Rapid Communication

Plasma Homocysteine Levels in Rabbits Fed a High Cholesterol Diet

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Introduction

Homocysteine, an intermediate metabolite of methionine is reported to be one of the risk factors for atherosclerosis^{1,2}. Homocysteine is metabolized to cysteine by cystathionine- β -synthase. Cysteine is then further metabolized and degraded to taurine, which is subsequently excreted.

Plasma homocysteine levels are remarkably elevated in patients with homocysteinuria³. Precocious atherosclerosis and cerebral infarction has been reported in these patients⁴. Furthermore, plasma homocysteine levels are higher in patients with atherosclerosis such as cardiovascular disease⁵⁻⁷ and cerebral infarction⁸ than in normal healthy controls. The prevalence of hypercholesterolemia in patients with coronary artery disease is higher than in subjects free of cardiovascular disease⁹. But, it remains uncertain whether plasma cholesterol is essentially involved in the metabolism and the plasma levels of homocysteine.

In this study, we measured plasma homocysteine levels in rabbits fed a high cholesterol diet to clarify the relationship between cholesterol and homocysteine.

Methods

Twelve male New Zealand White rabbits weighing 2.4–2.5 kg at the beginning of the study were obtained from Sankyo Labo Service Co. (Tokyo, Japan). The rabbits were randomly allocated to two different dietary regimens. One group (N group) was fed a normal control diet of commercially available pellets RC-4 (Oriental Yeast Co., Tokyo) and the other group was maintained on RC-4 supplemented with 0.2% cholesterol (Wako Pure Chemical Industries, Ltd. Osaka, Japan) for 22 weeks. Water was

taken *ad libitum*. Animals were housed in a temperature controlled ($24 \pm 1^\circ\text{C}$) and humidity maintained (55%) room with a 12 hour cycle of light and dark. Blood was taken from an auricular vein at two-week intervals after an overnight fast and the plasma was stored at -20°C until analysis. The plasma concentrations of total cholesterol¹⁰ and triglyceride¹¹ were determined enzymatically by commercially-available kits (Wako Pure Chemical Industries, Ltd.). Both total homocysteine and cysteine in the plasma were determined by LC-6A HPLC system (Shimadzu Co., Kyoto, Japan) by the modified method of Araki et al.¹² It is shown as follows. Briefly, Tri-n-butyl-phosphine (TBP), trichloroacetic acid (TCA), and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulphonate (SBD-F) were obtained from Wako Pure Chemical Industries Ltd. Ten percent TBP in dimethyl-formamide was added to the plasma to decouple thiols from the plasma protein. The solution was mixed with 10% TCA containing 1 mM EDTA to remove the protein. After centrifugation at 1,000 g for 5 minutes, the supernatant was mixed with SBD-F in a 2.5 M borate buffer (pH 10.5), which is a precolumn labeling reagent. The solution was applied to a Shim-Pack CLC-ODS column (Shimadzu Co.). The elution buffer was a 0.1 mM acetate buffer (pH 4.0) containing 2% methanol (v/v). The fluorescence intensities of the eluate was measured with excitation at 385 nm and emission at 515 nm. The correlation coefficient was $r=0.99$, $p<0.01$ over a range of the calibration curve for homocysteine from 0.1 pMol to 10 pMol. All data are presented as mean $\pm$ SD. Student's t-test was used to evaluate the statistical differences. A value of $p<0.05$ was considered significant.

Results

Serum cholesterol increased in Ch group, and it was significantly higher (709.3 mg/dl, $p<0.01$) at the fourth week compared to the initial value (53 ± 7.6 mg/dl). It was maintained at an elevated level until the last day of the experiment. Serum tri-

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Table 1 Serum levels of total cholesterol and triglyceride, and plasma levels of homocysteine and cysteine in the rabbits fed a high cholesterol diet

Weeks	0	2	4	6	8	12	16	20	22
n	6	6	6	6	6	6	6	6	6
TC (mg/dl)	53.0±7.6	461.5±41.9 <0.01	709.3±141.7 <0.01	967.3±186.2 <0.01	687.7±64.1 <0.01	706.1±184.5 <0.01	787.3±150.3 <0.01	829.1±163.8 <0.01	
TG (mg/dl)	62.9±39.7	43.5±11.2 NS	68.4±16.4 NS	42.5±10.5 NS	61.1±15.8 NS	97.2±42.7 NS	46.3±11.4 NS	85.1±31.2 NS	
cys (nmol/ml)	240.9±23.8	147.8±31.5 NS	181.4±45.9 NS	233.1±17.2 NS	208.1±6.3 NS	239.9±22.8 NS	185.3±12.5 NS	151.2±39.1 NS	
h cys (nmol/ml)	17.5±3.8	11.8±2.4 NS	14.9±3.1 NS	13.1±1.8 NS	11.3±4.4 NS	9.9±3.4 NS	12.6±1.9 NS	14.3±3.0 NS	

Abbreviations: cys, cysteine; h cys, homocysteine; NS, not significant. (Mean±SD)

Fig. 1 Plasma levels of homocysteine and cysteine in the control and cholesterol-fed groups at the final day of the experiment.

glyceride levels in the Ch group were unchanged throughout the experiment. Under these lipid conditions, neither the levels of plasma cysteine nor homocysteine changed significantly (Table 1).

Serum cholesterol was markedly higher in Ch group (829.1 ± 163.8 mg/dl, $p < 0.01$) compared with that in the N group (55.4 ± 12.1 mg/dl) at the end of the experiment. Serum triglyceride level was 58.2 ± 20.6 mg/dl at the first day of the experiment and remained unchanged (50.7 ± 14.9 mg/dl, NS) through the end of the experiment in the normal control group. No remarkable difference was observed in the serum triglyceride level between Ch group and the N group at the end of the experiment. Under these lipid conditions, there was no difference in plasma homocysteine or cysteine levels between the two groups at the final day of the experiment (Fig. 1).

Discussion

Homocysteine has recently been considered to be one of the risk factors for atherosclerosis^{1,2)}. Homocysteine is indeed harmful to endothelium *in vitro*¹³⁾ and *in vivo*. It was reported that a homocysteine infusion in baboons produced loss of endothelial cells from the intimal surface and circulating endothelial cells were found in peripheral blood samples¹⁴⁾. Platelet aggregation is also reported to be increased by homocysteine. Graber et al.¹⁵⁾ reported increased conversion of arachidonic acid to TXB₂, but no effect on the conversion of arachidonic acid to PGI₂.

Olszelski et al.^{6,7)} reported that plasma homocysteine levels correlated with total cholesterol and triglyceride levels in 52 male subjects. Of these 26 subjects had both coronary heart disease and hyperlipidemia, while the other 26 subjects had no coronary artery disease or hyperlipidemia. It is difficult to clarify in their report whether hyperlipidemia or

coronary artery disease was the cause of the elevated levels of homocysteine.

Recently, Araki et al.⁸⁾ reported no significant correlation between serum cholesterol and plasma homocysteine levels in normal subjects, whereas they found a significant correlation between these two factors in patients with cerebral infarction.

In the present study, we reared rabbits on a diet supplemented with cholesterol to confirm the extent to which serum cholesterol alone affects the level of plasma homocysteine. We found no change in the levels of homocysteine or cysteine, regardless of the extent of the cholesterol elevation.

These findings indicate that serum cholesterol itself is concluded not to be an important factor in influencing the plasma homocysteine level. We can conclude that plasma homocysteine is thought to be an independent risk factor for atherosclerosis.

It is known that vitamin B₆ deficiency¹⁶⁾ as well as a lack of enzyme activity necessary for homocysteine metabolism, such as cystathionine β -synthase¹⁷⁾, leads to elevate plasma homocysteine levels, while the mechanism of the homocysteine elevation in patients with arteriosclerosis is not clear. Further study is necessary to investigate the factors which affect the modulation of homocysteine levels in case of cardiovascular diseases.

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Summary

Homocysteine, a metabolite of the sulfur amino acids, is reported to be one of the risk factors for atherosclerosis. It has been reported that accumulation of homocysteine produces atherosclerosis in young patients with homocysteinuria. Indeed, plasma levels of homocysteine were statistically higher in patients with myocardial infarction and those with cerebral infarction. However, the relationship between cholesterol and homocysteine levels remains unclear. In the present study, we reared rabbits on a diet supplemented with 0.2% cholesterol for 22 weeks to confirm the extent to which serum cholesterol

affects the level of plasma cysteine and homocysteine. Plasma homocysteine, cysteine, serum cholesterol and triglyceride levels were estimated at two week intervals. No significant change in the plasma homocysteine or cysteine levels were noted compared to that in normal controls, despite a remarkable increase in serum cholesterol levels.

These findings indicate that the homocysteine level is independent of the serum cholesterol level.

Key words: homocysteine, cysteine, cholesterol, atherosclerosis.