Supplementary Materials

Pioglitazone Improves Endothelium-Dependent Vasodilation in Hypertensive Patients with Impaired Glucose Tolerance through a Decrease in Oxidative Stress

Methods

Study protocol 1. Endothelial function in healthy subjects and hypertensive patients with IGT

_Hypertensive patients with IGT_: Hypertension was defined as a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg, measured in a sitting position, on at least three different occasions, in the outpatient clinic of Hiroshima University. All patients underwent a 75 g oral glucose tolerance test. Fasting serum glucose level of less than 7.0 mmol/L and serum glucose level of more than 7.8 mmol/L but less than 11.1 mmol/L indicated IGT. Secondary hypertensive patients were excluded on the basis of a complete history and physical examination, radiologic and ultrasound examinations, and urinalysis. Plasma renin activity, aldosterone, angiotensin II and catecholamine concentrations, and serum creatinine, potassium, calcium and free thyroxine concentrations were determined, and 24-hr urinary excretions of catecholamines, 17-hydroxycorticosteroids, 17-ketogenic steroids, and vanillylmandelic acid were measured. All hypertensive patients with IGT had been treated for hypertension with 5mg of amlodipine for at least three months (from 3 months to more than 2 years). None of the patients had cardiac disease, retinopathy or neuropathy and none of the patients had received any drugs other than amlodipine.
**Healthy subjects:** Normal blood pressure was defined as systolic blood pressure of \(<130\) mm Hg and diastolic blood pressure of \(<80\) mm Hg. The healthy control subjects had no history of serious medical problems. None of the patients in either group was a current smoker or had a history of smoking.

We measured the forearm blood flow (FBF) responses to intra-arterial infusion of acetylcholine (ACh), an endothelium-independent vasodilator, and sodium nitroprusside (SNP), an endothelium-independent vasodilator, in all subjects. Subjects fasted the previous night for at least 12 hours. The study began at 8:30 AM. They were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of \(22^\circ\)C to \(25^\circ\)C) throughout the study. A 23-gauge polyethylene catheter (Hakkow Co.) was inserted into the left brachial artery for the infusion of vasoactive agents and to record arterial pressure with an AP-641G pressure transducer (Nihon Kohden Co.) under local anesthesia (1\% lidocaine). Another catheter was inserted into the left deep antecubital vein to obtain blood samples. Thirty minutes after maintaining the supine position, basal FBF was measured. Then FBF responses to ACh (Daiichi Pharmaceutical Co.), an endothelium-dependent vasodilator, and SNP (Maluishi Pharmaceutical Co.), an endothelium-independent vasodilator were measured. ACh was infused at doses of 3.75, 7.5 and 15 \(\mu g/\text{min}\) for 5 minutes, and SNP was infused at doses of 0.375, 0.75 and 1.5 \(\mu g/\text{min}\) for 5 minutes. These studies were carried out in a randomized fashion. Each study proceeded after FBF had returned to baseline.

To examine the effect of pioglitazone on release of NO, we measure FBF in the presence of the NO synthase inhibitor \(N^\circ\)-monomethyl-L-arginine (L-NMMA,
CLINALFA Co.). The responses of forearm vasculature to ACh after intra-arterial infusion of L-NMMA (8 µmol/min for 5 minutes) were evaluated.

Baseline fasting serum concentrations of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, creatinine, glucose, HbA1C and electrolytes, plasma concentrations of insulin, adiponectine and angiotensin II, and plasma activities of renin were obtained after a 30-minute rest period before the study. The 24-hr urinary excretions of 8-hydroxy-2’-deoxyguanosine (8-OHdG) were measured.

Analytical Methods

Samples of venous blood were placed in tubes containing sodium EDTA (1 mg/mL) and in polystyrene tubes. The EDTA-containing tubes were chilled promptly in an ice bath. Samples were stored at -80°C until the time of assay. Plasma concentrations of adiponectin were measured using the radioimmunoassay kits (Linco Research, St. Charles, MO). Serum concentrations of total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, glucose, and electrolytes were determined by routine chemical methods. Serum insulin was measured using an automated radioimmunoassay technique. Fasting concentrations of insulin and glucose were used to determine homeostatic model assessment (HOMA) parameters of insulin resistance using a program based on the HOMA algorithm (HOMA resistance=insulin/22.5e-1.5glucose). The urinary concentration of 8-OHdG was assayed by enzyme-linked immunosorbent assay using 8-OHdG kits (Nihon Yushi Co.).