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Research article

EFFECT OF VITAMIN C IN EXPERIMENTALLY INDUCED ATHEROSCLEROSIS

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ABSTRACT: Endothelial dysfunction outcome in atherosclerosis and may be associated with vessel-wall thickening due to a build-up of fatty materials and plaque. Atherosclerosis is response of chronic immune-inflammatory within the arterial wall developing from the influx of lipids, cells and extracellular matrix that underlies several adverse vascular occasions similar stroke, peripheral arterial disease and coronary artery damage. However, deficiency of Vitamin C has lead to occur many diseases, including cardiovascular disease, atherosclerosis, diabetes, and hypertension etc. Vitamin C (Ascorbic acid) also known as L-ascorbic acid is a necessary nutrient for humans. It is required for metabolic reactions in all plants and animals. Accumulating evidence from experimental, epidemiological, and clinical studies suggest that Vitamin C may also be associated with several indices of vascular function, including the development and progression of atherosclerotic cardiovascular disease. In our study have been shown orally administration of high fat induced dietary supplementation of vitamin C 30mg/ day/ kg of body weight in animal model reduce hyperlipidemia in atherosclerosis properties. And also supplementation of Vitamin C has beneficial effects of anti-inflammatory and insulin resistance was shown in (MEAN±SD) biochemical parameters.

Key words: atherosclerosis, inflammation, hypertension

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INTRODUCTION

Endothelial dysfunction outcome in atherosclerosis and may be associated with vessel-wall thickening due to a build-up of fatty materials and plaque (Menown IA, Shand JA 2010). Atherosclerosis is response of chronic immune-inflammatory within the arterial wall developing from the influx of lipids, cells (endothelial, smooth muscle, macrophages, and T lymphocytes) and extracellular matrix that underlies several adverse vascular occasions including coronary artery disease, stroke, and peripheral arterial disease (Victor VM et al, 2009). The inflammatory process involved in atherosclerosis is mediated by both pro-inflammatory and anti-inflammatory cytokines, which are responsible for the igniting events of plaque rupture and thrombosis.

Atherothrombotic disease, plaque formation is frequently a sign of widespread plaque formation affecting more than one vascular bed (Libby P et al, 2009). This theory is supported by the finding that the development of atherosclerosis is accompanied by an accumulation of oxidize LDL proteins in the arterial pole, that oxidized lipids in vitro induced a number of events putatively involved in atherosclerosis such as increasing adherence of inflammatory cells to endothelial cells and including vascular cell death and proliferation. Increasing the antioxidant defense against lipid peroxidation therefore has been proposed to retard the development of atherosclerosis (Chisolm GM, Steinberg D 2000). Atherosclerosis is the most important manifestation of cardiovascular disease (CVD) (Diaz MN et al, 1997). Oxidative stress to lipids is an elementary cause of atherosclerosis. There is a connection between atherosclerotic risk factors and efficaciousness vascular production of reactive oxygen species (ROS) (Keaney JF Jr. Vita JA 1995).

ROS, known to be cytotoxic and mutagenic agents, induce an oxidative stress response. High levels of ROS are strong inducers of the intrinsic apoptotic pathway and tissue injury in pathophysiological conditions as an integral part of atherosclerosis plaque stabilization (Mannick E.E et al, 1996). Upon repeat exposure to atherogenic stimuli such as Oxidized low-density lipoprotein (LDL) and ROS may directly cause endothelial dysfunction by reducing endothelial nitric oxide (NO) bioavailability (Eiserich J.P, Hristova M 1998), the vasoprotective effect of endothelial cells is diminished resulting in the proliferation and migration of vascular smooth muscle cells (VSMCs) (Mc Dowell IF et al, 1994). Reactive oxygen species production associated with CVD pathology is the mitochondrial respiratory chain, nicotinamide adenine dinucleotide phosphate oxidases, xanthine oxidase, lipoxygenase, uncoupled nitric oxide synthase and myeloperoxidase (Navarro A et al, 2004). The most important atherosclerosis risk factors are heredity, age, hypertension, dyslipidemia, diabetes and smoking (Heitzer T et al, 1999).

Animal experimental studies are documented in chronological order (Berliner JA, Heinecke JW 1996). Drugs that may have antioxidant properties include nitric oxide (NO), calcium channel blockers, statins, dobutamine and some angiotensin- converting enzyme (ACE) inhibitors (Carr AC, Frei B 1999). The mechanisms of oxidative damage and antioxidants in patients with atherosclerosis have been evaluated in extensive animal experiments and clinical research. There is a relationship between atherosclerosis risk factor (ARF) and among the main alterations ascribable to endothelial dysfunction is the reduced availability of nitric oxide (NO) and increased vascular production of reactive oxygen species (Edge R, and sTruscott, T.G. 1997). The pathogenesis of atherosclerosis are further related to immunresponce, inflammation and the proliferative process (Ross R 1993). Endothelial denuding injury leads to platelet aggregation and releases platelet-derived growth factor, which triggers the proliferation of smooth muscle cells forming the nidus of the atherosclerosis plaque in the arterial intima, implicating inflammatory changes in the development of the disease (Sundaresan M et al, 1995).. Trace elements like zinc, selenium, chromium, has also shown the protective and regression of atherosclerosis in experimental animals (Subrahmanyam G, Vijaya J 1997).

Vitamin C

Vitamin C (Ascorbic acid) also known as L-ascorbic acid is an essential nutrient for humans (Subrahmanyam G, Vijaya J 1997). It is required for metabolic reactions in all plants and animals (Meister A 1994). Ascorbic acid is one of the vital water-soluble vitamins. It is a juice for collagen, carnitine and neurotransmitter biosynthesis (Tomoda H 1996). Most of the plants and animals synthesize ascorbic acid for their own requirement. Vitamin C reaches every cell of the body and plays role in the making and protecting of our connective tissue that holds the body together. It helps the cardiovascular system by facilitating fat metabolism (Levine GN 1996). Deficiency of Vitamin C causes mainly scurvy, and increased risk factors hypertension, diabetes, eye diseases, myocardial infarction, cardiovascular disease, muscle weakness and nervous disease (Gaziano JM 1992). Vitamin C plays an important role in atherosclerosis implies that the development and progression of atherosclerosis, vascular disease can be inhibited by antioxidants (Ting HH et al, 1997). Thus, dietary antioxidants such as ascorbate (the chemically and biologically most active form of vitamin C) can protect against the development and progression of atherosclerosis in experimental model (Huang A et al, 2003).

MATERIALS AND METHODS

Experimental Animals

In the present study healthy 18 New Zealand white rabbits, weight about 1.8kg±0.2 were used. The rabbits were derived into the following groups, each group contains 6 Rabbits. The experimental study was approved by institutional committee.

Group-I: Rabbits were fed with standard diet (rabbit diet) procured from VK the Institute of Nutrition Pune, India.
Group –II: Rabbits fed with Group I diet and 2% cholesterol containing Rabbit diet procured from VK the Institute of Nutrition Pune, India.
Group-III: Rabbits fed with Group II diet plus Vitamin-C 30 mg / day/ kg body weight

Collection of blood and preparation of serum

Blood sample was collected following an overnight fast and were collectively analyzed in 3 groups of animals. Blood sample of 2ml was obtained from the Rabbits via marginal vein. Blood was collected into two polypropylene tubes, one for plasma and one for serum. The blood for plasma was collected in heparin. Serum was prepared by allowing the blood to clot at 37 degrees centigrade and centrifuge at 3000 RPM for 10 min. serum sample was stored in a freezer at -20 degrees for further biochemical analysis.

Biochemical analysis

The Blood Sample was analyzed for fasting glucose, serum Creatinine, Total cholesterol, LDL-cholesterol, Triglycerides, analyzed by using Humastar 300(GmBh) Autoanalyser. Serum insulin level was determined by using Chemiluminescence immunoassay (Beckmann coulter, USA). The homeostasis model assessment (HOMA) index was used to estimate insulin resistance and calculated as fasting serum insulin (μU/mL) × fasting plasma glucose (mM)/ 22.5. High sensitive-reactive protein (hsCRP) was estimated by turbidometry method using Humalyzer 3000 chemistry analyzer. Circulatory Cytokine interleukin-6 (IL-6) was measured by ELISA by using the ELISA kit procedure from Diaclone France.

Statistical Analysis

All the data values of variables expressed in mean and standard deviation (Mean±SD) using SPSS software version 8.0. The student unpaired *t* test was used to assess the significance of difference between the groups in rabbits. Correlation analysis was out using a person's test. A 'p' value less than 0.05 was considered statistically significant.

RESULTS

In the present study biochemical parameters of experimental rabbit groups were shown in Table-1, statically significant difference major change in the plasma glucose, Total cholesterol, HDL and LDL-cholesterol, serum insulin, serum Creatinine, 25-OH vitamin-C, and liver enzymes AST and ALT levels before and experiment. Whereas the significance difference in weight gained observed in high fat diet group (G-II) rabbits when compared to control (G-1) and Vitamin-C supplemented group (G-III) as shown in Table-1. Group-III animals showed decreased levels above mentioned parameters, then Group-II animals, Plasma lipids levels show significant difference between G-II and G-III. As shown in table-1 Triglycerides, plasma Total cholesterol, and LDL-cholesterol were significantly decreased in G-III when compared to G-II after the experiment. Figure-1 shows insulin resistance decreased significantly in group-III when compared to Group-II. Group-III animals are also shown in Figure-2&3 decreased levels of IL-6 and High sensitive C-reactive protein levels than the Group-II.

Table:1 Showing biochemical parameters

Variable	Group1	Group2	Group3
Weight	1.85±0.12	2.41±0.2	2.0±0.1
Glucose	136±6	208±18.0	159±5.6
Serum Creatinine	1.26±0.02	1.54±0.01	1.32±0.01
Serum AST (IU/L)	33±3.5	78.3±8.6	40.3±1.5
Serum ALT (IU/L)	36.6±6.5	73.6±7.6	54.6±4.7
Serum total cholesterol	31±5.2	800.3±50.0	670±36.7
Serum HDL- Cholesterol (mg/dl)	8.6±2.5	238.3±12.4	138.6±9.6
Serum LDL- Cholesterol (mg/dl)	19.6±2.5	541±24.5	365±15.9
Serum Triglycerides (mg/dl)	21.6±2.0	96±4.9	32.6±2.5
Serum fasting Insulin (mIU/ml)	0.96	3.1	0.87
Serum vitamin C (ng/ml)	0.33	0.21	1.48

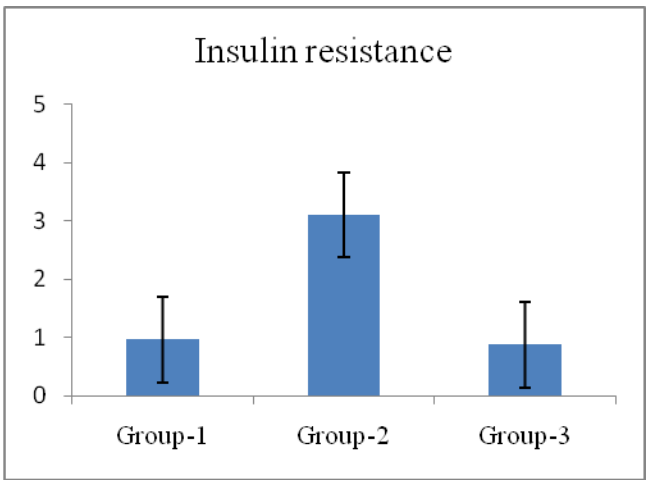

Figure-1. Insulin resistance

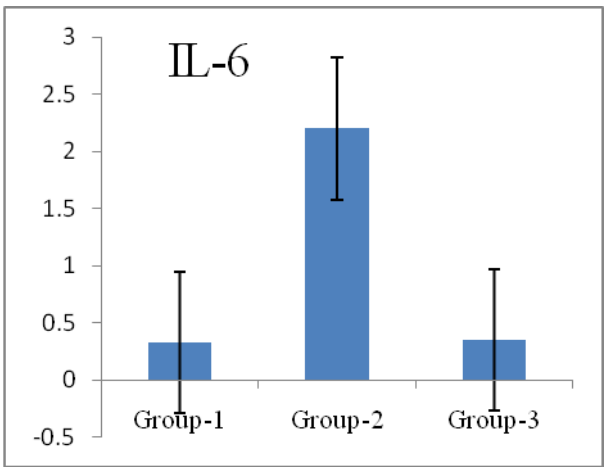

Figure-2. Interlequin-6

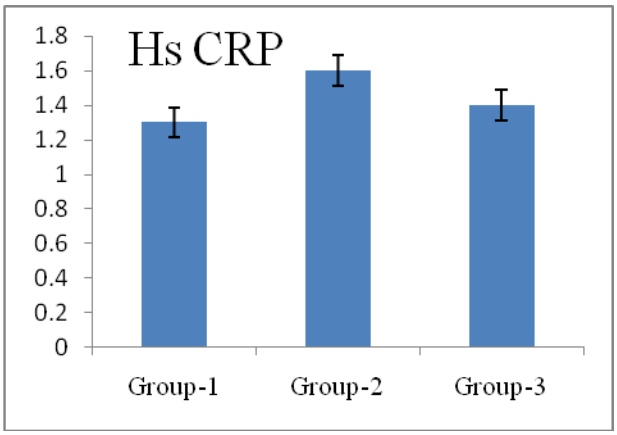

Figure-3. Highly sensitive C-reactive protein level (mg/dl)

Biosynthesis of Vitamin C in Atherosclerosis:

Several studies have shown that vitamin C plays a vital role in cardiovascular disease (Guo ZM et al, 2002). Usual experimental animals and humans can synthesize vitamin C and observe decreased collagen content in the atherosclerotic lesions, impair the biomechanical ability of the plaque and make it extensible prone to rupture (Papparella I et al, 2007).

In addition, vitamin C has potent antioxidant properties. Physiological concentrations of vitamin C can inhibit in vitro oxidative modification of LDL, a critical event during atherosclerosis (Libby P et al, 2002). Vitamin C readily scavenges reactive oxygen and nitrogen species and may thereby inhibit oxidative stress to important biological macromolecules such as DNA, lipids, and proteins (Freyschuss A, Stiko-Rahmt A 1993). The following proposal has been put forward guessing the mechanisms of vitamin C which has been shown to prevent apoptosis caused by cytokines in cultured endothelial cells exposed to hydrogen peroxide and UV-visible light (Tanner FC et al, 1991).
Experimental data on the effect of vitamin C supplementation of animal models in vitro LDL oxidation are sparse, mainly because ascorbate is removed from low-density lipoprotein during isolation from plasma (Amento EP et al, 1991). Ascorbate prevents oxidative modification of low-density lipoprotein primarily by scavenging free radicals and alternative reactive species in the aqueous milieus. Physiological concentrations of ascorbate powerfully inhibit LDL oxidation by vascular cells and neutrophils, as well as in cell-free system (Guo ZM et al, 2001). The interaction of vitamin C with 'free radicals' catalytically active metal ions could contribute to oxidative stress through the production of hydroxyl and alkoxyl radicals, where these mechanisms occur in vivo (Sasahara M et al, 1994).
The ascorbate-dependent addition of the polar hydroxyl group to the side chain proline and lysine may aid the self assembly and stability of the collagen fibril by forming interchain hydrogen bonds. The absence of sufficient vitamin C, a needed compound for prolylhydroxylase, thus impaired the construction of stable collagen (Witztum JL, Steinberg D 2001). Ascorbate may indirectly magnify endothelium dependent vasodilation by sparing intracellular thiols, which in turn stabilize endothelial nitric oxide (ENNO) through the formation of biologically active S-nitrosothiols (Pieper GM et al, 1997). Vitamin C has other anti-inflammatory effects as well, including increased synthesis and activities of nitric oxide (NO) in culture endothelial cells by increasing intracellular tetrahydrobiopterin (Stamler JS et al, 1992). Thus, a very likely mechanism by which intracellular ascorbate stimulates nitric oxide synthase (NOS) activity is the regeneration of tetrahydrobiopterin from the trihydrobiopterin radical. Vitamin C inhibits activation of nuclear factor -B (NF-B) (Heitzer T et al, 2000), a key regulator of decreased renal inflammatory cytokines and chemokines, renal immune cells, and arterial pressure and improved renal function and damage gene expression bioavailability (Marumo T et al, 1997).
Two recent studies have investigated the role of ascorbate in inhibiting leukocyte cell-cell adhesion to the vascular wall elicited and modification of the protein moiety of LDL (apolipoprotein B-100) (Dupont GP et al, 1992), either directly by leukocyte-derived oxidants such as hypochlorous acid or indirectly by lipid hydro peroxide breakdown products such as 4-hydroxynonenal and malonaldehyde (Carr AC et al, 2000,), results in a form of LDL that is internalized by macrophages via the scavenger receptor pathway leading to foam cell formation of oxidatively modified lipids with platelet- activating factor-like activity (Yang H et al, 2003). Although redox-active transition metal ions appear to play a pivotal role in cell-mediated LDL oxidation. Administration of ascorbate prevented the accumulation of this platelet activating factor and the subsequent leukocyte- endothelial cell interactions (Parthasarathy S et al, 1986).

DISCUSSION

Emerging scientific investigation has revolutionized our understanding of vitamin C and its crucial role in the modulation of cardiovascular, immunological, and metabolic process (Lehr HA et al, 1997). Researchers are investigating the possibilities that vitamin C may play a vital role in reducing risk of plaque buildup (Wannamethee SG et al, 2006). Vitamin C is an essential co-factor involved in many biochemical functions and act as an electron donor or reducing agent. Vitamin C effectively scavenges singlet superoxide, oxygen, hydroxyl, water-soluble peroxyl radical and hypochlorous acid. It is also reported to be an excellent source of electrons and therefore can donate electron to free radicals such as hydroxyl and super oxide radicals and quench their activity (Carr AC, Frei B, 1999). In addition to Vitamin C is a hydrophilic substance that acts as a nutritional effect on bone health and it is one essential in preventing scurvy (Upritchard JE et al, 2000). Therefore, vitamin C is a traditional effect on bone health and it is one of the important and essential vitamins for human health. It is needed for many physiological functions in human biology (Kushi LH et al 1996). Thus, though Vitamin C (ascorbic acid) was discovered in 17th century, the role of vitamin C important vitamin in human health and disease still remains a mystery in the view of many beneficial claims and contradictions. There are several hypotheses regarding the occurrence and prevention of atherosclerosis (Bendich A, Langseth L, 1995). Vitamin C can to some degree prevent the consequence of oxidized LDL, and provides NO synthase activity (Frei B et al, 1989). In our study total cholesterol and LDL cholesterol levels are decreased affect vitamin C supplementation.

C-reactive protein (CRP) in the blood is a marker of systemic inflammation. Higher levels of CRP may increase inflammation, blood pressure through a variety of biological effects in endothelial cells, which ultimately results in vasoconstriction and increased production of endothelial-1 (Sarah de F, Nader R 2002). It is synthesized and secreted primarily in hepatocytes and regulated by interleukin-1, interleukin-6, and tumor necrosis factor-alpha (TNF-α) (Kushner I, Feldmann G 1978). Additionally, the vitamin c was shown to suppress the secretion of anti inflammatory and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin (IL-6) and also it inhibits the cell adhesion process and decreases the expression of various adhesion molecules, leukocytes and chemokines by endothelial cells, both in vitro and in vivo. Elevated levels of serum CRP have been reported in atherosclerosis and diabetes other cardiovascular diseases (Carr AC, Frei B 1999).

Moreover, the ability of vitamin C to decrease apoptosis in oxidative and inflammatory conditions has been shown to be the result of prevention of cytochrome C release from mitochondria and prevention of the activation of caspase 9 (Wells W.W, and Jung C. 1997). Controversy exists as to whether free fatty acid (FFA) or glucose is the primary fuel source in the over nourished muscle and adipose tissue (Baron AD 1994). An increase in intracellular FFA, in turn, leads to reduced GLUT4 translocation to the plasma cell, resulting in resistance to insulin stimulated glucose uptake in muscle and adipose tissue (Hirashim O et al, 2000). Glucose and Free fatty acid overload may be supposed to influence endothelial cells, as well as cells, producing an endothelial dysfunction thorough an oxidative pressure (Paolisso G, Giugliano D 1996). Impaired insulin secretion has been associated with an FFA-induced increase in ROS, both in vitro and in vivo (Gopaul NK et al, 2001). In this setting, insulin resistance may be considered a compensatory mechanism that protects the cells against further insulin stimulated glucose and fatty acid uptake and therefore oxidative damage. Insulin resistance is obtained with impaired endothelial function (DeFronzo RA et al, 1979). Interestingly, it has been reported that both free fatty acids and glucose may impair insulin secretion in cells by activating uncoupling of protein 2 (Perticone F et al, 2001).

Guinea pig and rabbit develop atherosclerosis in vitamin-C deficiency model (Witztum JL 1991). Anti-atherosclerosis benefits of vitamin C supplementation on atherosclerosis in rabbits were observed (Ginter E, 1978). An animal study that vitamin C can show the progression of experiment in cholesterol induced atherosclerosis Rabbits. Rabbits develop atherosclerosis with high level of serum β VLDL levels. Vitamin C may decrease serum cholesterol and increase HDL. Vitamin C is needed for cholesterol synthesis and the conversion of cholesterol to biological mechanism. It modulates lipoprotein lipase thereby affecting triglyceride levels, decrease LDL and LDL oxidation. It has shown beneficial effects on platelet and endothelial- derived relaxing factor (EDRF) (Lynch S.M et al, 1996,). Our study has shown a reduction of total serum LDL cholesterol, triglycerides there also decrease levels of measured IL-6 and CRP. This may be due to decreased LDL oxidation, resulting anti inflammatory (Sean M et al, 1996,).. Insulin resistance may be reduced into anti-inflammatory effect (Yudkin JS et al, 1999). In some studies suggest that the atheroprotective effects of vitamin C are not restricted in the carotid artery wall, nor was it able to attenuated coronary atherosclerotic progression (Carr AC et al, 2000).

Our study was aimed to assess various biological parameters for investigating to determine whether this observation is relevant to animal study, we tested the hypothesis that vitamin C anti-inflammatory and hypolipidemic effect developing the insulin sensitivity with a beneficial role in atherosclerosis.

CONCLUSION

Vitamin C acts as an antioxidant under biochemical conditions in atherosclerosis. There are several hypotheses regarding the occurrence and prevention of atherosclerosis. Oxidized LDL appears to contribute to endothelial dysfunction by reducing endothelial NO bioavailability and producing ROS.

Conflict of interest:

The author has none to declare.

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