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Evaluation of the treatment with resveratrol-loaded nanoparticles in intestinal injury model caused by ischemia and reperfusion

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ABSTRACT

The gastrointestinal tract is extremely sensitive to ischemia and reperfusion (I/R). Studies have reported that resveratrol (RSV) is able to combat damage caused by intestinal I/R. Because of its effectiveness in increasing the permanence and bioavailability of resveratrol in the intestinal epithelium, we investigated whether the effect of resveratrol-loaded in poly(anhydride) nanoparticles reduce oxidative stress and promote myenteric neuroprotection in the ileum of rats subjected to I/R. Physicochemical evaluations were performed on nanoparticles. The animals were divided into nine groups (n = 6/group) and treated every 48 h. Treatments with resveratrol (7 mg/kg of body weight) were applied 5 days before surgery and continued for 7 days after surgery (reperfusion period). The superior mesenteric artery was occluded to cause I/R injury. Oxidative stress, myeloperoxidase, nitrite, aspartate aminotransferase, alanine aminotransferase, immunolabeling of myenteric neurons and glial cells, and gastrointestinal transit was evaluated. Both nanoparticle formulations presented negative charge with homogeneous distribution, and the payload, showed an encapsulation efficiency of 60%. Resveratrol administered in free form prevented alterations that were caused by I/R. The results of the groups treated with RSV-loaded nanoparticles presented similar results to the group treated with free resveratrol. Treatment with empty nanoparticles showed that poly(anhydride) is not an ideal nanocarrier for application in *in vivo* models of intestinal I/R injury, because of hepatotoxicity that may be caused by epithelial barrier dysfunction that triggers the translocation of nanoparticles.

1. Introduction

Ischemia/reperfusion (I/R) is a pathological condition that is initially characterized by the restriction of blood flow, followed by subsequent restoration (Eltzschig and Eckle, 2011). Several clinical events, such as surgery, transplantation, accidents, and visceral atherosclerosis, can result in I/R. The gastrointestinal tract is extremely sensitive to I/R. Injury to the intestines mainly affects the mucosa and enteric nervous system (Borges et al., 2016; Marosti et al., 2015), including enteric glial cells, the expression of vasoactive intestinal polypeptide (VIP), neuronal nitric oxide synthase (nNOS)-immunoreactive nitrergic neurons (Calcina et al., 2005), and the general population of HuC/D-immunoreactive neurons (Borges et al., 2016), which altogether can impair gastrointestinal transit (Calcina et al., 2005; Rivera et al., 2012). In addition to the activation of proinflammatory cytokines (Eltzschig and Eckle, 2011), I/R induces the production of free radicals that can overwhelm the neutralizing capacity of endogenous antioxidants. These free radicals exert their deleterious (Bhattacharyya et al., 2014; Grace, 1994) actions by promoting lipid peroxidation and generating oxidative stress (da Silva de Souza et al., 2015).

Previous studies have reported that oral treatment with 10 mg/kg resveratrol (RSV) in rats combats oxidative stress in animals that are subjected to intestinal I/R (Borges et al., 2016; da Silva de Souza et al., 2015). Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) is a polyphenol that is found mainly in grape seeds and red wine and has antioxidant and antiinflammatory activity (Kumar and Sharma, 2010). Kumar and

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Sharma (2010) suggested that RSV eliminates free radicals and increases the levels of antioxidant enzymes by neutralizing oxidative stress and promoting neuroprotection. One of the main difficulties of using RSV orally is its short half-life, in which it is rapidly metabolized (Walle et al., 2004). In order to solve this drawback, the use of nanoparticles with bioadhesive properties have been proposed (Penalva et al., 2015). In fact, these nanocarriers may be useful to significantly prolong the residence time of the dosage form in close contact with the absorptive epithelium and, in addition, control the release of the cargo. As a consequence, these nanoparticulate drug delivery systems may be of interest to improve the efficacy of resveratrol as potential treatment for gastrointestinal sections affected by I/R.

In this context, the main objective of this work was to evaluate the capability of resveratrol nanoencapsulated in poly(anhydride) nanoparticles as treatment to reduce the oxidative stress and to afford myenteric neuroprotection in the ileum of rats subjected to I/R. For this purpose, nanoparticles based on the combination between Gantrez® AN [poly(anhydride)] and hydroxypropyl-β-cyclodextrin (HPβCD) were selected as delivery systems for resveratrol. On one hand, poly(anhydride) nanoparticles based on Gantrez® AN display high bioadhesive properties (Agüeros et al., 2011). On the other hand, the use of the oligosaccharide is based on its ability to promote the payload of low absorption compounds in polymer nanoparticles (Agüeros et al., 2011) as well as on its capability to inhibit the effect of intestinal P-glycoprotein and cytochrome P450 enzymatic complexes (Agüeros et al., 2010, 2011), that have the ability to metabolize and promote the extrusion of drugs and other compounds from the epithelium to the intestinal lumen (Agüeros et al., 2010, 2011).

2. Materials and methods

2.1. Preparation of RSV-loaded nanoparticles (RSV-NP)

Resveratrol (Galena. Campinas, SP, Brazil; lot no. C20110928) and HPβCD (Sigma-Aldrich. Steinheim, Germany), (0.3:1 molar proportion) were dispersed in 2 mL of ethanol for 15 min and then in 5 mL of acetone that contained 100 mg of previously dissolved poly(anhydride) (Poly(methyl vinyl ether-co-maleic anhydride) (poly[anhydride]; Gantrez® AN 119; molar weight, 200,000) ISP (Koln, Germany)). The mixture was magnetically stirred for 15 min at room temperature. Nanoparticles were formed by the addition of 20 mL of a mixture with ethanol:ultrapure water (1:1, v/v). The organic solvents were evaporated under vacuum at 45 °C in a rotary evaporator (Büchi R-144, Switzerland). The resulting nanoparticles were purified by both slow and rapid centrifugation (3,000 × *g* for 4 min and 21,000 × *g* for 20 min). The supernatants were removed and the pellets resuspended in water. The formulations were frozen and lyophilized (Genesis 12EL, Virtis, USA) using 5% sucrose (p/p) as the cryoprotectant. As control, empty nanoparticles (NP) were prepared in the same way as described above but in the absence of RSV.

2.1.1. Physicochemical characterization

The mean hydrodynamic diameter of the nanoparticles and their zeta potential were determined by photon correlation spectroscopy and electrophoretic laser Doppler anemometry, respectively, using a Zetaplus analyzer (Brookhaven Instruments, USA). The diameter of the nanoparticles was determined after dispersion in ultrapure water (1:10) and measured at 25 °C by dynamic light scattering angle of 90 °C. The polydispersity index (PDI), which indicates the homogeneity of this distribution, was also calculated. The zeta potential was determined as follows: 200 µL of the samples were diluted in 2 mL of a 0.1 mM KCL solution. The yield of the process was calculated by gravimetry as described previously by Arbós and colleagues (Arbós et al., 2003). The formulations were measured in triplicate, and the results are expressed as mean ± standard error.

2.1.2. Morphological analysis

The morphology of the nanoparticles was analyzed and photographed using a scanning electron microscope (Zeiss DMS 940A SEM; Oberkochen, Germany) with a digital image capture system (Point Electronic GmBh, Halle, Germany). Lyophilized nanoparticles were resuspended in ultrapure water and centrifuged at 27,000 × *g* for 20 min at 4 °C. The supernatants were then discarded, and pellets were assembled on a glass plate and adhered with double-sided adhesive tape on dry metal bases under warm airflow. Finally, the nanoparticles were covered with a thin 12 nm layer of gold using an Emitech K550 cathode pulverizer device (Emitech, UK). The micrographs were obtained under the following conditions: 10 kV and 5000X of 9 mm distance.

2.1.3. Resveratrol quantification

Samples of lyophilized nanoparticles were centrifuged, and the pellets were solubilized in acetonitrile (3/4, v/v) for nanoparticle rupture. The amount of encapsulated RSV was determined by an ultraviolet-visible spectrum spectrophotometer at 305 nm using a calibration curve performed under a range of 0.6–7 µg/mL ($r^2 > 0.997$) in acetonitrile (3/4, p/v). The results are expressed as µg of RSV/mg of nanoparticles. The encapsulation efficiency (EE) was calculated using the following equation: EE (%) = (Encapsulated RSV weight/Initial RSV weight) × 100.

2.2. Animals and experimental protocol

We used 54 male albino Wistar rats (*Rattus norvegicus*; 255 ± 2.704 g). The animals were obtained from the Central Bioterium of the Universidade Estadual de Maringá and housed in the Sectorial Room of the Department of Morphological Sciences. During the treatment period, they remained in an environment at 22 °C ± 2 °C with a 12 h/12 h light/dark cycle. The animal procedures were approved by the Committee of Ethics in the Use of Animals of the University State of Maringá (opinion no. 149/2013) and were in accordance with the ethical principles adopted by the Brazilian Society of Science in Laboratory Animals (SBCAL/COBEA).

The animals received standard rodent chow (NUVILAB, recommended by the National Research Council and U.S. National Institutes of Health) and water *ad libitum*. The animals were divided into nine groups ($n = 6$/group) and treated every 48 h. Treatment began 5 days before surgery and continued for 7 days after surgery (reperfusion period). The animals were treated by gavage with 7 mg/kg resveratrol (in free or nanoencapsulated form; Table 1). Free resveratrol was diluted in 10% grain alcohol and 90% water. The nanoparticles were diluted only in water. The C, SC, and IRC groups were treated with a solution that contained only vehicle: 10% grain alcohol and 90% water before and after surgery.

2.3. Induction of ischemia

Prior to surgery, all of the animals were fasted for 15 h. After the

Table 1
Experimental groups and treatments. S: sham; IR: ischemia/reperfusion.

Groups		SMA[a]	Treatment
C	Not operated	–	Vehicle
SC	Underwent surgery	Not occluded	Vehicle
STR	Underwent surgery	Not occluded	Free resveratrol (unencapsulated)
STEN	Underwent surgery	Not occluded	Empty nanoparticle
STRN	Underwent surgery	Not occluded	Resveratrol – loaded nanoparticle
IRC	Underwent surgery	Occluded	Vehicle
IRTR	Underwent surgery	Occluded	Free resveratrol (unencapsulated)
IRTEN	Underwent surgery	Occluded	Empty nanoparticle
IRTRN	Underwent surgery	Occluded	Resveratrol – loaded nanoparticle

[a] SMA superior mesenteric artery.