Diagnostic Value of Lipoprotein (a) in Cardiovascular Disease due to Atherogenic Diet in Rats

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ABSTRACT

This research was conducted to evaluate cardiovascular disease caused by atherogenic diets, based on immunological assay by measuring the concentration of lipoprotein (a)/Lp (a). Eighty male Sprague Dawley rats, 150-200 grams of body weight and three months of age were used in this research. The rats were randomly alloted into four groups, 20 of each. Group I as control was fed normal diet, group II was fed diet containing high cholesterol, group III was fed diet containing high fat and group IV was fed diet containing high cholesterol and high fat (atherogenic). After 2, 4, 8, 16 weeks on experimental diet, 20 rats were selected randomly (5 rats of each group), and blood sample were withdrawn for Lp (a) analysis. All animal were then killed and the heart were taken out for histopathological analysis. The statistical analysis for Lp (a), data showed that there were significant differences (p<0,05) among of all the treatments, high fat diet had the greatest influence on Lp (a) concentration. Treatment period and interaction between treatment period and diet did not influence Lp (a) concentration.

It can be concluded that Lp (a) concentration could be influence by high fat diet, but not by period of treatment. Lp (a) concentration seems connected with the incidence of atherosclerosis in rats. For this reason, evaluation of Lp (a) concentration should be considered as a routine procedure in general health evaluation.

INTRODUCTION

Cardiovascular disease are still major public health problems and the first cause of death in modern countries, especially in United States and other Western countries. In Indonesia, based on Health Departement survey in 1986, cardiovascular disease has become the third cause of death after acute pneumonia and diarrhea. However, in 1992, cardiovascular disease has become the first cause of death (Anonim, 1993).

Atherosclerosis is a complex disease, because no single factor is an absolute cause of cardiovascular disease. The risk factor include: cigarette smoking, hypertension, obesity, psychosocial factor, physical inactivity, and diabetes mellitus (Kaplan, 1979). In fact, atherosclerosis is a slowly progressive disease of the large arteries that begins early in life, but rarely produces symtoms until middle age. Often the disease goes undetected until the time of the first heart attack which usually fatal. Based on those facts, a routine procedure in general health evaluation became important to prevent progresivisity of the atherosclerosis.

In 1963, Berg described a new antigenic component, Lp (a), among human plasma lipoproteins. Innitially it was thought that the antigen was present in about one-third of individuals. With the advent of more sensitive assay, it is apparent that the serum of virtually all humans contains this antigen, but in highly varies amount (Kane and Havel, 1990). Detailed studies of the structures of Lp (a) lipoproteins were initially hampered by the tendency of these lipoproteins to become denatured under conditions which do not appear to affect the structure of LDL. From initial immunochemical studies of the Lp (a) lipoproteins it was known that they contain material cross-reactive with apo B as well as Lp (a) specific antigen. Further studies then established that there were two principal proteins, one is corresponding to the apo B of LDL and the other is unrelated, heavily glycosylated protein responsible for the Lp (a) reactivity ((Enholm et al. 1972). In recent years, Lp (a) become interesting object for investigators. They believed that Lp (a) concentration have strong correlation with early atherosclerosis (Dahlen et al. 1993). The amount of Lp (a) in plasma varies from less than 1 mg/

dl to greater than 100 mg/dl, with a two-fold increase in coronary artery disease (Armstrong, 1986).

The present study was conducted to evaluate atherosclerosis, cause by atherogenic diet, based on immunological assay by measuring the concentration of Lp (a), using rats as animal models.

MATERIALS AND METHODS

Eighty male Sprague Dawley rats, 150-200 grams of body weight and three months of age were used in this research. The rats were randomly alloted into four groups, 20 of each. Group I as control was fed normal diet, group II was fed diet containing high cholesterol, group III was fed diet containing high fat and group IV was fed containing high cholesterol and high fat (atherogenic). After 2, 4, 8, 16 weeks on experimental diets, 20 rats were selected randomly (5 rats of each group), and blood sample were withdrawn for Lp (a) analysis. All animal were then killed and the heart taken out for histopathological analysis.

Analytical methods

Lipoprotein (a) concentration was assayed by using a commercial h-lipoprotein (a) ELISA kit produced by Boehringer Mannheim[1] and measured at 450 nm using ELISA reader.

Data of the experiments were analyzed statistically using Multifactorial Randomized design. The difference was considered to be significant if $p<0,05$.

RESULTS AND DISCUSSION

The effects of various diets and treatment periods on rats plasma Lp (a) concentration are presented on Figure 1. Statistical analysis showed that there were significant differences ($p<0,05$) among all of the treatments, high fat diet had the greatest influence on Lp (a) concentration.

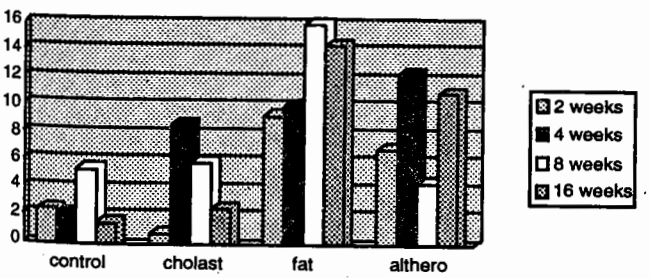

Figure 1. The effects of various diet for 2, 4, 8, 16 weeks on Lp (a) concentration

Although it has been generally accepted that Lp (a) is mainly under genetic control and therefore hardly sensitive to dietary change, a few studies show that specific dietary fatty acids do affect the Lp (a) concentration (Tholstrup *et al.*, 1995). Nestel *et al.* (1992) showed that elaidic acid (a trans isomer to oleic acid) raised Lp (a) concentration more than oleic acid, palmitic acid and butter. Mensink *et al.* (1992) reported that substitution of saturated fatty acid for oleic acid and trans-fatty acids caused the Lp (a) concentration to rise. Finally Hornstra and co-workers (1990) have demonstrated that ingestion of palm oil causes the Lp (a) concentration to falls to levels below those achieved with a habitual diet.

In this study, statistical analysis showed that treatment period and interaction between treatment period and diet did not influence Lp (a) concentration. This result showed that Lp (a) is mainly under genetic control. This result is in agreement with previous report saying that plasma Lp (a) levels are under genetic control, with clearcut ethnic differences in the frequency distribution. In healthy Caucasian populations the distribution is typically skewed, most of the subjects having plasma Lp (a) levels in individuals with genetic lipid disorders like familial hypercholesterolemia (FH) (Werba, 1993).

Lipoprotein (a) concentration in this experiment varies from 0,5 mg/dl to 39,41 mg/dl. This result showed that Lp (a) concentration have a great range like in human, although as not great as in human.

Incidence of cardiomiopathy and atheroma in this experiment showed the high level of Lp (a) concentration (>20 mg/dl), except in 7 rats. The result showed that Lp (a) concentration seems connected with the incidence of atherosclerosis in rats.

Incidence of atheroma with high level concentration of Lp (a) is in agreement with previous study that showed the covalent structure of apolipoprotein (a) has a striking similarity to plasminogen. The atherogenic potential of Lp (a) has been attributed to competition with plasminogen receptor on endothelial cells and monocytes as well as for binding site on fibrinogen and fibrin (Gotto, 1983).

Incidence of atheroma from group III (high fat diet) is a specific marker for atherosclerosis. Studies of atherosclerotic lesions with modern techniques of cell and molecular biology has revealed that each lesion contains significant elements of three cellular phenomena. These are smooth-muscle proliferation; formation by the proliferated cells of larrge amounts of connective

tissue matrix including collagen, elastic fibers, and proteoglycans; and accumulation of intracellular and extracellular lipid. The lesions of atherosclerosis occur principally within the innermost layer of the artery wall, the intima. They include fatty streak, and the fibrous plaque. Secondary changes have been noted in the media of the artery underlying the lesion, principally in association with the more advanced lesions of atherosclerosis (Ross, 1993).

Incidence of cardiomiopathy which showed low level of Lp (a) concentration in this research it might be caused by multiple form of apoprotein (a), with variation in molecular weight among the apo (a) proteins. The apo (a) molecule contains a variable amount of kringle 4 repeats, accounting for the considerable apo (a) size heterogeneity, with isoform varying between 400 and 700 kDa (Leus *et al.*, 1994). Utermann *et al.* (1983) first described the existence of six apo (a) isoform. They classified the isoform according to their electrophoretic mobility relative to apo B-100 as F (faster than apo B-100), B (similar to apo B-100), S1, S2, S3 and S4 (all slower than apo B-100). Phenotype B, S1, S2 showed an association with high level of Lp (a) concentration, and S3, S4 phenotype showed an association with moderate level of Lp (a) concentration.

CONCLUSION

Based on the present study result, it can be concluded that : (1) Lp (a) concentration could be influenced by high fat diet, but not by period of treatment. (2) Lp (a) concentration seems connected with the incidence of atherosclerosis in rats. For this reason, evaluation of the Lp (a) concentration should be considered as aroutine procedure in general health evaluation.

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