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ORIGINAL ARTICLE

Streptozotocin-induced vascular and biochemical changes in rats: Effects of rosiglitazone vs. metformin

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Abstract The aim was to investigate rosiglitazone and metformin effects on some vascular and biochemical changes associated with streptozotocin (55 mg/kg; i.p.)-induced hyperglycaemia in rats. Isolated aortas were used to evaluate their reactivity towards norepinephrine, acetylcholine, and sodium nitroprusside. Blood samples were used to assess the biochemical changes of some parameters viz., plasma lipid peroxides and nitric oxide levels and erythrocytic glutathione peroxidase activity. Hyperglycaemic animals orally received rosiglitazone (0.5 mg/kg) or metformin (150 mg/kg) daily for 2 weeks and their effects were determined 24 h after the last dose. Our results revealed that streptozotocin-induced hyperglycaemia is associated with impaired vascular reactivity towards various agents, increased lipid peroxides level, decreased glutathione peroxidise activity, and decreased nitric oxide level. Both drugs further decreased norepinephrine-induced contraction and improved acetylcholine- and sodium nitroprusside-induced relaxations. Rosiglitazone restored the alterations in all tested biochemical parameters while metformin restored only glutathione peroxidise activity. In conclusion both drugs show beneficial effects against the vascular dysfunction associated with hyperglycaemia which might be related to their euglycaemic activity in addition to anti-oxidant property of rosiglitazone and a direct effect of metformin on vascular smooth muscle.

1. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycaemia resulting from defects in insulin secretion and/or insulin action. The chronic hyperglycaemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, heart, and blood vessels.[1,2] DM is among the silent killers, since many people are not aware that they have the disease until they develop one of its life-threatening

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complications. The prevalence of DM increases with age.[3] DM is associated with an increased risk of micro- and macrovascular complications, causing considerable morbidity and mortality.[4] Blood glucose control can reduce the risk of these vascular complications but does not prevent them altogether.[5]

Endothelial dysfunction, a non-traditional cardiovascular risk marker, has been strongly associated with the reduced vascular reactivity occurring in patients with type 2 DM, thereby playing a major role in the development of complications of the micro- and macrocirculation.[6] Studies in experimental animals designed to investigate the mechanisms involved in such vascular dysfunction have implicated various factors including destruction of endothelial cells by oxidative stress and free radicals that lead to altered release of endothelium-derived constricting and relaxing factors.[7]

Nitric Oxide (NO) is an important endogenous regulator of blood vessel tone by promoting vasodilatation and therefore it plays an important role in the control of blood pressure (BP).[8] It is generated from the amino acid L-arginine within healthy endothelium by endothelial NO synthase (eNOS).[9] Inefficient utilization of the substrate L-arginine by NOS and decreased availability of NO due to scavenging by advanced glycated end-products resulting from excessive hyperglycaemia have been proposed to participate in impaired endothelial cell function.[10,11]

There is considerable evidence that oxidative stress resulting from increased production and/or inadequate removal of free radicals including reactive oxygen species (ROS) play a key role in the pathogenesis of late diabetic complications.[12] It has been reported that in uncontrolled diabetes, the levels of endogenous anti-oxidants such as superoxide dismutase, vitamin E, and lipoic acid are markedly reduced.[13]

The present study was devoted to investigate the influence of 2 commonly used anti-diabetic drugs, namely, rosiglitazone (ROSI) and metformin (MET) on some vascular and biochemical alterations that associate experimentally-induced hyperglycaemia in rats. Hyperglycaemia was induced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ). Both drugs were administered orally once per day for 14 consecutive days and their effects were evaluated 24 h after the administration of the last dose. For the assessment of hyperglycaemia-induced vascular changes, the responsiveness of the isolated thoracic aortic rings towards various vasoactive agents namely, norepinephrine (NE), acetylcholine (Ach), and sodium nitroprusside (SNP) was tested. For the assessment of hyperglycaemia-induced biochemical changes, the blood levels of some relevant biomarkers for oxidative stress and NO were determined. Plasma lipid peroxides level (measured as malondialdehyde; MDA) and erythrocytic glutathione peroxidase (GSH-Px) activity were taken as *in vivo* reliable indices for the contribution of free radical generation and in turn, oxidative stress in STZ-induced hyperglycaemia. Plasma nitrate/nitrite level was used as a convenient marker for NO formation.

2. Materials and Methods

2.1. Drugs and chemicals

Rosiglitazone maleate (GlaxoSmithKlein Company, Egypt) and metformin hydrochloride (Cid Company, Egypt) were used in the present investigation. Both drugs were freshly prepared in distilled water and given orally. The concentration of either drug was adjusted so that each 100 g animal body weight received 0.5 ml, containing the required dose. Streptozotocin, acetylcholine perchlorate and *N*-(1-Naphthyl) ethylene-diamine dihydrochloride (NEDD) were purchased from Sigma–Aldrich, USA. Norepinephrine hydrochloride, sodium nitroprusside, and sulphanilamide were purchased from Fluka (Italy), Oxford Laboratory (India), and Merck (Germany), respectively. All other chemicals were of the highest commercially available grade.

2.2. Animals

Adult male albino rats, weighing 180–250 g, were used in all experiments of this study. They were obtained from the Animal House Colony of the National Research Center (Dokki, Giza, Egypt), and were housed under conventional laboratory conditions throughout the period of experimentation. The animals were fed a standard rat pellet diet and allowed free access to water. The study was conducted in accordance with ethical procedures and policies approved by the Animal Care and Use Committee of National Research Center.

2.3. Induction of hyperglycaemia

Hyperglycaemia was induced by a single i.p. injection of STZ (55 mg/kg).[14] Briefly, rats were weighed and injected with STZ dissolved in a citrate buffer (0.1 M, pH 4.5). After 48 h blood samples were withdrawn from the retro-orbital venous plexus under light ether anaesthesia and the plasma was separated by centrifugation for the determination of glucose level. Only rats with plasma glucose levels more than 250 mg/dl were selected and considered as hyperglycaemic animals that have been subjected to further experimentation.

2.4. Experimental design

Hyperglycaemic rats were weighed and randomly allocated into 3 groups (8–10 rats each). One group served as hyperglycaemic control, while the other 2 groups were treated orally with ROSI (0.5 mg/kg/day)[15,16] and MET (150 mg/kg/day)[17] for 14 consecutive days, respectively. Drug treatment was started 48 h after STZ injection (time at which hyperglycaemia was confirmed). In addition, a universal normal group which received only the citrate buffer (8–10 rats) was used. Twenty-four hours after the last dose of either drug treatment, animals were weighed and then sacrificed by cervical dislocation. Blood was collected and prepared for the biochemical determination of MDA and NO levels as well as GSH-Px activity. Rings from isolated thoracic aortas were then prepared and suspended in an organ bath to test their reactivity towards the selected vasoactive agents, namely, NE, Ach, and SNP.

2.4.1. Assessment of vascular reactivity

The vascular reactivity towards NE as a vasoconstrictor, Ach as an endothelium-dependent vasodilator, and SNP as an endothelium-independent vasodilator was assessed using the isolated aortic ring preparation described by Cocks et al.[18] Briefly, segments of thoracic aortas were rapidly placed in warm Krebs' solution and dissected free of surrounding tissue before being cut into transverse rings of 3–5 mm length. An aortic ring was mounted in 10 ml water jacketed automatic