CROSS OVER PLACEBO CONTROL TRIAL OF DILAZEP IN BETA-THALASSEMIA/HEMOGLOBIN E PATIENTS

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Abstract. An attempt was made to find better symptomatic treatment for beta-thalassemia/hemoglobin E (β-thal/Hb E) patients in order to reduce their blood demand. Oral administration of dilazep was prescribed for these patients and a clinical trial was conducted over a 2-year period as a cross over placebo control study. Seventeen β-thal/Hb E patients were enrolled in the study. All of them received dilazep and placebo for 10 months at different periods of time and were taken care of by the same doctor throughout the study. The blood demand of the same patients during the period of receiving dilazep with the period of receiving placebo, was 1.5 ± 1.8 U/10 months versus 2.2 ± 2.6 U/10 months, respectively. Thus dilazep showed a benefit in decreasing the blood demand by about 50% although the results did not reach statistical significance ($p = 0.1$). There was a statistical difference in hemoglobin concentration of the patients receiving dilazep compared with placebo ($p = 0.038$). While receiving dilazep the mean ± SD hemoglobin level was 5.82 ± 0.8 g/dl, significantly higher than while receiving placebo (5.66 ± 0.9 g/dl) ($p = 0.038$). The liver, and renal function tests, and cardiac enzyme levels of the patients showed no significant changes throughout the study. However, one case had a problem with bleeding following tooth extraction whilst receiving dilazep and needed 1 unit of blood transfusion. In conclusion, administration of dilazep to patients with β-thal/Hb E increased the patients' hemoglobin and reduced their blood demand with few side effects.

INTRODUCTION

Beta-thalassemia/hemoglobin E (β-thal/Hb E) is a genetic disease causing serious health problems in Thailand and Southeast Asia generally (Panich *et al,* 1992). Some patients are in a severe anemic state and need regular transfusion. The best symptomatic treatment of choice now is multiple transfusion with an iron chelator. However, the cost of the iron chelator available on the market in Thailand is very high. Only a few patients can afford it. Without iron chelator, multiple transfusion will cause iron overload in recipients, resulting in damage to many organs. Also, the risk of getting infection from repeated blood transfusions, especially HIV infection, is another serious problem. An attempt was made to find better symptomatic treatment for these patients in order to reduce their blood demand. Dilazep {1,4-Bis-[3-(3,4,5-trimethoxybenzoyloxy)-propyl]-perhydro-1,4-diazepine} is a synthetic membrane stabilizer that is widely used in Japan for patients suffering from ischemic heart disease and cerebrovascular disease (Ishihana *et al,* 1974; Sambhi *et al,* 1989). It is an adenosine uptake inhibitor and has effects on erythrocytes, platelets and on the circulation (Saito *et al,* 1985; Sano *et al,* 1972; Yamamoto *et al,* 1983; Yasunaga *et al,* 1980). Dilazep was prescribed for β-thal/Hb E patients and a clinical trial was conducted prospectively over a 2-year period as a cross over placebo control study.

MATERIALS AND METHODS

Patients

β-thal/Hb E patients were recruited from the Division of Hematology, Department of Internal Medicine, Siriraj Hospital, Mahidol University, Thailand. The inclusion criteria were; 1. Patients aged over 15 years old, 2. Either sex, 3. Hemoglobin levels were less than 7 g/dl. All patients were given verbal explanation and gave written consent to participate in the study. This study was given ethical approval by the Committee of Human Rights in Research Involving Human Subjects of the Faculty of Medicine Siriraj Hospital, Mahidol University.

Medication

Dilazep and placebo were packed in the same way. Dilazep 4-5mg/kg/day (200mg/day) was prescribed. The placebo was composed of lactose. This prospective study was conducted over a 2-year pe-

riod. Each patient received placebo for 10 months and received dilazep for another 10 months with a washout period of 1 month whilst changing medication. The medicine was taken orally twice a day. Dilazep or placebo were randomly allocated in the first period using a randomized table. So in the first period, 7 patients received dilazep and 10 patients received placebo (Opartkiattikul *et al*, 1997). In the second period, the patients who received dilazep in the first period received placebo and the patients who previously got placebo received dilazep. The patients did not know what type of medication they were receiving during the two periods.

Data collection

Clinical data were evaluated every 2 months by Dr S who did not know which type of medication the patients were receiving. Blood transfusion was given to the patients according to their clinical symptom and hemoglobin level (less than 5 g/dl). Laboratory data (hemoglobin level, white blood cell number, platelet number, liver function tests, kidney function tests and CPK) were analyzed by fully automatic machines : - Technicon H1 for hematological data and Hitachi 717 for blood chemistry analysis every 2 months.

Data analysis

The results from the same patient were compared for the periods receiving dilazep and placebo by using paired Student's *t*-test.

RESULTS

Patients

Seventeen β-thal/Hb E patients were enrolled in the study. Twelve patients were post splenectomized cases and 5 patients were non splenectomized ones. The mean age of the patients was 32 ± 5.4 years. The detailed information of the patients is shown in Table 1

Table 1
Patient characteristics.

Total number	17
Diagnosis	
Non splenectomized β-thal/Hb E	5
Post splenectomized β-thal/Hb E	12
Sex	
Male	5
Female	12
Age (year)	32 ± 5.4

Table 2
The blood demand and mean hemoglobin concentration of the patients before and whilst receiving dilazep or placebo (n=17).

	Before medication	Receiving dilazep	Receiving placebo	p-value
Blood demand (U/10 months)	4.2 ± 4.1	1.5 ± 1.8	2.2 ± 2.6	0.1
Hemoglobin concentration (g/dl)	5.68 ± 1	5.82 ± 0.8	5.66 ± 0.9	0.038

Table 3
Comparison of blood chemistry of the same patients whilst receiving dilazep or placebo (n=17).

	Normal range	Dilazep	Placebo	p-value
BUN (mg/dl)	7 - 20	13.9 ± 5.5	14.0 ± 4.3	0.96
Creatinine (mg/dl)	0.5 - 1.5	0.64 ± 0.2	0.68 ± 0.2	0.23
Uric acid (mg/dl)	2.4 - 7.0	8.1 ± 2.1	7.5 ± 2.1	0.07
AST (U/l)	0 - 37	77.7 ± 44	74.1 ± 43	0.6
ALT (U/l)	0 - 40	63.6 ± 32	59.9 ± 30	0.55
Alkaline phosphatase (U/l)	39 - 117	149 ± 68	182 ± 137	0.12
Total bilirubin (mg/dl)	0.3 - 1.2	4.4 ± 2.1	4.1 ± 1.9	0.23
Direct bilirubin (mg/dl)	0 - 0.5	1.1 ± 0.7	1.0 ± 0.5	0.5
Albumin (g/dl)	3.5 - 5.5	4.4 ± 0.5	4.5 ± 0.5	0.46
CPK (U/l)	20 - 195	24.7 ± 10	23.3 ± 10	0.24

Table 4
Patient opinions.

Opinion	No. of patients
Dilazep was better than placebo	7
Dilazep and placebo were equally good	4
Dilazep and placebo had no effect	3
Placebo was better than dilazep	3

Outcome

All 17 patients received both dilazep and placebo at different periods of time and were taken care of by the same doctor throughout the study. It was therefore possible to compare directly their blood demand and their laboratory data for each type of medication.

Blood demand and hemoglobin level

When we compared the blood demand of the same patients whilst receiving dilazep and placebo which was 1.5 ± 1.8 U/10 months versus 2.2 ± 2.6 U/10 months respectively, dilazep showed benefit by decreasing the blood demand by about 50% although the results did not reach statistical significance ($p = 0.1$). There was a significant difference in the hemoglobin concentration of the patients receiving dilazep and placebo ($p = 0.038$): while receiving dilazep the mean ± SD of hemoglobin level was 5.82 ± 0.8 g/dl which was significantly higher than during receiving placebo (5.66 ± 0.9 g/dl). The mean hemoglobin level of 13 out of 17 patients was higher during receiving dilazep than while receiving placebo. (Table 2, Figs 1, 2).

Blood chemistry analysis

The liver and renal function tests and cardiac enzyme measurement of the patients showed no significant changes throughout the study (Table 3).

Opinions of the patients

The opinions of the patients were obtained by interviewing them with open end questions. The interviewer asked every patient to compare their feeling about each medication that they received at different periods of time. The patients did not know which kind of medication they got at each period. Their opinions are shown in Table 4.

Side effects

During the 2 years of the study, there was one case that suffered from a side effect of dilazep. Whilst receiving the medication she had a problem with bleeding after tooth extraction and needed 1 unit of blood transfusion.

DISCUSSION

The main objective of this trial was to study the effect of dilazep on the symptomatic treatment of b-thal/Hb E patients. The study was designed as

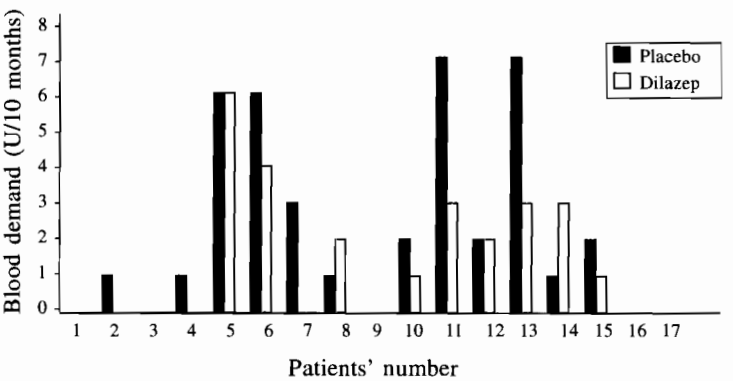

Fig 1–Comparison of blood demand whilst receiving placebo or dilazep of the same patients (n = 17).

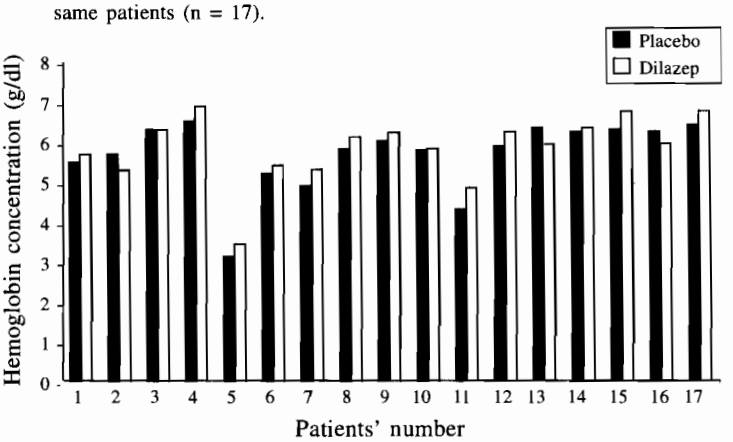

Fig 2–Comparison of mean hemoglobin levels whilst receiving dilazep or placebo of the same patients (n = 17).

a cross over placebo control study in order to compare the effect of dilazep and placebo received at different periods in the same patients and throughout the study the patients were taken care of by only one physician, so that the data of each patient could be compared by using a paired Student's *t*-test. The patients and the doctor did not know which type of medication the patients were receiving. The results indicated that dilazep had good effect on anemia. The hemoglobin levels of the patients when receiving dilazep were significantly higher than when receiving placebo ($p = 0.038$). Even though the actual values were not greatly different, this difference correlated with differential blood demand: Dilazep decreased the blood demand by about 50% although the results were not statistically significant ($p = 0.1$). Dilazep probably increases hemoglobin levels by its membrane stabilizing action and improvement of the deformability of thalassemic erythrocytes (Saito *et al*, 1985; Yamamoto *et al*, 1983). This reduces the destruction of thalassemic red blood cells so that the life span of thalassemic red blood cells increases. In addition, their microcirculation may be improved.

One case had severe bleeding after tooth extraction. This could be explained by the antiplatelet activity of dilazep (Funahara *et al*, 1984; Sumiyoshi *et al*, 1983; Yasunaga *et al*, 1980). To avoid this side effect, the patients should stop medication before any operation. The blood chemistry reflecting liver, renal and cardiac function of the patients whilst receiving dilazep or placebo was not significantly different.

From interviewing the patients for their opinion about the medication, 7 out of 17 patients felt that dilazep was better than placebo and 3 out of 17 patients felt that placebo was better than dilazep. One patient in the latter group was the one that had a bleeding problem after tooth extraction.

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REFERENCES

Funahara Y, Dhrama R, Ogawa K, Miki M, Hirata M, Kitaguchi H. Enhancing effect of collagen on platelet aggregation in whole blood. *Blood Vessel* 1984; 15: 533-6.

Ishihana H, Nakajima K, Kimata H, Cuzuki H, Shirai K, Yonemitsu M. Absorption, distribution, excretion and metabolism of 1,4 Bis [3-(3,4,5-trimethoxybenzoyloxy) propyl] perhydro-1,4-diazepine dihydrochloride monohydrate. *Clin Report* 1974; 8: 50-65.

Opartkiattikul N, Sukpanichnant S, Wanachiwanawin W, Fucharoen S, Funahara Y, Sumiyoshi A. A double blind placebo control trial of dilazep in beta thalassemia/Hb E patients. *Southeast Asian Trop Med Public Health* 1997; 28 (suppl 3): 167-71.

Panich V, Pornpatkul M, Sriroongrueng W. The problem of thalassemia in Thailand. *Southeast Asian Trop Med Public Health* 1992; 23 (suppl 2): 1-6.

Saito H, Saito A, Ogawa H. The effect of dilazep on red cell survival. *Clin Hematol* 1985; 26: 15-9.

Sambhi MP, Kannan R, Thananopavan C, Ooktens M, Gudenzi M. Therapeutic tolerance, hemodynamic effects and oral dose kinetics of dilazep dihydrochloride in hypertensive patients. *J Pharm Sci* 1989; 78: 281-4.

Sano N, Katasuki S, Kawada M. Enhancement of coronary vasodilator action of adenosine by 1,4 Bis [3-(3,4,5-trimethoxybenzoyloxy)-propyl]- perhydro-1,4-diazepine (Dilazep I N,N). *Drug Res* 1972; 22: 1655-8.

Sumiyoshi A, Hayashi T. The inhibitory effect of dilazep on *in vivo* accumulation of platelet onto the damaged aorta in rabbit. II Morphological analysis. *Thromb Res* 1983; 39: 37-42.

Yamamoto S. A possible mechanism of dilazep on enhancement of red cell deformability. *Jpn Pharmacol Ther* 1983; 11: 108-13.

Yasunaga K, Kanzaki M, Kimura M. Inhibition of platelet aggregation by dilazep dihydrochloride. *Jpn Arch Intern Med* 1980; 27: 99-101.