Pathology – Research and Practice 208 (2012) 452–457

Contents lists available at SciVerse ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

Glucose and blood pressure lowering effects of Pycnogenol® are inefficient to prevent prolongation of QT interval in experimental diabetic cardiomyopathy

Stanislava Jankyova[a,*], Jana Kmecova[a], Hana Cernecka[a], Lucia Mesarosova[b], Peter Musil[a], Zuzana Brnoliakova[c], Jan Kyselovic[a], Pavel Babal[d], Jan Klimas[a]

[a] Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic
[b] Department of Pharmacology, Jessenius Faculty of Medicine, Comenius University, Martin, Slovak Republic
[c] Institute of Experimental Pharmacology & Toxicology, Slovak Academy of Sciences, Bratislava, Slovak Republic
[d] Department of Pathology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

ARTICLE INFO

Article history:
Received 24 August 2011
Received in revised form 22 March 2012
Accepted 8 May 2012

Keywords:
Diabetes mellitus
Blood pressure
QT interval
Pycnogenol®

ABSTRACT

Diabetic cardiomyopathy shows ECG alterations related to cardiac repolarization and manifested by increased duration of QT interval. Although the mechanism is unknown, it is widely believed that the reduction of hyperglycaemia might prevent such alterations. To test this hypothesis, we used the standardized extract of French pine bark – Pycnogenol® (PYC) with hypoglycaemic and antioxidant properties in 8–9 week old rats with experimentally (streptozotocin) induced diabetes mellitus (DM). PYC was administered orally for 6 weeks in three different doses (10, 20, and 50 mg/kg b.w., resp.). Experimental DM was manifested by hyperglycaemia (four to six-fold increase in plasma glucose concentration; $p < 0.05$) and significantly increased mean arterial blood pressure (by 19%; $p < 0.05$) measured using catheterization of carotid artery *in vivo*. Both abnormalities were dose-dependently reduced by PYC. In addition, diabetic cardiomyopathy was associated with a significant increase in left ventricular weight to body weight ratio (by 21%; $p < 0.05$) and a significant decrease of the width of cardiomyocytes (by 23%; $p < 0.05$) indicating cardiac edema on the one side, and hypotrophy of cardiomyocytes on the other. Both of these changes were not affected by PYC. Consequently to metabolic and hemodynamic alterations, significant prolongation of QT interval (by 20%; $p < 0.05$) was present in diabetic rats, however, PYC failed to correct it. Conclusively, PYC fails to correct QT prolongation in spite of dose-dependent reduction of glycaemia and high blood pressure in streptozotocin-induced diabetic cardiomyopathy.

Introduction

Increase of QT interval duration (and heart rate corrected QTc), as a manifestation of impaired cardiac repolarization, is one of the most important cardiac parameters defining cardiovascular risk in a large number of diseases, including diabetes mellitus (DM) [28]. Diabetic cardiomyopathy, associated with increased risk of cardiac arrhythmias, ventricular fibrillations and sudden cardiac death, is becoming a major cause of mortality in diabetic patients [2]. Data from a population-based study demonstrate that impaired fasting glucose and hyperinsulinemia are associated with prolongation of QTc interval, and this is associated with increased risk of sudden cardiac death [29]. Likewise, Marfella et al. [20] have shown that acute hyperglycaemia leads to significant prolongation and increase of dispersion of QTc. Moreover, QT prolongation is associated with hypertension and left ventricular hypertrophy, both conditions increasing cardiovascular risk of DM [25]. As QT prolongation in diabetic patients is directly related to triggering of polymorphic ventricular tachycardia (torsades de pointes), which can turn into ventricular fibrillation, syncope and sudden death [4,8], the reduction of QT interval is an important therapeutic goal. Consequently, glycaemia reducing substances might have significant benefit in reduction of repolarization disturbances in diabetic heart and reduction of overall cardiovascular risk as well.

Pycnogenol® (PYC) is a standardized water-soluble extract of the bark of French maritime pine (*Pinus pinaster* ssp. *atlantica*) with significant antioxidant and hypoglycaemic properties [23]. It is the patented trade name of the Horphag Research Ltd., UK. In our previous experiments, PYC showed positive effects on neuronal

Abbreviations: ECG, electrocardiography; PYC, Pycnogenol®; DM, diabetes mellitus; STZ, streptozotocin; CON, non-diabetic, control rats; GO, glucose oxidase; LV, left ventricle; sBP, systolic blood pressure; dBP, diastolic blood pressure; PP, pulse pressure; MAP, mean arterial pressure; b.w., body weight; SEM, standard error of the mean.

* Corresponding author at: Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Odbojarov 10, 83232 Bratislava, Slovak Republic. Tel.: +421 2 50 117 364; fax: +421 2 50 117 100.
E-mail addresses: jankyova@fpharm.uniba.sk, jankyova@gmail.com (S. Jankyova).

0344-0338/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.prp.2012.05.010

activity in diabetic neuropathy [16] and on cardiac performance in diabetic cardiomyopathy [17]. Similarly in human, PYC diminished diabetic microangiopathy [5] and reduced cardiovascular risk factors [32]. Based on these findings, we investigated the dose-dependent influence of treatment with PYC on QT (QTc) duration in the experimental model of streptozotocin induced DM in rat.

Materials and methods

Animals. For experiment, 8–9-week-old male Wistar rats (Department of Toxicology and Laboratory Animal breeding, Dobra Voda, Slovak Republic) were used. They were housed in a quarantine facility for eight days before use. During the experiment, rats were housed in groups of four in cages of the type T4 Velaz (Prague, Czech Republic) with wood shaving bedding (exchanged daily). Animals had free access to a standard commercial diet and water. The animal room was air-conditioned, and the environment was continuously monitored for the temperature of $23 \pm 1\,^{\circ}C$ and relative humidity of 40–70%. Animals were kept under a stable regimen of 12 h light/12 h' darkness. All procedures involving the use of experimental animals were approved by the State Veterinary and Food Administration of the Slovak Republic. The investigation conforms to the Guide for the Care and Use of Laboratory Animals: Eight Edition (2010) published by the US Committee for the Update of the Guide for the Care and Use of Laboratory Animals; National Research Council and to the EU adopted Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for experimental and other scientific purposes.

Treatment. Animals were randomized into five groups ($n = 7$–8 rats per group). Experimental DM was induced by intraperitoneal bolus injection of streptozotocin (STZ; Sigma, St. Louis, MO, USA) at the dose of 25 mg/kg of body weight repeated for three consecutive days, as described previously [16]. STZ was dissolved in 0.1 M citrate buffer (pH 4.5) before use. Non-diabetic control rats (CON) received vehicle. Animals were fasted overnight prior to STZ administration. Water and food were available immediately after dosing. Two weeks later, after development of experimental DM, commercially available PYC (Horphag Research, Geneva, Switzerland; kindly donated by Generica s.r.o., Piestany, Slovak Republic) was administered in drinking water to the animals in three experimental groups. Three different doses were applied during eight weeks: 10 mg/kg b.w./day (STZ-P10), 20 mg/kg b.w./day (STZ-P20) or 50 mg/kg b.w./day (STZ-P50). Control groups (CON and STZ) received vehicle.

Plasma glucose analysis. Glycaemia was measured using spectrophotometric analysis from blood plasma. Blood (approximately 1 ml) was collected from the tail vein under both preprandial and postprandial conditions. After centrifugation, plasma glucose levels were measured using the commercial Glucose (GO) Assay Kit (Sigma–Aldrich, St. Louis, MO, USA) with spectrophotometric analysis.

ECG measurements. Standard 12-lead electrocardiography was performed in rats anaesthetized with tribromoethanol (15 μl of 2.5% solution/g/b.w.) and placed on a 37 °C table as described before [18]. Needle electrodes were used. The center of chest electrodes was 1.5 cm from the xyphoid process on the sternum. The legs were fixed in the ventral position by elastic cords. Heart rate was evaluated as an ECG cycle length (RR duration). Duration of QT was determined from the onset of QRS complex to the end of T wave semimanually in either of the simultaneously recorded leads (as exemplary shown in six limb leads in Fig. 1). Six consecutive beats were evaluated, and the arithmetic means of RR and QT were obtained. Duration of QT was corrected according to Kmecova and Klimas [19] as QTc (in ms) = $QT/(RR/f)^{1/2}$; $f = 150$. All animals were affected in the same manner, and the blood pressure measurements were performed consecutively.

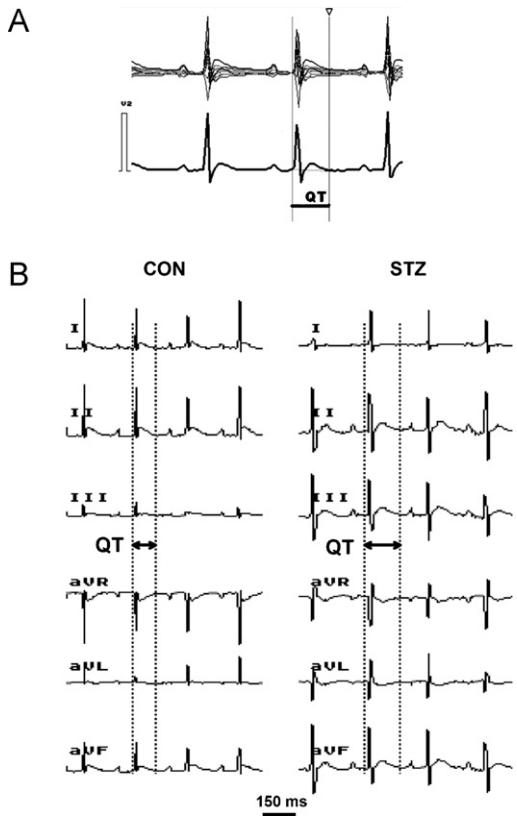

Fig. 1. ECG recording with depiction of QT interval duration measurement (A), and representative electrocardiograms (B) in non-diabetic, control rat (CON) and diabetic rat (STZ).

Intraarterial BP measurements. Direct cannulation of the carotid artery was performed using custom-fashioned polyethylene tubing in anaesthetized rats. Polyethylene tubing (PE50, 0.58 mm ID × 0.96 mm OD, Portex, England) was inserted into the carotid artery and pressure waveform was monitored, recorded and analyzed after 10 min of stabilization using S.P.E.L. Advanced Haemosys system (Experimetria Ltd., Hungary). Data of systolic and diastolic blood pressure (sBP, dBP) were collected. Consecutively, values of pulse pressure (PP) and mean arterial pressure (MAP) were calculated. Pulse pressure was defined as PP = sBP − dBP. Mean arterial pressure was defined as MAP = [(2 × dBP) + sBP]/3.

Post mortem gravimetry

Left ventricular weight was determined gravimetrically. After BP recordings, the animals were sacrificed and hearts were excised. Left ventricles (including the septal portion) were blotted dry and weighed. Two parameters were used as measures of pathological changes: absolute weight of LV and the LV-to-body weight ratio.

Histology. Paraffin-embedded left ventricular samples were used. Sections of left ventricles stained with hematoxylin–eosin were examined on a randomized basis for the number of nuclei per standardized area (as a measure of potential change of cardiomyocytes number) where only nuclei of cardiomyocytes were taken into account, and for the width of cardiomyocytes (as a measure of potential changes in cell size). Cardiac interstitial fibrosis was revealed with picric acid Sirius red staining [10]. The positively (appears as red structures) stained fibrotic area was measured using two dimensional image analysis and expressed as relative collagen density, *i.e.* percentage of positively stained interstitial field to total area. For microscopic imaging, a Meopta microscope was used (Finchley, UK). The detection system was based