EFFECTIVENESS OF GRANULOCYTE COLONY-STIMULATING FACTOR IN REDUCTION OF ATHEROSCLEROTIC LESIONS IN RABBIT

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ABSTRACT

Objective: To determine the effectiveness of granulocyte colony-stimulating factor (G-CSF) in the reduction of atherosclerotic lesions in a rabbit animal model.

Methodology: In this experimental study, 12 New Zealand rabbits were placed on normal regimen diet supplemented with 2% wt/wt of cholesterol for 3 months. Then the rabbits were assigned randomly to two groups: six rabbits received G-CSF 100 µg/kg/day subcutaneously for 7 days and six rabbits were considered as control group. Blood lipid profile and size of coronary artery lumen and atherosclerotic plaque were compared between the two groups.

Results: In each group the levels of triglycerides (TG), cholesterol, high density lipoprotein (HDL) and lipoprotein A (LPA) significantly increased after 90 days of feeding with cholesterol rich regimen. However, the levels of TG (465.66 ±81.12 vs. 499.00 ±129.96, p =0.60), cholesterol (2449.83 ±165.68 vs. 2455.00 ±143.58, p =0.95), HDL (124.33 ±8.93 vs. 125.00 ±5.32, p =0.87) and LPA (13.16 ±1.72 vs. 14.16 ±2.63, p =0.45) did not show significant difference between the two groups. Seven days after treatment with G-CSF the difference between two groups in size of lumen (p =0.20) and plaque (p =0.12) was not significant.

Conclusion: Granulocyte colony-stimulating factor did not significantly alter the blood lipid profile or the lumen/plaque size in the animal model studied.

Keywords: Granulocyte colony-stimulating factor, Cholesterol-rich diet, Atherosclerosis

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INTRODUCTION

Pharmacologically, a significant role is expected for granulocyte colony-stimulating factor (G-CSF) to play in vascular pathologies and progression of atherosclerosis[1,2]. Differentiation of bone marrow-derived progenitor stem cells to endothelial cells[3,4] and vascular smooth muscle cells (VSMCs)[5,6] have been indicated after administration of G-CSF in animals. These cells contribute to angiogenesis and the formation of microvessels and neointima[7-9]. A study on rabbits indicated that G-CSF reduces the neointima thickness by nearly 60%[1]. Moreover, a study on patients with myocardial infarction revealed better cardiac function and promoted angiogenesis after G-CSF therapy along with the intracoronary infusion of peripheral blood stem cells[2]. Another study on C57BL/6 mice signified that treatment with G-CSF decreases neointima formation following vascular injury and improves re-endothelialization[10]. The G-CSF-induces mobilization of bone marrow derived c-Kit+/Flk-1+ cells and help in the regeneration of endothelial cells in the rabbits[11]. Progenitor cells play an important role in some pathophysiological states such as atherosclerosis, vascular ischemia, and pulmonary hypertension[12-17]. It has been established that high-dose GCSF stimulates neointimal proliferation via cell mobilization and excessive inflammation[18]. On the other hand, studies revealed that in animals the G-CSF and granulocyte macrophage-colony stimulating factor (GM-CSF) may mobilize endothelial progenitor cells[19-20]. Furthermore, studies in humans revealed that GM-CSF is an important angiogenesis factor and improves the cardiac function in patients with myocardial infarction[21]. However, the results of studies are not consistent and a study in mice by Kong et al[22] did not reveal any positive effect of G-CSF or GM-CSF on atherosclerosis, instead it was shown by the study that G-CSF and GM-CSF induce atherosclerosis in mice. Furthermore, some studies de-

clared that G-CSF may induce Kawasaki Disease[21,23]. To date, scarce studies have evaluated the potential therapeutic effects of G-CSF administration on atherosclerosis. Therefore, we conducted this experimental study to determine the effect of G-CSF on the reduction of atherosclerotic lesions in rabbits. The results of this study may help physician and patients to consider G-CSF in the treatment of atherosclerotic lesions in men, because G-CSF is safe and available treatment for myocardial infarction.

METHODOLGY

This randomized controlled trial was conducted in the Tehran Heart Center, Tehran University of Medical Sciences, during March 2011 to March 2012. The Tehran Heart Center approved the study protocol. In this study, 12 New Zealand rabbits (average weight of 2 kilograms and 12 weeks old) were placed on normal regimen diet supplemented with 2 %wt/wt cholesterol for 3 months. At the start of the study and after three months of feeding with cholesterol rich regimen, the blood samples were taken and the level of triglycerides (TG), cholesterol, high-density lipoprotein (HDL) and lipoprotein A (LPA) was measured in both groups.

On the day of treatment, all 12 subjects were placed in a dark cage and one rabbit was removed by an individual other than research team, then another person other than research group, perform a coin toss. The head was considered as A and the subjects were transferred to the cage that signed as cage A and received G-CSF 100 µg/kg/daily subcutaneously for 7 days (interventional group, n=6) . The tail was considered as B and the subjects were placed in cage B and G-CSF was not given (control group, n=6). After seven days the two groups were sacrificed and blood samples were taken again from both groups and the level of TG, cholesterol, HDL and LPA were measured. After sacrificing, the samples from aorta were taken and were fixed in formalin and sent to the pathology laboratory and the size of lumen and plaque were measured and compared between two groups.

Sample size was based on the study by Sinha et al[24], the plaque of atherosclerosis in GCSF and control groups (S1=0.005,S2=0.014,µ1=0.058, µ2=0.039), n=6, for each group, as following:

$$N = \frac{(Z_{1-\alpha/2} + 1 - \beta)^2 \times (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)^2}$$

$$Z_{1-\alpha/2} = 1/96 \quad 1 - \beta = 1.28$$

Data were analyzed using IBM SPSS (Ver. 22, IBM Corporation, Armonk, NY, USA). Continuous variables such as levels of TG, cholesterol, HDL, LPA and sizes of plaque and lumen were reported as mean ±SD. The Student's t-test and the paired t-test were used to compare continuous variables. Differences were considered significant when $p < 0.05$.

RESULTS

At the start of the study the levels of TG (p =0.37), cholesterol (p =0.42) and HDL (p =0.45) were not different between two groups; however, the LPA in G-CSF group was significantly more than control (P =0.01). Moreover, in each group the levels of TG, cholesterol, HDL and LPA significantly increased after 90 days of feeding with cholesterol rich regimen (Table 2). However, the levels of TG (p =0.60), cholesterol (p =0.95), HDL (p =0.87) and LPA (p =0.45) did not show significant different between two groups (Table 1).

Seven days after treatment with G-CSF, the difference between two groups in size of lumen (p =0.20) and plaque (p =0.12) was not significant (Table 3).

DISCUSSION

We detected that G-CSF did not affect the level of lipid profiles and the size of plaque or lumen of the vessels. A comprehensive study, by Hu et al[25] revealed that the lesion area of the thoracic aorta and the plasma levels of total cholesterol (TC) and low-density lipoprotein (LDL) increased in the group of white New Zealand rabbits receiving G-CSF. Furthermore, they detected

Table 1: Hyperlipidemic rabbits before and after G-CSF administration in both treatment and control groups

Before G-CSF administration on 90th day (n=6)				After G-CSF administration		
Parameter	**G-CSF Treatment Group (A)**	**Control Group (B)**	**P value**	**G-CSF Treatment Group (A)**	**Control Group (B)**	**P value**
TG	86.16 ±49.99	65.66 ±21.46	>0.05	465.66 ±81.12	499.00 ±129.96	>0.05
Cholesterol	52.16 ±18.92	64.83 ±31.93	>0.05	2449.83 ±165.68	2455.00 ±143.58	>0.05
HDL	18.16 ±6.91	20.83 ±4.62	>0.05	124.33 ±8.93	125.00 ±5.32	>0.05
LPA	8.16 ±0.75	6.83 ±0.75	<0.01	13.16 ±1.72	14.16 ±2.63	>0.05

TG= Triglyceride; HDL= High density lipoprotein; LPA= Lipoprotein A

Table 2: Blood lipid profile of rabbits before and after receiving a cholesterol-rich diet for 90 days

Treatment Group, A (n=6)				Control Group, B (n=6)		
Parameter	**Before**	**After**	**P value**	**Before**	**After**	**P value**
TG	86.16 ±49.99	465.66 ±81.12	<0.001	65.66 ±21.46	499.00 ±129.96	<0.001
Cholesterol	52.16 ±18.92	2449.83 ±165.68	<0.001	64.83 ±31.93	2455.00 ±143.58	<0.001
HDL	18.16 ±6.91	124.33 ±8.93	<0.001	20.83 ±4.62	125.00 ±5.32	<0.001
LPA	8.16 ±0.75	13.16 ±1.72	<0.001	6.83 ±0.75	14.16 ±2.63	<0.001

TG= Triglyceride; HDL= High density lipoprotein; LPA= Lipoprotein A

Table 3: Plaque and lumen sizes in G-CSF and control groups

Parameter	Groups	n	Mean	SD	P value
Plaque 1	G-CSF (A)	6	436582	294742	>0.05
	Control (B)	6	384720	236076	
Plaque 2	G-CSF	5	502669	240754	>0.05
	Control	5	405505	196194	
Plaque 3	G-CSF	4	672833	238036	>0.05
	Control	5	349750	202884	
Plaque 4	G-CSF	1	658545		
	Control	0			
Lumen 1	G-CSF	6	1199459	587046	>0.05
	Control	6	982344	293196	
Lumen 2	G-CSF	5	1170358	590391	>0.05
	Control	5	1063482	226586	
Lumen 3	G-CSF	4	1555915	624314	>0.05
	Control	5	953108	251485	
Lumen 4	G-CSF	1	1540934		
	Control	0			
Atherosclerosis 1	G-CSF	5	0.36	0.16	>0.05
	Control	6	0.30	0.20	
Atherosclerosis 2	G-CSF	5	0.44	0.15	>0.05
	Control	6	0.30	0.19	
Atherosclerosis 3	G-CSF	4	0.44	0.04	>0.05
	Control	6	0.30	0.20	
Atherosclerosis 4	G-CSF	1	0.4200		
	Control	0			
Total lumen	G-CSF	4	4022980	1522912	>0.05
	Control	5	2998012	619732	
Total plaque	G-CSF	4	1761478	575100	>0.05
	Control	5	1143722	489152	
Total atherosclerosis	G-CSF	4	1.33	0.17	>0.05
	Control	6	0.89	0.52	

that G-CSF contributes to arterial endothelial damaging and aggravation of apoptosis. In line with our findings a study by Hill et al[26] on patients with CAD indicated that, although GM-CSF mobilizes endothelial progenitor cells from bone marrow, however, it does not improve cardiac function significantly and conversely, it contributes to some serious adverse events in these patients. In another study in 2007 by Haghighat et al[27] in mice model, supported this report and indicated that G-CSF or GM-CSF did not have any beneficial therapeutic effect on atherosclerosis. Moreover, they showed that G-CSF and GM-CSF contribute to some adverse effects and resulted in a worsening of atherosclerosis. While another study by Takai et al[28] in 2008 on swine, emphasized that G-CSF did not have any adverse effect on atherosclerosis. The possible explanations for this deleterious effect of G-CSF on atherosclerosis may be related to stimulation of inflammation by G-CSF and GM-CSF on the vessel wall[29] and promotion of neovascularization in the arterial wall by these stem cells[27].

Katsaros et al[30] in their work, measured G-CSF on 280 patients with stable coronary artery disease and followed them for 30 months. It was shown that in patients with cardiac events, the level of G-CSF was significantly higher than other patients. Also, this study demonstrated that patients with higher level of G-CSF had a 2-fold increased risk for major adverse cardiovascular events including death, myocardial infarction and re-hospitalization. The author concluded that endogenous G-CSF may predict cardiovascular events independently of established cardiac risk factors and is related to an increased risk of in-stent restenosis after the establishment of bare metallic stents in these patients.

The results of previous studies were not consistent and as opposed to our experience Sinha et al[24] in 2014 conducted a controlled trial in Apo-E-deficient mice and treated them with G-CSF or vehicle for 9 weeks and reported that G-CSF decreased the level of serum LDL and the size of atherosclerotic plaque. Furthermore, they indicated that the lesions in mice treated with G-CSF contained fewer lipid and macrophages. Also, a research on rabbits with myocardial infarction and balloon injuries by Hasegawa et al[31] showed that G-CSF inhibits the atherosclerosis progression. Additionally, a meta-analysis in 2017 reported that in animal models, G-CSF treatment inhibits the atherosclerosis progression[32]. The different outcome of current practice and some studies reported here may be related to the difference in the dose, the animal model of G-CSF treatment and difference in methodology.

LIMITATIONS

There are a number of limitations to this study that warrants mention, including small sample size and short duration of follow-up that limit us to generalize our reported results. Further larger studies with longer follow-up are needed to prove the exact role of G-CSF in atherosclerosis.

CONCLUSION

Granulocyte colony-stimulating factor 100 µg/kg/day subcutaneously for seven days did not affect the lipid profile and the size of the plaque and lumen of the vessels in the rabbits fed with high cholesterol diet for 3 months.

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CONTRIBUTORS

SR conceived the idea, planned the study and drafted the manuscript. SHA, MAB and MSA helped acquisition of data and did statistical analysis. MN and AMG did editing, critically revised the manuscript and final approval of manuscript. All authors contributed significantly to the submitted manuscript.