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2,3-Diphosphoglyceric Acid Changes in Uremia and During Hemodialysis

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The present study is an investigation of the mechanism of changes in erythrocyte 2,3-diphosphoglyceric acid (2,3-DPG) in patients with chronic renal failure during hemodialysis.

The study was conducted on 7 Korean and 6 American patients on maintenance hemodialysis. The plasma pH of Korean patients was 7.31±0.02 before hemodialysis and 7.40±0.04 after hemodialysis ($p<0.001$). The pH of erythrocyte also increased from 7.13±0.02 to 7.20±0.03. The concentration of hemoglobin 2,3-DPG in Korean patients was 10.86±2.89 μmol/g before hemodialysis and 19.93±2.89 μmol/g after hemodialysis ($p<0.001$).

Similar results were obtained in American patients. Hemoglobin 2,3-DPG was 12.54±2.53 and 18.76±6.73 μmol/g before and after dialysis respectively. Despite the presence of substantial anemia, hemoglobin 2,3-DPG prior to hemodialysis was significantly lower than the values obtained in the normal controls (17.45±4.3 μmol/g).

The blood glucose increased from 93.3±8.5 mg/dl before dialysis to 117.1±6.1 mg/dl after hemodialysis in Korean patients but no significant change was detected in American patients. The increased blood glucose with hemodialysis observed in Korean patients was probably attributable to the lower baseline glucose concentration and the gain of glucose from dialysate, which had a glucose concentration of 186±34.1 mg/dl.

The results suggest that the increase in 2,3-DPG with hemodialysis is probably caused by an increase of pH and an increased glucose utilization.

Key Words: *Hemodialysis, Anemia, Red blood cell 2,3-DPG, Glucose.*

INTRODUCTION

In chronic anemia, oxygen supply to the vital tissues is facilitated by decreasing the intracorpuscular oxygen binding capacity.

Chronically anemic patients in general show a little or sometimes no systemic symptoms. This suggests that there must be some mechanisms to compensate for the decreased partial pressure to oxygen in arterial blood. One important mechanism is the increase of the concentration of 2,3-diphosphoglyceric acid (2,3-DPG), an intracorpuscular phosphorylated compound resulting in decrease of the oxygen affinity of hemoglobin.[1-3] Thus 2,3-DPG plays an important role in unloading oxygen to the tissues.

Since the introduction of hemodialysis in 1940 by Kolff for the treatment of acute renal failure, not only prolongation of the lifespan of the patients and the return to the social life have became possible, but also many problems following hemodialysis as well as complications of chronic renal failure itself have become evident.

During hemodialysis the patients may experience cardiopulmonary symptoms such as dyspnea, chest discomfort, decrease of blood pressure, arrhythmia, tachycardia and muscle cramp. There are several proposed mechanisms to explain about these symptoms, but they are still controversial.[4-15]

Bergstroem et al.[16] attributed these non-

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physioligical phenomena to hypovolemia and decrease of plasma osmolarity due to hemodialysis. Rodrigo et al.[17] reported that the patients with stood the dialysis better and had less decrease of plasma osmolarity after changing the glucose concentration in dialysate from 278 to 714 mg/dl.

On observation of electrolytes and biochemical changes in patients with chronic renal failure and on maintenance hemodialysis, authors noticed that despite the severe anemia (hemoglobin 5-9g%), intracorpuscular 2,3-DPG concentration was lower than the normal value, and whole blood and hemoglobin 2-3-DPG concentration increased after hemodialysis. The purpose of this investigation is to determine the mechanism changes in 2,3-DPG in patients with chronic renal failure undergoing hemodialysis treatment.

SUBJECTS AND METHODS

Healthy volunteers without any evidence of kidney disease, 7 males and 4 females between 27 and 60 years of age, served as the control. The study group consisted of 13 patients (7 Korean and 6 American) on maintenance hemodialysis with relatively stable chronic renal failure. They were dialyzed using a hollow fiber dialyzer (Cobe comp.) for 4 hours, 2 to 3 times a week.

Five milliliters of blood samples were collected from the patients before and after dialysis. Blood cell counts were done with the Coulter counter, blood glucose measurement with a Beckman analyzer, intracorpuscular pH measurement by Peter Hilpert method[16] and arterial blood gas analysis with Radiometer-Copenhagen acid-base cart machine. The concentration of 2,3-DPG was measured in accordance with enzymatic methods in Sigma technical bulletin No. 35-UV as follows: One milliliter of blood sample was mixed with 3.0ml of 8% trichloroacetic acid and centrifuged for 10 min. at 3000 rpm to obtain the supernatant. One milliliter of NADH was dissolved in 8.0 ml of 0.2M triethanolamine buffer and 2.5 ml of this NADH solution was mixed with 0.1 ml of ATP and 0.25 ml of supernatant in a 3 ml cuvette.

To this reaction mixture, 0.2ml of GAPD/PGPD (glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate phosphokinase) and 0.2 ml phosphoglycerate mutase were added. The optical density of the reaction mixture was measured after 5 min. at 340 nm.

One-tenth milliliters of phosphoglycolic acid was added to the above reaction mixture and allowed to stand for 30 min. at room temperature. The optical density was measured again at 340 nm. The blood 2,3-DPG concentration (μmol/ml) thus measured was expressed as μmol per gm of hemoglobin.

RESULTS

1. **Concentration of 2,3-DPG of whole blood, packed cells and hemoglobin in control group**

   The mean hemoglobin and hematocrit value of control groups were 14.2±1.6% and 41.9±4.5%. The mean 2,3-DPG value of whole blood, packed cell and hemoglobin were 2.43±0.6, 5.81±1.4 μmol/ml and 17.45±4.3

Table 1. Blood findings and 2,3-diphosphoglycerate level in normal persons

	Age/Sex	Hg(g/dl)	Ht(%)	2,3-DPG (μmol/ml)		
				W.blood	P.cell	Hg(umol/g)
1.	28/M	16.5	51.6	2.01	3.89	12.2
2.	44/M	12.6	39.0	2.79	7.15	22.2
3.	31/M	12.8	39.6	2.74	6.92	21.4
4.	32/M	15.7	45.5	3.55	7.80	22.6
5.	29/M	13.7	40.6	3.24	7.98	23.7
6.	30/M	13.5	39.5	2.52	6.38	18.7
7.	27/M	17.5	48.2	2.52	5.20	16.8
8.	30/F	13.6	38.8	1.89	4.80	13.5
9.	31/F	14.2	40.8	1.65	4.00	11.6
10.	58/F	11.8	35.0	1.60	4.58	13.6
11.	60/F	14.4	43.0	2.25	5.23	15.6
M±SD	36.4	14.2±1.6	41.9±4.5	2.43±0.6	5.81±1.4	17.45±4.3

μmol/g. respectively (Table 1).

2. The change of partial pressure of arterial blood gas in patients on hemodialysis

Seven out of 13 patients with chronic renal failure showed significant increase of partial pressure of oxygen (pO_2) after hemodialysis. The predialysis PO_2 was 92.3±14.4 but increased to 105.9±14.3 mmHg after dialysis. Partial pressure of CO_2 (PCO_2) showed significant decrease from 31.9±8.5 to 28.4±6.5 mmHg after dialysis (Table 2).

3. pH change of whole blood and intraerythrocyte

pH of arterial blood was measured because the change of 2,3-DPG may have been caused by the change of pH. There was a significant increase of pH from predialysis value of 7.31±0.02 to 7.40±0.04 ($p<0.01$). There was also a significant intraerytrocyte pH change from 7.13±0.02 to 7.20±0.03 (Table 2).

4. Change of 2,3-DPG concentration during hemodialysis in patients with chronic renal failure

The mean predialysis hemoglobin and hematocrit values of 7 Korean patients with chronic renal failure on maintenance hemodialysis were 6.4±1.3g% and 19.4±1.6%, respectively. They showed little clincal symptoms of hypoxia in peripheral tissues. The mean 2,3-DPG value before dialysis was 10.86±2,89 μmol/g (range from 4.73 to 14.15 μmol/g) and increased to 19.93±5.27 μmol/g (range from 12.62 to 28.57 μmol/g) after dialysis. This is very

Table 2. Comparison of blood pH, O_2 tension (PO_2),CO_2 tension (PCO_2),bicarbonate (HCO_3^-) and intraerythrocyte pH(pHi) in pre-and post chronic hemodialysis patients

	Blood pH		PO_2 mmHg		PCO_2 mmHg		HCO_3^- mEq/ℓ		pHi	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1.	7.28	7.38	91	131	35	30	15.7	17.2	7.10	7.18
2.	7.28	7.45	76	93	32	17	14.4	11.5	7.11	7.24
3.	7.33	7.39	110	91	25	24	12.6	14.0	7.14	7.19
4.	7.33	7.40	117	123	19	30	9.6	18.0	7.14	7.20
5.	7.35	7.45	82	103	29	28	15.5	19.0	7.16	7.24
6.	7.30	7.31	80	95	35	30	16.5	14.1	7.12	7.13
7.	7.31	7.40	90	105	48	40	23.5	24.0	7.13	7.20
M±SD	7.31	7.40	92.3	105.9	31.9	28.4	15.4	16.8	7.13	7.20
±0.02	±0.04	±14.4	±14.3	±8.5	±8.5	±6.5	±3.9	±3.8	±0.02	±0.03

Table 3. Comparison of BUN, hemoglobin (Hg) and 2,3-diphosphoglycerte (2,3-DPG) in pre-and post chronic hemodialysis patients

	Age/Sex	BUN(mg%)		Hg(g/dl)		2,3-DPG					
						Blood(μmol/ml)		P. cell (μmol/ml)		Hg(μmol/d)	
		Pre	Post	Pre	Post	Pres	Post	Pre	Post	Pre	Post
1.	56/M	80.0	57.0	6.0	6.0	0.61	1.21	3.81	7.33	10.13	20.13
2.	45/M	131.8	89.7	7.0	7.0	0.89	2.00	4.81	9.44	12.71	28.57
3.	67/M	106.6	68.8	5.8	6.0	0.72	1.08	4.36	5.35	12.33	17.96
4.	42/M	86.7	54.6	6.1	6.8	0.29	1.78	1.60	8.56	4.73	26.16
5.	17/M	112.0	64.4	5.0	5.0	0.48	0.63	5.03	4.99	9.54	12.62
6.	46/M	121.8	70.1	5.8	5.8	0.82	1.09	5.80	5.78	14.15	18.86
7.	46/M	75.0	50.1	9.4	9.5	1.17	1.40	4.22	5.19	12.40	15.23
M±SD	45.6	101.9	64.9	6.4	6.5	0.71	1.31	4.23	6.66	10.86	19.93
		±20.1	±12.1	±1.3	±1.3	±0.27	±0.43	±1.23	±1.66	±2.89	±5.27

significant increase ($p<0.01$) (Table 3)

5. The relationship of blood glucose and 2,3-DPG changes in hemodialysis patients

The concentration of blood glucose was measured as a function of time during hemodialysis in 7 Korean patients because the change of 2,3-DPG may be caused by the change of blood glucose concentration during the hemodialysis. The results are shown in Figure 1. Predialysis basal value of blood glucose was 98.3±8.5 mg/dl and increased to 114.1±6.5 mg/dl at 15 min. 117.7±3.9 at 30 min. 106.6± 2.9 at 60 min. 110.7±4.2 at 120 min and 117.4±6.1 mg/dl at 4 hours of hemodialysis which is higher than the basal level. The glucose concentration of dialysate was 186.1 mg/dl. Therefore, the change of 2,3-DPG concentration was considered to be effected by blood glucose.

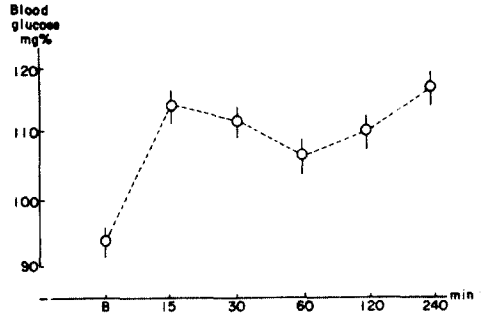

Fig. 1. *Sequential changes of blood glucose level during hemodialysis on chronic renal failure (Korean, N;6).*

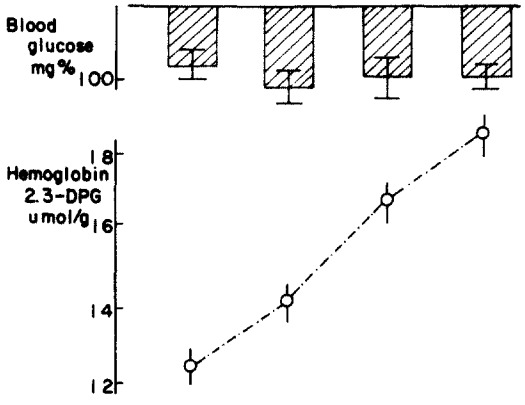

Fig. 2. *Relationship between blood glucose and 2,3-DPG changes in blood, packed cell and hemoglobin with chronic renal failure (American, N:6)*

We also examined the change of blood glucose in 6 American patients with chronic renal failure. The value was 98.5±9.5 mg/dl before dialysis, 105.9±3.9 at 1 hour of dialysis, 105.2±6.5 at 2 hours and 103.1±6.1 mg/dl at 3 hours. The predialysis glucose concentration of American patients was higher than those of Korean patients.

6. The change of 2,3-DPG concentration after the glucose load in normal controls

To investigate the relationship between the changes of 2,3-DPG and blood glucose, we studied 5 normal controls to determine changes in 2,3-DPG concentration of blood as a function of time after oral administration of 50 gm glucose. The blood glucose at a fasting state was 87.5±2.9 mg/dl and hemoglobin 2,3-DPG was 13.04±1.53 μmol/g, but 145.0±5.2 mg/dl and 13.16±2.66 μmol/g at 1 hour after the glucose change in 2,3-DPG during hemodialysis was a load, 85.0±4.9 mg/dl and 13.39±1.17 μmol/g at 2 hours and 87.5±7.2 mg/dl and 14.73±3.75 μmol/g at 3 hours, respectively.

The comcentration of 2,3-DPG increased in accordance with blood glucose at the first hour and continued increasing while blood glucose returned to a normal level 2 hours after the glucose loading.

DISCUSSION

There was a marked increase of hemoglobin 2,3-DPG concentration after hemodialysis in anemic

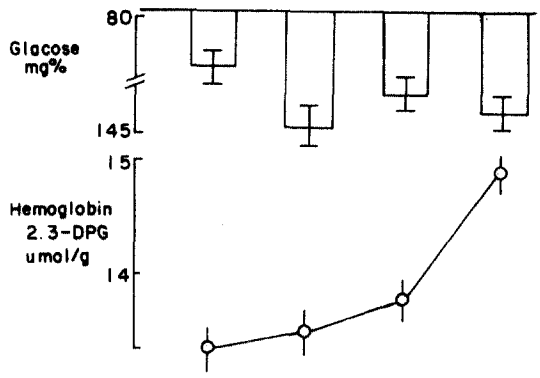

Fig. 3. *Changes in blood, packed cell and hemoglobin 2,3-DPG after glucose loading 50 g/orally in normal persons (N:5).*

patients with chronic renal failure. This is thought to be caused by an increase of pH and the blood glucose level effected by dialysate glucose through the dialysis membrane. There are several proposed mechanisms to explain such a rapid increase of intraerythrocyte 2,3-DPG concentration in response to hypoxia. Intraerythrocyte 2,3-DPG, an intermediate of glycolysis, is an organic phosphate converted from 1,3-DPG in Rapoport-Luebering shunt.[19] The increase of 2,3-DPG concentration after hypoxia was caused by either decrease of 2,3-DPG degradation due to inhibition of 2,3-DPG phosphatase or increase of 2,3-DPG synthesis through the accelerated 2,3-DPG mutase reaction. The amount of 2,3-DPG synthesis depends on how much 1,3-DPG entered Rapoport-Luebering cycle and how much 2,3-DPG mutase reacts. The hormones known to increase the intraerythrocyte 2,3-DPG are thyroxine,[20-21] androgen,[22-24] aldosterone[25-26] and glucocorticoid.[27-29] Especially hydrocortisone and thyroxine are known to activate 2,3-DPG mutase and thus increase the conversion of 2,3-DPG from 1,3-DPG[20,29]

In this study the mean 2,3-DPG concentration in patients with chronic renal failure was 10.86 ± 2.89 μmol/g, much less than 17.45 ± 4.3 μmol/g in normal controls. Theoretically, the intraerythrocyte 2,3-DPG level in severe anemic state must be higher than normal controls to compensate for the decreased amount of oxygen available to tissues.[30] But there was no clear explanation for this low 2,3-DPG level in anemic patients with chronic renal failure. On the other hand, intraerythrocyte 2,3-DPG concentration increased with hemodialysis by 54.5% from 10.86 ± 2.89 μmol/g to 19.93 ± 5.27 μmol/g. What caused this increase of 2,3-DPG concentration after hemodialysis? Intraerythrocyte pH change had an important role in controlling 2,3-DPG concentration.[31] In metabolic acidosis the level of 2,3-DPG decreases, and this lowers the oxygen affinity of hemoglobin. In this study the increase of 2,3-DPG may be explained by the fact that intraerythrocyte pH increased from 7.31 of predialysis to 7.40 of postdialysis.

Increase in pH activates phosphfructokinase and pyruvate kinase, resulting in increase 2,3-DPG concentration by inducing the intraerythrocyte glycolysis. Another possible mechanism for the change in 2,3-DPG during hemodialysis was a change in blood glucose. There was a significant increase of blood glucose from 93.3 mg% of predialysis to 117.1 mg% of postdialysis in 7 Korean patients (Fig. 1). We also observed the change of blood glucose and 2,3-DPG during dialysis than baseline in 6 American patients (Fig. 2). The increasing pattern of 2,3-DPG in American patients was similar to that observed in Korean patients, but the glucose levels did not change significantly.

The relatively high glucose concentration of dialysate, 186.1 ± 34.1 mg%, may have produced an increased blood gludose levels of Korean patients, which in turn accelerated the intraerythrocyte glycolysis and increased the 2,3-DPG concentration. There was a significant increase of 2,3-DPG after oral glucose load in 5 normal controls (Fig. 3). The concentration of intraerythrocyte 2,3-DPG continued to increase while blood glucose returned to normal at 120 minutes. It deserves in vitro experiments in the future.

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