Atherosclerosis towards Rat Relating with High Cholesterol Feed

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Abstract

Atherosclerosis or arteries hardening was a condition wherein occurred a plaque buildup in the arteries and often related to high-cholesterol foods. Aims: The research was intended to prove the high-cholesterol feed could increase the occurrence of atherosclerosis towards rat aorta walls. Methods: The Randomized Post-test Only Control Group Design was conducted in two groups of male Wistar rats 10-12 weeks old, weight from 140 to 150 grams. Each group consisted of 10 rats, one group was fed a standard feed and the other group was fed a high-cholesterol feed. Histopathological preparations were to use Hematoxylin-eosin staining (HE). The data were analyzed by computer, with α 0.05. Results: Eighty percent of a rat who was fed a high-cholesterol feed was found a plaque in its aorta while the rat fed a standard feed only 10%. It was obtained a relationship between high cholesterol feed with the plaque formation in the rat's aortic wall (p 0.003). Conclusion: There was a relationship between the feed provisions of high-cholesterol with the occurrence of atherosclerosis towards rat aortic.
1. Introduction

Cardiovascular disease is the leading cause of morbidity and mortality throughout the world, mainly due to atherosclerosis (Van Lammeren et al., 2011). Atherosclerosis or arteries hardening is a condition wherein a plaque buildup in the arteries. The plaque is made included cholesterol, fatty substances, cellular waste products, calcium, and fibrin (clotting substance in the blood). It partially or whole can block the blood flows through the arteries to the heart, brain, pelvis, legs, arms or kidneys. Some diseases that can develop as a cause of atherosclerosis disease, including coronary heart, angina (chest pain), carotid artery, peripheral artery, and chronic kidney. The arterial walls thickened causing an artery narrows lumen and the blood flow is reduced, thereby reducing oxygen supplies.

The occurrence of atherosclerosis is a complex process. The plaque begins to be formed due to damage of arteries inner walls (endothelium). There are three possible causes of damage to the arterial wall are increased cholesterol levels and triglycerides in the blood, hypertension, smoking a cigarette. The cholesterol level increasing, especially, the concentration of low-density lipoprotein (LDL), a high plasma is directly correlated with the development of coronary artery disease. (Rudel & Kesäniemi, 2000; Penalva et al., 2008), and a mild rise in triglycerides lead a risk of coronary and the development of coronary artery disease, as well as, the formation of new lesions (Hokanson & Austin, 1996). A consumption excess food is often related with a high cholesterol and atherosclerosis occurrence hypercholesterolemia due to the fatty will stick and cause inflammation in the arterial wall. There are several steps of atherosclerotic lesions development, i.e. Ross (1993); Du et al., (2004) the initial lesion is a fatty streak, which is the aggregation of lipid-rich macrophages and T-lymphocytes in the intima layer of the arteries. Fatty streak evolves become a medium lesion into consisting of a layer of foam cell macrophage and the smooth muscle cells then grows more complex and become occlusive lesions, as well as, a fibrous plaque. Based on the above description, therefore, the research is conducted regarding the effects of feed on high cholesterol towards the occurrence of atherosclerosis in a rat aorta.

2. Materials and Methods

The present study is experimental research. It is designed by the Randomized Post-test Only Control Group Design. The treatment towards an experiments animal is conducted in the Unit Laboratory Animal (ULA). The Department of Pharmacology, Medical Faculty, Udayana University, and histopathologic examination in the Pathology Laboratory, Faculty of Veterinary, Medical Faculty, Udayana University. The study is conducted about 20 weeks, the samples are 20 male Wistar rats, 10-12 weeks old, the weight is 140 to 150 grams. The samples are divided into two groups, each of them consisting of 10 rats. One group of rat (G1) is fed the standard feed of pellets 594 as many as 15 g/rat/day and given an ad libitum.

The second group (G2) meanwhile were fed a high-cholesterol feed and water and an amount equal to the first group. The high-cholesterol feed was made by mixing standard feed with a high-fat feed with the following composition includes 50% standard feed, 31.8% wheat, 1% cholesterol, 0.2% cholic acid, 10% lard, 2% pig brain, 5% egg yolk. The rats were placed in individual cages with size is 40 cm x 15 cm x 10 cm. At the end of the study, it was conducted in the rat furthermore, a decision aorta euthanasia was dissected. An aorta is put in a formalin buffer solution next make preparations for histopathological examination. The histopathological aorta preparation is routinely stained by Hematoxylin-eosin (HE) and observed under a microscope at 100x and 400x magnification in order to know a presence or mota plaque that is formed on the arterial walls.

3. Results and Discussions

The research was conducted towards two groups of rat i.e. the group was fed a standard feed (G1) and the group that was given a high-cholesterol feed (G2), each group consisted 10 male rats. Eighty percent of the rat that was given a high-cholesterol feed (G2) during five-month was found a plaque, whereas, upon the group that was not given a high-cholesterol feed (G1) merely 10%. In figure 1 is shown that the number of rats occurs atherosclerosis is more than G2 i.e. 10 rats, however, G1 is vice versa.

Figure 1. Number of rats that occurs atherosclerosis towards the group within standard feed (G1) and the group on the high cholesterol feed (G2)

Statistical analysis is to show, there is no relationship ($p > 0.003$) between the high-cholesterol feed provisions with the occurrence atherosclerosis to the rat aortic. The observation upon the microscopic appearance of the rat aorta for groups G1 and G2 showed a highly significant difference (Figure (2a & b)). In the group G1, the aorta lumen appeared to be empty, there is no atheroma plaque and intima as well as still intact.

Figure 2. The rat aorta histopathology

The rat aorta histopathology for group G1 that is fed a standard food, there is no atheroma in the aortic wall (a) whereas, in the group G2 that is given a high cholesterol feed appears belonging atheroma plaques (arrows). 100x magnification, HE Staining. In term of this, it is supported by the microscopic observation on the higher magnification i.e. 400 times (Figure 3a & b). In the group G1, the intima tunica itself is characterized by irregular of arranging endothelial cells, there are no endothelial cells which necrosis and erosion. However, in the group G2, it is observed there is endothelial dysfunction, unlike degeneration to necrosis is accompanied by belonging of an atheroma plaque in the aortic wall that almost covered a lumen.
The differences of the rat aorta histopathology image between the group G1 that is given a standard feed (a) with the group G2 that is given a high cholesterol feed. It is a reality in the presence of endothelial dysfunction (arrows) and the formation of atheroma plaques that fill the lumen of the aorta (asterisk) (b). 400 x magnification, HE Staining.

Discussion

The research results showed that atherosclerosis is the most occurred in the rat’s aorta that was fed a high-cholesterol feed compared with the rat aorta that was fed a standard feed. Atherosclerosis describes an association of fatty degeneration and blood hardening of vessels (Crowther, 2005).

Cholesterol is an essential component of cell membranes, as well as a precursor molecule for the steroid hormones synthesis, vitamin D, and bile salts. It comes from the feed will be absorbed in the intestine about 30-60% (Charlton-Menys & Durrington, 2008). The high cholesterol feed can increase the cholesterol in the blood is known as hypercholesterolemia, due to an imbalance of two basic homeostatic of cholesterol mechanisms. The imbalance of two systems can be caused by three terms included genetic factors, nutritional factors and combination factors both earlier. The nutritional factor is a high cholesterol feed degrades or can inhibit the action of the receptor for LDL (Joossens, 1988), thus there are many free lipid, especially, a free LDL in the blood vessels, further, it is localized and occurred a lipids accumulation in the vascular endothelium.

A lipid accumulation is required for definitive plaque development wherein lipid deposition is begun with the LDL movement from the blood into the blood vessel walls. Once in the blood vessel wall, there are three things that can happen to LDL, namely: moving back into the bloodstream, it becomes oxidized (through the free radicals action or leukocytes activity direct) or captured by monocytes/macrophages which eventually become foam cells. Oxidized LDL is a very atherogenic and unlike a chemotactic for monocyte-makrofag (Crowther, 2005).

The macrophages bind to LDL in the intima tunica through a new receptor that is known as scavenger receptors, which recognize LDL only after it has been oxidized. LDL absorption is oxidized resulted the movement of macrophages is reduced, thereby increasing the accumulation of macrophages cells with related to the lipid-laden (foam cells) in the intima. The foam cells maintain the metabolic activities and secrete a cytokines varieties and inflammatory mediators. Their activation results including the recruitment and proliferation of smooth muscle cells, further LDL oxidation, the cell monocytes recruitment/extra foam and a decreasing in endothelial function.

The previous research has shown that the high-cholesterol feed provisions in the rats for 8 weeks can increase the blood cholesterol and found the foam cells towards the arch aortic, however, it was not mentioned what percentage of rats that occurred atherosclerosis (Murwani et al., 2006).

4. Conclusion

Provide a statement that what is expected, as stated in the "Introduction" chapter can ultimately result in "Results and Discussion" chapter, so there is compatibility. Moreover, it can also be added the prospect of the development of research results and application prospects of further studies into the next (based on result and discussion).
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