EVALUATION OF THE ANTI-ISCHEMIC EFFECT OF THE PPARγ AGONIST, (ROSIGLITAZONE) IN PERMANENT FOCAL CEREBRAL ISCHEMIA IN DIABETIC RATS.

By

Shalaby A. and Boshra V.

From

Clinical Pharmacology Department

Faculty of Medicine - Mansoura University

ABSTRACT

Diabetes mellitus (DM) is a clear risk factor for stroke. Furthermore, diabetes has been shown to be a strong determinant for the presence of multiple lacunar infarcts in stroke patients. There has been a recent appreciation that peroxisome proliferator-activated receptors (PPARs) and their ligands may play an important role in the brain. An increasing number of studies have reported on the effects of PPAR agonists in animal models of neurological damage and disease, including the excitatory damage that occurs in stroke. The PPARγ agonist, rosiglitazone, a synthetic ligand of the thiazolidinedione class was currently used as an antidiabetic agent because of its insulin-sensitizing effect. So, the purpose of this study was to determine whether rosiglitazone may be neuroprotective in a model of permanent focal cerebral ischemia in diabetic rats. Methods: This study was carried on 96 albino rats. Rats were divided into 8 equal groups: Group (1): Control group, Group (2): Diabetic control group, Group (3): Non-treated ischemic group, Group (4): Diabetic and non-treated sham operated group, Group (5): Diabetic and non-treated permanent left middle cerebral artery occlusion group, Group (6): Treated ischemic group, Group (7): Diabetic and treated sham operated group, Group (8): Diabetic and treated permanent middle cerebral artery occlusion group. Treated groups

were received rosiglitazone at a dose of 3mg /kg /day IP for successive 7 days. Neurobehavioral evaluation was started on the next day after surgery and carried out for successive 6 days. On the 7th day of occlusion, fasting venous blood glucose level was measured, and then the rats were killed by decapitation. The forebrains of the rats were removed and randomly classified to two groups. One group was for measuring the infarction size using TTC stain, and the other was for determination of the levels of nitric oxide (NO) and malondialdehyde (MDA) in the forebrain tissue as oxidative stress markers. Results: Treatment with rosiglitazone caused significant improvement in the somatomotor function detected by significant improvement of the neurological score, significant attenuation of the infarction size. Also, it caused significant decrease in the blood glucose level, brain NO and MDA in comparison to the non-treated diabetic ischaemic group. Conclusion: The present study suggests that PPAR agonist may be useful in the glycemic control of the macrovascular complications of diabetes mellitus, including stroke.

Key words : diabetes mellitus, stroke, PPAR, rosiglitazone, neurological score, NO, MDA.

INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that function as ligand-activated transcriptional regulators of genes controlling lipid and glucose metabolism [1]. There has been a recent appreciation that PPARs and their ligands may play an important role in brain. Whereas a handful of early reports described expression patterns of PPAR mRNAs and proteins in brain [2]. An increasing number of studies have reported on the effects of PPAR agonists in animal models of neurological damage and disease, including the excitatory damage that occurs in stroke [3-5]. Research has implicated PPAR in macrophage biology, cell cycle regulation, cellular differentiation, and atherosclerosis [5&6]. Together, these additional "insulin-independent" properties and functions of PPAR agonists and activation have sparked a rapidly increasing interest in their potential therapeutic use. In vivo, PPAR agonists have been shown to modulate inflammato-

ry responses in the brain [6] and to reduce infarction size against transient focal ischemia [5,7]. Cerebral ischemia is frequently accompanied by inflammation, which can worsen neuronal injury. Activation of PPAR reduces inflammation and the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [8]. In addition, PPAR activators increase levels of Cu Zn-superoxide dismutase (SOD) in cultured endothelium, suggesting an additional mechanism by which it may exert protective effects within the brain [9].

Diabetes is a clear risk factor for stroke [10]. Diabetes mellitus (DM) was significant independent predictors of recurrent stroke in different studies of stroke [11, 12] .In the evaluation of 2-year stroke recurrence in the Stroke Data Bank, patients at the lowest risk had no history of diabetes [13]. Furthermore, diabetes has been shown to be a strong determinant for the presence of multiple lacunar infarcts in 2 different stroke cohorts [14, 15]. Glycemic control had shown to reduce the occurrence of microvascular complications (nephropathy, retinopathy, and peripheral neuropathy) in several clinical trials [16]. Data on the efficacy of glycemic control on macrovascular complications, including stroke, are more limited.

The purpose of this study was to determine whether a PPAR agonist may be neuroprotective in a model of permanent focal cererbral ischemia in diabetic rats. So that, we used the PPAR agonist rosiglitazone which is a synthetic ligand of the thiazolidinedione class and currently used as an antidiabetic agent because of its insulin-sensitizing effect. Rosiglitazone is soluble in water and can cross the blood brain barrier [17].

MATERIAL AND METHODS

Drug used :

Selective PPAR agonist: rosiglitazone, (Avanda 4 mg- GlaxoSmith Kline)

Animals used :

This study was carried on 96 male albino rats weighting 250 grams/ rat. Animals were having free access to food and water. They were exposed to the same housing conditions of heat and humidity.

Animal grouping :

Rats were divided into 8 equal groups (12 rats for each).

Group (1): Control group (1c.c saline).

Group (2): Diabetic control group (1c.c saline).

Group (3): Non-treated ischemic group (left MCA occlusion + 1c.c saline)

Group (4): Non-treated diabetic and sham operated group (anaesthesia+ labarotomy+1c.c saline)

Group (5): Non-treated diabetic and ischemic group (left MCA occlusion + 1c.c saline)

Group (6): Treated ischemic group (left MCA occlusion + rosiglitazone)

Group (7): Treated diabetic and sham operated group (anaesthesia+ labarotomy+ rosiglitazone)

Group (8): Treated diabetic and ischemic group: (left MCA occlusion + rosiglitazone).

Induction of diabetes mellitus :

Diabetes type II was induced by a single IP injection of streptozotocin (35 mg/kg, Sigma chemical Co.) dissolved in 0.1mmol/l sodium citrate buffer, pH 4.5. 72 hrs after streptozotocin injection, diabetes mellitus was confirmed by measuring venous blood glucose. Rats with fasting blood glucose level less than 300mg /dl were excluded from the experiment [18].

Surgical procedures :

Either diabetic or non-diabetic rats were exposed to left permanent middle cerebral artery (MCA) occlusion described by Tamura et al., [19]. Each animal was anesthetized by thiopental sodium at a dose of 30 mg /kg body weight intraperitoneally [20]. Briefly, the MCA was approached through a temporal incision, and the bone overlying the vessel was removed using a dental drill. The dura was opened, and the arachnoid membrane was gently removed. The vessel was occluded by bipolar coagulation, from a point proximal to the lenticulostriate branches to the rhinal fissure. The incision was closed, and the rats were returned to their home cage after full recovery from anaesthesia. The sham-operated rats were subjected to the same surgical procedure as focal cerebral ischae-

mia groups but without diathermic occlusion of the middle cerebral artery.

The animals received saline or medications within 5 minutes after surgery and then once per day for successive seven days intraperitoneally. The doses of drugs were dissolved in saline in order to obtain a dose required for each animal in 1c.c saline. The dose of rosiglitazone was 3mg /kg /day IP for successive 7 days [21].

Consequent steps after left MCA occlusion :

I- Neurobehavioral evaluation :

Neurobehavioral evaluation was started on the next day after surgery and carried out for successive 6 days. It consisted of the following six tests [23].

1- *spontaneous activity :* The animal was observed for 5 minutes in its normal cage. The rat's activity was assessed by its ability to approach all four walls of the cage. Scores indicate the following: Score 3: Rat moved around, explored environment, and approached at least three walls of the cage. Score 2: Slightly affected rat moved about the cage but did not approach all sides and hesitated to move, although it reached at least one upper rim of the cage. Score 1: Severely affected rat did not rise up at all and barely moved in the cage. Score 0: Rat did not move at all.

2- *Symmetry in the movement of four limbs :* The rat was held in the air by the tail to observe symmetry in the movement of the four limbs. Scores indicate the following: Score 3: All four limbs extended symmetrically. Score 2: Limbs on right side extended more or less slowly than those on left. Score 1: Limbs on right side showed minimal movement. Score 0: Limbs on right side did not move at all.

3- *Forepaw out stretching :* The rat was brought up to the edge of the table and to walk on forelimbs while being hold by the tail. Symmetry in the outstretching of both forelimbs was observed while the rat reached the table and the hindlimbs were kept in the air. Scores indicate the following: Score 3: Both forelimbs were outstretched, and the rat walked symmetrically on forepaws. Score 2: Right side

outstretched less than the left, and forepaw walking was impaired. Score 1: Right side moved minimally. Score 0: Right forelimb did not move.

4- *Climbing :* The rat was placed on the wall of a wire cage .Normally the rat uses all four limbs to climb up the wall. When the rat was removed from the wire cage by pulling it off by the tail, the strength of attachment was noted. Scores indicate the following: Score 3: Rat climbed easily and gripped tightly to the wire. Score 2: Right side was impaired while climbing or did not grip as hard as left side. Score 1: Rat failed to climb or tended to circle instead of climbing.

5- *Body propioception :* The rat was touched with a blunt stick on each side of the body, and the reaction to the stimulus was observed .Scores indicate the following: Score 3: Rat reacted by turning head and was equally anxious by the stimulus on both sides. Score 2: Rat reacted slowly to stimulus on right side. Score 1: Rat did not respond to stimulus placed on right side.

6- *Response to vibrissae touch :* A blunt stick was brushed against the vibrissae on each side ; the stick was moved toward the whiskers from the rear of animal to avoid entering the visual fields. Scores indicate the following: Score 3: Rat reacted by turning head or was equally startled by stimulus on both sides. Score 2: Rat reacted slowly to stimulus on right side. Score 1: Rat did not respond to stimulus on right side.

The score given to each rat at the completion of the evaluation is the summation of all six individual test scores. The minimum neurological score is 3 and the maximum is 18.

II-Blood glucose level : On the 7th day of occlusion venous blood samples were obtained from the tail veins of rats that were fasted over night to measure the fasting blood glucose level according to glucose oxidase method of Trinder [22]. Then the rats were killed by decapitation.

III. Evaluation of ischaemic area by 2,3,5 tripheny1 tetrazolium chloride (TTC) staining of the brain [24].

After decapitation, six brains of

each group were quickly removed and placed in ice-cold saline for 5 minutes. Both hemispheres were cut into 2-mm. coronal slices. Sections were incubated in TTC-containing saline solution (Sigma chemical Co.) for 20 minutes. Then, the slices were refrigerated in 10 % formalin over night. The infracted areas were outlined in white [25]. The longitudinal and transverse axes were measured in mm. between the farthest two points. The percentage of infracted size was measured by adobe photoshop-5 program.

IV- Assay of oxidative stress markers in the forebrain tissue :

Parasagital brain slices of both hemispheres of each forebrain of the other six rats were weighed and equally divided for the assay of nitric oxide (NO) and malondialdehyde (MDA).

A. Assay of nitric oxide metabolites (nitrate and nitrite) level in the forebrain tissue :

The parasagital slices were placed in a 5c.c plastic tube containing 1 ml krebs-Ringer's solution and preincubated in a water bath at 37° for 1 hour with continuous carbogen (5% O_2 / 95% CO_2) areation. Krebs-Ringer's solution had the following composition (mM): Nacl 129, Mg $S0_4$ 1.3, Na HCO_3 22.4, KH_3PO_3 1.2, KCl 4.2, glucose 10.0 and $CaCl_2$ 1.5. Following the completion of incubation, the slices were transferred into another 5c.c plastic tube containing 0.5 ml of ice-cold tris buffer (50mM, pH 7.4). The slices were homogenized, sonified and the homogenates were centrifuged at 2500 xg (Heraeus Pepatech Biofuge 17RS) for 30 min at 4°c. Resultant supernatants were kept at- 40°c until assay within 6 weeks [26]. NO was quantified in the brain tissue via the nitrite method based on the Griess reaction [27]. The nitrate present in the sample is reduced to nitrite by reduced nicotinamide adenine dinucleotide phosphate in the presence of the nitrate reductase. The nitrite formed reacts with sulfanilamide and N- (1-naphthyl)-ethylenediamine dihydrochloride to yield a red violet diazo dye, which is measured on the basis of its absorbence in the range of 546 nm using UV. visible spectrophotometer. Known concentrations of sodium nitrite (1μmoL/ μL) were included as standards.

B. Assay of lipid peroxidation products (malondialdehyde) level in the forebrain tissue :

The parasagital brain slices were transferred to ice-cold tris buffer (100 mM, pH 7.4). The tissue was then homogenized, sonified and centrifuged at 1200 xg (Heraeus Pepatech Biofuge 17RS) for 4 min. at room temperature. Resultant supernatant was stored at-20°c until assay within two weeks [28]. At the time of assay, the supernatant was deproteinized with trichloroacetic acid (TCA) and subjected to incubation with thiobarbituric acid (TBA) 38 mM at 95°c for 15 min. to determine the extent of lipid peroxidation to thiobarbiturate-reducing substances (TBARS) according to Draper & Hadley, [29]. Quantification was achieved spectophotometrically by measuring the optical density at 532 nm with a microplate reader, and the amounts of TBARS were calculated using a standard curve prepared with malondialdehyde in different concentrations. Protein levels were determined according to Lowery et al., [30] by standard microassay methods (DC protein Assay; Bio Rad. Munich, Germeny). Protein forms a colored complex with cupric ions in an alkaline medium. Colorimetric assay was achieved by measuring the optical density at 546 nm. MDH production was expressed as nanomoles per mg protein.

Statistical analysis :

The statistical analysis was performed with the aid of personal computer Pentium II 300 using Statistical Package for Social Science (SPSS) program version 6.01. Because our data are of multiple groups (more than two) and of parametric quality, one-way ANOVA analysis of data was done followed by post hoc test of Tukey HSD between pairs of results ($P < 0.05$ was considered to be significant) (31). Data were presented in the form of mean, and standard error (SEM) of mean.

RESULTS

I- Effect of rosiglitazone on the neurological score in permanent focal cerebral ischemia (MCA occlusion): [table 1]

The mean neurological scores of control and sham groups (either diabetic or non-diabetic) were 18 starting from the second postoperative day and maintained till the end of duration

of follow up (7days). The mean neurological scores were significantly decreased in the focal cerebral ischaemic group as compared to sham groups starting from the second postoperative day until time of sacrifaction. There was a significant increase in the mean neurological scores starting on the 3rd day of occlusion and manifest until time of sacrifaction in comparison to the neurological score on the 2nd postoperative day in all focal cerebral ischaemic groups (either treated or non- treated). Treatment with rosiglitazone caused significant increase in the mean neurological score starting on the 3rd postoperative day and till the time of sacrifaction in the ischaemic groups (diabetic or non diabetic; gps 6,8) as compared to the non-treated ischaemic groups (gps 3,5).

II- Effect of rosiglitazone on the mean levels of fasting venous blood glucose in permanent focal cerebral ischemia (MCA occlusion): [table 2]

There was significant increase in the mean level of fasting venous blood glucose in the non- treated diabetic groups (gps 2,4,5) in comparison to that of control group (gp 1). There were non significant differences in the mean levels of fasting blood glucose between the non-treated control, sham or ischaemic diabetic groups.

There were significant decrease in the mean levels of fasting blood glucose in the rosiglitazone treated diabetic sham or ischemic groups (gps 7,8) in comparison to that of the non-treated diabetic groups (gps 4,5). This decreased level of fasting blood glucose was not significantly differed than that of the control group.

III- Effect of rosiglitazone on the infarction size [stained by 2,3,5 tripheny1 tetrazolium chloride (TTC)] of brain sections in permanent focal cerebral ischemia (MCA occlusion) [Table3, fig.1]

The infracted size was significantly decreased in the diabetic or non diabetic MCA occluded groups treated with rosiglitazone (gps 6,8) versus that of non-treated MCA occluded groups (gps 3,5).

1V- Effect of rosiglitazone on the mean levels of oxidative stress markers (nitric oxide or malondialdehyde)

in the forebrain tissue in permanent focal cerebral ischemia (MCA occlusion): [table 2]

There were significant increase in the mean levels of nitric oxide (NO) or malondialdehyde (MDA) in the forebrain tissue of the non-treated diabetic groups (gps 2,4) in comparison to that of control group (gp1). Also, these oxidative stress markers were significantly increased in the non-treated ischemic group (gp 3) versus the control group (gp1). There were significant increase in the mean levels of NO or MDA in the forebrain tissue of the non-treated ischaemic diabetic group (gp 5) versus that of sham diabetic group (gp 4). The co-existence of diabetes and focal cerebral ischemia (gp 5) led to significant increase of the oxidative stress markers in comparison to that of either diabetic (gp2) or focal ischemic group (gp3) alone.

There were significant decrease in the mean levels of NO or MDA in the forebrain tissue in the rosiglitazone treated non diabetic ischemic, diabetic sham or diabetic ischemic groups (gp 6,7,8) in comparison to that of the non-treated groups (gps 3,4,5). These decreased levels of NO and MDA were not significantly differed than that of the control group.

Table (1) : Effect of rosiglitazone (3mg/Kg IP for successive 7 days) on neurological score after 7 days of permanent focal cerebral ischemia (MCA occlusion) in diabetic rats
(Mean±SEM, , n=12)

	Non-ischemic groups (gps 1,2,4,7)	Non-treated ischemic group (gp3)	Non-treated diabetic ischemic group (gp 5)	Rosiglitazone treated ischemic group (gp6)	Rosiglitazone treated diabetic ischemic group (gp8)
2nd day	18± 0	4.0± 0.2 a	3.9± 0.3 a	4.3± 0.1 a	4.5± 0.3 a
3rd day	18± 0	5.6± 0.3 a*	5.5± 0.2 a*	8.6± 0.7 abc*	8.8± 0.6 abc*
4th day	18± 0	5.6± 0.3 a*	5.5± 0.2 a*	11.9± 0.8 abc*#	12.1± 0.4 abc*#
5th -7th day	18± 0	5.6± 0.3 a*	5.5± 0.2 a*	12.8± 0.9 abc*#	13.2± 0.3 abc*#

P<0.05

a versus non-ischemicgroup

b versus non-treated ischemic *group*

c versus non-treated diabetic ischemic *group*

** versus the 2nd day* *# versus the 3rd day*

Table (2): Effect of rosiglitazone (3 mg/Kg IP for successive 7 days) on blood glucose (mg/dl), brain nitric oxide (nmol/gm tissue) & malondialdehyde levels (nmol/gm protein) after 7 days of permanent focal cerebral ischemia (MCA occlusion) in diabetic rats (Mean±SEM)

Groups	Blood glucose (mg/dl) n=12	Nitric oxide (nmol/gm tissue) n=6	Malondialdehyde (nmol/gm protein) n=6
Control group (gp 1)	124.2± 3.1	92.9±4.8	9.7±0.3
Diabetic control group (gp 2)	418.8±25.3 [a]	215.8±3.5 [a]	17.9±0.7 [a]
Non-treated ischemic group (gp 3)	126.5 ±7.2 [b]	290.6 ±7.2 [a]	20.1± 1.1 [a]
Non-treated diabetic sham group (gp 4)	450.4±26.1 [ac]	210.97±2.9 [a]	16.2±0.5 [a]
Non-treated diabetic ischemic group (gp 5)	430.8±19.1 [ac]	470.0±7.9 [abcd]	31.4±2.3 [abcd]
Rosiglitazone -treated ischemic group (gp 6)	127.4 ± 5.3 [bde]	110.8 ± 5.2 [bcde]	10.3 ± 0.5 [bcde]
Rosiglitazone -treated diabetic sham group (gp 7)	142.8 ± 4.5 [bde]	102.5 ± 3.6 [bcde]	11.5± 0.8 [bcde]
Rosiglitazone -treated diabetic ischemic group (gp 8)	143.9±9.7 [bde]	100.8±4.3 [bcde]	12.1±0.4 [bcde]

P<0.05

a versus control group *b versus control diabetic group*

c versus non-treated ischemic group *d versus non-treated diabetic sham group*

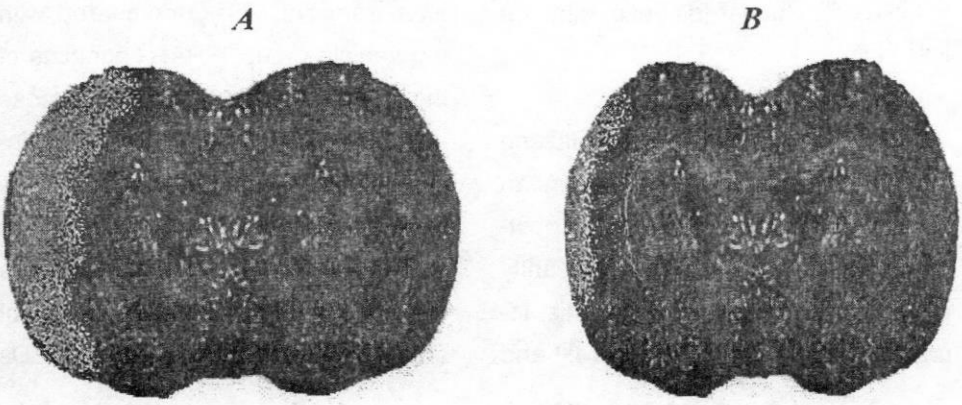
e versus non-treated diabetic ischemic group

Table (3) : Effect of rosiglitazone (3 mg/Kg IP for successive 7 days) on the infracted size of brain sections stained by 2,3,5 triphenyl tetrazolium chloride (TTC) stain after 7 days of permanent focal cerebral ischemia (MCA occlusion) in diabetic rats (Mean±SEM, , n=6)

	Percentage of total infracted area
Non-treated ischemic group (gp 3)	17.2 ± 0.8
Non-treated diabetic ischemic group (gp 5)	16.7 ± 0.9
Rosiglitazone -treated ischemic group (gp 6)	6.7 ± 0.5 *+
Rosiglitazone -treated diabetic ischemic group (gp 8)	6.3 ± 0.2*+

P<0.05 * versus non-treated ischemic group
+ versus non-treated diabetic ischemic group

Fig [1] : TTC stain of non-treated(A), rosiglitazone treated (B) ischemic forebrain section of rats.



DISCUSSION

This study demonstrates that treatment with rosiglitazone at a single IP daily dose of 3 mg/kg IP after permanent occlusion of the left MCA for successive 7 days caused significant decrease in the fasting blood glucose level as well as improvement of the markers of cerebral ischemia compared with the non- treated ischaemic diabetic group.

Treatment with rosiglitazone caused significant decrease in the mean level of fasting blood glucose level in the diabetic groups in comparison to that of the non-treated diabetic groups. The improvement of glucose tolerance by the thiazolidinediones, the synthetic agonists of PPAR-γ, was explained by enhancing insulin sensitivity and restoring the function of β-cells in either diabetic subjects [32,33] or Zucker diabetic fatty rat [34].

Treatment with rosiglitazone caused improvement of the mean neurological score since the 2nd postoperative day, which was significantly increased by the 3rd day. This increase was maximum by the 4th and 5th postoperative days, a level that was maintained till the 7th day. These levels of scores were significantly higher than those obtained in the non-treated ischaemic group, but still significantly less than those of non-ischemic groups. Pantoni et al., [35] stated that the neurological score designed by Garcia et al., [23] represents a useful tool for the assessment of functional outcome in animals with ischaemic brain damage. It does not require training of the rats or purchase of expensive equipment. In addition, results could be expressed in a numerical score compatible with analysis by statistical means.

The neurobehavioral result was agreed with the result of Shimazu et al., [7] who noted that, the pioglitazone exhibited significant improvement in neurological score one day after transient MCAO compared with the vehicle group. PPAR agonists of the TZD family can modify glucose metabolism in brain glial cells by increasing astrocyte glucose uptake from the media and lactate production and release from the cells [36]. Since the astrocytes are the main source of energy for neuronal activity [37], TZDs

can be beneficial in the neurological conditions e.g stroke [3] where glucose availability is reduced through modification of astrocyte metabolism and mitochondrial function. Also, Sundararajan et al., [8] reported that administration of troglitazone or pioglitazone 24 h before and at the time of cerebral infarction dramatically reduced infarction volume and improved neurological function following middle cerebral artery occlusion in rats. They argued that the beneficial effects of these drugs were likely due to reduced expression of the inflammatory mediators such as interleukin-1beta, cyclooxygenase-2 and inducible nitric oxide synthase which were known to exacerbate ischemic injury following stroke.

Treatment with rosiglitazone also caused significant decrease in the infracted size in the diabetic or non-diabetic ischemic groups versus the non-treated ischemic groups. This result was in accordance to Sundararajan et al.,[38] who administered troglitazone, a PPAR- agonist, to non diabetic rats twenty-four hours before and again at the time of MCA occlusion in doses of 35, 70 or 100 mg/kg . They found that there was significant reduction in infarct volume in rats treated with 35 mg/kg and 70 mg/kg troglitazone. Troglitazone, reduces infarct size following cerebral ischemia was likely due to the drug anti-inflammatory properties. As the immunoreactivity against the proinflammatory cytokines Il-1β and TNF α was reduced in the peri-infarct region of troglitazone treated rats. Furthermore, immunoreactivity against other markers of inflammation, intracellular adhesion molecule, major histocompatibility complex antigen I and cyclooxygenase-2 was also reduced in troglitazone treated animals compared with vehicle treated animals. Also, Shimazu et al., [7] noted that treatment with pioglitazone reduced the total infarct volume after transient MCAO compared with vehicle-treated rats. They hypothesized that the PPAR- agonist pioglitazone induces CuZn-superoxide dismutase, and this increase in the antioxidant CuZn-SOD is responsible for the decrease in infarct size in cerebral ischemia. Moreover, Pereira et al., [39] tested the effect of TZD-unrelated PPAR gamma agonist L-796,449 after permanent middle cerebral artery occlu-

sion (MCAO) in the rat brain. They showed that L-796,449 decreases MCAO-induced infarct size and improves neurologic scores via inhibition of MCAO-induced brain iNOS expression and reactive oxygen species production. Zhao et al., [40] also found that intracerebroventricular injection of pioglitazone promotes neuroprotection by attenuation of neuronal cyclooxygenase-2 overexpression after focal cerebral ischemia in rats. Furthermore, Park et al., [41] noted that both pioglitazone and rosiglitazone significantly decreased the lesion size after spinal cord injury (SCI) in adult rats. The treatment was effective only when the first injection was given by 2 h after SCI. Either pioglitazone or rosiglitazone significantly decreased the induction of inflammatory genes [interleukin (IL)-6, IL-1 , monocyte chemoattractant protein-1, intracellular adhesion molecule-1, and early growth response-1] compared with the vehicle group. They also significantly enhanced the post-SCI induction of neuroprotective heat shock proteins and antioxidant enzymes. Pretreatment with a PPAR antagonist, 2-chloro - 5-nitro - N- phenylbenzamide (GW9662), prevented the neuroprotection induced by pioglitazone or rosiglitazone.

In our study, the diabetic ischemic group showed more significant increase in the generation of MDA (a net product of oxidative stress) and nitric oxide in the brain tissue in comparison to that of either ischemic or diabetic group only. These results were according to Mastrocola et al., [42] who found that alongside enhanced reactive oxygen species (ROS) levels, both nitric oxide (NO) levels and mitochondrial nitric oxide synthase expression were found to be increased in brain mitochondria, in STZ diabetic rats. And so, oxidative and nitrosative stress may cause direct injury of neuronal cells by damaging brain mitochondria.

Treatment with rosiglitazone caused significant decrease in the mean level of NO or MDA in the forebrain tissue in the treated ischemic diabetic or non-diabetic groups in comparison to that of the non-treated groups. This result was in agree with that of Collino et al., [43] who investigated the effects of two PPAR-γ agonists, rosiglitazone and pioglitazone,

on oxidative stress and inflammatory response induced by ischemia/ reperfusion in the rat hippocampus after occlusion of common carotid artery for 30 min followed by 1 h reperfusion. The ischemia resulted in a significant increase in the generation of reactive oxygen species, nitric oxide and the end products of lipid peroxidation as well as markedly reduced endogenous antioxidant glutathione levels and up-regulated superoxide dismutase activity. Pretreatment with either rosiglitazone or pioglitazone significantly reduced oxidative stress and nitric oxide. Also, Heneka et al., [44] demonstrated that 7 day oral treatment of PPAR agonist pioglitazone resulted in a reduction in the number of activated microglia and reactive astrocytes in the hippocampus and cortex of mice. Drug treatment reduced the expression of the proinflammatory enzyme inducible nitric oxide synthase (iNOS) in parallel to the suppression of inflammatory markers of neurodegenerative diseases. It is becoming increasingly clear that oxidative stress and excessive inflammatory response are implicated in the pathogenesis of ischemia and reperfusion injury to many organs, including the brain [45]. The brain is very susceptible to the damage caused by oxidative stress, due to the high rate of oxidative metabolic activity, high polyunsaturated fatty acid contents, relatively low antioxidant capacity and inadequate neuronal cell repair activity [46]. Overproduction of reactive oxygen species results in oxidative damage, including lipid peroxidation, protein oxidation and DNA damage, which can lead to cell death [47]. Furthermore, reactive oxygen species can activate diverse downstream signalling pathways, such as mitogen-activated protein kinases (MAPKs) or the transcription factor nuclear factor-κB (NF-κB), thus regulating expression of genes encoding a variety of proinflammatory proteins. Overexpression of cyclooxygenase-2 (COX-2) and of inducible nitric oxide synthase (iNOS) have recently emerged as important determinants of post-ischemic inflammation, which contributes to the progression of brain damage [48].

Finally, we noted that the improvement of the neurological score was the same in both non-diabetic and diabetic ischemic groups (gps 6&8 re-

spectively) indicating that the anti-ischemic effect of rosiglitazone did not depend on its anti-diabetic effect. Also, treatment with rosiglitazone caused significant decrease in the mean level of both NO or MDA in the forebrain tissue and blood glucose level in the diabetic ischemic or non-ischemic groups (gp 8,7) in comparison to that of the non-treated group. While the decrease in the NO &MDA was not associated with decrease in blood glucose level in the non-diabetic ischemic group (gp6) indicating that the anti-oxidative effect and the anti-ischemic effect of rosiglitazone did not depend on its anti-diabetic effect.

The pleiotropic effects of PPARγ explain why its activation has been tested as a neuroprotective agent in cerebral ischemia [49]. The neuroprotection observed after treatment with PPAR agonists is related to several mechanisms including both oxidative stress modulation and anti-inflammatory effect. PPARγ agonists are able to prevent neuronal death resulting from NMDA (N-methyl-D-aspartate) excitotoxicity induced in brain in vitro or in vivo [40]. PPARγ is able to inhibit macrophage and microglial activation that contribute to many degenerative, ischaemic or inflammatory processes leading to neuronal death [50]. PPARγ agonists inhibit both post-glutamate-and low-potassium-induced neurotoxicity in cerebellar granule neurons [3]. PPARs are also able to inhibit the entry of inflammatory cells into the CNS (central nervous system) from the periphery by inhibition of chemokines, adhesion molecules and metalloproteinases [50]. PPARγ agonist-induced neuroprotective effect is also associated with a decrease in cerebral oxidative stress depending on the increase in activity of numerous antioxidant enzymes, in particular Cu/Zn superoxide dismutase and glutathione peroxidase [7]. This modulation of antioxidant enzymes is responsible for a decrease in ischemia-induced reactive oxygen species production and lipid peroxidation. This effect on oxidative stress could be related to a direct effect on antioxidant enzymes expression, because PPREs (PPAR-response elements) have been found in the gene of Cu/Zn superoxide dismutase [43]. The neuroprotective effects of PPAR agonists are also relat-

ed to inhibition of ischemia-induced inflammatory markers (interleukin-1β, COX-2 and inducible nitric oxide synthase) [43]. There is a link between PPAR-induced modulation of oxidative stress and inflammation, since prevention of COX-2 induction results from oxidative stress inhibition [40]. The cellular target of these anti-inflammatory effects is probably microglial cells, since PPARγ agonists, such pioglitazone, are able to decrease microglial activation when administered intracerebrally [51]. The key target of this anti-inflammatory effect is NF-kB, which plays a crucial role in neuronal death [52]. PPARg activation is responsible for inhibition of the NF-kB p65 monomer as well as induction of IkBa (inhibitory kB) [39]. The role of suppression of activation of p38 mitogen-activated protein kinase has also been demonstrated recently [43].

Beyond this direct effect on ischemia-induced deleterious pathways explaining neuroprotection, the challenge will be to demonstrate that a part of the neurological improvement induced by PPAR activators could be the result of neurorepair, since PPARγs are also involved in the regulation of neural stem cell proliferation and differentiation [53].

In conclusion, we showed that the PPAR-γ agonists rosiglitazone exert neuroprotective effects against cerebral ischemic injury by reducing oxidative stress and inflammatory response. This anti-ischemic effect does not depend on its anti-diabetic effect. So, this protective effects of the PPAR-γ agonists rosiglitazone might suggest a potential role of PPAR-γ agonists in modulating the events occurring late after permanent ischemic attacks that occur as a vascular complication of diabetes mellitus.

REFERENCES

1.**Vamecq, J. and Latruffe, N.(1999)** : Medical significance of peroxisome proliferator-activated receptors. Lancet.; 354: 141-148.

2.**Cullingford, T.E., Bhakoo, K., Peuchen, S., Dolphin, C.T., Patel, R.& Clark, J.B. (1998)** : Distribution of mRNAs encoding the per-

oxisome proliferator-activated receptor alpha, beta, and gamma and the retinoid X receptor alpha, beta, and gamma in rat central nervous system. J. Neurochem.; 70: 1366-1375.

3.**Uryu, S., Harada, J., Hisamoto, M. & Oda, T. (2002) :** Troglitazone inhibits both post-glutamate neurotoxicity and low-potassium-induced apoptosis in cerebellar granule neurons. Brain Res.; 924(2):229-36.

4.**Wanderi, E., Gamboa, J.L., Victor, N.A., Landreth, G., Lust, W.D. & Sundararajan, S. (2002) :** Expression of the transcription factor, peroxisome proliferator-activated receptor-gamma, is increased in ischemic rat brain. Abstr. Soc Neurosci.; 694: 13.

5.**Gamboa, J.L., Victor, N.A., Landreth,G.& Sundararajan, S. (2003) :** Pioglitazone, a PPAR gamma agonist, reduces infarct size and improves neurologic function in transient focal ischemia. Soc Neurosci.;102: 17-22.

6.**Heneka, M.T., Klockgether, T.& Feinstein, D.L. (2000) :** Peroxisome proliferator-activated receptor-gamma ligands reduce neuronal inducible nitric oxide synthase expression and cell death in vivo. J Neurosci.; 20: 6862-6867.

7.**Shimazu, T., Inoue,I., Araki, N., Asano, Y., Sawada, M., Furuya, D., Nagoya, H. & Greenberg, J. (2005) :** A peroxisome proliferator-activated receptor-gamma agonist reduces infarct size in transient but not in permanent ischemia. Stroke; 36:353-359.

8.**Sundararajan, S., Gamboa, J.L., Victor, N.A., Wanderi, E.W., Lust, W.D. & Landreth, G.E. (2005) :** Peroxisome proliferator-activated receptor-gamma ligands re-

duce inflammation and infarction size in transient focal ischemia. Neuroscience; 130: 685-696.

9.**Inoue, I., Goto, S., Matsunaga, T., Nakajima, T., Awata,T., Hokari, S., Komoda, T.& Katayama, S. (2001) :** The ligands/activators for peroxisome proliferator-activated receptor alpha (PPARalpha) and PPARgamma increase Cu2+,Zn2+-superoxide dismutase and decrease p22phox message expressions in primary endothelial cells. Metabolism; 50: 3-11.

10.**Burchfiel, C.M., Curb, J.D., Rodriguez, B.L., Abbott, R.D., Chiu, D.& Yano, K. (1994) :** Glucose intolerance and 22-year stroke incidence: the Honolulu Heart Program. Stroke; 25: 951-957.

11.**Petty, G.W., Brown, R.D., Whisnant, J.P., Sicks, J.D., O'Fallon, W.M. & Wiebers, D.O. (1998) :** Survival and recurrence after first cerebral infarction: a population-based study in Rochester, Minnesota, 1975 through 1989. Neurology; 50: 208-216.

12.**Hillen, T., Coshall, C., Tilling, K., Rudd, A.G., McGovern, R.& Wolfe, C.D. (2003) :** Cause of stroke recurrence is multifactorial: patterns, risk factors, and outcomes of stroke recurrence in the South London Stroke Register. Stroke; 34: 1457-1463.

13.**Hier, D.B., Foulkes ,M.A., Swiontoniowski, M., Sacco, R.L., Gorelick, P.B., Mohr, J.P., Price, T.R. & Wolf, P.A. (1991) :** Stroke recurrence within 2 years after ischemic infarction. Stroke; 22: 155-161.

14.**Mast, H., Thompson, J.L., Lee, S.H., Mohr, J.P.& Sacco, R.L. (1995) :** Hypertension and diabetes mellitus as determinants of multiple lacu-

nar infarcts. Stroke; 26: 30-33.

15.**Arauz, A., Murillo, L., Cantu, C., Barinagarrementeria, F.& Higuera, J. (2003) :** Prospective study of single and multiple lacunar infarcts using magnetic resonance imaging: risk factors, recurrence, and outcome in 175 consecutive cases. Stroke.; 34: 2453-2458.

16.**Ohkubo, Y., Kishikawa,H., Araki, E., Miyata, T., Isami, S., Motoyoshi, S., Kojima, Y., Furuyoshi, N. & Shichiri, M.(1995) :** Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. Diabetes Res. Clin. Pract.; 28: 103-117.

17.**Cox, P.J., Ryan, D.A., Hollis, F.J., et al. (2000) :** Absorption, disposition, and metabolism of rosiglitazone, a potent thiazolidinedione insulin sensitizer, in humans. Drug Metab Dispos.; 28: 772-780.

18.**Ho, R.S., Aranda, C.G., Tillery, S.L. et al., (1988) :** In-vivo and in-vitro glucose metabolism on a low dose streptozocin rat model on noninsulin- dependent diabetes. In: Shafrir, E., Renold, A.E., eds. Lessons from Animal Diabetes. Libbey; 202 .

19.**Tamura, A., Graham, D.I., McCulloch, J. & Teasdale, G.M. (1981) :** Focal cerebral ischemia in the rat: Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J.Cereb.Blood Flow Metab.;1: 53-59.

20.**Wixson, S.K. and White,W.J. (1987) :** A comparison of pentobarbital, Fentany1-Droperdiol, Ketamin-xylazine and ketamine-

diazepam anesthesia in adult male rats. Lab. Animal. Sci.; 37:726-730.

21.**Tian-Li, Y., Weike, B., Juan-Li, G., Jianqi C., Ling, T., Xin-Liang, M., Eliot, H. & Beat, M. (2005) :** Rosiglitazone treatment in Zucker diabetic fatty rats is associated with ameliorated cardiac insulin resistance and protection from ischemia/reperfusion-induced myocardial injury. Diabetes 54:554-562.

22.**Trinde, P. (1969) :** Enzymatic determination of glucose.Ann. Clin.biochem;6:24-29.

23.**Garcia, J.H, Wanger, S., Liu, K. & Hu, X. (1995) :** Neurological deficit and extent of neurological necrosis attributable to middle cerebral artery occlusion in rats. Stroke; 26: 627-635.

24.**Gurosy-Ozdemir, Y., Bolay, H., Saribas, O. & Dalkara, T. (2000) :** Role of endothelial NO generation and peroxynitrite formation in reperfusion injury after focal cerebral ischemia. Stroke; 31:1974-1981.

25.**Goldlust, E.J., Paczynski, R.P., He, Y.Y., Hsu, C.Y. & Goloberg, M.P. (1996) :** Automated measurement of infarct size with scanned image of TTC stained rat brains. Stroke; 27:1657-1662.

26.**Green, L,C., Wanger, D.A., Skipper, J.P. et al., (1982) :** Analysis of nitrate, nitrite and [15N] nitrite in biological fluids. Anal. Biochem.; 126:131-138.

27.**Ma, T. and Zhu, X. (1997) :** IL-6 increases the levels of cGMP and nitrite in rat hippocampal slices. Eur. J. pharmacol.; 321:343-347.

28.**Vedder, H., Anthes, N., Stumm, G., Wurz, C. & Kreig J. (1999) :** Estrogen hormone reduces lipid peroxidation in cells and tissues of the cen-

tral nervous system. J. Neurochem.; 72(6): 2531-2538.

29.**Draper, W. and Hadley, M. (1990) :** Indirect determination of oxygen free radicals. Method. Enzymol.; 186 : 421-431.

30.**Lowery, O.H, Rosebrough, N.J. & Randall, R.J. (1951) :** Protein measurement with the folinphenol reagent. J. Biol. Chem.; 193:265-275.

31.**Armitage, P. and Berry, G. (1994) :** Comparison of several groups. In: Statistical methods in medical research, 3rd ed. London, Blackwell Scientific Publication, Ch.7; P:189-225.

32.**Nolan, J.J., Ludvik, B., Beerdsen, P., Joyce, M. & Olefsky, J. (1994) :** Improvement in glucose tolerance and insulin resistance in obese subjects treated with troglitazone. N Engl J Med.; 331:1188-1193.

33.**Cavaghan, M.K., Ehrmann, D.A., Byrne, M.M.& Polonsky, K.S. (1997) :** Treatment with the oral antidiabetic agent troglitazone improves beta cell responses to glucose in subjects with impaired glucose tolerance. J. Clin. Invest.;100 :530 - 537.

34.**Sreenan, S., Sturis, J., Pugh, W., Burant, C.F. & Polonsky, K.S. (1996) :** Prevention of hyperglycemia in the Zucker diabetic fatty rat by treatment with metformin or troglitazone. Am JPhysiol; 271 : E742 -E747.

35.**Pantoni, L., Bartolini, L. Pracucci, G. & Inzitari, D. (1998) :** Interrater agreement in a simple neurological score in rats. Stroke; 29: 871-872.

36.**Russo, C.D., Gavrilyuk, V., Weinberg, G., Almeida, A., Bolanos, J.B., Palmer, J., Pelligrino, D., Galea, E., & Feinstein, D.L. (2003) :** Peroxisome proliferator-activated receptor thiazo-

lidinedione agonists increase glucose metabolism in astrocytes J.Biol. Chem.; 278: 5828 - 5836.

37.Pellerin, L. and Magistretti,P.J. (1994) : Glutamate Uptake into Astrocytes Stimulates Aerobic Glycolysis: A Mechanism Coupling Neuronal Activity to Glucose Utilization. Proc. Natl. Acad. Sci. U. S. A.; 91: 10625-10629.

38.Sundararajan, S., Lust,W.D. & Landreth, G.E. (2000) : A PPAR- agonist reduces proinflammatory cytokine immunoreactivity and infarct size following transient focal ischemia in rats. Stroke: 32:354.

39.Pereira, M.P., Hurtado, O., Cardenas, A., Alonso-Escolano, D., Bosca, L., Vivancos, J., Nombela, F., Leza, J.C., Lorenzo, P., Lizasoain, I. & Moro MA. (2005) : The nonthiazolidinedione PPAR gamma agonist L-796,449 is neuroprotective in experimental stroke. J. Neuropath. Exp. Neurol.; 64(9):797-805.

40.Zhao, Y., Patzer, A., Herdegen, T., Gohlke, P. & Culman, J. (2006) : Activation of cerebral peroxisome proliferator-activated receptors gamma promotes neuroprotection by attenuation of neuronal cyclooxygenase-2 overexpression after focal cerebral ischemia in rat. The FASEB Journal; 20:1162-1175.

41.Park, S-W, Yi, J-H, Miranpuri, G., Satriotomo, I., Bowen, K., Resnick, D.K & Vemuganti, R. (2007) : Thiazolidinedione Class of Peroxisome Proliferator-Activated Receptor - Agonists Prevents Neuronal Damage, Motor Dysfunction, Myelin Loss, Neuropathic Pain, and Inflammation after Spinal Cord Injury in Adult Rats. JPET; 320:1002-1012.

42.Mastrocola, R., Restivo, F., Ver-

cellinatto, I., Danni, O., Brignardello, E., Aragno, M. & Boccuzzi, G. (2005) : Oxidative and nitrosative stress in brain mitochondria of diabetic rats. Journal of Endocrinology; 187: 37-44.

43.Collino, M., Aragno, M., Mastrocola, R., Gallicchio, M., Rosa, A.C., Dianzani. C., Danni, O.,Thiemermann, C.& Fantozzi, R. (2006) : Modulation of the oxidative stress and inflammatory response by PPAR-γ agonists in the hippocampus of rats exposed to cerebral ischemia/reperfusion. Eur. J. Pharmacol.; 430, 70-80.

44.Heneka, M. T., Sastr, M., Dumitrescu-Ozimek, L., Hanke, A., Dewachter, I., Kuiperi, C., O'Banion, K., Klockgether, T., VLeuven, F. & Landreth, GE. (2005) : Acute treatment with the PPARγ agonist pioglitazone and ibuprofen reduces glial inflammation and Aβ1-42 levels in APPV717I transgenic mice. Brain;128 (6):1442-1453.

45.Schaller, B., Graf, R., (2004) : Cerebral ischemia and reperfusion: the pathophysiologic concept as a basis for clinical therapy. J. Cereb. Blood Flow Metab.; 24: 351-371.

46.Traystman, R.J., Kirsch, J.R. & Koehler, R.C. (1991) : Oxygen radical mechanisms of brain injury following ischemia and reperfusion. J. Appl. Physiol.; 71:1185-1195.

47.Floyd, R.A. (1999) : Antioxidants, oxidative stress, and degenerative neurological disorders. Proc. Soc. Exp. Biol. Med.; 222: 236-245.

48.Candelario-Jalil, E., Gonzalez-Falcon, A., Garcia-Cabrera, M., Alvarez, D., Al-Dalain, S., Martinez, G., Leon, O.S. & Springer, J.E. (2003) : Assessment of the relative contribution of

COX-1 and COX-2 isoforms to ischemia-induced oxidative damage and neurodegeneration following transient global cerebral ischemia. J. Neurochem. 86, 545-555.

49.**Bordet R., Ouk T., Petrault O., Gele P., Gautier S., Laprais M., Deplanque D., Duriez P., Staels B., Fruchart J.C and Bastide M. (2006) :** PPAR: A new pharmacological target for neuroprotection in stroke and neurodegenerative diseases. Biochem. Soc. Trans. : 34:1341-1346.

50.**Kielian, T. and Drew, P.D. (2003) :** Effects of peroxisome proliferator-activated receptor-gamma agonists on central nervous system inflammation. J.Neurosci. Res.; 71: 315-325.

51.**Bernardo, A., Gasparini, L., Ongini, E. and Minghetti, L. (2006) :** Dynamic regulation of microglial functions by the non-steroidal anti-inflammatory drug NCX 2216: implications for chronic treatments of neurodegenerative diseases. Neurobiol. Dis.; 22: 25-32.

52.**Hu, X., Nesic-Taylor, O., Qiu, J., Rea, H.C., Fabian, R., Rassin, D.K. & Perez-Polo, J.R. (2005) :** Activation of nuclear factor-kappaB signaling pathway by interleukin-1 after hypoxia/ischemia in neonatal rat hippocampus and cortex. J. Neurochem.; 93:26-37.

53.**Wada, K., Nakajima, A., Katayama, K., Kudo, C., Shibuya, A., Kubota, N., Terauchi, M., Miyoshi, H., Kamisaki, Y., Mayumi, T. et al. (2006) :** Therapeutic potential of thiazolidinediones in activation of peroxisome proliferator-activated receptor gamma for monocyte recruitment and endothelial regeneration. J. Biol. Chem.; 281; 12673-12681.

تقييم تأثير "روزيجليتازون" كمنشط لمستقبلات البيروكسيزوم جاما فى حالة قصور بؤرى دائم للدورة الدموية المخية فى الجرذان البيضاء المصابة بمرض البول السكرى.

امانى عبدالرحمن شلبى ، فيفيان بشرى عبدة.

قسم الفارماكولوجيا الإكلينيكية - كلية الطب - جامعة المنصورة

يعد مرض البول السكرى من المسببات الهامة للسكتة الدماغية.وقد تبين الدور الذى تلعبة مستقبلات البيروكسيزوم جاما فى مناطق مختلفة من المخ والتى تؤدى لتقليل الضرر الناتج من السكتة الدماغية.

و لذلك جاءت فكرة هذا البحث لتقييم تأثير دواء "روزيجليتازون" و هو من مجموعة ثيازوليديندیون المنشطة لمستقبلات البيروكسيزوم جاما فى حالة قصور بؤرى دائم للدورة الدموية المخية (عن طريق ربط الشريان المخى الأوسط الأيسر) فى الفئران البيضاء المصابة بمرض البول السكرى.

وقد أجرى هذا البحث على ٩٦ فأراً بيضاء حيث قسمت الى ٨ مجموعات متساوية: تتلقى هذه المجموعات محلول الملح أو دواء روزيجليتازون ٣ مجم / كجم وزن الجسم عن طريق الحقن فى التجويف البريتونى يوميا بعد عملية ربط الشريان مباشرةً لمدة ٧ ايام متتالية:

١ -مجموعة ضابطة.

٢-مجموعة ضابطة مصابة بمرض البول السكرى.

٣- مجموعة مصابة بقصور بؤرى دائم للدورة الدموية المخية و غير معالجة.

٤-مجموعة قياسية مصابة بمرض البول السكرى و غير مصابة بقصور بؤرى دائم للدورة الدموية المخية و غير معالجة.

٥-مجموعة مصابة بقصور بؤرى دائم للدورة الدموية المخية فى الفئران المصابة بمرض البول السكرى و غير معالجة.

٦-مجموعة مصابة بقصور بؤرى دائم للدورة الدموية و معالجة بدواء روزيجليتازون

٧- مجموعة قياسية مصابة بمرض البول السكرى و غير مصابة بقصور بؤرى دائم للدورة الدموية المخية و معالجة بدواء روزيجليتازون

٨- مجموعة مصابة بقصور بؤرى دائم للدورة الدموية المخية فى الفئران المصابة بمرض البول السكرى معالجة بدواء روزيجليتازون

وقد تم تقييم تأثير الدواء المستخدم على الخلل الناتج في الوظائف نتيجة لقصور الدورة الدموية للمخ في هذا النموذج التجريبى في الفئران بدراسة:

• السلوك الحركى للفئران من اليوم التالى لربط الشريان المخى الأوسط لـ ٧ أيام متتالية و ذلك لتقييم القدرة القيادية للمخ على الجهاز الحركى.

• فى اليوم السابع، تم قياس نسبة السكر الصائم فى الدم ثم اخذ المخ من كل مجموعة وتقسيمهم الى مجموعتين تحتوى كل منهما على ٦ عينات: أحداهما لتحديد حجم التنكرز باستخدام صبغة ٢،٣، ٥ ترای فينيل تترازوليم كلورايد ، والأخرى لقياس كل من اكسيد النتريك و المالونداياالدهايد كدلالات الجهد الأكسيدى الناتج فى أنسجة المخ.

وكانت النتائج هى نجاح الروزيجليتازون فى خفض ذو دلالة إحصائية فى تقليل الإصابة سواء من ناحية السلوك الحركى أو حجم التنكرز أو دلالات الجهد الأكسيدى هذا الى جانب تقليل نسبة السكر فى الدم. لذلك كان هذا التأثير الفعال مدعاة لأهمية دواء الروزيجليتازون فى تقليل نسبة الإصابة بمضاعفات مرض البول السكرى مثل السكتة الدماغية.