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Original article

Effect of curcumin and quercetin on lysosomal enzyme activities in streptozotocin-induced diabetic rats

Mallikarjun B. Chougala [a], Jamuna J. Bhaskar [a], M.G.R. Rajan [b], Paramahans V. Salimath [a,*]

[a] Dept. of Biochemistry and Nutrition, Central Food Technological Research Institute, CSIR, Mysore - 570 020, India
[b] Laboratory Nuclear Medicine Section, Tata Memorial Hospital Annexe, Parel, Mumbai - 400 012, India

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SUMMARY

Background & aims: Diabetes causes impairment of various enzyme activities in the physiological system, including lysosomal enzymes. The effect of feeding curcumin, quercetin and aminoguanidine on lysosomal enzyme activities viz., N-acetyl-β-D-glucosaminidase, β-D-glucuronidase, β-D-galactosidase and acid phosphatase were studied in different tissues of streptozotocin-induced diabetic rats.
Method: Rats were divided into four control groups and four diabetic groups. Experimental groups were fed with diet supplemented with curcumin (0.5%) or quercetin (0.1%) or aminoguanidine (0.05%). Lysosomal enzyme activities were determined in various tissues.
Results: The specific activity of N-acetyl-β-D-glucosaminidase in liver of diabetic rats was decreased when compared to control rats and was ameliorated with curcumin and quercetin treatment by 67% and 78%, respectively. On the other hand, β-D-glucuronidase activity was higher in the brain of diabetic rats (0.90 ± 0.04 nmol/mg protein/min), when compared to control rats (0.45 ± 0.02 nmol/mg protein/min) and was decreased in curcumin (0.75 ± 0.05 nmol/mg protein/min) and quercetin (0.74 ± 0.11 nmol/mg protein/min) treated rats. β-D-galactosidase activity in spleen of curcumin and quercetin fed diabetic group rats was ameliorated by 68% and 58%, respectively, in comparison to diabetic rats. Acid phosphatase activity in diabetic rats decreased in testis when compared to control.
Conclusion: Curcumin and quercetin feeding modulated lysosomal enzyme activities in different tissues during diabetes and the effect was comparable to well-known anti-glycative agent - aminoguanidine.

1. Introduction

Diabetes is one of the major metabolic disorders affecting large number of people around the globe. Diabetes is marked by sustained hyperglycemia that causes severe complications and deleterious effects on various organs in the body. The clinical manifestation of diabetes is known to have far more reaching implications than the disease per se. Microvascular and macrovascular complications of diabetes induced by high glucose in blood confer substantial morbidity and impair patient's quality of life.[1] Changes occur in various extracellular matrix components which comprises predominantly of glycoconjugates. Glycoconjugates are polymeric compounds in which sugars are covalently linked to either proteins or lipids.[2] They are ubiquitous in nature and are fundamental to the existence of life. Progress in cell biology has revealed that sugar moieties of glycoconjugates play key roles in many crucial cellular events.[3]

Lysosomal enzymes which are hydrolytic enzymes, play a key role in glycoconjugate metabolism. They catalyze hydrolytic cleavage of glycosidic bonds of glycosaminoglycans, glycoproteins and glycoplipids both in serum and tissues and are crucial in many biological processes.[4] Changes have been observed in the lysosomal enzyme activities in serum and tissues of both experimental animals and human diabetics.[5] It is reported that correlation exists between microvascular complications and glycoconjugate metabolism resulting in an increased rate of glycoprotein catabolism.[6]

Studies have also shown, stress-associated changes in the lysosomal systems in different organs.[7] Oxidative stress, a result of sustained glycemia is one of the factors contributing to diabetic complications. Advanced glycation end products (AGEs) formed by non-enzymatic glycation of reducing sugars such as glucose with amino groups of proteins/lipids/nucleic acids generate excessive amount of free radicals which in turn increases oxidative stress.[8] Hence, combating oxidative stress is one of the strategies available to fight diabetes. There is an increasing demand for natural products with potential antidiabetic activity, with minimum use of insulin and/or oral hypoglycemic drugs.[9] Medicinal plants are being

* Corresponding author. Tel.: +91 821 2514876; fax: +91 821 2517233.
E-mail addresses: paramahans1954@gmail.com, salimath1954@gmail.com (P.V. Salimath).

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used for centuries for treatment of various diseases including diabetes with no adverse side effects.[10] Phytochemicals isolated from plant sources are used for the prevention and treatment of diabetes, cancer, heart disease, high blood pressure, etc.[11]

Curcumin is a well known antioxidant from the rhizome of *curcuma longa* and possesess anti-hyperglycemic activity.[12] Several studies in recent years have shown that curcumin is an effective inhibitor of tumor initiation *in vivo*[13] and possesses anti-proliferative activity against tumor cells *in vitro*.[14] Quercetin is a bioflavonoid that is widely distributed in fruits and vegetables, scavenges free radicals and directly inhibits biomolecular oxidation and alters antioxidant defence pathways *in vivo* and *in vitro*.[15–17] The present study was carried out to determine the effect of phytochemicals such as curcumin and quercetin and a known advanced glycation end products (AGEs) inhibitor - aminoguanidine on various lysosomal enzyme activities namely N-acetyl-β-D-glucosaminidase, N-acetyl-β-D-glucuronidase, N-acetyl-β-D-galactosidase and acid phosphatase in different tissues of control and diabetic rats.

2. Materials and methods

2.1. Materials

Streptozotocin, quercetin, aminoguanidine, PNP-N-acetyl-β-D-glucosaminide, PNP-N-β-D-glucuronide, PNP-N-β-D-galactoside and PNP-acid phosphate were purchased from Sigma, St. Louis, USA. GOD/POD kit was from Span Diagnostics Limited (Surat, India). Curcumin (95% pure) was procured from Flavours and Essences, Mysore, India. Other reagents and chemicals used were of analytical grade.

2.2. Animals and diet

This study had the approval of Institutional Animal Ethical Committee. Male Wistar rats [OUTB-Wistar- IND cft (2c)] weighing around 140–160 g were taken for the experiment from Institute Animal House Facility and were fed with AIN-76 based diet. Rats were divided into following experimental groups; Starch fed control (SFC), starch fed diabetic (SFD), curcumin fed control (CFC), curcumin fed diabetic (CFD), quercetin fed control (QFC), quercetin fed diabetic (QFD), aminoguanidine fed control (AFC) and aminoguanidine fed diabetic (AFD). Streptozotocin (45 mg/kg body weight), prepared in 0.1 mol/L citrate buffer, pH 4.5 was injected to rats in diabetic group. Control rats were injected with citrate buffer. Animals were kept in cages with free access to 5% glucose solution for 24 h subsequent to the injection and thereafter were replaced by tap water.SFC and SFD groups received starch-based diet and CFC and CFD groups received diet supplemented with 0.5% curcumin and QFC and QFD groups received diet supplemented with 0.1% quercetin and AFC and AFD groups received diet supplemented with 0.05% aminoguanidine. The animals were fed with respective diet for 16 weeks after induction of diabetes. At the end of experimental period control groups had 6 rats each and diabetic groups had 6 to 8 rats.

2.3. Analytical methods

One week after streptozotocin injection, blood samples were collected in tubes containing sodium heparin salt (20 IU/mL) from retro-orbital plexus. Fasting blood sugar was measured by glucose oxidase method using commercially available kit. Rats having plasma glucose levels >200 mg/dL were considered diabetic and selected for the study. Urine was collected for a period of 24 h under a layer of toluene after keeping the animals in metabolic cages. Urine sugar was measured by 3, 5-dinitrosalycilic acid method.[18]

2.4. Measurement of lysosomal enzyme activities

Tissue homogenates (1:10) were prepared at 4 °C with acetate buffer (0.1 M, pH 4.5) using homogenizer (REMI) fitted with a Teflon plunger. The supernatant obtained by centrifuging the homogenate at 6000 g for 10 min at 4 °C was taken as a source of enzyme. The enzyme activity was derived as a measure of release of paranitrophenol from the paranitrophenol phosphate (PNP) conjugated substrate (10 mM) prepared in 0.1 M acetate buffer, pH 4.5. The substrate for N-acetyl-β-glucosaminidase (EC3.2.1.30), N-acetyl-β-D-glucuronidase (EC3.2.1.31), N-acetyl-β-D-galactosidase (EC3.2.1.23) and acid phosphatase (EC3.1.32) were PNP-glucosaminide, PNP-glucuronide, PNP-galactoside and PNP-phosphate, respectively. Enzyme substrate reaction mixture (500 µl) was incubated at 37 °C and was terminated by the addition of 2 mL of Na_2CO_3 (2%). The volume of enzyme and time of incubation for each enzyme assay are given in Table 2. Activities of enzymes are expressed in nmol PNP/mg protein/min. The colour intensity developed was read at 400 nm in a spectrophotometer. The protein content was determined by Lowry's method.[19]

2.5. Statistical analysis

All data were expressed as mean ± SD of control, diabetic and curcumin and quercetin treated rats. Statistical analysis of data was performed using one-way analysis of variance (ANOVA) with a Tukey's multiple comparison post-test and significance at $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

3. Results

3.1. Effect of curcumin, quercetin and aminoguanidine on fasting blood sugar, urine volume and urine sugar

Curcumin, quercetin and aminoguanidine showed antidiabetic effect and the data are presented in Table 1. The levels of fasting blood sugar, urine sugar and urine volume were elevated significantly in starch fed diabetic rats in contrast to control rats. Fasting blood sugar increased by four folds in diabetic rats (SFD), when compared to control rats (SFC). Feeding diet supplemented with curcumin (CFD), quercetin (QFD) and aminoguanidine (AFD) to diabetic rats ameliorated increase in fasting blood glucose levels significantly by 37%, 48% and 43%, respectively, when compared to SFD rats ($P < 0.001$). The amelioration of fasting blood sugar by curcumin and quercetin was comparable to aminoguanidine.

Table 1
Effect of curcumin and quercetin on urine volume, urine sugar, fasting blood sugar in control and diabetic rats.

Groups	Final FBS (mg/dL)	Urine sugar (g/24 h)	Urine volume (mL/24 h)
SFC	92.4 ± 8.13	0.018 ± 0.004	15.2 ± 2.05
SFD	373.1 ± 17.70$^{\psi}$	10.08 ± 0.870$^{\psi}$	72.3 ± 4.86$^{\psi}$
CFC	92.5 ± 7.16	0.032 ± 0.003	16.1 ± 3.12
CFD	268.4 ± 16.10***	6.610 ± 1.450***	53.5 ± 1.22***
QFC	88.2 ± 6.35	0.013 ± 0.002	14.8 ± 2.59
QFD	237.7 ± 20.10***	6.51 ± 1.460***	57.2 ± 3.21***
AFC	95.3 ± 9.37	0.021 ± 0.003	15.1 ± 1.50
AFD	250.6 ± 17.10***	5.99 ± 0.400***	45.9 ± 5.03***

Values are expressed as mean ± SD of control (n=6), diabetic (n=6) and treated rats (n=6). Starch fed control (SFC), starch fed diabetic (SFD), curcumin fed control (CFC), curcumin fed diabetic (CFD), quercetin fed control (QFC), quercetin fed diabetic (QFD), aminoguanidine fed control (AFC) and aminoguanidine fed diabetic (AFD).
Values are expressed as mean ± SD of control, diabetic and treated rats.
$^{\psi}P<0.001$ indicates significant values when SFD was compared with SFC.
***$P<0.001$ indicates significant values when SFD was compared with CFD, QFD, and AFD groups.