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Evaluation of cardio-protective effect of soybean oligosaccharides

Zhang Meng [a,b], Cai ShangLang [a,*], Ma JiangWei [c,*]

[a] Department of Cardiology, Affiliated hospital of Qingdao University, Qingdao, 266021, China
[b] Department of Cardiology, Affiliated Hospital of Jining Medical University, Jining, China
[c] Department of Cardiology, Fengxian Branch of Shanghai 6th People's Hospital, Shanghai 201400, China

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ABSTRACT

The aim of the present study was to investigate whether soybean oligosaccharides (SO) protects heart function against myocardium ischemia reperfusion (MIR) injury. Hearts were 20 min global ischemia and 50 min reperfusion. Rats were fed for 30 days with saline (sham and MIR groups) or the SO (200 or 400 mg/kg body weight, daily). At the end of 30 days, the left main coronary artery was occluded for 30 min, followed by 24 h reperfusion, in anesthetized rats. Sham operated animals were subjected to the same surgical procedures, except that the suture under the left anterior descending coronary artery was not tied. Results showed that SO decreased malondialdehyde (MDA) level and increased antioxidant enzymes activities in the SO-treatment group. Pretreated with SO it showed a significant recovery in cardiac contractile function, reduction in infarct size, and decrease in creatine kinase (CK), aspartate transaminase (AST) and lactate dehydrogenase (LDH) activities. Moreover, SO also significantly increased the expression of p-JAK2 and p-STAT3 proteins in rat heart. However, no significant change in JAK2 and STAT3 levels was observed. Activation of JAK2/STAT3 pathway showed a significant protective role in the SO-treatment group. Perhaps, the altered activation of the JAK2/STAT3 pathway in ischemic myocardium is one mechanism by which SO is cardioprotective.

1. Introduction

Cardiovascular disorders remain one of the important causes of death around the world (Wattanapitayakul and Bauer, 2001). Specifically, cardiac ischemia is a main reason for leading to heart dysfunction, hypoxia, and apoptosis, and necrosis. I/R-induced injury is known to increase the levels of reactive oxygen species (ROS) several-fold which can lead to apoptosis (Jacobson, 1996; Ryter et al., 2007). Various reports suggest that antioxidant therapy after I/R would help the myocardium to recover from ROS-induced damage (Chen et al., 1996; Du et al., 2007). Thus there is a need to understand and identify suitable antioxidant interventions to salvage the myocardium from IR-mediated tissue damage and dysfunction.

Soy products have superior nutritional characteristics in terms of high protein contents and amino acid quality. Soy is also considered to have a potential role in the prevention of chronic diseases such as atherosclerosis, cancer, osteoporosis, and menopausal disorders (Liu et al., 2002). Soybean oligosaccharides (SO), which are isolated from the soybean seeds, are "potential prebiotic material" and approved by the Food and Drug Administration as GRAS (Generally Recognized As Safe) ingredient in USA (Kim et al., 2003; Zhou et al., 2012). SO have been shown to be a promising candidate for the prevention of many chronic diseases such as cancer, osteoporosis, atherosclerosis and menopausal disorders (Espinosa-Martosy and Rupérez, 2006; Mateos-Aparicio et al., 2008). It has been reported that SO treatment reduces oxidative stress and abnormal blood lipid levels induced by high fat diets. However, the effect of SO on antioxidant enzyme activities and infarction in IR rats remains not clear.

Therefore, the aim of the present study was to define the effects of SO on cardiac function against IR injury and to find out its mechanism using a MIR rat model.

2. Material and method

2.1. Material

Soybean oligosaccharides were prepared in our laboratory.

2.2. Animals

Forty-eight male Wistar albino rats with mean weight of 250 ± 300 g were included in the study. The study protocol was approved by the Committee of Experimental Animals of Jining Medical University. All experimental procedures complied with the Guide for the Care and Use of Laboratory Animals. Rats were housed in an air-conditioned room with 12 h light and dark cycles, and a constant temperature (22 ± 2 °C). The rats were housed in cages, and allowed free access to

Abbreviations: SO, soybean oligosaccharides; MIR, myocardium ischemia reperfusion; CK, creatine kinase; AST, aspartate transaminase; LDH, lactate dehydrogenase.

* Corresponding authors.
E-mail addresses: jhgjkut@sohu.com (S.L. Cai), jiangwma013@163.com (J.W. Ma).

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Table 1
Effect of SO on LVSP, LVEDP, $+dp/dt_{max}$ and $-dp/dt_{max}$ in hearts.

Group	LVSP (mm Hg)	LVEDP (mm Hg)	$+dp/dt_{max}$ (mm Hg/s)	$-dp/dt_{max}$ (mm Hg/s)
Sham	127.51 ± 11.07	6.81 ± 0.72	4527.07 ± 415.62	−4137.42 ± 400.63
MIR	97.25 ± 8.83**	15.71 ± 1.62**	3091.14 ± 289.64**	−3126.58 ± 294.26**
SO (200 mg/kg)	113.28 ± 10.25#	11.72 ± 1.59#	3837.42 ± 335.27##	−3528.43 ± 314.35##
SO (400 mg/kg)	123.15 ± 13.04##	8.03 ± 0.84#	4301.59 ± 407.44##	−3904.35 ± 336.16##

** $p < 0.01$, compared with sham group.
# $p < 0.05$, compared with MIR group..
$p < 0.01$, compared with MIR group.

standard rat chow and water before the experiments. The animals were fasted overnight before the experiments, but were given free access to water.

2.3. Myocardial ischemia and reperfusion in rats

To induce myocardial infarction, the myocardial ischemia/reperfusion model was prepared according to the protocol of Takada et al. (2004) with minor modifications. Briefly, rats were anesthetized with 50 mg/kg sodium pentobarbital i.p. The trachea was intubated, and the animal was artificially ventilated with room air by an animal respirator (SN-480-7; Shinano, Tokyo, Japan) with a frequency of 54 strokes/min and a tidal volume of 1.5 ml/100 g. Left thoracotomy at the fifth intercostal space and pericardiotomy were performed, a 6/0 braided silk suture was placed around the left anterior descending coronary artery, and the coronary artery was occluded by pulling on the suture. A standard limb lead II electrocardiogram was monitored with a cardiograph (PowerLab; AD Instruments, Otago, New Zealand) as described (Takada et al., 2004). After thoracotomy of the left side, the proximal portion of the left anterior descending artery was surgically occluded for 30 min with a suture (size 6/0 NESCO suture; Azwell Inc., Osaka, Japan). The onset of ischemia was confirmed by development of cyanosis and typical elevation of the ST segment of the electrocardiogram. At 30 min after occlusion, the heart was reperfused by releasing the ligature, and the thoracotomy was closed. Sham operated animals were subjected to the same surgical procedures, except that the suture under the left anterior descending coronary artery was not tied.

2.4. Experimental protocol

The rats were assigned one of four groups. 1) Sham (n = 12): the ligature was placed under left anterior descending coronary artery without occlusion; 2) Ischemia and reperfusion (control) group (n = 12), rats pretreated with vehicle (saline) for 30 days before ischemia; 3) Ischemia and reperfusion + SO (200 mg/kg, n = 12); 4) Ischemia and reperfusion + SO (400 mg/kg, n = 12). SO (200 or 400 mg/kg, once daily) were orally administered for 30 days before ischemia. Blood samples (0.3 ml in each group) were collected in tubes containing heparin at baseline and 24 h of reperfusion for measurement of myocardial enzymes concentration. The entire sample was immediately centrifuged. Plasma was collected and frozen under −70 °C until analyzed.

2.5. Measurement of myocardial infarct size

At the end of 24 h reperfusion, all of the rats were sacrificed with overdose sodium pentobarbital, and the area at risk (AAR), infarct size, and viable area were evaluated by Evans Blue/TTC staining as previously described (Ertracht et al, 2011). Briefly, Evans blue stained areas indicated non-ischemia area. White parts in the heart indicated the infarct size. Red parts, which were stained by TTC, represented for ischemic but viable tissue. White plus red part was AAR.

2.6. Antioxidant assays

Malondialdehyde (MDA), which is a measure of lipid peroxidation, was spectrophotometrically measured by using the thiobarbituric acid assay (Ohkawa et al., 1979; Padurariu et al., 2010). 200 μL of supernatant was added and briefly mixed with 1 mL of 50% trichloroacetic acid in 0.1 M HCl and 1 mL of 26 mM thiobarbituric acid. After vortex mixing, samples were maintained at 95 °C for 20 min. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatants were read at 532 nm. A calibration curve was constructed using MDA as standard and the results were expressed as nmol/mg protein.

The amount of the reduced glutathione (GSH) and oxidized glutathione (GSSG) was determined from the extent of 5′-dithiobis(2-nitrobenzoic acid) reduction for GSH and from consumption of reduced nicotinamide dinucleotide phosphate (NADPH) in the presence of glutathione reductase for GSSG (Tietze, 1969). Briefly, 30 mg of the frozen tissue was mixed with 300 μL meta-phosphoric acid solution containing 1% meta-phosphoric acid. The samples were left on ice for 15 min and centrifuged at 10,000 g for 15 min, and the supernatants were used for analyses.

Superoxide Dismutase (SOD) activity in duodenal tissue was assayed as described in the Randox-Ransod enzyme kit. This method employs xanthine and xanthine oxidase to generate superoxide radicals, which react with INT (iodophenyl–nitrophenol–phenyltetrazolium) to form a red formazan dye. SOD activity was measured by the degree of inhibition of this reaction measured by the absorbance at 505 nm and 37 °C and expressed as U/mg protein.

Catalase (CAT) activity was measured at 37 °C by following the rate of disappearance of hydrogen peroxide (H_2O_2) at 240 nm ($\varepsilon 240 = 40\ M^{-1}\ cm^{-1}$) (Luck, 1963). One unit of CAT activity is defined as the amount of enzyme catalyzing the degradation of 1 lmol of H_2O_2 per min at 37 °C and specific activity corresponding to the transformation of substrate (in lmol) (H_2O_2) per min per mg protein. CAT activity was expressed as U/mg protein in the tissue.

Table 2
Effect of SO on MDA and GSH levels in hearts.

Group	MDA (nmol/mg)	GSH (mg/g)
Sham	2.27 ± 0.16	42.69 ± 3.78
MIR	6.03 ± 0.57**	21.53 ± 1.93**
SO (200 mg/kg)	4.58 ± 0.44##	30.54 ± 3.37##
SO (400 mg/kg)	3.31 ± 0.31##	39.72 ± 3.61##

** $p < 0.01$, compared with sham group.
$p < 0.01$, compared with MIR group.

Table 3
Effect of SO on SOD, CAT and GSH-Px levels in hearts.

Group	SOD (U/mg)	CAT (U/mg)	GSH-Px (U/mg)
Sham	219.04 ± 19.57	59.82 ± 4.83	74.38 ± 8.35
MIR	121.49 ± 11.42**	23.17 ± 2.05**	31.22 ± 3.52**
SO (200 mg/kg)	173.28 ± 15.39##	44.18 ± 5.42##	51.53 ± 6.03##
SO (400 mg/kg)	205.11 ± 18.05##	55.24 ± 6.11##	70.56 ± 7.21##

** $p < 0.01$, compared with sham group.
$p < 0.01$, compared with MIR group.

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