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Original Article

Klotho protein lowered in elderly hypertension

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Abstract: We intend to identify the relationship between Klotho protein and elderly hypertension. Serous Klotho protein and nitric oxide (NO) which gathered from 79 elderly hypertensive patients, 30 elderly non-hypertensive patients and 25 non-elderly hypertensive patients were detected by ELISA and nitro-reductase method, respectively; and the comparison came from the former group and the last two groups. The results were as follows: Klotho protein absorbance (0.303 ± 0.096) and NO concentrations (43.95 ± 21.85 μmol/L) in elderly hypertensive group were lower than the elderly non-hypertensive group (0.489 ± 0.216) and (62.63 ± 21.26 μmol/L). So it shows that there was significant difference between the two groups ($P < 0.01$). And the result suggested that, except of the contribution of Klotho protein to the calcification of vessel wall and reduction of vascular elasticity, elderly hypertension may partially attributes to the reduction of serous Klotho protein, which leads to the shrinkage of endothelial function companied with decrease of NO.

Keywords: Klotho protein, nitric oxide (NO), elderly, hypertension

Introduction

Hypertension is a clinical syndrome featured with the elevation of arterial blood pressure. Elderly Hypertension, a special type, has unique characteristics in the pathogenesis, clinical manifestations, treatment and rognosis etc. Gillessen [1] found that the extent of atherosclerosis and age are positively correlated, and the degree of vascular calcification is closely related to the increasing of systolic blood pressure and pulse pressure in patients. London [2] mentioned that systolic blood pressure compared with that of patients without calcification was significantly higher, while diastolic blood pressure is relatively low, which suggested that atherosclerosis and hypertension in the elderly have causal relationship. Klotho is a newly discovered gene associated with aging. Study shows that arteriosclerosis and ectopic calcification presented in Klotho-knockout mice within 4weeks after the birth, and deteriorated with increasing of age [3]. OLETF, the mouse model of atherosclerosis, with symptoms of high blood pressure, obesity, high blood sugar, high blood cholesterol shows endothelial dysfunction, lower nitroxide (NO) production, lower blood pressure, intimal thickening and vascular fibrosis, which could be alleviated by adenovrus-mediated Klotho gene [4]. In this study, we tested serous Klotho protein and NO in elderly hypertensive group and non-hypertension groups to explore the relationship between Klotho and elderly hypertension.

General materials

The cases, range from January to June in 2009, total 134, provided by The Department of Geriatric of The First Affiliated Hospital of Xi'an Jiaotong University School of Medicine. According to blood pressure, 79 cases including 41 males and 38 females (mean age 64 ± 3) being the elderly hypertensive group (age ≥ 60 years). 30 patients consist of 17 males and 13 females (mean age 63 ± 2) and 25 cases including 20 males and 5 females (mean age 50 ± 2 years) admitted to elderly non-hypertensive group and non-elderly hypertensive group (age < 60), respectively. Inclusion criteria: ① Blood pressure, measured by mercury sphygmomanometer, 140/90 mm Hg differs hypertensive and non-hypertensive. ② Blood, urine, stool, liver and kidney function, electrolytes,

Table 1. Comparisons of general parameters ($\bar{x} \pm s$)

Project	A	B	C
Cases	79	30	25
Gender (male)	41	17	20
Age (years)	64 ± 3	63 ± 2	50 ± 2*
TC (mmol/L)	4.57 ± 0.55	4.61 ± 0.54	4.54 ± 0.52
LDL-C (mmol/L)	2.64 ± 0.31	2.71 ± 0.28	2.59 ± 0.32
TG (mmol/L)	1.52 ± 0.20	1.49 ± 0.31	1.51 ± 0.24
HDL-C (mmol/L)	1.08 ± 0.20	1.06 ± 0.22	1.07 ± 0.23
BS (mmol/L)	5.33 ± 0.42	5.40 ± 0.32	5.36 ± 0.37
ALT (u/L)	38.25 ± 3.33	37.41 ± 2.97	38.05 ± 3.24
AST (u/L)	35.27 ± 2.21	36.01 ± 2.43	35.31 ± 2.33
BUN (mmol/L)	5.46 ± 0.19	5.51 ± 0.11	5.44 ± 0.20
CRE (μmol/L)	92.52 ± 3.68	92.58 ± 4.17	92.49 ± 3.68
UA (μmol/L)	373.14 ± 11.46	371.57 ± 10.96	374.32 ± 11.22

Compare respectively with non-hypertensive group and the elderly hypertensive group. *P < 0.05, A = elderly hypertensive group; B = elderly non-hypertensive group; C = non-elderly hypertensive group.

lipids, blood glucose tests were normal. ③ The blood pressure in patients with history of hypertension is still ≥ 140/90 mmHg. Exclusion criteria: ① The patients with history of hypertension blood pressure now is less than 140/90 mmHg. ② The patients have acute and chronic hepatitis, nephritis, cancer, cerebrovascular disease, peripheral vascular disease. ③ Blood, urine, stool, liver and renal function, blood lipids, blood glucose were not in the normal range. ④ Secondary hypertension.

Materials and methods

The supernatant of specimen, 3 ~ 5 mL, which collected from elbow venous blood of patients, were centrifuged under 2000 r/min for 10 min and stored at - 80°C. Klotho Protein Detection: using ELISA method. Diluting the supernatant of specimen according to 1:10 and then adding it to 96-wells plate, each well 0.1 mL, 4°C overnight. The next day, discarding all the liquids of 96-wells plate and washing five times with the Tween - 20 PBS, after then, adding anti-Klotho antibody (ab76356, provided by British Abcom company) diluted by PBS which contains bovine serum albumin according to 1/12500 to the plates, each well 0.1 mL, following putting into 37°C incubator for 1 h. After that, again, discarding all the liquids of 96 - wells plate and washing five times with the Tween - 20 PBS, after then, adding horseradish peroxidase-labeled by goat anti-rabbit antibody diluted by PBS according to 1/ 2000 to the plate, each well 0.1 mL. For the last time, discarding all the liquids of 96 - wells plate and washing five times with the Tween - 20 PBS, after then, adding freshly prepared TBM to the plate, each well 0.1 mL. Finally, putting the plate into 37°C incubator for 30 min and then adding stop solution to terminate the reaction. The values of absorbance were measured by microplate reader (Model 550, produced by United States) and repeatedly tested for at least 2 times. NO Determination: employ nitro-reductase method. The kits were provided by Southern built Bio Co., Ltd. The steps were strict accordance with the instructions. The results were detected by UV - Vis spectrophotometer (model SUV - 2120) of Korean Scinco, and calculated according to formula from instructions.

Statistical analysis

Data are provide as $\bar{x} \pm s$, with SPSS 17.0/PC package for statistical analysis. Comparison in two groups and multiple groups were compared by t test, and F test respectively. $P < 0.05$ was considered statistically significant.

Results

Comparison of data

There are no significant differences among liver function, kidney function, blood glucose and lipid parameters between general age group and the non-elderly age group (**Table 1**).

Kloth protein absorbance and NO concentration

Klotho protein absorbance and NO concentrations in elderly hypertensive group were lower than the elderly non-hypertensive group. The difference was statistically significant ($p < 0.01$). Compared with the non-elderly hypertensive group, the difference also was also statistically significant ($P < 0.05$), while the NO concentration was no significant difference between the two groups. Klotho protein absorbance and NO concentration of non-elderly

Table 2. Comparison of Klotho protein and NO in different groups ($\bar{x} \pm s$)

group	cases	Klotho (A450nm)	NO (μmol / L)
A	79	0.303 ± 0.096	43.95 ± 21.85
B	30	0.489 ± 0.216$^{*,\Delta}$	62.63 ± 21.26$^{*,\Delta}$
C	25	0.384 ± 0.136$^{\#}$	44.28 ± 21.74

A = elderly hypertensive group B = elderly non-hypertensive group; C = non-elderly hypertensive group; Compare with the elderly hypertensive group, $^{*}p < 0.01$, $^{\#}p < 0.05$; and with non-hypertensive group, $^{\Delta}p < 0.01$.

hypertensive group were lower than the elderly non-hypertensive group. The difference was statistically significant (P < 0.01) (**Table 2**).

Discussion

Hypertension in elderly is known as Systolic Hypertension as well. The decreasing vascular wall elasticity and compliance, in company with stiffness increasing, constitute the main features of mechanism of Systolic Hypertension. Reduction of arterial elasticity is mainly due to atherosclerosis. However arteriosclerosis is chiefly resulted from calcification of middle artery intima. Klotho is a new gene discovered by KURO and his companions in the study of spontaneous hypertension in 1997 [6]. It locates on the chromosome 13q12 region [7, 8] and is composed of 5 exons and 4 introns. The total length is 50 kb. Exon length of Human, mouse and rat were 3022 bp, 3036 bp, 3042 bp respectively. Homology exists among human, mouse and rat Klotho gene (Human and mouse 80%, human and rat 83%). Studies have shown that there is an intrinsic splice site in Klotho mRNA exon. If it accepts 50 bp fragment insertion, the production would be secreted proteins including approximately 549 amino acids [9]. If not, the production turns to membrane proteins. Further study found that serous Ca, P, and 1, 25 - (OH) 2D levels of Klotho gene mutant mice were increased. Some also disclosed that Klotho protein could reduce the expression of 1α - hydroxylase gene, which finally leads to the reduction of active vitamin D3 level; and a negative feedback involves in 1α – hydroxylase gene expression [10]. Klotho gene has close relationship with arterial calcification as its negative effect on 1α – hydroxylase gene expression. This study found that Klotho protein absorbance of elderly hypertensive group was significantly lower than elderly non - hypertensive group (P < 0.01) and non-elderly hypertensive group (P < 0.05). It shows that Klotho protein plays a very important role in the pathogenesis of hypertension in the elderly.

NO released by endothelial cells, plays a crucial role in physiological regulatory through its effect of vasodilation, inhibition of platelet aggregation and adhesion. Studies show that Klotho protein could reduce peroxide–induced endothelial cell apoptosis and aging, and enhance vascular endothelial cell activity and decrease caspase - 3, caspase – 9 activity [11].

NO concentration measured in this study pointed out that NO concentration in elderly hypertensive group was significantly lower than elderly non-hypertensive group (P < 0.01). While, there is no significant difference between elderly hypertensive group and non-elderly hypertensive group (P > 0.05). The results suggest that NO were reduced in elderly hypertensive group and non-elderly hypertensive group. With age increasing, reduction happens to the vivo Klotho protein, which leads to the shrinkage of endothelial function companied with decrease of NO and enhancement of vasoconstriction. On the other hand, contribution of Klotho to the calcification of vessel wall and reduction of vascular elasticity may be an important reason to elderly hypertensive incidence. Given the limited information in this study, the specific mechanism needs further research to clarify.

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Disclosure of conflict of interest

None.

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