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Effects of açaí and cilostazol on skin microcirculation and viability of TRAM flaps in hamsters

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ABSTRACT

Background: Tissue necrosis caused by insufficient perfusion is a major complication in flap transfer. This study evaluated whether treatment with cilostazol or hydroalcoholic extract of seeds of *Euterpe oleracea* Mart. (açaí) protects the transverse rectus abdominis myocutaneous (TRAM) flap against ischemic damage in hamsters.

Materials and methods: Fifty-four hamsters were divided into three oral treatment groups: placebo, açaí, or cilostazol. Caudally based, unipedicled TRAM flaps were raised, sutured back, classified into four vascular zones (I-IV), and evaluated for tissue viability, capillary blood flow (CBF), perfused vessel density (PVD), and microvascular flow index (MFI) by orthogonal polarization spectral imaging at three time points: immediately postoperatively (IPO), 24 h postoperatively (24hPO), and 7 d postoperatively (7POD).

Results: Comparing to placebo, açaí increased PVD at IPO and açaí and cilostazol increased CBF and PVD at 24hPO in zone I; cilostazol increased CBF, PVD, and MFI at IPO, and CBF at 24hPO in zone II; açaí and cilostazol increased CBF at all time points and PVD and MFI at IPO and 24hPO in zone III; cilostazol increased CBF at IPO and 7POD, açaí increased CBF at 7POD, and both increased PVD and MFI at all time points in zone IV; and açaí and cilostazol increased the percentage of viable area in zones III and IV.

Conclusions: Açaí and cilostazol treatments had a protective effect against ischemic damage to TRAM flaps in hamsters, improving microvascular blood flow and increasing the survival of flap zones contralateral to the vascular pedicle (zones III and IV).

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Introduction

Flap transfer is one of the pillars of plastic surgery. Tissue necrosis is a major complication in flap transfer that can lead to treatment failure due to ischemia caused by insufficient perfusion in distal areas of the flaps.[1] Ischemic complications represent a significant problem after reconstructions using the transverse rectus abdominis myocutaneous (TRAM) flap with a single superior pedicle. Much of the scientific research on TRAM flap has focused on finding methods to increase reliability and safety of its use by improving the blood flow and decreasing the risk of necrosis. Among these, vascular delay of the TRAM flap is the most reliable method.[2] However, vascular delay is a two-stage procedure, showing disadvantages from a medical and economic point of view. Pharmacological therapy may represent an alternative treatment strategy to replace the surgical procedure of vascular delay or save a flap with signs of ischemia.[3]

Various plant polyphenols exert important effects on the cardiovascular system and may be potential natural sources for new drugs for the treatment of cardiovascular and metabolic diseases.[4] The açaí berry (*Euterpe oleracea* Mart.) is a fruit that has gained great popularity as functional food due to its significant amount of polyphenols, which provide potent antioxidant[5] and important endothelium-dependent vasodilator effects,[6] increasing the bioavailability of nitric oxide (NO) in endothelial cells and preventing or improving endothelial dysfunction,[7] and have antiinflammatory properties.[8]

Cilostazol is a potent selective inhibitor of phosphodiesterase (PDE) 3 that increases cyclic adenosine 3′:5′-monophosphate (cAMP), decreasing intracellular calcium in smooth muscle cells, causing cellular relaxation and vasodilatation,[9] and promoting inhibition of platelet activation and aggregation, with reduction of thrombosis.[10] It is considered one of the most effective treatments of intermittent claudication, a symptom related to peripheral occlusive arterial disease.[11]

Açaí polyphenols and cilostazol may improve blood perfusion of ischemic flaps, increasing their survival. Previous studies have reported beneficial effects of cilostazol on protection against ischemia-reperfusion injury[12] and of both, açaí and cilostazol, on survival of random flaps in rats,[13,14] but their effects on flap microcirculation or on a TRAM flap model have not been studied.

The aim of this study was to evaluate whether treatment with either cilostazol or hydroalcoholic extract of açaí seeds administered preoperatively and continued postoperatively can protect the TRAM flap against ischemic damage in hamsters by improving microvascular blood flow and increasing flap survival.

Materials and methods

Animals

The study protocol was approved by Institutional Animal Care and Use Committee (approval no. 059/2010). All animals received humane care in strict compliance with Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (Colégio Brasileiro de Experimentação Animal), and all animal experiments complied with the Animal Research: Reporting of *In Vivo* Experiments guidelines and were carried out in accordance with the U.K. Animals (Scientific Procedures) Act, 1986, and associated guidelines. This study was conducted at the Laboratory of Clinical and Experimental Research in Vascular Biology of the State University of Rio de Janeiro (Brazil) and animals were obtained from Animais de Laboratório Criação e Comércio, Paulínia (São Paulo, Brazil).

Fifty-four adult male hamsters (*Mesocricetus auratus*) weighing of 125 ± 5 g were used. They were housed in pairs in polypropylene cages during the preoperative treatment and in individual cages postoperatively, under standard laboratory conditions on 12:12 h light-dark cycle, mean temperature of 22°C, and fed standard chow and water ad libitum. After 7 postoperative d (7POD), the animals were sacrificed with intracardiac injection of sodium pentobarbital (Hypnol, Syntec, Cotia, São Paulo, Brazil).

Study groups

The hamsters were randomized into three groups of 18 animals each and treated with either placebo (control group), hydroalcoholic extract of açaí seeds[15] (açaí group), or cilostazol (cilostazol group; Vasogard, Biosintética, Guarulhos, São Paulo, Brazil). The control group received 0.1 mL of filtered water, orally, once daily, for 30 preoperative and 7POD. The açaí group was treated with hydroalcoholic extract of açaí seeds (100 mg/kg body weight [bw]/d) in 0.1 mL of filtered water, orally, once daily, for 30 preoperative and 7POD. The cilostazol group was administered 0.1 mL of filtered water, orally, once daily for 23 d, followed by cilostazol (30 mg/kg bw/d; Biosintética) in 0.1 mL of filtered water, orally, twice daily, for 7 preoperative and 7POD.[16]

Surgical procedure

Animals were anesthetized intraperitoneally with sodium pentobarbital (30 mg/kg bw; Syntec). The ambient temperature was maintained between 20°C and 22°C, and body temperature was maintained at 33.5°C by a rectal thermistor-controlled heating pad (LTB 750 Thermostat System, Uppsala, Sweden).[17] In each hamster, a right-sided unilateral, single-pedicle TRAM flap, based on caudal epigastric vessels was raised measuring 3.5 × 4.5 cm. The TRAM flap, including the panniculus carnosus, was centered on the abdomen and placed 1.0 cm caudal from the xiphoid process (Fig. 1).[18] The flap was returned to its bed and sutured with 4-0 black silk thread, initially with ten simple reference stitches placed at each corner of the flap, midline, and outer edge of the rectus abdominis, both cranially and caudally, followed by a uniform, tensionless, continuous suture (Fig. 2).

Evaluation of microcirculation

Microcirculation was evaluated using an orthogonal polarization spectral imaging system (Cytoscan model, Cytometrics, Philadelphia, PA, USA) equipped with a manually positioned probe having a 10× objective lens. The probe was covered with