European Journal of Pharmacology 774 (2016) 20–24

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Cardiovascular pharmacology

Phytic acid suppresses ischemia-induced hydroxyl radical generation in rat myocardium

Toshio Obata [a,*], Michiko Nakashima [b]

[a] School of Nursing, Faculty of Health Sciences, Osaka Aoyama University, 2-11-1 Niina, Mino City, Japan
[b] Department of Nursing, School of Health Sciences, Asahi University, 1851 Hozumi, Mizuho City, Gifu, Japan

ARTICLE INFO

Article history:
Received 17 November 2015
Received in revised form
23 December 2015
Accepted 24 December 2015
Available online 25 December 2015

Keywords:
Phytic acid
Iron(II)
Norepinephrine
Ischemia
Hydroxyl radical
Microdialysis

ABSTRACT

The present study examined whether ischemia-reperfusion-induced hydroxyl radical (·OH) generation was attenuated by myo-inositol hexaphosphoric acid (phytic acid). A flexibly mounted microdialysis technique was used to detect the generation of ·OH in *in vivo* rat hearts. To measure the level of ·OH, sodium salicylate in Ringer's solution (0.5 mM or 0.5 nmol/μl/min) was infused directly through a microdialysis probe to detect the generation of ·OH as reflected by the nonenzymatic formation of 2,3-dihydroxybenzoic acid (2,3-DHBA). To confirm the generation of ·OH by Fenton-type reaction, iron(II) was infused through a microdialysis probe. A positive linear correlation between iron(II) and the formation of 2,3-DHBA (R^2=0.983) was observed. However, the level of 2,3-DHBA in norepinephrine (100 μM) plus phytic acid (100 μM) treated group were significantly lower than those observed in norepinephrine-only-treated group (n=6, $^*p<0.05$). To examine the effect of phytic acid on ischemia-reperfusion-induced ·OH generation, the heart was subjected to myocardial ischemia for 15 min by occlusion of the left anterior descending coronary artery (LAD). When the heart was reperfused, the normal elevation of 2,3-DHBA in the heart dialysate was not observed in animals pretreated with phytic acid. These results suggest that phytic acid is associated with antioxidant effect due to the suppression of iron-induced ·OH generation.

1. Introduction

Myo-inositol hexaphosphoric acid (phytic acid) is present in plants, particularly in cereals, nuts, oil seed, legumes, pollen, and spores (Gupta et al., 2015; Midorikawa et al., 2001). Phytic acid has been found to chelate metal ions such as iron and calcium (Kim et al., 2010). Phytic acid has been used as an antioxidant (Beck et al., 2014) and could conceivably be a protective agent in the human diet (Phillippy and Graf, 1997). Although the role iron on myocardial cellular injury is unclear, it has been implanted in cell degeneration more often than any other metal (Obata and Yamanaka, 2002). The antiradical effect of phytic acid occurs by chelating iron required for the generation of hydroxyl radical (·OH) via the Fenton-type reaction (Obata, 2003). Abnormal levels of extracellular noradrenaline and/or intraneural calcium triggered by ischemia-reperfusion may be detrimental to the functioning of adrenergic nerve terminals in the myocardium. The ·OH was generated by the presence of norepinephrine and oxygen. In mammalian cells, phytic acid and the lower inositol phosphate are present as intracellular molecules (Gibson, 2012). The present study examined the antioxidant effect of phytic acid on myocardial ischemia-reperfusion injury caused by ·OH generation. To achieve goal, we measured ·OH formation in in vivo hearts, with the use of a flexibly mounted microdialysis technique that we developed (Obata et al., 1994). The ·OH reacts with salicylate and generates 2,3- and 2,5-dihydroxybenzoic acid (DHBA) (Chiueh et al., 1994; Obata et al., 1994), which can be measured electrochemically in picomole quantities by a high-performance liquid chromatographic-electrochemical (HPLC-EC) procedure. Obata et al., 1994). The formation of DHBA at after systemic administration of salicylate is used as an index of ·OH generation in heart. The 2,3-DHBA can be nonenzymatically formed by the aromatic hydroxylation of ·OH and can provide an assay for ·OH formation (Chiueh et al., 1994; Wang et al., 2005).

2. Materials and methods

2.1. Chemicals

Phytic acid was purchased from Wako Pure Chemical

Abbreviations: Phytic acid, Myo-inositol hexaphosphoric acid; ·OH, hydroxyl radical; DHBA, dihydroxybenzoic acid; CPK, creatine phosphokinase

* Corresponding author.
E-mail address: t-obata@osaka-aoyama.ac.jp (T. Obata).

http://dx.doi.org/10.1016/j.ejphar.2015.12.045
0014-2999/

Industries, Ltd. (Osaka Japan). Norepinephrine and ferrous ammonium sulfate, and sodium salicylate, its hydroxylated metabolites were purchased from Sigma Chemical Co. (St. Louis, MO. USA). All other chemicals were obtained from Wako Pure Chemical Industries (Osaka, Japan). An appropriate volume of these stock solutions was added to Ringer's solution consisting of 147 mM NaCl, 2.3 mM $CaCl_2$, and 4 mM KCl (pH 7.4) immediately before use, as indicated in the Results.

2.2. Animals

Adult male Wistar rats weighing 200–300 g were kept in an environmentally controlled room (20–23 °C, 50–60% humidity, illuminated from 07:00 to 19:00 h) and fed with food and water *ad libitum* for 4 days prior to our experiments. The rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and the level of anesthesia was maintained by intraperitoneal injection of chloral hydrate (20 mg/kg, i.p.). Artificial ventilation was maintained by constant-volume respiration with room air mixed with oxygen. The heart rate, arterial blood pressure, and electrocardiogram (ECG) were monitored and recorded continuously. At the end of the experiments the rats were killed by an overdose of anesthetic. All procedures in dealing with the experimental animals met the guideline principles stipulated by the Physiological Society of Japan and the Animal Ethics Committee of the Asahi University.

2.3. Experimental protocol

Details of the flexibly mounted microdialysis technique and its application to measure biological substances in the interstitial space have been described previously (Obata et al., 1994). We created a suitable microdialysis probe. The probe was implanted from the epicardial surface into left ventricular myocardium to the depth of 3 mm and perfusate through the inlet tube. The synchronized movement of the tip of the microdialysis probe with the beating ventricle minimized the tissue injury that would otherwise be caused by friction between the probe and the muscle tissue (Fig. 1). The drugs were dissolved in Ringer's solution for perfusion (1 μl/min) through a microdialysis probe into the myocardium. For trapping ·OH radicals (Chiueh et al., 1994; Wang et al., 2005) in the myocardium, sodium salicylate in Ringer's solution (0.5 mM or 0.5 nmol/μl/min) was perfused by a micro-injection pump (Carnegie Medicine, CMA/100 Stockholm, Sweden) and the basal level of 2,3-DHBA during a definite period time was determined (Fig. 2). Samples (1 μl/min) were collected after 15 min into small collecting tubes containing 15 μl of 0.1 N $HClO_4$. In the preparation of ischemic rat, after the microdialysis probe implantation in the ischemic zone, the left anterior descending coronary artery (LAD) branch was clamped by a thread through a tube surrounding the coronary artery. The heart was subjected to regional ischemia for 15 min by the occlusion of the LAD coronary artery followed by reperfusion for 60 min. In order to ascertain the protective effect of phytic acid on myocardial infarction and reperfusion damage, the serum creatine phosphokinase (CPK) assay was measured. Blood was collected from catheterization of the carotid artery analyzed for CPK using 705 CK-NAK (Tanabe Seiyaku, Japan).

2.4. Analytical procedure

The dialysate samples were immediately injected for analysis into an HPLC-EC system equipped with a glassy carbon working electrode (Eicom, Kyoto, Japan) and an analytic reverse-phase column on an Eicompak MA-5ODS column (5 μm 4.6 × 150 mm^2; Eicom). The working electrode was set at a detector potential of 0.75 V. Each liter of mobile phase contained 1.5 g 1-heptansulfonic acid sodium salt (Sigma), 0.1 g Na_2EDTA, 3 ml triethylamine (Wako Pure Chemical Industries, Japan) and 125 ml acetonitrile (Wako) dissolved in H_2O. The pH of the solution was adjusted to 2.8 with 3 ml phosphoric acid (Wako).

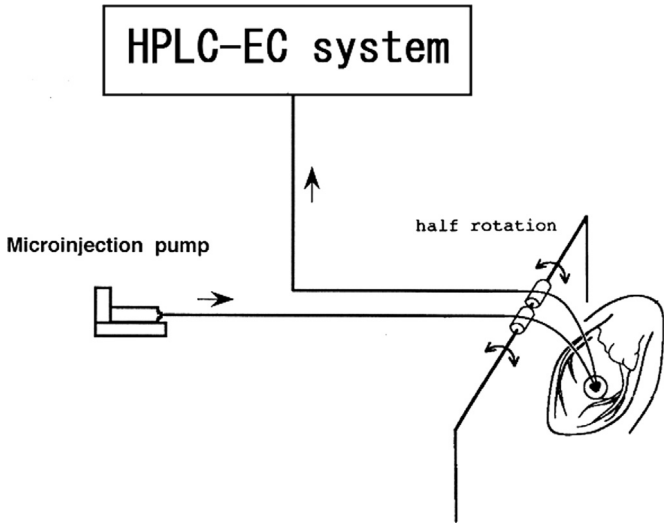

Fig. 1. Microdialysis probe-holding system in heart. Dialysis probe was implanted in area of left anterior descending artery (LAD) and perfused with Ringer's solution by a microinjection pump.Microdialysis probe-holding system involves loose fixation of the tube and synchronization of movement of the heart and probe, the probe being supported by a stainless steel axis. This system increased recovery rate without an accompanying increase in tissue damage. The dialysis probe was implanted in the left ventricular myocardium. The inlet tube of microdialysis probe was connected to a microinjection pump, and outlet tube led to the dialysate reservoir. The dialysate adenosine concentration was measured directly by the high-performance liquid chromatographic-electrochemical (HPLC-EC) system.

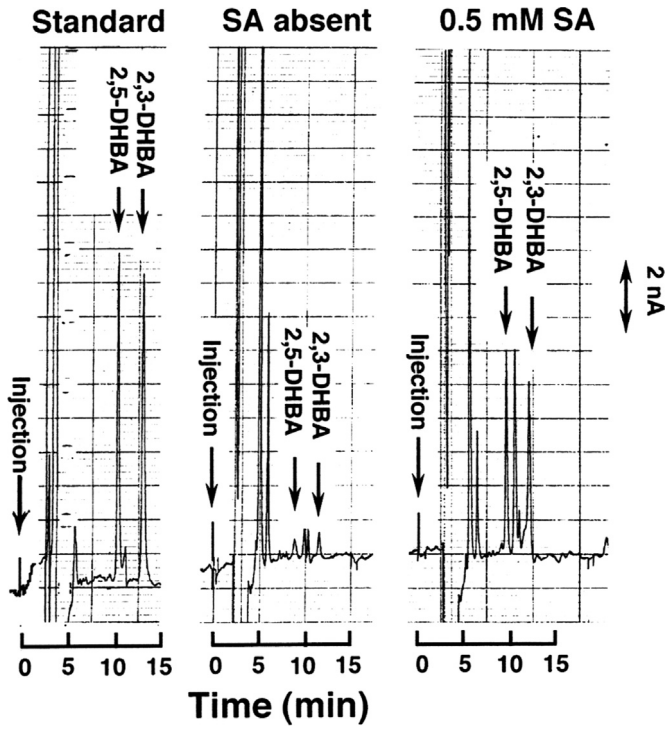

Fig. 2. Elution pattern by an HPLC-EC detection. Separation of a standard mixture of in picomole 2,3- and 2,5-DHBA. In the absence of salicylic acid (SA absent) and presence of 0.5 mM (or 0.5 nmol/μl/min) SA, the concentration of one picomole 2,3-DHBA in the dialysate within the first 15 min after implantation of the microdialysis probe was indicated.