REVIEW

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Therapeutic approach to chronic venous insufficiency – clinical benefits of red-vine-leaf-extract AS 195 (Antistax®)

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Background: Chronic Venous Disorders or Diseases (CVD) of the lower extremities are a common finding affecting almost 90 % of an adult population. CVD includes varicose veins with a prevalence of approx. 25 %, overlapping with Chronic Venous Insufficiency (CVI) with a prevalence of approx. 17% in the adult population. CVI is characterized by venous pathology and objective signs like edema, skin changes or venous leg ulcers. *Objective:* To review and evaluate non-clinical and clinical data on a standardised herbal product containing flavonoids (AS195; Antistax®) and to put them into a perspective with the pathophysiology of CVD. *Methods:* Literature available on non-clinical pharmacology and clinical studies with AS195 in CVI of the lower extremities was reviewed and described. *Conclusion:* Antistax® is a well-described herbal product with standardised starting materials and manufacturing process. Its active ingredients accumulate in the venous intima, preserve the endothelial barrier function, and inhibit the inflammatory and prothrombotic cascade behind the progression of CVD. Its efficacy was analysed in adequately planned and executed clinical trials in patients with mild to moderately severe CVD (CEAP C1s to C4). AS195 showed a statistically significant and clinically relevant efficacy over placebo: in objective endpoints like volumetry of lower leg edema, but also in outcomes directly relevant for patients like tension and heaviness of the legs, tingling, and pain. Supportive studies confirmed and validated these results also for the broader population treated in daily practice. AS195 was well tolerated in studies and in everyday therapy. There are no known interactions with other medications. In the later stages, it can be used in combination with compression, complementing the beneficial haemodynamic effects of compression at a cellular level. AS195 is an addition to compression and closes a therapeutic gap especially in patients, who cannot use compression stockings, but still require CVD therapy.

1. Introduction

1.1. Clinical picture and aetiology of Chronic Venous Disorders (CVD)

Chronic Venous Disorders or Diseases (CVD) of the lower extremities are a common finding affecting almost 90 % of an adult population. CVD includes varicose veins with a prevalence of approx. 25 %, overlapping with Chronic Venous Insufficiency (CVI) with a prevalence of approx. 17 % in the adult population. CVI is characterized by venous pathology and objective signs like edema, skin changes or venous leg ulcers (Rabe et al. 2003; Evans et al. 1999; Bergan et al. 2006; Eberhardt and Raffetto 2014). Risk factors for varicose veins and CVI include genetic factors, age, female sex, obesity, pregnancy, prolonged standing, and greater body height (Evans et al. 1999; Rabe et al. 2003; Jawien et al. 2003; Lacroix et al. 2003; Fowkes et al. 2001). Subjective symptoms traditionally ascribed to CVD include pain, discomfort, heaviness and a feeling of swelling, cramps, itching, tingling and restless legs (Bergan et al. 2006; Eberhardt and Raffetto 2014). It is easily understood that patients with CVD have a reduced quality of life, which is related to symptoms and signs and to an affected physical function and mobility. Furthermore, CVDs have been associated with depression and social isolation (Bergan et al. 2006; van Korlaar et al. 2003; Onida and Davies 2016).

Early forms of CVD are described by symptomatic teleangiectases or reticular veins, whereas more advanced chronic venous disease is characterized by varicose veins, edema without or with skin changes including permanent fibrotic processes, and skin changes with healed or with active ulceration (Eberhardt and Raffetto 2014; Porter and Moneta 1995; Eklöf et al. 2004). To provide a uniform basis for reporting, diagnosing, and treating CVD, classification scales for the observed symptoms were developed. An earlier classification, used especially in German speaking countries, was developed by Widmer et al. (1978). In 1995, an international consensus conference developed the Clinical, Etiology, Anatomic, Pathophysiology (CEAP) classification scheme (Porter and Moneta 1995). The widely used "C classes" of that classification describe changes of the clinical condition and are based on venous symptoms and signs of CVD. They are generally applied in daily phlebological practice and range from category C0 to C6 (Bergan et al. 2006; Eberhardt and Raffetto 2014) (see Table). If compared to the Widmer classification, CEAP stage C3 is the nearest counterpart to Widmer Stage I. The latter describes paraplantar phlebectasis with or without edema. Widmer Stage II corresponds to CEAP C4, characterized by skin changes. The most severe classes of CVD are categorized as Widmer Stage III with healed or active venous ulceration, corresponding to CEAP C5 or C6.

The term Chronic Venous Diseases covers all forms of venous pathology which need further diagnosis and treatment, and it is frequently used for C0s to C6 patients. Chronic Venous Insufficiency (CVI) is defined as C-classes C3 to C6 including edema, skin changes and venous ulcers. However, the different clinical signs

Table: CEAP Classification of Chronic Venous Disease (Eberhardt and Raffetto 2014), adapted

Clinical classification (C)*†		Nearest Widmer stage
C0	No visible sign of venous disease	
C1	Telangiectases or reticular veins	
C2	Varicose veins	
C3	Edema	I
C4	Changes in skin and subcutaneous tissue‡	II
A	Pigmentation or eczema	
B	Lipodermatosclerosis or atrophie blanche	
C5	Healed ulcer	III
C6	Active ulcer	

*Telangiectases are < 1 mm, reticular veins are between 1 and 3 mm, and varicose veins are > 3 mm measured in the upright position; however, in the Venous Clinical Severity Score, varicose veins are considered to be > 4 mm. The Revised Venous Clinical Severity Score considers varicose veins to be ≥ 3 mm in the standing position.
†The descriptor A (asymptomatic) or S (symptomatic) is placed after the C clinical class.
‡C4 is subdivided into A and B, with B indicating higher severity of disease and having a higher risk for ulcer development.

and symptoms as well as their progression over time share venous hypertension as the common, underlying pathology (Eberhardt and Raffetto 2014). Venous hypertension is generally caused by reflux through incompetent valves in the venous system. Furthermore, venous hypertension may result from venous obstruction, caused by chronic deep vein thrombosis, venous stenosis, or by extrinsic compression, e.g. related to obesity. Muscular pump dysfunction especially of the calf muscles leads to ineffective venous blood return from the distal lower extremity and exacerbates these factors; it is frequently related to leg immobility (Bergan et al. 2006; Eberhard and Raffetto 2014; Labropoulos 2003; Kistner et al. 1996). Disturbed hemodynamics in the large veins may furthermore lead to turbulent or even reversed blood flow and thus reduce the physiological shear stress to the venous walls and valves. Locally reduced shear stress modifies the expression of numerous genes and promotes an inflammatory and thrombotic milieu (Bergan et al. 2006; Traub and Berk 1998; Berk et al. 2001; Passerini et al. 2003; Sorescu et al. 2003; Stücker et al. 2016). Studies have shown that venous valves can tolerate high venous pressure for a limited period of time. However, prolonged venous hypertension induces inflammation. Inflammation in turn leads to valve remodeling, loss of valve function, and venous reflux, as well as a restructuring of vein walls. These restructured vein walls show a disturbed balance and arrangement of collagen, elastin, and smooth muscle cells, which in turn contribute to the weakness and reduced elasticity of varicose veins (Bergan et al. 2006; Takase et al. 2004 a,b; Pascarella et al. 2005; Travers et al. 1996; Porto et al. 1998; Wali and Eid 2002; Sansilvestri-Morel et al 2001; Rabe et al. 2013).

1.2. Treatment of CVD

The pathophysiology behind CVD shows the way towards its rational therapy. Early treatment should aim at preventing venous hypertension, reflux, and inflammation. It could thus avoid or slow down the progression to higher stages (Bergan et al. 2006). In a recent consensus statement published in the Journal of the German Society of Dermatology, Stücker et al. reviewed randomised controlled clinical trials and review articles/meta-analyses as well as international therapeutic guidelines on chronic venous diseases (Stücker et al. 2016). They conclude that a rational, symptom-based therapy is possible and relies on three "therapeutic pillars": invasive therapy, compression therapy, and oral pharmacological treatment. If feasible, invasive management of symptomatic varicose veins is recommended as the treatment of first choice, including thermal ablation, chemical ablation, or surgical methods. Surgery aimed at preventing venous reflux can aid healing and prevent the recurrence of ulcers (Barwell et al. 2004; Tenbrook et al. 2004).

If this approach is not feasible due to contraindications or patients' requests, compression therapy as well as pharmacological treatment should be considered; this applies also to patients with residual or recurrent symptoms after interventional therapy. Pharmacological and compression therapy are recommended either as monotherapy or in combination. Compression therapy improves venous hemodynamics, reduces edema and skin discoloration, and improves the quality of life in patients with CVI (Bergan et al. 2006; Ibegbuna et al. 2003; Motykie et al. 1999; Andreozzi et al. 2005).
Proactive pharmacological treatment targeted to inhibit inflammation in the early stages of CVD may offer the opportunity to prevent patients developing intractable ulcers and other disease-related complications (Bergan 2006; see Fig.). However, long-term prospective studies on this aspect are still missing. Currently available drugs like standardized red vine leaf extract (AS195), standardized horse chestnut extract, and oxerutin can attenuate various elements of the inflammatory cascade, particularly the leukocyte-endothelium interactions that are important in many aspects of the disease. AS195 shows additional activity at the venular endothelial gaps, thereby inhibiting capillary leakage and edema. The mentioned drugs possess good clinical evidence regarding their symptomatic efficacy (Stücker et al. 2016; Bergan et al. 2006; Takase et al. 2004 a,b; Boisseau 2000; Eberhardt and Raffetto 2005; Nicolaides 2003; Nees et al. 2014, 2016).

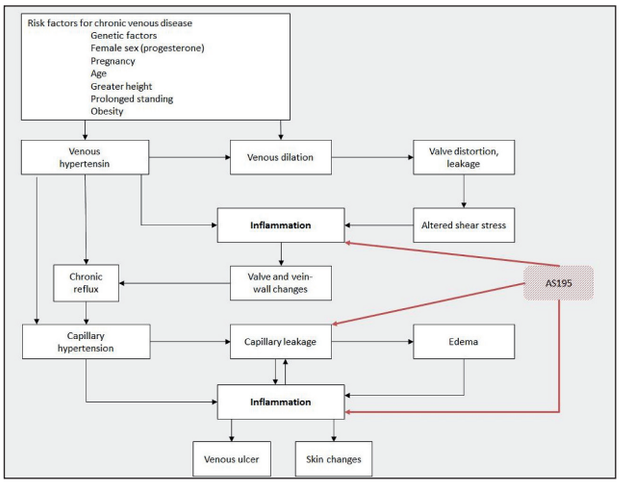

Fig.: Role of inflammation in the development of venous hypertension and CVI as well as effects of AS195 (modified from Bergan et al. 2006).

1.3. Pathophysiology of CVI

The endothelial surface constitutes a complex boundary layer catalyzer that maintains vascular blood fluidity and inhibition of thrombus formation, and in addition counteracts intravascular inflammatory processes. Due to the continuous release of anti-thrombogenic and anti-inflammatory substances from healthy endothelium, activation of leucocytes, thrombocytes and coagulation factors is prevented (van Hinsbergh 2012; Yau et al. 2015; Nees et al. 2016). The released substances are short-lived and must be replaced continuously. These events are most pronounced in the microvasculature, given its large inner vascular surface area in relation to the volume of passing blood (Busch et al. 1982). Varicose veins are overfilled and show reduced blood velocity, and thus a low contact surface related to blood volume passing. The concentration of the endothelium-derived inhibitors may thus fall below a critical level, resulting in the initiation of inflammatory and coagulatory processes by the release of inflammatory mediators, activated platelets and polymorphonuclear leucocytes (PMN) (Nees et al. 2013).
An important characteristic of the vein wall is its own extensive nutritive microvascular system — the vasa venarum (VV) (Nees et al. 2013; Kachlik et al. 2007, 2008; Lametschwandner et al. 2004).

These postcapillary venules are characterised by a highly specialized endothelium and a network of pericytes in their adventitia. The VV's drainage enters the vein they supply (Crotty 2007). Thus, stasis or reflux in this vein affects also its VV, with similar pathophysiological consequences in the VV as in the larger veins themselves.
Findings from recent studies in vascular physiology show that the pericytes in the VV and intima form the focus of vascular inflammation and trigger coagulatory processes (Kloc et al. 2015; Osterud 2010; Osterud and Bjorklid 2006; Rao and Pendurthi 2012). The original goal of these metabolic processes probably was the rapid and permanent closure of wounds and the isolation of inflammatory foci. Consequently, these processes show accelerated autocatalytic properties and the release of cytokines typical for inflammation and are accompanied by the characteristic signs of CVI: thrombophlebitis, thrombosis, remodeling of venous valves and tissues and, finally, necrotic processes in the surrounding tissue, i.e. venous ulcers (Eriksson et al. 2005).
When exposed to the inflammatory cytokines, the venules of the microvasculature open gaps between their endothelial cells at a microscopic level (Nees et al. 2003; Nees et al. 2016; Juchem et al. 2016). These gaps permit the outflow of plasma (measured as "hydraulic conductivity" in the quoted references) and thus the formation of edema, one of the early signs of CVI. At the microvasculature level, this edema disturbs blood flow, induces the expression of further inflammatory and pro-thrombogenic mediators and thus initiates a vicious circle.
To counteract these processes, specific pharmacological therapy should prevent the acceleration and spread of the inflammatory reactions in and at the vein wall as early as possible (Nees et al. 2014).

2. Red vine leaf extracts – active ingredients

The use of red vine leaf extracts in European medicine can be traced back to the Hippocratic school of the 5th and 4th century BC and later to Celsus and Galen in the 1st and 2nd century. Eventually, French winegrowers made pastes from leaves collected at the time of the grape harvest and used them for the treatment of swollen, painful legs (EMA 2010 a,b). Nowadays, modern standardized herbal medicines have been developed with these extracts, predominantly for the treatment of venous disorders like CVI.
Polyphenols are typical components of grapes and belong to the most complex substance groups occurring in plants. In red vine leaves (*Vitis vinifera*), more than 100 different compounds are found. Most of these substances belong to one of the four main groups: the flavonoids (e.g. flavonols), anthocyanes, procyanidins, or phenol-carbonic acids. All of them are closely related, as they are synthesized through a common biological pathway. The composition of these substances depends on the variety of the plant, the exposure to sunlight, the plant's stage of development, i.e. the time of the harvest, on their position in the plant, and for some components also on the stress-factors the leaves were exposed to. Especially the biosynthesis of kaempherol and quercetin, the main flavonoids in AS195, is influenced by UV-B radiation. Quercetin is one of the pharmacologically most important polyphenols in AS195/Antistax®. Compared to the marc of the grapes, vine leaves contain more than 20-fold the concentration of vasoactive polyphenols (EMA 2010a; Esperester et al. 2011; Leyva-Lopez et al. 2016; Li et al. 2016; Marki et al. 2015; Middleton and Kandaswami 1992; Monsieur and Van Snick 2006; Mouradov and Spangenberg 2014; Nees et al. 2003b). Thus, it is important to distinguish the plant's parts from which the flavonoids were sourced. To provide a consistently high quality of the extract, i.e. the final herbal drug AS195, leaves are collected and selected per narrow specifications, generally narrower than permitted by drug monographs (EMA 2010a; Ph. Franc. X. 1996). This precludes the usual supply through herb vendors, but requires the setup of own supply chains for AS195 from contracted partners submitted to quality audits.

3. Mode of action of AS195

In-vitro and *in-vivo* studies suggest that much of the clinical activity of flavonoids is related to their anti-inflammatory properties, which include inhibition of signaling through nuclear factor NF-κB (a central intracellular mediator of inflammation), the down-regulation of pro-inflammatory markers and mediators, an inhibition of prostaglandin synthesis, the scavenging of oxygen radicals, the neutralization of oxidants, the inhibition of leukocyte adhesion, activation and emigration, and the protection of the endothelium from damage by activated leukocytes (Rabe et al. 2013; Nicolaides et al. 2008; Perrin and Ramelet 2011; Gohel and Davies 2009; Nees 2003a; Rafetto 2016; Nees et al. 2016; Juchem et al. 2016).
Some flavonoids have been found to be highly effective (Mouradov and Spangenberg 2014; Middleton and Kandaswami 1992; Leyva-Lopez et al. 2016; Li et al. 2016; Flaumenhaft et al. 2015; Devi et al. 2015). Two of these are quercetin and kaempherol compounds, the main flavonoid fractions in AS195 (Antistax®). Both flavonoids accumulate in the human venous intima (Nees et al. 2016; Juchem et al. 2016; Terao et al. 2011; Papadopoulou et al. 2005). In the intima of leg veins and in the venules of their VV these substances from AS195 specifically inhibited the contractility of the cells in the vascular endothelium in the presence of inflammatory mediators. In the experimental setup, these flavonoids normalized the hydraulic conductivity of the endothelium and a filtration index for albumin. At the microscopic level, they closed the gaps between the endothelial cells and thus protected and maintained an effective barrier between prothrombogenic and proinflammatory pericytes and the blood (Nees et al. 2003a, 2014, 2016). At the same time, also the aggregability and metabolic activity of the thrombocytes and PMN were inhibited (Juchem et al. 2016). From the pharmacological point of view, flavonoids preserve the essential endothelial barrier function in the venous intima and VV.
Endothelial gaps permit the outflow of plasma and the formation of edema. On the other hand, endothelial gaps may also permit the contact of the endothelium with the prothrombotic tissue factor (TF), which is expressed by the intimal and microvascular pericytes. TF leads to the formation of prothrombinase (prothrombinase assembly PA) and thrombin, and successively to platelet activation, whereas normally the endothelium is completely free of TF and PA. In recent experiments, Nees et al. (2014, 2016) examined the antithrombotic activity of quercetin and kaempherol glucuronide regarding this prothrombogenic pathway. They found that both components almost completely counteracted the prothrombogenic activity. As quercetin and kaempherol also close the endothelial gaps, this suggests that they might stop the vicious circle initiated by inflammation.

4. Pharmacology of AS195 in patients

4.1. Improved microcirculation and oxygen supply

In a crossover study with 71 patients suffering from CVI of stages I or II (Widmer classification), Kalus et al. (2004) examined microvascular blood flow in the skin vessels of the calf with a Doppler laser. Reflected light was analysed in terms of intensity and frequency by Fourier transformation, indicating blood flow in skin vessels of different diameters. Furthermore, transcutaneous oxygen pressure (tcpO2) at the calf and changes of ankle and calf circumference were determined. Patients received Antistax® 360 mg or placebo once daily over two crossed over treatment periods of six weeks each, separated by a treatment free interval of four weeks.
Under therapy with Antistax, the velocity of microvascular blood flow almost doubled compared to placebo ($p < 0.0001$). Transcutaneous oxygen pressure was significantly higher in the Antistax group compared to the placebo group (+9 mmHg, starting from baseline values of approximately 31 mmHg). In line with the pharmacodynamics parameters, also the ankle and calf circumference decreased to a clinically relevant and statistically significant extent in the Antistax group (0.7 cm vs. placebo).
Kalus et al. (2004) observed an increase of transcutaneous oxygen pressure by AS195. The mechanism behind this induction was then further evaluated by Grau et al. (2016). Patients with microvascular disorders tend to show increased oxidative stress which limits nitric

oxide (NO) bioavailability. Human umbilical vein endothelial cells and red blood cells (RBC) were exposed to an oxidant (*tert*.-butylhydroperoxide, TBHP), to AS195, or to both substances. The study found that AS195 generally counteracted the oxidant effects of TBHP, including its effects on the deformability of RBC. AS195 increased NO synthase activation and decreased oxidative stress. Both mechanisms increase NO bioavailability, improve cell function, and may thus account for enhanced microcirculation and the increased transcutaneous oxygen pressure observed by Kalus et al. (2004).

In a small pilot study, Shevchenko et al. (2013) followed 33 patients over 15 months for recurrence of varicosis after surgery. Pharmacodynamic parameters were assessed which are known to be indicators of inflammatory processes in the microvasculature, including circulating endothelial cells as markers of endothelial desquamation as well as markers of endothelial dysfunction (VCAM-1, P-selectin, t-PA, Endothelin). Twenty-one patients received Antistax 360 mg once daily, 11 patients not receiving Antistax served as control. Intradermal and subcutaneous varicosis progressed in 14 % of patients in the Antistax group and in 91 % of controls. In the Antistax group, the number of circulating endothelial cells remained constant, whereas it increased significantly in the non-treated patients. Markers of endothelial dysfunction showed a tendency towards normalisation in the Antistax group, indicating anti-inflammatory effects as seen in non-clinical experiments (Nees et al. 2013a, 2014, 2016; Juchem et al. 2016; Middleton and Kandaswami 1992; Leyva-Lopez et al. 2016; Li et al. 2016; Flaumenhaft et al. 2015; Devi et al. 2015). Such effects were lacking in the non-treated patients; changes within and between groups were partly significant.

4.2. Pharmacokinetics

A clinical study on the pharmacokinetic properties of Antistax showed that the active polyphenols are absorbed to the blood after oral ingestion of the commercially available formulation. The study included 6 female and 6 male healthy volunteers and examined the uptake of quercetin and kaempherol glucuronides after a single dose 1800 mg Antistax over a period of 24 hours (De Mey 2006). Two peaks were observed for quercetin and kaempherol after 1 h (median: 16 and 38 ng/mL, resp.) and after 4 h (19 and 15 ng/mL, resp.). Data indicate a rapid absorption and complex pharmacokinetic properties. Plasma concentrations of both glucuronides declined within 12 h to levels at or below approx. 10 % of their peak concentrations. The $AUC_{0\text{-}24\ h}$ for quercetin and kaempherol was comparable (123 and 125 ng x h/mL, resp.).

Thus, this study proved that the active principles of AS195 reach the circulation and the proximity of the vascular target structures. The uptake of the polyphenols into the venous intima and their mode of action at this site have been examined and confirmed extensively in non-clinical studies, also in studies with human saphenous vein bypass remnants or endothelial cells and pericytes prepared from human veins (Nees et al. 2016; Juchem et al. 2016; Terao et al. 2011; Papadopoulou et al. 2005).

4.3. Clinical efficacy

4.3.1. Methods for evaluating efficacy

In the year 2000, a working group of phlebologists worked out a guideline for clinical efficacy studies with phleboactive drugs (Vanscheidt et al. 2001). Studies should follow Good Clinical Practice (GCP), be randomized, placebo-controlled and double-blind and follow-up the patient for at least three months. One primary endpoint the guideline recommends is the reduction of lower leg swelling and edema, determined by water displacement volumetry: the volume of the lower leg, immersed to a defined point, reflects the water volume replaced by the leg and flowing from the volumeter. The principle is simple, but errors in executing volumetry may negatively affect the outcome of clinical studies. Centers therefore require thorough training on the use of the volumeter. Volumetry should be performed under constant environmental, temporal, and procedural conditions (Rabe et al. 2010). If performed in a standardized setting, volumetry thus constitutes a valid scientific experiment establishing an objective effect of phleboactive drugs. However, the guideline recommends that studies should not only determine and follow-up lower leg volume, but that patients themselves should assess subjective endpoints related to quality of life, like pain and a feeling of heaviness or swelling/tension of the legs. Patients with CVD frequently complain of these symptoms and an improvement possesses direct validity and clinical relevance (Rabe et al. 2003). Thus, the pivotal studies with AS195 determined not only lower leg volume, but also subjective endpoints related to the patient's quality of life.

4.3.2. Significant reduction of edema and clinically relevant improvement of leg pain, heaviness and tension with AS195 360–720 mg (Antistax®)

The proof efficacy of Antistax in CVI is based on four pivotal randomized, double-blind, placebo-controlled studies using water volumetry. All studies used a largely identical design with the aim of proving edema reduction in addition to confirming an improvement of clinical symptoms related to quality of life.

Kiesewetter et al. (2000) performed a, multi-centre study (Kiesewetter et al. 2000). They included 260 male and female outpatients aged 25 to 75 years with stage I to stage II CVI (Widmer classification; i.e. patients without extensive trophic changes), who were not treated with compression stockings, diuretics or other drugs affecting fluid balance. Patients were randomly assigned to treatment with placebo, 360 mg or 720 mg Antistax once daily for 12 weeks, preceded and followed by a single-blind 2-week placebo treatment for baseline run-in and end-of-trial washout, respectively. Study criteria were evaluated at baseline, after 6 and 12 weeks of treatment and 2 weeks after discontinuation of treatment. The intention-to-treat analysis (N = 257) showed that the lower leg volume of the placebo patients increased by a mean of 33.7 g (displaced water mass) after 12 weeks compared to baseline. In contrast, the lower leg volume decreased in patients treated with Antistax. After 12 weeks of treatment, the difference in mean lower leg volume between the active treatment groups and the placebo group was -75.9 g and -99.9 g for the group treated with 360 and 720 mg Antistax, respectively. The changes in calf circumference showed a similar pattern: In patients treated with Antistax, both doses resulted in a clear and statistically significant reduction in circumference over time, with an advantage of up to approx. 1 cm for the higher dose, whereas circumference remained largely unchanged in placebo patients. The reductions in ankle circumference were qualitatively similar but quantitatively less marked. Subjectively, there was an improvement in key CVI symptoms (heavy and tired legs; sensation of tension; tingling; pain) at 6 weeks with all treatments, but a further improvement at week 12 was seen only in the active treatment groups. At 12 weeks, the changes compared to baseline were significantly greater ($p < 0.001$) in both active treatment groups than in the placebo group and reached a difference to placebo of approx. 2 cm on a 0–10 cm analogue scale.

Two further randomised, double-blind, placebo-controlled studies provided further findings on the beneficial effects of 360 mg Antistax per day (Esperester et al. 2013). Compared to the Kiesewetter study, the patient groups were markedly less homogeneous and included also patients with mild CVI symptoms. This may have been caused by switching the inclusion criteria from the Widmer to the CEAP classification (see Table 2), with investigators being less familiar with the latter (Rabe et al. 2010).

The first of these studies provided data of 245 patients. Fifty-six patients presented severe CVI, with edema of the legs or varicose veins classified as severe by the investigator (verbal rating scales), or with skin atrophy or eczema. In these patients, treatment with Antistax reduced lower leg volume by 66 mL more than placebo; this difference was statistically significant ($p = 0.028$) and comparable to the results of the study by Kiesewetter et al. The improvement of subjective symptoms in patients with severe CVI as well

as the investigators' and patients' assessments were in line with the results of the earlier study: leg pain, feeling of tiredness of the legs, and feeling of tension decreased by 1.0–2.6 cm vs. placebo on the 10-cm analogue scale, and the investigators' global assessment was good or very good in 57 % of patients who received Antistax vs. in 19 % of placebo patients.

The second of these studies with 360 mg/day Antistax included 202 patients overall. Again, a clinically relevant efficacy of Antistax in patients with severe CVI symptoms was demonstrated. As a *post hoc* sensitivity analysis, different classifications of severe CVI were applied, based on clinical signs and symptoms: severe leg edema or varicosis and at least moderate hyperpigmentation of the skin; CEAP grade 4a; ≥1 CVI symptom with a severity ≥5 of 10 on a visual analogue scale; leg pain ≥5 of 10 on a visual analogue scale. In these subgroups, the edema volume under Antistax decreased by 46 to 98 mL as compared to placebo. Thus, the effect was of the same order of magnitude as in the previous studies.

In both studies, this inhomogeneity obviated that significant advantages of Antistax in edema reduction *vs*. placebo were detected. Numerically, Antistax reduced lower leg volume approximately 10 to 11 mL more than placebo, which was not statistically significant. *Post hoc*, however, the wide range of patient characteristics allowed the identification of patient subgroups, which experienced a particularly pronounced reduction in the leg volume ("responders") when treated with Antistax. In both studies, patients responded best to Antistax who showed pronounced CVI at the start of treatment (*post-hoc* analyses, no confirmatory value).

The fourth randomised, double-blind study included a total of 248 patients, who were treated with Antistax 720 mg/day or with placebo (Rabe et al. 2011). Patients had stable lower leg edema (CEAP grade 3) or, in addition to leg edema, skin discolorations or venous eczema typical for CVI CEAP grade 4a. To be included in the study, patients had to demonstrate moderate to severe varicosis and leg pain with a severity of at least 3 on a 10-cm scale. After 6 and 12 weeks of treatment, the lower leg volume in the placebo group showed little change versus baseline, whereas in the Antistax group it was significantly reduced compared to placebo. At the study end after 12 weeks, treatment with Antistax had decreased the leg volume by 20±9 ml more than placebo (mean ± standard error, $p = 0.027$). The subjective symptoms of CVI improved significantly more with Antistax than with placebo: statistically significant advantages were observed for tension and pain (differences of approx. 7 mm vs. placebo, and baseline values of approx. 6 cm on the 10 cm scales). In the subjective efficacy assessment of the investigators, Antistax was also significantly superior to placebo.

4.3.3. Validation of confirmatory studies in daily clinical practice – AS195 (Antistax®) improves clinical symptoms related to CVD

Clinical studies are carefully planned scientific experiments. They are standardized for patient selection criteria, therapies, dosages, schedules and target criteria. In addition, the uncertainty to which treatment group a patient is assigned in a double-blind study is missing in everyday treatment. Therefore, it is desirable to review and validate the clinical effects of a drug, which has shown its efficacy in standardized clinical trials, also in clinical practice, i.e. in non-interventional studies.

Such studies were performed for Antistax. One of these followed the requirements of the Swiss regulatory authority and included 65 patients between 25 and 82 years of age with CVI of stage I or II according to the Widmer classification (Schäfer et al. 2003). Treatment with Antistax 360 mg once daily lasted for 6 weeks. As in the clinical studies, subjective symptoms of CVI were assessed on a 10-cm scale, the general efficacy and tolerability judgment of physician and patient on four-stage scales.

All subjective symptoms (tired, heavy legs, tense, tingling, pain) showed highly significant improvements by about 2–3 cm *versus* baseline over the study (10 cm scale; $p = 0.0001$). Approximately 80 % of physicians or patients assessed efficacy as good or satisfactory, over 90 % gave a positive assessment of tolerability.

Another non-interventional study was carried out in France (Monsieur and Van Snick 2006). This six-week study included 39 patients aged 28-72 years, approximately 80 % were women. All patients suffered from CVI of Widmer stage I or II. In this study, as in the other non-interventional study, the dose was 360 mg Antistax per day (2 x 180 mg). In addition to seriousness and pain, this study assessed leg volume by means of water displacement and calf circumference.

Over the six-week treatment with Antistax, leg volume decreased by 33 mL compared to the initial value, the calf circumference was reduced by 4.4 mm. Heaviness and leg pain improved by 2.6 cm on the 10-cm scale after 6 weeks. All changes *versus* baseline were statistically highly significant ($p < 0.001$). For all the parameters collected, a clear effect was seen already after two weeks of therapy with Antistax, indicating a rapid effect.

In a more recent study, investigators from Russia assessed the effectiveness of a 6-month administration of Antistax 360 mg/day in 62 women with CVI following long-term hormone replacement (Tsukanov and Tsukanov 2014). Calf and ankle circumference demonstrated a clear and significant reduction by 0.5–0.8 cm over time. The major part of the effect appeared in first 3 months and stabilised thereafter, despite ongoing hormone therapy. Subjective symptoms like leg heaviness, swelling at the end of the day and pain were significantly reduced after 3 months, while cramps completely resolved. These improvements were again maintained over the following 3 months. In addition, the quality of life as measured with the CIVIQ2 scale improved highly significantly by approx. 12 points (scale from 20–100).

The results of these non-interventional studies thus supplement, confirm and validate the data from the controlled clinical studies. Overall, the clinical studies in CVI addressed objective and subjective endpoints. Objective endpoints included volume of leg edema, measured by water displacement plethysmography, or malleolar and/or calf circumference, as easier-to-measure surrogates for edema. These endpoints showed a statistically significant superiority of Antistax vs. placebo overall, but the clinical relevance of reducing edema by a certain number of millilitres or of circumference by millimetres is difficult to judge. Thus, the consensus statement on the symptom-based treatment of chronic venous diseases recommends that in addition to objective parameters subjective symptoms should be evaluated, like pain and the sensation of heaviness and swelling (Stücker et al. 2016). These patient reported outcomes thus validate the objective endpoints and permit an assessment whether the patients have experienced an overall improvement in their quality of live. Not all drugs used for CVI have passed this hurdle, whereas Antistax has shown this subjective benefit in several studies (Stücker et al. 2016).

5. Expert opinion

Currently, there is no causal therapy preventing the development of CVI, i.e. no primary prophylaxis (Stücker et al. 2016). Therapy does not start when risk factors are recognized, but when clinical signs or symptoms appear. The choice of therapy should consider individual symptoms, patient preferences, but also the knowledge that the disease as such is progressive and will generally require long-term commitment to contain symptoms (Pannier 2015). Invasive procedures, compression therapy and pharmacological treatment frequently are applied successively over the course of the disease, but nevertheless they are not competitive, but complementary measures, which may be used in parallel (Stücker 2016).

New insights into the pharmacology of flavonoids allow a new perception of their role in the therapeutic armamentarium. They accumulate in the human venous intima, preserve the endothelial barrier function, and inhibit mediators at several parts of the inflammatory cascade. Thus, they reduce the outflow of plasma, i.e. the precursor of clinically apparent edema. In addition, the preserved barrier reduces the contact of plasma with extravascular prothrombotic tissue factor, the starting point of thrombophlebitis. Initiated early, flavonoids possess the potential to counteract the relevant processes driving the progredient course of CVI, thus maintaining the physiological function of the venous vascular system.

In patients with already apparent varicosis, non-pharmacological measures are indicated to normalize venous blood flow and to prevent venous stasis. Normal blood flow with physiologic shear forces is essential for the continuous release of antithrombogenic and anti-inflammatory substances (van Hinsbergh 2012; Yau et al. 2015; Nees et al. 2016). These non-pharmacological measures include ablation or surgery for effectively removing varicose veins from the circulation, but also compression therapy. Compression increases venous blood flow and shear forces. Like flavonoids at the microscopic level, it also reduces the outflow of plasma, i.e. swelling, as well as the contact of plasma with prothrombotic factors. Contrary to positive short-time data on symptoms in earlier or later stages of CVI, there is no data showing that compression might stop or reverse CVI at the stages where it is commonly used. Not all patients are suitable candidates for using compression, like patients with skin disorders or with peripheral arterial diseases. Furthermore, patients may be unwilling or not able to put on compression stockings. Ineptitude or adiposity-related immobility may preclude their use in many patients (Stücker and Larenz 2015). Flavonoids as contained in AS195 are included in consensus recommendations as a suitable monotherapy in CVI stages I to II (corresponding to C1 to C4) or in combination with compression (Stücker et al. 2016). AS195 showed a pronounced effect not only on objective endpoints, but also on clinically relevant symptoms of CVI and patient-reported outcomes. Its efficacy was larger in patients with more pronounced symptoms and it possesses a favourable risk profile with no known interactions. This makes AS195 suitable for long-term therapy – vital in a disease progressing without therapy. Pharmacological therapy with flavonoids closes a therapeutic gap especially in adipose patients, who frequently cannot use compression, but require CVI therapy most (Stücker and Larenz 2015).

6. Clinical safety

The safety results obtained from the clinical studies showed that the oral use of Antistax was well tolerated by most patients. No drug-related serious adverse events were reported during the clinical trials. Non-serious adverse events suspected by the investigators as related to Antistax were generally confined to gastrointestinal disorders and hypersensitivity reactions. Events reported were mild to moderate and transient. Antistax has been marketed since 1971 and its favourable safety profile is underlined by long-term experience and the quantitative aspects of its use (EMA 2010a,b). This profile is reflected by the Summary of Product Characteristics of "Antistax® extra Venentabletten", containing 360 mg AS195. It states the following side effects: occasional nausea, gastric pain and other gastrointestinal complaints; occasional itching exanthema; and urticaria, hypersensitivity reactions, and headache of unknown frequency (SmPC 08/2015).

7. Conclusion

The pharmacological effects of red vine leaf extract have been examined in various non-clinical experiments with different extracts (EMA 2010i). The EMA Assessment Report on *Vitis vinifera* L. *folium* recognizes its anti-inflammatory and anti-edematous effects derived from reducing hydraulic conductivity of endothelial monolayers, scavenging properties against radical oxygen species, and anti-lipoperoxidant activity. Recent research has additionally underlined the importance of the vascular endothelium as the drug's target for inducing anti-inflammatory and anti-thrombogenic substances. These are continuously released to the blood stream and prevent the activation of leucocytes, thrombocytes and coagulation factors.

Pharmacologic studies performed with AS195 suggest that the drug, if used in early CVI stages without permanent tissue damage, might stop the vicious circle including venous hypertension, inflammation, and thrombogenic factors, which drive the disease through its clinical stages. In later stages, when tissue damage is irreversible, AS195 may still improve symptoms, and CVI treatment guidelines suggest that pharmacological therapy may also be combined with compression to achieve the desired clinical effect.

Translating non-clinical pharmacological and especially vascular effects, Antistax has shown clinically relevant efficacy on edema (leg volume) as well as, more relevant for the patients, on subjective symptoms of CVI in recent clinical studies. Subjective endpoints are important, as they show the influence of CVI on the patients' quality of life. As shown by the "Bonner Venenstudie", the symptoms reported by most patients were the symptoms which improved most in the clinical studies with AS195. Furthermore, subjective endpoints validate the clinical relevance of the objective criteria. In addition to the choice of appropriate endpoints, it is relevant that the efficacy of Antistax – unlike that of some other phleboactive drugs – has been confirmed in several independent studies. Furthermore, non-interventional studies have shown results in line with the interventional, confirmatory ones. Interventional studies constitute a strictly experimental setting, including patient groups with characteristics not reflecting everyday practice. Thus, these studies require a validation in real life, which Antistax has passed. The clinical as well as non-interventional studies with Antistax showed its efficacy in diverse patient groups with both mild and with severe CVI. This ensures that study results are applicable to daily practice.

Herbal medicines show a complexity of identity and manufacturing comparable to biotechnologically produced drugs. The method of extracting influences the final composition of the medicine (EMA 2010a). It requires standardized, reproducible and well-described procedures as well as dedicated analytics to control and check the process, the identity of the final product, and its consistency over batches (Zündorf and Dingermann 2014). Similar to the complex development of biosimilar medicines, there is no easy way to develop a generic herbal medicine. Its unique active ingredients and efficacy profile precludes the extrapolation of data to other extracts without extensive analytical and clinical data (Zündorf 2014; Stücker et al. 2016). The nonclinical and clinical data on AS195/Antistax thus confirm its own mode of action and efficacy, but do not provide such proof for other 'similar' extracts.

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