PROSTACYCLIN PRODUCTION IN VASCULAR ENDOTHELIUM
OF PATIENTS WITH BLACKFOOT DISEASE

Oi-Tong Mak

Department of Biology
National Cheng Kung University
Tainan 70101, Taiwan, Republic of China

INTRODUCTION

Blackfoot disease is an endemic disease of the peripheral vascular system reported more than fifty years ago in the southwest coast of Taiwan, Republic of China. The symptoms are similar to those of the Buerger's disease or thromboangiitis obliterans. It is characterized pathologically by intravascular clot formation and inflammation of the vascular wall, which lead to partial or complete occlusion of the vessels involved. The area distributed by the diseased vessel then becomes discolored and gangrenous. The lesion often occurs on the lower extremities, hence the name Blackfoot disease. Other findings include pigmentation, atherosclerosis and a high level of high density lipoprotein (HDL), but a low level of low density lipoprotein (LDL). According to the statistics, 97% of Blackfoot disease patients will end up with either surgical amputation or natural disjointment due to gangrene. After almost thirty years of investigations, no specific treatment has been found. The primary cause of Blackfoot disease still remains elusive despite some intensive studies. Chen et al. reported that the artesian well water of endemic areas contained high concentrations of arsenic (0.1 – 0.35 ppm). Lu and Ling also found that ergotamine compounds were unusually high in level in the artesian well water. Both groups of investigators proposed that high concentrations of arsenic and ergotamine compounds in the drinking water in the endemic areas might be the main cause of Blackfoot disease. However, the installation of new water supply and drainage system since 1960 in the endemic areas has not eradicated or significantly reduced the incidence of the disease. In the present report, study of prostaglandins, particularly on 6-keto-POF<sub>1a</sub> and thromboxane B<sub>2</sub> (TXB<sub>2</sub>), the stable natural metabolic intermediates of prostacyclin (PGI<sub>2</sub>) and thromboxane A<sub>2</sub> (TXA<sub>2</sub>) respectively, had been carried out among the patients. PGI<sub>2</sub> is an important factor in platelet anti-aggregation and blood vessel dilatation, and plays an important role in the prevention of atherosclerosis and arterial thrombosis. TXA<sub>2</sub> exerts opposite physiological effects of PGI<sub>2</sub>. The enzyme activities of prostacyclin synthase, the prime enzyme for the synthesis of PGI<sub>2</sub>, and 15-hydroxyprostaglandin dehydrogenase (15-OH-PGDH), the enzyme involved in the conversion of PGI<sub>2</sub> to an inactive form 15-OH-PGI<sub>2</sub>, were also studied. The aim is to determine whether there is any change in PGI<sub>2</sub> and TXA<sub>2</sub> metabolism in Blackfoot disease patients.

MATERIALS AND METHODS

Materials

All reagents and solvents used were of analytical grade supplied by Merck Chemical (Darmstadt, Federal Republic of Germany) unless otherwise stated. Glass distilled water was used throughout. Prostaglandin compounds were purchased from Upjohn Company (Kalamazoo,
Radioactive prostaglandins and fatty acids were obtained from Amersham (Amersham, England). RIA kits for 6-keto-PGF₁α and TXB₂ were purchased from New England Nuclear (Boston, Mass. U.S.A.). Scintillation cocktail was obtained from Lumac (Schaesberg, The Netherlands). Polypropylene test tubes and pipet tips were purchased from Gilson (Villiers-le-Bel, France). Arachidonic acid, NAD⁺, heparin, aspirin (acetylsalicylic acid), and hematin were obtained from Sigma Chemical Company (St. Louis, Miss., U.S.A.). Sheep vesicular microsome was obtained from Hilran Biochemicals Ltd. (Tel-Aviv, Israel). TLC plates were purchased from Merck Chemical (Darmstadt, Federal Republic of Germany). Human arterial tissues were obtained from the Provincial Chiayi Hospital (Chiayi, Taiwan R.O.C.).

Preparation of human plasma

Fasting blood samples (2 ml) from 16 Blackfoot disease patients and 15 normal people were collected in polypropylene test tubes containing preweighed heparin (50 μg) and aspirin (acetylsalicylic acid, 100 mg). Platelets and blood cells were removed immediately by centrifugation at 1,000 × g for 10 min. The supernatants were collected and kept in ice bath for testing of 6-keto-PGF₁α and TXB₂ concentrations.

Radioimmunoassay

The levels of 6-keto-PGF₁α and TXB₂ of the samples were determined by radioimmunoassay (RIA) as described by the manufacturer (NEN, Boston, Mass.). The concentrations of various 6-keto-PGF₁α and TXB₂ standards used to construct the calibration curve were 50, 100, 250, 500, 1,000 and 2,500 pg/ml respectively. 100-μl of the standard or sample was added into RIA solution and mixed thoroughly, and the mixture was incubated overnight (16 hr) at 4°C. At the end of incubation, to all test tubes were added 500-μl pre-cooled charcoal suspension and the tubes were vortexed thoroughly. Suspensions were allowed to stand in ice bath for 15 min and centrifuged at 200 × g for 10 min. The supernatants were decanted without disturbing the charcoal residue, and the radioactivity measured in a LKB liquid scintillation counter.

Preparation of 15-OH-PGDH from human blood plasma

Human blood samples (2 ml) from Blackfoot disease patients and normal people were collected in polypropylene test tubes containing preweighed heparin (50 μg) and aspirin (acetylsalicylic acid, 100 mg). Platelets and blood cells were removed immediately by centrifugation at 1,000 × g for 10 min and the platelet-poor plasma (PPP) were kept at 4°C. To the PPP was added ice-cold 1 M acetic acid, dropwise with stirring and cooling until the pH was 5.2. The precipitate was then spun for 1 hr at 10,000 r.p.m. (14,000 × g, Centrikon H-401, Kontron, Sweden) at 4°C. The supernatant was stored at 4°C and used for the assay of 15-OH-PGDH activity.

Enzyme assays of 15-OH-PGDH

Enzyme activity of 15-OH-PGDH in human blood plasma was measured spectrofluorometrically by the method of Mak and Chen with excitation at 347 nm and emission at 468 nm in a Hitachi spectrofluorometer with prostaglandin E₂ (27 μM), NAD⁺ (440 μM), glycerol (1.7%, v/v), ethanol (1.7%, v/v) and 50 mM potassium phosphate buffer, pH 7.4 at 37°C. One unit was defined as the transformation of 1 nmol of substrate in 1 min per ml of enzyme solution at 37°C.

Preparation of human arterial endothelial prostacyclin synthase

Samples of arterial vascular tissues from the Blackfoot disease patients or normal people were immediately kept in ice after amputation and brought to the laboratory for further study. The fat tissues were removed thoroughly from the artery, and the artery was washed with 100 mM Tris-HCl buffer, pH 8.0. The tissues were rapidly frozen in liquid nitrogen and immediately homogenized into a fine powder by using a stainless steel blender. The powder was resuspended in 100 mM Tris-HCl buffer, pH 8.0 (1:4, w/v) and homogenized at high speed by using a Potter-Elvehjem tissue grinder. The homogenate was then centrifuged at 10,000 X g for 30 min at 4°C. The supernatant was collected after centrifugation and used for the prostacyclin synthase assays.