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Effects of amlodipine and valsartan on glibenclamide-treated streptozotocin-induced diabetic rats

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ABSTRACT

Diabetes mellitus (DM) is a spectrum of metabolic disorders, arising from pathologic mechanisms, resulting in hyperglycaemia. Diabetes and hypertension frequently occur together and are leading risk factors for cardiovascular complications. This study examined the effects of amlodipine and valsartan on glibenclamide-treated streptozotocin-induced diabetic rats.

Male albino rats (200–350 g) were fasted overnight and DM was induced by a single dose 40 mg/kg of streptozotocin (i.p.). After 48 h, DM was confirmed (blood sugar ≥200 mg/dl) and the animals were grouped into normal rats with no drug treatment, untreated diabetic animals and groups treated with glibenclamide, glibenclamide plus amlodipine, and glibenclamide plus valsartan. After six weeks treatment, animals were sacrificed under chloroform anaesthesia. Kidney, liver, lung, heart and blood were collected for histology, haematological and biochemical analyses.

Untreated diabetic rats had 100% mortality before 6 weeks but addition of valsartan to glibenclamide improved survival rate (71.4% compared with 57.4% in glibenclamide-treated) and blood glucose control but this was not so with glibenclamide plus amlodipine-treated group with 50% survival rate. Treatment ameliorated pathologic changes and there was histologic evidence of organ protection among the various treatment groups when compared with the untreated diabetic group. Addition of valsartan to glibenclamide improved treatment outcome compared to when glibenclamide was used alone but this was not so with the addition of amlodipine to glibenclamide.

1. Introduction

Diabetes mellitus (DM) is a spectrum of common metabolic disorders arising from a variety of pathologic mechanisms, all resulting in hyperglycaemia. Diabetes mellitus can be further defined as elevated blood glucose associated with absent or inadequate pancreatic insulin secretion with or without concurrent impairment of insulin action [1]. Insulin is a major anabolic hormone that binds to specific receptors to produce several metabolic effects which include transmembrane transport of glucose via glucose transport units (GLUTs), glycogen formation in the liver and skeletal muscles (glycogenesis), protein synthesis, increased triglyceride storage and its inhibition of glycogenolysis, gluconeogenesis and lipolysis. A subject with a random glucose concentration greater than 200 mg/dl, with classical signs and symptoms and a fasting glucose concentration greater than 126 mg/dl on more than one occasion is said to be diabetic [2]. Diabetes mellitus is pandemic and its prevalence is increasing with aging of the populace and the lifestyle changes associated with rapid urbanization and westernization [3]. Previous estimates from the International Diabetes Federation (2013) put the global number of diabetic patients at 381 million. The number is projected to almost double by 2030 [4]. As at 2015, an estimated 415 million people globally are diabetic and that accounts for 8.3% of the adult population and according to the latest 2016 data from World Health Organization [5], the number is now estimated to be about 422 million adults who are living with DM. In the United States, DM was listed as the 7th leading cause of death in 2007. It was also reported as the 8th leading cause of death worldwide. From 2012 to 2015 diabetes was estimated to have resulted in 1.5 to 5 million deaths [6]. Also between 2010 and 2030, there will be a 69% increase in the number of adults with diabetes mellitus in developing countries and 20% in developed countries [7].

The etiological classification of diabetes has now been widely accepted, type 1 and type 2 are the two main types. Although the prevalence of both type 1 and type 2 DM is on the increase worldwide, the prevalence of type 2 diabetes is rising much more rapidly. Reports have shown that type 2 diabetes constitutes 85–95% of all diabetes in high

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income countries [8]. The introduction of insulin therapy represented a major breakthrough in type 1 diabetes management and recent developments which include whole pancreas transplant or pancreatic islet transplant, stem cell, gene therapy and islets encapsulation have all formed a beacon of hope for a better management of this disease condition. The management of type 2 diabetes has been based on drugs that stimulate insulin secretion (sulphonylureas and rapid-acting secretagogues), reduce hepatic glucose production (biguanides), delay digestion and absorption of intestinal carbohydrates (alpha glucosidase inhibitors) or improve insulin action (thiazolidinediones).

Diabetes and hypertension frequently occur together and are two of the leading risk factors for atherosclerosis and its complications [9]. There is substantial overlap between diabetes and hypertension in etiology and disease mechanisms. Obesity, inflammation, oxidative stress and insulin resistance are thought to be common pathways. Recent advances in the understanding of these pathways have provided new insights and perspectives. Knowing the common causes and disease mechanisms allow a more effective and proactive approach in their prevention and treatment [9]. However, the association and frequent co-existence of diabetes and hypertension put the patient at a higher risk of mortality compared to singular existence of one. Therefore, there is need for the development of newer and better management regimens to prevent the increase in mortality rate. Antihypertensive drugs can also significantly influence the probability that otherwise healthy individuals will develop metabolic syndrome or type 2 diabetes. While diuretics and beta blockers have a prodiabetic effect, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers may protect against diabetes more effectively than the metabolically neutral calcium channel blockers [10]. Though there are evidences that agents that interrupt the renin-angiotensin system (RAS) provide greater protective effects, the data are not conclusive. However, most diabetic subjects will require combination therapy to reach goal blood pressure. Given that diabetes is an important cardiovascular risk factor, there is the potential for reductions in risk due to reduced blood pressure to be offset by increased risk due to development of diabetes. Such concerns should be considered in the selection of antihypertensive therapy [9].

As a proactive measure this study evaluated the effects and treatment outcomes of the combinations of some antihypertensive drugs mainly amlodipine and valsartan with glibenclamide in the management of streptozotocin-induced diabetes in rats. The drugs for this study were rationally selected: glibenclamide being a prototype in sulphonylurea group and one of the most prescribed hypoglycaemic agents. On the other hand, amlodipine and valsartan - a calcium channel blocker and an angiotensin II receptor blocker respectively, were the antihypertensives selected. Knowing that diabetes mellitus is a risk factor for hypertension and that when they co-exist in a patient there is a worsened prognosis, this study was designed to know if the addition of either of these antihypertensive drugs (amlodipine and valsartan) would improve the diabetes treatment outcome when combined with glibenclamide compared to when glibenclamide is used alone.

2. Materials and methods

2.1. Materials

Mechanical grinder, volumetric flask, measuring cylinder, test tubes, spatula, plastic cages, standard feed, syringes (1, 2 and 5 ml), oral-intubation tube, surgical dissecting kits, plain sample bottles, EDTA sample bottles, cotton wool, surgical gloves, ruler, Hettich Centrifuge (Rototix 32 A, Germany), human automated haematology system analyzer (ERMA PCE 210, ERMA, Japan), automated clinical system (VIS-7220 G, Biotech Engineering Management Company Limited, UK), analyzer ISE 4000 (SFRI, France), and optical photomicroscope (Leica MC170 HD, Leica Biosystems, Germany), scout pro digital balance (OHAUS Corporation, USA).

2.2. Animals

Male Wistar rats weighing (200–350 g) were obtained from the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The rats were kept in plastic cages and housed at room temperature (about 24 °C) and humidity. They were allowed free access to dry rodent pellet feeds (Top Feeds Limited, Ibadan, Nigeria) and water. The bedding materials (wood shavings) of the cages were changed daily. All experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of laboratory Animals (NIH Publications No. 80-23) revised in 2002.

2.3. Induction of diabetes mellitus

The animals were fasted overnight and diabetes mellitus was induced by a single dose intraperitoneal injection of streptozotocin (40 mg/kg body weight) dissolved in freshly prepared 0.1 M citrate buffer, pH 4.5. After administration the animals were allowed free access to feed and water. After 48 h, the animals were tested for diabetes using the Accu-Chek® Active glucometer (Roche, USA) and any animal with blood sugar level ≥200 mg/dl was considered diabetic [11].

2.4. Experimental design

The animals were selected into eight groups of at least seven rats each and treated orally with freshly prepared drugs (dissolved in measured volume of distilled water) for 6 weeks as follow;

Group 1: Non-diabetic animals given 0.2 ml distilled water daily.

Group 2: Diabetic animals given 0.2 ml distilled water daily.

Group 3: Diabetic animals treated with 5 mg/kg body weight dailydose of glibenclamide alone.

Group 4: Diabetic animals treated with 5 mg/kg and 2.5 mg/kg body weight daily dose of glibenclamide and amlodipine respectively.

Group 5:Diabetic animals treated with 5 mg/kg and 30 mg/kg body weight daily dose of glibenclamide and valsartan respectively.

2.5. Determination of blood glucose

Blood samples for measuring the glucose level were collected from the tail vein of the fasted rats before 9 a.m. The tail was cleaned with methylated spirit and allowed to dry. The lateral tail vein was pricked using a sterile lancet and droplet of the blood was placed on the glucose test strip and read using the Accu-chek® Active glucometer [12].

2.6. Determination of change in body weight

The weights of the animals were taken weekly using a sensitive weighing balance and the changes in weight during the experiment were determined [13].

2.7. Haematological and biochemical analyses

After 6 weeks treatment, blood samples were obtained from the aorta of the rats under chloroform anesthesia into an ethylenediamine tetra-acetic acid (EDTA) and plain sample bottles for haematological and biochemical analyses respectively. The samples were evaluated for changes in haematological parameters, liver function, renal function and lipid profile.

2.8. Histopathological study

The various organs (liver, kidney, lung and heart) excised after humane sacrifice of the animals under chloroform anesthesia were fixed in 10% formal-saline. Fixed tissues were completely dehydrated with absolute ethanol followed by 96% ethanol, 70% ethanol and then