CHAPTER III

Materials and Methods

This study was conducted at the Chulalongkorn Headache Clinic, Chulalongkorn University Hospital. Four groups of subjects were participated in this study. These were patients with migraine, migraine with depression, Parkinson's disease and normal controls.

Migraine group

Ten migraine patients and ten migraine patients with depression were studied. The diagnosis of migraine and depression was made according to the International Headache Society criteria 1988 (Headache Classification Committee of the International Headache Society, 1988). All migraine participants suffered from migraine without aura (MwA) and were headache free for at least one week before the study. Age, sex , length of attack and duration of disease are shown in Table 5.

Parkinson's disease

This group consited of ten patients with idiopathic Parkinson's disease. The diagnosis of Parkinson's disease was defined by the presence of at least

two of the following four features : tremor, regidity, bradykinesia and postural imbalance. Patients were excluded if there was evidence implicating other causes for the Parkinsonism (eg, phenothiazine exposure or history of encephalitis)

Control group

This group consisted of ten healthy volunteers, who were non-smokers and who were screened to eliminate central nervous system disease.

Excluding migraine patients with depression and patients with Parkinson's disease, all participants abstained from drugs for at least two weeks prior to the study.

Methods

1. Specimen collection

Ten milliliters of venous blood was collected from the antecubital vein of the subjects. The blood was divided into two portions, the first part was transferred into a polypropylene test tube which contained acid citrate dextrose (ACD) as anticoagulant 1:10 v/v. Platelets of this portion were referred to as resting platelets. The second part was placed into a glass tube

containing ACD. Since glass surface has the tendency to activate platelets, from this point platelets were referred to as the activated form(Factor et al., 1994). The blood in both glass and plastic test tubes was incubated at 37°C in a shaking waterbath for 30 minutes.

2. Platelet rich plasma preparation

Platelet rich plasma (PRP) was obtained by gentle centrifugation at 190g for 15 minutes at room temperature. At this centrifugation rate, red blood cell and white blood cell sedimented, while platelets still remained suspended in plasma.

3. Preparation for transmission electron microscopy

Obtained PRP from each specimen was mixed with an equal volume of 0.1 M whiter saline (pH 7.4) containing 0.6% glutaraldehyde and was kept at room temperature for 15 minutes. These partially fixed platelets were then centrifuged at 800g for 15 minutes at room temperature to form a pellet. The supernatant was discarded and replaced with 3% glutaraldehyde. The mixture was left standing at room temperature for 2 hours. The pellet was then washed in cacodylate buffer containing sucrose (0.2 mol/L). After 3 changes of buffer (10 minutes each), the pellets were exposed to a solution of 2% buffered osmium tetroxide for 1 hour. Then the pellets were rinsed

with distilled water and reacted with a 4% aqueous solution of uranylacetate for 45 minutes. The pellets then were dehydrated in a graded series of ethanol, embedded in an Epon 812 epoxy resin and polymerized at 60°C for 72 hours. Five plastic blocks were prepared from each pellet.

The plastic blocks were sectioned with an LKB ultramicrotome, thin sections were mounted on a 200 mesh copper grid and doubly stained with uranylacetate and lead citrate. The sections were examined with an electron microscope (JEM-1210). At 22,000 magnification, ten platelets were randomly selected from each sections.

The criteria for platelet selection for morphometric evaluation included, first, the cell to be cut in the equatorial plane; secondly, all organelles were clearly discernible; and thirdly, the cell to be fully enclosed by the grid.

Platelet organelles including dense granules, alpha granules, mitochondria and the surface connected canaliculi system (SCCS) were examined and measured. The numbers of each organelle per platelet were determined. As the average diameter of canaliculi in normal platelets was 183.1± 20.3 nm (averaged over 10 control subjects), canaliculi with a diameter above 200 nm were defined as being dilated and counted. The same morphometry was applied to both resting and activated platelets.

Statistical Analysis

Result are given as mean±SE. Analysis of the difference betaween two means was performed by using Student's T-test. Significant difference of more than two groups was performed by analysis of varience, followed by the Student 's T-test.

Table 5. Clinical features of patients with migraine, migraine with depression and Parkinson's disease

	Control	Migraine	Migraine with depression	PD
Age range (year)	27-45	24-45	24-47	51-74
Mean age (year)	34.7	39.3	34.1	65.2
Number	10	10	10	10
Duration of migraine (year)	-	1-15	1-27	