The Effects of Dipyridamole on Serum and Urine Calcium and Phosphorus Levels in Healthy Volunteers

Sağlıklı Gönüllülerde Dipridamolun Serum ve İdrar Kalsiyum Fosfor Düzeylerine Etkisi

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ABSTRACT Objective: It has been demonsrated that dipyridamole (D), increases serum phosphorus (P) levels and decreases urinary P excretion in some patients with hypophosphatemia. In this study we investigated the effects of D on the serum and urine P levels in healthy volunteers. **Material and Methods:** Twenty-six healthy volunteers older than 18 years were included. D was given orally 3 times 75 mg daily for 7 days. In blood and urine samples biochemical tests were done before and after D. **Results:** After D therapy: Serum Ca and Mg levels and urinary Ca excretion increased significantly ($p< 0.05$ for all). Daily urinary K excretion decreased ($p< 0.069$). Glomerular filtration rate (GFR), serum P level and daily urinary P excretion did not change. Tubular phosphorus reabsorption (TPR) and serum P and kidney threshold for P excretion (TmP/GFR) decreased ($p<0.05$ for all). **Conclusion:** D treatment did not change serum P levels and urinary P excretion, decreased TPR and renal P threshold in healthy volunteers These results can be related with duration of D therapy, normophosphatemia condition and our study was relatively small. D increased serum Ca level and daily urinary Ca excretion while serum PTH concentration and serum vitamin D level remained unchanged in healthy volunteers and renal tubular phosphorus reabsorption and renal phosphorus threshold decreased with oral D treatment. In spite of GFR did not change, tubular functions such as reabsorbtion and threshold of P, urinary excretion of K and Ca, can be affected with D treatment. We need similar studies exploring probable mechanisms in larger groups.

Key Words: Dipyridamole; phosphorus; electrolytes

ÖZET Amaç: Dipridamol (D)'un, hipofosfatemili bazı hastalarda serum fosforunu yükselttiği ve idrarla P atılımında azalmaya yol açtığı bilinmektedir. Bu çalışmada, sağlıklı gönüllülerde dipridamolun serum ve idrar P düzeylerine olan etkisini araştırdık. **Gereç ve Yöntemler:** On sekiz yaşından büyük 26 sağlıklı gönüllüye 3 x 75 mg/gün D verildi. Tedavinin başlangıcında ve sonunda kan ve idrar örneklerinde biyokimyasal testler yapıldı. **Bulgular:** D tedavisi sonrası serum Ca ve Mg seviyesi ve idrarla günlük Ca atılımı arttı ($p< 0.05$ hepsi için). Günlük idrar K atılımı azaldı ($p< 0.069$). Glomeruler filtrasyon hızı (GFR), serum P düzeyi ve günlük üriner P atılımı değişmemişti. Tübüler fosfor geri emilimi (TPR) ve P atılımında serum P ve böbrek eşiği (TmP/GFR) azalmıştı ($p<0.05$, hepsinde). **Sonuç:** Sağlıklı gönüllülerde 7 günlük D tedavisi ile serum P seviyesi ve idrarla P atılımı değişmedi. Fakat D, TPR ve böbrek P eşiğini azalttı. Bu sonuçlar D tedavi süresinin sadece 7 gün olması, serum P düzeyinin normal olması ve çalışmadaki olgu sayısının az olması ile ilgili olabilir. Bu etkiler serum P düzeyi düşük veya GFR'si azalmış hastalardakinden farklı idi. Ayrıca PTH ve D vitamini düzeyi değişmedi ancak serum Ca seviyesi ve idrarla Ca atılımı artış gösterdi. D tedavisi ile GFR değişmediği halde tübüler P geri emilimi ve P eşiği ve idrarla Ca ve K atılımı gibi bazı tübüler fonksiyonlar etkilenebilir. Daha büyük gruplarda olası mekanizmaların araştırılması ile bu konu daha iyi değerlendirilebilecektir.

Anahtar Kelimeler: Dipridamol; fosfor; elektrolitler

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The inhibitor mechanisms of the dipyridamole (D) on the platelet aggregation and adhesion are not known exactly. It has been proposed that it inhibits the re-uptake of adenosine of erythrocytes, increases 3.5 adenosine monophosphate by inhibiting phospho-diesterase in platelets and prohibits the synthesis of thromboxan A2 which is activator of platelets.[1] D causes vasodilatation by inhibiting adenosine destruction and for this reason it has been used in heart-vessel diseases since long time. In addition to these known effects, it has been reported that it decreases urine P excretion and increases serum P level in patients with primary hyperparathyroidism and in cases with low serum P and low renal P threshold.[2] It was reported that adenosine stimulates phosphate and glucose transport in opossum kidney epithelial cells.[3] In a similar way we observed an increase in serum P level with D therapy in renal transplant receivers with hypophosphatemia.[4] In addition we detected an increase in serum PTH level in chronic renal failure (CRF) (stage 2, 3, 4) patients treated with D.[5] The aim of this study was to investigate the changes in urine P excretion in healthy volunteers treated with D.

MATERIAL AND METHODS

Healthy volunteers were included in this study. Inclusion criteria: Age older than 18, without abnormal findings including physical examination, medical history and laboratory measurements and drug usage, acceptance of the volunteer to enter the study and to receive study drug D for 7 days. They were on their standard diet.

Blood samples were taken after 12 hours starvation and hemoglobin (Hb), hemotocrit (Hct), platelet glucose, blood urea nitrogen (BUN), creatinine (Cr), sodium (Na), potassium (K), chlorur (Cl), phosphorus (P), calcium (Ca), magnesium (Mg), total protein, albumin, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanineaminotransferase (ALT), gamma glutamine transpeptidase (GGT), parathormone (PTH), Vitamin D2, platelet aggregation tests (collogen, ristocetin, thrombin) were tested. Daily urine samples simultaneously were analyzed for BUN, Cr, Na, K, Cl, Mg, Ca, P, Cr and protein. Blood samples were taken to standard tubes and EDTA containing tubes. Roche modular DPP system was used for biochemical analyses. Enzymatic colorimetric method used for AST, ALT, ALP and GGT measurements. Colorimetry (photometry) was used for glucose, BUN, Cr, Na, K, Cl, Mg, Ca, P, total protein, albumin, PTH and Vitamin D_2. Beckmanın LH-750 Coulter-Counter system was used for complete blood count. Platelet agregation tests were performed by using Chrono-log 500-vs machine and Agro-link system.

Creatinine clearance (CCl) was calculated with Cockcroft-Gault formula. Fractionated sodium excretion (FEx Na), Tubular P reabsorbtion (TPR), Fractionated P excretion (FEx P) were calculated with formulas shown below:

- FEx Na= FEx Na: Urine Na X Plasma Cr/Urine Cr X Plasma Na,
- TPR: 1-(Urine P X Serum Cr/Urine Cr X Plasma P),
- FEx P: (Urine P X Plasma Cr/Plasma P X Urine Cr) x100.
- Renal P threshold (TmP/GFR) was calculated with Walton RJ, Bijvoet OLM nomogram.[6]

After basal evaluation, D (Drisentin 75 mg-Sanovel), 3 x 75 mg daily dose, was given orally for 7 days to healthy volunteers. Blood and urine tests were repeated at the end of 7 day. Seven of 33 volunteers were excluded for intolerance (headache, hypotension and leg pain) to D.

Ethical approval has been taken from Çukurova University Ethic Council (Date 07/06/2006). Patients were informed about the possible side effects of the drug

STATISTICAL ANALYSIS

Results have been given as minimum, maximum values and mean ± standard deviation. SPSS v.14 has been used for statistical analyses. Dependent t test has been used for the serum parameters (Ca, P, Cr, Na) before and after drug using. Wilcoxon test was used in nonparametric measurements. p value <= 0.05 was used for statistical significance.

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RESULTS

Study group was consisted of 26 healthy volunteers. Male/female ratio was 15/11 Mean ages for male and female were 36.67 ± 8.51 (Range 26-55) and 35.55 ± 9.76 (R 21-55), respectively. Test results before and after 7 days D therapy, have been shown in Table 1. Figure 1 shows the changes in serum Mg, Ca and P levels. Figure 2 shows the decreases in TmP/GFR (from 3.60 ± 0.60 mg/dL to 3.30 ± 0.57 mg/dL (p= 0.039) and TPR (from 0.88 ± 0.35 to 0.84 ± 0.56 (p= 0.001). TmP/GFR change was important according to the t test but not according to the Wilcoxon Signed Rank Test (p value 0.051). Figure 3 shows urinary P [from 0.61 ± 0.32 g/day to 0.65 ± 0.31 g/day (p= 0.485)] and urinary Ca levels (from 100.31 ± 49.09 mg/day to 134.73 ± 68.98 mg/day [p= 0.015]).

DISCUSSION

Phosphate plays an important role in cell metabolism and bone mineralization. In physiologic conditions serum P is higher than 0.8 mmol. Kidney is the essential organ in P homeostasis. In adults 80% of filtreted P is reabsorbed from proximal tubule and this re-absorption is under PTH control.[7,8] P loss from urine, as seen in primary hyperparathyroidism and PTH related peptide excretion, may be associated with excess PTH secretion. Additionally urinary P loss increases in Fanconi syndrome, congenital or acquired renal tubular acidosis and oncogenic osteomalacia and even it may be idiopathic.[9,10] P loss from urine causes hypophosphatemia, urolithiasis or bone demineralization.[2,3] Renal P loss is treated with increased P intake and/or vitamin D. However this therapy may stimulate occurrence of renal stone. Michaut et al. reported that D decreases renal P loss in human.[2] Although any changes in serum P, TmP/GFR, PTH, 1.25 dihydroxy vitamin D, alkaline phosphatase, osteocalcin and urinary Ca and cAMP excretion has not been found in 6 cases with X-linked hypophosphatemia treated by D for 14 weeks.[11] An increase in serum P and kidney threshold for P excretion (TmP/GFR), a decrease in fractionated P excretion with 30 minutes D infusion have been shown in 48 cases (12 normal, 9 primary hyperparathyroidism, 27 cases with hypophosphatemi unrelated with PTH).[2] We also found an important increase in serum P and TPR and a decrease in urinary P excretion in 11 renal transplant recipients treated by D for 3 weeks.[4] There was a positive correlation bet-

TABLE 1: Test results before and after 7 days dipyridamole therapy.

	Before D (mean ± SD) (min-max)	After D (mean ± SD) (min-max)	P
Na (mmol/L)	142.42 ± 3.31 (137-148)	138.96 ± 4.69 (126-146)	0.003
Ca (mg/dL)	9.84 ± 0.35 (9.1-10.5)	10.15 ± 0.64 (9.1-11.6)	0.008
Mg (mg/dL)	2.05 ± 0.13 (1.8-2.3)	2.32 ± 0.52 (1.8-3.5)	0.042
Urine Ca (mg/day)	100.31 ± 49.09 (35-257)	134.73 ± 68.98 (29-280)	0.015
Urine K (mmol/day)	46.24 ± 20.35 (14.6-112.2)	38.98 ± 12.87 (7-65)	0.069
TPR	0.88 ± 0.35	0.84 ± 0.56	0.001
F ExP	11.42 ± 3.87	16.30 ± 6.07	0.001
TmP/GFR (mg/dL)	3.6 ± 0.60 (2.6-5)	3.3 ± 0.57 (3.2-4.4)	0.039
K (mmol/L)	4.53 ± 0.41 (3.76-5.34)	4.40 ± 0.29 (3.94-4.96)	0.106
P (mg/dL)	3.58 ± 0.51 (2.6-4.7)	3.64 ± 0.43 (3-4.8)	0.509
CaXP	35.15 ± 4.95 (26.5-46)	36.86 ± 4.27 (31-49)	0.218
PTH (pg/mL)	42.26 ± 20.06 (22.47-91.72)	42.78 ± 21.10 (13.17-88.85)	0.909
VTt.D2 (ng/mL)	41.00 ± 26.42 (10-118)	36.79 ± 22.89 (6-101)	0.137
Urine P (g/day)	0.61 ± 0.32 (0.2-1.45)	0.65 ± 0.31 (0.05-1.27)	0.485
Urine Na (mmol/day)	162.38 ± 64.58 (68-315)	165.88 ± 60.71 (44-327)	0.751
CrCl (ml/min)	124.31 ± 25.30	124.92 ± 26.82	0.826
F Ex Na	1.05 ± 0.55	1.02 ± 0.45	0.909

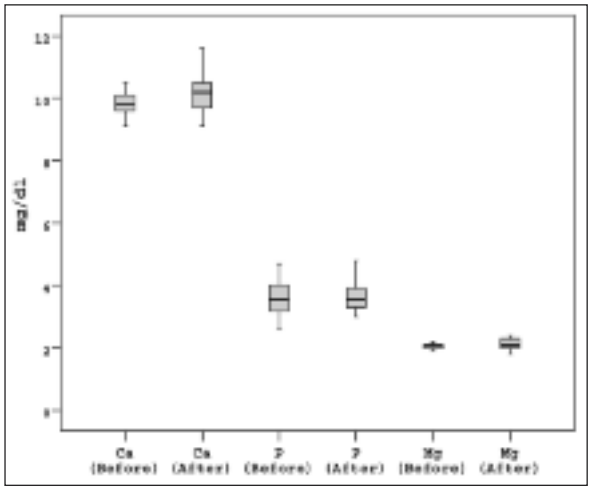

FIGURE 1: Serum Ca, P and Mg levels before and 7 days after dipyridamole (p= 0.008, p= 0.509, p= 0.042 respectively).

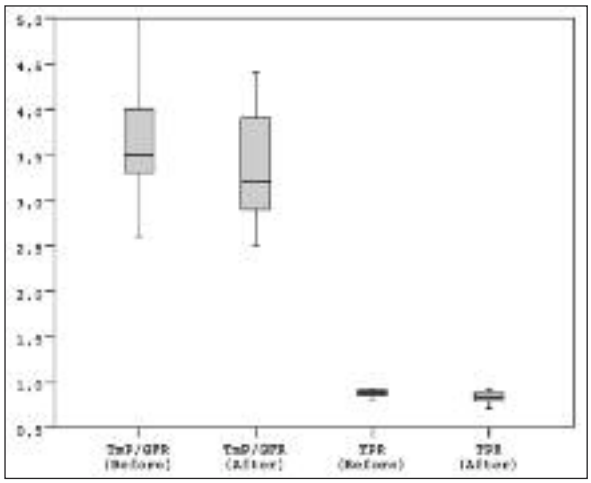

FIGURE 2: TmP/GFR and TPR measurements before and after 7 days of dipyridamole (p= 0.039, p= 0.001 respectively).

ween TPR and serum P increase. Although ongoing hyperparathyroidism causes to hypophosphatemia in renal transplant recipients it has been suggested that phosphatonins contribute to this hypophospatemia.[12] It was found that oral P support caused hypophosphatemia in hypophosphatemic patients.[13] This effect was explained as oral phosphorus decreases vitamin D and stimulates secretion of PTH.[12] In another our study we did not detect any change in serum P, Ca, Na, urinary P, Ca, TPR in 30 cases with CRF (grade III, IV) treated with D for 4 weeks but we found an increase in PTH.[5] For this reason it can be thought that D may increase the peripheral resistance to PTH. It is known that PTH activates adenylatecyclase and peripheral effects occur with cAMP.[2] D prevents adenosine re-uptake and decreases the intracellular cAMP and so D may cause resistance to the peripheral effects of PTH. We detected also in the present study that D caused change of TPR and TmPGFR in healthy volunteers. However, decrease of TPR and TmPGFR secondary to D therapy, was contrast to the results of other studies. But the TPR and TmPGFR after D, were not abnormal level. Although we did not detect a change in platelet functions, PTH level and renal functions tests, we detected important changes in serum Na, Ca, Mg and urinary Ca, K, and TPR with D therapy. The increase in urinary Ca was important while there was no change in vitamin D_2 or PTH. As known, PTH shows its effect with cAMP. It has been found that D does not change urinary cAMP/ceatinine ratio in cases with low renal P threshold.[14] But another study showed that urinary cAMP excretion was found to be decreased with D therapy.[2]

In our study, decreases in urinary K and serum Na may be related with changes in aldesteron. It has been reported that D inhibits renin in hypertensive cases.[15] We did not measure the levels of intracellular K and serum aldesteron and blood pH. In our study, changes in serum and/or urinary Ca, Mg, K, Na concentrations suggest the importance

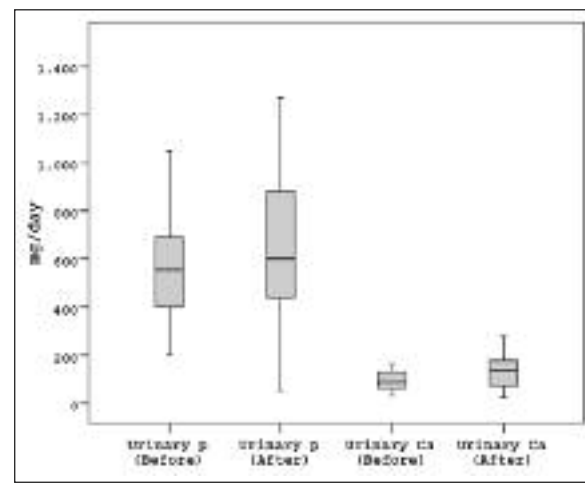

FIGURE 3: Urinary P (from 0.61 ± 0.32 g/day to 0.65 ± 0.31 g/day (p= 0.485) and Ca levels (from 100.31 ± 49.09 mg/day to 134S.73 ± 68.98 mg/day (p= 0.015) before and 7 days after dipyridamole.

of the effects of D in renal tubules and other cells. In contrast to the other studies different result for P excretion may be due to the D schedule which 30 minutes D infusion was made in another study. In other study, data was obtained from low TPR patients treated with 12 months D. In second study the increase in TPR was most prominent from the beginning until 9 months and there was not an additional increase after this time. In this study patients had hypophosphatemi and low TPR in contrast to our healthy volunteers. There was not a change with 12 weeks D therapy in serum P, PTH, 25(OH)2, Vitamin D, osteocalcin, TmP/GFR and urinary excretion of Ca and 3,5 cAMP.[11] D effects on TPR and TmP/GFR were different in various studies. In contrast to the literature information, we found a decrease in TPR and TmP /GFR in healthy volunteers treated by D with 3 x 75 mg dosage. However we previously found different results in other our studies related with D in patients with renal transplantation and chronic renal failure. In our first study we found an increase in TPR in hypophosphatemic renal transplant recipients treated with D.[4] In our second study, we did not find a change in TPR in patients with CRF. What is the meaning of the increase in serum and urinary Ca and no change in Vitamin D2 and PTH in healthy volunteers as observed in patients with CRF? It can be related with change in the response to PTH, or it may be due to the D effect on the Ca, P absorption via type II Na-P co-transporter receptors in small intestine. The net effects of D can be different in physiologic and pathologic conditions and dose and duration of D therapy.

In summary in healthy volunteers oral D therapy for 7 days schedule caused, a decrease in TPR and Tm/GFR, increase in fractional excretion of P and no change in serum P and urinary P excretion. Additionally we found an increase in serum Ca, Mg and urinary Ca, a decrease in serum Na and daily urinary K loss. These effects were different than that of in patients with lower serum P level or decreased GFR. While GFR did not change, tubular functions such as renal tubular reabsorbtion and threshold of P, urinary excretion of K and Ca, can be affected with D treatment. Similar study with larger number of volunteers will be more informative about the results and we believe that the evaluation of possible mechanisms may define the exact causes of these variable results.

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