LINFAVENIX: improvement of signs and symptoms of chronic venous insufficiency and microangiopathy

M. R. CESARONE, G. BELCARO, M. G. GROSSI, L. PELLEGRINI, A. LEDDA, G. VINCIGUERRA, A. RICCI, G. GIZZI, E. IPPOLITO, F. FANO, M. DUGALL, M. CACCHIO

Irvine[3] Vasc Lab and Physiology
Dept of Biomedical Sciences
Chieti-Pescara University, Chieti-Pescara, Italy
San Valentino Vascular Screening Project
Faculty of Motory Sciences
L'Aquila University, Italy

The aim of this independent study was to demonstrate the rapidity of the efficacy of an oral venotropic compound (Linfavenix, including natural elements) in patients with chronic venous insufficiency (CVI). Two groups of patients with chronic venous insufficiency (CVI) ankle swelling) were treated with Linfavenix or with below-knee elastic compression. The average ambulatory venous pressure (AVP) at inclusion (both groups)was 56.2 (range 48-55) with a refilling time (RT) shorter than 10 seconds. These parameters indicated a severe level of venous hypertension. There were no significant differences in AVP and RT between the two groups. The two groups of subjects with CVI were comparable; in the Linfavenix group there were 14 patients (age 44.5; sd 4; range 34-55; 7 females); in the elastic compression group there were 12 patients (45.4;5; range 36-56; 7 females). The clinical picture and microcirculatory parameters at inclusion were comparable. RF was comparable at inclusion in the two groups. At two weeks, the differences in RF (between goups) were not significant (the flux decreased in both groups, indicating improvement) while at 4 weeks the difference was larger (but non significant between the two groups) with a significant decrease in RF in the Linfavenix group. The RAS was also comparable at inclusion. Both groups had a significant decrease at 2 and 4 weeks. The decrease produced by Linfavenix after 4 weeks in RF was larger and significant ($p<0.05$) in comparison with the elastic compression group. Also the differences observed in ASLS were significant in both groups with an important, significant difference in favour of Linfavenix at 4 weeks (op<0.05) visibile as edema reduction. The decrease in edema was relevant in both groups at 2 ($p<0.05$) and 4 weeks ($p<0.05$) with a minimal but significant difference ($p<0.05$) between the Linfavenix and the elastic compression group. These variations in microcirculatory parameters indicate that the treatment with Linfavenix is, in its microcirculatory efficacy, at least comparable than elastic compression with is considered a standard therapeutic option in these patients. A significant level of improvement was reached with Linfavenix, in most patients (10/14) at 2 weeks for RF, at 7 days for the RAS and also at 2 weeks in almost all patients (13/14) considering ASLS and edema. No side effects due to treatment were observed. Com-pliance and tolerability were very good (no patient had to stop treatment; there were no drop-outs). In conclusion venous microangiopathy and edema were improved by the treatment with Linfavenix (better in comparison with compression) in a few days.

Key words: **Venous Disease, Varicose Veins, Ulcerations, Elastic Compression, Edema, Veins, Venous Microangiopathy**

Conflicts of interest—The authors have no financial interest with the companies quoted in this article. None of the authors has received or will receive any financial gain and involvement from/in the products quoted in this article.

Several natural compound, based on traditional and natural medicine, have been

used to treat or prevent chronic venous insufficiency (CVI), its signs and symptoms associated with varicose veins and deep venous disease and to improve capillary function and the microcirculation in venous microangiopathy.

Several studies have reported symptomatic relief and a decreased capillary filtration after the administration of several, different, oral preparations. In many studies, in experimental settings and in clinical reports the efficacy of these natural products (rutosides, pycnogenol, horse chestnut, bilberry, vine et cet) – generally produced or directly derived by plants - could be shown in days after the beginning of therapy.

The evolution of microcirculatory methods [1] allows now to study and define, in a quantitative way, microcirculatory changes produced by pharmacological treatment at the areas and levels most affected by chronic venous disease and venous hypertensive microangiopathy (VHM) [5-13].

The quantitative evaluation of the effects of treatments on edema and microcirculatory changes associated with CVI and microangiopathy, is possible by evaluation of dynamic microcirculatory parameters and dynamic capillary/microcirculatory responses by noninvasive tests (laser Doppler flowmetry, transcutaneous PO_2/PCO_2, capillary filtration) [11-14]. These noninvasive tests can be repeatedly performed to evaluate the rapidity of action of venoactive compounds and signs/symptoms.

In subjects with CVI and venous microangiopathy the sin flux at rest in the perimalleolar region (RF) is generallly increased. The VAR (the venoarteriolar response, namely the reflex vasoconstriction measured after the passage from the supine to the standing position) is altered and other microcirculatory changes, depending on the degree of CVI and its duration, may be present (i.e transcutaneous PO_2 is decreased, the PCO_2 is increased and capillary filtration – clinically visible as edema, and measurable with strain-gauge plethysmography – is greatly increased).[1-14] These parameters may change in days and even hours on the basis of several factors (i.e temperature, protracted standing) and if and when appropriate treatment is used.

The aim of this registry study was to evaluate how quickly a liquid, oral soluble preparation of Linfavenix is effective in relieving signs and symptoms in subjects with severe CVI and venous microangiopathy.[15]

Material and methods

Inclusion criteria

Evaluation methods for chronic venous disease described in this article have been reported in previous publications.[10-14]

Patients

Patients with CVI were evaluated. They had a moderate/severe level of chronic venous insufficiency and venous microangiopathy.

Noninvasive investigations

Venous reflux in the popliteal vein had been shown – before inclusion - by color duplex. The increase in venous pressure had been measured by AVP.

AVP (ambulatory venous pressure)

AVP [3, 4] was significantly increased all patients at inclusion (AVP was higher than 60 mmHg in all included limbs due to combined superficial and deep venous incompetence). AVP values after exercise had not been completely normalized by a tourniquet excluding the superficial venous system (this indicated combined, superficial and deep venous incompetence). Superficial venous incompetence in all studied limbs was associated to deep venous incompetence (most of venous hypertension was due to deep incompetence).

The average ambulatory venous pressure (AVP) at inclusion (both groups) was 56.2 (range 48-55) with a refilling time (RT) shorter than 10 seconds. These parameters indi-

cated a severe level of venous hypertension. There were no significant differences in AVP and RT between the two groups.

The duration of CVI was 3.3 years (SD 1.1) in the Linfavenix group and 3.2 years (SD 1.3) in the elastic compression group..

Exclusion criteria

No other clinical cardiovascular disease, requiring treatments, was present. Diabetic patients and those with bone or joint disorders and any systemic disease requiring medical treatment were excluded. We also excluded, with the use of ultrasound, the presence of recent thrombosis (12 months). Patients with a confirmed or even suspected clinical history of thrombosis in the previous 24 months were excluded. Also subjects with any defined allergy to the compounds present in the study product were excluded as well with all subjects with history of allergy to comparable products.

Linfavenix

The composition of Linfavenix includes several natural elements used in traditional, natural medicine to improve venous 'tone', the microcirculation and to control venous-related edema.

In the daily dose of 3 ml solution (40% alcool) Linfavenix includes:

— Aesculus hippocastanum	25%=ml 0.75=mg	187
— Hamamelis virginiana	20%=ml 0.60=mg	120
— Vaccinium Myrtillus	20%=ml 0.60=mg	120
— Ruscus aculeatus	15%=ml 0.45=mg	68
— Pyrus sorbus Gaertner	10%=ml 0.30=mg	30
— Castanea sativa	10%=ml 0.30=mg	30

and other trace components (Mn-Co, fluoride, Mg).

The composition had been defined on the basis of experimental data _____

The mild alcoholic solutions allows a better solubilisation of the different elements and their better penetration through the intestinal-blood barrier. The absorptions occurs in minutes after the administration.

Treatment schedule

Patients in the Linfavenix group received 3 ml/day of the solution, once daily at 8 a.m, for 4 weeks). In the elastic compression group any drug was forbidden (excluding possible emergencies).

Comparative treatment: elastic compression

Boston, below- knee (Boston, Sanagens, Treviso, Italy) elastic stockings were used as the comparative treatment. The stockings were used at least 8 hours/daily (between 8 and 20) during the standing/working hours.

Measurements

All measurements were made in microcirculation rooms at constant temperature (21-22°C) before 10 a.m. to avoid the effect of standing, and after 30 minutes of acclimatization in a resting, supine position.

LDF (laser Doppler flowmetry) resting flux (RF), namely the skin flux in the supine resting position, capillary filtration - measured as the rate of ankle swelling (RAS) by strain-gauge plethysmography (Hokanson, USA)- were measured at inclusion and repeated after 4 weeks of treatment.

Strain-gauge-derived RAS (rate of ankle swelling)

In brief, his test quantifies capillary filtration at the ankle. While the patient is resting supine after 30 minutes a strain-gauge is applied at the ankle level and relationship between the strain-gauge and the volume/section of the limb are calibrated. The strain-gauge is placed at the minimum circumference of the ankle, just proximal to the medial malleolus. The patients is then asked to move to a standing position supporting the weight onto the opposite leg and just touching the floor with the leg under examination. The increase in volume (corresponding to the increase in section measured by the strain-gauge) is then recorded for ten minutes. The first phase (3-4 minutes) is associated to an increase in volume due to venous filling (in venous patients the filling happens mainly

0==========X=========10	
SCORE COMPOSITION:	SCORE
1. Edema	0-2
2. Pain	0-2
3. Restless limbs	0-2
4. Subjective swelling	0-2
5. Skin alterations/redness	0-2
TOTAL	0-10

Figure 1.—A composite analogue score (based on signs/symptoms: edema, pain, restless limbs, subjective swelling, skin alterations/redness) ranging from 1 to 10 was considered and evaluated by patients at inclusion and after the 4 weeks of treatment by marking on the analogue scale line the level of discomfort. Patients indicated a value for each item (0= no symptom; 1 symptom present, not continuously; 3= symptom severe, continuous).

from the proximal to the distal level for reflux). The second phase is associated to venous stretching (3-4 minutes). After a period variable from 7 to 9 minutes a steady state with very limited increase in volume is reached. The tangent to the volume increase curve measured between 7 and 10 minutes is considered to be mainly due to capillary filtration (passage of fluid from the intra-vascular to the extra-vascular components).

Reversing the position of the patient to the supine position and elevating the leg, the first components of venous volume can be eliminated in seconds (venous filling and stretching) while the small quantity of fluid passed into the interstitial fluid remains in the extra-vascular compartment and its volume remains visible. In all patients the leg with more severe degree of CVM was studied.

Laser-Doppler

A TSI-Vasamedics laser Doppler flowmeter (Vasamedics and TSI, St Paul, USA) was used to measure skin flux at the internal perimalleolar region, the area mostly affected by venous hypertension and microangiopathy and often a frequent localization of ulcerations in patients with CVH.

All methods used were according to the methods and procedures described in detail in previous publications.[3, 4, 11] Measure-ments were only performed if all the components of the skin were intact (even with the presence of CVM).

A composite, analogue clinical score based on signs and symptoms (edema, pain, restless limbs, subjective swelling, skin alterations/redness) and ranging between 1 and 10 was recorded by patients—after careful briefing and teaching them its meaning—at inclusion and after 4 weeks of treatment by marking on an analogue scale line the level of discomfort (Figure 1).

Edema evaluation

The evaluation of edema was also made by the observing physician considering a value of 0 when no edema was present and 10 when severe, pitting, edema was clearly visible (i.e. the value of 5-6 was associated to moderate edema without clinical signs and symptoms).

Statistical analysis was performed with the Mann-Whitney U-test. The proportion of included samples in the two groups were calculated in groups of at least 10 patients in each group to detect significant variations in the microcirculatory measurements before-after 4 weeks. A spontaneous variation around 5-10% in most microcirculatory measurements is possible as a consequence of spontaneous variations in the capillary system even under standardized conditions both in normal subjects and in patients with CVI. Therefore an arbitrary cutoff point of at least a >15% variation in parameters was considered to be valid to define changes due to treatment.

Results

The two groups of subjects with CVI were comparable.

In the Linfavenix group (lin in Table 2) there were 14 patients (age 44.5; sd 4; range 34-55; 7 females) and in the elastic compression group (ec in the table) there were 12 patients (45;4; sd 5; range 36-56; 7 females). The clinical picture and microcirculatory parameters at inclusion were also comparable in the two groups (Table I).

Perimallolar RF was comparable at inclusion in the two groups. At two weeks basi-

TABLE I.—*variations in microcirculatory parameters: resting flux (RF), rate of ankle swelling (RAS), analogue symptomatic score (ASLS) and edema.*

Time WEEKS		Inclusion 0	Control 2	Final evaluation 4	P value (between groups)
RF	Lin	2,7;0,1	1,9;0,2	1,5;0,2	ns
	Ec	2,6;0,1	2;0,2	1,8;0,5	
RAS	Lin	2,112;0,01	1,713;0,05	1,469;0,04	*
	Ec	2,121;0,02	1,811;0,04	1,694;0,06	
ASLS	Lin	8,1;1,1	2,8;1,2	2,7;1	*
	Ec	8; 2,1	4,4;2	4;2,1	
EDEMA	Lin	8,4; 1,1	3,2;2,1	3,1;2	*
	Ec	8,2; 1	5,1;1,2	4,1;1	

lin= Linfavenix treatment; ec= elastic compression. KEYS: RF = resting flux: RAS= rate of ankle swelling; ASLS= analogue scale line score; EDEMA= scale 0-10
Edema in ASLS was evaluated by patients.
EDEMA as a separate item: evaluated by the research physicians.

cally the differences in RF were not significant while at 4 weeks the difference was larger (but non significant between the two groups) with a significant decrease in RF in the Linfavenix group.

The RAS was also comparable at inclusion. Both groups had a significant decrease at 2 and 4 weeks. The decrease produced by Linfavenix after 4 weeks in RF for was larger and significant ($p<0.05$) in comparison with the elastic compression group.

Also the differences observed in ASLS were significant in both groups with an important, significant difference in favour of Linfavenix at 4 weeks ($op<0.05$) visibile as edema reduction.

The decrease in edema was relevant in both groups at 2 ($p<0.05$) and 4 weeks ($p<0.05$) with a minimal but significant difference ($p<0.05$) between the Linfavenix and the elastic compression group.

These variations in microcirculatory parameters indicate that the treatment with Linfavenix is, in its microcirculatory efficacy, at least comparable if not clinically better than elastic compression with is considered a standard therapeutic option in these patients.

Actually, theoretically, the two treatment options could be combined.

Level/timing of improvement

A significant level of improvement was reached with Linfavenix, in most patients (10/14) at 2 weeks for RF, at 7 days for the RAS and also at 2 weeks in almost all patients (13/14) considering ASLS and edema. Therefore the effects of Linfavenix were faster and clinically evident even in comparison with elastic compression (most patients felt the clinical effects of compression in 7-14 days).

Safety

No side effects due to treatment were observed. Compliance and tolerability were very good (no patient had to stop treatment). A number of patients in the comparative group treated with stockings had several, different kind of minor problems of tolerability of the stockings (including problems due to an abnormally higher temperature) and was partially unable to wear them according to the schedule, missing a number of hours and days of compression.

Comment

RAS shows a decrease in 6-9 hours (in selected subjects with very severe edema) (Grossi MG, Belcaro G, Cesarone MR: Linfavenix file: data on file).

Considering the clinical efficacy of Linfavenix in a chronic disease—such as CVI—the fast decrease in RAS and RF documented in days in most subjects with CVI is clinically remarkable.

The clinical decrease in edema and ASLS is a further important observation of great clinical value in severe CVI.

Discussion

Microcirculatory methods are useful to quantify and follow up the evolution of venous hypertensive microangiopathy and the effects of treatments [10-11] on the microcirculation.

Venous ulceration and CVI are always associated with important, microcirculatory changes.[15-20] Alterations in skin flux and other microcirculatory dynamic parameters (21,22) are important, quantitative measurements in the evaluation of venous microangiopaty associated to the development of edema.

The quantitative evaluation of capillary filtration is relatively more complex but very effective in defining the degree of venous microangiopathy [23] and its changes in time with treatment as an increased capillary filtration, clinically present as edema, is usually the most important, often the single, initial sign present in CVI.

This independent study indicates that oral treatment with Linfavenix is very effective and fast in improving the microcirculation and signs and symptoms in patients with CVI. In these patients venous microangiopathy is generally characterized by high skin flux and increased RAS and edema in the perimalleolar region.

The clinical and microcirculatory effects of venoactive compounds on skin flux, edema, ankle swelling and on capillary filtration may be observed even in a limited sample of patients after a few days of treatment and the final results—after only a few weeks of treatment—confirm the positive trend.

The decrease in capillary filtration clinically evident as edema are rapidly associated with symptomatic improvement.

In a microcirculatory model including the examined variables some 60% of the global effects of Linfavenix are obtained within 10 days from the beginning of treatment (in 60% of patients in 8-10 days).

In previous iontophoresis studies it has been observed that Linfavenix is effective on the microcirculation by reducing istamine-induced skin vasodilatation (physiologically comparable to the picture of venous hypertensive microangiopathy). This may be obtained both by previous administration of the compund (limiting the following istamine-vasodilatation) and even as a therapeutic effect (acting on established vasodilatation, Linfavenix reduced its level).

Linfavenix data

The final treatment of CVH—if technically possible—is, generally, either conservative (elastic compression) or definitive (surgery and/or sclerotherapy).[3]

However in most patients, medical and conservative measures are effective and may be also used in association with interventional (surgery, sclerotherapy) methods. Medical treatment is effective and should be available in most patients in these conditions, particularly when edema is significant.

Linfavenix is safe, is very well tolerated and has been prescribed without problems for several years.

New applications are under investigations and may offer, in the next future, important indications both for normal subjects prone to edema and patients with venous disease.

Theoretically, edema present in diabetics may also be controlled with Linfavenix.

In conclusion this study confirms the rapid clinical efficacy of Linfavenix in chronic venous insufficiency and venous microangiopathy.

The study indicates its important role in the treatment and control of this common clinical problem.

Riassunto

L-venix: miglioramento dei segni e sintomi dell'insufficienza venosa cronica e della microangiopatia. Uno studio prospettico sul modello acuto

L'obiettivo di questo studio indipendente è stato di dimostrare la rapida efficacia di un composto venotropo somministrato per via orale (Linfavenix, contenente elementi naturali) in pazienti con insufficienza venosa cronica (*chronic venous insufficiency*, CVI). Due gruppi di pazienti con CVI e gonfiore alle caviglie sono stati trattati con Linfavenix o con calze. La pressione venosa media (*ambulatory venous pressare*, AVP) all'inclusione era, per entrambi i gruppi, pari a 56,2 (range 48-55), con *refilling time* (RT) <10 secondi. Questi parametri indicavano un grave livello di ipertensione

venosa. Tra i due gruppi non vi erano differenze significative (AVP, RT). I due gruppi erano paragonabili; il gruppo trattato con Linfavenix era composto da 12 pazienti (età: 44,5 anni, SD: 4; range 34-55; 7 di sesso femminile); il gruppo trattato con calze era composto da 12 pazienti (età 45,4 anni, SD: 6; range: 35-58; 7 di sesso femminile). L'aspetto clinico e i parametri microcircolatori all'ingresso nello studio erano paragonabili. A due settimane le differenze di RF non erano significative (il flusso era diminuito in entrambi i gruppi, indicando un miglioramento), mentre a 4 settimane la differenza è stata maggiore (ma non significativa tra i due gruppi), con una diminuzione significativa di RF nel gruppo trattato con Linfavenix. Anche la RAS era paragonabile all'inclusione. Entrambi i gruppi hanno evidenziato una diminuzione significativa a 2 e a 4 settimane. La diminuzione di RT provocata da Linfavenix dopo 4 settimane è stata maggiore e significativa (P<0,05) rispetto alla compressione. Anche le differenze osservate per ASLS erano significative in entrambi i gruppi, con un'importante, significativa differenza in favore di Linfavenix a 4 settimane (P<0,05) dimostrata dalla riduzione dell'edema . La diminuzione dell'edema è stata rilevante in entrambi i gruppi a 2 e a 4 settimane (P <0,05) con una differenza minima ma significativa (P<0,05) tra il gruppo trattato con Linfavenix e quello trattato con calze. Queste variazioni dei parametri microcircolatori indicano che il trattamento con Linfavenix è, per quanto riguarda l'efficacia sulla microcircolazione, paragonabile alle calze elastiche considerate l'opzione terapeutica standard in questi pazienti. Nella maggior parte dei pazienti trattati con Linfavenix (10/14) è stato raggiunto un significativo miglioramento a 2 settimane per RF, a 7 giorni per RAS e a 2 settimane in quasi tutti i pazienti (13/14) prendendo in considerazione ASLS ed edema. Non sono stati osservati effetti collaterali. Compliance e tollerabilità sono state molto buone (nessun paziente ha interrotto il trattamento, non ci sono stati drop-outs). In conclusione, la microangiopatia venosa e l'edema sono migliorati dopo trattamento con Linfavenix (in misura maggiore rispetto al trattamento con calze) in giorni.

Parole chiave: Insufficienza venosa - Vene varicose - Ulcere - Compressione elastica - Edema - Vene - Microangiopatia venosa.

References

1. Linfavenix paper (basic composition, rationale).
2. Linfavenix paper (biochemistry/pharmacology).
3. Linfavenix investigator's file.
4. De Sanctis MT, Cesarone MR, Incandela L, Belcaro G, Acerbi G. Methods of evaluation and quantification of microangiopathy in high perfusion microangiopathy (Chronic venous insufficiency and diabetic microangiopathy). J Cardiovasc Pharmacol Therap 2002;7 (Suppl. 1):3-5.
5. Belcaro G, Nicolaides AN. Noninvasive investigations in vascular disease. Imperial College press. London 2001.
6. Almond NE *et al*. A laser Doppler blood flowmeter used to detect thermal entrainment in normal persons and patients with Raynaud's phenomenon. In: Spence VA, Sheldon CD, editors. Practical aspects of skin blood flow measurement. London: Biological Engineering Society; 1985.
7. Anning ST. The cause and treatment of leg ulcers. Lancet 1952;ii:789-91.
8. Anning ST. Leg ulcers. Their causes and treatment. London: J & A Churchill Ltd, 1954.
9. Anning ST. The historical aspect. In Dodd H, Cockett FB, editors. The pathology and surgery of the veins of the lower limb. Churchill-Livingstone, Edinburgh, 1976
10. Belcaro G. The role of transcutaneous PCO_2 measurements in association with laser Doppler flowmetry in venous hypertension. Phlebology 1988;3:189-93.
11. Belcaro G, Grigg M, Rulo A, Nicolaides AN. Blood flow in the perimalleolar skin in relation to posture in patients with venous hypertension. Ann Vasc Surg 1989;1:5-8.
12. Belcaro G, Christopoulos D, Nicolaides AN. Skin flow and swelling in postphlebitic limbs. Vasa 1989;18:136-8.
13. Belcaro G, Nicolaides AN, Volteas N, Leon M. Skin flow, the venoarteriolar response and capillary filtration in diabetics. A three-year follow-up study. Angiology 1992;43:490-4.
14. Belcaro G, Bollinger A, Hoffman U, Nicolaides AN. Laser Doppler Flowmetry. London: Med-Orion, 1994.
15. Bergan JJ, Schmid-Schonbein GW, Takase S. Therapeutic approach to chronic venous insufficiency and its complications: place of Daflon 500 mg. Angiology 2001;52 (Suppl 1):S43-5.
16. Bollinger A, Jager K, Geser A, Sgier F, Seglis J. Transcapillary and interstitial diffusion of Na-Fluorescein in chronic venous insufficiency with white atrophy. Int J Microcirc 1982;1:5-8.
17. Fagrell B. Local microcirculation in chronic venous incompetence and leg ulcers. Vasc Surg 1979;13:217-9.
18. Fagrell B. Microcirculatory disturbances - the final cause for venous leg ulcers? Vasa 1982;11:101-3.
19. Fagrell B. Problems using laser Doppler flowmetry on the skin in clinical practice. In: Belcaro G, Hoffman U, Nicolaides AN, Bollinger A, editors. Laser Doppler. London: Med Orion; 1994. p. 49-53.
20. Franzeck U, Bollinger A, Huch R, Huch A. Transcutaneous oxygen tension and capillary morphologic characteristics and density in patients with chronic venous incompetence. Circulation 1984;70:806-9.
21. Hendriksen O. Local reflex in microcirculation in human subcutaneous tissue. Acta Physiol Scand 1976; 97:446-9.
22. Rayman G, Hassan A, Tooke JE. Blood flow in the skin of the foot related to posture in patients with diabetes mellitus. Br Med J 1986;292:87-91.
23. Thulesius O. Capillary filtration under normal and pathological conditions. Angiologia 1973;10:198-201.