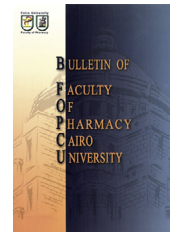Cairo University

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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

multi-chamber organ bath system (Model No. ML870B6/C, Panlab, Spain) containing Krebs' solution of the following composition (g/l): NaCl 6.9, KCl 0.35, KH_2PO_4 0.16, $MgSO_4$ $7H_2O$ 0.3, $CaCl_2$ $2H_2O$ 0.37, $NaHCO_3$ 2.1, and glucose 1.05. The organ bath solution was continuously aerated with carbogen (a mixture of 95% O_2 and 5% CO_2) and its temperature was kept at 37 °C. The mounted aortic ring was suspended horizontally between 2 hooks passed through its lumen, care being taken not to injure the luminal surface. The bottom hook was attached to a support leg while the upper one was attached to a force–displacement transducer (Model No. MLT0201, Panlab, Spain) connected to an amplifier (Power-Lab, AdInstruments Pty. Ltd.) which is connected to a computer. The chart for windows (v 3.4) software was used to record and elaborate data. The preparation was allowed to equilibrate for about 2 h under a resting tension of 2 g during that time any change in the resting tension was readjusted.

NE, Ach, and SNP were freshly prepared by dissolving them in Kerbs' solution. Serial dilutions of each vasoactive agent were prepared such that cumulative additions to the bath gave a final bath concentration ranging from 10^{-9} M to 10^{-4} M. For testing the relaxant effect of Ach or SNP, precontraction with NE was carried out first with a concentration that produces approximately 60–70% of the maximum contractile response. Contractile responses to NE were expressed as percentage of maximal response while relaxant responses to the vasodilators Ach and SNP were expressed as percentage relaxation of the pre-contraction value.

2.4.2. Assessment of biochemical parameters

From each 18 h food-deprived rat, 2 blood samples were withdrawn from the retro-orbital venous plexus using heparinized capillary tubes and collected into 2 tubes containing EDTA. One blood sample was centrifuged at 3000 rpm for 10 min and the plasma was obtained. An aliquot of the separated plasma was then used for the determination of glucose level while the rest was stored at −70 °C for the subsequent determination of MDA and NO levels. The other blood sample was centrifuged at 3000 rpm for 10 min at 4 °C using the cooling centrifuge (Laborezentrifugen, 2k15, Sigma, Germany) and the plasma was drawn off. Blood cells' sediment was then washed once with 10 volumes of cold saline (0.9% w/v NaCl). Lysis of sedimented erythrocytes was performed by adding 4 volumes of cold deionized water to the estimated pellet volume. Centrifugation at 3000 rpm for 10 min at 4 °C was then carried out and the resultant clarified erythrocyte lysate was collected and stored at −70 °C for the subsequent determination of GSH-Px activity.

2.4.2.1. Determination of glucose level.
Glucose level was determined as quinineamine using a test reagent kit (Stanbio, USA) according to the method of Trinder.[19] The absorbance was measured at 510 nm and the results were expressed as mg/dl.

2.4.2.2. Determination of lipid peroxides level.
Lipid peroxides level was determined as thiobarbituric acid (TBA)-reactive substances using a test reagent kit (Biodiagnostic, Egypt) according to the method of Ohkawa et al.[20] The absorbance was measured at 534 nm and the results were expressed as nmol/ml.

2.4.2.3. Determination of NO level.
The total amount of NO was indirectly estimated in terms of its main metabolites,

nitrate and nitrite by the Griess reaction using NEDD and sulphanilamide as described by Miranda et al.[21] after deproteinizing the sample with absolute ethanol. The absorbance was measured at 540 nm and the results were expressed as nmol/ml.

2.4.2.4. Determination of GSH-Px activity.
GSH-Px activity was determined by a kinetic assay at 37 °C using a test reagent kit (Biodiagnostic, Giza, Egypt) according to the method described by Paglia and Valentine.[22] The absorbance was measured at 340 nm and the results were expressed as mU/ml.

2.4.3. Assessment of body weight changes

Each rat was weighed individually twice, first at the beginning of the experiment (initial weight) and second 24 h after the administration of the last dose of either drug (final weight). The difference in body weight of each rat was calculated and expressed as percentage change according to the following:

$$\% \text{ change in body weight} = \frac{\text{final weight} - \text{initial weight}}{\text{initial weight}} \times 100$$

2.5. Statistical analysis

Data are expressed as means ± SEM. Statistical significance was taken as $P < 0.05$, using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test to judge the difference between various groups.

3. Results

3.1. Vascular reactivity experiments

3.1.1. Effect of ROSI and MET on NE-induced contractions
Cumulative concentrations of NE, starting from 10^{-9} M to 10^{-4} M, produced a concentration-dependent contraction of aortic rings isolated from normal rats. A maximum contraction was achieved at a concentration of 10^{-5} M NE, reaching about 98.12% ±0.58 of the maximal response. Aortic rings isolated from hyperglycaemic rats (16 days after STZ administration) showed an attenuation of the vascular responsiveness towards almost all concentrations of NE, especially at concentrations from 10^{-9} M to 10^{-6} M, as compared to the respective values of normal group. Oral treatment of hyperglycaemic rats with either ROSI (0.5 mg/kg/day) or MET (150 mg/kg/day) for 2 weeks tended to further decrease the vascular responsiveness of aortic rings as compared to the respective values of hyperglycaemic-untreated group. The decreased responsiveness was, however, quite significant at high concentrations of NE starting from 10^{-6} M (Fig. 1a).

3.1.2. Effect of ROSI and MET on Ach-induced relaxations
Cumulative concentrations of Ach, starting from 10^{-9} M to 10^{-4} M, elicited a dose-dependent relaxation of aortic rings isolated from normal rats (pre-contraction was achieved with 10^{-6} M of NE). A maximum relaxation was attained at a concentration of 10^{-4} M Ach, reaching about 89.40% ±5.62 of the pre-contraction value. Aortic rings isolated from hyperglycaemic rats (16 days after STZ administration) showed a decrease of the vascular responsiveness towards almost all
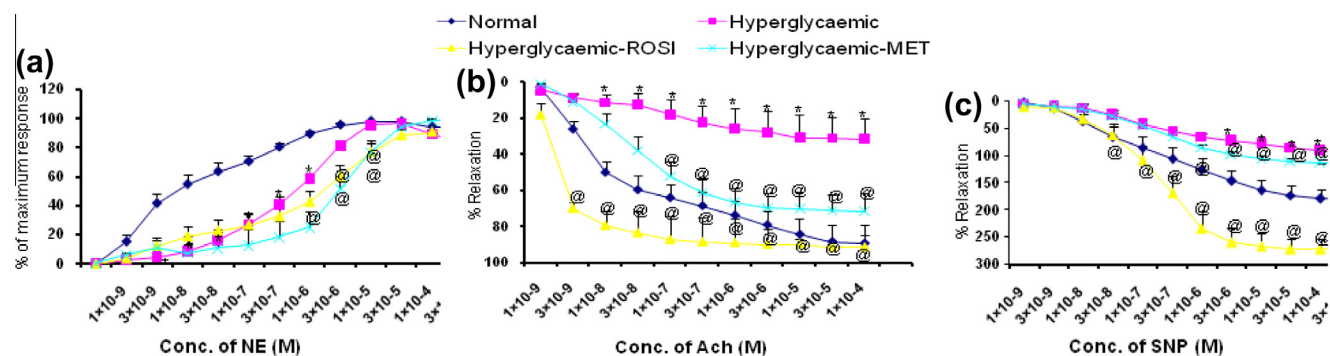
Figure 1 Effect of rosiglitazone and metformin on NE-induced contractions (a), Ach-induced relaxations (b), and SNP-induced relaxations (c) of aortic rings isolated from streptozotocin-treated rats. Rats were rendered hyperglycaemic by a single i.p. injection of streptozotocin (STZ; 55 mg/kg). Rosiglitazone (ROSI; 0.5 mg/kg/day; p.o.) and metformin (MET; 150 mg/kg/day; p.o.) were administered 48 h after STZ injection for 2 weeks. Aortas were isolated and suspended as rings (length, 3–5 mm) 24 h thereafter. Relaxation was induced by applying cumulative concentrations on aortic rings pre-contracted with 10^{-6} M of NE. Results are expressed as means $\pm$ SEM ($n = 6$–10). *Significant difference from normal rats at $P < 0.05$. @Significant difference from hyperglycaemic rats at $P < 0.05$.

concentrations of Ach, as compared to the respective values of normal group. A maximum relaxation of 31.71% ± 9.62 of the pre-contraction value was achieved at a concentration of 10^{-4} M Ach. Oral treatment of hyperglycaemic rats with ROSI (0.5 mg/kg/day) for 2 weeks showed an enhancement of the vascular responsiveness of aortic rings towards almost all concentrations of Ach as compared to the respective values of hyperglycaemic-untreated group. A maximum relaxation of 91.57% ± 3.29 of the pre-contraction values was attained at 3×10^{-5} M Ach. A similar treatment of hyperglycaemic rats with MET (150 mg/kg/day) restored the attenuated vascular responsiveness of aortic rings towards almost all concentrations of Ach. A maximum relaxation of 71.70% ± 10.34 of the pre-contraction values was attained at 10^{-4} M Ach (Fig. 1b).

3.1.3. Effect of ROSI and MET on SNP-induced relaxations

Cumulative concentrations of SNP, starting from 10^{-9} M to 10^{-4} M, produced a concentration-dependent relaxation of aortic rings isolated from normal rats (pre-contraction was elicited with 10^{-6} M of NE). A maximum relaxation was achieved at a concentration of 10^{-4} M SNP, reaching about 178.82% ± 17.28 of the pre-contraction value. Aortic rings isolated from hyperglycaemic rats (16 days after STZ administration) showed an attenuated vascular responsiveness towards SNP, as compared to the respective values of normal group, especially towards high concentrations of SNP starting from 10^{-6} M. A maximum relaxation of 91.34% ± 3.44 of the pre-contraction value was attained at a concentration of 10^{-4} M SNP. Oral treatment of hyperglycaemic rats with ROSI (0.5 mg/kg/day) for 2 weeks produced an enhancement of the vasorelaxant effect towards SNP, as compared to the normal group. A maximum relaxation of 272.35% ± 19.51 of the pre-contraction value was attained at a concentration of 3×10^{-5} M SNP. Similar treatment of hyperglycaemic rats with MET (150 mg/kg/day) tended to restore the attenuated vascular responsiveness towards SNP. A maximum relaxation of 113.92% ± 7.74 of the pre-contraction value was elicited at a concentration of 10^{-4} M SNP (Fig. 1c).

3.2. Biochemical parameters experiments

3.2.1. Effect of ROSI and MET on plasma glucose level
A single i.p. injection of STZ (55 mg/kg) produced an elevation of plasma glucose level which was evidenced 48 h after administration. The elevation was found to be persistent during the period of investigation and reached the average value of 376% of the normal one, 16 days after STZ administration. Oral treatment of hyperglycaemic rats with either ROSI (0.5 mg/kg/day) or MET (150 mg/kg/day) for 2 weeks succeeded to cause a decrease in the elevated plasma glucose level reaching nearly the normal values (Table 1).

3.2.2. Effect of ROSI and MET on erythrocytic GSH-Px activity
Induction of hyperglycaemia with a single i.p. injection of STZ (55 mg/kg) was associated with a decreased erythrocytic GSH-Px activity. The decreased activity reached about 41% of the normal values, 16 days after STZ administration. Oral treatment of hyperglycaemic rats with either ROSI (0.5 mg/kg/day) or MET (150 mg/kg/day) for 2 weeks provoked an increase in the decreased enzyme activity reaching about 71% and 73% of the normal values, respectively (Table 1).

3.2.3. Effect of ROSI and MET on plasma MDA level
Induction of hyperglycaemia with a single i.p. injection of STZ (55 mg/kg) was encountered with an elevated plasma MDA level. This elevation reached about 167% of the normal values, 16 days after STZ administration. Oral treatment of hyperglycaemic rats with ROSI (0.5 mg/kg/day) for 2 weeks caused a decrease in the elevated plasma MDA level reaching about 119% of the normal value. A similar treatment of hyperglycaemic rats with MET (150 mg/kg/day) did not show any significant change in the elevated plasma MDA level as compared to the hyperglycaemic group (Table 1).

3.2.4. Effect of ROSI and MET on plasma NO level
Induction of hyperglycaemia with a single i.p. injection of STZ (55 mg/kg) was accompanied by a decreased plasma NO level.

Table 1 Effect of rosiglitazone and metformin on blood glucose, MDA, and NO levels as well as GSH-Px activity of streptozotocin-treated rats. Rats were rendered hyperglycaemic by a single i.p. injection of streptozotocin (STZ; 55 mg/kg). Rosiglitazone (ROSI; 0.5 mg/kg; p.o.) and metformin (MET; 150 mg/kg; p.o.) were administered for 2 weeks. Treatment with either drug was started 48 h after STZ injection. Twenty-four hours after the last dose of either drug, blood samples from 18 h food-deprived animals were withdrawn using heparinized capillary tubes and collected into tubes containing EDTA. Separated plasma was used for glucose, MDA, and NO estimation while erythrocyte lysate was used for GSH-Px estimation. Results are expressed as means ± SEM ($n = 6–10$).

Groups	Glucose (mg/dl)	GSH-Px (mU/ml)	MDA (nmol/ml)	NO (nmol/ml)
Normal	114.05 ± 5.01	141.73 ± 15.53	6.40 ± 0.03	60.41 ± 2.04
Hyperglycaemic	429.00 ± 8.08[*]	58.36 ± 5.62[*]	10.75 ± 0.86[*]	22.30 ± 2.45[*]
Hyperglycaemic + ROSI	101.34 ± 10.47[@]	101.16 ± 11.78[@]	7.60 ± 0.65[@]	43.49 ± 3.30[*,@]
Hyperglycaemic + MET	95.69 ± 2.33[@]	103.75 ± 5.62[@]	9.65 ± 0.98[*]	30.73 ± 1.67[*]

[*] Significant difference from normal rats $P < 0.05$.
[@] Significant difference from hyperglycaemic rats $P < 0.05$.

This decrease reached about 37% of the normal values, 16 days after STZ administration. Oral treatment of hyperglycaemic rats with ROSI (0.5 mg/kg/day) for 2 weeks caused an elevation in the decreased plasma NO level reaching about 72% of the normal value. A similar treatment of hyperglycaemic rats with MET (150 mg/kg/day) did not affect the decreased plasma NO level as compared to the hyperglycaemic group (Table 1).

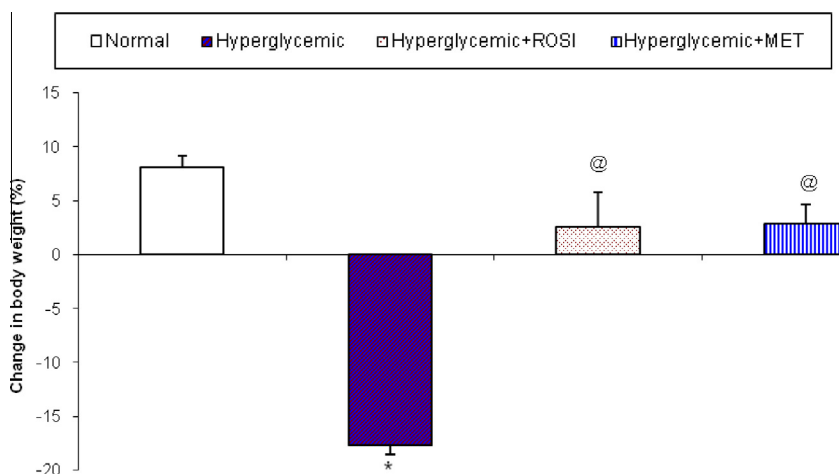### 3.3. Effect of ROSI and MET on body weight change

Induction of hyperglycaemia with a single i.p. injection of STZ (55 mg/kg) was associated with a loss in body weight by an average of about 26% of the normal values, 16 days after administration of STZ. Oral treatment of hyperglycaemic rats with either ROSI (0.5 mg/kg/day) or MET (150 mg/kg/day) for 2 weeks decreased body weight loss and showed a gain by about 2.55% and 2.8%, respectively (Fig. 2).

4. Discussion

Results of the present study revealed that STZ-induced hyperglycaemia in rats was accompanied by some vascular and biochemical changes. The vascular changes were evidenced by alterations in the reactivity of isolated aortic rings towards a vasoconstrictor as well as endothelium-dependent and -independent vasodilators. The biochemical changes, on the other hand, were evidenced by an increased level of plasma MDA concomitantly with the decreased activity of erythrocytic GSH-Px and level of plasma NO. STZ-induced hyperglycaemia was also encountered with a profound loss in body weight. These findings were in accordance with previously reported data.[23–25]

Aortic rings isolated from hyperglycaemic rats showed a decreased reactivity towards NE, an α_1-adrenoceptor agonist. Several mechanisms are proposed to be implicated for such vascular impairment, including non-enzymatic protein glycation, sorbitol myoinositol changes, generation of ROS, and activation of diacylglycerol protein kinase C pathway.[23] It is well documented that hyperglycaemia that associates DM results in endothelial cell damage leading to vascular reactivity changes. Previous studies concerned with the contractile response of α_1-adrenoceptor agonists showed, however, inconsistent results, while in some increased reactivity was demonstrated[26], in others decreased[27] or unchanged responsiveness[28] was shown. This discrepancy has been related to the duration and/or the severity of the induced hyperglycaemia.[29]

Figure 2 Effect of rosiglitazone and metformin on body weight of streptozotocin-treated rats. Rats were rendered hyperglycaemic by a single i.p. injection of streptozotocin (STZ; 55 mg/kg). Rosiglitazone (ROSI; 0.5 mg/kg; p.o.) and metformin (MET; 150 mg/kg; p.o.) were administered for 2 weeks. Treatment with either drug was started 48 h after STZ injection. Each rat was weighed individually at the beginning of the experiment and 24 h after the last dose of either drug, the change in body weight was calculated as percentage. Results are expressed as means ± SEM ($n = 6–10$). [*]Significant difference from normal rats $P < 0.05$. [@]Significant difference from hyperglycaemic rats $P < 0.05$.

In the present study, treatment of hyperglycaemic rats with either ROSI or MET for 2 weeks showed a further decrease in the vascular responsiveness towards NE as compared to the respective hyperglycaemic-untreated group. It has been observed that ROSI is able to reduce BP in diabetic patients,[30,31] hypertensive transgenic mice,[32] spontaneously hypertensive rats,[33] and in Zucker fatty rats.[34] ROSI may directly regulate vessel tone and thus potentially contribute to the improved BP.[32] Moreover, it has been found that MET significantly reduced the contractility towards the α_1-adrenoceptor agonist, phenylephrine and reduced the elevated BP in diabetic rats.[17]

Acetylcholine is a vasoactive substance that requires the presence of endothelial cells to produce its relaxant effect on the vasculature. It has been documented that this relaxation is due to the release of NO from the vascular endothelium, an effect which is absent in rubbed or denuded vessels.[35] Results of the current study revealed that hyperglycaemic rats showed a decreased vascular responsiveness towards Ach. It has been previously reported that endothelium-dependent relaxation response to Ach is impaired in diabetic rat aorta.[30,36] Furthermore, reduction in endothelium-dependent relaxation is a common feature known to occur in the arteries of diabetic animals.[37] Many mechanisms were proposed for the reduced Ach-induced NO-dependent vasodilatation in diabetic animals including decreased NO release and/or decreased reactivity of vascular smooth muscle (VSM) to NO.[38] Moreover, it has been proven that DM is associated with an increase in oxidative stress, which causes endothelium damage due to increased production of ROS. Damaged endothelium cannot produce vasodilators such as NO with the possibility of increasing the production of vasoconstrictors such as endothelin-1.[39]

In the current study, treatment of hyperglycaemic rats with either ROSI or MET for 2 weeks caused an enhancement of the vascular responsiveness towards Ach as compared to the respective hyperglycaemic-untreated group. This observation was supported by Ryan and his colleagues[31] who showed that ROSI improves the endothelial-dependant relaxation of the carotid artery in hypertensive transgenic mice. Furthermore, histological evidence implies that leukocyte infiltration into atherosclerotic or ischaemic tissues which are a marker of an inflamed and dysfunctional endothelium is reduced with ROSI treatment.[30,40] Some authors attributed the beneficial effects of ROSI to its anti-oxidant property[41] while others related it to the reduction of the inflammatory response.[42] On the other hand, the improving effect of MET on Ach-induced vasorelaxation in diabetic rats has been suggested to be due to elevated cyclic-GMP level which in turn leads to a decreased intracellular calcium level.[17,43]

Sodium nitroprusside exerts its relaxant effect on VSM cells *via* a spontaneous release of NO, without the involvement of vascular endothelium.[44] Results of the current study revealed that hyperglycaemic rats showed a decreased vascular responsiveness towards SNP and their treatment with ROSI for 2 weeks caused an enhancement of SNP-induced relaxation even more than that of normal animals. This may be attributed to its anti-oxidant property.[41] It has been reported that the drug has the ability to restore the relaxant action of insulin in resistant arteries and thus preventing the development of hypertension. ROSI was documented to improve aortic diastolic function of insulin resistant-hypertensive rats, a mechanism that might be associated with an increase in NO, a

decrease in BP and serum insulin as well as an improvement of insulin resistance.[45] On the other hand, oral treatment with MET for 2 weeks tended to restore the attenuated vascular responsiveness towards SNP. This was in accordance with other studies where the drug improved the microvascular function in type 2 diabetic model. This effect of MET has been reported to be due to its direct effect on VSM.[17]

Oxidative stress and overexpression of NOS have been discussed as interrelated contributing factors in pancreatic β-cell dysfunction and destruction associated with diabetic conditions that lead to micro- and macrovascular complications. Hyperglycaemia is considered one of the factors responsible for the development of oxidative stress that results from enhanced formation of ROS by glucose auto-oxidation.[46]

Streptozotocin-induced hyperglycaemia selectively destroys the islets of Langerhans by oxidant production and by producing inappropriate NO response.[47,48] It has been previously shown that toxic action of STZ is due to the generation of free radicals[49] which results in enhanced lipid peroxidation.[50,51]

In the present study, STZ-induced hyperglycaemia was accompanied by an elevated plasma lipid peroxides level, suppressed erythrocytic GSH-Px activity, and decreased plasma NO level. It is well known that GSH-Px is among the endogenous scavenging anti-oxidant enzymes that remove the toxic free radicals *in vivo*.[52] GSH-Px is essential to reduce the levels of ROS such as superoxide anion and hydrogen peroxide which are considered to be the major causes of lipid peroxidation. Erythrocytic GSH-Px, activity has been reported to be lowered in diabetic conditions.[24,53]

Treatment of hyperglycaemic rats with ROSI for 2 weeks showed a decrease in the elevated plasma MDA level and an increase in the decreased erythrocytic GSH-Px activity which might indicate that ROSI has a radical scavenging activity.[41] MET treatment, on the other hand, provoked an increase in GSH-Px activity but did not affect MDA level. This may be probably because treatment of hyperglycaemia neutralizes oxidative stress and thus restores anti-oxidant enzymatic activity of GSH-Px. It has been previously reported that ROSI decreases blood MDA level while its level is not affected by MET treatment.[54] Other observations reported, however, that MET reduced the oxidative stress in various animal models.[55]

Hyperglycaemic rats subjected to treatment with ROSI for 2 weeks caused an elevation of plasma NO level. This elevation of NO has been previously reported to be due to stimulation of NO synthesis and/or bioavailability in aortic endothelial cells (*via* AMP-activated protein kinase)[56,57] and in peripheral skin.[6] Treatment with MET, on the other hand, did not affect plasma NO level as compared to hyperglycaemic group. It has been assured that MET unlike ROSI does not activate AMP-activated protein kinase and does not stimulate NO release in human aortic endothelial cells.[58,59]

5. Conclusions

The findings of the current study revealed that oral treatment with either ROSI or MET for 2 weeks exerted beneficial effects against some vascular and biochemical changes associated with STZ-induced hyperglycaemia in rats. These beneficial effects might be related to their ability to improve hyperglycaemia in addition to the anti-oxidant property of ROSI and the direct effect of MET on VSM cells.

6. Conflict of interest

None.

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