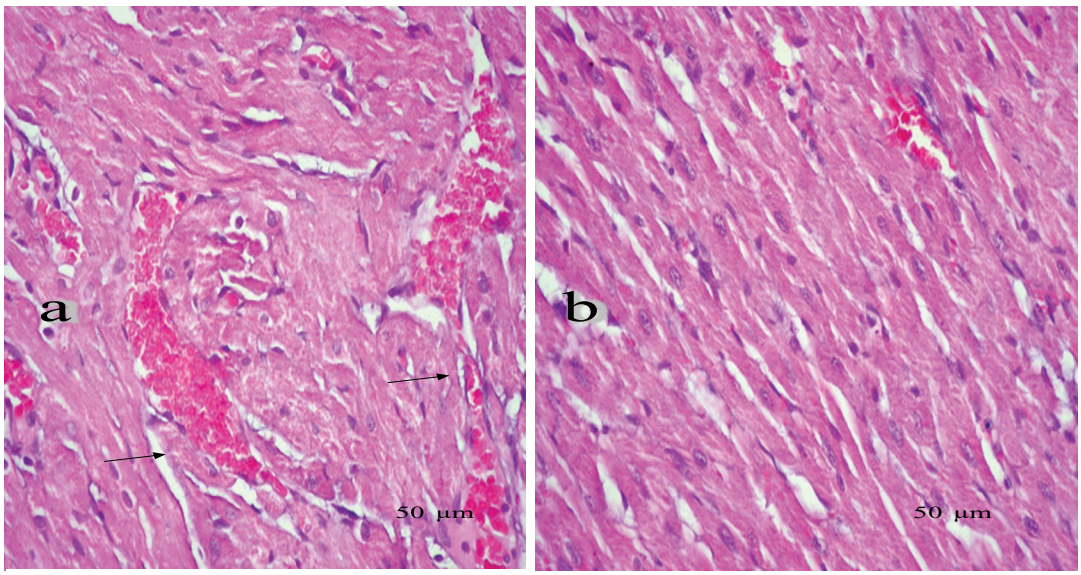


Figure 3: Histopathological Examination of the Hearts of the Rats Treated with Isoprenaline + Rosuvastatin. (a) Congestion Showed by Black Arrows. (B)Normal Myofibers and Congestion Showed by Arrows.

Congestion denotes the buildup of blood within blood vessels, potentially signifying compromised blood flow or elevated back pressure in the circulatory system. Normal myofibers are present in the heart, indicating that rosuvastatin may provide a protective effect against Isoproterenol-induced injury. Nonetheless, congestion persists, indicating that the protection is inadequate. The study indicates that rosuvastatin does not entirely inhibit the effects of Isoproterenol, but it does mitigate them to some extent. The existence of normal myofibers in certain regions of the heart indicates that rosuvastatin may confer a degree of protection against Isoproterenol-induced injury. It is crucial to ascertain if this congestion occurs in the same places as typical myofibers or in other sites.

Histopathological Examination of the Hearts of the Rats Treated with Isoprenaline + Vitamin D

Analysis of H&E-stained sections from the hearts of rats administered isoprenaline and vitamin D revealed thrombosis of blood vessels across rather normal cardiac fibers (Figure 4ab).

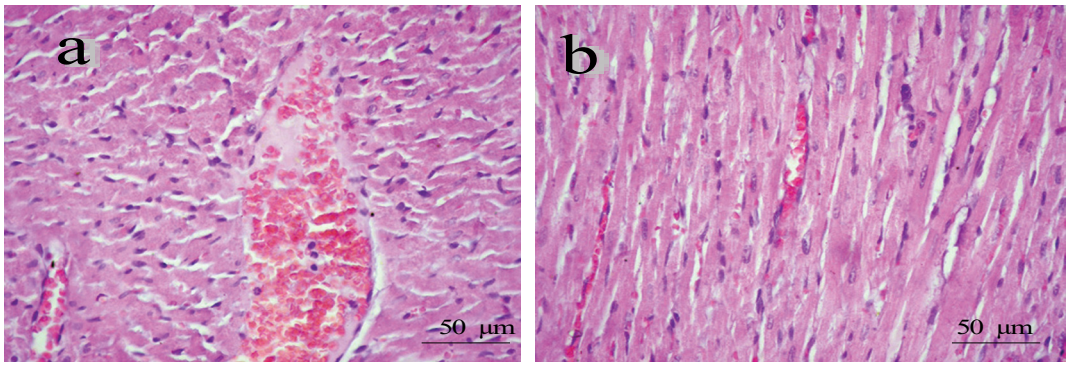


Figure 4: *Histopathological Examination of the Hearts of the Rats Treated with* Isoprenaline + Vitamin D Showing Formulated Thrombosis with more or Less Normal Myocardial Fibers.

Vitamin D may offer protection against Isoproterenol-induced myocardial injury, partially preserving the integrity of cardiac myocytes. Nonetheless, it does not entirely mitigate the detrimental effects of Isoproterenol, as indicated by the occurrence of thrombosis. The developed thrombosis may exacerbate damage, even in regions where the cardiac fibers seem reasonably intact. The conjunction of "formulated thrombosis" and "relatively normal myocardial fibers" indicates a mixed result.

Effect of Treatment with rosuvastatin on the Contractile Response of the ISO Untreated Rats' Aortae to Norepinephrine

Cumulative concentration-response curves induced by norepinephrine on aortic ring preparations from normal rats, ISO untreated rats, and ISO rats administered rosuvastatin (15 mg/kg) for 10 days. The results indicate that the contractile response of the aortae was significantly elevated ($P< 0.001$) in ISO untreated rats compared to normal rats, and significantly diminished ($P< 0.05$) in ISO rats treated with rosuvastatin relative to ISO untreated rats. However, there remains a significant ($P< 0.05$) increase in the contractile response of the aortae in ISO rats treated with rosuvastatin when compared to the aortae of normal rats. Cumulative concentration-response curves induced by norepinephrine on aortic ring preparations from normal rats, ISO untreated rats, and ISO rats administered vitamin D (100 mg/kg) for 10 days. The results indicate a significant increase ($P< 0.001$) in the contractile response of the aortae in ISO untreated rats compared to normal rats. Furthermore, there was a significant decrease ($P< 0.05$) in the contractile response of ISO rats treated with vitamin D relative to ISO untreated rats. However, the contractile response of ISO rats treated with vitamin D remained significantly elevated ($P< 0.05$) compared to that of normal rats.

Table 4: Effect of Treatment with Rosuvastatin or Vitamin D on the Contractile Response of the ISO Untreated Rat's Aortae to Norepinephrine.

Groups	Normal Untreated	ISO Untreated	ISO Rats Treated with Rosuvastatin	ISO treated with vit D
-Log. molar conc. of NE	*Contraction(g)*	*Contraction(g)*	*Contraction(g)*	*Contraction(g)*
$1x10^{-8}$	0.00 ± 0.00	0.8± 0.05*	0.27 ±0.03*#	0.33 ±0.03 *#
$3x10^{-8}$	0.05 ± 0.02	0.98 ± 0.03*	0.35 ± 0.04*#	0.4± 0.03 *#
$1x10^{-7}$	0.28 ±0.03	1.19± 0.04*	0.67± 0.04*#	0.73± 0.05 *#
$3x10^{-7}$	0.51± 0.05	1.63± 0.06*	0.96± 0.04*#	1.11± 0.05 *#
$1x10^{-6}$	0.79± 0.06	1.98± 0.09*	1.32± 0.06*#	1.41± 0.06*#
$3x10^{-6}$	1.01± 0.06	2.61± 0.06*	1.68± 0.05*#	1.82 ± 0.05 *#
$1x10^{-5}$	1.45± 0.07	2.91± 0.08*	2.03± 0.07*#	2.11± 0.07*#
$3x10^{-5}$	1.45± 0.07	2.91± 0.09*	2.03± 0.08*#	2.11 ± 0.08*#

Each value represents mean ± SE of 7 – 9 rats. * Significant difference from the normal untreated rats ($P< 0.01$). # Significant difference from the ISO untreated rats ($P< 0.01$).

The research indicated that rats administered rosuvastatin and vitamin D had a marked decrease in the contractile response to norepinephrine relative to the untreated cohort, implying possible vascular dysfunction or heightened sensitivity to norepinephrine. The most significant disparity between the untreated and treated groups was observed at the highest norepinephrine values. The rosuvastatin cohort exhibited marginally reduced contractile responses compared to the vitamin D cohort across most norepinephrine values; however, statistical analysis is required to ascertain the significance of this difference. The findings indicate that even in rats not exposed to isoproterenol-induced myocardial infarction, there is a noted enhancement in aortic contractility in response to norepinephrine, potentially indicating underlying vascular alterations. Rosuvastatin and vitamin D both significantly reduced this heightened contractility, indicating its potential to enhance vascular function. The diminished contractile response indicates that these treatments may enhance endothelial function, decrease vascular smooth muscle sensitivity to norepinephrine, or influence other variables affecting vascular tone. The findings indicates that both therapies positively influenced the vascular system, potentially impacting the management of hypertension and other cardiovascular disorders linked to heightened vascular reactivity.

Cumulative concentration-response curves induced by Ach on NE precontracted aortic ring preparations from normal rats, ISO untreated rats, and ISO rats administered rosuvastatin (15 mg/kg) for 10 days. The findings indicate that the relaxant response of the aortae was significantly diminished ($P < 0.001$) in the ISO untreated rats compared to the normal rats. Conversely, there was a significant increase ($P < 0.01$) in the relaxant response in the ISO rats treated with rosuvastatin relative to the ISO untreated rats. However, a significant decrease ($P > 0.05$) in the response of the aortae in the ISO rats treated with rosuvastatin persisted when compared to the aortae of the normal rats. Cumulative concentration-response curves induced by Ach on NE precontracted aortic ring preparations derived from normal rats, ISO untreated rats, and ISO rats administered vitamin D (100 mg/kg) for 10 days. The results show that the relaxant response of the aortae was decreased significantly ($P< 0.001$) in the ISO untreated rats in comparison with the normal rats, and increased significantly ($P< 0.01$) in the ISO rats treated with vit D in comparison with the ISO untreated rats, but still there was a significant ($P > 0.05$) decrease in the response of the aortae of the ISO rats treated with vit D as compared to the normal rats aortae as shown in Table 5.

Table 5: Effect of Treatment with Rosuvastatin or Vitamin D on the Relaxant Response of the ISO Treated Rats Isolated Aortae to Acetylcholine.

Groups	Normal	ISO Untreated	ISO rats Treated with Rosuvastatin	ISO Treated with vit D
-Log. molar conc. of Ach	*Relaxation (% of residual tone)*	*Relaxation (% of residual tone)*	*Relaxation (% of residual tone)*	*Relaxation (% of residual tone)*
$1x10^{-8}$	80.1 ± 1.4	100.0 ± 0.0 *	89.1 ± 1.4*#	92.4 ±1.3*#
$3x10^{-8}$	70.2 ± 1.3	97.8 ± 1.1 *	83.1 ± 1.4*#	88.4± 1.2*#
$1x10^{-7}$	50.2 ± 1.2	88.5 ± 1.2 *	71.3 ± 1.3*#	80.1± 1.5*#
$3x10^{-7}$	41.4 ± 1.2	79.8 ± 1.3 *	55.4 ± 1.4*#	63.1± 1.4*#
$1x10^{-6}$	30.3 ± 1.4	69.4 ± 1.3 *	47.4 ± 1.4*#	54.4± 1.5*#
$3x10^{-6}$	23.6 ± 1.5	65.5 ± 1.4 *	43.1 ± 1.5*#	50.3 ± 1.6*#
$1x10^{-5}$	17.2 ± 1.3	59.1 ± 1.4 *	35.8 ± 1.6*#	42.1± 1.5*#
$3x10^{-5}$	17.2 ± 1.2	59.1 ± 1.2 *	35.8 ± 1.4*#	42.1 ± 1.3*#

Each value represents mean ± SE (standard error) of 7 – 9 rats.
*Significant difference from the normal untreated rats ($P< 0.01$).
Significant difference from the ISO untreated rats ($P< 0.01$).

The study revealed that ISO therapy compromised endothelium-dependent relaxation, indicating endothelial dysfunction. The "ISO untreated" group demonstrated a markedly diminished relaxation response to acetylcholine across all concentrations in comparison to the "Normal" group, indicating endothelial dysfunction. Both rosuvastatin and vitamin D demonstrated a marked enhancement in the relaxation response to acetylcholine relative to the "ISO untreated" group. The relaxation response intensified with diminishing doses of acetylcholine, as anticipated. The rosuvastatin cohort exhibited a marginally enhanced relaxation response compared to the vitamin D cohort at the majority of acetylcholine doses, indicating that rosuvastatin may have conferred a modestly superior improvement in endothelial function. Nonetheless, a statistical study comparing the two therapy groups is necessary to validate this. The findings indicate that ISO therapy caused endothelial dysfunction, as demonstrated by the diminished relaxation response to acetylcholine. Rosuvastatin and vitamin D exhibited substantial protective benefits, enhancing endothelium-dependent relaxation in ISO-treated rats. These data indicate that both therapies may augment nitric oxide bioavailability, boost endothelial cell function, or influence other aspects that facilitate vascular relaxation. These findings have ramifications for the therapy of cardiovascular disorders linked to endothelial dysfunction, including hypertension and atherosclerosis.

Discussion

Myocardial infarction occurs when there is an interruption in coronary blood supply to a major part of the heart

muscle.[26] Cardiovascular disease (CVD) is a major cause of mortality worldwide, and myocardial infarction (MI) is the end results.[27] Epidemiologic reports have indicated that an inverse correlation exists between serum 25-hydroxyvitamin D (25(OH)D) levels and the prevalence of cardiovascular disease, including myocardial infarction.[6] The human heart is known to express both vitamin D receptors and the 25(OH)D-1α-hydroxylase enzyme, a discovery that can be attributed to the research of tissue-specific 1,25-dihydroxyvitamin D (1,25(OH)2D) production.[28] The aim of the present study was to evaluate the cardioprotective effects of vitamin D2 and rosuvastatin on isoproterenol-induced myocardial infarction in albino rats.

Our study revealed that rats treated with ISO demonstrated a significant ($p<0.01$) increase in serum aspartate aminotransferase. Increase of AST and ALT is a regular adverse effect of the induction of Myocardial infarction with ISO. These findings were also achieved by the study of Takase *et al*.[28] and the study of Arozal *et al*.[29]. On the other hand, vitamin D effect on hepatic enzymes was previously mentioned in agreed prior studies amongst is the study of Al-Bayyari *et al*.[30] and the study of Guo *et al*.[31]. The role of Rosuvastatin is similar, as it has the potential to decrease these enzymes as well. This was mentioned earlier in the study conducted by Sikder *et al*.[32]. Statins are known for their lipid-lowering effect and prevention against thrombosis and hypertension, which are important for preserving the proper blood supply to the myocardium.[33] In addition, recent reports show that some statins, despite their lipid-lowering efficacy, also have a positive effect on heart tissue.[34] This effect of the statins could be due to their antioxidant, anti-inflammatory, anti-apoptotic, and immunomodulatory effects.[35]

However, the molecular mechanisms of these actions of statins at the level of the myocardium are understood in reducing cardiac hypertrophy.[36] The vitamin D from these compounds increases the expression of eNOS and stimulates an increase in its activity and the intracellular signaling molecule SIRT1.[37] Upstream of the Ser133 site of PGC-1α, an increase in p-AMPK and the Ser635/637 site of PGC-1α holds GSK3 beta, a regulatory kinase for cardiac hypertrophy. Besides, they activate the phosphorylation of p38 MAPK, which in turn inhibits the calcineurin/NFAT pathway.[38] Vitamin D2 and ROS decrease the phosphorylation of p42/44 kinase, responsible for the activation of NF-κB, which inhibits apoptosis in myocardial infarction.[39]

It is difficult to ascertain definitively which is superior between vitamin D and rosuvastatin based on the existing results of our study. Both treatments demonstrated a significant reduction in many markers of cardiac injury relative to the ISO-induced group. However, neither treatment completely reinstated the effects of ISO, since all measures remained considerably elevated compared to the normal control group. These findings go with finding revealed by Renke *et al*.[38] who found that vitamin D had a positive effect on cardiac parameters but was not able to normalize its values may be due to short study period or it may be due unadjusted dosage of vitamin D

The study conducted by Yu *et al*.[39] reveled contradicting results regarding rosuvastatin. The study evaluated the cardioprotective effects of rosuvastatin against isoproterenol-induced myocardial infarction in rats. The rats were administered isoproterenol for two days and subsequently treated with rosuvastatin for eight weeks. Research demonstrated that rosuvastatin diminished the extent of myocardial infarction, enhanced histological changes, and decreased enzymes indicative of myocardial damage. It also reinstated lipid peroxidation, diminished antioxidants, lowered pro-inflammatory cytokines, and blocked the activation of the NLRP3 inflammasome, demonstrating its ability to alleviate ISO-induced myocardial infarction injury.

Knowledge Gap

The research investigates the distinct cardioprotective benefits of vitamin D2 and rosuvastatin, together with their synergistic impact. It recognizes antioxidant processes and cardiac enzyme regulation as essential components of these medicines' cardioprotective actions. The research additionally investigates the effects on vascular function via the application of norepinephrine and acetylcholine. Nonetheless, it does not conclusively determine whether the combined effect is synergistic or additive. Additional investigation is required to elucidate the precise molecular processes implicated.The study's emphasis on short-term effects is constrained, and the long-term influence of vitamin D2 and rosuvastatin on cardiac remodeling, fibrosis, and functional recovery post-myocardial infarction remains ambiguous. Longitudinal research are essential to fill this gap. The research employed particular amounts of vitamin D2 and rosuvastatin; however, ascertaining the ideal dosage and time for maximal cardioprotective effectiveness necessitates additional inquiry. The study's translational significance for humans remains ambiguous, necessitating additional research in larger animal models and clinical trials. The genetic determinants affecting the response to these therapies are still unidentified, which is essential for personalized medicine strategies. The study did not examine the interaction of these substances with other cardiovascular medications, including beta-blockers, ACE inhibitors, or antiplatelet treatments. The study also did not investigate particular biological pathways, such the NF-kB pathway or the P38MAPK pathways.

Implications

The research demonstrated that vitamin D2 and rosuvastatin conferred notable cardioprotective benefits against isoproterenol-induced myocardial infarction in albino rats, as indicated by decreased serum AST levels and possible alteration of additional cardiac damage indicators. These findings corroborate prior research emphasizing the distinct advantages of these drugs. The noted decreases in AST levels are significant, as AST serves as a sensitive

indicator of myocardial injury, with its rise in ISO-induced myocardial infarction signifying cardiomyocyte necrosis. The pleiotropic mechanisms of rosuvastatin, encompassing its lipid-lowering, anti-inflammatory, and antioxidant attributes, are well-documented.

The cardioprotective benefits of Vitamin D2, likely mediated by its interactions with the vitamin D receptor and the regulation of signaling pathways, align with the current literature. The observed impact on liver enzymes indicates possible systemic advantages in addition to the direct cardiac effects. Although neither treatment completely normalized cardiac indicators to control levels, this may be due to the severity of the ISO-induced damage or the constraints of the research duration. Enhancing dosage and doing extended research may uncover more significant impacts.

The practical consequences and potential application to human studies encompass clinical relevance, personalized medicine, public health considerations, and future research directions. Preliminary human investigations should concentrate on establishing the ideal dosage and administration routes of vitamin D2 and rosuvastatin in patients with or at risk of myocardial infarction. Clinical trials must test the efficacy and safety of these substances in the prevention or treatment of myocardial infarction, encompassing the evaluation of clinical outcomes, subgroup analyses, long-term follow-up, and combination studies.

Exploration of personalized medical approaches that account for individual genetic variants and risk factors is essential, including the customization of treatment regimens based on an individual's vitamin D status, lipid profile, and other pertinent biomarkers. The public health ramifications for cardiovascular disease prevention may be considerable due to the prevalent lack of vitamin D. Future study should examine the molecular pathways that contribute to the cardioprotective effects of vitamin D2 and rosuvastatin, especially for combination therapy, and assess potential interactions with other cardiovascular medicines.

Future Directions

Myocardial infarction (MI) is a critical global health issue. Although rosuvastatin and vitamin D2 have distinct cardioprotective properties, their synergistic effectiveness in alleviating isoproterenol (ISO)-induced myocardial infarction in albino rats requires additional research. The notion that the concurrent administration of vitamin D2 and rosuvastatin may provide synergistic cardioprotection against ISO-induced myocardial infarction is based on their complimentary modes of action.

Possible synergistic mechanisms encompass anti-inflammatory effects, antioxidant effects, anti-apoptotic effects, anti-remodeling effects, and enhancements in endothelial function. Rosuvastatin reduces the expression of pro-inflammatory cytokines, including TNF-α and IL-6, whereas vitamin D2 attenuates NF-κB activation. The synergistic impact may lead to a more effective inhibition of inflammatory pathways, reducing myocardial injury.

The antioxidant properties are significant, as ISO-induced myocardial infarction is marked by oxidative stress. Rosuvastatin augments antioxidant enzyme activity, but vitamin D2 diminishes the generation of reactive oxygen species (ROS). Their dual dosing may offer improved protection against cardiomyocyte apoptosis.

The anti-remodeling actions are essential for sustained heart function, as both rosuvastatin and vitamin D2 mitigate cardiac fibrosis and hypertrophy. This combinatorial modulation may enhance long-term heart function. Future study should concentrate on comprehensive mechanistic investigations, optimal dosage and timing, long-term effects, translational studies, clinical trials, genetic polymorphisms, and combination therapies with other treatments. Preclinical investigations in larger animal models, meticulously structured clinical trials, and genetic polymorphisms in vitamin D receptors and HMG-CoA reductase will facilitate the validation of findings and the evaluation of the safety and efficacy of combination therapy prior to human trials.

In conclusion, the possible synergistic cardioprotective effects of vitamin D2 and rosuvastatin in alleviating isoproterenol-induced myocardial infarction in albino rats necessitate additional research and translational relevance for clinical application.

Study Limitations

The study employed treatment regimens consisting solely of pretreatment with rosuvastatin and vitamin D2, without post-treatment interventions. The fixed dosages of rosuvastatin and vitamin D were not examined, and the routes of administration differed. The study featured restricted biochemical and histological evaluations, with few markers for inflammatory cytokines, growth factors, or components of signaling pathways. The study's singular time point and homogenized tissue obscure certain localized effects.

Conclusion

Vitamin D and rosuvastatin were able to reverse the effect of ISO and prevent the cardiac damage which indicates their capability as cardioprotective agents against myocardial infarction in animal models induced by isoproterenol-induced in albino rat. Both Vitamin D and rosuvastatin were not capable to normalize the hepatic enzymes and cardiac parameters in this animal model. These findings formulate a question about the cardioprotective effect of combination protocol of Vitamin D and rosuvastatin against myocardial infraction.

Ethical Issues

This study's research and protocol were conducted according to the all-experimental protocols that approved by the "Research Ethics Committee of the Faculty of Medicine for Girls at Al-Azhar University" and were conducted in accordance with the "Guide for the Care and Use of Laboratory Animals" as outlined in "National Institutes of Health" (NIH, 1978). The research ethics

committee of the Faculty of Medicine for Girls, Cairo, Al-Azhar University (FMG-IRB) met at the Faculty of Medicine for Girls, Nasr City (ethical code #IRB The study was approved by research ethics committee at Faculty of Medicine, Al-Azhar University. Under number (0_0000016). This manuscript hasn't been published elsewhere, and its plagiarism has been checked.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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