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Original Article

Comparative Effects of Metformin and Glibenclamide on Aortic Reactivity to Vasodilator and Vasoconstrictor Agents in STZ-Induced Diabetic Rats

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Abstract

Diabetes is an important risk factor for cardiovascular events. Endothelial dysfunction is the main cause of disability and death in diabetic patients. The present study investigates the effects of metformin and glibenclamide on vasoconstrictive and vasodilative responses in the diabetic rat aorta. Rats were divided into four experimental groups (control, STZ-diabetic, metformin and glibenclamide treated diabetic rats). The treated rats received metformin (300 mg/kg) or glibenclamide (5 mg/kg) daily by gavage for 6 weeks.Thoracic aortic rings were mounted in an organ bath system, then contractile and dilatation responses induced by acetylcholine (ACh), phenylephrine (PE), potassium chloride (KCl), and sodium nitroprusside (SNP) were evaluated in different situations.Blood glucose level in glibenclamide group in days 24 and 45 were significantly lower than diabetic group. Metformin and glibenclamide significantly reduced the contractile responses to higher concentrations of PE (10^{-6} - 10^{-5} M) compared to diabetic group. Metformin and glibenclamide significantly reduced the contractile responses to concentrations of KCl (50 and 60 mM) compared to diabetic group. The relaxation responses to Ach 10^{-8} M, was increased in metformin and glibenclamide groups compared to the diabetic group. The relaxation responses to Ach 10^{-7} - 10^{-5} M were significantly higher in both treated groups compared to diabetic group. The chronic administration of metformin or glibenclamide has a significant hypoglycemic effect and improves aortic reactivity to vasoconstrictor and vasodilator agents in STZ-induced diabetic rats. No significant difference was found regarding the effects of metformin and glibenclamide on vasoconstrictive and vasodilative responses in aorta.

Key words: Diabetes mellitus, STZ, Metformin, Glibenclamide, Aorta, Rat

1. Introduction

Diabetes mellitus is a multi-systemic disease and can result in a multiple organ damage through several mechanisms. Vascular diseases are the main contributors to the morbidity and mortality associated with diabetic patients [1, 2]. Diabetic patients have an increased risk for the three major types of macrovascular diseases such as peripheral vascular disease, coronary heart disease and

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stroke [3]. Endothelial dysfunction is the main cause of death and disability in diabetic patients [4]. Several mechanisms for endothelial dysfunction have been reported, such as impaired signal transduction, impaired release of endothelium-derived relaxing factors (EDRF), increased destruction of EDRF, enhanced release of endothelium-derived constricting factors and decreased sensitivity of the vascular smooth muscle to EDRF [5]. In most subjects with diabetes mellitus two defects coexist: defective insulin sensitivity and defective insulin secretion. Both of these abnormalities contribute to hyperglycemia. Therefore, oral therapy with either biguanide metformin, which improves sensitivity of peripheral tissues to insulin, or sulfonylureas, which stimulate insulin secretion, are rational approaches to diabetes mellitus [6].

Metformin is one of the most frequently used drugs to treat patients with type 2 diabetes. In addition to decreasing hyperglycemia and improving insulin sensitivity, it also independently promotes vasculoprotection [7]. Metformin, a biguanide family member generally used in treatment of type 2 diabetes, appears to increase liver and peripheral tissue sensitivity to insulin as well as decrease hepatic glucose production; however, its exact mechanism remains unclear [8]. It could lower glycaemia by reducing glucose absorption in the intestine, stimulating insulin secretion from the pancreatic B-cells, decreasing glucose output in the liver and increasing glucose uptake in peripheral tissues, for example in the muscle and fat [9]. Although this biguanide derivative has been used for more than 50 years, its mechanism of action has not been fully elucidated. The antihyperglycemic mechanisms of metformin include: decreasing glucose absorption in the small intestine, increasing glucose transport into cells, decreasing the plasma free fatty acid concentrations and inhibition of gluconeogenesis. Activation of AMP-activated protein kinase (AMPK) plays an important role in these processes. The latest discoveries have revealed mechanisms of anti-atherosclerotic, hypotensive and anticancer action of metformin and its impact on vein endothelial function [10]. Metformin treatment is capable of reducing the oxidative stress as well as glycation and early inflammation observed in this animal model, and these actions were associated with a recovery in NO bioavailability and endothelial function in aorta [7].

Sulfonylurea antidiabetic agents such as glibenclamide, long used in treatment of non-insulin dependent diabetes mellitus, are known to promote insulin secretion through inhibition of ATP-sensitive K^+ (K_{ATP}) channels in the pancreatic cells [11]. Apart from the antidiabetic property, glibenclamide has also been reported to exhibit anti-nociceptive, anti-

tumor and platelet aggregation inhibitor activities [12]. Glibenclamide was found to act as both a selective ATP-sensitive K^+ channel blocker and a vasorelaxant. Glibenclamide-induced endothelium-dependent relaxation involves nitric oxide release and this effect may be related to its stimulatory effect on endothelial Ca^{2+} levels. However, the glibenclamide-induced endothelium-independent relaxation may be associated with its inhibitory effect on Ca^{2+} influx through Ca^{2+} channels and on the protein kinase C-mediated contractile mechanism [13].

The present study was designed to investigate the comparative effects of metformin and glibenclamide on aortic reactivity to vasodilator and vasoconstrictor agents in STZ-induced diabetic rats.

2. Materials and Methods

PE, ACh, KCl, SNP, STZ, metformin, and glibenclamide were obtained from Sigma (Germany). Moreover where needed for all drugs, the Krebs solution was used as the solvent. Blood glucose was measured by Easygluco Glucometer (Korea).

Male Wistar rats (250–280 g, 10 weeks old) were housed on a 12 hr light-dark cycle, under constant temperature (22 ± 1°C) and were allowed free access to standard laboratory diet and drinking water.

Diabetes was induced by a single intraperitoneal (i.p) injection of STZ (60 mg/kg)[14]. Three days after the STZ injection, we confirmed the development of diabetes by measuring fasting blood glucose levels in the blood samples taken from the tail vein. Rats with the blood glucose levels ≥ 250 mg/dl were considered to be diabetic.

The rats were randomly divided into four groups (n = 10 in each group): control (C), diabetic (D), diabetic-metformin (DM), diabetic-glibenclamide (DG). Normal saline was administered orally by gavage to the C and D groups, metformin (300 mg/kg) [15] to the DM group and glibenclamide (5 mg/kg) to the DG group daily by gavage for 6 weeks [16].

At the end of the treatment period, all the rats were euthanized with decapitation by guillotine. The descending thoracic aorta was rapidly dissected out and immersed in 95% O_2/5% CO_2-gassed (carbogen) ice-cold Krebs solution (pH 7.4) with the following composition (mM): NaCl (118.0), KCl (4.7), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $MgSO_4$ (1.6), glucose (11.1), $NaHCO_3$ (25.0). The aorta was removed free of connective tissue and fat and then cut into ring segments of 2–3 mm in length, and care was taken to avoid any damage to the endothelium. The aortic rings were individually suspended on stainless steel rods in 10 ml organ bath containing Krebs solution gassed with carbogen at 37°C. After a resting tension of 2 g, the vessel segments were allowed to stabilize for 1 h with the bath fluid being changed every 15 minutes to prevent metabolite interference. Changes in tension were recorded by isometric transducers connected to a data acquisition system (AD instrument, Australia).

The fasting blood glucose was measured in three different periods of the experiment:

before STZ injection, 3 and 24 days after the STZ injection (when the diabetes was confirmed), and 6 weeks after the STZ injection (day 45).

To test the contractile responses of aortic rings, cumulative concentrations of PE (10^{-9} - 10^{-5} M) or KCL (20 - 60 mM) were added to the organ bath and the contraction responses were recorded.

To evaluate the vasorelaxant responses of aortic rings, after induction of contraction by PE (10^{-6} M), cumulative concentrations of Ach (10^{-8} - 10^{-5} M) or SNP (10^{-9} - 10^{-6} M) were added to the organ bath and the relaxation responses were recorded.

The results are expressed as mean ± SEM. Statistical analyses were made using one-way ANOVA followed by the Tukey's test, Post hoc. Statistical significance was defined as $P < 0.05$.

3. Results and Discussion

Fasting blood glucose levels in days 3, 24, and 45 in the group D were significantly higher than that of the control group ($P < 0.001$). Our results showed that blood glucose levels in the group DM and DG in days 24 and 45 were significantly lower than that of the diabetic group ($P < 0.05$, $P < 0.01$) (Figure 1).

The contractile responses of aortic rings to cumulative concentrations of PE (10^{-9} to 10^{-5} M) were shown in Figure 2. Metformin and glibenclamide significantly reduced the contractile responses to higher concentrations of PE (10^{-6} - 10^{-5} M) compared to diabetic group ($P < 0.05$ to $P < 0.01$) (Figure 2).

The cumulative concentrations of KCl (20-60 mM) increased the contractile responses of aortic rings concentration dependently. In higher concentrations of KCl (50 and 60 mM) the increase in the aortic responses were significant between group D compared to group C ($P < 0.05$). Metformin and glibenclamide significantly reduced the contractile responses to concentrations of KCl (50 and 60 mM) compared to group D ($P < 0.05$) (Figure 3).

In the aortic rings pre-contracted with PE, Ach relaxation was impaired in group D compared to those from normal rats. The highest relaxation responses of aortic rings were observed in DM and DG groups. The relaxation response to Ach 10^{-8} M, was increased in DM and DG groups compared to that of group D ($P < 0.05$). The relaxation responses to Ach 10^{-7} - 10^{-5} M, were significantly higher in treated groups compared to group D ($P < 0.001$) (Figure 4).

In the aortic rings pre-contracted with PE, the relaxation responses to SNP (10^{-9}-10^{-6} M) were not significantly different between the groups (Figure 5).

The results of the present study showed that chronic administration of metformin and glibenclamide has a hypoglycemic effect and improves aortic reactivity to vasoconstrictor and vasodilator agents in STZ-induced diabetic rats. No significant difference in the hypoglycemic effect and improved aortic reactivity was observed between metformin and glibenclamide. Hyperglycemia plays a role

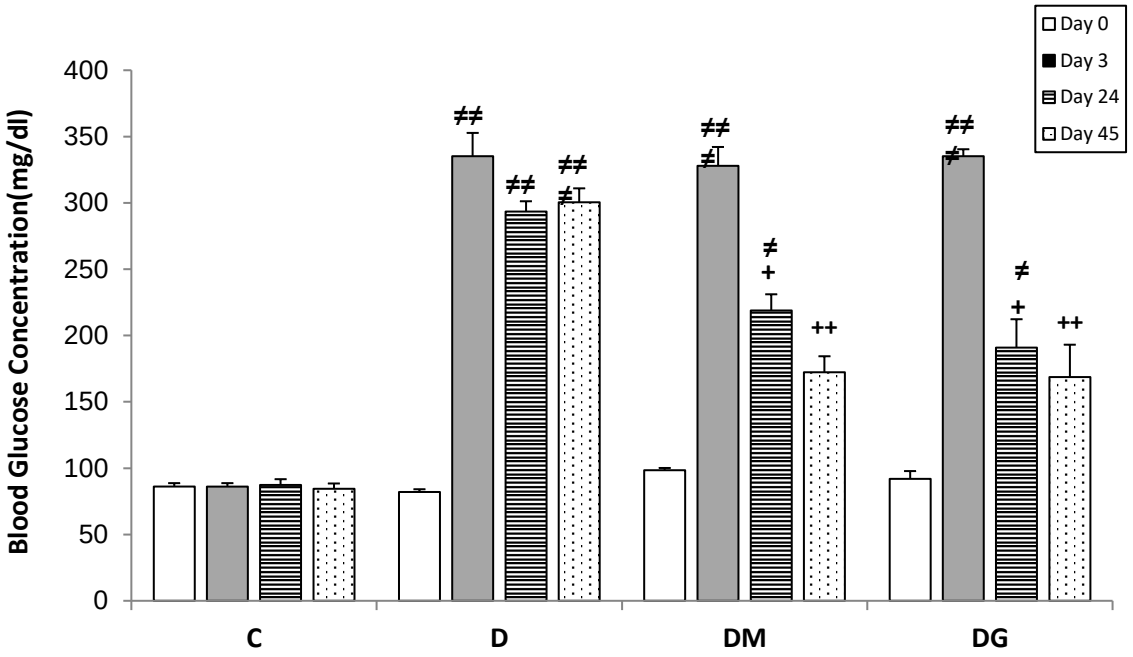

Figure 1. Average of fasting blood glucose level (mg/dl) in **the** control (C), diabetic (D), diabetic + metformin (DM) and diabetic + glibenclamide (DG) groups. Values are means ± SEM (n = 8). $^{\neq}P < 0.05$, $^{\neq\neq}P < 0.001$ compared to control group and $^{+}P < 0.05$, $^{++}P < 0.01$ compared to diabetic group.

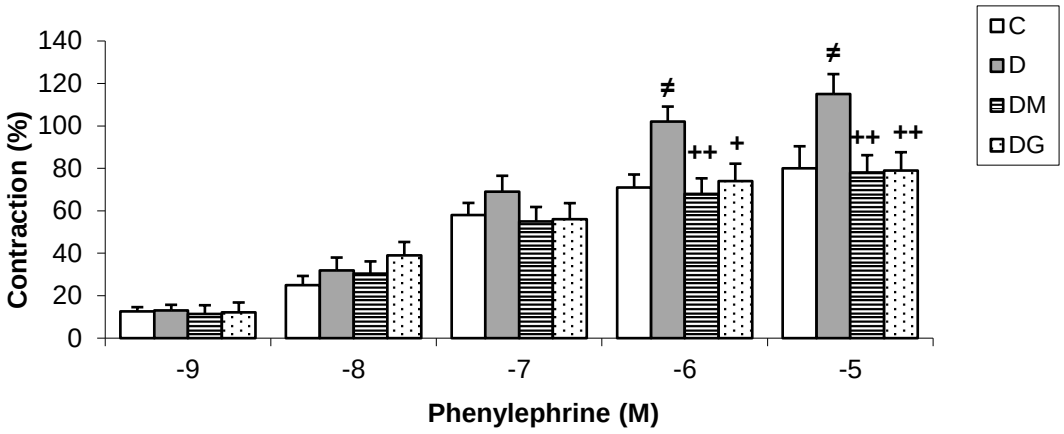

Figure 2. The contractile response of aortic rings to cumulative concentrations of phenylepherine (10^{-9}- 10^{-5} M) in control (C), diabetic (D), diabetic + metformin (DM) and diabetic + glibenclamide (DG) groups. Values are means ± SEM (n = 8). $^{\neq}P < 0.05$compared to control group and $^{+}P < 0.05$, $^{++}P < 0.01$ compared to diabetic group.

to the direct effects of glucose and other sugars on proteins, including glycation and non-enzymatic glycosylation [17].

Previous studies demonstrated that enhanced vascular reactivity to vasoconstrictor agents [18] or impairment of the vasodilation [19] contributes to the cardiovascular complications related to diabetes mellitus. Promoted vascular reactivity to alpha-1 adrenoreceptor agonists was indicated in

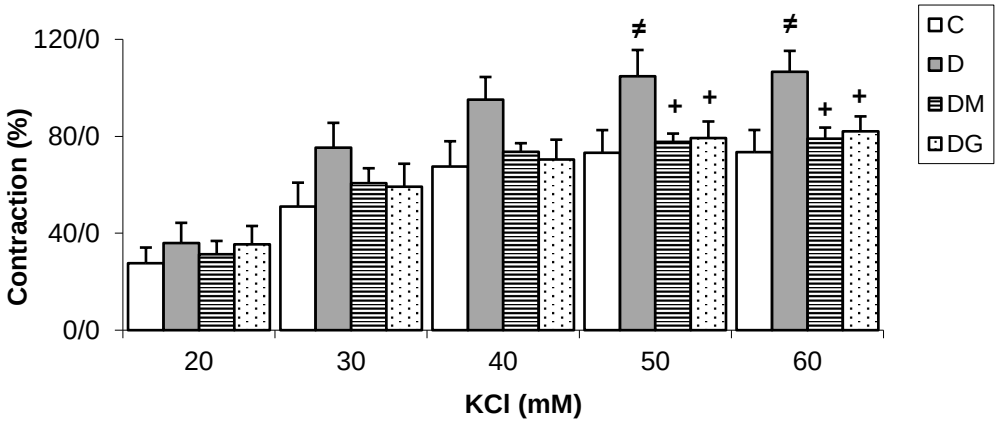

Figure 3. The contractile response of aortic rings to cumulative concentrations of KCL (20-60 mM) in control (C), diabetic (D), diabetic + metformin (DM) and diabetic + glibenclamide (DG) groups. Values are means ± SEM (n = 8). $^{\neq}$ P < 0.05 compared to control group and $^{+}$ P < 0.05 compared to diabetic group.

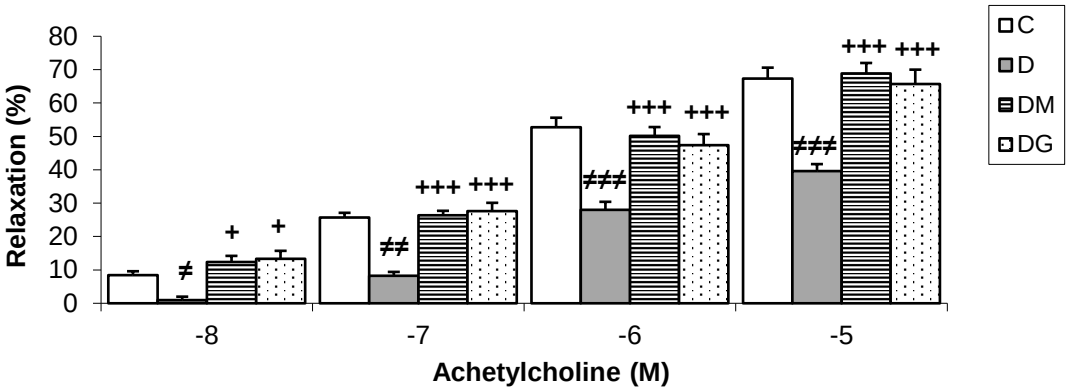

Figure 4. The relaxation responses of aortic rings to cumulative concentrations of acetylcholine (10^{-8}- 10^{-5} M) in control (C), diabetic (D), diabetic + metformin (DM) and diabetic + glibenclamide (DG) groups. Values are means ± SEM (n=8). $^{\neq}$P < 0.05, $^{\neq\neq}$P < 0.01, $^{\neq\neq\neq}$ P < 0.001 compared to control group and $^{+}$ P < 0.05, $^{+++}$ P < 0.001 compared to diabetic group. Statistical analyses were made using the one-way ANOVA followed by the Tukey's test, Post hoc.

different vascular beds from diabetic animals [20]. The increased aortic contractile responses of diabetic rats may be due to impaired endothelial function [21], increased calcium influx through voltage-dependent L-type Ca^{2+} channels [18], increased myofilament Ca^{2+} sensitivity [22], increased vasoconstrictor prostanoids due to increased superoxide anions and increased sensitivity to adrenergic agonists [23] and oxidative stress [24].

PE, an adrenoreceptor agonist, causes aortic contraction by Ca^{2+} influx via receptor-operated Ca^{2+} channels (ROCCs) and by the release of Ca^{2+} from the sarcoplasmic reticulum [25, 26]. The latter pathway involves PE stimulation of phospholipase C to produce diacylglycerol (DG) and 1, 4, 5 triphosphate

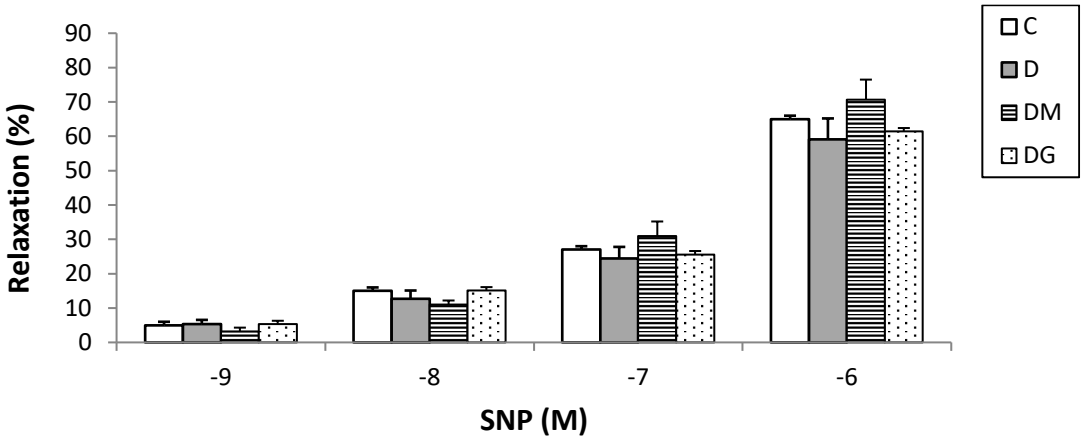

Figure 5. The relaxation responses of aortic rings to cumulative concentrations of sodium nitroprusside (10^{-9}- 10^{-6} M) control (C), diabetic (D), diabetic + metformin (DM) and diabetic + glibenclamide (DG) groups. Values are means ± SEM (n = 8).

inositol (IP_3), and subsequently DG activates the light chain of myosin by activation of protein kinase C (PKC), and IP_3 induces Ca^{2+} release from the sarcoplasmic reticulum through opening the IP_3 receptors [25].

The results of the previous studies have shown that the voltage-dependent Ca^{2+} channels (VDCCs) are involved in the KCl-induced contraction [27]. Our study showed that metformin and glibenclamide significantly reduced the contractile responses to concentrations of KCl (50 and 60 mM) compared to diabetic group. The effect of these drugs on PE and KCl-induced vasoconstriction of the diabetic rat aortic rings may be due to VDCCs.

In the endothelial cells of most vascular beds, Ach can stimulate formation and release of endothelial-derived relaxing factors including Nitric Oxide (NO), prostacyclin (PGI_2), endothelium-derived hyperpolarizing factor and in this pathway causes the relaxation of vascular smooth muscle in an endothelium-dependent manner [28].

NO is one of the most important factors released by the endothelium. Diabetes-induced endothelial dysfunction is characterized by the decrease of NO bioavailability in the vessel wall. NO, an important regulator of vascular tone, is produced by the activity of endothelial NO synthase (eNOS) [29] and impaired endothelium-dependent vasodilatation has been well indicated in the diabetes mellitus [5].

The Ach-induced relaxation response is endothelium-dependent and NO-mediated [30]. The results of the present study indicated that the relaxant response was decreased in aortas from STZ-induced diabetic rats and this decreased relaxation was profoundly recovered by metformin and glibenclamide. The impairment of Ach-induced relaxation would suggest a possible common pathophysiologic mechanism that there is an attenuation of NO release in the diabetic group, thus metformin

may improve NO pathway in endothelial cells in diabetic rats.

The impaired endothelium-dependent relaxation in STZ-induced diabetic rat might be due to enhanced fasting blood glucose level and reduced blood insulin level. It has been shown that hyperglycemia leads to tissue damage by several mechanisms, including the advanced glycation end product (AGE) formation, increased polyol pathway flux, apoptosis and reactive oxygen species (ROS) formation [31]. The initial trigger by which the high glucose concentrations change the vascular function is the imbalance between NO bioavailability and accumulation of ROS, leading to endothelial dysfunction. Indeed, hyperglycemia-induced production of superoxide anion (O_2^-) inactivates NO to form peroxynitrite ($ONOO^-$), a potent oxidant which easily penetrates across phospholipid membranes and induces substrate nitration [32].

Our results showed that metformin and glibenclamide treatment could have hypoglycemic effects in STZ-induced diabetic rats. Therefore, its beneficial effect on aortic tissue of diabetic rats may be in part due to its hypoglycemic effects. In our study, metformin decreased the fasting blood glucose level in diabetic rats. Similar data were obtained in the previous studies [7]. A possible important target for the effect of metformin is AMPK. When the activation of AMPK occurs, inhibition of glucose production by the liver occurs and improves the insulin sensitivity and glucose uptake by the muscle and induces fatty acid oxidation. AMPK is a major cellular regulator of lipid and glucose metabolism [21, 33].

Metformin may be effective in the prevention of diabetic complications, not only through lowering the plasma glucose, but also directly by inhibiting the advanced glycation end product (AGE) formation [18].

Moreover, administration of metformin led to decreased MDA concentration in the aorta and heart tissues as well as increased total thiol content [34]. Metformin has been suggested to have antioxidant effects [22] by reducing NAD (P)H oxidase and mitochondrial respiratory chain–induced activities in the aortic endothelial cells-stimulated by glucose, subsequently decreasing intracellular ROS [23]. Metformin improved aortic reactivity of the diabetic rats, which may be attributed to the improved glycation and antioxidant defense and diminished Rho kinase activity [35]. Metformin improves vascular function in insulin-resistant rats [36].

In this study, we also evaluated the vascular effect of SNP, a general NO donor and an endothelium-independent vasodilator. The relaxation responses to SNP were similar and there was insignificant differences between our experimental groups. The majority of previous studies support our findings that the response of the tissues to SNP is not impaired in the diabetics versus control [37, 38].

Glibenclamide used orally is available for the treatment of type 2 diabetes mellitus [39]. In our study, glibenclamide significantly decreased the fasting blood glucose levels in the diabetic rats. Glibenclamide may potentiate the insulin effects, either by increasing the insulin

secretion, increasing the release of bound insulin, enhancing transport of the blood glucose to peripheral tissues or inhibiting the degradation of insulin in the vascular endothelial cells [40]. The drug molecules bind to a specific receptor (sulphonylurea receptor) identified as adenosine triphosphate sensitive potassium (K_{ATP}) channel that is present on the pancreatic beta-cell membrane causing depolarization by reducing conductance of K_{ATP} channels [41], This results in the opening of voltage-gated calcium channel causing Ca^{2+} influx and degranulation with the release of pre-formed insulin [42]. Sulfonylureas selectively inhibited K_{ATP} channels in cardiac myocytes, vascular smooth muscle, skeletal muscle and central neurons. Glibenclamide-induced endothelium-dependent relaxation involves NO release and this effect may be related to its stimulatory effect on endothelial Ca^{2+} levels. Therefore, the glibenclamide-induced endothelium-independent relaxation may be associated with its inhibitory effect on Ca^{2+} influx through Ca^{2+} channels and on the protein kinase C-mediated contractile mechanism [13].

Glibenclamide may act through multiple sites in either endothelium or the underlying arterial smooth muscle and thus serve as a non-selective muscle relaxant at high concentration. Although there are increasing numbers of studies in this area of research, conflicting data still exists regarding the mechanism of action of glibenclamide as an inhibitor of contractile agonists [43]. Finally, there were no significant differences in the effects of metformin and glibenclamide on the improvement of blood glucose and aortic responses. A study on diabetic patients also showed that metformin and glibenclamide produced similar glycemic control. Glibenclamide therapy is accompanied by greater systolic blood pressure responses to norepinephrine and angiotensin II and higher plasma norepinephrine levels than those that occur in metformin therapy [44].

4. Conclusion

Our study indicated that chronic administration of metformin or glibenclamide has a significant hypoglycemic effect and improves aortic reactivity to vasoconstrictor and vasodilator agents in the STZ-induced diabetic rats. Also, no significant difference in the hypoglycemic effect and improved aortic reactivity was observed between metformin and glibenclamide. Based on the results of this study, the effect of metformin has a more vasodilatory effect than glibenclamide, but the difference was not significant.

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