Reparil®
Product Monograph
INDEX

p.7  1. Introduction

p.9  2. Escin: the active principle and its pharmaceutical developments

p.11 3. Chemistry Drug extraction and pharmaceutical formulations

p.13 4. Pharmacology

p.17 5. Pharmacokinetics

p.19 6. Toxicology

Clinical use

p.21 7. Oral administration Reparil® dragées

p.23 8. Topical applications Reparil® Gel

p.29 9. References
CHAPTER 1
Introduction

The chemical and pharmacological Madaus Research Laboratories (Cologne, Germany) first succeeded in showing that the saponin named “escin” was the active pharmacological compound in horse chestnut (*Aesculus hippocastanum L.*) seeds. These findings were also considered an important technological advancement for the acquired know-how in isolation of a pure, chemically well-defined, active principle from a medicinal plant. (Lorenz et al. 1960; Patt et al. 1960).

The subsequent technological development of the industrial scale-up of therapeutic escin preparations (intravenous, oral and topical formulations) has been an important breakthrough, leading to worldwide launch of escin-based drugs under the trademark REPARIL®.
The specific effect of horse chestnut extract on the vascular wall tissue had been generally reported as protection against capillary vessels fragility. However, it had not yet been possible to clearly define the active principle from a chemical and pharmacological point of view. Moreover, the coumarine derivative esculin, sometimes considered to be the main responsible for escin pharmacodynamic properties (notwithstanding lack of preclinical data showing a consistent pharmacological effect of esculin itself), is actually not found in the seeds of horse chestnut. The quick-onset pharmacological effects on vascular wall tissue, observed with horse chestnut raw extract, were indeed not shown–up to then–for any of the already isolated and chemically defined molecules. It was then found, by Madaus, that the horse chestnut seeds, and their well-standardised extracts, contained a fraction consisting in a mixture of triterpenic saponins, which could be chemically isolated without denaturation, for instance by means of cationic exchange (Winkler et al. 1960). Such a component, in its original formulation, was named escin and subsequently it was also named β-escin. In order to ensure its optimal extraction, a quick desiccation of the seeds was needed immediately after harvesting; this was also preventing the enzymatic degradation (favoured by the high water content of seeds, reaching up to 40% of their weight) of its original glicosidic content.
CHAPTER 3
Chemistry

The complex of triterpenic glucosides internationally known under the name of escin is characterised by the presence of penta-cyclic triterpenic sapogenins protoescigenin and barringtonenol. The glicosidic component linked to the 3-OH residue is a trisaccharoid (glucose, xilose, galactose). Domains C21 and C22 are esterified with an organic acid, e.g. angelic, tiglinic or acetic acid (Hoppe et al. 1968; Wulff et al. 1969). Such a complex steric and molecular configuration is the reason for non succeeding in achievement of the chemical synthesis of the active compound. The main isomers are represented by β-escin esterified in C21 and C22 (relatively water-insoluble, isolated by Madaus as the basis of pharmaceutical preparations of Reparil®), and kryptoescin esterified with acetic acid, easily hydrosoluble but much less active. The molecular formula of escin is C55H86O24, its M.W. 1131,27

Molecular structures

<table>
<thead>
<tr>
<th>Protoescigenin</th>
<th>R1</th>
<th>R2</th>
<th>GLucose</th>
<th>Galactose</th>
<th>Xylose</th>
<th>R6</th>
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<tr>
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<td>(1)</td>
<td>H</td>
<td>CH2OH</td>
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<td>H</td>
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</tbody>
</table>
Pharmaceutical preparation and development of the formulations

The raw escin 2.5% is extracted with metanol/water from a purified, concentrated homogenised preparation of horse chestnut seeds. The raw escin is subsequently further purified and crystallised as pure escin.

Dragées with orally absorbable escin

Crystallised escin has a <0.01% solubility in water and, consequently, a low bioavailability after oral administration. For this reason, Madaus developed a specific production technology aimed at isolating an escin formulation suitable for oral administration. Comparative technical and biological analyses, conducted also in collaboration with Prof. Schulman, a Surfaces Chemist and Physicist of Columbia University, NY, have shown the advantages of transforming into the amorphous state the crystalline structure of escin, with achievement of water solubility at 2% (Rosoff et al. 1967). With a Madaus-patented process of industrial grinding of pure crystallised escin in an agata mortar, amorphous escin was produced, to be used as the active principle of gastroresistant dragées of Reparil® 20 mg and 40 mg, the first oral form of absorbable escin. Clinical indications were: post-traumatic cephalgia, post-thrombotic syndrome and static oedema.

In the ‘70s, the processing by grinding was substituted by the more efficient spray-drying process (Madaus 1975).

Escin-gel, for transdermic action

Between 1960 and 1980, several companies tried to develop low-molecular weight compounds with heparin-like activity, for topical treatments of thrombosis and haematoma. For this purpose, Madaus had developed sodium escin-polysulphate (APS), by means of a polysulphation of sodium escinate. APS showed antithrombotic and fibrinolytic properties and, with a molecular weight of about 1/10 with respect to heparin, was much better absorbed into the cutaneous tissues.

Reparil® Gel, a pharmaceutical preparation with 1% APS, 1%, escin and 5% diethylaminosalicylate (DEAS) in a iso-propylalcohol-based gel formulation was therefore developed, with clinical indications for treatment of localised oedema, blunt lesions, haematoma, superficial thrombophlebitis and vertebral painful syndrome. In the mid-‘80s, new studies of clinical pharmacology showed that APS was not providing a determinant additional contribution to the therapeutic action of the core-association escin/DEAS. For this reason, APS was subsequently removed from the formulation.
CHAPTER 4
Pharmacology

The inflammation process is ultimately associated with an increased permeability of blood vessels, with fluids extravasation as well as with activation of specialised mediators of inflammation which may either recruit leukocytes to limit tissue damages or, depending on the time interval from the initial lesion, favour cell debridement prior to the tissue granulation process.

In view of these physio-pathologic phases, escin has been extensively studied in animal models on several parameters of the inflammatory process.

**Rat leg oedema model and increase of lymphatic influx in rabbit**

Escin was injected intravenously 16 hours before induction of leg oedema by subcutaneous injection of irritant agents such as ovalbumine, carragenin, histamine or serotonin. Results showed that escin, at dosages of 0,3 mg/Kg and 0,4 mg/Kg, was effective in reducing up to 30% the size of oedemas, with respect to controls (oedemas induced and measured the day before in the contralateral leg, Vogel et al. 1970).

In the anaesthetised rabbit, infusion with prostaglandin E2 immediately induces an increase in the lymphatic flux, as measured by draining lymphatic vessels content corresponding to the affected hind leg. The concomitant infusion with escin (0,3 mg/kg) reduced by 30% the lymphatic flux increase (Rothkopf et al. 1976).

These pharmacological data demonstrated that the mechanism of action of escin is based on the counteraction of the pathological increases in the vascular permeability.

**Vascular permeability and fluids extravasation**

The efficacy of escin in reducing the vascular permeability -experimentally increased in the cutaneous and connective tissues- has also been demonstrated in the following animal models. Evans blue dye was injected in rabbits and a local inflammation was subsequently induced in the abdominal skin surface by application, after shaving, of chloroform-soaked tapes. The resulting change in color, expression of the artificially induced vascular damage, was measured by means of a reflectometer. Escin, at dosage ranges of 0,5 – 2 mg/Kg (intravenous administration) and 10-40 mg/kg (oral administration) was capable to reduce capillary permeability in a dose-dependent way (Hampel et al. 1970).

These preclinical experiments are examples which document the specific properties of escin in reducing vessels permeability in an
inflamed tissue, thereby inhibiting oedema formation. Other data on the vessel-protection activity of escin resulted, for example, from the following ex-vivo experiments. In just-isolated umbilical veins, hypoxic conditions elicit an increase in neutrophil adhesiveness. Following a 2-6 hours incubation of umbilical vein segments with escin at concentrations ranging from 100 to 750 µg/ml, the hypoxia-induced damages were prevented, in comparison with the related control groups. Besides these inflammation markers, the hypoxia-induced superoxyde anions and leukotrien B4 formation was almost completely prevented (Bougelet et al. 1988).

**Human endothelial cells**

In a model of hypoxia, human endothelial cells (extracted from umbilical vein) have been shown to undergo a marked loss of cellular ATP, together with a dramatic increase in the activation of phospholipase A2. Such changes could be prevented, in a dose-dependent way, by preincubation with escin (Arnould et al. 1996). In a second set of experiments, human endothelial cells (taken from umbilical cord) have been exposed to a model of hypoxia by incubation in CoCl2. As critical factors of endothelial cells viability and functionality during exposure to the toxic microenvironment induced in the hypoxic model, the following parameters were assessed: expression of cell-adhesion molecules, reduction of platelet-endothelial cells adhesion molecules and alteration in cytoskeleton molecules patterns. As an additional model of inflammation, the IL-6 production following E. coli –LPS damage on the human endothelial cells was also assessed. All damages affecting the endothelial cells were reduced, in a dose-dependent way, by escin pre-treatment in comparison with respective controls, as shown on the basis of biochemical markers and also fluorescent microscopy immunostaining (Monstopoli et al. 2007). As an example of escin protection of the critical intercellular adhesion molecule PECAM1, the figure (c) shows that hypoxia (CoCl2) disrupts the normal abundancy and distribution of this molecule (immunostaining with antibody anti-PECAM1). Escin (d) prevents such a damage: this could explain the reason why escin is effective in preventing the pathological increase in blood vessels permeability (i.e., by preventing loss of endothelial cells intercellular junctions).
As an overall summary, escin demonstrates, in several pharmacological studies both *in vitro* and *in vivo*, its capability to inhibit the following physiopathological processes leading to oedema formation: fluid extravasation, tissue swelling, formation of leukotrienes and other inflammation mediators. Due to the general shortage of pharmacological models sufficiently predictive of the human inflammatory process, it has always been necessary to study the efficacy of escin on the blood vessels permeability in selective, discrete models.

**Schematic representation of fluids exchange within the capillary vessels micro environment**

*Physiological fluids exchange*

*Capillary vessels increase and oedema formation*
CHAPTER 5
Pharmacokinetics

Since it is not possible to synthesize escin, the radioactive 3H-compound for the pharmacokinetic studies has been produced starting from the natural one. The main limitations of such an instable radiolabelling were well known and, for this reason, additional thin layer chromatography was also performed on samples in order to clearly identify the labelled compounds, thereby excluding occurrence of volatile radioactivity.

On the basis of assays carried out on mice and rats and, in part, also on pigs, the following properties were highlighted:

**Oral administration**

Following oral administration with a gastric probe, 13-16% of the dose is absorbed (non volatile radioactivity), with a t\text{max} achieved about 4 hours after administration and with about 75% subsequent bile excretion.

Thin layer chromatographies on blood, bile and urine samples showed that, following oral administration, escin was undergoing major metabolisation. For example, in mice, only 1/3 to 1/4 of non volatile radioactivity measured in blood was related to native escin; in rats only 1/6 to 1/7 of the absorbed dose was excreted as unmodified escin (Lang et al. 1977; Lang 1981).

**Topical application**

For transcutaneous absorption studies, the 3H-escin was applied on dorsal and ventral skin in mice, rats, guinea pigs and pigs, and the total radioactivity, the non-volatile radioactivity and the thin-layer chromatographies were assessed, at different time-courses, in several tissues and organs. Following the topical administration, excretion in bile and urine was always confirmed (Lang 1974; Lang 1977).

In all animal species, the total clearance (on the basis of excretion over 2 days) was rather low, in 1-3% range. High escin concentrations were however found under the site of application, even in deeper muscle structures. In pigs, for instance, non volatile radioactivity measured 24 hours following topical application was 50 times higher, in subcutaneous tissues and muscles under the site of application, with respect to the blood level.

Maximal concentration in skin and subcutaneous tissue was reached about 6 hours after application. Subsequent time-course showed reduction in concentration in these tissues, due to progressive diffusion of escin, with relative increase in concentration
in the underlying muscle structures. The different experiments have therefore clearly demonstrated that escin is absorbed through the skin: high, therapeutically effective concentrations are therefore reached under the site of application, whereas in peripheric lymphatic and blood circulations, as well as in internal organs, there is no detectable escin diffusion.
CHAPTER 6
Toxicology

Escin has been widely tested, during its pharmaceutical development, from the toxicological point of view.

**Orally administered amorphous escin**

Oral toxicity of amorphous escin, with a LD50 at 100-800 mg/kg, was much lower than parenteral toxicity, also as a consequence of a limited gastrointestinal absorption. Chronic toxicity studies in animals did not show any pathological alterations in organs. Embryotoxicity and theratogenesis studies showed that escin does not induce malformations. Mutagenic potential was also tested and excluded both *in vitro* and *in vivo*.

**Topical administration of escin**

Subchronic and chronic cutaneous tolerability researches were conducted in rats, guinea pigs, rabbits and pigs, in view of a development of escin as a gel for topical application. With the highest dosages, a transient reddening of the skin at site of application, as well as some aspecific cutaneous reactions, were observed; such reactions however occurred also with application of the “placebo” gel (only containing the excipients). No systemic effect ascribable to escin was ever observed.

*In summary, it can be confirmed that escin, either orally administered as Reparil® Dragées or topically applied as Reparil® Gel, does not show any toxicological problem.*

**Clinical use**

Both oral and topical forms of escin have undergone a wide clinical investigation in their different field of application; it has always been quite difficult to achieve consistent demonstrations of clinical efficacy, particularly in view of the new clinical and statistical methodologies. Several examples, taken from a large variety of clinical researches, will be here summarised for either oral (Reparil® Dragées) and topical (Reparil® Gel) escin administration.
CHAPTER 7
Oral administration (Reparil® Dragées)

Traumatology

With the following two studies, the clinical efficacy of Reparil® Dragées in inhibiting oedema formation in orthopaedic patients has been demonstrated.

In a clinical study published in 1977, 295 patients with post-operative or post-traumatic soft tissue swellings were recruited in a controlled double-blind 3-arms trial (Tsuyama et al.1977). Treatments were: Reparil® Dragées 20 mg t.i.d. or placebo or the fibrinolitic drug Serratiapeptidase (Danzen® Takeda Pharma). Contusion, distorsion, fracture traumas were included in the diagnoses, and the treatment was protracted over a period of 14 days. The reduction of the tissue swellings was assessed by measuring their circumference and diameter at treatment days 0, 2, 3, 5, 7 and 14. Moreover, on a 5-points scale, improvements of oedema, pain, local inflammation, pain at pressure and general conditions were also registered. The reduction of oedema was much higher in the group treated with Reparil® Dragées, in comparison with the other 2 groups. A statistical significance p<0.005 was already achieved at an early stage, at day 3, even in comparison with patients treated with Serratiapeptidase. This results highlight the specific antiexudative effectiveness of escin, with respect to other antiphlogistic agents, in early prevention of fluids extravasation within the lesioned tissues.

As regards the general improvement of subjective evaluation criteria, at the end of the 14-day treatment Reparil® Dragées provided significantly better scoring.

Another placebo-controlled clinical trial recruited 100 orthopaedic patients, half of them with post-plastering oedema and the remaining with reactive post-traumatic oedema (Hernandez Carbajal 1971). 40 patients, in either group, were treated with Reparil® Dragées (2 x 20 mg t.i.d.); 10 patients in each group were treated with the related placebo. The circumference of leg or arms, the plethysmographic measurements (water volume increases), the presence/absence of spontaneous pain were evaluated. Volume measurements and circumference were taken weekly for 5 weeks; the pain intensity was registered by means of an analogic scale. A significant improvement, particularly in the first 3 weeks of treatment, was observed with both types of oedema after treatment with Reparil® Dragées. The mean difference in water volume change, after 4 weeks of treatments, was -750 ml with Reparil® Dragées and -450 ml with placebo.
Phlebology

80 patients with saphenous vein varices (degree II and III) were included in a placebo-controlled randomised clinical trial. They were treated with Reparil® Dragées (2 x 20 mg t.i.d.) or placebo. At days 0, 14 and 28 the venous filling state and the venous filling time were measured by means of light-reflection rheography; subjective parameters such as pain, swelling, stretching sensation were also taken into account (Hoffman 1988).

The basal values of filling times were, in both groups, well below the physiological value of about 25 seconds. At day 14, the filling time in the Reparil® Dragées-treated group had increased to 31 seconds, from the initial 13 seconds. On the contrary, no significant difference was observed in the placebo group. The difference in results between the two groups was clinically relevant and highly statistically significant (p<0,0001).

The results on the filling status were similar. As regards the symptomatic criteria, the improvements observed with 14 days of treatment with Reparil® Dragées were quite relevant, whereas no significant change had occurred in patients treated with placebo.

In another placebo-controlled clinical trial, 195 female patients with venous insufficiency in pelvic area and in legs were included (Vazquez Camacho 1975). The daily treatment, protracted over 3 months, was Reparil® Dragées (2 x 20 mg t.i.d.) or placebo. Efficacy was evaluated on the basis of an observer-blind assessment of symptoms improvement, classified as “very good”, “good”, “moderate”, “bad”.

The results of the 148 patients, who finished the treatment and were finally evaluated, showed that in the Reparil® Dragées-treated group there was an 85% increase in the “very good” and “good” scoring, in contrast with a 12% increase observed in the placebo-treated group.

Even if phlebopathies are traditionally regarded as the primary indication for horse chestnut derivatives, and hence particularly for escin, it is important not to forget that escin has shown a specific tropism for the endothelial cells, where its efficacy in contrasting the vascular wall damage and the oedema formation is more evident. For this reason, even better clinical results may be expected when, by means of a topical application, an higher concentration of escin on the injured body area can be reached.

In summary, the oral treatment with Reparil® Dragées proved to be effective in different pathologic conditions (post-traumatic, post-operative, phlebopathic etc.) which are associated with local and painful oedema formation.
CHAPTER 8
Topical administration (Reparil® Gel)
In view of its well documented antiexudative properties, as demonstrated in several pharmacological studies, escin should be directly applied, with topical administration, in case of blunt acute tissue injuries; the endothelial cells damage represents in fact the most specific and direct target for the pharmacological action of escin. The quick-onset action of escin in protecting the loss of structure in endothelial cells (e.g. loss of intercellular adhesion molecules like PECAM1, or alteration in its structural cytoskeleton proteins, see for reference Montopoli et al. 2007) represents the most likely mechanism of action, by which escin not only reduces initial fluids extravasation but also attenuates the humoral and cellular inflammatory cascade, thus effectively preventing or reducing oedema formation.

Traumatology
Legs are most frequently affected in accidents occurring in sport activities or at work or even in the domestic activities. Tissue swellings and oedema formations are also frequently observed following diagnostic or therapeutic surgical interventions. The typical sport lesions are represented by contusions, distortions, stretchings and crushings with or without consequent hematoma formation. Following mechanical lesion of blood vessels in the lesioned area, and the consequent increase in vascular permeability, inflammatory mediators (e.g. prostaglandins, bradykinin and histamine) -locally produced- usually start the classical inflammatory process. From the clinical perspective, this phase is characterised by presence of swelling and pain, by functional limitations and reduced mobility.

The model of human clinical pharmacology: subinjection hematoma
The so-called subinjection hematoma has been developed in collaboration with specialists of Sport Medicine (Giannetti and Pabst) with the objective to create an in vivo model, for clinical pharmacology researches, characterised by good correlation with the physiopathological evolution of a post-traumatic lesion with hematoma formation, swelling and pain (Pabst et al. 1986). This model proved to be effective in quantifying and comparing efficacy of topical pharmaceutical preparations indicated for those type of lesions. The specific advantage of the model relates to the possibility
to induce well-standardised local lesions by means of subcutaneous injections of the patient’s own blood; moreover, all patients can be treated also with the placebo, thus reducing the inter-patients variability.

In this model it was possible to assess repetitively and for many days, with algometric and colorimetric parameters, the specific symptomatologic criteria such as pain or change in color in the affected skin area.

By using this human clinical pharmacology model, it has been possible to conduct many comparative efficacity studies.

Reparil® Gel has been compared with placebo in a double blind randomised clinical trial in patients with blunt sport lesions (Rothhaar et al 1982), mostly affecting ankle and knee. The topical treatment was protracted, as an average, for 9 days; the results observed on the 81 patients were as follows.

The mobility of the affected limb - with respect to the contralateral healthy one - increased from 50 to 89% with Reparil® Gel and from 50% to 67% with placebo. The circumferences, as measured in legs with swellings, did not show any change after placebo whereas, with Reparil® Gel, they almost went back to the value of the contralateral unaffected one.

In general, effectiveness of the therapy with Reparil® Gel was reported to be “very good” or “good”, in contrast to “weak” or “non effective” with placebo.

Another double-blind controlled clinical trial in 305 patients with “early-stage” contusions and distortions from sport injuries had demonstrated that the Madaus 2-component formulation (escin 1% + diethylaminesalicylate 5%) performed significantly better, against pain at pressure and spontaneous pain, with respect to similar mono-component gels (Hess et al 1996).

A double-blind comparative clinical pharmacology study of Reparil® Gel vs. Voltaren® gel in 140 patients with subinjection hematoma has shown that pain at pressure was restored to the normal threshold (value before induction of the hematoma) significantly quicker with Reparil® Gel (Bonnekoh et al. 1992).
Phlebology

The typical, progressive alterations which are observed in the skin and subcutaneous tissue, mostly in lower leg segments of patients with chronic venous insufficiency, are caused by hypoxia due to venous blood stasis. This pathological condition is characterised by the presence of varices and by chronic, painful increase in local venous pressure. The hypoxic damage to the endothelial cells, together with the increased intravenous pressure, contributes to create a chronic condition of fluids extravasation, which is clinically observed as lower leg oedema. The inflammatory cascade aggravates the biochemical unbalance, leading to further fluids extravasation.

To demonstrate clinical efficacy of a tested drug, in this complex pathological condition, the above described human clinical pharmacology model of subinjection hematoma is particularly appropriate (Pabst et al. 1986).

A randomised, double-blind, placebo-controlled clinical trial was conducted with Reparil® Gel in 50 patients with saphenous vein varices (stages II and III). Treatment was applied b.i.d. for 3 (Hoffmann et al 1988). The subjective symptoms improved in both groups, probably due to the cooling effect of the common isopropyl alcohol base. The essential parameter to evaluate the real clinical benefit was the venous filling time of the cutaneous plexus, assessed by means of light-reflection rheography. Reparil® Gel-treated group showed an improvement (=prolongation) of the venous filling time, expression of the restored venous tone, whereas the placebo-treated group did not show any improvement.
Mode of action of escin and Reparil® Gel

The synergic action of the two components of Reparil® Gel: escin and diethylamine salicylate (DEAS).
CHAPTER 9
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