Original Article

Simvastatin's Intrinsic Calcium Channel Antagonistic Activity on Vascular Smooth Muscle Cells

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ABSTRACT

Objective: To determine the intrinsic calcium channel antagonistic activity of Simvastatin on vascular smooth muscle cells.

Study design: Experimental animal study on Rabbits aortic strips.

Place and duration of study: This study was conducted at Pharmacology department lab "Ayub Medical College, Abbottabad" from Jan, 2017 to Jan, 2018.

Material and Methods: In tissue organ bath of Power lab, different molar concentrations of Simvastatin were applied on the rabbit's aortic strips to record its relaxing effects on the Spontaneous, KCl-induced and NE-induced contractions. Calcium chloride response curves (CCRCs) were drawn by decalcification of rabbit's aortic strips and then the known concentration of calcium chloride was provided to draw control calcium chloride curves to compare it with simvastatin treated tissues; calcium chloride curves for simvastatin were constructed by using calcium channel blocker Verapamil as standard.

Results: Our study showed significant results for intrinsic calcium channel antagonistic activity of Simvastatin on Vascular smooth muscles (VSMCs), in comparison with Verapamil as standard.

Conclusion: This study demonstrates that Simvastatin have an intrinsic calcium channels (L-Type) antagonistic activity on vascular smooth muscle cells (VSMCs), besides its normal lipid lowering effects.

Key Words: Simvastatin. Vascular smooth muscle cells (VSMCs), Verapamil, Cardiovascular diseases (CVDs), CCB (Calcium channel blocker), Potassium chloride (KCl), Nor-Epinephrine (NE), CCRCs (Calcium chloride response curves)

Citation of articles: Ali W, Bukhari S, Adeel M, Imran K. Simvastatin's Intrinsic Calcium Channel Antagonistic Activity on Vascular Smooth Muscle Cells. Med Forum 2019;30(4):70-73.

INTRODUCTION

Cardiovascular diseases are the most prevalent cause of mortality and morbidity[1]. Most important cause of cardiovascular disease is high blood cholesterol levels[2]. Lipid lowering drugs which are used to lower the blood cholesterol levels in patients with hypercholesterolemia include Simvastatin (Statins) as first line drugs[3], prevention of atheromateous lesion and prevention of subsequent development of atherosclerosis is the virtue of Statins. Mechanism of action via which Simvastatin lowers blood cholesterol level is HMG-COA reductaseinhibition, with inhibition ofDe-novo synthesis of cholesterol as well[4]. Simvastatin also increases the LDL receptors that can combine and internalize circulating LDLs, so the plasma levels of cholesterol is reduced by inhibiting the cholesterol synthesis and raise of LDL catabolism. Simvastatin also have pleotrophic effects[5]. In 1990, three different Statins were introduced to the market that are Simvastatin, Lovastatinand Pravastatin[6]. Valuable effects of these Statins were seen in Hypercholestrolemics but with the passage of time side effects were seen. GIT system side effects of Statins include Constipation, dyspepsia, abdominal pain, nausea, vomiting, heart burn and flatulence[7]. These side effects of Statins are proved to be due to the Calcium channels blocking activity of Statins on smooth muscles of GIT[8]. Statins also deregulate the calcium channels that stimulate differentiated phenotype of vascular smooth muscle cells. A study also reports that Statins also up-regulate the L-type calcium channels but this up-regulation takes time as this study was performed in cell lines[9].In another study it was noted that the long term use of Statins in hypertensive patients normalized blood pressure[10,11]. In the light of above studies and the approval of Statins side effects on GIT due to calcium channel blocking activity, so we designed our study to check the calcium channel blocking activity of Simvastatin in Smooth muscle cells (SMCs) of vascular system thus our objective was to check the possible inhibitory effects of Simvastatin on L-Type voltage gated calcium channels in vascular system[12] that may

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Received: December, 2018
Accepted: March, 2019
Printed: April, 2019

describe possible rationale for blood pressure lowering effects of Simvastatin (Statin).

MATERIALS AND METHODS

We conducted this study in lab of Pharmacology department, Ayub Medical College, Abbottabad, from Jan, 2017 to Jan, 2018. Our experimental models for the study were Rabbit aortic strips. The rabbits were kept in Pharmacology lab, water and standard diet was freely available. Rabbits were slaughtered and dissection was done, the aorta was carefully extracted and then divided into small strips. These were mounted in the tissue organ baths of Power lab, Carbogen gas was continuously supplied with Kreb's solution in each organ bath, temperature of each organ bath was kept at 37C.[13]

Simvastatin in different molar concentrations (10^{-8} M to 10^{-2}M) were applied directly first on spontaneously contracting aortic tissues, then KCl-induced contacting aortic tissues, then Nor epinephrine (NE)-induced aortic tissues, to study the relaxing effects of Simvastatin. All the above observations were repeated three times using the Lab Chart 7 software of Power lab.[13,14,15]

Finally Calcium Chloride Curves (CCRC), were constructed for Simvastatin to determine its effects on calcium channels in aortic tissues and possible mechanism of its blood pressure lowering effects by following the following procedure, the aortic strips were maintained in Kreb's solution. After stabilization of tissues in organ baths of Power lab, the aortic tissues were exposed to a series of wash with Kreb's normal (Calcium free) solution, followed by exposure to K-rich Kreb's solution[17]. This led to the decalcification of tissues and control CCRC were constructed in absence of Statins. Then CCRC were constructed in the presence of different molar concentrations of Simvastatin following incubation period of 1 hour. Similarly curves were constructed in the absence and presence of Verapamil, a standard calcium channel blocker. The CCRCs were compared for any possible right shift[8,13,14,15,17].

RESULTS

The relaxing effects of different molar concentrations of Simvastatin were noted as follows:-

1-Simvastatin's Effects On Spontaneous Contractions: The relaxing effects of Simvastatin on spontaneous activity of aortic strips started at 10^{-7}M and reached maximum at 10^{-4}M. The mean EC_{50} value of Simvastatin for spontaneous contractions of aortic strips was $1.94\pm0.5\times10^{-5}$M.

2-Simvastatin's Effects On KCl-induced

KCl-induced contractions in aortic strips started relaxing at a concentration of 10^{-6}M of Simvastatin and reached maximum on 10^{-2}M. Mean EC_{50} of Simvastatin for KCl-induced contractions were $4.74\pm 0.02 \times 10^{-4}$M.

3-Simvastatin's Effects On NE-induced Contractions:

NE-induced contractions in aortic strips were started relaxing at a concentration of 10^{-6}M and reached maximum at about 10^{-3}M of Simvastatin. Mean EC_{50} value of Simvastatin for NE-induced contractions were $8.35\pm 0.03 \times 10^{-5}$M, as shown in the table 1.

MIN, MAX & MEAN MOLAR EC_{50}(MEAN±SD) CONCENTRATION(n=3) OF SIMVASTATIN.

Table No.1:To Show the Effects of Simvastatin on Spontaneous, KCl-induced & NE-induced contractions in isolated Rabbit's aortic Strips.

Types of contractions	Simvastatin (Min Relaxing Conc)	Simvastatin (Max Relaxing Conc)	Simvastatin (EC_{50}value)
Spontaneous	10^{-7}M	10^{-4}M	$1.94\pm0.5\times10^{-5}$M
KCl-Induced	10^{-6}M	10^{-2}M	$4.74\pm0.22\times10^{-4}$M
NE-Induced	10^{-6}M	10^{-3}M	$8.35\pm0.33\times10^{-5}$

4-Calcium Chloride Response Curves:

CCRCs were constructed for Simvastatin according to standard protocoal which showed that EC_{50} of Simvastatin for control curve is -2.8±0.04 [log (Ca++) M], while rabbits aortic strips pretreated with 1.33×10^{-5}M of Simvastatin, EC_{50} is -1.77±0.03 [log (Ca++)M], which showed us that the Simvastatin shifted the calcium chloride curve to right that were similar to the effects of Verapamil(A standard CCB).

Calcium Channels Response Curves (CCRs)

Table No.2:To represent the EC_{50} values in absence (Control) and presence of different concentrations of test Simvastatin.

Statins	CCRCs Specifications	EC_{50} Log[Ca++]M
Simvastatin	Control	-2.8±0.004
	Test Conc 1 (1.33×10^{-5}M)	-1.77±0.03
	Test Conc 2 (2.6×10^{-5}M)	-3.54±0.02

When the dose of the pretreated Simvastatin was doubled that is 2.6×10^{-5}M, then the EC_{50} was -3.54±0.02 [log (Ca++)M], which also showed that the Simvastatin shifted the Calcium chloride curve further to the right, which follows the pattern of Verapamil. The results of different molar concentrations of Simvastatin for CCRCs are shown in table 2.

DISCUSSION

The findings of our study have proven that Simvastatin (A Statin) have intrinsic inhibitory effects on the voltage gated calcium channels on vascular smooth

muscles of blood vessels. It means that Simvastatin causes its vasodilatory effects by blocking the L-Type calcium channels in the vessels leading to lowering of blood pressure in addition to its normal lipid lowering effects.

Simvastatin's intrinsic calcium channels inhibitory effects are very crucial interms of the use of Simvastatin (Statins) along with other antihypertensives because the combined effects may lead to increased lowering of blood pressure in patients who are hypertensive and hypercholestrolemics simultaneously (Obese Patients).

Our study was supported by the results of research work conducted by ALI N, which proved calcium channel antagonistic activity of Statins on the Gastro-intestinal (GIT) smooth muscles[8], another study by Clunn GF, which shows the Statins up-regulates calcium channels in vascular smooth muscles[9] and another study by ALI W, which shows potentiating effects of Simvastatin and Amlodipine in hypertensive rats[18, 19].

Conclusion: Based on the findings of our experimental works, it is concluded that Simvastatin intrinsic calcium channel antagonistic activity on Vascular smooth muscles and when it is used in combination with other antihypertensives, in patients suffering from hypertension and hypercholestrolemia simultaneously, then Simvastatin may have additive or potentiating effects on blood pressure (BP) which explicit the pharmacodynamicinteractions of Simvastatin.

This may sometime distort the therapeutic or pharmaceutical cure plan defined for patients who have hypercholestrolemia or are hypertensives, because the dose has to be adjusted according to the combined effects of the two drugs.

CONCLUSION

This study demonstrates that Simvastatin have an intrinsic calcium channels (L-Type) antagonistic activity on vascular smooth muscle cells (VSMCs), besides its normal lipid lowering effects.

Author's Contribution:

Concept & Design of Study:	Wajid Ali
Drafting:	Saima Bukhari, Muhammad Adeel
Data Analysis:	Saima Bukhari, Muhammad Adeel
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Final Approval of version:	Wajid Ali

Conflict of Interest: The study has no conflict of interest to declare by any author.

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