Cardioprotective Effects of Quercetin Against Ischemia-Reperfusion Injury Are Age-Dependent

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Summary

Quercetin, a polyphenolic compound present in various types of food, has been shown to exert beneficial effects in different cardiac as well as non-cardiac ischemia/reperfusion (I/R) models in adult animals. However, there is no evidence about the effects of quercetin on I/R injury in non-mature animals, despite the fact that efficiency of some interventions against I/R is age-dependent. This study was aimed to investigate the effects of chronic quercetin treatment on I/R injury in juvenile and adult rat hearts. Juvenile (4-week-old) as well as adult (12-week-old) rats were treated with quercetin (20 mg/kg/day) for 4 weeks, hearts were excised and exposed to 25-min global ischemia followed by 40-min reperfusion. Functional parameters of hearts and occurrence of reperfusion arrhythmias were registered to assess the cardiac function. Our results have shown that quercetin improved post-ischemic recovery of LVDP, as well as recovery of markers of contraction and relaxation, +(dP/dt)max and −(dP/dt)max, respectively, in juvenile hearts, but not in adult hearts. Quercetin had no impact on incidence as well as duration of reperfusion arrhythmias in animals of both ages. We conclude that the age of rats plays an important role in heart response to quercetin treatment in the particular dose and duration of the treatment. Therefore, the age of the treated subjects should be taken into consideration when choosing the dose of quercetin and duration of its application in prevention and/or treatment of cardiovascular diseases.

Key words

Quercetin • Heart • Ischemia-reperfusion • Age

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Introduction

Ischemia-reperfusion (I/R) injury of the heart belongs to leading causes of morbidity and mortality in developed countries. One of the major mechanisms participating on the development of subcellular defects in the heart due to I/R injury is oxidative stress (Dhalla *et al.* 2000). Numerous studies have shown that prevention and/or treatment with various types of antioxidants, such as vitamin E (Saleh and Saleh 2011, Dianat *et al.* 2014), N-acetylcysteine, N-mercaptopropionylglycine (Saini-Chohan and Dhalla 2009), stobadine (Knezl *et al.* 1999, Brosková *et al.* 2013) as well as various natural plant-derived antioxidant substances including flavonoids (Bartekova *et al.* 2010, Khandelwal *et al.* 2011), have a potential to increase the resistance of the heart to I/R injury. Quercetin is a naturally occurring polyphenolic substance with proposed positive effects on cardiovascular system (CVS). Main natural sources of quercetin are various types of food such as vegetables, fruits or red wine (Hertog *et al.* 1996). This flavonoid possesses various biological activities including anti-oxidative, anti-apoptotic, anti-inflammatory, and oxygen radical-scavenging activities (Erden Inal and Kahraman 2000, Wu *et al.* 2014). Quercetin has been shown to induce several positive effects in CVS such as blood pressure lowering effects (Perez-Vizcaino *et al.* 2009,

Larson *et al.* 2012) or protective effects on hearts exposed to I/R injury (Ikizler *et al.* 2007, Bartekova *et al.* 2010, 2015). It has been also documented that quercetin exerts protective effects in non-cardiac I/R models such as renal (Singh *et al.* 2004, Chen *et al.* 2014), brain (Yao *et al.* 2012, Lei *et al.* 2015) or liver (Su *et al.* 2003, Tokyol *et al.* 2006) ischemia. It has been demonstrated that the efficiency of certain protective interventions against I/R injury may be age-dependent. Recently, it has been shown that the potency of ischemic preconditioning in isolated perfused rat hearts is age-dependent (Griecsova *et al.* 2015). In this respect, the knowledge about the efficiency of quercetin in the hearts of various age could be of potential interest and importance for its application in the prevention and treatment of I/R injury. However, protective effects of quercetin on I/R injury have been documented only in adults, there is no evidence on age-dependence of quercetin effects on I/R. Therefore, the aim of our present study was to compare the effects of prolonged treatment with quercetin in juvenile rat hearts and rat hearts isolated from adult rats.

Material and Methods

Experimental model

Juvenile (4-week-old, weighted 80-100 g) and adult (12-week-old, weighted 200-250 g) male Wistar rats were used in the experiment.

Animals were divided into 4 groups: adult controls (AC), adults treated with quercetin (AQ), juvenile controls (JC) and juvenile rats treated with quercetin (JQ). The number of animals was the same in each group (n=6). The rats were housed in a room with controlled temperature (22-24 °C), 12/12 light-dark cycle and were fed with regular pellet diet. All animal experiments were performed in accordance with the rules issued by the State Veterinary Administration of the Slovak Republic, project No. 241/08-221, and with the regulations of the Animal Research and Care Committee of Institute for Heart Research SAS. The groups AQ and JQ received quercetin in a dose of 20 mg/kg/day for 4 weeks. Quercetin (Sigma, USA) was administered to the rats in drinking water as described previously (Mézešová *et al.* 2010). Briefly, quercetin was dissolved in a small amount of ethanol and then dissolved in 0.1 mol·l^{-1} phosphate buffer, pH 6.0 to keep maximal stability of quercetin in solution (previous experiments have shown maximal stability of quercetin at pH 6.0) so

that the final concentration of ethanol in drinking solution was 1 % (vol./vol.). The control animals received 0.1 M phosphate buffer, pH 6.0 with 1 % ethanol as drinking solution. The weight of animals and the amount of water consumption were measured every day and the dose of quercetin was counted according to these data every day. This dose of quercetin, as well as the duration of treatment and mode of delivery, was chosen according to our preliminary experiments where this particular dose of quercetin blocked increase of blood pressure in juvenile spontaneously hypertensive rats and prevented development of hypertension (Barteková *et al.* 2008). After 4 weeks of treatment with quercetin, the animals were anesthetized and the hearts were excised and perfused according to Langendorff to test their resistance against I/R injury.

Perfusion technique and determination of heart function

After rats were anesthetized with thiopental (50 mg/kg, i.p.) and heparinized (500 IU, i.p.), the hearts were rapidly excised, placed in ice-cold perfusion buffer, cannulated *via* the aorta and placed on the Langendorff setup (ADInstruments, Germany) for perfusion at a constant perfusion pressure of 73 mm Hg and temperature of 37 °C. Perfusion solution was a modified Krebs-Henseleit buffer gassed with 95 % O_2 and 5 % CO_2 (pH 7.4) containing (in mmol/l): NaCl 118.0; KCl 3.2; $MgSO_4$ 1.2; $NaHCO_3$ 25.0; KH_2PO_4 1.18; $CaCl_2$ 2.5; glucose 7.0. This solution was filtered through the 5 μm porosity filter to remove contaminants. An epicardial electrogram was registered by means of two stainless steel electrodes attached to the apex of the heart and aortic cannula. Left ventricular pressure was measured by means of a water-filled balloon inserted into the left ventricle *via* the left atrium (adjusted to obtain end-diastolic pressure of 1-5 mm Hg) and connected to a pressure transducer. Left ventricular developed pressure (LVDP, systolic minus diastolic pressure), maximal rates of pressure development and fall, +(dP/dt)max and −(dP/dt)max, as the indexes of contraction and relaxation were used to assess cardiac function. Recovery of these parameters after ischemia/reperfusion was expressed as a percentage of pre-ischemic baseline values. Heart rate (calculated from EG) and coronary flow were measured during the whole experiment as well. Occurrence and duration of ventricular fibrillation as well as the incidence of ventricular tachycardia (VT), which was defined as a run of four or more consecutive ectopic beats and

characterized by both the number of its episodes and its duration, were monitored to assess the reperfusion arrhythmias. Global ischemia was maintained for 25 min, followed by a 40 min reperfusion. Functional parameters of hearts were measured during the whole reperfusion, reperfusion arrhythmias were monitored during the first 10 min of reperfusion.

Statistical evaluations

Data are presented as means ± SEM. Statistical significance between two groups was tested by Student's unpaired *t*-test, the effects of quercetin were always compared to the corresponding control groups (JQ vs. JC, AQ vs. AC). Statistical significance of differences among all groups was analyzed by two-way ANOVA using the Holm-Sidak *post-hoc* test. Differences were considered significant at p<0.05 in all the tests.

Results

Effect of quercetin on functional parameters of isolated hearts exposed to I/R

Our results have shown that 4-week chronic treatment with quercetin had no effect on pre-ischemic values of functional parameters of hearts in juvenile as well as in adult rat hearts (Table 1). We have shown that quercetin treatment significantly improved post-ischemic recovery of functional parameters of isolated hearts in juvenile animals. This improvement was manifested by significantly improved recovery of +(dP/dt)max (45.92±6.56 % in JQ vs. 18.85±4.88 % in JC, P=0.016) (Fig. 1A), −(dP/dt)max (60.92±8.57 % in JQ vs. 27.13±8.92 % in JC, P=0.04) (Fig. 1B), as well as LVDP (57.48±6.74 % in JQ vs. 23.05±9.93 % in JC, P=0.031) (Fig. 1C) in the 40th minute of reperfusion. In contrast to

findings in juvenile hearts, quercetin had no effect on post-ischemic recovery of functional parameters in adult rat hearts (25.3±8.23 % in AQ vs. 27.86±9.28 % in AC, P=0.84 for +(dP/dt)max; 28.54±8.69 % in AQ vs. 33.14±10.58 % in AC, P=0.75 for −(dP/dt)max; 27.06±8.19 % in AQ vs. 29.34±10.03 % in AC, P=0.86 for LVDP) (Fig. 1A-C). Quercetin failed to prevent I/R-induced increase in end-diastolic pressure in both adult and juvenile hearts. However, the two-way ANOVA revealed the increase of end-diastolic pressure in the reperfusion to be age-dependent with lower I/R-induced increase in juvenile hearts (Fig. 1D). Finally, quercetin did not affect heart rate and coronary flow in all animals of both ages during whole experiment (data not shown).

Effect of quercetin on reperfusion arrhythmias

Occurrence and total duration of ventricular fibrillation as well as total number of episodes and total duration of ventricular tachycardia were monitored in the first 10 min of reperfusion to assess ischemia-induced reperfusion arrhythmias in all experimental groups of hearts. Our results have shown no occurrence of ventricular fibrillation in all hearts included in experiment (data not shown); however, we have detected ventricular tachycardia in all experimental groups. Quercetin failed to decrease the total number of episodes of ventricular tachycardia (Fig. 2A) as well as to decrease the total duration of ventricular tachycardia (Fig. 2B) in both juvenile and adult rat hearts when compared with corresponding controls. However, the two-way ANOVA revealed significant difference between total duration of ventricular tachycardia between juvenile and adult hearts, independently of the quercetin treatment with its longer total duration in adults (Fig. 2B).

Table 1. Pre-ischemic values of functional parameters of isolated hearts.

Group	JC	JQ	AC	AQ
HR (beats/min)	283 ± 21	265 ± 25	270 ± 6	249 ± 11
CF (ml/min)	6.12 ± 0.36	5.88 ± 0.62	7.36 ± 0.40	6.96 ± 0.59
LVDP (mm Hg)	76.6 ± 7.50	83.72 ± 4.54	89.64 ± 9.51	89.52 ± 8.94
+(dP/dt)max (mm Hg/s)	3295 ± 281	3366 ± 220	3105 ± 583	3283 ± 404
−(dP/dt)max (mm Hg/s)	2054 ± 214	2125 ± 146	2079 ± 290	2179 ± 258

JC – juvenile controls, JQ – juvenile treated with quercetin, AC – adult controls, AQ – adult treated with quercetin.
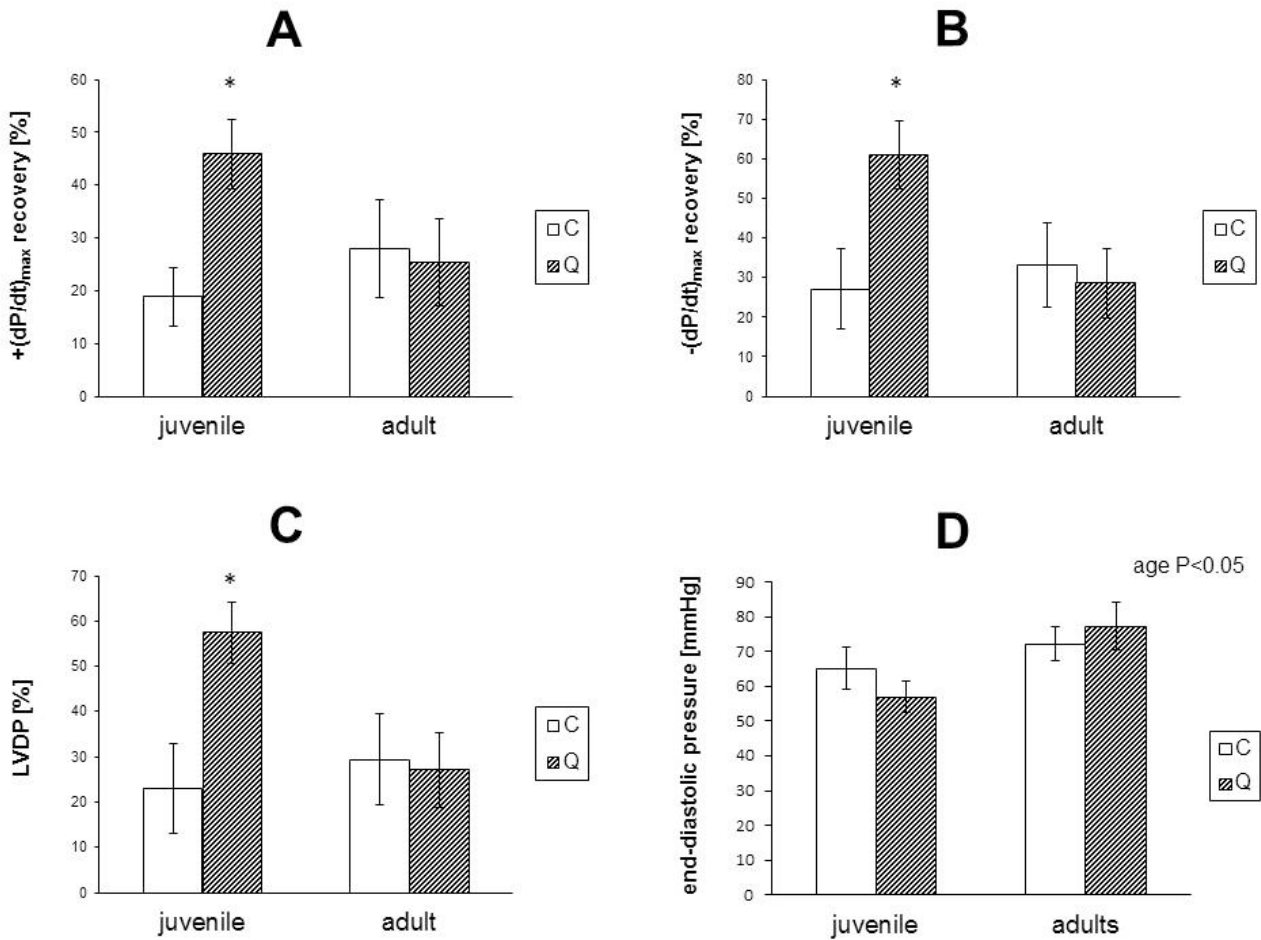
Fig. 1. Effects of quercetin on functional parameters of juvenile and adult rat hearts in the 40th minute of post-ischemic reperfusion. (**A**) Post-ischemic recovery of the maximal rate of pressure development +(dP/dt)max, (**B**) Post-ischemic recovery of the maximal rates of pressure fall −(dP/dt)max, (**C**) Post-ischemic recovery of the left ventricular developed pressure LVDP, (**D**) Post-ischemic levels of the end-diastolic pressure. Functional parameters were determined after 25 min lasting global ischemia followed by a 40-min reperfusion. The recovery of parameters was expressed as a percentage of pre-ischemic baseline values, the levels of end-diastolic pressure are presented in mmHg. Each bar represents mean ± SEM. *P<0.05 vs. corresponding control.
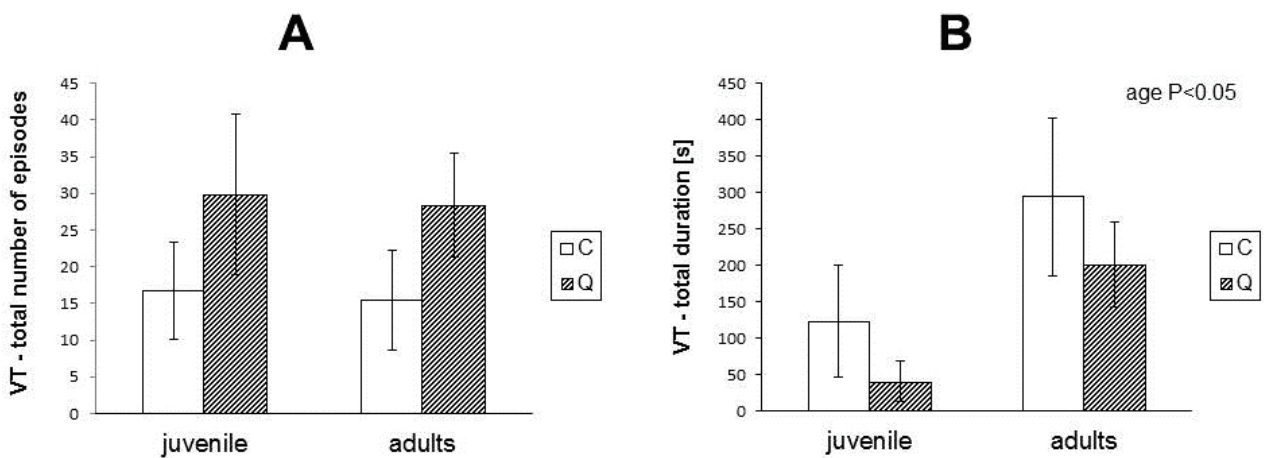

Fig. 2. Effects of quercetin on reperfusion arrhythmias in juvenile and adult rat hearts. (**A**) Total number of episodes of ventricular tachycardia, (**B**) Total duration of ventricular tachycardia. Arrhythmias were determined after 25 min lasting global ischemia during the first 10 min of reperfusion. Each bar represents mean ± SEM. Statistical significance is revealed by two-way ANOVA.

Discussion

In the present study, we have documented for the first time that cardioprotective effects of quercetin against I/R injury are age-dependent. We have shown that 4-week´s chronic treatment of the rats with quercetin at the dose of 20 mg/kg/day led to significant improvement of post-ischemic recovery of functional parameters LVDP, +(dP/dt)max and −(dP/dt)max in Langendorff-perfused juvenile rat hearts exposed to 25-min global ischemia and consequent 40-min reperfusion, while no protective effects were documented in adult hearts. On the other hand, quercetin failed to modify post-ischemic increase of end-diastolic pressure in hearts of both ages. Finally, our data have shown no effects of quercetin on total number of episodes and total duration of ventricular tachycardia during post-ischemic reperfusion in both juvenile and adult hearts.

It has been shown previously that age, as well as other co-morbidities, belongs to the important factors that may influence efficiency of preventive interventions against I/R injury (Ferdinandy et al. 2014, Griecsová et al. 2015). Quercetin has been shown to exert protective effects against I/R injury after both acute and chronic administration (Ikizler et al. 2007, Barteková et al. 2010, Jin et al. 2012, Liu et al. 2014); however, its efficiency in relation to the age of the treated subject has not been studied yet. Here we have shown that cardioprotective effect of quercetin on post-ischemic recovery of functional parameters of isolated rat hearts is age-dependent with its better efficiency in young animals. This finding is in concordance with findings of Griecsova et al. (2015) where the authors have shown that efficiency of another cardioprotective intervention against I/R injury, ischemic preconditioning, is also age-dependent, supporting the view that the age of the treated subject might be the determining factor for success in different interventions against I/R injury.

It is surprising that our data have shown no protective effect of quercetin in adult hearts even if other authors have shown quercetin to be protective against I/R injury in adult hearts (Ikizler et al. 2007, Jin et al. 2012, Liu et al. 2014). However, their findings were obtained by using either higher doses of quercetin (Ikizler et al. 2007, Liu et al. 2014), or different duration of the treatment (Bartekova et al. 2015), or different mode of the quercetin delivery (Jin et al. 2012). It seems that not only the dose and the duration of the treatment, but also the mode of the drug delivery may play a crucial role in efficiency of quercetin against I/R injury. In concordance with this view are the findings of Carlstrom et al. (2007) showing that quercetin added to the feeding diet had no effect on the onset or the severity of cardiovascular complications that develop in spontaneously hypertensive rats, while the administration of quercetin via oral gavage lowered arterial blood pressure in these rats.

It has been shown previously that pretreatment with quercetin exerts antiarrhythmic effects in anesthetized rats exposed to I/R when applied 2 min prior to reperfusion (Xiao et al. 1993) as well as in peroxynitrite-induced arrhythmias in isolated cardiac tissues and anesthetized animals (Soloviev et al. 2002). However, our data demonstrated no protective effect of quercetin on total number of episodes of ventricular tachycardia as well as on total duration of ventricular tachycardia in the rat hearts of both ages. The reason of this discrepancy may result from the different experimental models used as well as different dose and mode of quercetin delivery. On the other hand, our results have shown that increasing age of animals significantly increases total duration of ventricular tachycardia during post-ischemic reperfusion of isolated hearts, independently of quercetin treatment. These findings are in concordance with previous studies where the aging has been shown to be an important factor that increases susceptibility of hearts to reperfusion arrhythmias (Ledvényiová et al. 2013). Anyway, potential protective effects of quercetin on occurrence and severity of reperfusion arrhythmias remain the matter of doubt and should be studied intensively in the future.

Numerous mechanisms, including activation of PI3K/Akt pathway, increased activity of endogenous antioxidant systems, up-regulation of connexins (Bartekova et al. 2015), as well as attenuation of apoptosis via inhibition of JNK and p38 mitogen-activated protein kinase signaling pathways (Li et al. 2016) have been suggested to be involved in cardioprotective effects of quercetin against I/R injury. On the other hand, the molecular basis of age-dependency of quercetin efficiency against I/R injury is not clear. Based on the literature we can speculate that the age-dependent level of activation of Akt kinase due to preventive interventions against I/R injury (Griecsova et al. 2015) might be one of the factors participating in the cardiopriotective effects of quercetin in the treatment of subjects of different ages. Unfortunately, our present study has not solved this question as the molecular mechanisms have not been studied.

Conclusions

Our study demonstrated for the first time that cardioprotective effects of flavonoid quercetin against I/R injury are age-dependent with its better effects on functional recovery in young animals. On the other hand, quercetin had no effect on total duration and total number of episodes of ventricular tachycardia in the hearts of both ages. We conclude that the age of treated subject might be one of the determining factors in efficiency of quercetin treatment against I/R injury, and should be taken into consideration in quercetin application.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

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