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RELATION OF FAMILY HISTORY OF HYPERTENSION TO PLATELET AGGREGATION, RATIO OF TOTAL CHOLESTEROL TO HDL CHOLESTEROL AND URINARY KALLIKREIN EXCRETION

BY

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ABSTRACT

Some of the relatively easily measurable and possibly hypertension-associated parameters were evaluated in thirty normotensive young subjects divided into the PHT (either parent hypertensive) group and the PNT (both parents normotensive) group. In subjects of the PHT group, the platelet aggregating sensitivity to the arachidonic acid and the ratio of total cholesterol to HDL cholesterol were significantly ($p<0.05$) increased while urinary kallikrein excretion was decreased without simultaneously significant elevation of blood pressure. The enhanced platelet aggregating sensitivity to the arachidonic acid and the increased ratio of total cholesterol to HDL cholesterol suggest that subjects with a positive family histroy of hypertension might have a greater tendency to atherosclerosis and could contribute to the development of essential hypertension. Decreased urinary kallikrein excretion suggests that the vasodepressive activity of the kallikrein-kinin system might be inhibited in subjects with a positive family history of hypertension.

Key words: platelet aggregation, total to HDL cholsterol ratio, urinary kallikrein excretion, family history of hypertension

INTRODUCTION

Although many theories have been raised about the etiology of essential hypertension, the conclusion still remains uncertain. As most of them have estimated the established hypertensive subjects, the possibility that the secondary changes due to hypertension might be mistaken for its etiology shouldn't be neglected. Basing on the fact that heredity plays an important role in the development of essential hypertension (Stamler *et al.* [1]; Ayman [2]), the present study was designed to evaluate some of the relatively easily measurable and possibly hypertension-associated parameters such as platelet aggregation, urinary kallikrein excretion and serum lipids in normotensive young subjects either with or without a family history of hypertension.

MATERIALS AND METHODS

One hundred and forty-nine medical students of Tokyo Medical and Dental University were screened for their family history of hypertension. Thirty males of them participated in the study as volunteers. The volunteers were divided into the PHT (either parent hypertensive, $n=14$) group and the PNT (both parents normotensive, $n=16$) group according to their family history of hypertension.

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They were asked to collect their 24-hour urine in a polyethylene collecting bottle containing 3 m*l* of toluene solution and measure the amount of the collected urine at home on a specific day. The next morning, they visited the outpatient department of the university hospital in a fasting state, bringing 30 m*l* of the collected urine for the determination of urinary sodium, potassium, creatinine and kallikrein excretion. After bed rest for 30 minutes, the blood pressure and pulse rate were measured in a supine position. Then, 9 m*l* of the whole blood were collected from the cubital vein with a 21 gauge needle and plastic disposable syringe. No tourniquet was used in this procedure. The collected blood was well mixed with 1 m*l* of 3.8% sodium citrate solution for the determination of the platelet count and platelet aggregability. Again, 10 m*l* of the venous blood were collected for the analysis of blood biochemistry and hematocrit.

The blood mixed with sodium citrate was centrifuged at 1,000 rpm (revolutions per minute) for 10 minutes at room temperature to obtain the platelet-rich plasma (PRP) of the upper layer. The remaining was centrifuged again at 3,000 rpm for 10 minutes to obtain the platelet-poor plasma (PPP). Platelets suspended in the PRP were counted by a platelet counter (TOA PL-100) and their aggregability was estimated by a platelet aggregometer (NKK Hema Tracer 1, Model PAT-4A). The estimation of platelet aggregability was performed firstly by adjusting the recording pen of the aggregometer to 0% for PRP and 100% for PPP. Then, the maximal aggregation was determined by adding the aggregating agents of epinephrine (1 μM), collagen (3 μg/m*l*) or ADP (3 μM) into the PRP. In addition, the minimal (or threshold) concentration of arachidonic acid required to induce an irreversible platelet aggregation was also determined by the same aggregometer using 0.3 mM, 0.5 mM, 0.7 mM and 1.0 mM of arachidonic acid.

The estimation of urinary kallikrein excretion was based on the esterase assay method (Morita *et al.* [3]), using prolyl-phenylalanyl-arginine-4-methylcoumaryl-7-amide (Pro-Phe-Arg-MCA) as substrate. Blood urea nitrogen, creatinine and uric acid were determined by an automatic analyzer (Hitachi 712) while sodium and potassium were determined by a flame photometer (IL 343). Serum calcium was estimated by the titration method. Serum total cholesterol was determined by the cholesterol oxidase method, triglyceride by the glycerol phosphate oxidase (GPO) method and HDL cholesterol by the sodium phosphotungstete-Mg method.

The data were expressed as mean ± standard deviation (SD). Two-sample test (Mann-Whitney test) was used to evaluate the difference between groups. Spearman's rank correlation was used to examine the correlations. The comparison of proportions of the two groups was made by Fisher's exact method. A p value less than 0.05 was considered significant.

RESULTS

Table 1 showed the background of both groups. There were no significant differences in age, body weight, height, pulse rate and blood pressure between the two groups. The maximal platelet aggregation induced by 1 μM of epinephrine, 3 μg/m*l* of collagen or 3 μM of ADP was not significantly different between the two groups (Table 2). When 0.5 mM of arachidonic acid was added to the PRP, 8 subjects (61.5%) of the PHT group showed an irreversible aggregation while only 3 subjects (18.8%) of the

Table 1. Background of Both Groups

Background	PHT group	PNT group	p value
Age (yr)	23± 4	23± 3	NS
Body weight (Kg)	64± 6	63± 5	NS
Height (cm)	171± 5	170± 4	NS
Pulse rate (min^{-1})	61±10	67±10	NS
MAP (mmHg)	88± 9	87± 8	NS

Mean±SD. NS: Not significant; PHT: Parent hypertensive; PNT: Parent normotensive; MAP: Mean arterial pressure (=diastolic blood pressure +1/3 pulse pressure)

Table 2. Maximal Platelet Aggregation Induced by 1 μM of Epinephrine, 3 μg/m*l* of Collagen or 3 μM of ADP

Aggregating agent	PHT group	PNT group	p value
1 μM of epinephrine	54±27%	42±29%	NS
3 μg/m*l* of collagen	54±17%	45±34%	NS
3 μM of ADP	53±18%	53±23%	NS

Mean±SD. NS: Not significant

Table 3. Threshold Concentration of Arachidonic Acid and Number of Subjects Showing Irreversible Platelet Aggregation in Both Groups

Threshold concentration of arachidonic acid	PHT group (N=13)	PNT group (n=16)	
0.3 mM	0	0	
0.5 mM	8 (61.5%)	3 (18.8%)	$p<0.05$ ($\chi_s^2=3.91$)
>0.5 mM*	5 (38.5%)	13 (81.2%)	

* Threshold concentration greater than 0.5 mM include 0.7 mM, 1.0 mM and that greater than 1.0 mM.

PNT group showed such an aggregation. It took a greater concentration (0.7 mM, 1.0 mM or > 1.0 mM) of arachidonic acid to induce the irreversible aggregation in 5 subjects (38.5%) of the PHT group and 13 subjects (81.2%) of the PNT group. The platelet aggregating sensitivity to the arachidonic acid was significantly enhanced in the PHT group ($X_s^2=3.91$, $p<0.05$; Table 3).

In spite of the insignificant differences in total cholesterol, HDL-cholesterol and triglyceride between the two groups, the ratio of total cholesterol to HDL cholesterol (Tch/HDL-ch ratio) was significantly greater in the PHT group than in the PNT group (3.40±0.70 vs. 2.91± 0.48, $p<0.05$; Table 4). There were no significant differences in blood urea nitrogen, creatinine, Na, K, Ca, uric acid, platelet count and hematocrit between the two groups (Table 4).

Although there were no significant differences in urinary volume (UV) and urinary sodium excretion (UNaV) between the two groups, urinary kallikrein excretion (UKE) was significantly lower in the PHT group than in the PNT group (72±89 μ mole·min/24 hr vs. 180±186 μ mole·min/24 hr, $p<0.05$, Table 5).

As shown in Table 6, UKE did not correlate with UNaV or UV in the PHT

Table 4. Blood Biochemistry and Hematology

Blood biochemistry & hematology	PHT group	PNT group	p value
Total cholesterol (mg/dl)	168 ±34	159 ±24	NS
HDL-cholesterol (mg/dl)	50 ±10	55 ± 8	NS
Tch/HDL-ch ratio	3.40 ± 0.70	2.91 ± 0.48	<0.05
Triglyceride (mg/dl)	77 ±58	59 ±18	NS
Blood urea nitrogen (mg/dl)	15 ± 3	16 ± 4	NS
Creatinine (mg/dl)	0.767± 0.137	0.769± 0.145	NS
Na (mEq/L)	143.0 ± 2.0	142.7 ± 2.2	NS
K (mEq/L)	4.04 ± 0.16	4.08 ± 0.25	NS
Ca (mg/dl)	9.51 ± 0.35	9.46 ± 0.28	NS
Uric acid (mg/dl)	6.9 ± 1.0	6.3 ± 1.0	NS
Platelet count ($\times 10^4$/mm^3)	19.8 ± 3.1	17.4 ± 3.0	NS
Hematocrit (%)	43 ± 2	44 ± 2	NS

Mean±SD. NS: Not significant; Tch/HDL-ch ratio: Ratio of total to HDL cholesterol

Table 5. Urinary Volume and Biochemistry

Urinary volume & biochemistry	PHT group	PNT group	p value
Urinary volume (ml/24hr)	1245 ±316	1576 ±542	NS
UNaV (mEq/24hr)	238 ± 74	266 ± 64	NS
UKV (mEq/24hr)	59 ± 18	54 ± 22	NS
UCrV (g/24hr)	1.85± 0.38	1.71± 0.39	NS
Ccr (ml/min)	144 ± 46	135 ± 33	NS
UKE (μ mole·min/24hr)	72 ± 89	180 ±186	<0.05

UNaV: Urinary sodium excretion; UKV: Urinary potassium excretion; UCr: Urinary creatinine excretion; Ccr: Creatinine clearance rate; UKE: Urinary kallikrein excretion; Mean±SD. NS: Not significant

Table 6. Correlation Coefficients of UKE with UNaV and UKE with UV in Both Groups

Correlation	Both groups (PHT+PNT)	PHT group	PNT group
UKE vs. UNaV	r=0.523** (n=30)	r=0.332 (n=14)	r=0.431* (n=16)
UKE vs. UV	r=0.523** (n=30)	r=0.194 (n=14)	r=0.584* (n=16)

UKE: Urinary kallikrein excretion; UNaV: Urinary sodium excretion; UV: Urinary volume
** $p<0.01$, *$p<0.05$

group. However, it correlated significantly with UNaV ($r=0.431$, $p<0.05$) and UV ($r=0.584$, $p<0.05$) in the PNT group.

Discussion

Parameters such as platelet aggregation (Matsumoto *et al.* [4]), serum lipids (Kristensen [5]) and urinary kallikrein excretion (Margolius *et al.* [6]; O'Connor and Preston [7]; Lechi *et al.* [8]) have been observed to be different between hypertensive and normotensive subjects. It is, however, not well understood whether such differences in the parameters take place before or after the development of hypertension. As the incidence of hypertension in subjects with one hypertensive parent is higher than in subjects with normotensive parents

(28.3% vs. 3.1%, Ayman [2]), it may be possible to find out the factors associated with the development of hypertension by evaluating the relationship between the family history of hypertension and the possibly hypertension-associated parameters.

As it has been observed that there could be abnormalities of the red or white cell membrane in the hypertensives and/or their offspring (Parker and Berkowitz [9]), the possibility of the abnormalities of the platelet membrane might be considered as well. In subjects with essential hypertension, the platelet aggregating sensitivity to the arachidonic acid was enhanced (Matsumoto *et al.* [4]). The aggregating sensitivity to arachidonic acid might be an indicator of the biosynthesis of thromboxane A_2 and the activity of platelet cyclooxygenase might be associated with such aggregating sensitivity (Matsumoto *et al.* [4]). On the other hand, thromboxane A_2 has been suggested to be an important factor in the initiation and progression of atherosclerosis (Numano [10]). In the present study, there was enhanced aggregating sensitivity to the arachidonic acid in the PHT group as well. It suggests that there might be disorders of the platelet membrane with abnormal cyclooxygenase activity resulting in a greater tendency to atherosclerosis in the PHT group.

The ratio of total cholesterol to HDL cholesterol might be an indicator of atherosclerosis (Lipinska and Gurewich [11]) and this ratio was greater in subjects with essential hypertension (Kristensen [5]). In the present study, this ratio was greater in the PHT group than in the PNT group. It suggests that subjects of the PHT group might run a higher risk of atherosclerosis.

The enhanced aggregating sensitivity and the increased ratio of total cholesterol to HDL cholesterol without simultaneously significant elevation of blood pressure in the PHT group (Table 1, 3 and 4) suggest that there could be a greater tendency to atherosclerosis in subjects with a positive family history of hypertension. As the sclerotic changes of the blood vessels might take place before the development of hypertension and nephrosclerosis might be a factor associated with the etiology of hypertension (Moritz and Oldt [12]), the greater tendency to atherosclerosis might contribute to the development of essential hypertension.

There were no significant differences in urinary volume (UV) and urinary sodium excretion (UNaV) between the two groups. However, urinary kallikrein excretion (UKE) was significantly decreased in the PHT group. Although there are not so many studies on UKE in the prehypertensive stage, urinary kallikrein concentration in the children have been reported to be negatively correlated with mother's systolic blood pressure (Zinner *et al.* [13]). Decreased UKE without simultaneous elevation of blood pressure in the PHT group in the present study suggests that the vasodepressive activity of the kallikrein-kinin system (Röckel and Heidland [14]) might be inhibited in this group and could be associated with the etiology of essential hypertension.

In rats, UKE correlates with UV and UNaV (Carretero and Scicli [15]). In human subjects, a positive correlation between UKE and UNaV has also been reported both in the normotensives and hypertensives (Adetuybi and Mills [16]). Some investigators (Margolius *et al.* [17]; Seino [18]), however, showed data against such correlation. In the present study, although UKE correlated with UV

and UNaV in the PNT group, it correlated with neither UV nor UNaV in the PHT group. This suggests that the correlations of UKE with UV and UKE with UNaV in the normotensives might be genetically different and vary with their family history of hypertension.

Excluding the influence of hypertension, some of the possibly hypertension-related parameters were found to be abnormal in the PHT group. Abnormalities of platelet aggregation and serum lipids suggest that the greater tendency to atherosclerosis in the prehypertensive stage might be etiologically important in essential hypertension.

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