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Research Paper

Cardioprotective actions of two bioflavonoids, quercetin and rutin, in experimental myocardial infarction in both normal and streptozotocin-induced type I diabetic rats

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Abstract

Objectives Revascularization therapy is the mainstay of treatment in the management of myocardial infarction in normal and diabetic patients. We attempted to evaluate the cardioprotective actions of quercetin and rutin in ischaemia–reperfusion-induced myocardial infarction in both normal and diabetic rats.

Methods Myocardial infarct size was measured using the staining agent 2,3,5-triphenyltetrazoliumchloride. Serum and tissue malondialdehyde levels and superoxide dismutase and catalase in heart tissue were estimated spectrophotometrically. A lead II electrocardiogram was monitored at various intervals throughout the experiment.

Key findings Results demonstrated the larger infarct size, enhanced lipid peroxidation, partial depletion of antioxidant enzymes and drastic drop in heart rate in diabetic hearts subjected to in-vivo ischaemia–reperfusion in comparison to normal rats subjected to ischaemia–reperfusion. Furthermore, quercetin and rutin significantly limit the infarct size in both normal and diabetic animals in a similar fashion. However, rutin offered complete cardioprotection at a dose of 10 mg/kg in terms of limiting infarct size. Both flavonoids could partially but significantly attenuate the lipid peroxidation. In addition, treatment has shown moderate improvement in heart rate in both normal and diabetic rats.

Conclusions Our data suggest the possible cardioprotective effects of quercetin and rutin in ischaemia–reperfusion injury in both normal and diabetic rats, and that protection might be in part due to the attenuation of oxidative stress and moderate increment in antioxidant reserves.

Keywords cardioprotection; flavonoids; free radicals; quercetin; reperfusion injury

Introduction

Revascularization therapy is the mainstay of treatment of myocardial infarction in normal and diabetic patients. In the light of the poor clinical outcomes of revascularization procedures, reperfusion injury has gained more importance in cardiovascular research. Reperfusion may be considered a double-edged sword: although it is essential for the survival of the tissue, it paradoxically results in some new cellular damage that blunts the beneficial effects of reperfusion itself. One of the major therapeutic goals of modern cardiology is therefore to design strategies aimed at salvaging the myocardium from the ravages of reperfusion injury and improve the benefits of reperfusion therapy. Since diabetic patients represent a high-risk group for the development of ischaemic heart disease, they constitute a growing segment of the population undergoing coronary revascularization surgical procedures.[1–3] Recent reports indicate that patients with diabetes account for approximately 10–20% of patients currently undergoing coronary artery bypass grafting and percutaneous transluminal coronary angioplasty procedures.[1,3,4]

Of numerous mechanisms implicated in the pathobiology of reperfusion injury, oxidative stress has been suggested as a central mechanism of cellular damage in ischaemia–reperfusion (I/R) injury. Formation of free radicals in a biological system includes xanthine oxidase,[5] activated neutrophils,[6] direct donation of an electron from myocardial electron transport chain,[7] catecholamine oxidation,[8] cyclo-oxygenase and lipoxygenase enzymes.[9] Since free radical mediated injury is a potential threat to a viable myocardium,[10] oxidative damage should be limited and the antioxidant defence mechanism reinforced by treating with antioxidants. Indeed, several clinical studies[11–13] and also some

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experimental studies[14–16] have reported that administration of antioxidants resulted in beneficial outcomes in I/R injury.

Research on flavonoids has been triggered by the discovery of the 'French paradox' whereby populations with high red wine consumption have a relatively low cardiovascular mortality rate.[17] The flavonoids in red wine are responsible, at least in part, for this effect.[18] Furthermore, epidemiologic studies suggest a protective role of dietary flavonoids against coronary heart disease.[19] The association between flavonoid intake and the long-term effects on mortality was studied subsequently[20] and it has been suggested that flavonoid intake is inversely correlated with mortality due to coronary heart disease.

The high lipid solubility of flavonoids allows them easy access into the cell, where free radicals do most damage. In addition they have wide applicability (antioxidant, antiinflammatory, free radical scavenging and antiplatelet aggregatory effects) in cardiovascular research. These two factors prompted us to evaluate their cardioprotective activity in I/R injury.

Flavonoids such as quercetin and rutin have received much attention in the area of nutritional biology. In spite of the tremendous potential of these two compounds to protect the heart during I/R, only a few studies have reported on such effects. In addition, the cardioprotective mechanisms of quercetin and rutin have not been explored so far in I/R of diabetic hearts. The objective of the present investigation was therefore to evaluate the cardioprotective actions of the flavonoids quercetin and rutin against I/R injury in both diabetic and normal rats.

It may not always be feasible to administer flavonoids as a chronic pretreatment before revascularization procedures. An acute treatment model, just at the point of reperfusion, is therefore of greater interest; the present study used the acute treatment model.

Materials and Methods

Drugs and chemicals

Streptozotocin (STZ), quercetin, rutin and 1,1,3,3-tetraethoxypropane were purchased from Sigma Chemical Company (St Louis, USA). 2,3,5-Triphenyl tetrazolium chloride (TTC) was purchased from BDH Chemicals Ltd (UK). Thiopentone sodium was supplied by Abott Lab Ltd (Ankleshwar, India). Nitroblue tetrazolium, NADH, reduced glutathione, oxidized glutathione, 1-chloro-2,4-dinitrobenzene, Folin's phenol reagent, Ellman's reagent and TritonX-100 RS were purchased from Sisco Research Laboratories Pvt Ltd (Mumbai, India). Phenazine methosulphate was purchased from National Chemicals (Vadodara, India). All other chemicals and reagents were used of analytical grade.

Animals

Albino Wistar rats (National Institute of Nutrition, Hyderabad, India), of either sex, weighing 200–250 g, were selected. Animals were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity 50 ± 15% and normal photoperiod (12 h dark/12 h light). Commercial pellet diet (Rayon's Biotechnology Pvt Ltd, India) and water were provided *ad libitum*. The experimental protocol has been approved by the Institutional Animal Ethics Committee and by the Animal Regulatory Body of the Government (Regd. No. 516/01/A/CPCSEA).

Experimental design

The rats were randomly divided into 14 groups, of which seven were normal groups and seven diabetic groups. The usage pattern of number of rats for the determination of biochemical parameters was as follows: $n = 6$ for percentage left ventricle necrosis and malondialdehyde (MDA) levels, $n = 5$ for heart rate, superoxide dismutase (SOD) and catalase (CAT) levels. Group 1, sham control group; group 2, control I/R group treated with saline (0.2 ml); group 3, vehicle control I/R group treated with 0.2 ml of 50% dimethyl sulfoxide (DMSO); groups 4 and 5 were treated with quercetin 5 and 10 mg/kg, respectively; groups 6 and 7 were treated with rutin 5 and 10 mg/kg, respectively; group 8, diabetic sham control group; group 9, diabetic control I/R group treated with saline; group 10, diabetic vehicle control group treated with DMSO; groups 11 and 12, diabetic control I/R treated with quercetin at doses of 5 and 10 mg/kg, respectively; groups 13 and 14, diabetic control I/R treated with rutin at doses of 5 and 10 mg/kg, respectively. Quercetin and rutin were dissolved in 50% DMSO and administered intraperitoneally (i.p.) 10 min before reperfusion.

Induction of diabetes

Diabetes was induced by a single i.v. injection of STZ, 45 mg/kg of body weight, dissolved in citrate buffer (pH 4.5), into the tail vein of animals lightly anaesthetized with ether. Age-matched rats were injected with citrate buffer only. Diabetes was confirmed after the third day of STZ injection by estimation of serum glucose using an autoanalyzer (Dade Behring, USA). Following 2 weeks of diabetes induction, rats were subjected to the surgical procedure. About 20% mortality was observed in diabetic rats even before being subjected to I/R injury. Diabetic rats showing serum glucose concentration of more than 350 mg/dl were used for the experiment.

Surgical procedure

Rats were anaesthetized with thiopentone sodium (30 mg/kg, i.p.), tracheotomized and ventilated with room air by a Techno positive pressure mechanical respirator (animal respirator, Crompton Parkinson Ltd, UK). The right jugular vein was cannulated in order to inject drugs. A left thoracotomy and pericardiotomy were performed, and the left coronary artery was dissected free above the first diagonal branch and ligated just below the origin of the left circumflex artery with the help of a silk thread (6–0). The artery was occluded for 30 min by a knot. The silk thread was removed after 30 min with the help of two knot releasers to allow reperfusion of the heart for the next 4 h.

Electrocardiograph

After surgical preparation, rats were allowed 10 min for stabilization and then control electrocardiograph (ECG) measurements were taken. The rats were then subjected to a 30 min occlusion. At 15 min of occlusion the ECG was

taken again. Measurements of ECG were repeated immediately after release of the occlusion and at 1, 2, 3 and 4 h intervals for all the groups of animals. A lead II electrocardiogram was monitored using Cardiart 408 (BPL) with 20 mm/mV sensitivity at a paper speed of 50 mm/s. Heart rates were expressed as beats per minute.

Quantification of infarct size

In all the groups, after sacrificing the animal by giving excess anaesthesia, the heart was rapidly excised from the thorax and the greater vessels were removed. The left ventricle was separated from the heart and weighed. It was sliced parallel to the atrioventricular groove into 2–3 mm thick sections and the slices were incubated in 1% TTC solution, prepared in phosphate buffer pH 7.4, for 30 min at 37°C.[21] In the viable myocardium, TTC is converted by dehydrogenases to a red formazan pigment that stains tissue dark red.[22] The infarcted myocardium, which does not take TTC stain where the dehydrogenases are drained off, remains pale in colour. The pale necrotic tissue was separated from the stained portions and weighed on an electronic balance (Dhona 200D, Dhona instruments Ltd, India). Infarct size was calculated as a percentage of necrotic tissue of the left ventricle.

Determination of malondialdehyde levels in serum

In all the groups, before sacrificing the animal at the end of 4 h of reperfusion, 2 ml of blood sample was collected from the left ventricle for the estimation of MDA levels in blood serum. Serum MDA levels were estimated by the method developed by Yagi.[23] Tetraethoxypropane (0.5, 1, 2, 4, 6, 8 and 10 nmol) served as the external standard. MDA levels in serum were expressed as nanomoles per millilitre of serum.

Determination of malondialdehyde levels in myocardium

MDA levels in the myocardium were measured by the method developed by Ohkawa *et al*.[24] Briefly, the infarcted left ventricular tissues were homogenized with 1.15% KCl (10% w/v). The assay mixture consisted of 0.1 ml of tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of 20% acetic acid (adjusted to pH 3.5 with NaOH) and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA). This was heated for 60 min at 95°C. Thereafter, the mixture was cooled and extracted with 5 ml of a mixture of *n*-butanol and pyridine (15 : 1, v/v). After centrifugation at 4000 rpm for 10 min, the organic phase was assayed spectrophotometrically at 532 nm. Tetraethoxypropane (2, 4, 6 and 8 nmol) served as an external standard. MDA levels in myocardium were expressed as nanomoles per gram of tissue.

Determination of superoxide dismutase in myocardium

The SOD level was estimated by the method described by Kakkar *et al.*[25] The assay mixture contained 0.1 ml of sample, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of phenazine methosulphate (186 μM), 0.3 ml of 300 μM nitro blue tetrazolium and 0.2 ml of NADH (750 μM). Reaction was started by addition of NADH. After incubation at 30°C for 90 s, the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. The reaction mixture was stirred vigorously with 4.0 ml of *n*-butanol. The mixture was allowed to stand for 10 min, centrifuged and the butanol layer was separated. The colour intensity of the chromogen in the butanol was measured spectrophotometrically at 560 nm and the concentration of SOD was expressed as units per milligram of protein.

Determination of catalase in the myocardium

The CAT level was measured by the method of Aebi.[26] Supernatant (0.1 ml) was added to a cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240 nm. The activity of CAT was expressed as micromoles of H_2O_2 metabolized per milligram of protein per minute.

Estimation of protein

Protein was estimated by the method of Lowry *et al.*[27] The level of protein was expressed as milligrams of protein per gram of tissue.

Statistical analysis

The results are expressed as mean ± SD. Differences in infarct size, serum and tissue lipid peroxide levels, SOD and CAT were determined by factorial one-way analysis of variance. Individual groups were compared using Tukey's test. Heart rate data were tested for differences between groups by using a two-way ANOVA for repeated measurements followed by a Bonferroni post test when the differences were significant. Differences with $P < 0.05$ were considered statistically significant.

Study limitations

We could not quantify the free radicals for technical reasons and the area at risk was not measured to avoid the interference of TTC staining with MDA levels that were estimated in the necrotic part of TTC stained heart.

Results

Effect of quercetin and rutin on myocardial infarct size

In normal control animals (group 2) subjected to I/R injury the infarct size was found to be 49.59 ± 1.32% and statistically significant ($P < 0.05$) compared to sham control animals (group 1). In vehicle-treated animals, infarct size was found to be 48.04 ± 0.59% (group 3). There was no significant difference between the infarct size in the vehicle group (group 3) and the normal control group (group 2). Treatment with quercetin (5 and 10 mg/kg) (groups 4 and 5) significantly diminished the infarct size to 21.93 ± 1.05% and 9.77 ± 1.17%, respectively. Treatment with rutin (5 and 10 mg/kg) (groups 6 and 7) significantly diminished the infarct size to 20.90 ± 1.38% and 2.91 ± 0.58%, respectively. It is noteworthy that rutin at a dose level of 10 mg/kg

almost diminished the infarct size (2.91 ± 0.58%) to the level of the infarct size (4.89 ± 0.39%) of the sham control group. Furthermore, there was no significant difference between the infarct sizes of the group treated with rutin at 10 mg/kg (group 7) and the normal sham control group (group 1) (see Figure 1 and Table 1).

In diabetic control animals subjected to I/R injury, the infarct size was significantly increased to 58.73 ± 1.29% (group 9) when compared to normal control animals (49.59 ± 1.32%) (group 2) and diabetic sham control animals (9.46 ± 0.51%) (group 8). In vehicle-treated diabetic animals, infarct size was significantly reduced to 56.22 ± 1.64% (group 10). Treatment with quercetin (5 and 10 mg/kg) (groups 11 and 12) significantly diminished the infarct size to 31.76 ± 0.57% and 12.95 ± 0.69%, respectively. Treatment with rutin (5 and 10 mg/kg) (groups 13 and 14) significantly diminished the infarct size to 28.83 ± 1.27% and 7.45 ± 0.85%, respectively. A similar pattern of complete cardioprotection was also observed in diabetic animals. This was clearly evident in that there was no significant difference between infarct sizes in the rutin 10 mg/kg treated group (7.45 ± 0.85%) (group 7) and the diabetic sham control group (9.46 ± 0.51%, group 8) (Figure 1). It is noteworthy that rutin at a dose level of 10 mg/kg diminished the infarct size almost to the level of the infarct size (4.89 ± 0.39%) of the sham control group.

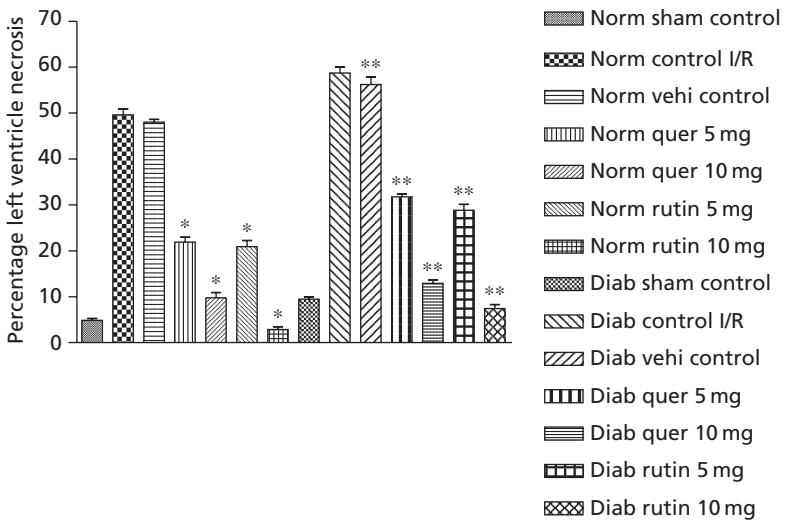

Figure 1 Effect of quercetin and rutin on left ventricle necrosis of both normal and diabetic experimental group animals subjected to ischaemia–reperfusion injury. All values are expressed as mean ± SD ($n = 6$). I/R, ischaemia–reperfusion; Norm vehi, normal vehicle-treated; Norm quer, normal quercetin; Diab vehi, diabetic vehicle-treated; Diab quer, diabetic quercetin. *$P < 0.05$ vs control I/R. **$P < 0.05$ vs diabetic control I/R.

Table 1 Consolidated data table

Group	PLVN (%)	Serum MDA (nmol/ml of serum)	Tissue MDA (nmol/g wet tissue)	SOD (U/mg protein)	CAT[a]
Norm sham control	4.89 ± 0.39	2.32 ± 0.28	4.94 ± 0.36	25.12 ± 0.62	26.98 ± 0.40
Norm control I/R	49.59 ± 1.32	26.66 ± 1.03	121.29 ± 1.09	13.32 ± 0.42	12.25 ± 0.23
Norm veh control I/R	48.04 ± 0.59*	26.19 ± 0.95*	100.47 ± 1.60	13.65 ± 0.40*	12.80 ± 0.58*
Norm quer 5 mg	21.93 ± 105	19.79 ± 0.92	60.57 ± 1.94	16.98 ± 0.40	16.52 ± 0.74
Norm quer 10 mg	9.77 ± 1.17	12.66 ± 0.50	26.56 ± 0.78	22.13 ± 0.74	22.44 ± 1.07
Norm rutin 5 mg	20.90 ± 1.38	18.52 ± 0.48	54.81 ± 1.64	17.88 ± 0.50	16.55 ± 0.57
Norm rutin 10 mg	2.91 ± 0.58***	10.70 ± 0.53	23.35 ± 2.18	22.70 ± 0.54	23.44 ± 0.61
Diab sham control	9.46 ± 0.51	6.73 ± 0.81	13.35 ± 0.65	22.03 ± 0.49	20.62 ± 1.02
Diab control I/R	58.73 ± 1.29	54.57 ± 0.50	154.74 ± 1.14	11.67 ± 0.42	11.24 ± 0.73*
Diab veh control	56.22 ± 1.64	49.42 ± 0.99	140.48 ± 1.93	11.87 ± 0.53†	12.01 ± 1.24†
Diab quer 5 mg	31.76 ± 0.57	33.89 ± 0.56	94.77 ± 1.29	13.84 ± 0.43	15.33 ± 0.67
Diab quer 10 mg	12.95 ± 0.69	17.93 ± 0.68	40.27 ± 1.82	18.94 ± 0.25	19.37 ± 0.87
Diab rutin 5 mg	28.83 ± 1.27	31.29 ± 0.69	90.08 ± 1.67	14.32 ± 0.62	15.77 ± 0.92
Diab rutin 10 mg	7.45 ± 0.85†††	16.30 ± 0.50	30.33 ± 1.51	19.23 ± 0.46	19.24 ± 0.65

[a]Units for catalase are expressed as micromoles of H_2O_2 decomposed per milligram of protein per minute. All values are expressed as mean ± SD ($n = 6$ for PLVN and MDA levels, $n = 5$ for SOD and CAT). PLVN, percentage left ventricle necrosis; MDA, malondialdehyde; SOD, superoxide dismutase; CAT, catalase; Norm, normal; Diab, diabetic; quer, quercetin; veh, vehicle. *not significant vs normal control I/R, **not significant vs normal vehicle control I/R, ***not significant vs normal sham control, †not significant vs diabetic control I/R, ††not significant vs vehicle diabetic control I/R, †††not significant vs diabetic sham control.

Effect of quercetin and rutin on lipid peroxidation

Malondialdehyde is the end product of lipid peroxidation. The reaction of lipid peroxides with TBA has been widely adopted as a sensitive assay method for lipid peroxidation. MDA levels in serum and heart tissue in normal control animals were found to be 26.66 ± 1.03 nmol/ml (Figure 2) and 121.29 ± 1.09 nmol/g tissue (Figure 3), respectively, and statistically significant ($P < 0.05$) compared to the sham control group. In the normal vehicle control group, MDA levels in serum and tissue were found to be 26.19 ± 0.95 nmol/ml and 100.47 ± 1.60 nmol/g tissue. In the vehicle-treated group there was a significant reduction in tissue MDA levels ($P < 0.05$) but the same was not observed for serum MDA levels. Quercetin treatment with 5 mg/kg significantly ($P < 0.05$) reduced the serum and tissue MDA levels to 19.79 ± 0.92 nmol/ml and 60.57 ± 1.94 nmol/g tissue, respectively. With quercetin at 10 mg/kg, there was a significant ($P < 0.05$) further reduction in the serum and tissue MDA levels to 12.66 ± 0.50 nmol/ml and 26.56 ± 0.78 nmol/g tissue, respectively. Rutin treatment at 5 mg/kg significantly ($P < 0.05$) reduced the serum and tissue MDA levels to 18.52 ± 0.48 nmol/ml and 54.81 ± 1.64 nmol/g

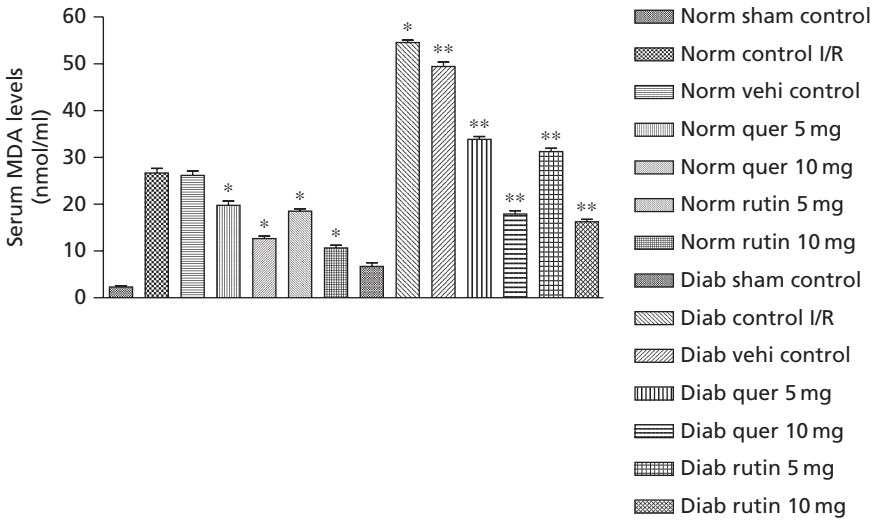

Figure 2 Effect of quercetin and rutin on serum malondialdehyde levels of both normal and diabetic experimental group animals subjected to ischaemia–reperfusion injury. All values are expressed as mean ± SD ($n = 6$). MDA, malondialdehyde; I/R, ischaemia–reperfusion; Norm vehi, normal vehicle-treated; Norm quer, normal quercetin; Diab vehi, diabetic vehicle-treated; Diab quer, diabetic quercetin. *$P < 0.05$ vs control I/R. **$P < 0.05$ vs diabetic control I/R.

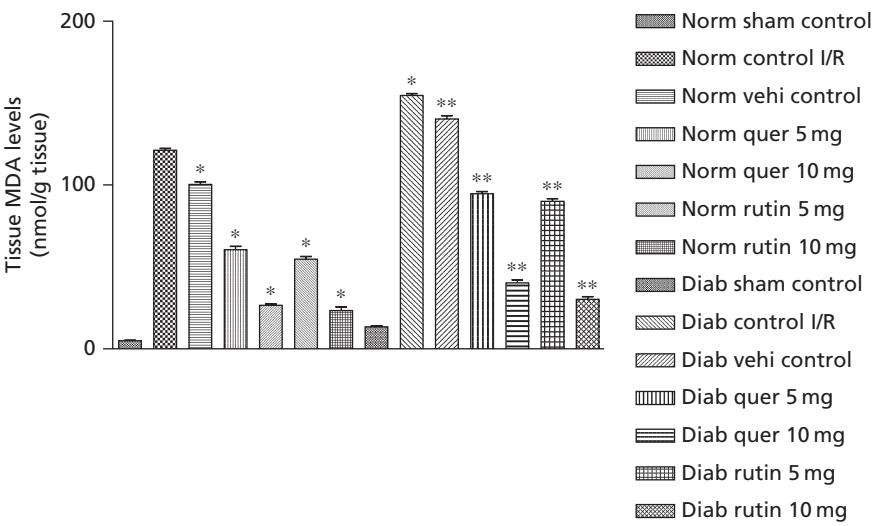

Figure 3 Effect of quercetin and rutin on tissue malondialdehyde levels of both normal and diabetic experimental group animals subjected to ischaemia–reperfusion injury. All values are expressed as mean ± SD ($n = 6$). MDA, malondialdehyde; I/R, ischaemia–reperfusion; Norm vehi, normal vehicle-treated; Norm quer, normal quercetin; Diab vehi, diabetic vehicle-treated; Diab quer, diabetic quercetin. *$P < 0.05$ vs control I/R. **$P < 0.05$ vs diabetic control I/R.

tissue, respectively. With rutin at 10 mg/kg, there was a significant ($P < 0.05$) further reduction in the serum and tissue MDA levels to 10.70 ± 0.53 nmol/ml and 23.35 ± 2.18 nmol/g tissue, respectively (see Table 1).

MDA levels in serum and heart tissue in diabetic sham control animals slightly increased to 6.73 ± 0.81 nmol/ml and 13.35 ± 0.65 nmol/g tissue when compared to the normal sham control group. MDA levels in serum and heart tissue in diabetic control animals were significantly raised to 54.57 ± 0.50 nmol/ml (Figure 2) and 154.74 ± 1.14 nmol/g tissue (Figure 3), respectively, when compared with normal control animals as well as the diabetic sham control group.

In diabetic animals subjected to I/R injury, quercetin treatment at 5 mg/kg significantly ($P < 0.05$) reduced the serum and tissue MDA levels to 33.89 ± 0.56 nmol/ml and 94.77 ± 1.29 nmol/g tissue, respectively. With quercetin at 10 mg/kg, there was a a significant ($P < 0.05$) further reduction in the serum and tissue MDA levels to 17.93 ± 0.68 nmol/ml and 40.27 ± 1.82 nmol/g tissue, respectively. Rutin treatment at 5 mg/kg significantly ($P < 0.05$) reduced the serum and tissue MDA levels to 31.29 ± 0.69 nmol/ml and 90.08 ± 1.67 nmol/g tissue, respectively. With rutin at 10 mg/kg, there was a significant ($P < 0.05$) further reduction in the serum and tissue MDA levels to 16.30 ± 0.50 nmol/ml and 30.33 ± 1.51 nmol/g tissue, respectively.

Effect of quercetin and rutin on antioxidant enzymes

In control group animals, the levels of endogenous antioxidant enzymes such as SOD and CAT in both normal and diabetic heart tissue were reduced to 13.32 ± 0.42, 12.25 ± 0.23, 11.67 ± 0.42 and 11.24 ± 0.73, respectively (Figures 4 and 5), and the differences were statistically significant ($P < 0.05$) compared to normal and diabetic sham control animals. In vehicle-treated animals, there was no significant increase in SOD and CAT in both normal and diabetic animals. Quercetin and rutin treatment significantly ($P < 0.05$) prevented the depletion of SOD and CAT in both normal and diabetic animals. The remaining data are shown in Figures 4 and 5, and Table 1.

Effect of quercetin and rutin on heart rate

In the normal control group, a continuous decrease in heart rate was observed during 30 min of coronary artery ligation and throughout the reperfusion period compared to the sham control group (Table 2). Similarly, in the diabetic control group, a significant ($P < 0.05$) and continuous decrease in heart rate was observed during the 30 min coronary artery ligation and throughout the reperfusion period compared to the diabetic sham control group. The groups treated with quercetin and rutin at doses of 5 and 10 mg/kg produced slight decreases in heart rate during the 30 min coronary artery ligation and thereafter gradual increases throughout the reperfusion period. There was no significant difference between the normal sham control group and the normal treated groups subjected to I/R. The effect in diabetic rats was similar: there was no significant difference between the diabetic sham control group and the treatment groups at 4 h after reperfusion. In fact, treatment could not restore heart rates to normal values at the end of the 4 h period in either normal or diabetic rats. This clearly indicates the moderate restoration of heart rate by the two flavonoids, quercetin and rutin. Data for heart rate recorded at various intervals of time during the experiment for all the groups are given in Tables 1 and 2.

Discussion

Reperfusion injury has been attributed in part to massive oxygen free radical formation with subsequent lipid peroxidation and membrane damage.[28] This hypothesis is supported by studies that demonstrate that infarct size can be reduced by

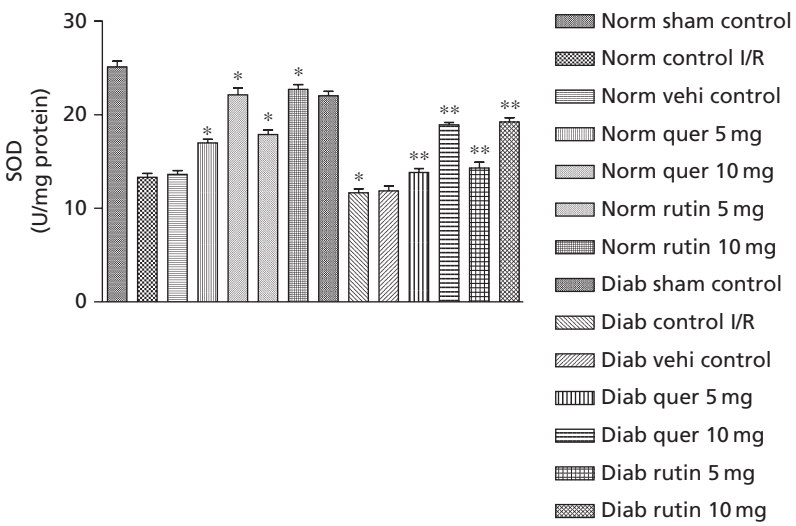

Figure 4 Effect of quercetin and rutin on superoxide dismutase levels of both normal and diabetic experimental group animals subjected to ischaemia–reperfusion injury. All values are expressed as mean ± SD ($n = 5$). SOD, superoxide dismutase; I/R, ischaemia–reperfusion; Norm vehi, normal vehicle-treated; Norm quer, normal quercetin; Diab vehi, diabetic vehicle-treated; Diab quer, diabetic quercetin. *$P < 0.05$ vs control I/R. **$P < 0.05$ vs diabetic control I/R.

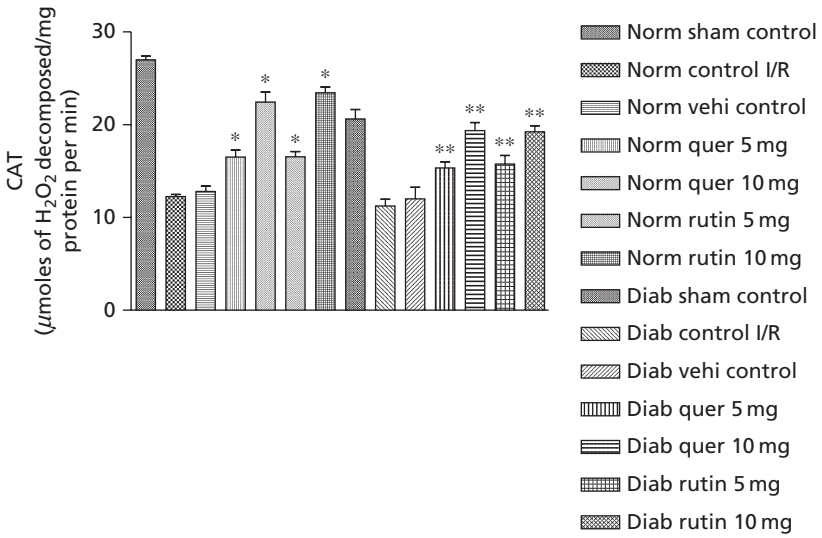

Figure 5 Effect of quercetin and rutin on catalase levels of both normal and diabetic experimental group animals subjected to ischaemia–reperfusion injury. All values are expressed as mean ± SD ($n = 5$). CAT, catalase; I/R, ischaemia–reperfusion; Norm vehi, normal vehicle-treated; Norm quer, normal quercetin; Diab vehi, diabetic vehicle-treated; Diab quer, diabetic quercetin. *$P < 0.05$ vs control I/R. **$P < 0.05$ vs diabetic control I/R.

Table 2 Heart rate recorded at various stages of myocardial occlusion and reperfusion

Groups	Heart rate (beats/min)						
	BO	MOP	IAR	1 h AR	2 h AR	3 h AR	4 h AR
Norm sham control	420 ± 15	418 ± 29	421 ± 24	425 ± 30	422 ± 24	423 ± 26	420 ± 28
Norm control I/R	414 ± 14	336 ± 17	326 ± 27	338 ± 27	338 ± 21	344 ± 25	342 ± 25
Norm veh control I/R	431 ± 22*	341 ± 19	343 ± 7	347 ± 18	339 ± 18	342 ± 22	353 ± 20
Norm quer 5 mg	422 ± 30*	325 ± 29	333 ± 28	349 ± 24	347 ± 29	362 ± 29*	375 ± 24*
Norm quer 10 mg	419 ± 27*	331 ± 22	336 ± 18	372 ± 36	369 ± 24	383 ± 21	386 ± 21*
Norm rutin 5 mg	407 ± 20*	320 ± 19	319 ± 21	339 ± 27	355 ± 24	365 ± 21	399 ± 23*
Norm rutin 10 mg	410 ± 20*	315 ± 18	344 ± 21	351 ± 26	368 ± 24	376 ± 14	392 ± 19*
Diab sham control	363 ± 42	361 ± 23	363 ± 25	360 ± 24	358 ± 34	362 ± 36	359 ± 26
Diab control I/R	355 ± 22**	272 ± 15	265 ± 20	270 ± 27	253 ± 15	251 ± 16	243 ± 12
Diab veh control	368 ± 19**	280 ± 18	289 ± 25	275 ± 22	260 ± 23	252 ± 15	248 ± 16
Diab quer 5 mg	387 ± 21**	290 ± 17	295 ± 19	299 ± 24	299 ± 20	310 ± 16**	314 ± 22**
Diab quer 10 mg	391 ± 18**	285 ± 21	289 ± 23	295 ± 32	286 ± 21	320 ± 19**	322 ± 30**
Diab rutin 5 mg	373 ± 25**	279 ± 28	280 ± 17	283 ± 19	302 ± 30	302 ± 22	315 ± 24**
Diab rutin 10 mg	390 ± 31**	250 ± 19	252 ± 19	268 ± 20	314 ± 23**	342 ± 31**	366 ± 29**

Values are expressed as mean ± SD and expressed as beats per minute. BO, before occlusion; MOP, middle occlusion period; IAR, immediately after reperfusion; AR, after reperfusion. Norm, normal; Diab, diabetic; quer, quercetin; veh, vehicle. *Not significant compared to normal sham control; **Not significant compared to diabetic sham control.

treatment with free radical scavengers administered during ischaemia and early reperfusion.[16,29,30] Recently there has been an upsurge of interest in the therapeutic potential of flavonoids as antioxidants in attenuating such free radical induced tissue injury in I/R injury.

Infarct size

In the present study, infarct size was found to be greater in the diabetic sham control group than in normal sham control group animals. A negligible amount of damage was observed in diabetic sham control animals, which can probably be explained by the diabetic sham control group having higher lipid peroxide levels in serum and heart tissue than normal sham controls. However, the damage was not due to the sham procedure itself. The present study demonstrates the significantly larger infarct size in diabetic rat hearts when compared to normal rat hearts subjected to I/R injury. The results help to explain the increased cardiovascular injury seen in the diabetic heart. Despite most recent clinical data indicating that the diabetic heart is more sensitive to myocardial I/R injury,[31,32] animal studies have produced inconsistent results regarding the sensitivity of diabetic hearts to ischaemic injury.[33,34] However, our results differ from the previous findings reported by Tosaki *et al.*,[35] in which a 2-week diabetic state produced a reduction in the incidence of ventricular fibrillation and tachycardia as well as improved contractile function relative to age-matched control rats. Earlier findings by Kam-Ming *et al.*[36]

demonstrated that susceptibility of diabetic hearts to I/R injury seemed to be dependent on the reperfusion time course. In their study, short-term (2-week) diabetic rat hearts were resistant to 20 min of ischaemia and 20 min of reperfusion but were sensitive to 20 min of ischaemia and 40 min of reperfusion. In our present study, the sensitivity of the diabetic heart to I/R injury is probably attributed to the prolonged period of reperfusion (30 min of ischaemia and 4 h of reperfusion) irrespective of various factors like length of diabetes,[37] dose of STZ,[38] or altered activity of the Na^+/H^+ exchanger, which can influence the sensitivity of the diabetic heart to I/R injury.[39]

The early stages of diabetes may not trigger increased levels of oxidative stress but make tissue more susceptible to I/R injury. It is well accepted that diabetes itself increases oxidative stress within the vascular system and myocardium.[40,41] Reactive oxygen species (ROS), notably superoxide anions, incapacitate endothelium-derived nitric oxide (NO) to form potent oxidant peroxynitrite, exacerbate local oxidative stress and ultimately lead to the development of endothelial dysfunction in diabetes.[42,43] Moreover, increased leukocyte accumulation in the diabetic heart coupled with enhanced diabetic polymorphonuclear neutrophil oxygen free radical production will probably cause an enhanced inflammatory response.[44] Hence, the pre-existing oxidative stress and endothelial dysfunction makes the diabetic heart more vulnerable to I/R injury. The greater degree of injury demonstrated in diabetic rat hearts is therefore most likely be due to exacerbated oxidative stress within the diabetic myocardium.

In the present study, the infarct size was significantly reduced by the flavonoids quercetin and rutin in both normal and diabetic I/R injury. The cardioprotective action of quercetin was supported by Kolchin *et al*.,[45] who carried out experiments on dogs. Our results are also in part supported by previous findings from our laboratory in which quercetin and rutin partially reversed the impaired cardiac function in streptozotocin-induced diabetic rats.[46] However, the observed differences in the cardioprotection of quercetin and rutin in both these studies should probably be attributed to differences in the experimental settings of diabetic cardiomyopathy[46] and myocardial I/R (present study). In addition, Ikizler *et al*.[47] reported that the cardioprotective effect of quercetin was found with chronic but not with acute treatment. In contrast, our results support the cardioprotection of quercetin on acute treatment just before reperfusion. A possible rationale for the discrepancies between these two studies is the route of administration of drug, which affects its availability in the circulatory system. In our study, quercetin was administered through the intraperitoneal route, which ensures better absorption of the drug than the oral route used by Ikizler. This is the most likely reason why, in our study, quercetin and rutin showed cardioprotection even with acute treatment. A previous study by Chopra and Manjeet[48] also supports the cardioprotective action of rutin in an in-vivo model of I/R. The protective capacity of quercetin against I/R injury is not limited to the myocardium but is also found in several other tissues, such as the kidney,[49,50] liver[51] and brain.[52] Considering the potential of the flavonoids as a whole, our result further supports previous studies reporting the limiting effect of flavonoids on infarct size.[53–55]

Lipid peroxidation and antioxidant reserves

In the present study, lipid peroxide levels in serum and heart tissue in the diabetic sham control group were significantly higher than in normal sham controls. This finding clearly indicates a mild degree of oxidative stress in sham control rats. Increased lipid peroxidation, in terms of MDA levels in serum and heart tissue of the diabetic control group compared to the normal control group, further confirms the exacerbated oxidative-mediated damage during I/R in diabetic hearts. Oxidant balance in the heart has a very important role in protecting the heart and allowing normal cardiac contractile performance. The myocardial cell has developed very effective antioxidant defence mechanisms. These mechanisms include SOD, CAT and glutathione in nuclear and lipid membranes.[10]

There is growing evidence that diabetes is associated with vascular complications, which could be due to oxidative stress coupled to increased production of ROS.[42,43] However, the antioxidant reserves can be inadequate in situations such as I/R and diabetes. During cardiac events in diabetics, the antioxidant levels may be depleted by oxidative stress and may compromise myocardial function. During ischaemia, the diabetic myocardium may not have the adequate antioxidant protection normally afforded, due to decreased antioxidant protection in the diabetic heart compared to the non-diabetic heart. The results of the present study also demonstrate the exaggerated oxidative stress in diabetic I/R injury, clearly evidenced by the increased MDA levels and decreased antioxidant enzymes such as SOD and CAT in the diabetic heart following I/R injury.

Quercetin and rutin treatment significantly reduced the serum and tissue MDA levels in both normal and diabetic I/R injury. Although quercetin does not reverse oxidative stress in other organs collected from diabetic rats,[56] numerous earlier studies reveal that quercetin attenuates oxidative stress in experimental settings of I/R.[51,53] Furthermore, quercetin has a well-proven antioxidant effect, *in vitro* as well as *in vivo*. The high lipid solubility of quercetin and rutin probably facilitates their easy access into the cell, where free radicals do their damage. However, our results also suggest that quercetin and rutin fail to reduce MDA levels completely. This indicates that a reduction in lipid peroxidation is not entirely responsible for the limiting effect of flavonoids on infarct size. Our results suggest the partial involvement of ROS in the cardioprotective effect offered by quercetin and rutin. In a similar fashion, an enhancement of the antioxidant enzymes SOD and CAT by quercetin and rutin also contributes to the size-limiting effect. By virtue of their antioxidant properties, flavonoids directly scavenge the superoxide anion (O_2^-) and protect the NO from being incapacitated by it.[57] They also prevent the formation of highly reactive peroxy nitrite. Girard *et al*.[58] suggested that the vasodilator effect of flavonoids can also be mediated by their scavenging of the superoxide anion and may thus prevent nitric oxide degradation by free radicals. Furthermore, flavonoids improve the endothelial function in spontaneous hypertensive rats, possibly because of an enhanced NO bioavailability.[59] They also increase the levels

of NO, as determined by electron paramagnetic resonance spectroscopy in rat brains during global ischaemia and reperfusion.[60] On the other hand, quercetin and related flavonoids also scavenge NO.[61] Although quercetin is found to have two opposing effects, earlier studies indicate that, under conditions of increased O_2^-, quercetin is a better scavenger of O_2^- than of NO.[62]

It is quite interesting to note that rutin confers almost complete cardiac protection at 10 mg/kg i.p. in the present study. In addition, rutin demonstrated better activity than quercetin in limiting the infarct size. This is probably due to better absorption of rutin in comparision to quercetin. Naturally occurring flavonols exist predominantly in a glycolysated form, e.g. rutin, rather than in their aglycone form. The forms of the flavonoids seem to influence the rate of absorption. Hollman *et al.*[63] suggested that the glycolysated forms of quercetin are absorbed more readily than the aglycone form, although catechin, which is not glycosylated in nature, is absorbed relatively efficiently.[64]

Heart rate

In the normal control group, a continuous decrease in heart rate was observed during the 30 min of coronary artery ligation and throughout the reperfusion period compared to the sham control group (Table 2). In the case of diabetic rats, there was a drastic drop in heart rate, particularly during the 30 min coronary artery ligation and throughout the reperfusion period. This result is in agreement with the previous study of Kolchin *et al.*,[45] in which heart rate decreased during I/R. The groups treated with quercetin and rutin at doses of 5 and 10 mg/kg showed a slight decrease in heart rate during the 30 min of coronary artery ligation, with a gradual increase throughout the reperfusion period. It was not possible to restore the heart rate to normal values at the end of 4 h in either diabetic or non-diabetic rats (Table 2). In another experimentally induced myocardial infarction in dogs by 60-min occlusion of the coronary artery with subsequent 24-h reperfusion, the administration of quercetin solution was found to improve the contractile function of the left ventricular myocardium, decrease the incidence of heart rate and conductivity disorders, limit the ischaemic damage area, promote the preservation of the vessels' integrity, improve coronary circulation and prevent intravascular thrombus formation.[45]

Conclusions

The data strongly suggest that there is a possible cardioprotective action of quercetin and rutin in I/R-induced myocardial infarction in normal and diabetic rats. Protection is due in part to the attenuation of oxidative stress and a slight increment in antioxidant enzymes. Further studies are warranted to explore other mechanisms that contribute to the cardioprotective effects of quercetin and rutin.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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