Morphology of Aortic Arch in Rabbits with Atherosclerosis Treated with Resveratrol

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KEY WORDS: morphology, aortic arch, rabbit, atherosclerosis, resveratrol.

ABSTRACT

Objective: Atherosclerosis is a chronic inflammatorycondition associatedwith the production of oxidative species. The phenoliccompound, resveratrol, seems to have cardioprotective activities preventing the oxidation of low-density lipoproteins.

In this study we investigated the effect of resveratrol on prevention of induced atheromatosis, through the morphological study of the segment of aortic arch in White New Zealand rabbits.

Study design: 20 rabbits were divided into four groups which received the following diet for 60 days: control group (CT) normal ration; resveratrol group (R) normal ration and resveratrol (3 mg/kg/day); cholesterol group (CL) 1.5% of cholesterol added to the ration; group cholesterol plus resveratrol (CR) 1.5% of cholesterol added to the ration and resveratrol (3 mg/kg/day). The analysis of the atherosclerotic lesions were performed by the means of appropriate histological techniques.

Results and conclusions: The animals belonging to group CL showed atherosclerotic lesions with tunica intima thickening due to the presence of foam cells, placed in several disorganized layers, and extracellular lipid droplets in subendothelial conjunctive tissue.

We also observed the invasion of foam cells in the beginning of tunica media. In animals belonging to group CR there were changes in the subendothelial of tunica intima, although in a minor degree of development as for the number of foam cells layers and extracellular lipid droplets. An invasion of foam cells in tunica media was observed in this group. We haven't seen any changes in tunica adventitia in any of the studied groups. There were not evident histological changes in any of the analised tunicas for groups CT and R

Conclusions: Tthis studymay help demonstrate that the phenolic compound, resveratrol, worksas a preventive agent in the development of atherosclerotic lesions.

INTRODUCTION

Presently, cardiovascular disease (CVD) is the main causes of morbidity and mortalityand is among the most important health problems facing the adult population in the developed world. Geographically, the incidence and mortality rates vary tremendously with the Mediterranean area hving the smallest rates in the world. The World Health Organization (WHO) predicts that

global economic prosperity and westernization may exacerbate the problem n developing countries (PEREZ et al.,[1] 1998).

Atherosclerosis is a pathologic condition that precedes most of the cardiovascular episodes, named myocardial infarction and stroke (COTRAN & SCHOEN,[2] 2000).

The atherosclerotic plaques are formed mainly by cells, including smooth muscle cells, macrophages and other leucocytes; extracellular matrix of conjunctive tissue, including collagen, elastic fibers, proteoglycans and intra and extracellular lipid deposits. The typical atheromata contain a relatively abundant quantity of lipids that invade tunica intima and tunica media and in advanced lesions tunic adventitia in medium and big-caliber arteries, especially those located in high blood pressure areas. The development of lesions is affected by many systemic factors, such as hyperlipidemia, high blood pressure, obesity, sedentary lifestyle, diabetes, hemodynamic factors, smoking, among others (COTRAN & SCHOEN,[2] 2000).

Studies have demonstrated that the formation of Oxygen Reactive Species (ROS) contributes to the atherogenesis and progression of cardiovascular diseases. The exposure of macromolecules, such as lipids: mainly low density lipoproteins (LDL), proteins and nucleic acids to ROS results in oxidative changes with potential harmful substances. (HALIWELL & GUTTERIDGE,[3] 1999).

The oxidative theory of atherosclerosis says that when the LDL is excessively oxidized it would promote the atherosclerosis, because the native LDL is not pathogenic. Since oxidized lipids were discovered in fatty striation and experiments with antioxidants have demonstrated to reduce the formation of lesions, it was evidenced that these lipids played an important role in atherogenesis. (CHEMELLO & GUERRA,[4] 2003). Therefore, we observed that diet antioxidants have been attracting some attention as preventive and therapeutic agents. Countless in-vitro and in-vivo studies with animal models show that the use of antioxidants may prevent and delay atherosclerosis progression.

The phenolic compounds, including resveratrol, may function as active antioxidants, donating hydrogen to free radicals, and also as prevention, avoiding lipid peroxidation (LDL) and inhibiting oxidative enzymes (phospholipase A2, cyclooxygenases and lipoxygenases). Besides that the phenols may actuate as protectors and regenerators of primary antioxidants from the organism such as ascorbic acid (vitamin C), tocopherol (vitamin E) and beta-carotene (vitamin A) (FUHRMAN & AVIRAM,[5] 2001).

The aim of this paper was to evaluate the effect of resveratrol as therapeutic agent in preventing induced atherosclerotic lesions in rabbits, through morphologic studies of intima, media and adventitia tunics in initial region of aortic arch. Besides that, its purpose was to provide information to professionals of human and veterinarian health areas, since some breeds of cats and dogs are susceptible to hypercholesterolemy and primary hypertriglyceridemia consequently may develop atherosclerosis.

MATERIAL AND METHODS

Chemical compounds: Resveratrol was acquired from Pharma Nostra (Rio de Janeiro, RJ), Cholesterol was acquired from Vetec (Duque de Caxias, RJ). The maintenance diet was acquired from Purina (Paulinia, SP) and diet containing 1,5% of cholesterol was formulated by UNESP Nutrition Department, the animals were kept in the biotery of Animal Physiology and Morphology Department of FCAV, Jaboticabal Campus – UNESP, during a period of 60 days. The experiment was realized according to the approval of COMISSÃO DE ÉTICA E BEM ESTAR ANIMAL (CEBEA) (Ethics and Animal Welfare Commission) from Universidade Estadual Paulista, Jaboticabal Campus – UNESP.

Methodology: In this study we used twenty adult male rabbits, with average weight of 2.5 kg, hybrid of White New Zealand breed, from March 15 to May 15

2007. The animals were previously fed with normal ration during one week. After, were then divided into four experimental groups formed by 5 animals each that received the following nourishment: white control group (CT) received maintenance ration; group (R) maintenance ration and simultaneous oral administration of resveratrol (3 mg/kg/day); group (CL) 1.5% of cholesterol added to the maintenance ration; group (CR) 1.5% of cholesterol added to the maintenance ration and simultaneous oral administration of resveratrol capsules (3 mg/kg/day). To prepare the hypercholesterolemic diet powder cholesterol was added to previously ground maintenance ration, which after this procedure was blended, pelletized again and kept into an environment protected from heat. The resveratrol dose used was based in previous studies performed by WANG et al.6 (2005). During the experiment water was supplied ad libitum and the quantity of ration for all the animals was 100 g/day.

When the experimental period finished, the animals were kept without food during 18 hours, after that they were euthanized with sodium pentobarbital and the aortic arch was removed. This arterial segment was carefully washed with saline solution and fixed in Bouin's solution during 24 hours in ambient temperature. After the macroscopic view of the plaques, a segment of the aortic arch was processed routinely and included in histosec. After the blocks of all samples were cut into 5 µm thick sections and the specimens were mounted on glass slides, which were then stained by Masson's trichrome and hematoxylin-eosin techniques (TOLOSA et al.,[7] 2003) observed through Leica DM 5000 B photomicroscope to perform the morphological analyses. In this study we evaluated the microscopic morphology of all the tunics from atherosclerotic areas of the aortic arch initial segment and we also classified the lesions we found. We prepared five glass slides with five histological cuts for each animal. (THE STATISTICAL TECHNIQUE WASN'T DESCRIBED)

RESULTS

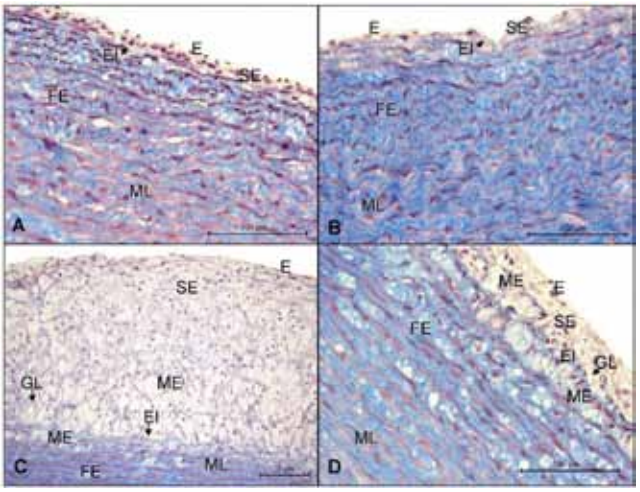
The analyses of aortic arch segment from animals belonging to group CT showed normal histological aspect for tunics intima, media and adventitia in this arterial portion. It was observed in tunica intima the presence of endothelium constituted by simple squamous epithelium, supported by loose conjunctive tissue, sub endothelium, followed by elastic inner limiting membrane. In tunica media were observed smooth muscle fibers among plenty of elastic fibers. It was also noticed the presence of elastic outer limiting membrane, which separates tunica media from tunica adventitia. The last, tunica adventitia, was composed mainly of by collagen fibers, lymphatic vessels, vasa vasorum, nervous fasciculus and simple squamous epithelium that covered it externally.

The analyses of aortic arch segment from animals belonging to group R showed histological characteristics which were similar to those found in group CT, regarding aspect and placement in all the structures mentioned,

The analyses of aortic arch segment from animals belonging to group CL demonstrated abnormality in tunica intima, because it presented a great quantity of foam cells, placed in disorganized layers, as well as extracellular lipid droplets in subendothelial conjunctive tissue, covered by discontinuous endothelium. We also observed, right under the inner elastic limiting membrane, or, in the beginning of tunica media, the invasion by foam cells. The rest of tunica media, elastic outer limiting membrane and tunica adventitia were typical.

The analyses of aortic arch segment from animals belonging to group CR has also indicated pathologic changing in sub endothelium of tunica intima, although in lower developmental level regarding the number of foam cells layers toward the light of the vessel, besides some extracellular lipid droplets in subendothelial conjunctive tissue , covered by discontinuous endothelium. We also observed the presence of elastic

***Figure 1**: Photomicrography of aortic arch segment of White New Zealand Rabbit belonging to groups CT, R, CL and CR depicted as letters A, B, C and D respectively, showing in tunica intima of groups CT and R the endothelium (E), the sub endothelium (SE) and the elastic inner limiting membrane (EI), in groups CL and CR besides these structures we can observe foam cells (ME) and lipid droplets (GL). In tunica media of groups CT and R the smooth muscle fibers (ML) intermingled with elastic fibers (FE), in the beginning of tunica media of groups CL and CR the invasion by foam cells followed by smooth muscle fibers (ME) intermingled with elastic fibers). 1, Staining: Masson's trichrome.*



inner limiting membrane and right under this one, in the beginning of tunica media, the invasion by foam cells. The rest of tunica media, outer limiting membrane and tunica adventitia were typical (Figure 1).

DISCUSSION

The histological analysis of the aortic arch of animals belonging to groups CT and R (fed with normal diet) showed normal histological characteristics for tunics intima, media and adventitia according to those described by JUNQUEIRA & CARNEIRO,[8] (2008).

In analyses of aortic arch segment from animals belonging to group CL there was an observed abnormality in tunica intima and because of it presented a great quantity of foam cells, placed in several disorganized layers, and also extracellular lipid droplets in subendothelial conjunctive tissue. The endothelium that covered the tunica intima was composed by a layer of discontinuous cells, in accordance with the reports by STARY et al.9 (1994), in human beings, these descriptions are also present in other species of pigeons and monkeys. The tunica media was invaded by foam cells and it was observed an indefinite arrangement of elastic inner limiting membrane. There were no changes in the components in the rest of this tunic.

In animals belonging to group CR there was pathologic change in sub endothelium of tunica intima, but in lower level of development regarding the number of foam cells layers toward the light of the vessel besides some extracellular lipid droplets in subendothelial conjunctive tissue. The endothelium presented itself as a descontinuous layer of cells. These lesions where similar to those found in the experiment performed by JUZWIADK et al. [10] (2005) using quercetin flavonoid. It was also observed the invasion in the beginning of medium layer. This kind of lesion allows us to classify it according to STARY et al. [9] (1994) as type III lesions, or, an intermediate level between type II and type IV (atheroma). Together with the lipid cells present in type II lesions, type III lesions contain spread extracellular lipid droplets that make the disruption of smooth muscle cells in tunica intima. These extracellular lipids are the forerunners to the formation of "lipid nucleus" which characterizes the type IV lesion. Type III lesions appear in some regions of normal adaptative thickening in intima (regions that are susceptible to the development of lesions). Atherosclerotic lesions in aorta, classified as type II are found in 99% of 2 to 15-year-old children, most frequently in aortic arch, abdominal and descendent thoracic aorta than in ascendent aorta. Still in hypercholesterolemic groups CL and CR we observed in some places of the wall of the vessel type II lesions and the so called "fatty striation" which consists of layers of foam cells.

There weren't evidences of lesions in

tunica adventitia for all the experimental groups; this information is according to STARY et al. [9] (1994), who report that only in advanced levels of atherosclerotic lesions, where a great accumulation of extracellular lipids occur, there is deformity in tunica adventitia.

The pattern of lesion found in this study to type III lesion, was similar to those found in human species and also those found in dogs (STARY et al.,[9] 1994; KAGAWA et al.,[11]1998).

It is possible to imagine that resveratrol reduced the development of atherosclerotic lesions based in some academic reports about this polyphenolic compound where it was observed a broad aspect of biological effects, such as antioxidant, antiplatelet, endothelium protective properties and in proliferation of smooth muscle cells (FRÉMONT,[12] 1999).

Therefore, regarding antioxidant effects, studies performed by WAKABAYASHI13 (1999) proved that the polyphenols from red wine were able to reduce LDL plasma oxidation. Also MORENO et al.[14] (2000) describe by means of an in-vitro study that the antioxidant effect caused by phenolic compounds was bigger than that caused by vitamins C and E HEATON et al.[15] (2002) showed that using antioxidants such as vitamins E, C, taurine, licopene and beta-carotene added to dogs' food, improved the plasma antioxidant capacity, reduced the lesion in DNA and still promoted a better immunologic response in animals.

With regards to platelet aggregation, WANG et al.[16] (2002) observed a significant inhibition of platelet aggregation in-vitro, which depended on the resveratrol concentration used. In white New Zealand rabbits with induced hypercholesterolemy and simultaneous administration of 4mg/kg of resveratrol, there was also inhibition of platelet aggregation ASCIAK et al.[17] (1995), when they studied in-vitro the effect of trans-resveratrol, some phenolic compounds that are derived from wine and several antioxidants, on platelet aggregation and the synthesis of eicosanoids in human cells, they noticed a stop in platelet aggregation and a reduction in eicosanoids synthesis, what, according to the authors, contributed to an cardioprotector effect of resveratrol, mainly in atherosclerosis and in patients with heart disease. Also, another explanation for cardioprotective activity of resveratrol would be the improvement of endothelial function, according to what was observed by ZOU et al.[18] (2003) in their papers, where the plasmatic level of endothelin had a reduction and the levels of nitric oxide increased promoting better blood vessel dilation. Another observation that could explain the effect of resveratrol in atherosclerosis is due to its effect in migration and proliferation of smooth muscle cells. Therefore in studies in-vitro with muscular cells taken from bovine aorta, it was noticed that with the use of resveratrol, in a dependent dose, there was a reduction in their proliferation, yet in another study using cells from mice there was also a reduction in intimal hyperplasia in vessel wall in induced lesions (ARAIM et al.,[19] 2002; GU et al.,[20] 2006). It's also pointed the beneficial effect of resveratrol in preserving the integrity of endothelium, because some papers affirm its inhibitor effect in the expression of VCAM-1, ICAM-1 and in the transcription factor NF- κβ, the last being responsible by modulatory pro-inflammatory effects (FERRERO et al.,[21]1998; MANNA et al.,[22] 2000; Wung et al.,[23] 2005).

Thus, we concluded in this study that resveratrol has performed as a preventive agent in the development of atherosclerotic lesion and it is supposed that using it as diet supplement for humans and animal species susceptible to atherosclerosis may be a possible alternative to help preventing the development of this disease.

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Source of Funding:

"No conflicts of interest have been declared"