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Protective Effects of Micronized Purified Flavonoid Fraction (MPFF) on a Novel Experimental Model of Chronic Venous Hypertension

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WHAT THIS PAPER ADDS

As clinical trials usually provide knowledge of established chronic venous disease (CVD), rodent models of chronic venous hypertension could be useful to investigate pathophysiological mechanisms and pharmacological effects. Unlike the arteriovenous (AV) fistula model, which induces venous hypertension and high blood flow, in this study a novel model was developed based on external iliac vein ligature, which elicits venous hypertension and low blood flow, reproducing the main hemodynamic changes of CVD. For the first time this model allows observation, *in vivo*, of microvascular alterations as a function of venous pressure increase during a 10 week period.

Objectives: To assess protective effects of micronized purified flavonoid fraction (MPFF) on microcirculation in an original chronic model of hind limb venous hypertension with low blood flow in small animals.

Methods: Vein ligatures were performed on male hamsters, as follows: A-right femoral vein; A + B-right femoral vein and its right branch; A + C-right femoral vein and its left branch; A + B + C-right femoral and its right and left branches; D-external right iliac vein. In sham operated groups, similar vascular dissections were performed without ligatures. Superficial (epigastric) and central (jugular) venous pressure evaluations were made during a 10 week period. Hamsters subjected to A + B + C and D ligatures were selected for leukocyte rolling and sticking, functional capillary density (FCD), and venular and arteriolar diameter observations. D ligature was selected to evaluate pharmacological treatment efficacy. MPFF (100 mg/kg), concomitant active flavonoids of MPFF (diosmetin, hesperidin, linarin, and isorhoifolin) (10 mg/kg), diosmin (100 mg/kg) or drug vehicle were administered orally during 2 weeks before vein ligature and 6 weeks thereafter.

Results: A, A + B and A + C models maintained venous return through collaterals. From the 2nd to the 10th weeks after vein ligatures, A + B + C and D models elicited a progressive increase of superficial venous pressure (3.83 ± 0.65 vs. 8.56 ± 0.72 mmHg, $p < .001$ and 4.13 ± 0.65 vs. 9.35 ± 0.65 mmHg, $p < .001$, respectively) with significant changes to the microcirculation. As D model significantly increased superficial venous pressure without affecting central venous pressure, it was used to evaluate the long-term effects of treatment. Compared with vehicle, MPFF, concomitant active flavonoids of MPFF, and diosmin, significantly decreased leukocyte-endothelium interaction and prevented FCD reduction. Only MPFF significantly prevented venular enlargement as observed in the vehicle treated group.

Conclusion: MPFF was more effective than diosmin in improving all microvascular variables. The superiority of MPFF over diosmin alone can be explained by the synergistic beneficial effects of the association between diosmin and active flavonoids of MPFF.

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INTRODUCTION

Chronic venous disease (CVD) encompasses a large spectrum of signs and symptoms that vary from asymptomatic telangiectasias to active venous ulcers.[1] The severity of CVD is closely related to the magnitude of venous hypertension,[2] which can be triggered by venous refluxor flow obstruction.[3] The high venous pressure induces adverse changes in

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the venous wall resulting in excessive dilatation and tortuosity of varicose veins (VVs).[4]

Another hallmark of CVD is the infiltration of leukocytes in the affected leg by capillary plugging or by adhesion to venular endothelium.[5] Leukocytes trapped in the microcirculation reduce capillary perfusion, induce reactive oxygen species (ROS), and proteolytic enzyme release leading to venous inflammation,[7] and later to skin damage and leg ulceration.[6]

As clinical trials provide knowledge of established CVD, experimental models were developed to better understand its pathophysiological mechanisms. However, few consist of ligature of large veins,[7–10] which induces venous pressure elevation and allows inflammatory variable assessment on the day of,[7] or maximally 14 days after the ligation procedure.[8–10]

Micronized purified flavonoid fraction (MPFF) is an oral veno-active drug, consisting of 90% diosmin and 10% of concomitant active flavonoids, of which the beneficial effects on CVD have been reported in several studies.[11–18]

The present study was designed to develop a novel model of chronic venous hypertension, allowing long-term monitoring (during a 10 week period) of hind limb venous pressure and its impact on the microcirculation, and to compare the therapeutic efficacy of MPFF and its constituents on microvascular damage evoked by chronic venous hypertension and low blood flow.

METHODS

Animals and procedures

Ethics statement. The experimental protocol and animal procedures were approved by the Ethical Committee of the State University of Rio de Janeiro, Brazil (CEA/215/2007) and are in accordance with the Guide for the Care and Use of Laboratory Animals.[19]

Animal housing. Animals, 422 male hamsters (*Mesocricetus auratus*) weighing 85–120 g were caged in a light, temperature (20–24 °C), and humidity controlled environment. All animals were fed with chow for small rodents and received tap water *ad libitum.*

Development of experimental model of chronic venous hypertension. Based on the model described by Hahn and co-workers,[9] an experimental model of high pressure and low blood flow on veins was developed, which allowed longer periods of observation to investigate venous pressure and microcirculatory changes over time.

Animals were anaesthetized by intraperitoneal injection of xylazine plus ketamine (10 mg/kg and 200 mg/kg, respectively, CevaSaúde Animal Ltda, Paulínia, SP, Brazil).

Groin incisions were made, and vein ligatures were performed, using suture threads, as follows: A-right femoral vein (above the origin of superficial epigastric vein); A + B-right femoral vein and its right branch at origin; A + C-right femoral vein and its left branch at origin; A + B + C-right femoral vein and its right and left branches at origin; D-upper third of right external iliac vein (Fig. 1). In sham operated animals, similar vascular dissections were performed without vein ligatures. Evaluations of venous pressures were made every 2 weeks (total of 10 weeks) in the fore and hindlimbs.

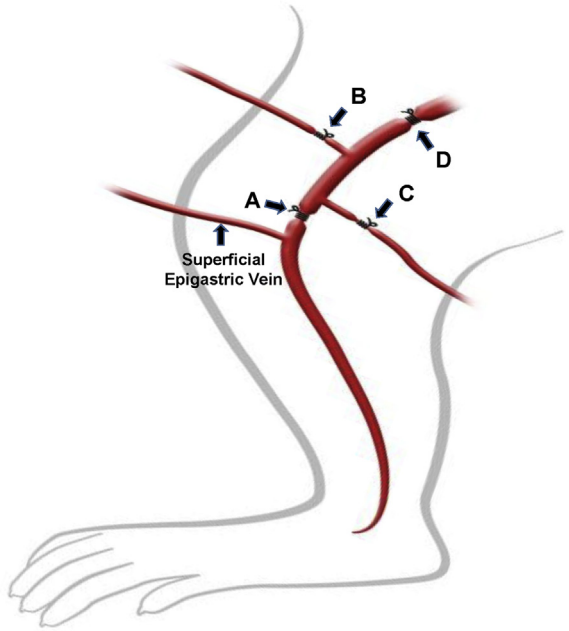

Figure 1. Schematic representation of ligature models. (A) Right femoral vein above the origin of superficial epigastric vein; (B) origin of right branch of femoral vein; (C) origin of left branch of femoral vein; and (D) upper third of right external iliac vein.

Measurement of venous blood pressure. Superficial and central venous pressure signals of anaesthetised hamsters were acquired by catheters placed in the right superficial epigastric and right jugular veins connected to PowerLab 4/35 System equipped with LabChart Pro (AD Instruments Ltd, Melbourne, Australia).

Intravital microscopy observation. For assessment of microvascular alterations induced by venous hypertension, separated groups of hamsters subjected to ligature models A + B + C and D were used. Models A, A + B, and A + C induced collateral venous outflow and, thus, were not used for this purpose.

Anesthesia was induced by an intraperitoneal injection of xylazine plus ketamine and maintained by 100 mg/kg of α-chloralose (Sigma–Aldrich, St. Louis, MO, USA) administered through a jugular vein catheter, also used for fluorescein isothiocyanate (FITC)-dextran (MW 150,000 Dalton, TdB Consultancy, Uppsala, Sweden) or rodhamine-6G (Sigma–Aldrich, St. Louis, MO, USA) administrations. Then, the hind limb was dissected to expose the subcutaneous microcirculation and the preparation was placed under an intravital microscope (Leica DMLFS, Wetzlar, Germany) coupled to a TV camera (Optronics, model 60,366–1, Goleta, CA, USA), with optical magnification of 50× for functional capillary density (FCD) and 100× for arteriolar and venular diameters and leukocyte rolling and sticking. The preparation was continuously superfused, at a rate of 4.0 mL/min by a HEPES supported HCO_3 buffered saline

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