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The influence of rutin on the extracellular matrix in streptozotocin-induced diabetic rat kidney

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Abstract

We previously reported that rutin administration to streptozotocin (STZ)-induced diabetic rats decreased plasma glucose and increased plasma insulin levels. In this study, we have examined the role of rutin on matrix remodelling in the kidney of STZ-induced diabetic rats. STZ was administered intraperitoneally (50 mg kg^{-1}) to male albino Wistar rats to induce experimental diabetes. Rutin (100 mg kg^{-1}) was orally administered to normal and STZ-induced diabetic rats for a period of 45 days and its influence on the content of hydroxyproline and collagen and on the activity of matrix metalloproteinases (MMPs) were studied. We have also studied the levels of tissue inhibitors of metalloproteinases (TIMPs) in the kidney. STZ-induced diabetic control rats showed increased content of hydroxyproline and collagen, decreased activity of MMPs and increased levels of TIMPs in the kidney. These changes were positively modulated by rutin treatment in STZ-induced diabetic rats, thereby protecting the kidney. In normal rats treated with rutin, none of the parameters studied were significantly altered. From the results obtained, we could conclude that rutin influences MMPs and effectively protects kidney against STZ-induced damage in rats. The effects observed are due to the reduction of plasma glucose levels by rutin.

Introduction

Diabetes mellitus is characterized by a series of complications that affect many organs. Hyperglycaemia is an important factor in the development of diabetic complications (Bretzel et al 1998). There are many biochemical pathways by which the effects of hyperglycaemia can be mediated. One of the possible mechanisms is the accumulation of advanced glycation end products (AGEs), which have been shown to correlate with the degree of diabetic complications (Monnier et al 1999). Extracellular matrix (ECM) plays an important role in the regulation of cell function (Mauch 1998), and changes in its composition and structure as a result of AGEs therefore could have profound pathophysiological implications in the genesis of diabetic complications (McLennan et al 2002). The remodelling of ECM both in normal and pathological conditions is controlled by a group of enzymes called matrix metalloproteinases (MMPs) (Nagase & Woessner 1999).

MMPs are a family of structurally and functionally related zinc endopeptidases that are capable of in-vitro and in-vivo degradation of all kinds of ECM protein components, such as interstitial basement membrane collagen, proteoglycans, fibronectin and laminin, and thus are implicated in connective tissue remodelling processes associated with various pathological conditions (Bissell 1998). The activity of MMPs is tightly regulated by tissue inhibitors of MMPs (TIMPs), and the MMP/TIMP ratio is critical for coordinating matrix production and degradation (Vincenti 2001). Streptozotocin (STZ) is widely used to induce diabetes in experimental animal models and is associated with the generation of reactive oxygen species (ROS) causing oxidative damage (Szkudelski 2001). MMP activity is reduced in glomeruli of rats with STZ-induced diabetes (Jacot et al 1996). The role of ROS in increased ECM synthesis in diabetic kidney has been well established (Ha & Lee 2000).

Rutin (5,7,3′,4′-hydroxy-3-rutinose), the glycosidic form of quercetin (3,3′,4′,5,7-pentahydroxy flavone), is one of the flavonoids most abundantly consumed in foods

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(Nakamura et al 2000). In Western diets, the richest sources of quercetin glycosides are onions (347 mg kg^{-1}), apples (36 mg kg^{-1}), tea (20 mg kg^{-1}) and red wine (11 mg kg^{-1}) (Hertog et al 1993). A wide variety of pharmacological actions of rutin have been reported – antitumour (Deschner et al 1991), anti-inflammatory (Aleksandrov et al 1986), anti-diarrhoeal (Di Carlo et al 1993), anti-mutagenic (Bear & Teel 2000), myocardial protecting (Pozin et al 1996), immunomodulatory (Chen et al 2000) and hepatoprotective activity (Janbaz et al 2002).

A study by Gao et al (2002) showed that rutin increased the antioxidant status in normal mouse liver. In an in-vitro study, Nagasawa et al (2003a) have shown that rutin and a rutin analogue exhibited significant antioxidant activity. Nagasawa et al (2003b) have also shown that 0.2% of G-rutin (a rutin-glucose derivative), supplemented in 20% casein diet for a period of one month to STZ-induced diabetic rats, decreased kidney thiobarbituric acid reactive substances (TBARS). Odetti et al (2003) have shown that oxerutin treatment reduced the accumulation of collagen-linked fluorescence in skin. It also reduced glomerular accumulation of N^{ε} -(carboxymethyl) lysine and protected against the increase in glomerular volume. Furthermore, the apoptosis rate was significantly decreased and the glomerular cell density was better preserved. A report by Krishna et al (2005) showed that STZ-diabetic rats treated with cyclodextrin complexes of rutin had significantly decreased serum triglycerides and cholesterol.

Previously, we have reported that in STZ-induced diabetic rats, rutin decreased plasma glucose, glycosylated haemoglobin, plasma TBARS and lipid hydroperoxides (HP) and increased the levels of plasma insulin, C-peptide, haemoglobin and non-enzymic antioxidants, such as reduced glutathione, vitamin C, vitamin E and ceruloplasmin (Kamalakkannan & Stanely Mainzen Prince 2006). In our laboratory, we have also reported that rutin possesses an antioxidant effect in isoproterenol-induced myocardial infarction in rats (Karthick & Stanely Mainzen Prince 2006). In this study, we investigated the role of rutin on the content of hydroxyproline and collagen in the kidney of STZ-induced diabetic rats. The activity of MMPs, along with the level of TIMPs, in the kidney was also studied.

Materials and Methods

Animals

The experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and the experimental protocol was approved by the Animal Ethical Committee of Annamalai University (Reg. No. 160/99/CPCSEA; vide No. 169, 2003). Male albino Wistar rats, 150–180 g (Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University), were used in this study. The rats were allowed free access to a standard pellet diet (Pranav Agro Industries, Pune, India) and water. The pellet diet consisted of 22.02% crude protein, 4.25% crude oil, 3.02% crude fibre, 7.5% ash, 1.38% sand silica, 0.8% calcium, 0.6% phosphorus, 2.46% glucose, 1.8% vitamins and 56.17% carbohydrates. It provided a metabolizable energy of 3600 kcal. The rats were maintained in a controlled environment (12-h light–dark cycle) and temperature ($30 \pm 2^{\circ}$C) and were acclimatized to the laboratory conditions before starting the experiment.

Chemicals

Rutin hydrate, STZ, Tris, acrylamide, bis-acrylamide, gelatin, sodium dodecyl sulfate (SDS), ammonium persulfate, 2,4,6-trinitrobenzene sulfonic acid (TNBSA) and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and biochemicals used in this study were of analytical grade.

Induction of experimental diabetes

STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5) and maintained on ice before use. The overnight-fasted rats were made diabetic with a single intraperitoneal injection of STZ (50 mg kg^{-1}). Control rats were injected with citrate buffer alone. Diabetes was confirmed in the STZ-treated rats by measuring the fasting plasma glucose levels 72 h post injection. After an overnight fast, blood was withdrawn by sinocular puncture (0.2 mL) from rats in tubes containing potassium oxalate and sodium fluoride as anticoagulant. Plasma was separated after centrifugation and glucose was estimated using a commercial glucose kit (Product No. 72101; Qualigens Diagnostics, Mumbai, India). Rats with plasma glucose levels above 13.89 mmol L^{-1} (250 mg dL^{-1}) were considered as diabetic (Kamalakkannan & Stanely Mainzen Prince 2006) and were used in the experiment. Treatment with rutin was started on the third day after STZ injection (i.e. after the estimation of plasma glucose).

Experimental design

Previously, a pilot study was conducted with three doses of rutin (25, 50 and 100 mg kg^{-1}) to determine the dose-dependent effect in STZ-induced diabetic rats. We found that 25 mg kg^{-1}, 50 mg kg^{-1} and 100 mg kg^{-1} of rutin significantly ($P < 0.05$) decreased plasma glucose levels and rutin at a dose of 100 mg kg^{-1} was more effective in reducing plasma glucose levels significantly ($P < 0.05$) after 45 days of experimental study. Hence, we chose the dose 100 mg kg^{-1} of rutin for further studies (Kamalakkannan & Stanely Mainzen Prince 2006).

For this study, four groups of rats were maintained, each containing 8 rats: Group I, normal control; Group II, normal + rutin (100 mg kg^{-1}) (Kamalakkannan & Stanely Mainzen Prince 2006); Group III, diabetic control; Group IV, diabetic + rutin (100 mg kg^{-1})

(Kamalakkannan & Stanely Mainzen Prince 2006). Rutin was suspended in carboxymethylcellulose (CMC) ($0.01\,g\,mL^{-1}$) and was orally administered to rats using an intragastric tube for a period of 45 days. Normal control and diabetic control rats received CMC alone.

After the last treatment (45 days), rats were fasted overnight and sacrificed by cervical decapitation. Blood was collected and plasma was obtained after centrifugation. Kidneys from the rats were removed, cleared of blood and collected in ice-cold containers containing Tris-HCl buffer (pH 8.9). The kidney tissue was homogenized in Tris-HCl buffer (pH 8.9) for the estimation of MMPs and TIMPs. For the estimation of hydroxyproline and collagen, kidneys were washed thoroughly in phosphate-buffered saline (PBS) containing protease inhibitors and the weighed kidneys were used for extraction.

Biochemical investigations

Estimation of plasma glucose and insulin

Plasma glucose was estimated as stated above. Plasma insulin was assayed by an enzyme-linked immunosorbent assay (ELISA) method using a commercial kit (Catalog No. SP-401) from United Biotech Inc. (Mountain View, CA, USA).

Estimation of hydroxyproline and collagen

The content of hydroxyproline was estimated by the method of Woessner (1961). The hydrolysate (2.0 mL) and a series of standards containing 2–10 μg of hydroxyproline in a total volume of 2.0 mL were mixed with 1.0 mL of chloramine-T. The contents of the tubes were mixed thoroughly and allowed to stand for 20 min at room temperature. Chloramine-T was then destroyed by adding 1.0 mL of perchloric acid to each tube. The contents were mixed and allowed to stand for 5 min. Finally, 1.0 mL of *p*-dimethylaminobenzaldehyde (PDAB) solution was added and the mixture was shaken. The tubes were incubated in a 60°C water bath for 20 min, then cooled and the colour developed was read spectrophotometrically at 557 nm. The collagen content of tissue samples was arrived at by multiplying the hydroxyproline content by a factor 7.46.

Estimation of total MMP activity

Total MMP activity was assessed by multiwell zymography (Sudhakaran & Ambili 1998). About 50–100 μL of the tissue homogenate was added to the wells of a multiwell plate. Gelatin was added to standard Laemmli acrylamide polymerization mixture, at a final concentration of $1.0\,mg\,mL^{-1}$ under non-reducing conditions. This was added quickly into each well and allowed to set. After the gel had polymerized, it was gently detached from the bottom of the well and incubated for 36 h with Tris-HCl (0.05 M, pH 7.0/5 mM $CaCl_2$). Each gel bit was then stained using Coomassie blue and destained in water. The total MMP activity was quantitated using a laser densitometer (Gel/chemi Doc, Bio-rad. Software: quantity one 4.4.1).

Estimation of individual expression of MMPs

The individual expression of MMPs was analysed by gelatin zymography (Ambili et al 1997). Gelatin was added to standard Laemmli acrylamide polymerization mixture at a final concentration of $1.0\,mg\,mL^{-1}$ under non-reducing conditions. Concentrated tissue extract was mixed with 3:1 with substrate gel sample buffer and 20–30 μL were loaded immediately into Laemmli acrylamide stacking gel on a cast vertical mini gel. Gels were run at 15 mA/gel while stacking and at 20 mA/gel during the separating phase at 4°C. Following electrophoresis, the gels were soaked in 2.5% Triton X-100 with gentle shaking for 30 min at room temperature with one change of detergent solution. Gel was rinsed and incubated overnight at 37 °C in Tris-HCl (0.05 M, pH 7.5/5 mM $CaCl_2$). The gel was then stained using Coomassie blue and destained in water. The individual activity of MMPs was quantitated using a laser densitometer (Gel/chemi Doc, Bio-rad. Software: quantity one 4.4.1).

Estimation of the activity of MMPs

MMP-2 and MMP-9 were estimated by the succinylation method (Baragi et al 2000). Gelatin was dissolved in 50 mM borate buffer, pH 8.5 at a concentration of $20\,mg\,mL^{-1}$. An equal amount of succinic anhydride was then gradually added to the solution and the pH of the reaction was maintained at 8.0–8.5 by the addition of 1.0 M NaOH. The succinylated gelatin was then dialysed extensively against 50 mM sodium borate buffer, pH 8.5. All assays were done in 96-well flat-bottom microtitre plates. MMP-2 was assayed in 50 mM borate buffer with 10 mM $CaCl_2$, pH 7.0, whereas MMP-9 was assayed in 50 mM borate buffer, pH 8.5. The total reaction volume was 150 μL, which contained enzyme (tissue homogenate) and 200 μg of succinylated gelatin. Blank without substrate but with appropriate buffer and enzyme was performed for each enzyme assay. The reaction was carried out at 37 °C for 30 min. Fifty microlitres of 0.3% TNBSA was then added to the reaction mixture and incubated at room temperature for 20 min. The optical density was determined at 420 nm. The activity of MMP-2 and MMP-9 was expressed as 0.01 change in optical density at 420 nm/min/mg protein.

Estimation of TIMPs

The levels of TIMP-1 and TIMP-2 were estimated by the technique of ELISA. One-hundred microlitres of tissue homogenate was coated in different wells in 24-well ELISA plates and incubated at room temperature for 3 h. After incubation, the wells were drained and washed with PBS twice. Then 200 μL of 0.05% Tween-20 was added and incubated at room temperature for 1 h. The plates were washed with 0.05% Tween-20 in PBS twice followed by the addition of 100 μL of primary antibody (TIMP-1 antibody, 1:500 in gelatin–Tween–PBS, and TIMP-2 antibody, 1:1000 in Tween–PBS), incubation at room temperature for 2 h, and washing in Tween-20 twice. Then secondary antibody (anti-rabbit IgG peroxidase, 1:1000 in Tween–PBS) coupled to horseradish peroxidase

was added and kept at room temperature for 1 h. Plates were washed again with Tween–PBS followed by PBS twice. Then 1.0 mL of o-dianisidine was added to all the wells and incubated in the dark for 30 min. The reaction was arrested by adding 5 M HCl and the optical density was measured at 450 nm. The levels of TIMP-1 and TIMP-2 were expressed as 0.01 change in optical density at 450 nm/min/mg protein.

Estimation of protein

Protein content in the tissue homogenate was determined by the method of Lowry et al (1951). Five-hundred microlitres of tissue homogenate was precipitated with 500 μL of 10% TCA, centrifuged for 10 min and the precipitate was dissolved in 1.0 mL of 0.1 M sodium hydroxide. A 0.1-mL volume was taken and made up to 1.0 mL with distilled water. Then 4.5 mL of alkaline copper reagent was added and allowed to stand at room temperature for 10 min. After incubation, 0.5 mL of Folin's-Ciocalteau reagent was added and the blue colour developed was read after 20 min at 620 nm. A standard curve was obtained using BSA. The protein content was expressed as mg (g tissue)$^{-1}$.

Statistical analysis

All the grouped data were analysed by one-way analysis of variance followed by Duncan's multiple range test (DMRT) using SPSS software package, version 9.05. $P < 0.05$ was considered as significant.

Results

Fasting plasma glucose levels were significantly ($P < 0.05$) increased in diabetic control rats (21.17 ± 1.62 mmol L^{-1}) when compared with normal control rats. Diabetic rats treated with rutin had significantly ($P < 0.05$) decreased plasma glucose levels (7.89 ± 0.60 mmol L^{-1}) in comparison with diabetic control rats. Normal rats treated with rutin did not show any significant ($P > 0.05$) change in plasma glucose levels (3.89 ± 0.30 mmol L^{-1}). The levels of plasma glucose in normal control rats was found to be 3.83 ± 0.29 mmol L^{-1}. A significant ($P < 0.05$) decrease in plasma insulin levels was observed in diabetic control rats (6.89 ± 0.22 μU mL^{-1}) when compared with normal control rats, and on treatment with rutin the levels significantly ($P < 0.05$) increased (10.92 ± 0.48 U mL^{-1}) compared with diabetic control rats. There was no significant change in the levels of plasma insulin in normal rats treated with rutin (13.74 ± 1.05 U mL^{-1}). The plasma insulin level in normal control rats was observed to be 13.67 ± 1.04 μU mL^{-1}.

Figure 1 shows the content of hydroxyproline and collagen in the kidney of normal and experimental rats. Significantly ($P < 0.05$) increased content of hydroxyproline and collagen in the kidney of diabetic control rats was observed when compared with normal control rats. Rutin-treated diabetic rats had a significantly ($P < 0.05$) decreased content of hydroxyproline and collagen in the kidney when compared with diabetic control rats.

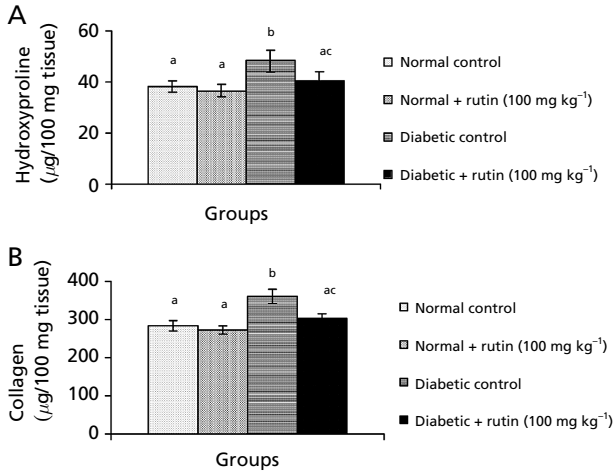
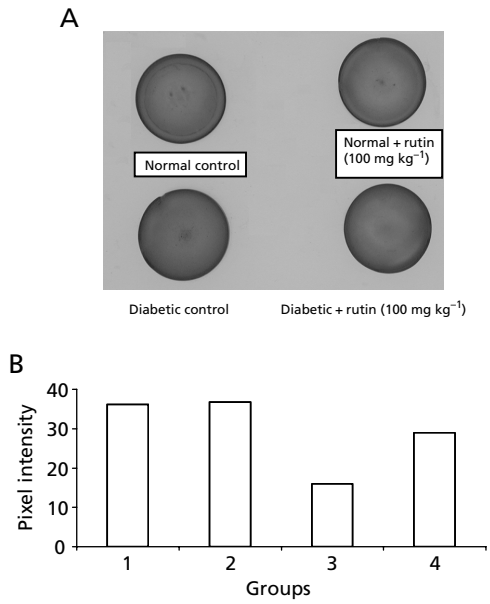


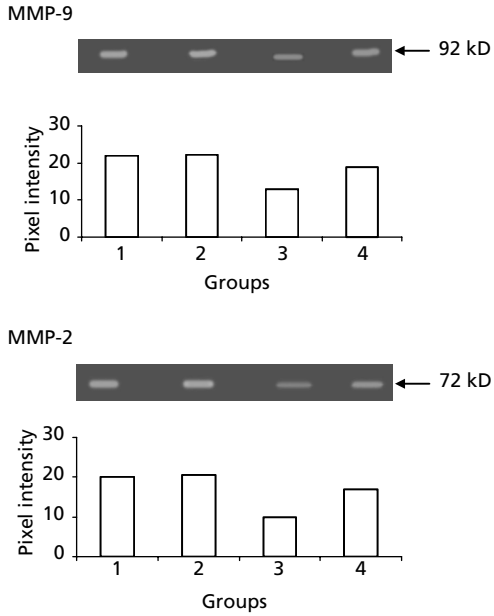
Figure 1 Effect of rutin on the content of hydroxyproline and collagen in the kidney of normal and diabetic rats. Data are presented as means ± s.d. for 8 rats in each group (n = 8). Columns that have a different superscript letter (a, b, c) differ significantly from each other ($P < 0.05$, DMRT).

Figure 2A shows the multi-well zymogram of MMPs and Figure 2B shows their relative pixel intensity. Figure 3 shows the individual activity of MMPs (MMP-2 (72 kD) and MMP-9 (92 kD)) and their relative pixel intensity. Decreased activity of the MMPs was observed in the kidney of diabetic control rats when compared with normal control



Group 1: Normal control; Group 2: Normal + rutin (100 mg kg^{-1}); Group 3: Diabetic control; Group 4: Diabetic + rutin (100 mg kg^{-1})

Figure 2 A. Multi-well zymography of kidney samples of normal and diabetic rats. B. Densitometry of the kidney multi-well zymogram.

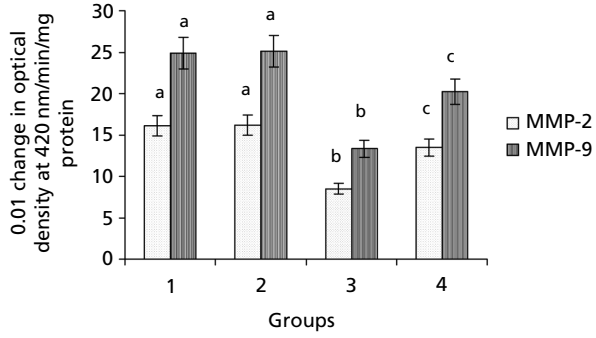


Group 1: Normal control; Group 2: Normal + rutin (100 mg kg^{-1});
Group 3: Diabetic control; Group 4: Diabetic + rutin (100 mg kg^{-1})

Figure 3 The zymogram and its relative pixel intensity of MMP-9 (92 kD) and MMP-2 (72 kD) in kidney of normal and diabetic rats.

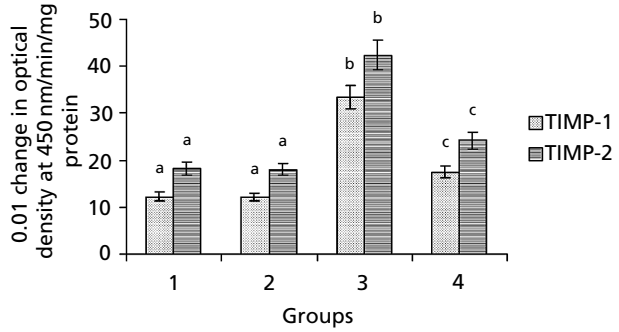
rats. Administration of rutin to diabetic rats increased the activity of MMPs when compared with diabetic control rats.

Figure 4 shows the changes in the activity of MMP-2 and MMP-9 in the kidney of normal and diabetic rats. The activity of these MMPs significantly ($P < 0.05$) decreased in diabetic control rats when compared with normal control rats. Administration of rutin to diabetic rats increased the activity of these MMPs significantly ($P < 0.05$) on comparison with diabetic control rats.



Group 1: Normal control; Group 2: Normal + rutin (100 mg kg^{-1});
Group 3: Diabetic control; Group 4: Diabetic + rutin (100 mg kg^{-1})

Figure 4 Effect of rutin on the activity of MMP-2 and MMP-9 in the kidney of normal and diabetic rats. Data are presented as means ± s.d. for 8 rats in each group (n = 8). Columns that have a different superscript letter (a, b, c) differ significantly from each other ($P < 0.05$, DMRT).



Group 1: Normal control; Group 2: Normal + rutin (100 mg kg^{-1});
Group 3: Diabetic control; Group 4: Diabetic + rutin (100 mg kg^{-1})

Figure 5 Effect of rutin on the levels of TIMP-1 and TIMP-2 in the kidney of normal and diabetic rats. Data are presented as means ± s.d. for 8 rats in each group (n = 8). Columns that have a different superscript letter (a, b, c) differ significantly from each other ($P < 0.05$, DMRT).

The levels of TIMP-1 and TIMP-2 were significantly ($P < 0.05$) increased in the kidney of diabetic control rats (Figure 5) on comparison with normal control rats. When rutin was administered to diabetic rats, the levels of TIMPs significantly ($P < 0.05$) decreased in the kidney compared with control diabetic rats.

Discussion

In our previous study, we observed that STZ administration to rats resulted in increased levels of plasma glucose and decreased levels of insulin. Treatment with rutin decreased plasma glucose and increased insulin levels in diabetic rats. Rutin, by its ability to scavenge free radicals and to inhibit lipid peroxidation, prevents STZ-induced oxidative stress and protects β-cells, resulting in increased insulin secretion and decreased plasma glucose levels (Kamalakkannan & Stanely Mainzen Prince 2006).

Effect of rutin on the content of hydroxyproline and collagen

A balance between ECM synthesis and degradation is a prerequisite for maintaining the structural and functional integrity of the glomerulus (Lenz et al 2000). The pathogenesis of both experimental and human renal disease has consistently implicated locally active growth factors, transforming growth factor (TGF) and platelet-derived growth factor (PDGF) (Johnson et al 1993; Border & Noble 1994). In-vitro studies have shown that AGEs induce increased ECM production via both TGF- and PDGF-dependent mechanisms (Throckmorton et al 1995).

Diabetes is associated with an increase in ECM, which because of its slow turnover is particularly susceptible to AGE accumulation (McLennan et al 2002). AGEs accumulate in diabetic tissues at an accelerated rate as a consequence

of increased glucose concentration in extracellular fluids (Kelly et al 2001). Deposition of AGEs also occurs in both the tubulointerstitium and glomerulus in STZ-induced diabetic rats (Kelly et al 2001). Increased content of hydroxyproline and collagen were observed in the kidney of diabetic rats in this study. Riva et al (1998) had reported that diabetes induced by STZ leads to an increased collagen deposition. Rutin-treated diabetic rats showed significantly decreased content of hydroxyproline and collagen in the kidney.

Effect of rutin on MMP activity and levels of TIMPs

Matrix metalloproteinases are a family of proteolytic enzymes that share several structural and functional characteristics, but have different substrate specificities (Nagase & Woessner 1999). They are synthesized as latent proenzymes, which are later activated by serine proteases, including trypsin and plasmin, active MMP-2 and membrane-type (MT)-MMPs (Nagase & Woessner 1999). MMPs are involved in several levels of ECM turnover, and thus play a crucial role in maintaining the balance between ECM synthesis and degradation (Lenz et al 2000). Of the currently known MMPs, MMP-1, MMP-13, MMP-3, MMP-2, MMP-9 and MT-1-MMP have been extensively studied in the kidney (Lenz et al 2000). MMP-2 and MMP-9 are often detected by gelatin zymography because of their high-gelatinolytic activity (Okada et al 1992). These two gelatinases (MMP-2 and MMP-9) have a gelatin-binding domain inserted between the catalytic domain and the active site domain (Kleiner & Stetler-Stevenson 1999). MMP-2 and MMP-9 form proenzyme complexes with TIMP-2 and TIMP-1, respectively (Gomez et al 1997), and both these TIMPs have been shown to be expressed in human glomeruli (Carome et al 1993).

Decreased activity of MMP-2 and MMP-9 and increased levels of TIMP-1 and TIMP-2 were observed in this study. An increase in ROS might contribute to MMPs/TIMPs imbalance (Campo et al 2004) due to the hyperglycaemic state observed in diabetic rats. Arthur (1990) has shown that large amounts of ROS were able to enhance TIMP generation, which in turn favoured collagen deposition by inhibiting MMP's activity. Oxygen-derived free radicals stimulate the synthesis of collagen, the main component of ECM (Lin et al 1993). Oxidative stress contributes to matrix accumulation either directly due to inhibition of MMP-2 (Mattana et al 1998), or by inducing a cytokine response (Studer et al 1997).

In STZ-induced diabetic rats, Nakamura et al (1994) have shown downregulation of MMPs. An upregulation of TIMP-1 was also observed by Wu et al (1997) in STZ-induced diabetic rats. Also, a decrease in the expression of MMP-2 activity was reported in the glomeruli of STZ-diabetic rats by Feng-qin et al (2004). A marked decrease in MMP-2 mRNA expression was detected in the glomeruli of diabetic patients (Del Prete et al 1997). High glucose concentrations have been shown to act on mesangial cells directly to decrease the activity of MMPs (Nagase & Woessner 1999). In-vitro studies of the effect of high ambient glucose on ECM turnover revealed an increased expression of matrix molecules, while the activity of MMP-2 and MMP-9 were decreased in mesangial cells (McLennan et al 1998). Other mechanisms that might also lead to the decreased activity of MMPs in diabetic rats include the excessive production of insulin-like growth factor-1 (IGF-1) in diabetes, which decreases both mRNA levels and MMP-2 activity (Lupia et al 1999) and hyperfiltration, hyper-perfusion and hypertension in capillaries appearing in the early stages of diabetes, which leads to decreased expression of MMP-2 mRNA (Yasuda et al 1996).

Administration of rutin to diabetic rats increased the expression of MMP-2 and MMP-9, as well as decreasing the levels of TIMP-1 and TIMP-2. This is achieved by decreasing the oxidative stress caused by hyperglycaemia by means of increased plasma insulin levels and improved antioxidant status in diabetic kidney. In this context, we have previously reported that rutin increased the plasma insulin levels in STZ-induced diabetic rats (Kamalakkannan & Stanely Mainzen Prince 2006). We have also observed that in rutin treated diabetic kidney there was a significant decrease in lipid peroxidative products, such as TBARS and HP and a significant increase in the activity of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase and reduced glutathione concentration. Rutin administration also improved renal function and reduced damage to the kidney in diabetic rats (data not shown). Also, Schaefer et al (1994) have reported that decreased gelatinase activity in STZ-treated diabetic rats can be restored by insulin treatment. Campo et al (2004) have shown that reduction in ROS might decrease expression of the TIMP gene and its activity. It has been observed that glycaemic control can prevent, or at least slow, the progression of diabetic nephropathy in a number of patients (The Diabetes Control and Complications Trial Research Group 1993). The effects observed are due to the reduction of plasma glucose levels by rutin.

The results of our study show that rutin is effective in decreasing the content of collagen and hydroxyproline and maintaining the balance between MMPs/TIMPs by increasing the activity of MMPs and decreasing the levels of TIMPs in the kidney of STZ-induced diabetic rats.

References

Aleksandrov, P. N., Speranskaia, T. V., Bobkov Iu, G., Zagorevskii, V. A., Zykov, D. A. (1986) Effect of rutin and esculamine on models of aseptic inflammation. *Farmakol. Toksikol.* **49**: 84–86

Ambili, M., Pillai, M. R., Sudhakaran, P. R. (1997) Characteristics of 60 kD gelatinase involved in rat mammary gland involution. *Indian J. Biochem. Biophys.* **34**: 347–353

Arthur, M. J. P. (1990) Matrix degradation in the liver. *Semin. Liver Dis.* **10**: 47–55

Baragi, V. M., Shaw, B. J., Renkiewicz, R. R., Kuipers, P. J., Welgus, H. G., Mathrubutham, M., Cohen, J. R., Rao, S. K.

(2000) A versatile assay for gelatinases using succinylated gelatin. *Matrix Biol*. **19**: 267–273

Bear, W. L., Teel, R. W. (2000) Effects of citrus flavonoids on the mutagenicity of heterocyclic amines and on cytochrome P450 1A2 activity. *Anticancer Res*. **20**: 3609–3614

Bissell, D. M. (1998) Hepatic fibrosis as wound repair; a progress report. *J. Gastroenterol*. **33**: 295–302

Border, W. A., Noble, N. A. (1994) Transforming growth factor beta in tissue fibrosis. *N. Engl. J. Med*. **331**: 1286–1292

Bretzel, R. G., Voigt, K., Schatz, H. (1998) The United Kingdom Prospective Diabetes Study (UKPDS): implications for the pharmacotherapy of type 2 diabetes mellitus (Editorial). *Exp. Clin. Endocrinol. Diabetes* **106**: 369–372

Campo, G. M., Avenoso, A., Campo, S., D'Ascola, A., Ferlazzo, A. M., Calatroni, A. (2004) The antioxidant and antifibrogenic effects of the glycosaminoglycans, hyaluronic acid and chondroitin-4-sulphate in a subchronic rat model of carbon tetrachloride-induced liver fibrogenesis. *Chem. Biol. Interact*. **148**: 125–138

Carome, M. A., Striker, L. J., Peten, E. P., Moore, J., Yang, C. W., Stetler-Stevenson, W. G., Striker, G. E. (1993) Human glomeruli express TIMP-1 mRNA and TIMP-2 protein and mRNA. *Am. J. Physiol*. **264**: F923–F929

Chen, S., Gong, J., Liu, F., Mohammed, U. (2000) Naturally occurring polyphenolic antioxidants modulate IgE-mediated mast cell activation. *Immunology* **100**: 471–480

Del Prete, D., Anglani, F., Forino, M., Ceol, M., Fioretto, P., Nosadini, R., Baggio, B., Gambaro, G. (1997) Down-regulation of glomerular matrix metalloproteinase-2 gene in human NIDDM. *Diabetologia* **40**: 1449–1454

Deschner, E. E., Ruperto, J., Wong, G., Newmark, H. L. (1991) Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis* **12**: 1193–1196

Di Carlo, G., Autore, G., Izzo, A. A., Maiolino, P., Mascolo, N., Viola, P., Diurno, M. V., Capasso, F. (1993) Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure-activity relationships. *J. Pharm. Pharmacol*. **45**: 1054–1059

Feng-qin, D., Hong, L., Wei-min, C., Jun, T., Qun, L., Yu, R., Fen-ping, Z., Zhe, Z. (2004) Effects of pioglitazone on expressions of matrix metalloproteinases 2 and 9 in kidneys of diabetic rats. *Chin. Med. J*. **117**: 1040–1044

Gao, Z., Xu, H., Huang, K. (2002) Effects of rutin supplementation on antioxidant status and iron, copper, and zinc contents in mouse liver and brain. *Biol. Trace Elem. Res*. **88**: 271–279

Gomez, D. E., Alonso, D. F., Yoshiji, H., Thorgeirsson, U. P. (1997) Tissue inhibitors of metalloproteinases: structure, regulation and biological functions. *Eur. J. Cell. Biol*. **74**: 111–122

Ha, H., Lee, H. B. (2000) Reactive oxygen species as glucose signaling molecules in mesangial cells cultured under high glucose. *Kidney Int*. **58**: S19–S25

Hertog, M. G., Hollman, P. C., Katan, M. B., Kromhout, D. (1993) Intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr. Cancer* **20**: 21–29

Jacot, T. A., Striker, G. E., Stetler-Stevenson, M. A., Striker, L. J. (1996) Mesangial cells from transgenic mice with progressive glomerulosclerosis exhibit stable, phenotypic changes including undetectable MMP-9 and increased type IV collagen. *Lab. Invest*. **75**: 791–799

Janbaz, K. H., Saeed, S. A., Gilani, A. H. (2002) Protective effect of rutin on paracetamol- and CCl_4-induced hepatotoxicity in rodents. *Fitoterapia* **73**: 557–563

Johnson, R. J., Floege, J., Couser, W. G., Alpers, C. E. (1993) Role of platelet derived growth factor in glomerular disease. *J. Am. Soc. Nephrol*. **4**: 119–128

Kamalakkannan, N., Stanely Mainzen Prince, P. (2006) The antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic Wistar rats. *Basic Clin. Pharmacol. Toxicol*. **98**: 97–103

Karthick, M., Stanely Mainzen Prince, P. (2006) Preventive effect of rutin, a bioflavonoid on lipid peroxides and antioxidants in isoproterenol induced myocardial infarction in rats. *J. Pharm. Pharmacol*. **58**: 701–707

Kelly, D. J., Gilbert, R. E., Cox, A. J., Soulis, T., Jerums, G., Cooper, M. E. (2001) Aminoguanidine ameliorates overexpression of prosclerotic growth factors and collagen deposition in experimental diabetic nephropathy. *J. Am. Soc. Nephrol*. **12**: 2098–2107

Kleiner, D. E., Stetler-Stevenson, W. G. (1999) Matrix metalloproteinases and metastasis. *Cancer Chemother. Pharmacol*. **43**: S42–S51

Krishna, K. M., Annapurna, A., Gopal, G. S., Chalam, C. R., Madan, K., Kumar, V. K., Prakash, G. J. (2005) Partial reversal by rutin and quercetin of impaired cardiac function in streptozotocin-induced diabetic rats. *Can. J. Physiol. Pharmacol*. **83**: 343–355

Lenz, O., Elliot, S. J., Stetler-Stevenson, W. G. (2000) Matrix metalloproteinases in renal development and disease. *J. Am. Soc. Nephrol*. **11**: 574–581

Lin, T. A., Lustig, K. D., Sportiello, M. G., Weisman, G. A., Sun, G. Y. (1993) Signal transduction pathways coupled to a P29 receptor in neuroblastoma x glioma (NG 108-15) cells. *J. Neurochem*. **60**: 1115–1125

Lowry, O. H., Rosebrough, M. J., Farr, A. L, Randall, R. J. (1951) Protein measurement with Folin-phenol reagent. *J. Biol. Chem*. **193**: 265–275

Lupia, E., Elliot, S. J., Lenz, O., Zheng, F., Hattori, M., Striker, G. E., Striker, L. J. (1999) IGF-1 decreases collagen degradation in diabetic NOD mesangial cells: possible role in mesangium accumulation. *Diabetologia* **43**: 642–648

Mattana, J., Margiloff, L., Sharma, P., Singhal, P. C. (1998) Oxidation of the mesangial matrix metalloproteinase-2 impairs gelatinolytic activity. *Inflammation* **22**: 269–276

Mauch, C. (1998) Regulation of connective tissue turnover by cell-matrix interactions. *Arch. Dermatol. Res*. **290**: S30–S36

McLennan, S. V., Yue, D. K., Turtle, J. R. (1998) Effect of glucose on matrix metalloproteinase activity in mesangial cells. *Nephron* **79**: 293–298

McLennan, S. V., Martell, S. K. Y., Yue, D. K. (2002) Effects of mesangium glycation on matrix metalloproteinase activities. Possible role in diabetic nephropathy. *Diabetes* **51**: 2612–2618

Monnier, V. M., Bautista, O., Kenny, D., Sell, D. R., Fogarty, J., Dahms, W., Cleary, P. A., Lachin, J., Genuth, S. (1999) Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA_{1C} as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial. *Diabetes* **48**: 870–880

Nagasawa, T., Tabata, N., Ito, Y., Nishizawa, N., Aiba, Y., Kitts, D. D. (2003a) Inhibition of glycation reaction in tissue protein incubations by water soluble rutin derivative. *Mol. Cell Biochem*. **249**: 3–10

Nagasawa, T., Tabata, N., Ito, Y., Aiba, Y., Nishizawa, N., Kitts, D. D. (2003b) Dietary G-rutin suppresses glycation in tissue proteins of streptozotocin-induced diabetic rats. *Mol. Cell Biochem*. **252**: 141–147

Nagase, H., Woessner, F. (1999) Matrix metalloproteinases. *J. Biol. Chem.* **274**: 21491–21494

Nakamura, T., Fukui, M., Ebihara, I., Osada, S., Tomino, Y., Koide, H. (1994) Abnormal gene expression of matrix metalloproteinases and their inhibitor in glomeruli from diabetic rats. *Ren. Physiol. Biochem.* **17**: 316–325

Nakamura, Y., Ishimitsu, S., Tonogai, Y. (2000) Effects of quercetin and rutin on serum and hepatic lipid concentrations, fecal steroid excretion and serum antioxidant properties. *J. Health Sci.* **46**: 229–240

Odetti, P., Pesce, C., Traverso, N., Menini, S., Maineri, E. P., Cosso, L., Valentini, S., Patriarca, S., Cottalasso, D., Marinari, U. M., Pronzato, M. A. (2003) Comparative trial of N-acetylcysteine, taurine, and oxerutin on skin and kidney damage in long-term experimental diabetes. *Diabetes* **52**: 499–505

Okada, Y., Gonoji, Y., Naka, K., Tomita, K., Nakanishi, I., Iwata, K., Yamashita, K., Hayakawa, T. (1992) Matrix metalloproteinase 9 (92 kD gelatinase/type IV collagenase) from HT 1080 human fibrosarcoma cells. Purification and activation of the precursor and enzymic properties. *J. Biol. Chem.* **267**: 21712–21719

Pozin, V. M., Skuratovskaia, S. G., Pocheptsova, G. A. (1996) Changes in the vascular wall and ischemic damages to the myocardium in reversible episodes of heart muscle ischemia. *Fiziol. Zh.* **42**: 10–16

Riva, E., Andreoni, G., Bianchi, R., Latini, R., Luvara, G., Jeremic, G., Traquandi, C., Tuccinardi, L. (1998) Changes in diastolic function and collagen content in normotensive and hypertensive rats with long-term streptozotocin-induced diabetes. *Pharmacol. Res.* **37**: 233–240

Schaefer, L., Schaefer, R. M., Ling, H., Teschner, M., Heidland, A. (1994) Renal proteinases and kidney hypertrophy in experimental diabetes. *Diabetologia* **37**: 567–571

Studer, R. K., Craven, P. A., DeRubertis, F. R. (1997) Antioxidant inhibition of protein kinase C-signaled increases in transforming growth factor-beta in mesangial cells. *Metabolism* **46**: 918–925

Sudhakaran, P. R., Ambili, M. (1998) Assay of matrix metalloproteinases in substrate impregnated gels in multiwells. *Indian J. Biochem. Biophys.* **35**: 317–320

Szkudelski, T. (2001) The mechanism of alloxan and streptozotocin action in β-cells of the rat pancreas. *Physiol. Res.* **50**: 536–546

The Diabetes Control and Complications Trial Research Group. (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **329**: 977–986

Throckmorton, D. C., Brogden, A. P., Min, B., Rasmussen, H., Kashgarian, M. (1995) PDGF and TGF-beta mediate collagen production by mesangial cells exposed to advanced glycosylation end products. *Kidney Int.* **48**: 111–117

Vincenti, M. P. (2001) The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase genes. *Methods Mol. Biol.* **151**: 121–148

Woessner, J. F. (1961) The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch. Biochem. Biophys.* **93**: 440–447

Wu, K., Setty, S., Mauer, S. M., Killen, P., Nagase, H., Michael, A. F., Tsilibary, E. C. (1997) Altered kidney matrix gene expression in early stages of experimental diabetes. *Acta Anat.* **158**: 155–165

Yasuda, T., Hondo, S., Homma, T., Harris, R. C. (1996) Regulation of extracellular matrix by mechanical stress in rat glomerular mesangial cells. *J. Clin. Invest.* **98**: 1991–2000