Suppressive effect of oral pyridoxine hydrochloride on platelet aggregation in essential hypertension

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The effect of oral pyridoxine-HCl on platelet functions of hypertensive patients was studied. The trial comprised 18 patients with essential hypertension (11 males, 7 females), aged between 24-62 years (46±9.72) and 12 normotensive volunteers (8 males, 4 females), aged between 23-58 years (43±7.8 yr) as control. When compared with the normotensive cases, the hypertensive group had a significant higher systolic and diastolic blood pressure (p<0.001). Also,hypertensive platelets were more sensitive to adenosine 5'-diphosphate (ADP) than normotensive platelets (p<0.05) at entry. On the other hand, there were no significant differences in platelet count, bleeding and clotting time between two groups. Pyridoxine inhibited ADP and epinephrine-induced aggregation by 15% and 12% (p<0.01, p<0.05, respectively), after 4 weeks of pyridoxine treatment. In addition, pyridoxine prolonged both bleeding and clotting time within the physiological limits. It had no effect on platelet count. These observations strongly suggest that pyridoxine with no effect on platelet count, not only inhibits platelet aggregation but also prolongs bleeding and clotting time in patients with essential hypertension. [Turk J Med Res 1995; 13(6):189-193]

Key Words: Platelets, Epinephrine, Hypertension, Pyridoxin, ADP

Vitamin B-6 is an important member of the B-complex vitamins involved in a wide variety of biochemical reactions. It exists in three forms-pyridoxine, pyridoxamine, and pyridoxal. They can also be phosphorylated. After ingestion, vitamin B-6 is first converted to pyridoxine phosphate and then to pyridoxal phosphate (PLP), the conversion being activated by a kinase and oxidase, respectively. PLP Is required for many enzymatically catalyzed reactions of aminoacid metabolism, glycogen catabolism, and porphyrin synthesis, in which it participates through formation of a Schiff base (1). In addition to its normal function as a cofactor for enzymes, such as transaminases and carboxylases, PLP, with its reactive aldehyde group, has been found to be an ideal reagent for chemical modification of enzymes and proteins as a means of identifying and studying the functional group (2-4). Plasma catecholamine levels are higher in essental hypertensive patients than in normotensive cases (5-7). In addition, epinephrine potentiates the effects of other aggregating agents, accelerates blood coagulation, shortens platelet survival, and may itself induce platelet aggregation (8,9). There are complex interactions between sympathetic activity and pyridoxine action. Many investigators find hyper-responsive platelets in hypertensive patients (10-12), while others do not (13). On the other hand, at a concentration >0.8 mM, PLP completely inhibited the second wave of aggregation in platelet-rich plasma (PRP) containing ADP and thrombin. A similar inhibitory effect on collagen-induced platelet aggregation was also observed when PLP was present at a concentration of >1.5 mM. The primary aggregation by ADP, thrombin and collagen was also abolished when PLP was present in PRP at a concetration higher than 2 mM (14). They have also suggested that vitamin B-6 prolongs clotting time.

In view of these findings, it is reasonable to suggest that PLP might interact with platelets and thereby modify platelet function. To investigate this probability, we studied the effect of PLP on the aggregation of platelets by ADP, epinephrine and collagen in the patients with essential hypertension.

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MATERIALS AND METHODS

Ambulatory patients with essential hypertension were recruited between 10 December 1993 and 30 March 1994 provided that no more than two occasions their systolic blood pressure (SBP) had been higher than 140 mmHg or their diastolic blood pressure (DBP) higher than 90 mmHg. eighteen patients with essential hypertension (11 men, 7 women, aged 46 ± 9.7 (SD) years (range 24-62 years) and their average body mass index 24.8±4.3 kg/m^2), as control were investigated. All patients were evaluated and followed up at the hypertension clinic of the Dicle Medical Center. The diagnosis of essential hypertension had to be established before the study based on the absence of any clinical evidence of secondary hypertension; normal laboratory findings including results of a complete blood count, estimations of serum glucose, creatinine, urea nitrogen, and sodium and potassium concentrations, and urine analysis. Hypertensive patients who were being treated with antihypertensive durgs were tapered off their medications and were studied after a minimum period of 10-days during which no antihypertensive medication were taken. They were treated by a single oral dose of 5mg /kg/day pyridoxine for 4 weeks. Control and hypertensive groups were evaluated and followed up at the hypertension clinic of the Dicle Medical Center at 08:30 a.m. after an overnight (12h) fasting subjects were also prohibited from the use alcohol, nicotine, and caffeine during this period. This study was approved by the University Human Ethics Committee and written informed consent was obtained from each participant. After a 5-minute rest, blood pressure was measured at three 1-minute intervals on the right arm of seated subjects with a mercury sphygmomanometer. First and fifth phase, Korotkoff sounds were recorded. The mean of the second and third blood pressure measurements was used in analyses (13). Heart rate was registered at the same time. Mean arterial blood pressure (MAP) was calculated as the DBP plus one third the pulse pressure. Venous blood samples were obtained on two occasions before treatment, and then after 1 month of treatment under basal conditions. These samples were collected between 09.00 and 10.00 a.m.; the subjects had fasted since at least midnight previously in the morning.

Chemicals: Pyridoxine-HCI tablet was obtained from Liba Laboratories (Istanbul, Turkey). Adenosine 5'-diphosphate (ADP) and epinephrine (lyophilised with buffer salts) were obtained from Sigma Chemical CO (St.Louis, MO, U.S.A.).

Platelet preparation: Blood was collected from antecubital vein with a plastic syringe from hypertensive group. The sample immediately mixed with a 3.8 % sodium citrate solution in ratio of 9 volumes blood/volume citrate. The gently mixed sample was centrifuged at 100 g for 20 minutes and the platelet-rich plasma (PRP) was transferred at 2000 g for at least 10 minutes to prepare platelet-poor plasma (PPP); and the PPP was removed to a plastic test tube and also stored at room temperature. At least 30 minutes was allowed to elapse between venipuncture and assay of platelet aggregation; no sample was run any later than 3 hours after venipuncture. Platelets' counts were determined on an S-Plus Coulter Counter (Coulter Electronics Inc.).

Platelet aggregation studies: Aggregometry was performed according to the method of Born (15) with use of a Lumi-Aggregometer (Chrono-Long Corporation, Haverford, PA) at 37°C with 1-ml silicon-treated cuvettes and rotating bars (1200 rpm). 450 (iL of PRP was added to an aggregation cuvette without stirring bar. PRP and PPP cuvettes were placed in corresponding instrument sample wells and followed manufacturer's instructions for setting base lines. The volume of aggregating agents (ADP, or epinephrine) added to the PRP cuvette was 50 uL. Platelet aggregation responses were recorded for minimum of 5 minutes. The Chorono-Log Lumi-Aggregometer automatically sets the PPP base line to 90 on the chart recorder when the PRP blank is set to 10. This allows the % aggregation to be calculated from the final chart reading [CR] as follows:

$$\%\text{Aggregation} = \frac{90 - CR}{90 - 10} \times 100 = \frac{90 - CR}{80} \times 100$$

Bleeding time was determined by Ivy (16) and clotting time was determined by Lee-White (17) method.

Student's t-test was used to evalute differences between controls and patients in the mean for blood pressure or platelet aggregation. A paired t-test was used to evaluate differences between base-line and treatment values in the patients, as well as differences between initial and final platelet aggregation. Results are expressed as means ±SD. A minimum level of significance was set at $p<0.05$.

RESULTS

The characteristics of the hypertensive and control groups at the time of entry into the study are shown in Table 1.

The results are described in Table 2. ADP-induced platelet aggregation was reduced from 85.4±12.8 %72.3±10.5% after pyridoxine treatment. Approximately a 15% inhibition of platelet aggregation was found to be statistically significant ($P<0.01$). Epinephrine-induced platelet aggregation was also inhibited by pyridoxine. An average decrease of 79.7% to 69.8% in epinephrine-induced aggregation index was observed after pyridoxine treatment. Approximate-

Table 1. Characteristics of hypertensive and normotensive subjects at entry (mean ±SD).

Characteristic	Hypertensive (n=18)	Control (n=12)
Age(yr)	46±9.72	43±7.8
Sex(%male)	61	66
Height (cm)	172+2.32	173+3.62
Weight (kg)	75±7.65	73±6.8
BMI(kg/m^2)	25.3±3.54	4.4±5.2
SBP (mmHg)	158+16.2*	30±7.8
DBP (mmHg)	103i.56*	88±4.9
MAP (mmHg)	121 ±10.4*	02±5.8

*p<0.001, pretreatment versus control values, BMI: Body mass index, SBP: Systolic blood pressure. DBP: Diastolic blood pressure, MAP: Mean arterial blood pressure.

Table **2.** Effect of pyridoxine on platelet aggregation, bleeding time, clotting time and platelet count (meantSD).

Parameter	Pretreatment (n-18)	Post-treatmen t(n=18)	Control (n=12)
ADP-induced aggregation index (%)	85.4±12.8*	72.3±10.5*	76.3±8.5
Epinephrineinduced aggregation index(%)	79.7±15.2	69.8±11.9*	71.6±9.8
Bleeding time (min)	1.42±0.28	1.68±0.31*	1.51±0.13
Clotting time (min)	4.37±1.37	5.79±1.29"	5.0H1.12
Platelet count (x10^6/L)	312+42	324±46	315±32

*P<0.05, preatreatment versus control and post-treatment values
**P<0.01, preatreatment versus post-treatment values

ly a 12 % inhibition of platelet aggregation was statistically significant (p<0.05). Pyridoxine prolonged bleeding and clotting time from 1.42±0.28 min and 4.37±1.37 min to 1.68±0.31 min and 5.79±1.29 min(p<0.05, p<0.01, respectively), but these values were not over the physiological limits. Platelet count was not affected by pyridoxine treatment.

DISCUSSION

Various report have indicated that pyridoxine deficiency in he adult rats leads to true arterial hypertension (18,19). Treatment of pyridoxine-deficient rats with pyridoxine restored blood pressure, hypothalamic PLP, gamma-aminobutyric acid, serotonin, and plasma norepinephrine and epinephrine levels to normal range within 24 hours (18). Plasma catecholamine levels are higher in essential hypertensive patients than in normotensive subjects (5-7). The concentration of norepinephrine and epinephrine in peripheral plasma can be taken as a valid reflection of sympathetic activity. Because norepinephrine and epinephrine stimulate the heart and constrict blood vessels, it has long been thought that increased sympathetic tone is involved in the etiology of hypertension (20). Recently, we reported that oral supplementation of pyridoxine (5 mg/kg body weight) to essential hypertensive human subjects markedly diminished arterial blood pressure and plasma cathecolamine levels (26). There are complex interactions between sympathetic activity and pyridoxine action. In addition, Ardlie et al. (8), and Mustard and Pacham (9) found that epinephrine potentiates the effect of aggregating agents, accelerates blood coagulation, shortens platelet survival, and may itself induce platelet aggregation.

The major findings of our study on platelet aggregation in hypertensive human subjects and the effects of oral pyridoxine (5mg/kg body weight) treatment were as follows: 1)There is an increased platelet aggregation in response to ADP and epinephrine in hypertensive subjects with MAP>120 mmHg. 2)Treatment with pyridoxine decreased the aggregatory response to ADP and epinephrine (p<0.01). 3) Also, treatment with pyridoxine prolonged did not affect platelet count. Platelet aggregation patterns in hypertensive subjects have been compared with those of normotensive subjects with conflicting results in a number of studies (10-12). In those studies where the MAP of the hypertensive subjects is less than 120 mmHg, no differences in aggregatory responses to ADP or epinephrine are found. However, in the studies where the MAP of patients is greater than 120 mmHg, platelets of hypertensive patients are more reactive than platelets of normotensive person. Pyridoxine deficiency causes arterial thrombosis, atherosclerotic lesions and arterial hypertension in monkey, dogs, rats and rabbits (18,19,21,22). Pyridoxine in moderate doses (1 mg/kg/day) prevents fatal thrombosis and pulmonary embolism in rabbits receiving high doses of methionine or homocystine thiolactone (23). Inherited homocystinurics manifest cardiovascular complications such as vascular thrombosis, thromboembolism and atherosclerosis. Clinical studies have shown that such patients could be successfully treated with vitamin B-6 (18,19,24). Subbarao et al. (25) reported that a single oral administration of 100 mg of pyridoxine-HCl to humans produced a mild inhibitory effect on platelet aggregation within 2 h and suggested that vitamin B-6 might also function as an antithrombotic agent in vivo. Inhibition of platelet aggregation by PLP -a derivative of vitamin B-6- is supported by these dta. Our findings support the previous observation indicated by Subbarao et al. (14). Kornecki et al. (24), and Sermet et al. (26). But they contradict with some of the available findings suggesting that vitamin B-6 has no effect on platelet aggregation (27,28). These observations strongly suggest that PLP is an antithrombotic agent

which not only alters platelet function but also prolongs clotting time.

In healthy volunteers intravenous PLP not only inhibited platelet aggregation but also prelonged the whole blood clotting time (14). The clotting time of PLP -bound fibrinogen was two to three times longer than that of normal fibrinogen. Similarly, the procoagulant activity of PLP - linked thrombin was much lower than that of native thrombin. Thus, binding of PLP to thrombin and fibrinogen results in a prolonged clotting time (14). Patients with essential hypertension have a higher concentration of free cytosolic calcium in their platelets, compared with normotensive subjects (29). Internal calcium flux is required for the internal platelet concentration and secretion induced by aggregation and release-inducing agents, and maybe PLP prevents this. The mechanisms involved in the inhibitory effect of vitamin B-6 are not fully understood at this time. However several hypotheses have been postulated related related to its mode of action: Increses in cyclic AMP concentration have been observed in response to various substances that inhibit platelet aggregation; thus its possible that PLP may be producing its inhibitory effect by raising platelet cyclic AMP. The mechanism by which PLP inhibits platelet function may be through the formation of a reversible Schiff base between PLP's aldehydic group and specific platelet surface amino groups or PLP may interact with the agonist-platelet system by an interaction with essential surface residues (e.g., sulfhydryl groups), or interaction with agonists to form a complex thereby thereby recucing available agonists concentration, or interaction with a necessary cofactor, e.g., fibrinogen and interaction with sites that are specific for agonist action (e.g., ADP receptor sites) (24).

It is concluded that PLP -derivative of vitamin B-6-may play an important role in the control of platelet sensitivity to different aggregating agents and also in the coagulation of plasma in the patient with essential hypertension.

Ağızdan alınan piridoxin hidroklorürün esansiyel hipertansiyonda trombosit agregasyonu üzerine baskılayıcı etkisi

Ağızdan alınan piridoxin hidroklorürün hipertansif trombosit fonksiyonu üzerine olan etkisi in vitro olarak incelendi. Çalışma yaşları 24-62 arasında değişen(46±9.72), 11 erkek, 7 kadından oluşmak üzere 18 esansiyel hipertansiyonlu ve 8 erkek, 4 kadından oluşmak üzere, yaşları 23-58 arasında değişen (43±7.8 yıl) 12 normal tansiyonlu bireyde kontrol grubu olmak üzere oluşmaktaydı. Normal tansiyonlu bireylerle karşılaştırıldığında hipertansiyonlu bireylerde sistolik ve diyastolik kan basıncı ciddi olarak daha yüksekti (p<0.001). Aynı zamanda, hipertansiyonlu hastalarda trombositler, çalışmaya başlarken, normal tansiyonlu hastalardaki trombositlere göre adenozin 5'-difosfata CADP) daha duyarlı idi (p<0.05). Diğer taraftan, trombosit sayısı, kanama ve pıhtılaşma zamanı açısından her iki grupta anlamlı fark yoktu. Pridoksin tedavisinden 4 hafta sonra, ADP ve epinefrinin neden olduğu agregasyon sırasıyla % 15 (p<0.01) ve % 12 (p<0.05) oranında azalmaktaydı. Ayrıca, pridoxin kanama ve pıhtılaşma zamanını uzatmaktaydı, ancak bu uzama fizyolojik sınırlar içindeydi. Trombosit sayısı üzerine etkisi yoktu. Bu gözlemler, pridoksinin trombosit sayısı üzerine etkisinin olmadığını ve yalnızca esansiyel hipertansiyonlu hastalarda trombosit agregasyonu inhibe etmediğini, aynı zamanda da kanama ve pıhtılaşma zamanını uzattığına güçlü bir şekilde işaret etmektedir.
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