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The vasodilating effect of a *Hintonia latiflora* extract with antidiabetic action

Christina Vierling[a], Christine M. Baumgartner[b], Melanie Bollerhey[c], Wolf D. Erhardt[c], Andreas Stampfl[d], Wolfgang Vierling[a,*]

[a] *Institute of Pharmacology and Toxicology of the Technical University Munich, Munich, Germany*
[b] *Centre of Preclinical Research of the Technical University Munich, Munich, Germany*
[c] *Institute of Experimental Oncology and Therapeutic Research of the Technical University Munich, Munich, Germany*
[d] *Institute of Toxicology, German Research Center for Environmental Health, Neuherberg, Germany*

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ABSTRACT

In the present study, it is shown for the first time that an extract of *Hintonia latiflora* (HLE) which is used as an antidiabetic herbal medicine, is not only able to decrease blood glucose concentration but additionally exerts a vasodilating effect. Accordingly, this extract might have a positive influence on diabetes-associated dysfunction of blood vessels.

The vasodilating effect was demonstrated *in vitro* in aortic rings of guinea pigs as well as *in vivo* in rabbits. Aortic rings pre-contracted with noradrenaline (NA) could completely be relaxed by HLE (EC_{50}: 51.98 mg/l). In contrast, potassium-induced contractions were not diminished by HLE. Therefore, it can be suggested that the vasodilating effect of HLE is primarily the result of an inhibition of G protein-induced increase in intracellular calcium and not of a blockade of voltage-operated L-type calcium channels.

The neoflavonoid coutareagenin (COU), a constituent of HLE which in part is responsible for the blood glucose-lowering effect of HLE, also relaxed NA-induced contractions of aortic rings (EC_{50}: 32.55 mg/l) and only weakly inhibited potassium-induced contractions.

Experiments in aortic rat cells revealed that calcium transients evoked by vasopressin were suppressed by 60 mg/l COU supporting the idea of an inhibition of G protein-induced intracellular calcium release by a constituent of HLE.

To study the effect of HLE on vascular tone under *in vivo* conditions, ultrasound measurements were carried out in conscious rabbits which received a single oral dose of HLE. Under the influence of HLE, a vasodilation combined with a lowering of blood flow velocity could be observed in the abdominal aorta and the common carotid artery. Additionally, a decrease in blood glucose concentration in the HLE group occurred.

The combination of a blood glucose-lowering with a vasodilating effect may be helpful for reducing angiopathies, typical long-term complications in patients with diabetes mellitus.

Introduction

Diabetes mellitus (DM) is a widespread disease with an increasing number of patients. The patients generally need medical supervision during lifetime. Type 2 diabetes accounts for almost 90% of all cases of diabetes in adults. Usually, the blood glucose level can more or less be well controlled in these patients and dangerous events such as heavy fluctuation of the blood glucose level and diabetic coma are rare. The main unsolved problems of DM include long term vascular complications which are the leading causes of death in type 2 DM. Chronic hyperglycaemia appears to be one of the central initiating factors responsible for the development of diabetic complications as microangiopathies like retinopathy, neuropathy, nephropathy or macroangiopathies like stroke and myocardial infarction (Brownlee, 2005). For example, glucose can bind to proteins of the body and induce formation of advanced glycosylated end products (AGE products; Brownlee, 2005). Consequences of this formation are dysfunction and destruction of blood vessel walls. Particularly, vascular relaxation will be

Abbreviations: COU, coutareagenin; DM, diabetes mellitus; HLE, *Hintonia latiflora* extract; NO, nitric oxide; NA, noradrenaline; ROS, reactive oxygen species; VP, vasopressin ([Arg^8]vasopressin).

* Corresponding author. Tel.: +49 89 4140 3282; fax: +49 89 4140 3305.
E-mail address: wolfgang.vierling@lrz.tu-muenchen.de (W. Vierling).

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impaired (Pannirselvam et al., 2003) due to a decrease in endogenous vasodilators and an increase in endogenous vasoconstrictors (Brownlee, 2005). Then, tissues might not be sufficiently supplied with nutrients and oxygen.

On the basis of this knowledge, it was of interest to investigate whether an extract of *Hintonia latiflora* (HLE) has, additionally to its blood glucose-lowering effect, an influence on the tone of blood vessels. An ethanolic-aqueous extract of *H. latiflora* (Copalchi) bark from South America is used as a phytoantidiabetic agent primarily for the treatment of patients with mild to moderate severe type 2 diabetes (Korec et al., 2000; Korecova et al., 2006). The antidiabetic effect of the extract was confirmed in several animal experimental studies (Kaiser and Geyer, 1955; Korec et al., 2000; Pinto et al., 1997; Slijepcevic and Kraus, 1997).

In the present study, aortic rings of guinea pigs were used to study the effects of HLE and COU in arterial blood vessels. Additionally, the action of COU on calcium movements in vascular smooth muscle cells was investigated. Finally, HLE was applied per gavage to rabbits and the influences on abdominal aorta and carotid artery, heart rate, blood pressure and blood glucose level were investigated.

Material and methods

Materials

For the *in vitro* experiments, ±noradrenaline (NA) and milrinone were obtained from Sigma, Germany. Cultivated rat arterial smooth muscle (A10) cells were obtained from DMSZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen), Germany. The fluorescence dye fura-2/AM was purchased from Microprobes, USA and VP ([Arg^8]vasopressin) from Sigma Aldrich, Germany.

H. latiflora bark native extract and COU were kindly provided by Gehrlicher GmbH, Eurasburg, Germany. *H. latiflora* (Sessé & Moc. ex DC.) Bullock belongs to the family of Rubiaceae and grows in the north of South America and in Central America. The used extract was an ethanolic-aqueous spissum extract (drug-extract relation 3:1; lot no. 7618) with a COU content of 12.9% determined by HPLC. A voucher specimen of the bark and the extract is deposited by Gehrlicher GmbH for future reference.

COU (5-hydroxy-7-methoxy-4-(3,4-dihydroxyphenyl)-2*H*-benzo-1-pyran-2-on) was synthesized by Gehrlicher GmbH, Eurasburg, Germany. For the *in vitro* experiments, *H. latiflora* extract (HLE) and COU were dissolved in water ethanol (1:1) mixture and further diluted with water. For *in vivo* experiments, HLE was dissolved in polyethylene glycol (macrogol 300; Caesar & Lorentz, Germany). NA, milrinone and VP were diluted in water.

Animals and study design

In vitro evaluation in isolated aortic rings of guinea pigs

Guinea pigs were euthanized by concussion (Close et al., 1997). The thoracic aorta was dissected and cut into 2–4 mm ring segments. Isometric force was measured. The bath solution was a modified Krebs–Henseleit buffer (KHB) containing (mM): NaCl, 115; KCl 2.8; $CaCl_2$ 2; $NaHCO_3$, 25; KH_2PO_4 1.2; $MgCl_2$ 1.2; glucose 10. HLE was added in a cumulative manner to the tissue bath. Alternatively, COU, milrinone or ethanol was added cumulatively. Additionally, the effects of HLE and COU on contractions induced by potassium (addition of 30 mM KCl) were observed.

In vitro evaluation of Hintonia latiflora extract in rat aortic (A10) cells

A10 cells were bred on coverslips which were fixed in an experimental chamber (filled with modified KHB with 1.2 mM Ca^{2+}) and put on an inverse microscope. Cells were loaded with 8 to 10 μM fura-2/AM and then superfused to remove extracellular fura-2/AM. For intracellular calcium measurements, cells were illuminated alternately with light of wave-lengths of 340 and 380 nm (AMKO LTI, Germany). The emitted fluorescence at 510 nm was recorded and the ratio between the emitted fluorescence signals was calculated as indicator for the intracellular calcium concentration. Addition of 2 nM VP led to a temporary increase of the intracellular calcium concentration (calcium transient). VP was added after a 45 min rest period and removed after 6 min. After a following rest period of 5 min, COU was added and equilibrated during 40 min. Then addition of VP followed. In control experiments, it was shown that in the absence of COU, the second VP-induced calcium transient did not differ remarkably from the first one.

In vivo evaluation of the effect of Hintonia latiflora by ultrasound

Ultrasound measurements of the abdominal aorta and the common carotid artery were carried out in 8 adult, white New Zealand rabbits (Charles River, Germany) using an ultrasound device (GE Vingmed Ultrasound A/S System VIVID FIVE, N-3191, Norway) fitted to a 10 MHz linear probe. Additionally, mean arterial blood pressure and heart rate were measured by using a special equipment (Data Ohmeda S/5, type F-CM1.00, Finland) connected *via* pressure sensor to Art. auricularis of the animal. Blood glucose was measured in blood of Art. auricularis by using Accu-Check, Roche Diagnostics, Germany. The following parameters were determined: diameter of the abdominal aorta and the common carotid artery, maximum systolic blood flow velocity in these vessels, mean arterial blood pressure, heart rate and blood glucose concentration. After preparing the animals for the experiments, the baseline values were determined. Then, the animals were anesthetized for a short time with 1% propofol (7 mg/kg; Fresenius Kabi GmbH, Germany). During anaesthesia, HLE (200 mg/kg) dissolved in macrogol 300 (1 ml/kg) was administered per gavage to one animal and only macrogol 300 (1 ml/kg) to the other animal. After recovering from anaesthesia, ultrasound measurements were performed at 0.5, 1, 2, 3 and 4 h. All investigations were carried out by a blinded examiner. The animal experiments were performed in concordance with local laws of animal protection and approved by the animal protection committee of the Government of Upper Bavaria, Munich, Germany.

Statistical analysis

Results are expressed as means ± standard error of the means (SEM). Concentration-effect curves were fitted to a sigmoidal relationship after logarithmic transformation of the concentrations and the EC_{50} values ± SEM determined using GraphPad Prism, USA. The *t*-test was used for the determination of significance in the fura experiments. In the *in vivo* studies, Friedman, Wilcoxon and Mann–Whitney tests were used. Differences were considered as significant at $p \leq 0.05$.

Results

Effects of Hintonia latiflora extract and coutareagenin on noradrenaline-induced contractions

In aortic rings pre-contracted with NA (10 μM), HLE concentration-dependently relaxed the rings (Fig. 1). Relaxation begun at concentrations >45 mg/l and was complete at 60 mg/l (by 99.18 ± 2.35%). The EC_{50} was 51.98 mg/l (51.41 to 52.60 mg/l). Addition of COU also led to a relaxation of the NA-induced contraction. However, relaxation begun at concentrations >15 mg/l and was almost complete at 60 mg/l (by 93.81 ± 5.62%). The EC_{50} was 32.55 mg/l (30.52 to 34.63 mg/l). Accordingly, the potency of the compound is about 1.6 times of that of the extract. Control experiments with ethanol which was used as solubilizers