AESCIN: PHARMACOLOGY, PHARMACOKINETICS AND THERAPEUTIC PROFILE

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Aescin, the major active principle from Aesculus hippocastanum (Hippocastanaceae) the horse chestnut tree, has shown satisfactory evidence for a clinically significant activity in chronic venous insufficiency (CVI), haemorrhoids and post-operative oedema. In one controlled trial aescin was shown to be as effective as compression therapy as an alternative to medical treatment for CVI. The therapeutic benefit is well supported by a number of experimental investigations in different animal models, indicative of clearcut anti-oedematous, anti-inflammatory and venotonic properties, mainly related to the molecular mechanism of the agent, allowing improved entry of ions into channels, thus raising venous tension in both in vitro and in vivo conditions. Other mechanisms, i.e. release of PGF$_2$ from veins, antagonism to 5-HT and histamine, reduced catabolism of tissue mucopolysaccharides, further underline the wide ranging mechanisms of the therapeutic activity of aescin. The excellent tolerability of aescin in the clinic indicates this treatment is of definite clinical benefit in patients with clinical conditions resulting in CVI, haemorrhoids or peripheral oedema formation.

KEY WORDS: aescin, horse chestnut, chronic venous insufficiency, haemorrhoids, post-operative oedema, review.

INTRODUCTION

Aescin is the major active principle from Aesculus hippocastanum (Hippocastanaceae) the horse chestnut tree, a plant widely distributed all over the world because of its excellent resistance to environmental conditions. The horse chestnut grows in Iran, Northern India, Asia Minor, South-East Europe, from the Balkans to the Caucasus, as well as in the USA [1]. It is also widely cultivated in parks and gardens, and along city streets. The parts of the plant used in medicine are the seeds and the bark of young branches. The fruit of the horse chestnut is a prickly small globular capsule which becomes leathery as it matures, containing from two to four large seeds. The seeds are round and smooth with large light base areas. The husk may contain from one to three achenes or nuts [2]. Chestnuts have a large base area, while having the remains of the perigonium and stigmas on top.

A number of reports dating from the early 18th century have indicated therapeutic properties for horse chestnut. These have ranged from anti-fever (Bon, 1720) to, at the end of the 19th century, anti-haemorrhoidal properties (Artault de Vevey, 1886).

ACTIVE COMPONENTS OF A. HIPPOCASTANUM

The seeds of A. hippocastanum contain a saponin mixture from which two crystallin products can be separated: 'aescin' (haemolytic) and 'prosapogenin' (non-haemolytic). A number of other products have been isolated from the chestnut seeds, i.e. bioflavonoids such as: quercetin, kaempherol and their diglycosyl derivatives, as well as anti-oxidants, such as proanthocyanidin A$_2$ and the coumarins esculin and fraxin [3]. However, all of these products can be found in larger amounts from other sources and, furthermore, during 1960 Lorenz and Marek [4] concluded that the anti-oedemigenous, anti-exudative and vasoprotective activities of hippocastanus extracts (HCE) are exclusively due to aescin.

Aescin is a natural mixture of triterpene saponins [5] (Fig. 1). The aglicos are derivatives of protoascigenin, acylated by acetic acid at C-22 and by either angelic or tiglic acids at C-21. Aescin exists in two forms, $\alpha$ and $\beta$, that can be distinguished by: melting point, specific rotation, haemolytic index and solubility in water. $\alpha$-aescin is formed through an acylic migration involving the hydroxyl groups at positions C$_{21}$, C$_{22}$, C$_{23}$ by simply heating to 100°C an aqueous solution of $\beta$-aescin.

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β-aescin appears to be the active component of the mixture and is the molecular form present in major available pharmaceutical products.

A number of specific assays have been developed in order to quantitatively determine the aescin content of various products. The aescin content in ointments can be determined by TLC-densitometry, whereas an HPLC method has been developed for the separation and assay of aescin saponins in extracts and in pharmaceutical preparations [6]. A fingerprint of the β-aescin composition has been finally obtained by liquid chromatography–mass-spectrometry (LC-MS) using a thermospray (TSP) interface [7]. This is successfully used for the monitoring of the saponin composition pattern in different industrial samples of β-aescin.

PHARMACOLOGY OF β-AESCIN

The pharmacological profile of β-aescin has received significant contributions in recent years, in order to establish the pharmacological basis for the major clinical indication of: treatment of chronic venous insufficiency (CVI).

Most of the studies have been carried out with HCEs containing around 70% of aescin. There is clear evidence that the higher the percentage content of the active component, the higher the therapeutic effectiveness [3]. From now on, all quoted products will be named ‘aescin’, indicating the content of active material.

At least three types of pharmacodynamic actions have been attributed to aescin:

1. anti-oedematous properties;
2. anti-inflammatory activities;
3. venotonic properties.

All of these appear to be due to a basic molecular mechanism, identified as a selective vascular permeabilization [8], allowing a higher sensitivity, of e.g. calcium channels, to molecular ions, resulting in increased venous and arterial tone [9]. These sensitizing effects to ions and other molecules, e.g. 5-HT, result probably in the enhanced venous contractile activity, and as a consequence, in the anti-oedematous property of the molecule. Aescin is now, in fact, widely quoted in the literature as a pharmacological tool to assess the sensitivity of vascular tissues to different agonists, in order to evaluate the mechanism of e.g. hypertension development in animal models [10]. Major mechanisms of aescin are summarized in Fig. 2.

ANTI-OEDEMATOUS PROPERTIES

Aescin has been shown to be effective in preventing the formation of oedema in models of inflammation that reproduce the initial exudative phase, such as oedema induced in the paw by a series of irritative agents (ovalbumin, dextran, cotton pellet, carrageenin and bradykinin), serous peritonitis induced in rats by the injection of formalin and in mice by carrageenin [11]. On the contrary, aescin is not effective in preventing oedema in models of inflammation that reproduce the late proliferative phase, such as oedema produced by formalin in rats’ paw and pocket-granuloma models; furthermore, it is not effective in experimental models of polyarthritis [11].

The mechanism of the anti-oedematous effect, in addition to the earlier described sensitization to Ca²⁺ ions, resulting in a ‘sealing effect’ on small vessels permeable to water, has also been related to a reduced hypoxia-induced activation of human endothelial cells [12]. In
inflammatory conditions, as well as during blood stasis, resulting in decreased oxygen supply, a reduction in ATP content may occur due to lowered mitochondrial oxidative phosphorylation [13]. This results in a cascade of metabolic events: release of prostaglandins and PAF, neutrophil recruitment, adherence and activation, all leading to venous stasis and oedema in the case of varicose disease. Aescin can well antagonize the reduction in ATP content and increased phospholipase A2 responsible for the release of precursors of inflammatory mediators [14]. There is, furthermore, a reduced neutrophil adherence/activation, all resulting in the protection of veins and reduced oedema [15].

In order to evaluate the effectiveness of aescin on the increased capillary pressure found in oedematous conditions of venous disease, a crossover clinical trial was carried out in 24 women with CVI stages I–III according to Widmer; the diagnosis was based on the outcome of Doppler sonography, light reflection rheology and physical examination [16]. Patients were placed supine, with an extended leg raised 10 cm above the heart, supported by the ankle, with the foot against a wall. Two cuffs were applied to the leg, one around the thigh connected to a compressor and the other around the calf, connected to a plethysmograph equipped with a recording device. A pressure equivalent to 60 mmHg was exerted by the compressor for 20 min and the changes in calf volume recorded by the plethysmograph. The first 6 min corresponded to the intravascular filling volume, the period thereafter to capillary filtration into the extravascular compartment. This procedure was repeated three times, at baseline and after either aescin (100 mg orally) or placebo administration. The intervals between sessions lasted 35 min; the total duration of the experiment was 4 h. In the 22 evaluable subjects the capillary filtration coefficient was significantly reduced in the aescin group 3 h after ingestion of medication, both clinically and statistically: 2% increase after placebo vs 22% decrease after aescin ($P = 0.006$).

Besides the described vascular ion sensitizing activity of aescin, the ‘sealing’ properties may rest on the ability to inhibit the enzymes elastase and hyaluronidase [17]. These are involved in the degradation of proteoglycans, important constituents of the capillary endothelium and major components of the extravascular matrix. Aescin may shift the balance between synthesis and degradation of proteoglycans towards net synthesis, strengthening the capillary wall and preventing leakage. This hypothesis has been well documented by electron microscopy in animals and by studies with sulphomucopolysaccharides labelled with $S^{35}$, showing that the degradation of mucopolysaccharides in rat connective tissue (xiphoid cartilage) is significantly reduced after intraperitoneal treatment with 1 mg kg$^{-1}$ daily of aescin for 3 weeks [18].

**ANTI-INFLAMMATORY PROPERTIES**

Aescin and, in general, HCE possess significant anti-inflammatory properties, well exemplified by a reduced
inflammatory granuloma in the rat [15]. The anti-inflammatory action can be separated with difficulty from the anti-oedematous effects in models, such as experimental pleurisy in rats and the acute lymphatic oedema developed after blockade of the leg lymphatic circulation [18]. Studies with the ‘skin window’ technique of Rebuck & Crowley, measuring leukocyte, neutrophil and macrophage density in the inflammatory exudate in patients with chronic venous insufficiency given aescin (5 mg i.v. every 12 h for 7 days), disclosed a 33% reduction of leukocyte density per surface unit; there were also qualitative changes, as macrophages diminished by about 50%, being replaced by neutrophils, increased by 46% [18]. The colloidal charcoal test, furthermore, showed that phagocytosis was unimpared.

These findings suggest that aescin can effectively interfere with the cellular phase of the inflammatory process, i.e. with leukocyte activation. This hypothesis is supported by the suppression of leucocyte migration into the pleural cavity in the previously reported experimental model of pleurisy in the rat and by studies on hypoxia-activated endothelial cells, which show that aescin can reduce the adhesiveness of neutrophils and associated release of inflammatory mediators [13, 15].

The inhibition of foot swelling induced by carrageenin and of carrageenin induced pleurisy in rats is accompanied by a significant reduction of lipid peroxidation in a concentration dependent manner [15]. These findings have mainly been carried out in studies with non-purified HCE but there is clear evidence for an improved activity of the purified product [3].

The typical reduction of oedema formation, reflecting the general cellular mechanism of the agent, is also well exemplified by a reduced rat skin capillary permeability, increased by 5-HT and histamine [15]. The effect of aescin as a potentially selective 5-HT antagonist is also supported by studies reporting an enhanced acceleration of gastrointestinal transit in mice [19] following oral aescin, due to the selective activation of the 5-HT2 receptors, with a relative antagonism for the pro-inflammatory 5-HT1 receptors [19].

VENOTONIC PROPERTIES

Exposure of dog veins to either aescin or HCE leads to a significant rise of venous contractility. Contractions increase up to 50% at concentrations of $10^{-5}$ M in dog veins exposed to noradrenaline, with a duration of increased contractility of over 5 h. The response is noted both in normal and pathologically altered veins. The effect can also be detected in veins perfused in the inverse direction: in these, with normal valves, flow is, in fact, significantly reduced, with a maximal effect close to that of noradrenaline [15].

In the anaesthetized dog a dose related increase of the rate of rise of venous pressure and final maximal pressure (+20.6 and +30%, respectively) can be achieved at injected doses between 25 and 50 mg of aescin [15]. With these, consequent to the basic mechanism of activity of the agent, a markedly increased lymphatic thoracic flow was observed (+70%).

In human saphenous veins in vitro, venotonic properties were well confirmed with purified β-aescin [20]. Achieved contractures are maximal at concentrations of $10^{-3}$ M but are already quite evident at $10^{-6}$–$10^{-7}$ M. Maximal contractions are achieved with noradrenaline $10^{-7}$ M. A dose–response curve obtained from 10 different human saphenous vein preparations indicated a 10–20% increase of venous tone, already clinically meaningful, well evident at concentrations below $10^{-7}$ M in the majority of preparations. Such concentrations can be safely obtained after oral intake of the agent (see later).

The mechanism of increased venous tone has been clearly associated with an enhanced generation of prostaglandin F2α in human veins, by the group of Berti in Milano [21]. Aescin, at concentrations of $10^{-3}$ M or lower resulted in a clear increase of contractility in human isolated saphenous veins. This activity was lost after exposure to indomethacin, with complete recovery of aescin’s activity 120 min after removal of the anti-inflammatory agent. Assay of the prostaglandin like material released by the saphenous vein during incubation in the presence of aescin, clearly established the presence of prostaglandin like material, later identified as PGE$_2$ [21] (Fig. 3). Increased PGE$_2$ depends either on the activation of phospholipases or of other acylhydrolases, and from stimulation of α-keto reductase, the enzyme converting PGE$_2$ to PGF$_{2α}$ present in many tissues and particularly in veins.

AESCIN TOXICOLOGY AND MECHANISMS SUPPORTING THE CLINICAL INDICATIONS

Aescin, as clearly indicated by all reported studies, is generally well tolerated. Reports of poisoning with horse chestnut seeds are consequent to the presence of the toxic principle esculose, not to aescin [22]. Aescin has undergone extensive toxicological studies in rodents. Toxic manifestations following i.v. administration of high doses are due to massive haemolysis. The DL$_{50}$ following i.v. administration in rodents is equivalent to 28 times the maximum dose recommended for therapeutic use in man; the DL$_{50}$ following oral dosing is over 100 mg kg$^{-1}$, equivalent to 59 times the recommended therapeutic range. In rabbits the continuous administration of 1.1 mg kg$^{-1}$ for 1 month (equivalent to about 10 times the therapeutic dose) is associated with minimal haemolysis, that can be detected only by an increased erythropoiesis, and does not manifest clinically. No important toxicity was found in subacute and chronic toxicity studies. Reproduction and fertility studies did not disclose any untoward effects on offspring or on male fertility [23, 24].
A number of basic mechanisms underlie the proposed therapeutic indications of aescin in major clinical conditions. Aescin exerts powerful anti-inflammatory properties due to a molecular effect of ion channel sensitization, improved disposal of fluids and enhanced contractility. Furthermore, the drug acts on the starting factor of inflammation/oedema, i.e. hypoxia of endothelial cells, followed by a decrease in ATP. This triggers the release of prostaglandins via phospholipase A2 and PAF, which initiates an inflammatory response, associated to oedema and neutrophil adhesion to the endothelium; both of these interfere with blood flow and exacerbate hypoxia. Neutrophils, furthermore, release enzymes, such as elastase and other degradative substances, that can cause parietal damage to veins and stimulate fibroblast growth factor, leading to vein enlargement [25].

Aescin may intervene at various steps in the process. It may prevent oedema and neutrophil adhesion, thus avoiding further hypoxia and reducing damage to the venous walls by antagonizing proteoglycan degradation and, finally, due to the basic sensitizing activity on Ca²⁺ ions [26], exert a venotoxic effect, potentially mediated by enhanced release of PGF₂α (Fig. 2).

These mechanisms can have a major role in the antagonism to post-operative oedema, again due to reduced tissue damage and necrosis, initiating the local inflammatory process associated with increased vessel permeability [27]. Aescin can well curtail the formation of post-operative oedema by blocking the increase in permeability and the local inflammatory process.

**ABSORPTION AND KINETICS OF HCE AND AESCIN**

Adequate bioavailability of natural molecules is of special interest in view of the growing use of these products in clinical medicine. For many molecules of natural origin, most frequently presented under the form of extracts or mixtures, bioavailability data are either absent or inadequate to support their clinical indication. This had been the case of HCE up to recent years, when the availability of purified products, particularly β-aescin formulations, has provided a more reliable source of pharmacologically active molecules. In addition, adequate methods for determination of blood levels have become available.

The significant advances in understanding of bioavailability and kinetics of β-aescin should be attributed to the development of a highly specific radioimmunoassay (RIA) allowing the detection of concentrations in the ng ml⁻¹ range (10⁻⁶ M), pharmacologically active, and supporting the therapeutic activity of the product [20].

With the RIA method, initial studies indicated that a newly developed film coated tablet with sustained release allowed a high bioavailability, i.e. 98.3–120.9%, compared to the AUC of reference preparations [28]. This method was also applied to comparisons between the bioavailability of aescin contained in different HCE formulations, again suggesting the excellent absorption of β-aescin also in HCE, but with a considerable dispersion of data [29].

Finally, a more recent study with a crossover design compared different β-aescin formulations, all containing 50 mg aescin in tablet form, on a series of 18 healthy Caucasian volunteers of both sexes [30]. The study was a randomized, open, multiple dose two-period switch-over trial with kinetic evaluations covering a 24 h cycle of two successive dose intervals. The two tested products proved bioequivalent with a Cmax (first dose) around 16–18 ng ml⁻¹ and a C average (Cav) around 10 ng ml⁻¹. Interestingly, at the second dose interval, the Cmax was somewhat reduced, to levels around 10–11 ng ml⁻¹, together with a small reduction in Cav (7 ng ml⁻¹). The lower Cmax after the second daily dose was well reproduced upon repeated treatments, being explained by the authors as possibly reflecting the effect of food, rather than of circadian rhythms [30]. There was essentially no change in kinetics by continuing doses bid up to 7 days, with Cav maintained at 8 ng ml⁻¹.

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**Fig. 3.** Prostaglandin-like material released by a human saphenous vein during a period of 30′ in the presence of aescin, as tested on rat stomach strips (RSS). PGF₂α bioassays are also indicated. A = 10 µg ml⁻¹ aescin superfused at 2 ml min⁻¹; P = superfusion with Krebs solution (5 ml); P + A: Perfusate in the presence of aescin 10 µg ml⁻¹. Aescin releases PG-like material, identified with PGF₂α; indomethacin abolishes the release [21].
Table I

Placebo-controlled studies with oral horse chestnut preparations containing aescin (modified from Pittler and Ernst, Reference 31)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Quality score</th>
<th>Study design</th>
<th>No of evaluable patients/dropouts</th>
<th>Treatments (standardized to mg aescin)</th>
<th>Primary end-point</th>
<th>Outcome (mean ↓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neiss &amp; Bohm (1976)</td>
<td>5</td>
<td>Cross-over</td>
<td>233/7</td>
<td>50 mg bid × 20 days</td>
<td>CVI symptoms</td>
<td>↓ oedema, pain, itching vs placebo (P &lt; 0.05)</td>
</tr>
<tr>
<td>Friederich et al. (1978)</td>
<td>4</td>
<td>Cross-over</td>
<td>118/23</td>
<td>50 mg bid × 20 days</td>
<td>CVI symptoms</td>
<td>↓ Calf spasm, pain, fatigue, tension vs placebo (P &lt; 0.05)</td>
</tr>
<tr>
<td>Bisler et al. (1986)</td>
<td>5</td>
<td>Cross-over</td>
<td>24/2</td>
<td>100 mg qd × 2 weeks</td>
<td>Calf volume changes under standardized pressure in CVI</td>
<td>↓ −22% vs placebo see text (Reference 16)</td>
</tr>
<tr>
<td>Lohr et al. (1986)</td>
<td>3</td>
<td>Parallel group</td>
<td>80/16</td>
<td>50 mg bid × 8 weeks</td>
<td>Leg volume changes</td>
<td>↓ 12.7 ml vs placeboa</td>
</tr>
<tr>
<td>Rudofsky et al. (1986)</td>
<td>5</td>
<td>Parallel group</td>
<td>40/1</td>
<td>50 mg bid × 4 weeks</td>
<td>Leg volume changes</td>
<td>↓ 10 ml vs placebo (P &lt; 0.001)</td>
</tr>
<tr>
<td>Pilz (1990)</td>
<td>4</td>
<td>Parallel group</td>
<td>30/2</td>
<td>50 mg bid × 20 days</td>
<td>Ankle circumference</td>
<td>↓ 0.7 cm vs placebo (P &lt; 0.05)</td>
</tr>
<tr>
<td>Steiner (1990)</td>
<td>3</td>
<td>Cross-over</td>
<td>20/nr</td>
<td>50 mg bid × 2 weeks</td>
<td>Leg volume changes</td>
<td>↓ 113 ml vs placebo (P = 0.009)</td>
</tr>
<tr>
<td>Diehm et al. (1992)</td>
<td>4</td>
<td>Parallel group</td>
<td>40/1</td>
<td>75 mg bid × 6 weeks</td>
<td>Leg volume changes</td>
<td>↓ 80 ml vs baseline placebo; ↓ 4 ml</td>
</tr>
</tbody>
</table>

a P value not reported.

This study confirms data from previous trials with less adequate methods of determination, i.e. a Tmax around 2 h after the first dose and a t1/2β around 6–8 h. It confirms, therefore, the excellent bioavailability of β-aescin and the clear potential to achieve steady-state levels adequate for therapeutic benefit when given bid [20], possibly distant from meals in order to avoid the latter effect on bioavailability.

THERAPEUTIC ACTIVITY IN CHRONIC VENOUS INSUFFICIENCY

A considerable number of trials have been carried out with oral HCE or aescin in patients with chronic venous insufficiency (CVI). This disease has a prevalence of 10–15% in adult men and of 20–25% in women. It reflects more than a ‘cosmetic problem’ because many patients require hospital and/or surgical treatment and at least two-thirds of leg ulcers have evidence of venous disease in the affected limb [25]. This disease leads, therefore, to a high degree of suffering and a high economic cost for society. Clinical studies with oral chestnut extracts (HCEs) containing aescin have been recently reviewed by Pittler and Ernst [31]. This review has been updated by Bielanski and Piotrowski [32] and quoted in a more recent overall review on the treatment of CVI [25], adding minimal further clinical information.

The search by Pittler and Ernst identified 11 randomized clinical trials of aescin vs placebo, three of which did not reach pre-set minimal quality criteria. One of the placebo-controlled trials has already been described in the previous section on the mechanism of action [16].

The overview of the eight placebo-controlled studies with aescin is provided in Table I. In the 521 evaluable patients given 100–150 mg aescin daily, generally bid, dosing for 2–8 weeks always led to a significant reduction in leg volume and symptoms (pain, fatigue, sensation of tension, itching) compared to placebo. In two of the four trials with reduction in leg volume as primary end-point, the difference vs placebo was clinically significant in two (80–113 ml) and in the remaining two it was of a lesser degree (10–12.7 ml). The authors comment that, in spite of some disagreement among experts from Europe and United States, the results of controlled trials are non-disputable and accompanied by modest side effects. Significant adverse reactions (ADRs) were reported in only three studies as not different from the incidence in the placebo group and, in a recent observational study (see later), occurring in not more than 0.6% of patients during treatment.

In the analysis of controlled investigations, of particular significance are studies presenting with
Comparisons between everyday treatment choices for patients and physicians. A randomized, three-armed controlled clinical trial, compared oral aescin (50 mg bid) with a matching placebo and with compression therapy in patients with CVI. The 240 selected patients were treated for 12 weeks with either compression stockings class II, 50 mg aescin bid, or placebo (one capsule bid) at a ratio of 2 : 2, 2 : 1. Patients allocated to the compression treatment arm also received a diuretic once daily for 7 days in order to achieve the best possible stocking fit. The primary efficacy end-point was logarithmically transformed leg volume data, after 12 weeks of treatment. All patients entered the period of randomized treatment and were available for the intention-to-treat analysis (compression therapy $n = 99$; aescin $n = 95$; placebo $n = 46$). The mean reduction in leg volume was $43.8 \pm 11.4$ ml with aescin, $46.7 \pm 8.2$ ml with compression therapy; leg volume actually increased with placebo on average by $9.8 \pm 15.0$ ml. The statistical analysis concluded that the therapeutic efficacy of aescin was equivalent to compression therapy preceded by diuretic, and both active treatments were significantly superior to placebo ($P = 0.005$ and $P = 0.002$, respectively) [33]. This study clearly indicates that patients with CVI have a choice between compression therapy, not accepted by more than 50% of patients, as reported in the literature, and $\beta$-aescin, in order to reduce oedema resulting from chronic venous insufficiency (Fig. 4).

Further double blind comparisons were carried out not vs placebo but vs another commonly used drug treatment, i.e. hydroethylrutoside (HR) in four randomized, double-blind, controlled clinical trials in a total of 237 patients given 50–75 mg bid aescin and dosages of HR ranging from 500 to 2000 mg daily [31]. With one exception, no statistical comparisons were performed between treatments generally showing similarity in effectiveness; the three studies not reporting detailed statistical comparisons will not be, therefore, further considered. The only well controlled trial was a large investigation carried out in 137 post-menopausal females, comparing two different HR dosage regimens (1000 mg daily for the whole treatment period vs 1000 mg daily loading dose for 4 weeks, followed by 500 mg daily for another 8 weeks) to 50 mg bid aescin under double-blind conditions [34]. The period of randomized treatment was preceded by a 2-week placebo run-in period and followed by a 6-week follow-up without medical treatment. The evaluable patient population (aescin $n = 51$; HR1000 $n = 51$; HR 1000 + 500 $n = 35$) aged less than 70 years old with stage II CVI, documented by Doppler sonography in the previous 6 months. The primary endpoint was the area under the curve between baseline and end of study of baseline-adjusted leg volume assessed by water displacement technique (AUC 0–18), measured twice daily at each visit at week 4, 8 and 12 during treatment and at week 15 and 18 during the follow-up period. Mean leg volumes were reduced in all three groups; the course of reduction is shown in Fig. 5. Aescin appeared to be as effective as HR in spite of a small, non-statistically significant better volume reduction with HR. It should be noted, however, that aescin resulted in a better mean subjective score for ‘tired heavy legs’. General tolerability was also better with aescin. A total of 11 patients reported, in fact, potential ADRs, i.e. three of 59 (5.1%) in the HR 1000 mg group, six out of 37 (16.2%) in the 1000–500 mg group, vs only two out of 62 (3.2%) in the aescin group. The symptoms reported were: non-specific gastrointestinal complaints, headache and dizziness of a transitory nature [34].

In addition to the randomized, double-blind, controlled clinical trials, one further trial is worthy of mention. The National Association of General Practitioners (GPs) [35] in Germany performed an observational study on the usage of aescin in CVI. The study assessed the impact of aescin on symptoms (pain, fatigue, tension, swelling, itching in the legs) and its tolerability profile in clinical practice. Eight hundred GPs took part in the trial and recruited 5429 patients (mean age: 55.3 years, range 41 to 70 years; 88% with CVI, 81.6% CVI with varicose veins, 32.5% CVI with skin lesions, 56.4% CVI with ‘other venous disorders’); 88.1% of the patients had already received treatment, but only 16% had benefited from it. Most patients were treated for 4–10 weeks with 75 mg oral aescin bid. All symptoms improved during the first week of treatment and their severity was reduced considerably by the end of treatment (Fig. 6). Also the percentage of patients free of symptoms at the end of treatment increased considerably (Fig. 7). Compliance was excellent, being close to 95%. Almost all GPs (94%) stated that the therapeutic efficacy of aescin was good or excellent. This being an open-label trial, improvement in symptoms may well have been a placebo effect; however, the improvement was remarkable, with minimal side effects and it would appear as unlikely to be related only to a placebo effect.
The overview of studies on CVI clearly concluded that aescin is effective in controlling oedema and related symptoms in CVI. It is as effective as compression therapy, but better tolerated and more acceptable to patients. In a very recent overview of major available treatments for CVI, i.e. aescin, hydroxyethylrutoside and daflon, it is noted that aescin appears to have a unique effectiveness, when compared to compression therapy, vs the other two agents and that, furthermore, aescin appears to exert a progressively better effect during continued therapy [25].

HAEMORRHOIDS

In the field of venous diseases, haemorrhoids have an important clinical position, and a limited therapeutic armamentarium is available. Most, if not all medications, are available for local use. One double blind placebo-controlled trial evaluated efficacy and safety of aescin in 80 patients suffering from acute symptomatic haemorrhoids [36]. Treatment was 40 mg aescin tid for a period of up 2 months, according to response. The therapeutic efficacy was assessed based on the subjective opinion of the patient, severity of symptoms and outcome of endoscopic evaluation. Thirty-eight patients allocated to aescin film-coated tablets and 34 patients allocated to placebo were evaluable. Endoscopic examination at baseline documented bleeding and swelling in 28 and 34 patients allocated to aescin, respectively, and in 18 and 33 patients allocated to placebo. Thirty-one patients (81.6%) reported a considerable improvement vs 11 in the placebo group (32.4%). The endoscopic examination disclosed notable improvement in bleeding in 26 patients in the aescin group (94.8%) and in 13 patients in the placebo group (61.8%); swelling improved in 29 patients with aescin (86.9%) and in 12 placebo patients (38.3%) (P < 0.01) [36]. Improvement in symptoms was reported on average after 6 days of treatment; endoscopic signs of improvement were recorded after 2 weeks (Fig. 8).

POST-OPERATIVE OEDEMA

Post-operative oedema occurs after a variety of procedures, particularly large ones at the lower limb level, and is mainly handled by local treatments. Two clinical trials assessed the efficacy of aescin in post-operative oedema. In the first, 72 in-patients subjected to herniotomy (n = 33), meniscectomy (n = 24) or surgery for lower leg fractures (n = 15), were allocated either to aescin or to no treatment [37]. Aescin treatment was given as 5 mg i.v. in the evening before surgery, 10 mg i.v. in the morning and...
5 mg i.v. in the evening on the day of surgery, in the first three post-operative days, and as 5 mg i.v. in the evening of the subsequent 3 days. The main efficacy endpoint was the difference in skin temperature between the operated part and the contralateral side. Temperature was always significantly lower only in the operated side treated with aescin, independent of the type of surgery.

In the second study carried out in patients undergoing various surgical procedures involving the hands [27], the same parameter—difference in temperature between the operated side and the other—was evaluated by infrared thermography. Twenty-seven of the patients were given 10 mg aescin bid and 26 were not given any treatment. The hand temperature course differed in the two treatment groups: in the aescin group peak hand temperature difference was recorded on the second post-operative day and then diminished rapidly. In the group on no treatment, peak hand temperature difference was reached on the fourth post-operative day and took longer to disappear; in the patients operated on for Dupuytren fibrosis a difference of 0.71 °C was still present on day 8. Eight standardized skin areas were identified for temperature measurements bid following the same procedure, until the difference had disappeared (5–8 days after surgery); the total number of measurements amounted to 7500. Considering the whole patient population, the difference reached statistical significance on the third day ($P < 0.001$).

Besides these two studies, both with i.v. aescin, extensive experience in the prevention and treatment of post-operative oedema within the setting of open-label studies has been reported in the literature, in some cases with oral aescin. Overall the number of cases exceed 1200 patients, who were subjected to a variety of operations, most reporting a positive outcome (Table II), [38–41].

### SAFETY

In the controlled trials in CVI the rate of adverse reactions ranged from 0.9 to 3.0%. Most ADRs were gastrointestinal symptoms, dizziness, headache and itching [28]. In the large German observational study adverse events were reported in only 0.6% of patients and consisted mainly in gastrointestinal disturbances [35]. A similar low range of side effects was observed in the papers describing the 1262 surgical cases treated with aescin for post-operative oedema (one case of nausea and vomiting, two cases of urticaria) and an unspecified number of cases of burning and/or feeling of heat after i.v. administration [37–40].

Post-marketing experience has included rare cases of acute anaphylactic reactions. Urticaria and dyspnea have been reported in a few cases of topically applied aescin. One severe case was reported in Spain, resulting in generalized urticaria and dyspnea, successfully handled by i.m. chloropheniramine and hydrocortisone [42].

Finally, a serious safety issue was raised more than 25 years ago, i.e. the risk of acute renal failure, when patients, who had undergone cardiac surgery, were given high doses of horse chestnut extract i.v. for post-operative oedema. The phenomenon was dose-dependent as no alteration in renal function was recorded with $340 \, \mu g \, kg^{-1}$, mild renal function impairment developed with $360 \, \mu g \, kg^{-1}$ and acute renal failure with $510 \, \mu g \, kg^{-1}$ [22]. This led to three clinical trials to assess the effects of aescin on renal function [43–45]. The total number of subjects studied was 83:

- 18 healthy volunteers. Ten were administered 10 mg i.v. daily for 3 days, eight were given 20 mg i.v. for 6 days;

#### Table II

<table>
<thead>
<tr>
<th>Surgical procedure</th>
<th>n</th>
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<tbody>
<tr>
<td>Varicose veins</td>
<td>160</td>
</tr>
<tr>
<td>Inguinal surgery</td>
<td>160</td>
</tr>
<tr>
<td>Haemorrhoids</td>
<td>147</td>
</tr>
<tr>
<td>Leg trauma</td>
<td>89</td>
</tr>
<tr>
<td>Fractures</td>
<td>83</td>
</tr>
<tr>
<td>Hydrocele</td>
<td>80</td>
</tr>
<tr>
<td>Anal surgery</td>
<td>72</td>
</tr>
<tr>
<td>Phimosis</td>
<td>64</td>
</tr>
<tr>
<td>Umbilical surgery</td>
<td>53</td>
</tr>
<tr>
<td>Facial plastic surgery</td>
<td>49</td>
</tr>
<tr>
<td>Gastroctomy</td>
<td>48</td>
</tr>
<tr>
<td>Amputations</td>
<td>42</td>
</tr>
<tr>
<td>Mastectomy/gynecomastia</td>
<td>37</td>
</tr>
<tr>
<td>Facial surgery</td>
<td>30</td>
</tr>
<tr>
<td>Hand surgery</td>
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<tr>
<td>Orthopaedics</td>
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</tr>
<tr>
<td>Miscellaneous</td>
<td>98</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>1262</strong></td>
</tr>
</tbody>
</table>

Fig. 8. Persistence of acute signs and symptoms in a double-blind, placebo-controlled, parallel group trial in patients with acute hemorrhoidal attacks after treatment with either 40 mg aescin tid orally ($n = 38$) or matching placebo ($n = 34$) [36]. ■ placebo; □ aescin.
40 in-patients (38 adults and two children aged 4 and 8 years) with intact renal function, given aescin (10 mg i.v. bid for 6 days—the highest recommended therapeutic dose—except in the two children, who received 0.2 mg kg\(^{-1}\) daily) for the treatment of post-operative oedema after reconstructive surgery of the hand and extremities following trauma;

12 patients with cerebral oedema and normal renal function, who were given a massive i.v. dose on the day of surgery (49.2 ± 19.3 mg) and 15.4 ± 9.4 mg daily for the following 10 days;

13 patients with impaired renal function due to glomerulonephritis or pyelonephritis, who were given 20–25 mg i.v. daily for 6 days.

In all studies renal function was monitored daily resorting to the usual tests of renal function: BUN, serum creatinine, creatinine clearance, urinalysis. In a selected number of cases para-aminomollipurate and labelled EDTA clearance were also measured. No signs of development of renal impairment in the patients with normal renal function or of worsening of renal function in the patients with renal impairment were recorded [43–45]. All of these studies were carried out with whole HCE. The recent use of purified β-aescin, as in EDEVEN tablets, rules out any significant risk of renal impairment with this product.

**CONCLUSIONS**

Studies with the horse chestnut extract and, more recently, with the active principle, β-aescin, have provided satisfactory evidence for a clinically significant activity in chronic venous insufficiency, haemorrhoids and post-operative oedema. The therapeutic efficacy, as shown in the international literature, includes studies published in top ranked journals, and is well supported by a large number of randomized controlled studies. In one trial aescin was shown to be as effective as compression therapy as an alternative to medical treatment for CVI. Comparison with other active principles, e.g. hydroxyethyluroside, shows similar activity and improved tolerability.

The therapeutic benefit is well supported by a number of experimental investigations in vivo in different animal models and in vitro, indicative of clearcut anti- oedematous, anti-inflammatory and venotonic properties. These are mainly related to the molecular mechanism of the agent, allowing improved response to Ca\(^{2+}\) ions [26], thus raising venous tension in both in vitro and in vivo conditions. This molecular mechanism has resulted in a wide use of β aescin as a tool in a variety of experimental studies investigating contractile properties of different vascular districts [10]. Other mechanisms, i.e. release of PGF\(_{2\alpha}\) from veins, antagonism to 5-HT and histamine, reduced catabolism of tissue mucopolysaccharides, further underline the wide ranging mechanism of therapeutic activity of aescin.

The excellent tolerability of aescin in the clinic, supported now by controlled studies involving many thousands of patients, indicates this treatment is of definite benefit in patients with clinical conditions resulting in CVI, haemorrhoids or peripheral oedema formation.

**REFERENCES**