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Full Length Research Paper

Minocycline on blood biochemical parameters and triglyceride levels in the model of alloxan-induced diabetes in rats

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Increasing evidence suggests that oxidative stress and inflammation play major roles in diabetes mellitus and its complications. Furthermore, hyperglycemia increases the production of free radicals, resulting in oxidative stress. Minocycline presents potent anti-inflammatory and antioxidant activities, as evaluated by *in vivo* and *in vitro* models. In the present study, the minocycline anti-diabetic effect was assessed in the model of alloxan-induced diabetes. Alloxan was injected to male Wistar rats (50 mg/kg, intravenously), and their blood was collected 48 h later and also after treatments, for measurements of glycemia, triglycerides, cholesterol and liver transaminases. Groups of untreated diabetic controls and diabetic treated with minocycline (1 to 50 mg/kg, peritoneally, p.o.) or glibenclamide (5 mg/kg, p.o., as reference), for different periods, were used. Furthermore, slices of pancreas, liver and kidney were submitted to histological and immunohistochemical analyses. While significant decreases in glucose and triglycerides were shown at the 5th and mainly at the 30th days after minocycline treatments, as compared to the untreated diabetic group, no changes were observed in total cholesterol, alanine aminotransferase (ALT) or aspartate aminotransferase (AST) levels. Histological analyses of pancreas, liver and kidney showed that minocycline significantly reversed tissue alterations, as those seen in untreated diabetic animals. Besides, minocycline also reduced tumor necrosis factor (TNF)-alpha, cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) expressions. The beneficial minocycline effects in diabetes could be due, at least partly, to its anti-inflammatory and antioxidant properties, indicating that this drug may be a therapeutic alternative in diabetes mellitus and other pathological conditions where inflammation plays a significant role.

Key words: Minocycline, diabetes, inflammation, hyperglycemia, hypertriglyceridemia.

INTRODUCTION

Diabetes mellitus consists of progressive hyperglycemia, insulin resistance and pancreatic β-cell failure, and is associated with the development of atherosclerosis. Atherosclerosis in diabetes is, at least in part, the result

of a chronic low-grade inflammatory process (Danesh et al., 2000; Pradham and Ridker, 2002). In addition, diabetes in elderly men is related to cyclooxygenase (COX)-mediated inflammation, reflected by enhanced prostaglandin formation (Helmersson et al., 2004).

The role of inflammation in the pathogenesis of diabetes and of vascular complications was confirmed by intervention studies (Zozulinska and Werusz-Wysocka, 2006), and the association of diabetes to inflammation opens new clinical perspectives for its diagnosis and treatment. Commonly, type 1 and type 2 diabetes mellitus are considered inflammatory processes, as there is a significant increase in pro-inflammatory cytokines in the blood of patients with the disease (Alexandraki et al., 2008; Erbagci et al., 2001; Esposito et al., 2002; Francés et al., 2013).

Insulin resistance and the vascular complications of diabetes include the activation of an inflammation cascade, endothelial dysfunction and oxidative stress. Diabetes comorbidities, as obesity, insulin resistance, hyperglycemia, hypertension and dyslipidemia, aggravate diabetic complications, while anti-hyperglycemic interventions tend to correct them. Although inflammation represents a protective response that controls infections and promotes tissue repair; it can also contribute to tissue damage in inflammatory diseases where pro-inflammatory cytokines and oxidative stress play a role (Kampoli et al., 2011).

Prostaglandins are mediators of inflammation, and prostaglandin $(PGF)_{2\alpha}$ is considered an indicator of *in vivo* inflammatory processes (Basu and Eriksson, 1998; Basu et al., 2000). These data suggest an ongoing COX-related low-grade inflammatory process, among patients with type 2 diabetes, both as an early event and as a later process in the disease development. This was confirmed by a study (Helmersson et al., 2004) suggesting the involvement of low-grade COX-mediated inflammation and oxidative stress in type 2 diabetes, where the appearance of chronic inflammation seems to be an early process, whereas oxidative injury may be a later and possibly a secondary process in the progress of the disease.

Furthermore, in diabetic patients, insulin resistance, oxidative stress and inflammation are among the mechanisms implicated to cause endothelial damage (Oudot et al., 2009; Park et al., 2009). The increased oxidative stress is due to a decreased antioxidant capacity, chronic exposure to ROS, increased plasma oxidation, peroxidetion and glycol-oxidation as well (Hartge et al., 2007; Goldin et al., 2014).

In addition, once activated, polymorphonuclear (PMN) cells release reactive oxygen species (ROS) and mediators of proteolytic tissue degradation, contributing to oxidative stress, subsequent inflammation and endothelial damage (Orekhov, 2013). Type 2 diabetes is shown (Shurtz-Swisrski et al., 2001) to be accompanied by a priming of PMN cells, resulting in oxidative stress and increased necrosis. Necrosis starts a chain of inflammatory reactions, causing cell recruitment and, in the long run, oxidative stress that may result in endothelial dysfunction.

Minocycline is a semi-synthetic 2nd generation tetracycline that exerts anti-inflammatory effects, distinct from its antimicrobial action (Ryan and Ashley, 1998). Clinical studies have shown that minocycline and related tetracyclines have beneficial effects in several inflammation-based diseases (Ryan et al., 1998). Tetracycline like minocycline has been reported to have a number of pharmacological actions, including the ability to inhibit matrix metalloproteinases, superoxide production from neutrophils, and inducible nitric oxide synthase (iNOS) expression in human cartilage and murine macrophages (Golub et al., 1998; Golub et al., 1999).

Minocycline possesses potent anti-apoptotic properties manifested by inhibition of caspase-1 and caspase -3 expressions and by direct blockade of cytochrome c release from mitochondria (Chen et al., 2000; Zhu et al., 2002). Furthermore, minocycline reduced cell apoptosis, decreased cytochrome c release and reduced up-regulation of p53 and Bax, in a model of ischemic renal injury in rats (Kelly et al., 2004).

The objectives of the present study were to demonstrate a possible action of minocycline on blood biochemical parameters, focusing mainly on blood glucose and triglyceride levels, in the model of alloxan-induced diabetes in rats, as related to untreated diabetic controls and diabetic rats treated with glibenclamide, as reference. Besides, histological analyses and immunohistochemical assays for tumor necrosis factor (TNF)-alpha, COX-2 and iNOS in pancreas, kidney and liver were also carried out.

MATERIALS AND METHODS

Drugs and reagents

Alloxan monohydrate was purchased from Sigma Chemical Co. (St. Louis, MO, USA), and glibenclamide, from Aventis Brasileiro Ltda. (GO, Brazil). Powdered minocycline was from Galena Laboratory (São Paulo, Brazil) and was dissolved in distilled water before use. All other drugs were of analytical grade.

Animals

Male Wistar rats (180 to 250 g) from the animal house of the Faculty of Medicine, Estácio of Juazeiro do Norte (Brazil) were housed under standard environmental conditions (22 ± 1°C,

humidity 60 ± 5%, 12 h light/12 h dark cycle), with access to a standard diet and water *ad libitum.* The experiments were performed according to the Guide for the Care and Use of Laboratory Animals, from the US Department of Health and Human Services, Institute of Laboratory Animal Resources, Washington DC, 1985, and approved by the Ethical Committee for Animal Experimentation, of the Federal University of Ceará.

Experimental protocol

The animals were divided into groups of 7 to 23 rats each. Diabetic groups (untreated and treated) fasted for at least 16 h, received alloxan (50 mg/kg, intravenously) through the penile vein. The diabetic state was assessed by measurements of serum glucose levels, 48 h later, when 10% deaths were registered, at the most. Only animals presenting glucose levels equal to or higher than 250 mg/dl were used and distributed into the following groups: diabetic rats administered with distilled water (untreated diabetic controls) and diabetic rats treated with minocycline (Mino; 1, 5, 10, 25 and 50 mg/kg, p.o.) or glibenclamide (GLI; 5 mg/kg, p.o.) as a standard drug. Two protocols were used. In the first one, 48 h after the diabetes induction, blood samples were collected and then the 5-day treatments started. After this period and 1 h after the last drug administration, blood was collected again for biochemical measurements. In the second protocol, the same experimental conditions were followed, except that only one dose of minocycline (25 mg/kg) or glibenclamide (5 mg/kg) was used, and the daily treatment continued up to 30 days. Under this condition, blood was collected 48 h after the alloxan injection and 5, 10 and 30 days after treatments, for biochemical analyses.

Determination of biochemical parameters in rat serum

Blood collected from the retro-orbital plexus was centrifuged at 3000 rpm, for 10 min, and the glucose level was determined by the glucose oxidase-peroxidase enzymatic method (Labtest, Brazil). Concentrations of serum total cholesterol (TC) and triglycerides (TG) were spectrophotometrically measured by standard enzymatic methods. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were also determined by standard procedures, according to the manufacturer's instructions (Labtest, Brazil).

Histological analyses

Pancreas, liver and kidney are organs very affected in diabetic conditions. These organs were excised from four groups of three animals each (untreated diabetic and diabetic rats treated for 30 days with minocycline, 25 and 50 mg/kg or glibenclamide, 5 mg/kg) after decapitation, and fixed in 10% buffered formalin. Paraffin blocks were prepared for routine microscopic slices processing (5 µ sections). Hematoxylin and Eosin (HE) staining was performed in all slides, examined afterwards using 400X magnifications.

Immunohistochemistry assays for TNF-alpha, COX-2 and iNOS

Considering that a chronic low-grade inflammation is present in diabetes mellitus and contributes to insulin resistance, we decided to perform imunohistochemistry assays for TNF-alpha, COX-2 and iNOS in pancreas, liver and kidney from untreated diabetic and diabetic rats after minocycline or glibenclamide treatments for 30 days. For that, tissue sections were deparaffinized, dehydrated in xylol and ethanol, and immersed in 0.1 M citrate buffer (pH 6) under microwave heating, for 18 min, for antigen recovery. The sections, after cooling at room temperature for 20 min, were washed with a phosphate buffered saline (PBS), followed by a 15 min blockade of endogenous peroxidase with 3% H_2O_2. They were then incubated overnight (4°C) with anti-TNF-α, anti-COX-2 p65 or anti-iNOS rabbit primary antibodies diluted in PBS-BSA, according to the manufacturers' instructions. The next day, the sections were washed in PBS and incubated for 30 min with each biotinylated rabbit secondary antibody (anti-IgG). Then, after washing in PBS again, they were incubated for 30 min with the conjugated streptavidin peroxidase complex (ABC Vectastain® complex, Vector Laboratories, Burlingame, CA, USA). After another washing with PBS, the sections were stained with 3, 3'diaminobenzidine-peroxide (DAB), counter-stained with Mayer hematoxylin, dehydrated and mounted in microscope slides for analyses.

Statistical analysis

The paired Student's t test was used in statistical analyses of biochemical parameters, for comparing means of each group before and after drug treatments. In other experiments, one-way analysis of variance (ANOVA) and the Newman-Keuls as the post hoc test were used for comparing the results among treatments. The significance level was set at $p < 0.05$.

RESULTS

Minocycline decreases blood glucose levels (mg/dl) in diabetic rats

In the 1st protocol, the oral treatment of diabetic rats with minocycline (5, 10, 25 and 50 mg/kg), for 5 days (starting 48 h after the alloxan injection), reduced glycemia by 48, 58, 46 and 47%, as compared to untreated diabetic controls (369.7±16.26). However, glycemia values were not brought to normal levels after this short treatment (5 mg/kg: 170.2±22.29; 10 mg/kg: 138.1±22.58; 25 mg/kg: 190.5±19.73; 50 mg/kg: 183.0±26.94). No significant difference from untreated diabetic controls was noticed with the dose of 1 mg/kg. On the other hand, a 47% reduction in glycemia (183.0±26.94) was seen with glibenclamide 5 (Figure 1A). In the 2nd protocol, the minocycline (25 mg/kg) treatment continued up to 30 days. Glycemia values were measured at three periods: 5, 10 and 30 days, in order to evaluate whether a longer treatment would bring values to normality. Figure 1B shows decreases of 38 (5 days: 164.2±19.45), 67 (10 days: 87.4±5.65) and 57% (30 days: 112.9±9.62), respectively, as compared to the same diabetic group before drug treatments (263.3±24.00). On the other hand, untreated diabetic controls did not show, as expected, any decrease in glycemia values, along the observation periods (starting value: 304.1±11.92; 5 days: 387.1±19.32; 10 days: 464.5±21.79; 30 days: 442.9±19.44).

Minocycline treatments of diabetic rats do not change total cholesterol, but decreases triglyceride levels

The 5-day minocycline treatments of diabetic animals

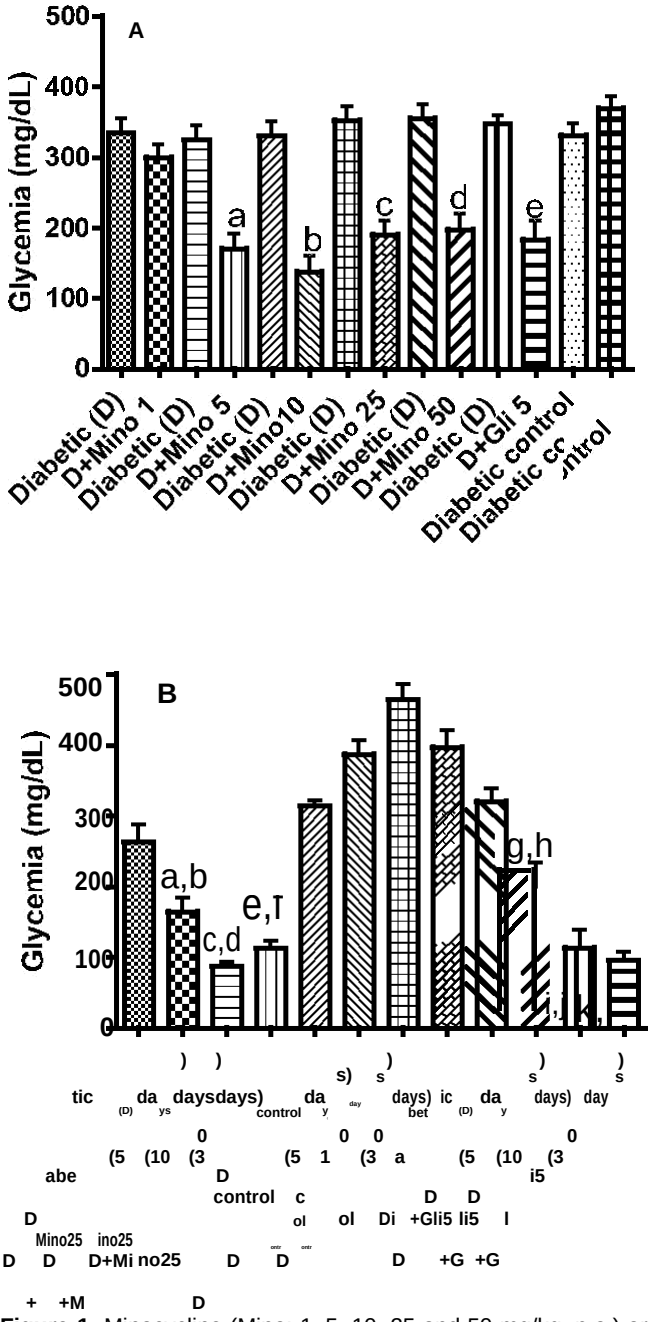


Figure 1. Minocycline (Mino: 1, 5, 10, 25 and 50 mg/kg, p.o.) or GLI 5 treatments of alloxan-induced diabetic rats reduced blood glucose (mg/dl), after daily administration for 5 days (A); which also occurred after treatments with Mino25 or GLI5, for 5, 10 and 30 days (B). In both cases, glibenclamide (GLI: 5 mg/kg, p.o.) was used as reference. Untreated diabetic rats (diabetic controls), under the same experimental conditions, are shown for comparisons. Each pair of bars represents the same diabetic group, before and after treatments with water (diabetic controls) or with Mino or GLI. The data are means ± standard error of mean (SEM) from 10 to 18 animals per group. Normal control values are: 95.9±2.62 mg/dl (data from 12 animals). (A) 5-day treatments - a, b, c, d and e: $p<0.0001$-0.008 vs. the same group before treatments (paired Student-t test). (B) 5, 10 and 30-day treatments - a, c, e, g, i and k: $p<0.01$-0.001 vs. the same group before Mino or GLI treatments, at each period of observation; b, d, f, h, j and l: $p<0.001$ vs. untreated diabetic controls, at the same period of observation (paired Student-t test or One-way ANOVA, followed by Newman-Keuls as the *post hoc* test for multiple comparisons).

significantly reduced triglyceride levels (mg/dl) by 43, 46, 32 and 35%, with 1 (113.0±14.38), 5 (104.0±15.22), 10 (116.6±5.88), 25 (110.8±2.05) and 50 mg/kg (167.2±22.65), respectively, as related to the same diabetic group before treatments (Figure 2A). Glibenclamide 5 presented a 49% reduction, as related to its starting value (starting value: 172.8±25.03; 5 days: 88.3±6.73). The values in untreated diabetic controls did not change (starting value: 170.6±15.65; 5 days later: 175.1±19.05). On the other hand, total cholesterol did not significantly change in any group, as related to starting values (diabetic rats before treatments) or to untreated diabetic controls (Figure 3A). In the longer treatment protocol (Figure 2B), a significant reduction (34%) of triglycerides was noticed after the minocycline 25 treatment, only at the 30th day (starting value: 148.4±28.43; 30th day: 97.8±13.07). The values for untreated diabetic controls did not significantly alter at the same period (starting value: 184.3±27.75; 30 days later: 144.2±13.73). Similarly, the cholesterol values were unaltered in all groups (Figure 3B).

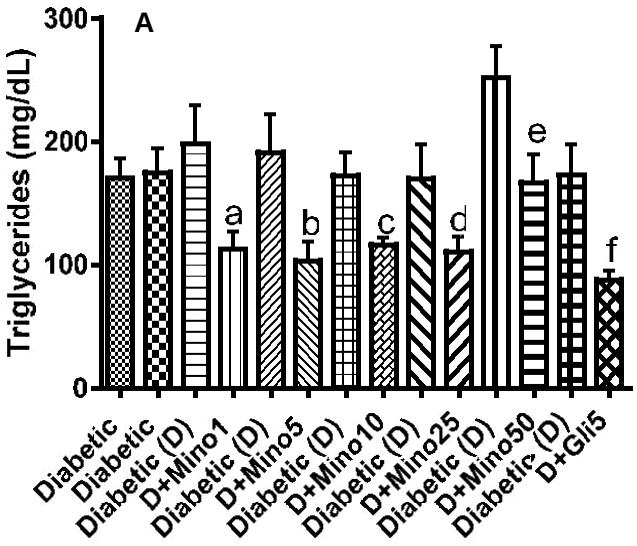
Minocycline treatments do not alter ALT or AST levels in diabetic rats

Our data show no significant changes in either ALT or AST values in diabetic groups, after 5-day treatments with minocycline (1, 5, 10, 25 and 50 mg/kg) or glibenclamide 5 (Figures 4A and 5A). Similarly, no changes were observed in diabetic rats, after minocycline 25 or glibenclamide 5 longer treatments (2nd protocol), for any of the liver transaminases (Figures 4B and 5B).

Minocycline 30-day treatments protect diabetic rat pancreas, liver and kidney tissues from the alloxan-induced cytotoxicity

The histological analyses (HE staining) showed damaged islets with vacuolated cells and reduced size, in the pancreatic tissue of untreated diabetic controls. On the other hand, diabetic rats treated with glibenclamide (5 mg/kg) showed restoration to a normal cellular population and absence of islet damage. Similarly, in diabetic rats treated with minocycline (25 or 50 mg/kg), there was a partial or total restoration, respectively, towards a normal islets profile.

In rat liver tissues from untreated diabetic controls, there was hyperplasia of the bile ducts and congestion of blood vessels. In the glibenclamide 5-treated diabetic group, hyperplasia of the bile ducts and congestion of blood vessels were still present. However, in liver tissues from diabetic rats treated with minocycline (25 mg/kg), there was no bile ducts hyperplasia, but congestion of blood vessels was still present. On the other hand, in liver tissues from diabetic rats treated with minocycline (50



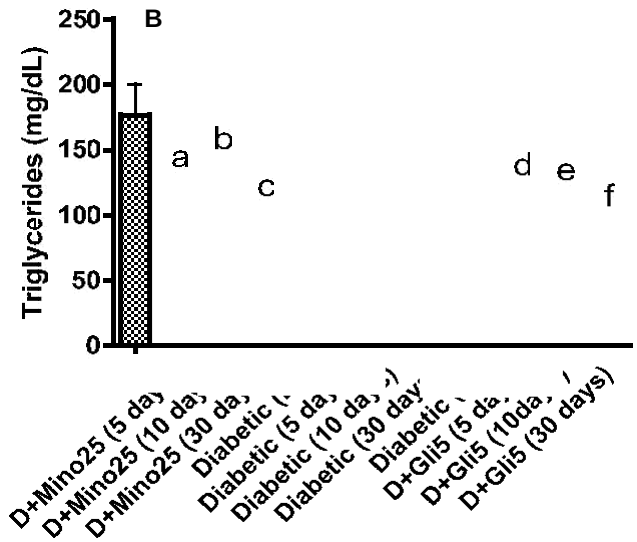
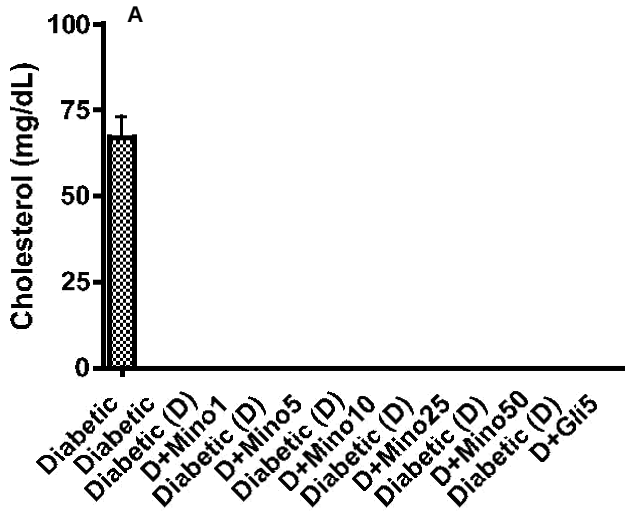


Figure 2. Minocycline (Mino: 1, 5, 10, 25 and 50 mg/kg, p.o.) or GLI5 treatments of alloxan-induced diabetic rats significantly reduced triglycerides levels (mg/dl), after daily administration for 5 days (A), which also occurred after treatments with Mino25 or GLI5, for 5, 10 and 30 days (Figure 2B). In both cases, glibenclamide (GLI: 5 mg/kg, p.o.) was used as reference. Untreated diabetic rats (diabetic controls), under the same experimental conditions, are shown for comparisons. Each pair of bars represents the same diabetic group, before and after treatments, with water (diabetic controls) or with Mino or GLI. The data are means ± standard error of mean (SEM) from 8 to 14 animals per group. Normal control values are: 51.2±7.63 mg/dl (data from 12 animals). (A) a: $p < 0.0094$; b: $p < 0.031$; c: $p < 0.0197$; d: $p < 0.05$; e: $p < 0.034$; f: $p < 0.0094$ vs. the same group before treatments. (B) a: $p < 0.042$; b: ns; c: $p < 0.029$; d: $p < 0.0127$; e: $p < 0.004$; f: $p < 0.005$; all vs. the same group before treatments.



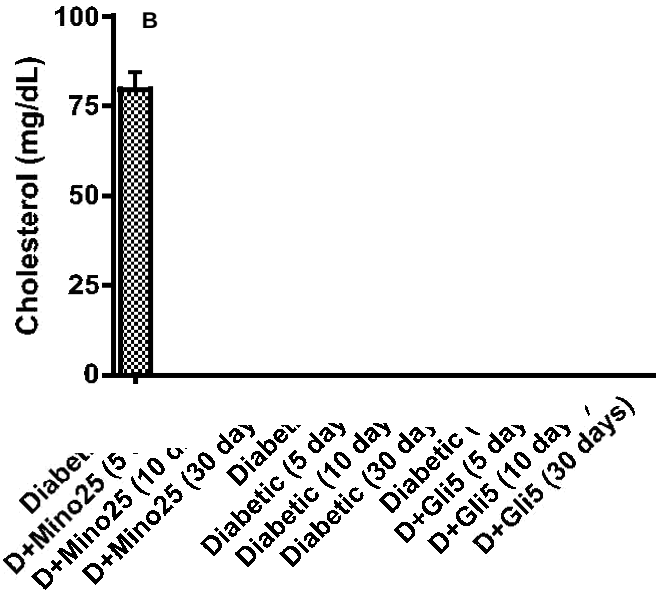


Figure 3. Minocycline (Mino: 1, 5, 10, 25 and 50 mg/kg, p.o.) or GLI5 treatments of alloxan-induced diabetic rats did not change total cholesterol levels (mg/dL), after daily administration for 5 days (A), which also occurred after treatments with Mino25 or Gli5, for 5, 10 and 30 days (B). In both cases, glibenclamide (GLI: 5 mg/kg, p.o.) was used as reference, and untreated diabetic rats (diabetic controls) are shown for comparison. Each pair of bars represents the same diabetic group, before and after treatments, with water (diabetic controls) or with Mino or GLI. Normal control values are: 62.0±3.21 mg/dl (data from 12 animals). The data are means ± standard error of mean (SEM) from 8 to 21 animals.

mg/kg), there were no histological alterations.

Representative photomicrographs of kidney tissues showed that, in the untreated diabetic group, there was a marked degeneration of glomeruli with inflammatory cell infiltration. However, in kidney tissues from diabetic rats treated with glibenclamide (5 mg/kg), there was no alteration, while diabetic rats treated with minocycline (25

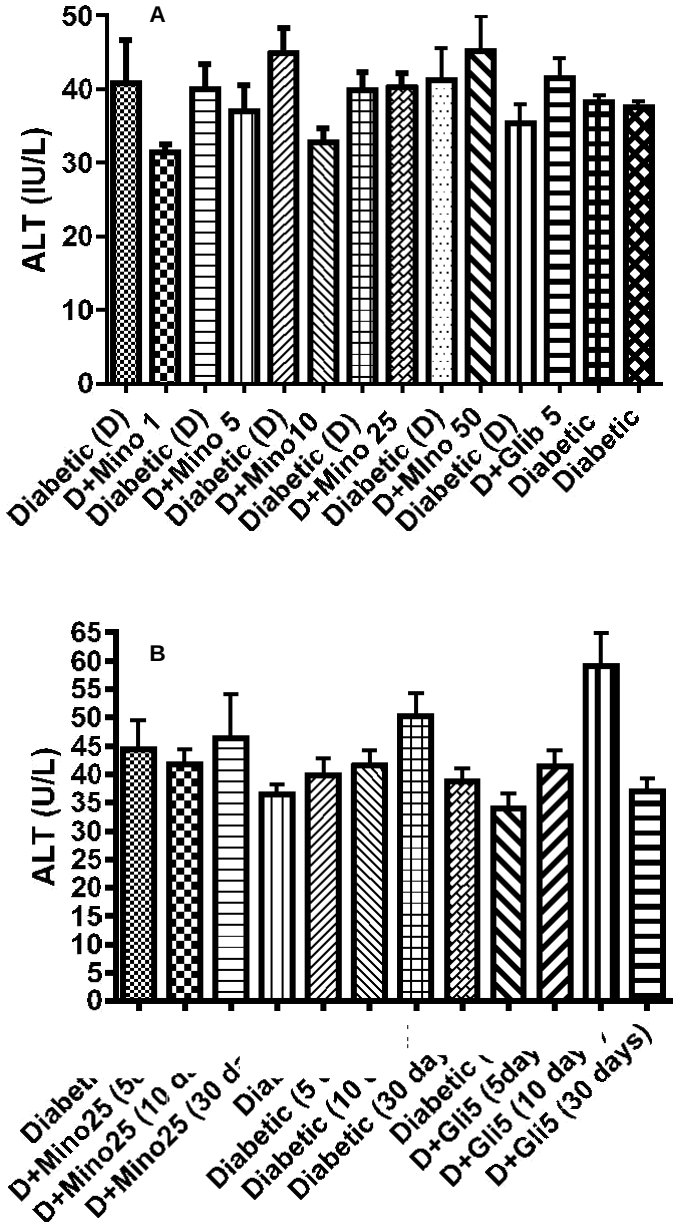


Figure 4. Minocycline (Mino: 1, 5, 10 and 25 mg/kg, p.o.) or GLI5 treatments of alloxan-induced diabetic rats did not change total ALT (U/L), after daily administration for 5 days (A), which also occurred after treatments with Mino25 or GLI5, for 5, 10 and 30 days (B). In both cases, glibenclamide (GLI: 5 mg/kg, p.o.) was used as reference, and untreated diabetic rats (diabetic controls) are shown for comparison. Each pair of bars represents the same diabetic group, before and after treatments, with water (diabetic controls) or with Mino or GLI. Normal control values are: 62.0±3.21 U/L (data from 12 animals). The data are means ± standard error of mean (SEM) from 8 to 21 animals.

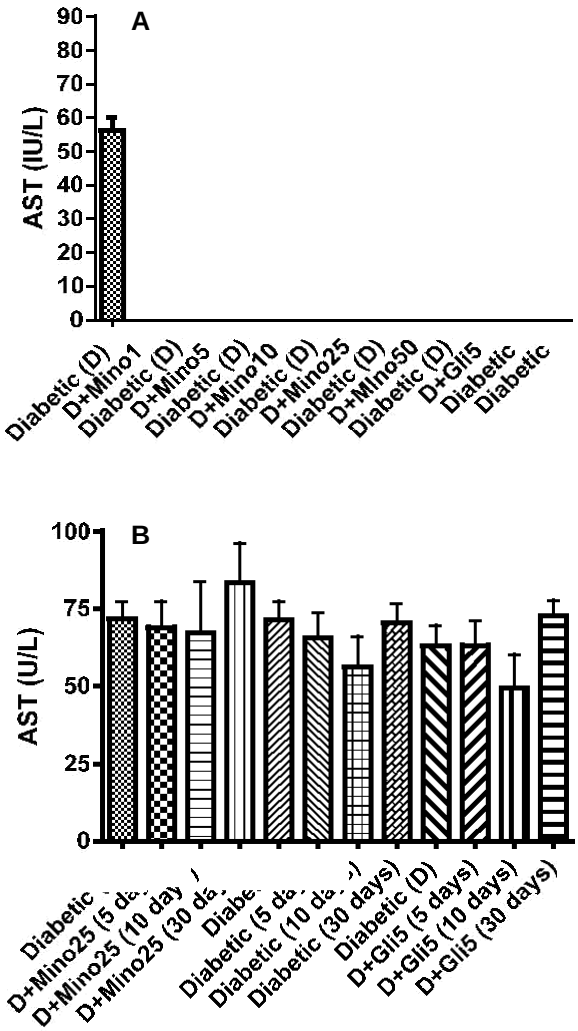


Figure 5. Minocycline (Mino: 1, 5, 10 and 25 mg/kg, p.o.) or GLI5 treatments of alloxan-induced diabetic rats did not change total AST (U/L), after daily administration for 5 days (A), what also occurred after treatments with Mino25 or GLI5, for 5, 10 and 30 days (B). In both cases, glibenclamide (GLI: 5 mg/kg, p.o.) was used as reference, and untreated diabetic rats (diabetic controls) are shown for comparison. Each pair of bars represents the same diabetic group, before and after treatments, with water (diabetic controls) or with Mino or GLI. Normal control values are: 62.0±3.21 U/L (data from 12 animals). The data are means ± standard error of mean (SEM) from 8 to 21 animals.

mg/kg) showed marked degeneration of the glomeruli with inflammatory cell infiltration. On the other hand, an almost complete restoration to a normal histology in the diabetic group treated with minocycline, at the dose of 50 mg/kg, was noticed.

Minocycline 30-day treatments reduce immunoexpression for TNF-alpha, COX-2 and iNOS, in pancreas, liver and kidney from diabetic rats

The immunohistochemical data demonstrated that minocycline 25 reduced the number of TNF-alpha immunostained cells, in diabetic pancreas and liver as related to untreated diabetic rats (Figure 6). A similar result was observed for diabetic rats after glibenclamide (5 mg/kg), used as reference drug (Figure 7). Minocycline (25 and 50 mg/kg) or glibenclamide 5 also decreased

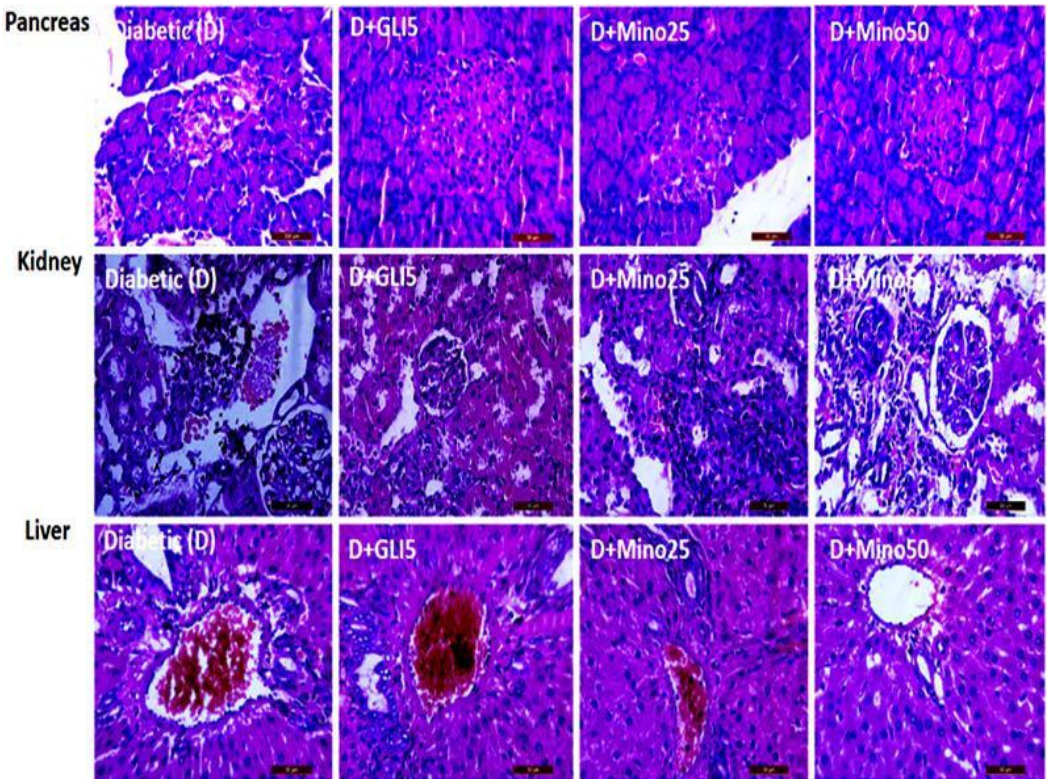


Figure 6. Photomicrographs showing that minocycline (Mino: 25 and 50 mg/kg, p.o.), administered daily for 30 days to diabetic rats, significantly protects pancreas, liver and kidney tissues from alloxan-induced damages, as related to untreated diabetic controls. Glibenclamide (GLI: 5 mg/kg) was used as reference (HE staining, 400× magnification).

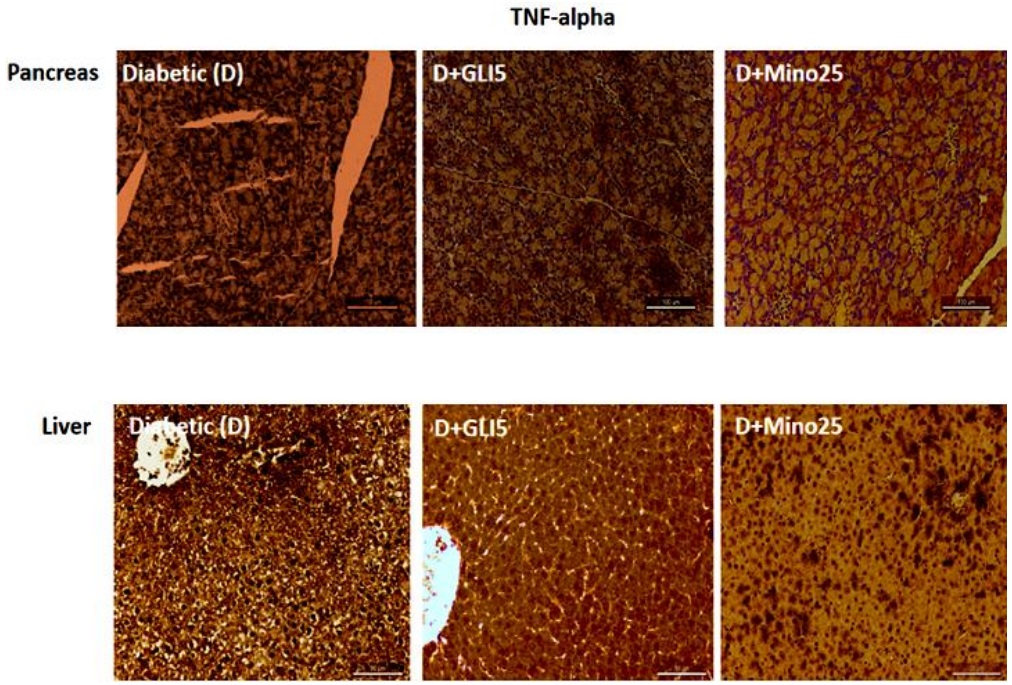


Figure 7. Representative photomicrographs showing that the minocycline (Mino: 25 mg/kg, p.o.), administered daily for 30 days to diabetic rats, reduces the number of TNF-alpha immunostained cells in pancreas and liver, as related to untreated diabetic controls. Glibenclamide (GLI: 5 mg/kg, p.o.) was used as reference (400× magnification).

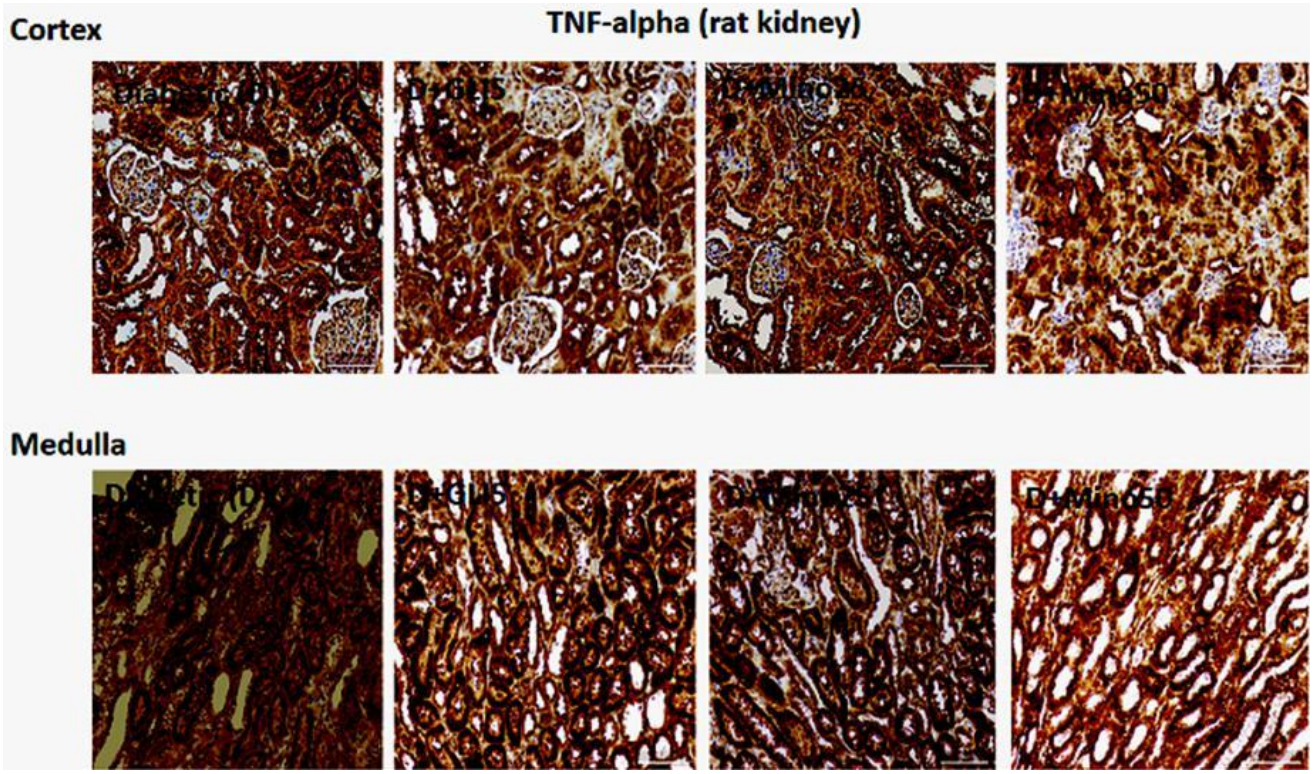


Figure 8. Representative photomicrographs showing that the minocycline (Mino: 25 or 50 mg/kg, p.o.), administered daily for 30 days to diabetic rats, reduces the number of TNF-alpha immunostained cells in kidneys (cortex and medulla), as related to untreated diabetic controls. Glibenclamide (GLI: 5 mg/kg, p.o.) was used as reference (400× magnification).

TNF-alpha expression in diabetic kidney, and these results were seen in kidney cortex and medulla as well (Figure 8). In addition, decreased immunostaining for COX-2 and iNOS were observed in diabetic pancreas after minocycline (25 mg/kg) and glibenclamide (5 mg/kg) treatments, as related to untreated diabetic controls (Figure 9).

DISCUSSION

This work evaluated the effects of minocycline on blood biochemical parameters, in the model of alloxan-induced diabetes in rats. Histological and immunohistochemical studies on diabetic pancreas, liver and kidney were also performed, attempting to elucidate the mechanism for minocycline action. As far as we know, these minocycline actions focusing on diabetes are shown here for the first time.

Despite the fact that hyperglycemia and insulin resistance are the main characteristics of diabetes, this multifactor disease is in part a consequence of a chronic low-grade inflammation (Helmersson et al., 2004). Lowering high blood glucose is the primary goal, and several drugs are known to do this, but only to a limited extent. In this sense, type 2 diabetes is a chronic hyperglycemic disorder caused by a defective action and/or secretion of insulin, manifesting complications that ultimately provoke most of its morbidity and mortality.

In a model of transgenic mice, diabetes pathogenesis and progression occur primarily through islet beta-cell dysfunction with subsequent beta-cell loss. Both onset and progression were significantly inhibited by the chronic treatment with tetracycline that interacts with aggregates of proteins implicated in amyloid-related diseases (Aitken et al., 2010; Forloni et al., 2012). In this study, a chemically-induced diabetes model was used. Alloxan is selectively toxic to pancreatic cells as it preferentially accumulates in beta-cells, and its cytotoxic action is mediated mainly by the generation of ROS (Rohilla and Shahjad, 2012). Furthermore, oxidative stress plays a pivotal role in the development of both microvascular and cardiovascular diabetes complications (Giacco and Brownlee, 2010).

Minocycline is known to present a variety of anti-apoptotic and anti-inflammatory effects, and these actions are manifested at low doses of the drug, as previously observed by several models of inflammation *in vivo* (Leite et al., 2011). Minocycline reduces microgliosis, the expression of iNOS, caspase-1 activity, formation of IL-1beta, metalloproteinase activity and the production of COX and PGs (Blum et al., 2004; Domercq and Matute, 2004). Others (Kraus et al., 2005) suggested that the direct antioxidant activity of minocycline may

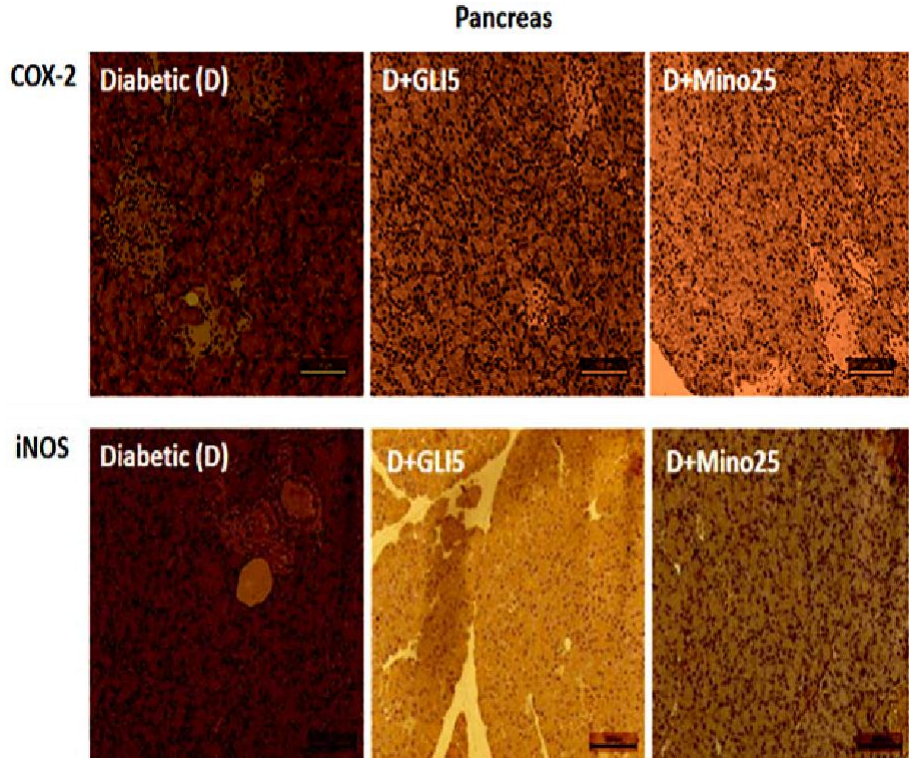


Figure 9. Representative photomicrographs showing that minocycline (Mino: 25 mg/kg, p.o.), administered daily for 30 days to diabetic rats, reduces the number of COX-2 and iNOS immunostained cells in pancreas, as related to untreated diabetic controls. Glibenclamide (GLI: 5 mg/kg, p.o.) was used as reference (400× magnification).

contribute to its neuroprotective effects in neuronal injury processes, as previously observed (Viana et al., 2010). The oxidative stress seems to be associated with macrovascular and microvascular diabetes complications (Folli et al., 2011; Schaffer et al., 2012). In a recent study (leite et al., 2011), a potent antioxidant action of minocycline *in vitro* was also demonstrated.

In this work, the hypoglycemic effect of minocycline, orally administered for 5 days to diabetic rats was studied, and it showed that it significantly lowered blood glucose levels. A longer treatment (up to 30 days) brought glycemia to normal values. Glucose is the driving force in microvascular complications of diabetes, and the glycemic control is a primary goal in the treatment of patients (Reusch, 2003). Micro-albuminuria represents an abnormally elevated urine albumin level, and predicts the worsening of renal disease to overt diabetic nephropathy and an elevated risk of cardiovascular disease (Mogensen, 1999). Minocycline, due to its anti-apoptotic and anti-inflammatory properties were shown to protect the renal function, in a model of ischemia-reperfusion in rats (Kelly et al., 2004).

Although, in the present study, minocycline did not change total cholesterol levels, it significantly reduced triglyceride levels. The increased risk of coronary artery disease in diabetic patients can be explained, in part, by their lipoprotein abnormalities. Hypertriglyceridemia and low levels of HDL are the most common lipid abnormalities (O'Brien et al., 1998). In a cohort study (Wiggin et al., 2009) carried out with diabetic neuropathic patients, elevated triglycerides were correlated to myelinated fiber density loss, independently of the disease duration, age, diabetes control and other variables, supporting the concept that hyperlipidemia is instrumental in the progression of diabetic neuropathy. Furthermore, a large proportion of ischemic cardiovascular diseases occur in individuals without hypercholesterolemia. Under this condition, triglycerides and low HDL-C should be addressed in the management of dyslipidemia, especially in the presence of low LDL-C (Pang et al., 2012). Our data, showing that minocycline decreased glycemia, as well as triglycerides in diabetic rats, points out to a potential use of this drug as a therapeutic alternative for diabetes control.

Data from Krady et al. (2005), demonstrated that minocycline represses diabetes-induced inflammatory cytokine production, decreases the release of cytokines from activated microglia, and significantly reduces measurable caspase-3 activity within the retina. Their results indicated that inhibiting microglial activity may be an important strategy in the treatment of diabetic retinopathy, and minocycline could be a good option for the

the delay or prevention of vision loss associated with the disease. We recently showed that TNF-α and iNOS positive cells appear in much lower number in the paw and periodontium of minocycline-treated rats, in models of carrageenan-induced edema and periodontal disease (Leite et al., 2011; Menezes, 2012).

Diabetes leads to depletion of the cellular antioxidant defense system and is associated with an increase in free radicals production (Shi and Vanhoutte, 2009). Minocycline depresses the release of oxygen radicals from various types of cells, including leukocytes, and the production of nitric oxide, through its effect on nitric oxide synthase (Amin et al., 1996). Data from *in vitro* rat brain assays show that minocycline is an effective antioxidant, with radical scavenging potency, similarly to vitamin E (Kraus et al., 2005), confirming previous results from our laboratory (Leite et al., 2011).

Furthermore, this study showed that minocycline offers a partial (25 mg/kg) or total (50 mg/kg) protection to rat pancreas, liver and kidney, in the alloxan -induced diabetes model, as assessed by histological HE staining. In the pancreas tissue, a complete restoration to normality of the Langerhans islets was seen, in diabetic rats treated with the higher minocycline dose. Similarly, either no alteration or almost a complete restoration was observed in liver and kidney histology, after minocycline treatments.

This study also demonstrated that minocycline decreased the immunoreactivity for TNF-alpha, in pancreas, liver and kidney of diabetic rats. This drug was reported to decrease TNF-alpha levels produced in human T-cell and microglia interaction. According to Giuliani et al. (2005), the effect is mediated by a direct action of minocycline on activated T-cells and on microglia, resulting in the decreased ability of T-cells to contact microglia. Others (Drabek et al., 2014) showed that minocycline attenuated TNF-alpha levels, in a model of hypothermic cardiac arrest in rats. Interestingly, minocycline effects on TNF-alpha levels were also observed in a model of hyperglycemia *in vitro*, where TNF-alpha- and glucose-induced oxidative responses in cultured osteoblasts were overcome by minocycline (Soory and Tilakaratne, 2006). Thus, this minocycline capacity to down-regulate pro-inflammatory cytokines, as TNF-alpha, constitutes a new target for therapeutic intervention.

Besides the TNF-alpha immunoreactivity decrease in diabetic pancreas, liver and kidney, this study showed that minocycline also reduced iNOS and COX-2 immunoreactivity in diabetic pancreas. Others (Lee et al., 2004) observed that it exerts a neuroprotective effect, associated with the inhibition of iNOS induction and NO production in glial cells that is mediated by the LPS-induced production of TNF-alpha. Furthermore, minocycline was shown to significantly reduce COX-2 protein levels, in a rat model of Aβ toxicity (Ryu et al., 2004), and the effectiveness of the drug in modulating microglia and COX-2 activities could contribute to its neuroprotective effect.

Evidences (Czerny et al., 2012; Schwartz et al., 2013) demonstrated that minocycline decreased liver injury and oxidative stress, after mice hemorrhage and resuscitation, and mitigated the damage caused by ischemia/reperfusion injury in cultured rat hepatocytes. As discussed earlier, a chronic low-grade inflammation is known to be present not only in type 2, but also in type 1 diabetes (Odegaard and Chawla, 2012), and contributes to insulin resistance. Furthermore, the accumulation of activated innate immune cells in metabolic tissues results in the release of inflammatory mediators, in particular IL -1beta and TNF-alpha, promoting insulin resistance and beta-cell damage.

Conclusions

Conclusively, our data strongly suggest that minocycline protective effects in pancreas, liver and kidney may be a consequence of the drug anti-inflammatory and antioxidant properties, as previously shown by Leite et al. (2011) and Viana et al. (2010). Furthermore, this study clearly demonstrated that the potent anti-inflammatory and antioxidant actions, inhibitions of pro-inflammatory cytokines and metalloproteinase activity, as assured by several laboratories, including ours, could certainly explain minocycline hypoglycemic and hypotriglyceridemic effects on diabetic rats. Thus, minocycline, due to its pleiotropic effects, appears as a potential and efficacious candidate for the treatment of several pathological conditions, as diabetes mellitus, where inflammation plays an important role.

Conflict of Interest

The authors declare they have no conflict of interest.

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