ORIGINAL ARTICLE

THE EFFECT OF EVOLOCUMAB ALONE AND IN COMBINATION WITH ATORVASTATIN ON LIPID PROFILE

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ABSTRACT

The aim: In this study, we try to investigate whether evolocumab or its combination with atorvastatin has potent effect on lipid profile?

Materials and methods: Forty local domestic male rabbits were included in this study, and categorized into four group, two untreated group (nohypercholostermic and untreated hypercholostermic) and treated groups (evolocumab treated group at dose 6.1mg/kg/2Wk and atorvastatin treated group at dose 3.5 mg/kg/day),the blood samples were analyzed at base line and after 5week and at the end of the study after 10 weeks for lipid profile by standard enzymatic methods.

Results: The serum levels of total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL-C),were increased after 10 weeks of administration of the atherogenic diet significantly ($p<0.05$) as compared with other groups (group I: 61.19 ± 14, group II: 1301 ± 443, group III 41.01 ± 5.81: 280 ± 50, group IV: 190 ± 38 group I: 46 ± 15.0, group II: 256.0 ± 24.0, group III: 101.0±28, group IV: 48.18 ± 15.27, group I: 29±14.50, group II: 929±251.0, group III: 283.0±36, group IV: 209.0±33mg/dl) respectively while the levels of high-density lipoprotein cholesterol (HDL-C) decrease (18.0±4.1 to 15.0±3.0mg/dl). Compared with evolocumab monotherapy, combination of evolocumab and atorvastatin reduce serum level of total cholesterol, triglyceride and low density lipoprotein more than that of evolocumab.

Conclusions: Preproteins convert as esubtilisin/kexin type 9 inhibitor regulates the serum levels of lipid and cholesterol by lowering LDL-C, and the results also indicate that combination of evolocumab and atorvastatin are more potent in lowering the lipid profile and then reduce progression of atherosclerosis than evolocumab alone in rabbits suggesting that this combination might be beneficial for treatment of atherosclerosis.

KEY WORDS: evolocumab, HMG-COA reductase inhibitors; atorvastatin; cardiovascular diseases, hypercholesterolemia

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INTRODUCTION

Dyslipidemia is a major risk factor for coronary heart disease (CHD) [1]. Dyslipidemia refer to elevation of one or more than lipid parameter which include total cholesterol (TC), low-density lipoprotein (LDL-C) and triglyceride (TG) [2]. Atherosclerosis is a significant risk factor of dyslipidemia, which recognized as the major risk factor for developing of coronary heart disease, stroke and peripheral arterial disease [3], individual with lipid disorder at high risk for developed coronary heart disease than Normolipidemic one [4]. There are big evidence that the low density lipoprotein (LDL-C) and high density lipoprotein (HDL-C) are a major lipoprotein that responsible for regulate cholesterol hemostasis in the body, through carrying cholesterol into peripheral tissue (i.e. coronary arteries) via low density lipoprotein (LDL-C) and in other side high density lipoprotein (HDL-C) carry cholesterol from peripheral tissue into liver in order to be excreted in bile [1]. Most dyslipidemia are associated with unhealthy diet and bad lifestyles [5]. Many studies have revealed that dyslipidemia is an important modifiable risk factor with a key role in CVD. Therefore, early screening and effective control of lipid levels can reduce the morbidity and mortality of CVD, which has useful social value [6]. Treatment guidelines of hyperlipidemia are well established. For decades, statins have been the gold standard of treatment for hypercholesterolemia. They inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase which is considered as a rate limiting step in cholesterol biosynthesis. However, even intensive statin therapy can only reduce cardiovascular events by about 35 percent in patients [7]. A four-fold increase in lipid-lowering medications has been noted and accounts for the decrease in CAD risk reduction in Americans between 1990 and 2010 [8]. Although statins are considered to be well tolerated, adverse drug events were seen in approximately 10–20% of participants, which in return limits their cardioprotective effect, there are multiple agents available to manage the lipid profile, these have been found to be associated with either intolerable side effects such as bile acid sequestrates, or have limited efficacy such as ezetimibe. New agents such as preproteins convert ase subtilisin/kexin type 9 inhibitors have also demonstrated significant results in treating hyperlipidemia. PCSK9 inhibitors such as evolocumab can treating high-risk patients and those without a reduction in LDL after statin use is well documented [9].

MATERIALS AND METHODS

Forty local domestic male rabbits were used in this study. Their weight ranged between (1-1.5 kg) and their age between (6-12) months were housed in the same experimental conditions with constant humidity and they were allowed to drink tap water and pellet diet *ad libitum.* After one week of adaption the animals were randomized in to four groups, ten rabbits each as fellow: Normal control group I were kept on standard chew diet and tap water, group (II): atherogenic high cholesterol diet, were kept on atherogenic diet (normal rabbit chew plus 2% cholesterol (wt/wt)and tap water during the duration of the study 10weeks;group (III) evolocumab treated group, kept on atherogenic diet and evolocumab was given (6.1mg/kg/day) by sub cuteness group, group IV: received combination of evolocumab plus atorvastatin in dose (6.1mg/kg/day S.C plus 3.5 mg/kg/day orally) respectively. From each rabbit about 3 ml of blood was collected from the central ear vein following an overnight fasting. Blood samples were collected at zero time, at the end of 5 weeks and at the end of 10 weeks of drugs treatment for each group of rabbits. Sera were removed for measurement of serum parameters.

PLASMA ASSAYS

Blood was drawn from the central ear vein of the rabbits that had been starved overnight for 12 h. The blood samples were collected at baseline (beginning of the study) and at weeks 5 and 10. The supernatant was collected after centrifugation at 4,000 rpm for 15 min. The serum levels of triglycerides (TG), total cholesterol (TC), HDL-C, and LDL-C were measured by enzymatic methods.

STATISTICAL ANALYSIS

Data are express as mean ± SD paired t-test are used to compare differences between the mean values within each group at different time. Chi-square test was also used to compare Histopathological finding in different group Statistical significance is consider as $p < 0.05$. Analyses performed by using SPSS software update version 21.

RESULTS

EFFECT OF HIGH CHOLESTEROL DIET ON LIPID PARAMETER

Three of four study groups show significant ($p<0.05$) elevation in all lipid parameters, group II which feed with 2% cholesterol for 10weeks this lead to elevation in total cholesterol (TC) from 63.94 ± 16.15 mg/dl at base line to1301 ± 443mg/dl after 10 weeks of treatment with high fat diet. Also high fat diet cause significant elevation ($p<0.05$) in serum level of triglyceride(TG) from 42.05 ± 3.51 mg/dl at the beginning of treatment with high fat diet to 256.0 ± 24.0mg/dl at the end of 10 weeks. There was a significant elevation ($p<0.05$) in low density lipoprotein (LDL-C) from 30.0±11.0 at the base line to 929 ± 251.0 mg/dl at the end of the study. In other side high fat diet cause not significant reduction ($p>0.05$) in serum level of high density lipoprotein (HDL-C) from18.0 ± 4.2 mg/dl at the beginning to the 16.0 ± 2.1 mg/dl. Also high fat diet cause a significant elevation ($P<0.05$) in serum level of both very low density lipoprotein(VLDL-C) and atherogenic index from 7.98 ± 1.12 mg/dl , 3.0±0.6 at the beginning of the study to 29.79 ± 3.30 mg/dl ,41.0 ± 5 respectively.

EFFECT OF EVOLOCUMAB ON LIPID PARAMETER

Three of four study groups show significant ($p<0.05$) reduction in all lipid parameters, group III which treated with evolocumab for 10 weeks this lead to reduction in total cholesterol (TC) from 1301 ± 443 mg/dl to291 ± 50 mg/dl. Also treatment with evolocumab cause significant reduction ($p<0.05$) in serum level of triglyceride (TG) from 256.0±24.0 mg/dl to 41.01 ± 5.81mg/dl at the end of 10 weeks. There was a significant reduction ($p<0.05$) in low density lipoprotein (LDL-C) from 929 ± 251.0 mg/dl to 283.0 ± 36.0 mg/dl at the end of the study. In other side evolocumab cause not significant elevation ($p>0.05$) in serum level of high density lipoprotein (HDL-C) from16.0±2.1 mg/dlto18.0±1.6mg/dl. Also evolocumab cause a significant reduction ($p<0.05$) in serum level of very low density lipoprotein (VLDL-C) and atherogenic index from 29.79 ± 3.30 mg/dl, 41.0 ±5 to 13.90 ± 1.30 mg/dl, 17.0 ± 2.9 respectively.

EFFECT OF COMBINATION OF EVOLOCUMAB AND ATORVASTATIN ON LIPID PARAMETER

Three of four study groups show significant ($p<0.05$) reduction in all lipid parameters, group IV which treated with evolocumab plus atorvastatin for 10weeks this lead to reduction in total cholesterol (TC) from 1301 ± 443mg/dl to190 ± 38mg/dl Also treatment with combination of evolocumab and atorvastatin cause significant reduction ($p<0.05$) in serum level of triglyceride (TG) from 256.0 ± 24.0 mg/dl to 48.18 ± 15.27mg/dl at the end of 10 weeks. Atorvastatin add on therapy with evolocumab cause a significant reduction ($p<0.05$) in low density lipoprotein (LDL-C) from 929 ± 251.0 mg/dl to 209.0±33mg/dl at the end of the study, in other side evolocumab in combination with atorvastatin cause not significant elevation ($p>0.05$) in serum level of high density lipoprotein (HDL-C) from16.0 ± 2.1 mg/dl to26.00 ± 4.2 mg/dl. Also evolocumab in combination with atorvastatin cause a significant reduction ($P<0.05$) in serum level of very low density lipoprotein (VLDL-C) and atherogenic index from 29.79 ± 3.30 mg/dl, 41.0 ±5 to 10.70 ± 1.10 mg/dl, 10.0 ± 1.1 respectively (Table I).

DISCUSSION

Diets have changed greatly in recent years, economic development facilitate these changes. This lead to implicate of

Table I. Changes of various atherosclerotic parameters in the four experimental groups, the data expressed as mean ± SD (N=10 in each group).

Group		Zero week	5 weeks	10 weeks
Group I	TC mg/dl	59.86 ± 15.16	61.56 ± 19.00	61.19 ± 14
Control	TG mg/dl	48.48±15.85	47±2.0	46±15.0
	LDL mg/dl	29±14.50	28±11.0	28.0±11.0
	HDL mg/dl	16.0±1.2	15.1.5±3.4	16.0±1.5
	VLDL	8.43±0.74	8.56±0. 81	8.51±0. 90
	AI	2.4 ±0.5	2.3±0.4	2.3±0.3
	IT µm		103.46± 13.85	
Group II	TC mg/dl	63.94 ± 16.15	717.64 ± 209	1301 ± 443
Atherogenic diet	TG mg/dl	42.05 ± 3.51	196.4±45.35	256.0±24.0
	LDL mg/dl	30.0±11.0	576.0±190	929±251.0
	HDL mg/dl	18.0±4.2	16.0±3.5	16.0±2.1
	VLDL	7.98±1.12	22.41±6.10	29.79±3.30
	AI	3.0±0.6	29.0±5	41.0 ±5
	IT µm		248.43± 11.11	
Group III	TC mg/dl	59.93 ± 18.63	442.00 ± 63	291 ± 50
Evolocumab	TG mg/dl	187.0± 41.25	97 ± 18	41.01 ± 5.81
	LDL mg/dl	33.0±8.0	486±42	283.0±36
	HDL mg/dl	17.0±2.0	16.00±1.3	18.0±1.6
	VLDL	8 ±0.60	22.82 ± 2*	13.90 ± 1.30α
	AI	2.0 ±0. 4	22.0±3.2*	17.0±2.9 *α
	IT µm		9.45±160.66	
Group IV	TC mg/dl	61.30 ± 16.50	374.00 ± 95	190 ± 38
Evolocumab & Atorvastatin	TG mg/dl	148.0 ± 22	70.0 ± 10	48.18 ± 15.27
	LDL mg/dl	35.00±9	429.0±48*	209.0±33
	HDL mg/dl	17.0±2.8	18.0±2.6	26.00±4.2
	VLDL	8.30 ± 0.58	21.70 ± 2.10*	10.70 ± 1.10α
	AI	2.2±0.2	20.0±2	10.0 ±1.1*α
	IT µm		121.79 ± 5.3	

fatty foods in much daily diet therefore continues congestion of fatty food leads to dyslipidemia [10]. High fat diet cause an elevation in total cholesterol (TC), the low density lipoprotein (LDL-C) and triglyceride (TG), while these high fat diet cause reduction in the high low density lipoprotein (HDL-C) [11], lipid metabolism disorders, inducing obesity, diabetes (T2DM), hypertension, dyslipidemia, nonalcoholic fatty liver, and atherosclerosis. Hyperlipidemia is classified as one of the major risk factors leading to cardiovascular disease [12]. In the current study, it was found that the ingestion of high fat diet cause significant elevation in the serum level of total cholesterol and these result in consistent with that demon explain the abnormal changes in serum lipids in obese people [13]. Feeding of rabbits with high fat diet results in significant elevation ($p<0.0.5$) in triglyceride serum level, this is come in agreement with that demonstrated by Timmers S al 2011 who said, that the saturated fats present in the high-fat diet are responsible for the increase in the glucose and lipid profile [14], subsequently, oxidative stress caused by the consumption of a high-fat diet is evident in most experimental models and patients with clinical conditions [15-16]. These results are compatible with previous studies that show the levels of the lipid peroxidation product malondialdehyde, nitric oxide and advanced protein oxidation products were increased in high-fat diet fed rats [17]. Evolocumab a fully human monoclonal antibody which used as second chose in treatment of dyslipidemia, its lower lipids via a unique and novel mechanism, which is blocking PCSK9, Consequently, it provides an additional effective choice to be implemented in the management of atherosclerosis, dyslipidemia, or hypercholesterolemia, in current study it was found that evolocumab cause significant reduction ($p<0.05$) in lipid parameters, and this is in agreements with other studies done by Sabatini MS et al which is confirm that evolocumab showed significant CV outcome benefits in high-risk patients with CVD [18-20], evolocumab could be extremely useful in cases of resistance or when developing intolerable side effects. In current study we found that administration of evolocumab in combination with atorvastatincause a significant reduction($p<0.05$) in all lipid parameters, and this effect is more potent and statistically

significant than when we give evolocumab alone, these finding are in agreement with previous studies done by Koren MJ et al which show that he PCSK9 levels are expected to increase with the use of statins, in return, this results in elevating LDL-C, hence by blocking PCSK9, this should be able to reduce LDL-C by counteracting the mechanism. Therefore, anti-PCSK9 is expected to be effective when administered alongside the statins [21-23].

CONCLUSIONS

Preproteins convert as esubtilisin/kexin type 9 inhibitor regulates the serum levels of lipid and cholesterol by lowering LDL-C, and the results also indicate that combination of evolocumab and atorvastatin are more potent in lowering the lipid profile and then reduce progression of atherosclerosis than evolocumab alone in rabbits suggesting that this combination might be beneficial for treatment of atherosclerosis.

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The Authors declare no conflict of interest.

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