

## Pharmacologic Aspects of a Phlebotropic Drug in CVI-Associated Edema

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### ABSTRACT

Several phlebotropic drugs, or edema-protecting drugs, are available, the most important of which are found in the  $\gamma$ -benzopyrone family (flavonoids).  $\gamma$ -Benzopyrones can be plant extracts, semisynthetic preparations, or synthetic preparations. This family is divided into two different groups: flavones and flavonols, and flavanes (flavanones). The flavone group contains various types of molecule and includes diosmin. Here we discuss the pharmacologic aspects in edema associated with chronic venous insufficiency (CVI) of one of the reference phlebotropic drugs, micronized purified flavonoid fraction (MPFF\*), a semisynthetic preparation from the diosmin group, which represents the latest improvement in flavonoid formulation. Before we detail the pharmacologic aspects, a brief summary of the pathophysiology of edema in CVI is necessary. Several factors are implicated: the veins, which create the conditions favorable to edema; the microcirculation, which is the site of fluid transfer into the interstitial tissue; and the lymphatics, which have a limited possibility to reduce edema. Major discoveries are currently being made in CVI and the microcirculation. Results of studies show that MPFF decreases capillary permeability and increases capillary resistance, which could partly be explained by inhibition of leukocyte activation, migration, and adhesion. This inhibition is linked to a significant decrease in plasma levels of endothelial adhesion molecules (VCAM-1 and ICAM-1) after MPFF treatment. Thus, the CVI-induced damage to the microcirculation is

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(Abstract continued)

counteracted by MPFF. The lymphatic system is also improved by MPFF treatment. The lymphagogue activity of MPFF has been demonstrated in experimental animal models and confirmed by microlymphographic measurement in patients suffering from severe CVI. The pharmacologic activity of MPFF in lymphedema was observed in a study using an animal model of acute lymphedema and in a study in patients with upper limb lymphedema secondary to breast cancer treatment. All these findings point to the importance of acting on each factor involved in the formation and maintenance of edema. This pharmacologic activity is indeed reflected by the clinical efficacy on edema observed during treatment with MPFF.

\*Daflon 500 mg, Alvenor, Ardiurn, Arvenum, Capiven, Detralex, Variton, Venitol.

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### Introduction

Pharmacologic therapy is of major value in phlebology, especially in treating edema associated with chronic venous insufficiency (CVI). The drugs used are called phlebotropic drugs or edema-protecting agents, and several families of drug exist, such as benzopyrones and saponins. These may be plant extracts or synthetic substances. The most important family is that of the benzopyrones, which can be divided into two groups:  $\alpha$ -benzopyrones including coumarin derivatives, which are used as oral anticoagulants; and  $\gamma$ -benzopyrones or flavonoids. Flavonoids can be further divided into two types of molecule:<sup>1,2</sup>

- flavones, flavonols, and derivatives (diosmin, diosmetin, rutin, etc);
- flavanes, flavanones, and derivatives (hesperidin, pycnogenol, etc).

This presentation is devoted to micronized purified flavonoid fraction (MPFF), a semisynthetic micronized preparation of the  $\gamma$ -benzopyrone family, which represents the latest improvement in flavonoid formulations. MPFF has improved pharmacologic properties due to a micronized formula that increases bioavailability<sup>3</sup> and thus has greater therapeutic efficacy than nonmicronized diosmin.<sup>4</sup> MPFF provides an excellent opportunity to discuss all the pharmacologic mechanisms by which edema may be reduced with a phlebotropic drug.

Before we analyze the mode of action of MPFF in CVI-associated edema, we should briefly recall the pathophysiology of edema. Several factors are involved in the formation of edema. First, of course, is venous hypertension, which is a consequence of venous reflux and/or the well-known risk factors of venous disease, such as prolonged standing position, heat, etc. Venous hypertension in the venous trunks is automatically transmitted to the venules and capillaries, for there is no post-capillary sphincter limiting the impact of venous hypertension on the capillaries. In the microcirculation, which is the site of fluid exchange with the interstitial tissue, the anomalies observed in the case of venous disease/venous hypertension are increased capillary permeability and decreased capillary resistance associated with a local inflammatory process characterized by leukocyte activation, adhesion, and migration. In the short term, this local inflammatory reaction leads to tissue damage. As a consequence, these pathological conditions lead to an increase in fluid outflow into the surrounding tissues. At the early stages of the disease, this can be compensated for by the lymphatic system, and no edema occurs. However, the lymphatic function has a limited capacity for compensation. If chronic venous disease persists and worsens, the lymphatic system becomes overloaded and is no longer able to handle the excess fluid. At this stage of the disease, edema appears and is the result of an imbalance between inflow of interstitial fluid and lymphatic drainage.

### Mode of Action of MPFF in CVI-Associated Edema

Fighting edema means counteracting all the mechanisms described above and in this regard the mode of action of MPFF is perfectly suited to the pathophysiology of this condition.

#### Venous Tone

MPFF has a demonstrated impact on venous tone. This mechanism has been observed in several different studies: *in vitro* using the isolated venous segments,<sup>5,6</sup> and *in vivo* using strain gauge plethysmography in CVI patients.<sup>7</sup> Recently, a new method involving calculation of the elastic modulus (K) with air plethysmography confirmed the improvement in venous tone after MPFF treatment.<sup>8</sup> This controlled study involved 25 women with abnormal venous elasticity/venous tone, divided into two groups: one group treated with MPFF (1,000 mg/day) for 4 weeks, and the other a control group receiving no treatment. Two measurements were made with air plethysmography (APG): one at the beginning of the study and a second 4 weeks later. All measurements were performed at constant temperature and at the same time of the day. The elastic modulus (K) was determined from the pressure-volume curve. The results showed that the elastic modulus (K) in the control group remained the same at the end of the study ( $p > 0.1$ ), while in the MPFF group, venous tone was reinforced as demonstrated by a significant increase in K ( $p < 0.02$ ).

#### Microcirculation

The microcirculation is a valuable research field in CVI, and in this context many studies with MPFF have been published.<sup>9-15</sup> The pharmacodynamic activity of MPFF on the microcirculation and, more particularly, capillary filtration, was studied in hamster cheek pouch preparation.<sup>9</sup> MPFF (20 mg/kg/day) was administered orally to the male hamsters for 10 days. The effect of MPFF on increased microvascular permeability induced by histamine, bradykinin, and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) was investigated by using intravital microscopy. A fluorescent macromolecular tracer (fluorescent isothiocyanate-labeled dextran 150 [FITC-dextran]) was given intravenously after the cheek pouch preparation. Histamine, bradykinin, and LTB<sub>4</sub> were applied topically and

caused an increase in the number of fluorescent vascular leakage sites in the postcapillary venules. The number of leaky sites per square centimeter was quantified by UV light microscopy. Compared with vehicle, MPFF significantly inhibited the effect on the macromolecular permeability of histamine ( $343.5 \pm 22.3$  vs  $207.5 \pm 32.0$ ;  $p < 0.01$ ), bradykinin ( $342.2 \pm 19.0$  vs  $206.2 \pm 21.6$ ;  $p < 0.01$ ), and LTB<sub>4</sub> ( $353.3 \pm 27.5$  vs  $242.7 \pm 33.6$ ;  $p < 0.05$ ). These data demonstrate a protective effect of MPFF against leakage of macromolecules after application of permeability-increasing substances to the cheek pouch microvasculature.

Recently some experimental models have been used to further study the mechanism of MPFF in the microcirculation. In one of these trials, a dorsal skin-fold chamber preparation of hamsters was used.<sup>12</sup> Animals received MPFF, or an equivalent volume of the vehicle, by gavage for 8 days. A 4-hour period of tourniquet ischemia was induced and the leakage of the macromolecule FITC-dextran (150 kd) was analyzed during reperfusion by intravital fluorescence microscopy. Ischemia reperfusion injury was elicited by significant macromolecular leakage from postcapillary venules in vehicle-treated animals. In the MPFF-treated group, the postischemic leakage was totally inhibited ( $p < 0.05$ ). These results indicate that MPFF prevents leakage of macromolecules from postcapillary venules. It was hypothesized that this effect may be linked to an inhibitory action on the migration of activated leukocytes. This hypothesis was confirmed in another study<sup>13</sup> that focused on postischemic leukocyte/endothelial cell interactions and microvascular barrier dysfunction in skeletal muscle in rats. In this trial performed in rat cremaster muscles, MPFF was shown to attenuate the postischemic increases in leukocyte rolling, adhesion, and migration. This was associated with a decrease in venular protein leakage ( $p < 0.05$ ).

To assess the protective effect of MPFF, an experimental model of venular occlusion/reperfusion in the rat mesentery was designed.<sup>14</sup> The degree of leukocyte/endothelium interaction and the extent of parenchymal cell death were determined. The results of this study demonstrated that when MPFF was administered for 7 consecutive days by gavage, it significantly reduced the consequences of 1-hour venular occlusion: parenchymal cell death, rolling of leukocytes, leukocyte adhesion, and migration across the venous wall were all significantly decreased ( $p < 0.05$ ).

The first clinical research with MPFF in this field was undertaken in 20 patients suffering from chronic venous disease, stages C2 to C4 and C5 (with a healed ulcer for at least 4 weeks) according to the CEAP classification.<sup>15</sup> All patients underwent venous duplex examination and photoplethysmography to establish the precise diagnosis of venous disease. Plasma markers of endothelial (E-selectin, P-selectin, vascular cell adhesion molecule [VCAM-1], intercellular adhesion molecule [ICAM-1], and von Willebrand factor) and leukocyte activation (lactoferrin) were measured by ELISA tests before and after a 60-day treatment with MPFF. Blood samples were taken from the long saphenous vein at the ankle before and after 30 minutes standing, which raises the venous pressure in the superficial veins of the leg to between 70 and 80 mm Hg. After treatment, VCAM-1 and ICAM-1 plasma levels were significantly reduced following therapy in all patients. Lactoferrin and von Willebrand factor levels were significantly reduced following therapy in a subgroup of patients with skin changes. The significant decrease observed in VCAM-1 and ICAM-1 plasma levels as a consequence of MPFF therapy is of major importance in the comprehension of the mode of action of the drug, because of the key role of these adhesion molecules in the endothelial interaction with neutrophils, monocytes, and lymphocytes. The finding that some of these molecules are specifically lowered in patients with skin changes indicates that MPFF may improve or prevent some of the skin damage induced by chronic venous disease and fight the microcirculation-damaging processes.

#### Lymphatic System

Fighting edema does not simply involve reinforcing venous tone and protecting the microcirculation. The third "actor" in CVI-associated edema, the lymphatic system, also plays a decisive role.

In an experimental study performed with MPFF, randomized groups of rats were given acute lymphostasis of the leg.<sup>16</sup> Two groups of animals received MPFF, at doses of 20 and 100 mg/kg/day. The control group received the vehicle. Several parameters were measured: weight of the thighs, thickness and total water content of the subcutaneous tissue, and transcutaneous oxygen pressure. Electron microscopy

was performed qualitatively and quantitatively. The results showed that MPFF decreased the weight of the thighs, the concentration of protein in tissue, and the number of fibroblasts, significantly and in a dose-dependent manner ( $p < 0.002$  for a high MPFF dose, and  $p < 0.02$  for a low MPFF dose). Moreover, the reduced transcutaneous oxygen pressures in lymphedema were largely restored toward normal in both MPFF groups.

The dose-dependent effect of MPFF on lymph drainage was also demonstrated in a study measuring the lymph flow.<sup>17</sup> This lymphagogue effect of MPFF was confirmed in unanesthetized sheep before and after 5 days of MPFF administration.<sup>18</sup> In this study, MPFF significantly increased the lymph flow (MPFF-treated group versus control group:  $p < 0.001$ ). These results show the direct stimulatory effect of MPFF on lymphatic contractility and on lymphatic flow, with, as a result, increased lymph drainage.

A clinical confirmation of these experimental data came from a study using microlymphography in CVI patients.<sup>19</sup> The microlymphographic parameters (diameter of lymphatic capillaries and number of functional lymphatic capillaries) were assessed in 24 patients with third-stage CVI before and after 28 days of treatment with MPFF (1 g/day), and 14 days after treatment was stopped. The intralymphatic capillary pressure was measured by the Servo Nulling Pressure System. The results show that the number of functional lymphatic capillaries significantly increased between D0 and D28 ( $p = 0.001$ ) and was unchanged at D42. The diameter of lymphatic capillaries and the intralymphatic pressure were significantly decreased at the end of the treatment and increased after treatment was stopped ( $p = 0.001$ ). This confirms the beneficial lymphatic effect of MPFF by the increasing microlymphatic density and improving microlymphatic drainage.

Pharmacologic data have confirmed the activity of MPFF on lymph drainage. These experimental results have since been confirmed by various clinical studies performed in patients with edema and lymphedema. One of these studies was performed with patients suffering from upper limb lymphedema secondary to breast cancer treatment.<sup>20</sup> Over 6 months of MPFF therapy (1,000 mg/day), lymphedema progressively reduced.

### Conclusion

The pharmacologic activity of MPFF has been demonstrated in many pharmacologic and clinical trials studying the various mechanisms responsible for edema formation and persistence: decreased venous tone, microcirculation-damaging processes, capillary hyperpermeability, and overloading of the lymphatic system. MPFF has been shown to improve each of these mechanisms. This has been confirmed clinically in var-

ious studies performed in patients suffering from edema<sup>21</sup> or lymphedema.<sup>20</sup> These results suggest that the mode of action of MPFF perfectly suits the pathophysiology of edema.

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