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Research Article

In vitro effects of alcesefoliside and mauritianin, isolated from *Astragalus monspessulanus* subsp. *monspessulanus*, on the contractility of *a. basilaris*

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Abstract

Flavonoids are one of the most popular antioxidants in plants. Their varied pharmacological activities are important for these compounds in order to add and to complement conventional therapy. Alcesefoliside and mauritianin are rare flavonol triglycosides, isolated from the overground part of *A. monspessulanus* subsp. *monspessulanus*. The aim of the study was to examine the *in vitro* effects of the isolated flavonoids on the contractility of *a. basilaris*. Administered alone, at concentration 10 μM, alcesefoliside and mauritianin did not influence the vascular tone of segment of *a. basilaris*. The combination of both compounds, at 10 μM, revealed an increased response of the vascular tone of *a. basilaris*. These effects of the flavonoids suggest their possible beneficial effect as further candidates in the complex therapy of neurodegenerative disease.

Keywords

Astragalus, flavonoids, vasoconstriction, vasodilatation, smooth muscle

Introduction

Flavonoids are a group of widespread secondary metabolites, known for their advantageous health effects. They can be found in plants and some beverages (Panche et al. 2016). Flavonoids represent the largest group of phenolic compounds occurring in *Astragalus* species (Fabaceae). They are well known for their antioxidant, antifibrotic, antimicrobial, hepatoprotective, hypotensive and anti-inflammatory effects (Xue et al. 2008; Wang et al. 2012; Bratkov et al. 2016). *Astragalus monspessulanus* L. (Montpellier Milk Vetch) is a clump- forming perennial herb, approximately 20–30 cm high, found in Bulgaria. Several flavonoids and flavoalkaloids were isolated from this species, including the rarely occurring flavonol triglycosides mauritianin and alcesefoliside (Krasteva et al. 2015). These compounds exhibited a varied pharmacological activity – *in vitro* antioxidant, cytoprotective (Krasteva et al. 2015) and *in vivo* antioxidant and neuroprotective effect (Simeonova et al. 2019).

Oxidative stress is directly connected to cell death, which is associated with certain neurodegenerative conditions. In recent years, it has been considered as one of the most significant factors in the pathogenesis of brain ischaemia and a number of neurodegenerative diseases such as Alzheimer's, Parkinson's disease, epilepsy, multiple sclerosis, aging, etc. Reactive oxygen species (ROS) such as H_2O_2, O_2^- and OH• are generated in cells by several pathways. O_2^- is generated by leakage of electrons from the mitochondria. O_2^- are also generated by NADPH cytochrome P450 reductase, hypoxanthine/xanthine oxidase, NADPH oxidase, lipoxygenase and cyclooxygenase. Superoxide dismutase converts O_2^- to H_2O_2. H_2O_2 produces the highly reactive OH• radical by either Fenton or Haber-Weiss reactions. Three processes in which ROS are likely to play a pathogenic role in blood vessels are hypertension, atherosclerosis, and vascular remodelling. Links between oxidative stress and hypertension have been established experimentally, namely that angiotensin-II increases ROS production by vascular smooth muscle cells (Griendling et al. 1994). It has recently been shown that angiotensin II-induced hypertension is associated with increased vascular O_2^- production and that treatment with liposome-encapsulated SOD lowers blood pressure by 50 mm Hg in angiotensin II-infused rats (Laursen et al. 1997). Similarly, liposome-encapsulated SOD enhanced *in vivo* responses to acetylcholine and *in vitro* responses to endothelium-dependent vasodilators in angiotensin II-treated rats (Laursen et al. 1997). These results suggest that hypertension caused by chronically elevated angiotensin II is mediated in part by O_2^-, possibly by degradation of endothelial NO. Inhibition of NADH/NADPH-oxidase (the predominant intracellular source of O_2^-) limits vascular smooth muscle cells hypertrophy stimulated by angiotensin II (Ushio-Fukai et al. 1996). Most studies on the effect of ROS and oxidative stress in vessels have focused on the coronary, carotid and cerebral arterial systems (Heitzer et al. 2001; Gagov et al. 2003; Jacobson et al. 2003; Guzik et al. 2004), particularly of NAD(P)H- oxidase activity (Görlach et al. 2000; Paravicini et al. 2002, 2004; Ellmark et al. 2005), effect of oxidative stress on endothelium (Johnson et al. 1996; Cai and Harrison 2000) and on cell cultures (Griendling et al. 1994). The basilar artery (*arteria basilaris*) carries oxygen-rich blood to the cerebellum, brainstem, and occipital lobes. Because of its location and the key role, it plays in providing oxygenated blood and nutritional substances to various essential portions of the brain, an interruption of the blood flow through the basilar artery can lead to severe brain damage, organ malfunction, or even death.

Effects of many natural antioxidants for cytoprotection in vessels have been investigated so far. Extracts of grape seeds and grape skin (Monagas et al. 2005), leaves of *Ginkgo biloba* (Zheng et al. 2021), isolated anthocyanins (Mazza 2007), olive oil (Waterman and Lockwood 2007), etc., have been proposed as antioxidants and free radical scavengers after many studies. Compiled, these facts suggest that powerful antioxidants, such as flavonoids, could have beneficial effect on the contractility of brain vessels, and could possibly attribute to improved circulation. Despite this, investigations of isolated flavonoids are scarce.

In continuation of our studies on the two rare flavonol triglycosides, isolated from *Astragalus monspessulanus* subsp. *monspessulanus* – alcesefoliside and mauritianin, the aim of this study was to identify their *in vitro* effects on the contractility of *a. basilaris*.

Materials and methods

Isolation of the flavonoids

The procedure of isolation of both compounds has been described previously (Krasteva et al. 2015). The overground parts of the plant were pulverised and extracted with 80% MeOH exhaustively. The extracts were combined, evaporated to dryness, suspended in lukewarm water and successively extracted with dichloromethane, ethyl acetate and *n*-butanol in a separatory funnel. The dried n-butanol extract (32 g) was separated by column chromatography (CC) oved Diaion HP-20 (Mitsubishi Chemicals, Japan) (H_2O:MeOH, 100%:0%→0%:100%) to produce 10 fractions. After TLC analysis (Silica gel plates F254, Merck, Germany; EtOAc:EtCOMe:HCOOH:H_2O, 5:3:1:1; Naturstoffreagenz A, 366 nm) fraction 4, containing flavonoids, was further separated over Sephadex LH-20 (MeOH) to give seven sub fractions (Sd1–Sd7). Sub fraction Sd4 was purified by repeated low pressure liquid chromatography (LPLC) over octadecyl silica gel eluted with MeOH-H_2O (40:60, v/v) and further subjected to semi preparative HPLC using MeCN-H_2O (14:86, v/v) to give alcesefoliside (125 mg) and mauritianin (136 mg). The structure of both compounds was proved by extensive mass spectral and NMR studies and compared to the previous report (Krasteva et al. 2015).

Dissection and mounting of vessels

Male Wistar-Kyoto rats were obtained from the Breeding Centre of the Bulgarian Academy of Sciences, Slivnitsa, Bulgaria. Ethical clearance for the experiment was obtained from Bulgarian Drug Agency and the methods used were approved by the Bulgarian Agency of Food Safety, following the principles, stated in the European Directive 2010/63/EU. The rats were killed by stunning and subsequently decapitated and the basilar artery was isolated. A 18–20 mm long piece of the artery was threaded on two 40 μm diameter stainless steel wires and mounted on a wire-myograph (model 410A, JP Trading, Denmark), containing physiological salt solution consisting (in mM) of: 120 NaCl, 4.5 KCl, 1.2 NaH_2PO_4, 1 $MgSO_4$, 1.6 $CaCl_2$, 0.025 EDTA, 5.5 Glucose, 26 $NaHCO_3$, 5 HEPES (at pH 7.4), which was continuously bubbled with carbogen. Isometric force was recorded with the program Myodaq (JP Trading, Denmark). In most experiments the endothelium was intact. Probes for temperature and pH were placed in the experimental chamber and these parameters were controlled throughout the experiment. After

the temperature reached 37.0 ± 0.5 °C, the vessels were stretched radially to their optimal lumen diameter do corresponding to 90% of the passive diameter of the vessel at 100 mmHg and were allowed to stabilize for 15 min. Thereafter, the reactivity of the vessel was tested with two applications of a solution containing 125 mM KCl. All samples were applied directly into the experimental chamber, dissolved in the medium (saline). The potassium-rich solution was made by an equimolar replacement of sodium.

Results and discussion

Alcesefoliside and mauritianin were successfully isolated from the plant source as reported before, in quantity, significant to perform the tests.

Administered alone, alcesefoliside revealed a decreased vascular tone. In other experiments, a transient increase of the vascular tone was observed, which however, was not statistically significant and it did not have an impact on the general observations (Fig. 1).

Administered alone, mauritianin revealed an insignificant increase in the vascular tone (Fig. 2).

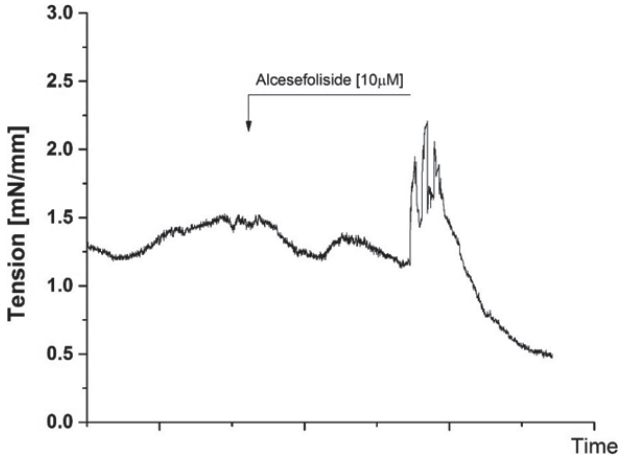

Figure 1. Original record, showing the effect of a single administration of alcesefoliside (10 μM) on *a. basilaris*.

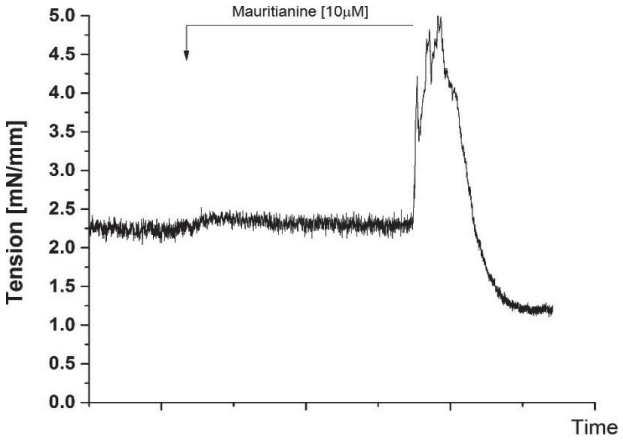

Figure 2. Original record, showing the effect of once administered mauritianin (10 μM) in a segment of *a. basilaris*.

The effects of alcesefoliside and mauritianin, administered alone, in the same concentration (10 μM) did not show statistically significant responses against a control segment of *a. basilaris* (Fig. 3).

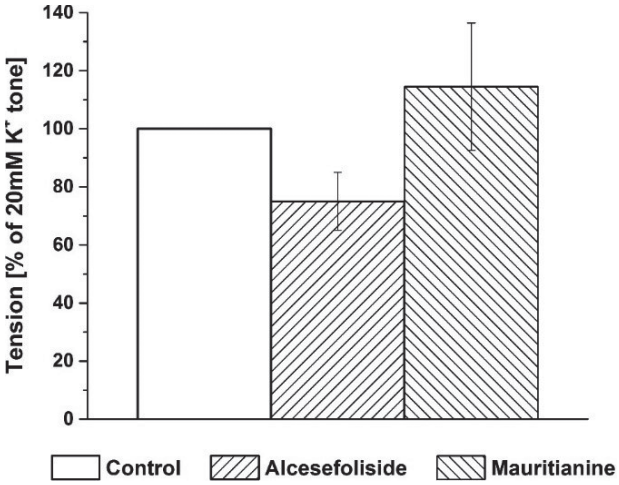

Figure 3. Effects of alcesefoliside and mauritianin, administered alone in concentration 10 μM, on the vascular tone of the segments of *a. basilaris*. Data represent mean ± SEM.

Applied in combination, alcesefoliside and mauritianin, at a concentration of 10 μM, revealed an increased response of the vascular tone (Fig. 4).

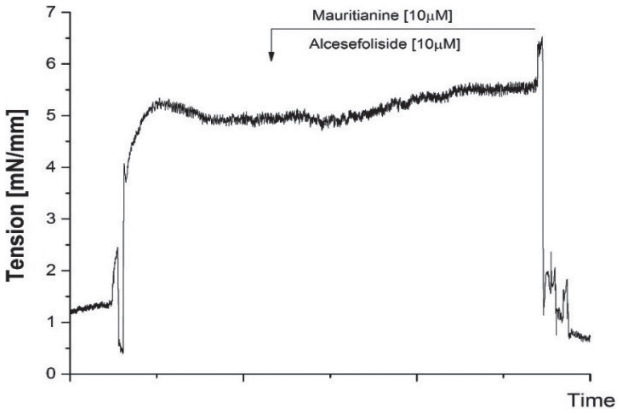

Figure 4. Original record, showing the effect of combination alcesefoliside and mauritianin, at a concentration 10 μM, in a segment of *a. basilaris*.

The effects of the combination alcesefoliside and mauritianin, at a concentration 10 μM, showed statistically significant responses against a control segment of *a. basilaris* (Fig. 5).

Our findings correlate to previously published data on flavonoids. Flavonoids and phenolic acids obtained from the aerial parts of *A. karakuschensis* significantly reduced arterial pressure in experimental animals compared to papaverine hydrochloride (Guzhva et al. 1990). A similar effect was found in the study of an extract containing flavonoids from *A. virgatus* (Guzhva, Luk'yanchikov, and Kazakov 1988). A well-pronounced protective effect of a total flavonoid mixture of *A. membranaceus* in ischemia was found (Wang et al. 1999).

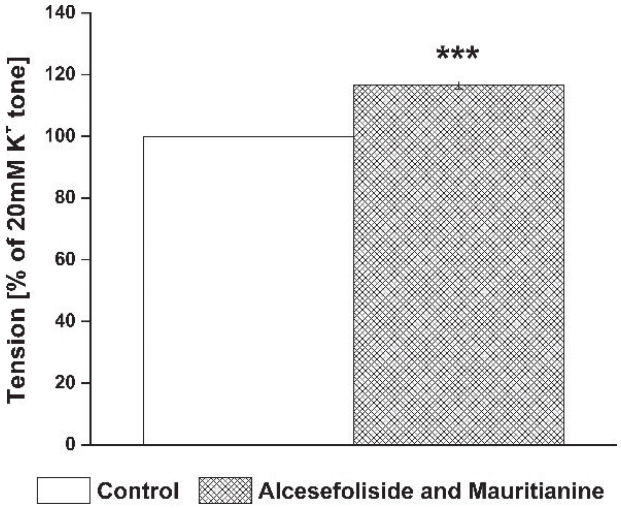

Figure 5. Effects of combination alcesefoliside and mauritianin, at a concentration 10 μM, on the vascular tone of segments of *a. basilaris*. Data represent mean ± SEM. *** $P < 0.001$ *vs* control (untreated segments).

When administered intravenously to urethane-anesthetized animals, flavonoids isolated from *A. centralpinus* caused a sustained decrease in arterial pressure (Bratkov et al. 2016). A total flavonoid mixture from *A. lasioglotis* lowered the level of cholesterol and triglycerides in animals with experimental hyperlipidaemia (Luk'yanchikov 1984). A flavonoid fraction from *A. complanatus* (FF) exhibited a well-defined hypotensive effect on spontaneously hypertensive rats without affecting heart rate and stroke volume. The observed action was mainly due to a significant reduction in peripheral resistance (Xue et al. 2002). These effects of the FF were further investigated and it was found to lower plasma angiotensin II levels (Li et al. 2005). In addition, FF has been shown to inhibit angiotensin II-induced portal vein contraction, with inhibition similar to that of valsartan (Xue et al. 2008). It was found that calycosin can generate an endothelium-independent vasorelaxant effect due to its action as a non-competitive calcium channel antagonist (Wu et al. 2006). Formononetin also induced vasodilation through endothelium/NO-dependent and endothelium-independent mechanisms in isolated rat aorta experiments (Wu et al. 2010).

Sodium formononetin-3'-sulfonate exhibited a protective effect in an *in vivo* model of cerebral ischemia/reperfusion injury (Zhu et al. 2014). Calycosin and formononetin, isolated from *Astragali mongholici radix*, increased the activity of neuronal NO-synthase and dimethylarginine dimethylaminohydrolase. Increased production of NO induced an antihypertensive effect and amelioration of endothelial and cardiovascular dysfunctions (Bai et al. 2013). A purified flavonoid fraction obtained from *A. mongholicus* was tested *in vivo* on rabbits with experimentally induced atherosclerosis. The results of the study showed that flavonoids significantly reduced plasma levels of total cholesterol and low-density lipoprotein, increased high-density lipoprotein levels, and reduced aortic fat deposits (Wang et al. 2012).

This is the first study on the contractility capabilities of alcesefoliside and mauritianin on *a. basilaris*.

Conclusion

Flavonoids are one of the most popular antioxidants in plants. Their varied pharmacological activities are important for these compounds in order to add and to complement conventional therapy. The outcomes of the study suggest that both flavonoids had a positive effect on the contractility of *arteria basilaris*. These findings could serve as the basis for further research on the possible beneficial effects of highly glycosylated flavonoids in neurological conditions.

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