

Physiological and Biochemical Studies of β -Carotene on Thrombosis and Thrombosis

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Abstract: β -Carotene is one of antioxidant micronutrients that can be favorably manipulated without side effects. Basic research provides a plausible mechanism by which antioxidant is associated with reducing the risk of atherosclerosis. The purpose of this study was to understand better the effects of β -carotene on atherosclerosis and thrombosis by using the atherosclerotic rabbit model. Twenty-four New Zealand White rabbits were divided into 4 groups: normal rabbits as control-control (Group I, n=4); atherosclerosis, non-thrombus triggered and non- β -carotene treatment as control (Group II, n=4); atherosclerosis, thrombus triggered by Russell's viper venom and histamine but non- β -carotene treatment (Group III, n=8); atherosclerosis, thrombus triggering and β -carotene (30 mg/kg, i.v.) treatment (Group IV, n=8). Atherosclerosis was induced by a balloon surgery with feeding a high cholesterol diet (1%). After rabbits were sacrificed isolated carotid arteries were placed in a dual perfusion chamber and tissues were kept in liquid nitrogen for biochemical measurements. Both carotid arteries from each rabbit were perfused with oxygenated physiologic buffered solution at 37°C and 60 mmHg. Baseline vasodilation was determined using norepinephrine (NE, 1×10^{-6} M) precontraction, and pharmacological challenge was performed with acetylcholine (Ach, 1×10^{-5} M) and sodium nitroprusside (SN, 1×10^{-5} M). Vessel diameter was measured by a computer planimetry system. Metallothionein, glucose, protein and glucose-6-phosphatase in the tissues and serum were measured after the rabbits were sacrificed. β -Carotene reduced thrombosis triggering. The vasodilation activity of artery responded to norepinephrine, acetylcholine and sodium nitroprusside was Group I>Group II>Group IV>Group III (inter-group ratio of the arterial vasodilation after pharmacological challenge was 1.2-3.5, $p < 0.01-0.05$). β -Carotene reduced liver, kidney and heart metallothionein content of atherosclerotic rabbits. Group III had higher serum glucose content than Group IV. There was no significant difference of glucose and protein content in aorta arteries among 4 groups. β -Carotene has potential pharmacological effects on atherosclerotic animal. [Nature and Science. 2006;4(3):58-64].

Keywords: atherosclerosis; β -carotene; thrombosis; vasoconstriction

Abbreviation: Ach: acetylcholine; EDTA: ethylenediamine-tetraacetic acid; G-6-Pase: glucose-6-phosphatase; LDL: low-density lipoproteins; MT: metallothionein; NE: norepinephrine; PBS: physiologic buffered saline solution; RVV: Russell's viper venom; SEM: scanning electron microscopy; SN: sodium nitroprusside; TEM: transmission electron microscopy

1. Introduction

The model of plaque disruption and thrombosis developed by Constantinides et al in the early sixties was based on simulating the conditions often thought to be the main triggers for acute cardiovascular events (Constantinides, 1981). These include a hypercoagulable state and vasospasm. Based on this hypothesis various agents were tested independently and in combination to achieve a disrupted plaque with a superimposed platelet rich thrombus overlying a disrupted plaque. These included epinephrine and vasopressin etc. However, the best combination includes histamine, a vasoconstrictor agent in rabbits and Russell's viper venom (RVV), an activator of the coagulation Factors Va and Xa. Each alone could not produce a significant number of events but in combination resulted in about 72% event rate (Hung,

2002). The original model called for a two-year preparation by feeding rabbits a high cholesterol diet alternating with a normal diet. This double lipemic exposure approach seemed to create a lesion similar to that seen in humans, a lipid rich core with a fibrous cap. At the end of this period, RVV and histamine were given at 48 and 24 hours prior to sacrifice to induce a trigger for plaque disruption and thrombosis. A modification of the model was made by endothelial debridement to accelerate the atherosclerotic process as described by Baumgartner et al (2000). Using this approach it was possible to prepare the rabbits within a 6 months period and maintain the same event rate demonstrated by Constantinides (1989). Because it is not entirely clear what effects the triggering has on the vasomotor function in this model, we tested arteries by pharmacological challenges in a dual organ chamber. β -

Carotene is one of antioxidant micronutrients that can be favorably manipulated without side effects. Basic research provides a plausible mechanism by which antioxidant is associated with reducing the risk of atherosclerosis. To understand better the effects of β -carotene on atherosclerosis and thrombosis, we used β -carotene on the atherosclerotic rabbit model to try and see if it could change the event rate and alter the vasomotor function.

2. Materials and Methods

2.1 Study Groups

Twenty-four, male, New Zealand White rabbits weighing between 2.5 to 3.2 kg were divided into 4 groups (Table 1). The method of establishing the atherosclerotic rabbit model and thrombus triggering were described previously.

2.2 Atherosclerosis Inducing

Briefly, the control-control group (Group I) consisted of four normal white rabbits that were fed a regular diet for 6 months. Rabbits in Groups II, III and IV (n= 4, 8 and 8, respectively) underwent balloon deendothelialization and were then maintained on a 1% cholesterol enriched diet alternating with regular diet every month for a total of 6 months. Under general anesthesia (ketamine 50 mg/kg and xylazine 20 mg/kg, i.m.) balloon-induced deendothelialization of the aorta was performed using a 4F Fogarty arterial embolectomy catheter introduced through the right femoral artery cutdown. The catheter was advanced in a retrograde fashion to the ascending aorta and pulled back three times.

2.3 Pharmacological Triggering

Only the atherosclerotic rabbits (Groups II, III and IV) had pharmacological triggering since previous studies have not shown thrombosis to occur in normal arteries. Thrombus triggering was induced by RVV (0.15 mg/kg, i.p., Sigma Chemical Co., St. Louis, MO, USA) and histamine (0.02 mg/kg, i.v., 30 min after RVV, Sigma Chemical Co., St. Louis, MO, USA) given at 48 and 24 hours prior sacrifice. In Group IV, β -carotene (30 mg/kg, i.v., BASF Corporation, Mount Olive, NJ, USA) given 8 days prior to sacrifice. Heparin sulfate (1000 IU/rabbit, i.v., Sigma Chemical Co., St. Louis, MO, USA) was given 30 min prior to sacrifice to prevent postmortem clotting. Rabbits were sacrificed with an overdose i.v. injection of pentobarbital (Abbot Laboratories, North Chicago, IL, USA). Tissue samples from the heart, liver and kidney were stored immediately in liquid nitrogen until biochemical measurements.

2.4 Quantitation of Thrombosis

The total surface area of the aorta, the surface area of aorta covered with atherosclerotic plaque and the surface area of aorta covered with *ante mortem* thrombus were measured. The surface area was measured from images obtained by a digital camera. The digitized images were calibrated by use of a graticule. Surface area was measured by use of a customized quantitative image analysis package. Also, the number of thrombi on the aortic arch to the distal common iliac branches was counted.

2.5 Artery Diameter Respond Evaluation

After rabbits were sacrificed both carotid arteries were isolated from each rabbit and placed in a dual organ chamber and perfused with oxygenated physiologic buffered solution (PBS) (NaCl 119 mM, KCl 4.7 mM, CaCl₂ 2 mM, NaH₂PO₄ 1.2 mM, MgSO₄ 1.2 mM, NaHCO₃ 22.6 mM, glucose 11.1 mM and Na₂EDTA 0.03 mM) at 60 mmHg and 2.5 ml/minute flow rate at 37°C. Baseline vasodilation was determined using norepinephrine (NE, 1×10^{-6} M) precontraction and pharmacological challenge was then performed with acetylcholine (Ach, 1×10^{-5} M) and sodium nitroprusside (SN, 1×10^{-5} M) successively. Vessel diameter was measured by a computer planimetry system (Figure 1). The data were calculated according to the formulas: Ach-NE (%)=(Ach-NE)/NE \times 100 and SN-NE (%)=(SN-NE)/NE \times 100 separately, where Ach, NE and SN represented the diameter (mm) of the arteries that were perfused by the PBS containing a corresponding chemical.

2.6 Metallothionein (MT)

Metallothionein concentration as an index of oxidation was measured with Cd-hemoglobin saturation method (Eaton, 1991). Tissues were removed and rinsed in ice-cold Tris-HCl buffer (0.05 M, pH 8.6) then homogenized in 3 volume of the Tris-HCl buffer. The homogenate was centrifuged at 8,000 \times g for 10 minutes at 4°C and the supernatant fraction was heated for 90 seconds at 100°C. The heated samples were centrifuged at 8,000 \times g for 5 minutes at 4°C to remove precipitates. 100 μ l of 400 ppm CdCl₂ solution was added into 200 μ l of the supernatant and allowed to incubate at room temperature for 10 minutes. 150 μ l of 2% bovine hemoglobin solution (w/v) was added into the sample, then the sample was mixed and heated in boiling water for 2 minutes. The boiled samples were placed in ice for 3 minutes and centrifuged at 8,000 \times g for 5 minutes at 4°C. Another 150 μ l of 2% hemoglobin was added into the supernatant, then heating, cooling and centrifuging were repeated, and the supernatant was collected. The Cd concentration in the supernatant was measured using a flame atomic absorption equipment and MT

concentration was calculated from the Cd concentration measured in the supernatant (1 mg Cd represented 8.93 mg MT).

2.7 Glucose-6-phosphatase (G-6-Pase)

G-6-Pase measurement was followed Harper method (Harper, 1965). Briefly, 0.1 ml of tissue homogenate (100 mg tissue/ml) in citrate buffer (0.1 M, pH 6.5) was added into a test tube and incubated at 37°C for 5 minutes. 0.1 ml of glucose-6-phosphate (0.08 M) was added and the sample was incubated at 37°C for 5 minutes, then 5 ml of trichloroacetic acid (10%, w/v) was added and centrifuged at 9,000 \times g at 4°C for 5 minutes. 1 ml of the supernatant was taken into a test tube and 5 ml of ammonium molybdate solution (2 mM) then 1 ml of reducing solution (42 mM 1-amino-2-naphthol-4-sulphonic acid, 560 mM SO₃) was added. The sample was incubated at room temperature for 30 minutes then absorption was measured at 660 nm.

2.8 Glucose Concentration

Sigma Glucose Diagnostic Kit (Sigma Chemical Co., St. Louis, MO, USA) was used. The method of the instruction by Sigma was followed for this evaluation.

2.9 Light and Electron Microscopy

Arterial tissue specimen were examined with light and electron microscope.

2.10 Statistical Analysis

With Jandel Scientific program, SigmaStat (Sigma Chemical Co., St. Louis, MO, USA) was used for data statistical analysis. $P < 0.05$ was considered statistically significant difference. Measured data were reported as mean \pm SD. The student t-test was used for comparison.

3. Results

3.1 Aorta

The measurement results of the rabbit aortas are showed in Table 2. The average surface area of aorta of the four groups was 1660 \pm 257 mm². There was no significant difference for surface area of aorta within the four groups (Table 2).

All the rabbits that were fed a 1% cholesterol diet and underwent endothelial debridement had visible diffuse atherosclerosis. Half of triggered rabbits developed thrombosis. The ratio of the thrombus surface area on the aorta in Group III rabbits to that on the aorta in Group IV rabbits was 1.56 (78.6 \pm 38.9 vs 50.3 \pm 25.9, $p < 0.05$). Group II rabbits developed atherosclerosis, but no thrombus because rabbits of this group had no thrombus triggering by RVV and histamine. Table 2 also showed that the number of thrombi in Group III rabbits was two times as that in β -carotene treatment

rabbits (Group IV) (5.0 \pm 4.3 vs 2.3 \pm 1.3, $p < 0.05$). Group I rabbits got neither atherosclerosis nor thrombus.

The average of glucose and protein content in aorta of the 4 rabbit groups were 1.3 \pm 0.3 mg/g and 10.1 \pm 0.8 mg/g respectively. There were no significant difference for glucose and protein content among the 4 rabbit groups (Table 2).

3.2 Artery Diameter

In this experiment, the contraction diameter of rabbit left and right carotid arteries using NE precontraction and pharmacological challenge with Ach and SN were measured (Table 3). There was no significant difference between left and right carotid arteries. The data in Table 3 were the average of left and right carotid arteries.

The vasodilation range of pharmacological challenge with Ach and SN to NE was 10-40% and it represented the following order: Group I > Group II > Group IV > Group III. Inter-group ratios of the arterial vasodilation after pharmacological challenge were 1.2 to 3.5 for Ach-NE ($p < 0.005$ to $p < 0.05$) and 1.1 to 3.2 for SN-NE ($p < 0.01$ to $p < 0.05$) (Table 3).

3.3 Metallothionein (MT)

MT in liver, kidney and heart tissues of Group III rabbits was significantly higher than that in Group I (ratios were: liver 3.3, $p < 0.03$; kidney 4.3, $p < 0.02$; heart 8.9, $p < 0.05$), Group II (ratios were: liver 1.9, $p < 0.05$; kidney 1.6, $p < 0.05$; heart 1.6, $p < 0.05$) and Group IV (ratios were: liver 2.5, $p < 0.03$; kidney 1.3, $p < 0.05$; heart 2.2, $p < 0.05$) rabbits. There were no significant differences for MT content among the 4 groups in aorta, carotid and femoral arteries (Table 4).

3.4 Glucose, Protein, Glucose-6-phosphatase (G-6-Pase) and Lipid-liver

The results of glucose in the blood, total protein in the blood and in liver tissue, the activity of G-6-Pase in the liver tissue and lipid-liver disease happening were evaluated and shown in Table 5.

The serum glucose content in the 4 groups was Group III > Group II > Group IV > Group I ($p < 0.05$ -0.01). Serum glucose of Group III rabbits was significantly higher than that of Group IV rabbits ($p < 0.03$) and there was no significant difference between Group II and Group III rabbits. There was no significant difference for the both serum and liver protein content among the 4 rabbit groups. Liver G-6-Pase of non- β -carotene rabbits was significantly higher than that of other 3 groups ($p < 0.05$) and there was no significant difference for liver G-6-Pase among the 3 groups. Serum cholesterol of Group I was significantly lower than that of the other 3 groups and there was no significant difference among the 3 groups. About a half of atherosclerotic rabbits (38-50%) got lipid-liver

disease and there was no lipid-liver happening in the Group I (Table 5).

3.5 Light and Electron Microscopy Observation

Thrombi were observed on the atherosclerotic rabbit aortas after the thrombus triggering. Using the electron microscopy observation, the plaques, irregular cell surface and ulceration with blood cells and clotting fibers could be seen on the lamina surface of the endothelium layer. It seems that there was no significant change could be observed between Group IV and Group III. Comparing with Group I, all treated groups could be

seen increased elastin number, degenerated organelles, irregular density, bundles of microfibrils, some longitudinal and others in cross-section. These changes were more prominent in Group III than Group IV. Endothelium can be seen clearly attaching on thick internal elastin lamella in Group II and Group IV, but it was hardly to be found in Group III. In Group IV, prominent vacuoles were presented which were considered as lipid. In Group III, the impression of morphological architectural and subcellular organs seems to be more irregular and imparity than that in the droplets of rest groups.

Table 1. Preparation of the four rabbit groups

Groups	Treatments	n	Normal Diet	Cholesterol Diet (1%)	Balloon Trauma	RVV and Histamine	β -Carotene
I	Control-control	4	Yes	No	No	No	No
II	Control	4	Yes	Yes	Yes	No	No
III	Non- β -carotene	8	Yes	Yes	Yes	Yes	No
IV	β -Carotene	8	Yes	Yes	Yes	Yes	Yes

Table 2. Evaluations of rabbit aortas

Groups	Surface Area (mm ²)	Weight (g)	Thrombus Surface (mm ²)	Thrombus Number	Glucose Content (mg/g)	Total Protein (mg/g)
I	1550±181	1.62±0.55	0	0	1.1±0.1	10.4±1.7
II	1881±233	3.92±0.28	0	0	1.0±0.4	10.0±2.0
III	1664±285	3.48±0.21	78.6±38.9	5.0±4.3	1.4±0.3	11.0±1.1
IV	1545±193	3.76±0.46	50.3±25.9	2.3±1.3	1.6±0.9	9.1 ±1.1
Average	1660±257	3.20±1.07	32.2±39.0	1.8±2.4	1.3±0.3	10.1±0.8

Table 3. Diameter of rabbit carotid artery response to vascular regulating chemicals (mm)

Groups	NE (1×10 ⁻⁶ M)	Ach (1×10 ⁻⁵ M)	SN (1×10 ⁻⁵ M)	Ach-NE (%)	SN-NE (%)
I	13.98±2.34	18.21±2.81	18.83±2.45	35.27±29.76	39.38±26.07
II	18.56±7.16	20.94±2.98	21.88±2.96	16.80±14.91	20.30±15.22
III	18.05±3.39	19.68±3.64	20.21±3.62	9.98±9.68	12.43±11.84
IV	16.52±3.22	18.75±3.32	19.16±3.29	14.30±10.02	16.93±11.37
Average	16.78±2.06	19.40±1.20	20.02±1.37	19.09±11.15	22.26±11.86

Ach-NE (%)=(Ach-NE)/NE×100; SN-NE (%)=(SN-NE)/NE×100. Ach, NE and SN here are diameter (mm) of the arteries perfused by the PBS containing a corresponding chemical.

Table 4. Metallothionein in rabbit tissues ($\mu\text{g-MT/g-wet tissue}$)

Groups	Liver	Kidney	Heart	Aorta artery	Carotid artery	Femoral artery
I	136.6 \pm 54.4	46.3 \pm 0.6	6.4 \pm 0.5	4.0 \pm 0.3	4.5 \pm 0.8	3.3 \pm 0.7
II	239.8 \pm 46.1	126.5 \pm 17.1	36.1 \pm 25.1	8.0 \pm 2.9	4.9 \pm 1.3	5.1 \pm 0.9
III	447.5 \pm 200.1	200.9 \pm 123.7	56.7 \pm 21.7	5.9 \pm 0.5	5.4 \pm 0.9	4.3 \pm 0.7
IV	189.5 \pm 50.5	149.3 \pm 15.4	25.5 \pm 2.6	4.2 \pm 0.3	5.8 \pm 1.1	4.1 \pm 0.9
Average	274.6 \pm 158.4	120.8 \pm 158.4	31.2 \pm 21.0	5.5 \pm 1.9	5.2 \pm 0.6	4.2 \pm 0.7

Table 5. Glucose, total protein, glucose-6-phosphatase and lipid-liver

Groups	Serum Glucose (mg/dl)	Serum Protein (mg/ml)	Liver Protein (mg/g)	Liver G-6-Pase (mol/min/g)	Serum Cholesterol (mg/l)	Lipid-liver Disease (%)
I	101.55 \pm 4.27	35.91 \pm 0.29	13.48 \pm 2.76	10.47 \pm 3.06	1837 \pm 306	0
II	151.59 \pm 34.75	32.44 \pm 4.61	11.37 \pm 1.14	11.28 \pm 2.67	4208 \pm 712	50
III	160.41 \pm 20.41	33.07 \pm 1.75	12.13 \pm 2.31	16.34 \pm 3.51	4196 \pm 615	38
IV	136.46 \pm 13.95	33.04 \pm 1.43	11.56 \pm 3.10	12.02 \pm 2.19	4257 \pm 683	38
Average	137.50 \pm 25.93	33.63 \pm 1.56	12.14 \pm 0.95	12.53 \pm 2.62	3625 \pm 1192	32

Discussions

Atherosclerosis, or "hardening of the arteries", is the process that causes heart attacks and most strokes. It is characterized by the progressive build-up of fatty plaques in blood vessels. One major component of the atherosclerotic plaque is cells loaded with cholesterol called foam cells. It is currently believed that cholesterol, especially the low-density lipoproteins (LDL), must be modified or oxidized before they can be taken up to cause foam cells. Antioxidants such as vitamin C, vitamin E, and carotenoids can prevent the oxidative modification of LDL in the laboratory. This has given rise to the concept that these vitamins could decrease the risk of heart disease by preventing oxidation of LDL in the body. Myocardial infarction in human cases a triggering activity such as physical exertion precipitates the acute onset of the disorder (Mittleman 1993; Muller 1989; Tofle1990), but it is difficult to be studied in human. Therefore, a suitable animal model has been created recently (Abela, 1995). This study demonstrated that vulnerable plaques could be produced, the plaque disruption and platelet-rich arterial thrombus formation could be triggered pharmacologically in the atherosclerotic rabbits that has been described in a previous paper (Abela, 1995). Rabbits in the three groups which were balloon induced arterial injury and then maintained in an alternative 1% of cholesterol diet for a total of six months clearly caught atherosclerosis. The aorta artery weight of the atherosclerotic rabbits was 2.25 times heavier than that of normal rabbits ($p < 0.01$) (Table 2). This model is a useful method to get atherosclerotic animal for the

related scientific research purpose.

When cells use oxygen for energy purpose, they produce by-products called free radicals. Free radicals damage cells and tissues during a process called oxidation - a factor in many chronic illnesses, including some forms of cancer, cataracts, arthritis and cardiovascular disease. LDL, known as the "bad cholesterol", is actually a protein that carries cholesterol throughout the body. The cholesterol carried by LDL deserves its bad reputation, however. It often ends up in our arteries, causing clots that can lead to heart attacks. Oxidation of LDL-cholesterol contributes to the plaque build-up in arteries, a process called atherosclerosis that can cause blockages and reduced blood flow. The process also plays a role in the loss of elasticity in arteries. Antioxidants help to neutralize free radicals and prevent them from causing cellular damage. Once oxidized, the cholesterol is less apt to be expelled by the body's cleaning mechanisms and more likely to be stored in arteries. Taking antioxidants, such as β -carotene, may lower a person's risk of developing heart disease. Free glucose in blood is the important energy resource and precursor resource for the various requirements including anti-oxidation in animal body. One of the problems for atherosclerotic rabbits is that their free radical and oxidation conditions are changed under the disease. Free radical modification of serum that is not the solely increased level of lipoprotein oxidation products in blood lipoproteins is an important cause for cholesterol accumulation in cells, and apparently for their transformation into foam cells during atherosclerosis (Jacob, 1996). Once altered by

free radical oxidation, plasma lipoproteins undergo dramatic change, both in the manner in which they can interact with cells and in the ways in which they influence cell functions (Chisolm, 1991). This study showed that the average aorta weight of Groups II, III and IV was about 2.25 times heavier than that of Group I ($p < 0.01$) and the average total aorta cholesterol of Groups II, III and IV was about 6 times as that of Group I ($p < 0.01$) (Table 2). One of the reasons that β -carotene improves the atherosclerotic condition in experimental rabbits maybe that β -carotene balances the adverse function of free radical and oxidation from high cholesterol.

In animal liver, glycogen is broken down into glucose-1-phosphate by liver phosphorylase and then converted into glucose-6-phosphate. Glucose-6-phosphate is dephosphorylated by G-6-Pase to yield free D-glucose, which passes into the systemic blood to be transported to other tissues. The non- β -carotene rabbits have higher free glucose concentration in the blood hints that they have higher requirement for the free glucose. For this phenomenon, the explanation is not clear, and the energy metabolism may be involved. The β -carotene treatment let the glucose concentration down hints that β -carotene compensates the requirement of the free glucose amount (Table 5).

MT is a ubiquitous class of low molecular weight and cysteine-rich proteins (about 1/3 of amino acid in MT is cysteine) binding unusually high amounts of metal ions, such as Ag, Cd, Cu, Hg and Zn. The most conspicuous biological feature of MT is its inducibility by a variety of agents and conditions. The biosynthesis of MT is induced by various factors such as heavy metals, certain hormones, cytokines, growth factors, tumor promoters, coldness, heat, hunger, radiation and diseases (Brady, 1982). Most of the inducing factors of MT biosynthesis are adverse factors. MT is thought to play an important role the homeostasis of metal ions and to be involved in the detoxification of heavy metals, scavenge free radical, etc. (Kagi, 1991). The accurate functions of MT in atherosclerosis are not clear. One study examined the supplementation of β -carotene on metal ion-dependent and metal ion-independent induced oxidation of LDL and it showed that β -carotene would not inhibit the LDL oxidation (Gaziano, 1995). The phenomenon that the non- β -carotene rabbits have higher MT level in the various tissues shows that the balloon-induced arterial injury, high cholesterol diet and injection of RVV/histamine could enhance the biosynthesis of MT or keep MT from the degradation. That the amount of MT in the Group IV was similar to that in Group II and Group I rabbits but much lower than that in Group III rabbits showed that β -carotene made some compensation function for the MT induced requirement, such as free radical scavenge and anti-

oxidation (Table 4).

In this study, the results showed that the arteries from β -carotene treatment rabbits had the similar contraction response to Ach and SN as the arteries from control-control and control rabbits (Table 3). Since those rabbits are in later atherosclerotic stage, it seems to that the β -carotene shows a protective function on the balloon-induced arterial injury and a 1% cholesterol diet treatment is not only in the early stage of atherosclerosis but also in the late stage of atherosclerosis. Explanation seems to be involved in endothelium function as reported before (Abela, 1995). The vascular endothelium plays a central role in the regulation of vascular function. In particular, the local release of endothelium-derived relaxing factor regulates vascular tone and prevents platelet adhesion to the vascular wall. The atherosclerotic rabbits with hyper-cholesterol ichemia can significantly induce endothelium dependent relaxation function (Jayakody, 1988). Also there was a report showing that imbalance vascular oxidative stress and antioxidant protection is involved in the development of the vascular dysfunction in atherosclerosis (Keaney, 1995). The antioxidative function of β -carotene seems to effecting on the activity of the endothelium in the atherosclerotic rabbit arteries.

The activity of G-6-Pase in liver of these four rabbit groups had the consistent magnitude result with glucose content in blood (Table 5). This hinted that the alteration of glucose level in blood was adjusted by the activity of G-6-Pase in liver. It was not clear whether the β -carotene inhibited G-6-Pase expression or inhibits the activity of G-6-Pase, or other more complex procedure.

Carotenoid pigments are widely distributed in nature, where they play an important role in protecting cells and organisms against oxidation and free radical (Palozza, 1992). As the most important one of the carotenoid pigments, β -carotene is the most well known antioxidant of the carotenoid family. Carotenoids are natural plant pigments that produce the bright and vibrant colors that associate with fresh fruits and vegetables. β -Carotene supplies the bright orange color of carrots, apricots, cantaloupe, sweet potatoes and acorn squash. In addition to giving plants a bright color, β -carotene is a potent antioxidant. Epidemiological studies have shown the more fruits and vegetables we eat, the lower our risk of heart disease se will get. A strong association between β -carotene consumption from foods and risk of heart disease has also been described.

Our study suggests that β -carotene has important function in prevention of atherosclerotic plaque disruption and arterial thrombosis in atherosclerotic rabbit model. It is provided pilot information for management in human atherosclerosis.

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