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Endothelium protectant and contractile effects of the antivaricose principle escin in rat aorta

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Abstract

The triterpene saponin escin is the active component of the extract of seeds of *Aesculus hippocastanum* used in the treatment of chronic venous insufficiency. Escin is also used experimentally to increase membrane permeability in isolated cells. Since endothelial dysfunction is postulated to be involved in venous insufficiency, the possible endothelium-protectant effect of escin was explored in rat aortic rings, a model widely used to study such effects with cardiovascular agents. Escin enhanced endothelium-dependent relaxation induced by acetylcholine when such relaxation had been reduced by exposure to the superoxide ion generator pyrogallol. This effect was attributed to enhanced nitric oxide production by endothelial nitric oxide synthase, a calcium-dependent enzyme, activated by the increased endothelial cell permeability to calcium induced by escin. Another effect of escin thought to contribute to its therapeutic activity is its ability to produce venous contraction. The compound was found to induce concentration-related contraction also in rat aortic rings. This response was partially inhibited by removal of the endothelium or by preincubation with indomethacin, and was completely abolished by incubation in a calcium-free perfusion fluid. Contraction was considered to be due mainly to the aforementioned effect on calcium permeability, with some mediation by release of endothelial vasoconstrictor prostanoids. It was concluded that, in rat aorta, escin possesses an endothelium-protectant action and a direct contractile effect. The former could contribute to its beneficial effect in the treatment of venous insufficiency, while the latter could constitute a limiting side effect.

Keywords: Escin; *Aesculus hippocastanum*; Endothelial dysfunction; Nitric oxide synthase; Calcium influx

1. Introduction

Escin is the major active principle of the seeds of *Aesculus hippocastanum*, the horse chestnut tree. Extracts of the seeds are widely used in the treatment of chronic venous insufficiency, a condition characterized by the appearance of varicose veins. Hippocastanum extracts have been subjected to detailed pharmacological studies, which provide the basis for such use (Sirtori, 2001), as well as to a number of clinical trials confirming their therapeutic activity (Pittler and Ernst, 1998; Sirtori, 2001). The antivaricose effect of the extract is attributed to its venoconstrictor, antiedematous, antiinflammatory and antioxidant properties (Guillaume and Padioleau, 1994).

Escin is a natural mixture of triterpene saponins; the aglycones are derivatives of protoescigenin, acylated by acetic acid at position 22 and by either angelic or tiglic acids at position 21 (Fig. 1). It exists in two forms, α and β, that can be distinguished by melting point, specific rotation, hemolytic index and solubility in water. The β form appears to be the active component of the mixture and was used throughout the present study.

Although venous valvular malfunction has traditionally been regarded as a major contributing factor in the physiopathology of chronic venous insufficiency, Michiels et al. (1993a) postulate that venous endothelial hypoxia consecutive to blood stasis initiates a cascade of events leading to the disorganization of the vessel wall, typical of chronic venous insufficiency. According to this hypothesis, hypoxia determines a decrease in ATP availability which activates endothelial cells to produce the proinflammatory molecule platelet-activating factor and to bind polymorphonuclear neutrophils. These responses lead to

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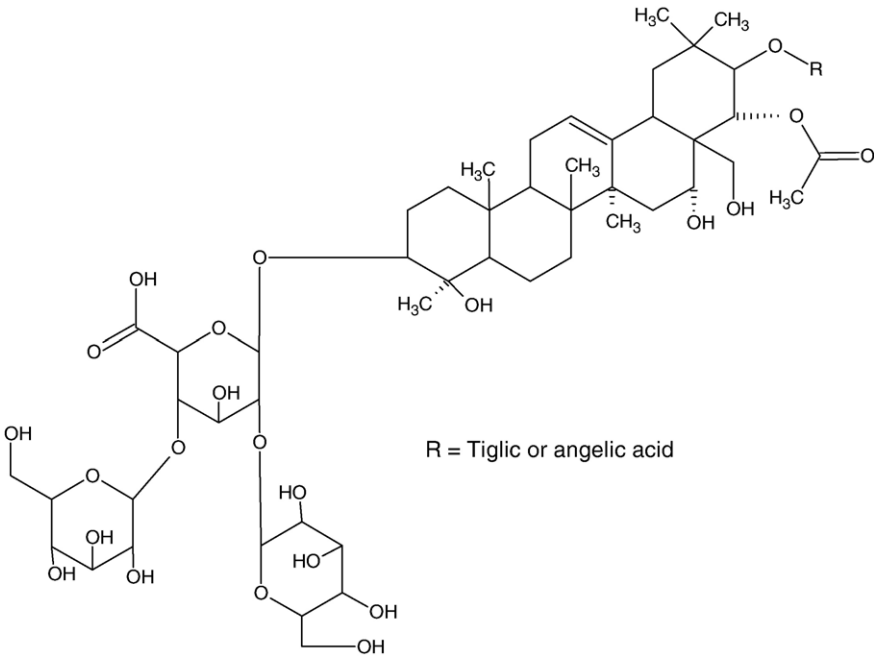

Fig. 1. Structural formula of β-escin.

infiltration of endothelial cells in the media of veins, thus affecting smooth muscle cells and connective tissue. It has been hypothesized that escin, through the pharmacological actions mentioned above, could interfere at several steps with this process (Sirtori, 2001). Accordingly, in human umbilical vein endothelial cells, the drug specifically reduces the hypoxia-mediated decrease in ATP production, as well as the increase in cell adhesiveness to neutrophils (Arnould et al., 1996).

The purported role of the endothelium in venous insufficiency can be considered as a modality of the association of arterial endothelial dysfunction, and the resultant deficiency in nitric oxide production, with cardiovascular disease (Cines et al., 1998; Giles, 2006). Reduced endothelium-dependent vascular relaxation, such as that elicited by acetylcholine, is considered a hallmark of endothelial dysfunction. The finding that a number of cardiovascular drugs, including modulators of the renin–angiotensin system, calcium channel blockers, β-blockers and statins, improve this response, has contributed to identify the endothelium as a pharmacological target for reducing cardiovascular risk factors (Giles, 2006). The present study was therefore carried out in rat aortic rings to determine whether escin behaves as other endothelium-protecting agents in influencing arterial relaxation induced by acetylcholine.

Another aspect of escin action evaluated in the present work was its vascular smooth muscle contracting effect. Venoconstriction has been confirmed in various experimental models (Guillaume and Padioleau, 1994), including human varicose veins (Annoni et al., 1979; Brunner et al., 2001) and attributed to release of prostaglandins (Berti et al., 1977). Experiments were conducted in the abovementioned model to determine whether escin also contracts arterial smooth muscle, a possibility hitherto apparently unexplored.

2. Material and methods

2.1. Animals

Adult male Wistar rats weighing between 200 and 300 g, raised in the animal facilities of the School of Medicine, Universidad Nacional Autónoma de México, were used in all experiments. Rats were kept in animal rooms illuminated from 07:00 to 19:00 (12-h light/12-h dark cycles) and maintained at 21–23 °C. The animals had free access to food pellets (Purina Chow, St Louis, MO) and tap water. Rats were brought daily to the laboratory for the experiments, which were conducted in accordance with the Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996) and were approved by the local Ethics Committee.

2.2. Relaxation and contraction experiments

Rats were anesthetized with sodium pentobarbital, 20 mg i.p. total dose, and subsequently sacrificed by cervical dislocation. The thoracic aorta was removed and segments 0.5 cm long were obtained and suspended in jacketed 20-mL organ chambers between two nickel–chromium wire hooks. One of the hooks was fastened to the bottom of the chamber and the other was attached to a Grass FT03 force transducer, which was connected in turn to a Grass Model 79 polygraph (Grass Instrument Division, Astro-Med, West Warwick, RI). The baths contained Krebs–Henseleit solution of the following composition: 127 mM NaCl; 4.7 mM KCl; 1.1 mM $MgSO_4$; 1.2 mM KH_2PO_4; 2.5 mM $CaCl_2$; 25 mM $NaHCO_3$; 11 mM glucose; and 0.02 mM EDTA. The solution was kept at 37 °C and bubbled with a mixture of 95% O_2 and 5% CO_2; pH was 7.4. The