Cardioprotective effect of 2',3,4'--trihydroxychalcone in preclinical experiment

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SUMMARY

Cardioprotective effect of 2',3,4'-trihydroxychalcone in preclinical experiment
2',3,4'-trihydroxychalcone is a newly synthesized substance with antioxidant properties. The aim of this pilot study was to monitor its effect during heart perfusion in the laboratory rat.
The study included two groups of animals of the same number (n = 10). The 1st group was pretreated with chalcone in a dose of 10 mg/kg p.o. during 15 days. The 2nd group was a placebo one. After i.p. administration of a heparin injection of 500 IU dose, the hearts were excised and perfused (a modified Langendorf's method). Working schedule: stabilization/ischaemia/reperfusion proceed at intervals of 20/30/60 min. Monitored parameters in the isolated heart: left ventricle pressure (LVP), end-diastolic pressure (LVEDP), contractility ($+dP/dt_{max}$). The treated hearts showed improved postischemic recovery, reaching LVP values of 101 ± 4% at the end of reperfusion, the placebo ones only 42 ± 6%. In the placebo hearts LVEDP increased from 10.0 ± 0.5 mm Hg to 32 ± 5 mm Hg after, in treated animals only about 10.5 ± 2 mm Hg. The treated hearts improved +dP/dtmax recovery during reperfusion to 92 ± 7 %. These values were significantly greater than those obtained from the placebo hearts. We conclude that the administration of 2',3,4'--trihydroxychalcone in laboratory rats has a cardioprotective potential against ischemia-reperfusion induced injury.
Key words: ischemia/reperfusion of heart – 2',3,4'-trihydroxychalcone – laboratory rat

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SOUHRN

Kardioprotektivní efekt 2',3,4'-trihydroxychalkonu v preklinickém experimentu
2',3,4'-trihydroxychalkon je nově syntetizovaná substance s prokázaným antioxidačním efektem. Cílem pilotní studie bylo sledovat jeho efekt za stavu ischémie/reperfuze srdce laboratorního potkana. Do studie byly zařazeny dvě skupiny zvířat (n = 10). První byl podáván chalkon v dávce 10 mg/kg p.o. po dobu 15 dnů. Druhá skupina byla placebo. Po i.p. podání heparinu (500 IU) byla odebrána srdce pro následnou perfuzi, metoda dle Langendorffa. Pracovní režim: stabilizace – ischémie – reperfuze: 20 – 30 – 60 minut. Sledované biomechanické parametry izolovaného srdce: systolický tlak v levé komoře (LVP), end-diastolický tlak v levé komoře (LVEDP) a kontraktilita ($+dP/dt_{max}$). Srdce zvířat premedikovaná chalkonem vykazovala návrat LVP na hodnoty 101 ± 4 % v porovnání s hodnotou výchozí, srdce ze skupiny placebo jen 42 ± 6 % hodnot výchozích. U srdcí z placebo skupiny nastalo zvýšení LVEDP z původních 10,0 ± 0,5 mm Hg na 32 ± 5 mm Hg po 60 minutách reperfuze. Toto zvýšení nebylo pozorováno u zvířat premedikovaných chalkonem. Předléčení chalkonem vedlo k návratu kontraktility (+dP/dtmax) na hodnotu 92 ± 7 % původního

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stavu, což je signifikantně vyšší než u placebo skupiny. 2',3,4'-trihydroxychalkon má kardioprotektivní potenciál u ischemicko/reperfuzního poškození.
Klíčová slova: ischémie/reperfuze srdce – 2',3,4'-trihydroxychalkon – laboratorní potkan

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Introduction

Myocardial infarction is one of the major causes of death in many countries. Although restoration of blood flow is the only way to save the myocardium from necrosis, reperfusion-induced injuries with the help of thrombolytic therapy are at the background of a high mortality rate [1]. Extensive studies show that myocardial ischemia/reperfusion injury is associated with increased generation of reactive oxygen species (ROS). These oxygen free radicals may result from depressions in contractile function, arrhythmias, depletion of endogenous antioxidant network, and membrane permeability changes [2]. The role of oxygen free radicals in the pathophysiology of ischemia/reperfusion injury is supported by an increased formation of lipid peroxides and other toxic products following such as injury [3]. The interaction of ROS with cell membrane lipids and essential proteins contributes to myocardial cell damage, leading to inflammatory reactions, and irreversible tissue injury [4].

In the search of the mechanisms of ischemia/reperfusion-induced pathways that may be amenable to manipulation, a number of potential candidates have been identified and have been subjected to many investigations. It is highly probable that a number of interaction mechanisms combine to determine the damage caused by ischemia/reperfusion in the myocardium, and a variety of such triggers have been postulated, including ionic disturbances and ion channels [5], fatty acid metabolism [6], α- and ß-adrenergic receptors [7], various gene expression [8], platelet-activating factor [9], endothelin [10], nitric oxide [11], heme oxygenase-1 and carbon monoxide [12, 13], and free radicals [14, 15]. It has been also shown that ischemia and reperfusion of the myocardium result in an activation of various pathways including caspase cascade, and it is hypothesized that a degree of caspase inhibition could be related to the recovery of postischemic function [16].

A large number of natural flavonoids with biological activity have been indentified in recent decades. One group of these products, the polyhydroxylated chalcones, exhibit antimicrobial [17], antiviral [18], antitumoral [19] and anti-inflammatory activities [20], and applications of therapeutic effects have been reported.

2',3,4'-trihydroxychalcone (C) was prepared by condensation of 2,4-dihydroxyacetophenone (A) (Fig. 1) and 3-hydroxybenzaldehyde (B) [21]. *In vitro* studies confirmed the scavenging potential of 2',3,4'--trihydroxychalcone in comparison with butylhydroxytoluene.

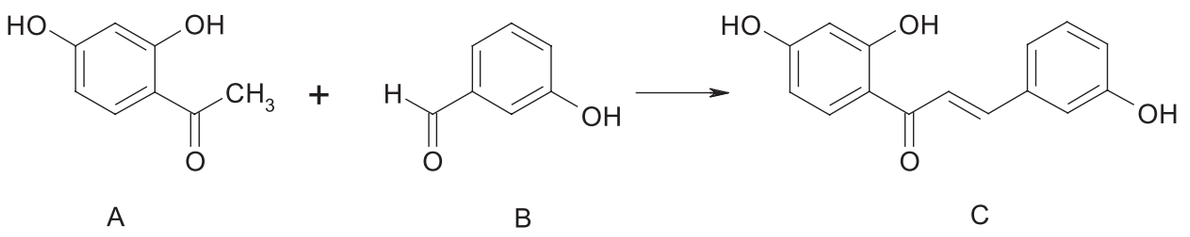

Fig. 1. Synthesis of 2',3, 4'-trihydroxychalcone

The aim of the study was to monitor the effects of the tested substance during heart perfusion of the laboratory rat.

The study and its experimental protocol were approved and monitored by the Ethics Committee of the University. The state of health of all animals was inspected regularly several times a day both during the acclimation of the animals and in the course of the whole experiment performed by the work group whose members are holders of the Eligibility Certificate issued by the Central Commission for Animal Protection pursuant to Section 17 of the Czech National Council Act No 246/1992 Coll. on animal protection against maltreatment.

EXPERIMENTAL PART

Methods

This study was performed on 20 male Wistar SPF (AnLab, Germany) laboratory rats of the same age (6 months) and comparable weight (345 ± 15 gr). The

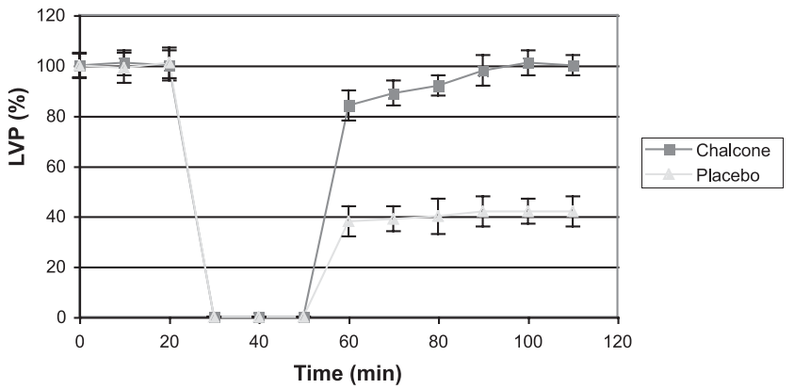

Fig. 2. Left ventricle pressure

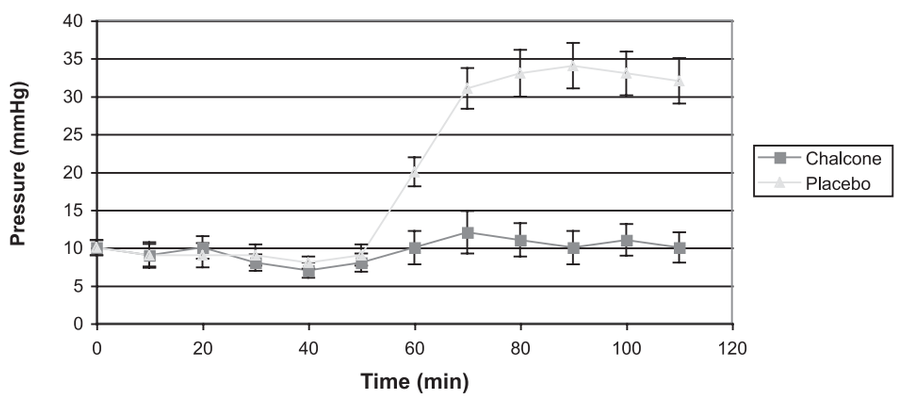

Fig. 3. End-diastolic pressure

animals were housed in a standard controlled temperature, fed the standard diet for small laboratory animals, and given water *ad libitum*. After a recovery period, the animals were divided randomly into 2 groups (n = 10).

The first group – the treated group – received chalcone in a dose 10 mg/kg solved in 2 ml of 0.5% Avicel solution orally by an intragastric tube. The second group – the placebo group – received only 0.5% Avicel in the same quantity (2 ml) and by the same application method as in the treated group.

At the end of the treatment period (15 days), the rats were anesthetized with an i.p. injection of an anaesthetical mixture (2% Rometar 0.5 ml + 1% Narkamon 10 ml, dose 0.5 ml solution/100 g body weight). After the i.p. heparin injection of 500 IU dose, the hearts were excised and perfused. In all experiments, a modified Langendorff method and a universal apparatus Hugo Sachs Electronic UP 100 (Germany HSE) were used. Schedule: stabili zation/ischemia/reperfusion proceeded at intervals of 20/30/60 min. Biomechanical parameters from the isolated heart: left ventricle pressure (LVP), end-diastolic pressure (LVEDP) and contractility (dP/dt_{max}) were measured using a ball filled with liquid (8–12 mm Hg), inserted through the left atrium into the left ventricle connected to an analog convertor (Isotec HSE, DIF modul HSE) [22].

RESULTS

In the hearts from the placebo animals, LVP recovered up to 42 ± 6% of the pre-ischemic values at the end of reperfusion. In the chalcone-pretreated animals, the hearts showed a significantly better postischemic recovery, reaching LVP values of 101 ± 4% at the end of the reperfusion (Fig. 2).

In the hearts from the placebo animals, LVEDP increased from 10.0 ± 0.5 to 32 ± 5 mm Hg after 60 min of reperfusion. This increase was diminished in the hearts from the chalcone-pretreated animals at the end of reperfusion (Fig. 3).

The pretreatment with chalcone improved $+dP/dt_{max}$ recovery during reperfusion to 92 ± 7% after 60 min of reperfusion. These values were significantly greater than those obtained from the placebo hearts (68 ± 5%) (Fig. 4).

DISCUSSION

The ischemia/reperfusion complex is a multidimensional process leading to the generation of

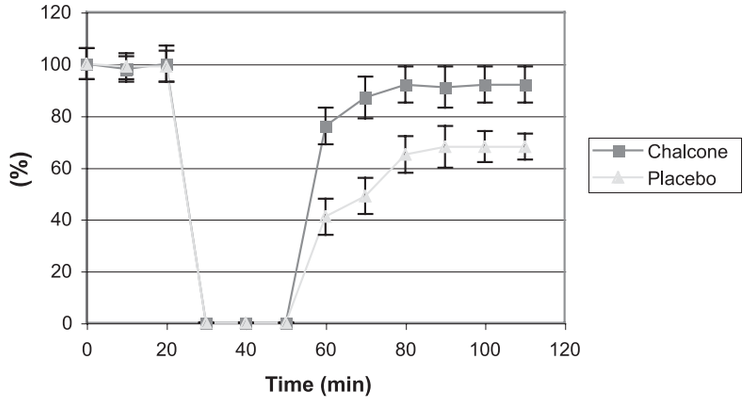

Fig. 4. Contractility

reactive oxygen species and oxidative stress, which results in severe tissue damage. Under normal conditions, ROS, which are generated during cellular functions, are eliminated by the intrinsic antioxidant enzyme systems such as superoxide dismutase, glutathione peroxidase and catalase [23]. Especially during the early stages of reperfusion, tissue concentrations of ROS increase partly due to the increased production and partly due to the insufficient levels of the antioxidant systems [24]. In the myocardial tissue, with the initiation of such process, increasing amounts of ROS result in ischemia/reperfusion damage and finally lead to myocardial injury [25]. Reactive oxygen species scavenging agents and antioxidant molecules have the capacity to partially reduce or eliminate deleterious effects induced by ischemia/reperfusion injury [23].

Flavonoids are polyphenolic compounds which constitute one of the most numerous and ubiquitous groups of plant metabolites and are an integral part of both human and animal diets. Recent interest has increased greatly owing to their antioxidant capacity with free radical scavenging properties attributed to the catechol or pyrogallol group. The redox properties of polyphenols allow them to act as enzyme inhibitors [23, 26], reducing agents, hydrogen donating antioxidants and in some cases they also chelate transition metal ions [26]. These properties play an important role in reducing the risk of free radical-related oxidative damage associated with degenerative disease such as in the treatment and prevention of cancer and cardiovascular disease [25, 27]. However, the compounds with the catechol moiety present a potent antioxidant activity in particular conditions. Therefore, research interest in this field has also been focused on the synthesis of modified flavonoids. One of them is 2',3,4'-trihydroxychalcone. The antioxidant properties of this substance were tested *in vivo* during the conditions of alloxan-induced diabetes mellitus in a pre-clinical experiment [21].

In our study, ischemia/reperfusion-induced cardiac dysfunction was evaluated by the measurement of left ventricle pressure (LVP), end-diastolic pressure (LVEDP) and contractility (dP/dt_{max}). These indices of myocardial dysfunction have been used in several studies in the isolated heart model of global ischemia/reperfusion [28–31]. The isolated rat, rabbit or guinea pig heart models provide an inexpensive and reproducible method to evaluate cardiac function and myocardial metabolic alterations during ischemia/reperfusion. In the isolated beating heart, cardiac function can be assessed independent of the influence of circulating blood cells and hormones, which may considered an important advantage of this model.

Left ventricular end-diastolic pressure is determined by volume and pressure load conditions, systolic performance of the heart, and diastolic pressure-volume relationships. Left ventricular end-diastolic pressure increases during angina in some patients with coronary artery disease. The mechanism of this increase, whether a decrease in left ventricular contractility or an alteration in pressure-volume relationship, remains controversial [32].

Under the conditions of global ischemia/reperfusion, a significant decrease in LVP and contractility and an increase in LVEDP were observed in animals without pretreatment with 2',3,4'-trihydroxychalcone.

From the results of our experiment it can be deduced that the administration of 2',3,4'-trihydroxychalcone in the laboratory rats has a cardioprotective potential against ischemia/reperfusion induced injury.

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