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Arctigenin improves vascular tone and decreases inflammation in human saphenous vein

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ABSTRACT

The goal of this study was to test the effects of bioactive phenylpropanoid dibenzylbutyrolactone lignan arctigenin (ATG) in vascular tone. Human bypass graft vessel, from a saphenous vein (SV), were set up in organ bath system and contracted with potassium chloride (KCl, 40 mM). Two concentration–response curves of noradrenaline (NE) (10 nM–100 μM) separated with an incubation period of 30 min without (Control) or with ATG (3–100 μM) were established. Inhibitors of nitric oxide, prostaglandins, K^+ related channels or calcium influx were used to delineate the molecular mechanisms beyond ATG effects. To investigate anti-inflammatory actions, SV were treated with 10 μM or 100 μM ATG and incubated for 18 h in the absence or presence of both interleukin-1beta (IL-1β) and lipopolysaccharide (LPS) to mimic the physiological or inflamed tissue conditions. Proatherogenic and inflammatory mediators İnterleukine-1 beta (IL-1β), Monocyte Chemoattractant Proteine-1 (MCP-1), Tumor Necrosis Factor- α (TNF-α), İnterleukine-6 (IL-6), Prostaglandin E_2 (PGE_2) and İnterleukine-8 (IL-8) in the supernatant were measured.

ATG significantly decreased vascular contractile response to NE. Moreover, it reduced contractions induced by KCl and cumulative addition of $CaCl_2$. The mediators were significantly increased in inflammatory conditions compared to normal conditions, an effect which was inhibited by ATG (10 and 100 μM). ATG reduces contractions in SV and decreases the production of proinflammatory-proatherogenic mediators, setting the stage for further evaluating the effect of ATG in cardiovascular diseases.

1. Introduction

ATG is a phenylpropanoid dibenzylbutyrolactone lignin isolated from the seeds of *Arctium lappa* L. (Gao et al., 2008). The anti-inflammatory action has been investigated in vivo and in-vitro studies, including LPS induced inflammation models in murine RAW264.7 or human U937 macrophage cells, bronchoalveolar fluid composition, gastric or colitis induced ulcers by showing reduced levels of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, iNOS and COX expressions and down-regulation of NF-κB, JAK-STAT and MAPK and activation of AMPK pathway (Cho et al., 1999, 2004; Dos Santos et al., 2008; Han et al., 2016; Hyam et al., 2013; Kou et al., 2011; Lee et al., 2011; Shi et al., 2014; Wu et al., 2015). ATG has in vitro antiviral activity against influenza A virus (Yang et al., 2005).

Moreover, it has shown also antiallergic action from inhibition of B and T cell mediated hypersensitivities and inflammation-related enzymes (Lee and Kim, 2010).

ATG has shown neuroprotective effects in the nervous system inflammation processes, with potential therapeutic implications in ischaemic stroke and through decreased formation of β-amyloid (Aβ) and senile plaques associated with Alzheimer's disease (Fan et al., 2012; Zhu et al., 2013). Moreover, ATG has also shown beneficial effects in other conditions predisposing to cardiovascular disease (CVD) such as diabetes, by improving glycemic control and ameliorat-

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ing metabolic disorders (Duan et al., 2015; Xu et al., 2015) and hypercholesterolemia, by promoting cholesterol efflux in oxidized low-density lipoprotein (oxLDL)-loaded THP-1 macrophages (Xu et al., 2013).

The cardioprotective effects of ATG have recently been shown in an experimental animal model of spontaneously hypertensive rats, which showed that ATG improves endothelial function by increasing eNOS phosphorylation and reducing systolic blood pressure (Liu et al., 2015).

These data suggest that ATG could also have a direct vascular effect; therefore we decided to investigate the pharmacological activity of ATG on the isolated human SV by exploring its vasoactivity mechanism and anti-inflammatory action.

2. Materials and methods

2.1. Isolated vascular tissue preparations and organ bath

All the procedures in this study were conducted according to guidelines in the Declaration of Helsinki and the study design was approved by Ethics Committee in Faculty of Medicine (Nr: 3625), University of Prishtina—Hasan Prishtina and University Clinical Center of Kosovo (Pristina, Kosovo). The study was performed on isolated segments of human SV obtained from patients undergoing coronary artery bypass grafting (CABG). The characteristics of the patients are shown in supplemental **(**Table S1**).** SV fragments isolated during surgery were placed in cold (4 °C) Krebs-Ringer bicarbonate solution (NaCl 118.5 mM; KCl 4.8 mM; NaHCO3 25 mM; $MgSO_4$·7H2O 1.2 mM; $CaCl_2$ 1.9 mM; KH_2PO_4 1.2 mM; glucose 10.1 mM; disodiumethylenediaminetetraacetic acid (EDTA) 0.026 mM; pH 7.4) and immediately transferred to the laboratory. Preparations were dissected from adhering fatty and connective tissues and rings of 2–4 mm diameter were cut. Tissue rings were mounted in an organ bath containing 10 ml of Krebs solution Tissue Organ Bath System (750TOBS, DMT-USA, Ann Arbor, MI, USA). The solution was bubbled with 95% O_2 and 5% CO_2 at 37 °C. A force-displacement transducer recorded changes in tension in isometric and continuous mode on a multichannel recorder polygraph model coupled with the software LabChart7 connected to power lab 4/35 data acquisition system (PowerLab 4/35, ADInstruments Pty Ltd., NSW, Australia). Each ring was initially stretched to an optimal load of ~ 2 g. Subsequently, preparations were equilibrated for 90 min with bath solution changes every 15 min.

2.2. Contraction/relaxation studies

After the equilibration period, the viability of the vessel specimens was assessed using potassium chloride (KCl, 40 mM). After KCl-induced contraction, preparations were washed until the basal tone was reestablished. Two consecutive KCl responses were obtained in each ring for the standardization of the preparations. After the last KCl contraction, the preparations were washed until the basal tone was reestablished. Initial concentration–response curves were obtained with norepinephrine (NE) (10 nM–100 μM). In SV preparations contracted previously by NE, when a maximal effect was obtained, the preparations were washed with Krebs solution until they returned to the resting tone. Subsequently, these preparations were incubated (30 min) with ATG (3–100 μM). Some preparations were incubated without compound and served as a time control. After this incubation period, a second concentration-response curve of NE was obtained. Each preparation was used for one protocol (two concentration–response curves of NE separated with an incubation period). In another set of experiments, after the equilibration period was finished, human SV preparations were precontracted with NE (1 μM) and ATG added to the baths in a cumulative manner (3–100 μM) following plateau. Concentrations chosen in our study appears to be within the physiological ranges reached after in vivo ATG administration and are comparable with previous pharmacological studies (Hayashi et al., 2010; He et al., 2012; Hyam et al., 2013; Kou et al., 2011). To determine the involvement ATG in vasorelaxation, these preparations were incubated separately for 20 min with inhibitors of either nitric oxide synthase (L-NO-arginine; L-NOARG; 100 μM), soluble guanylyl cyclase (^{1}H-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one; ODQ; 10 μM), cyclooxygenase (indomethacin; 10 μM),

ATP-sensitive K^+ channel (glibenclamide; GLI; 10 μM), voltage-sensitive K^+ channel (4-aminopyridine; 4AP; 1 mM), inward rectifier K^+ channel (barium chloride; $BaCl_2$; 100 μM),

calcium-activated potassium channel (Apamin and charybdotoxin; CbTX; 1 μM); (Coleman et al., 2017), non-selective K^+ channel (tetraethylammonium chloride; TEA; 1 mM). Additionally, we investigated also the role of ATG in the endothelium-denuded (E-) SV segments (Liu et al., 2000; Yang and He, 1997). The vasorelaxant effect of ATG was also investigated in the presence of different vasoconstrictors such as phenylephrine (PE, 1 μM); endothelin-1 (ET1, 100 nM); thromboxane A2 agonist (u46619) (10, 30 and 100 nM) and KCl (40 mM).

Acetylcholine (ACh) (10 μM) was used to test endothelium-dependent relaxation, while sodium nitroprusside (SNP) (100 μM) was used to test vascular relaxation capacity.

2.3. Effects of arctigenin on extracellular Ca^{2+} flux

In another set of experiments, the effect of ATG on contractions induced by $CaCl_2$ was investigated; concentration-response curves to $CaCl_2$ (from 10 μM to 10 mM) in the absence and presence of ATG 100 μM were obtained. After the equilibration period, tissue rings were washed three times at 15 min intervals with Ca^{2+}-free Krebs solution containing 0.1 mM (EDTA). Tissue rings were constricted first with 1 μM PE for 15 min to deplete intracellular Ca^{2+}. After three washes with Ca^{2+}-free Krebs solution, the tissue was preincubated with arctigenin 100 μM for 20 min, with PE 1 μM applied to induce stable contraction. Thereafter, $CaCl_2$ was added cumulatively (from 10 μM to 10 mM). The contractile responses induced by $CaCl_2$ in the presence and absence (control) of ATG pre-treatment were compared (Senejoux et al., 2013).

Nifedipine (L-type voltage-gated calcium channel blocker) (10 μM) was used as positive control (Babaei and Azarmi, 2008; Ford et al., 2006).

2.4. Proinflammatory mediator measurement

For the tissue culture experiments, SV rings were incubated in 12-well plates containing RPMI 1640 (Roswell Park Memorial Institute Medium) (PAN-Biotech). The volumes of the culture medium were adjusted to 1 ml per 70 mg of tissues.

These preparations were treated with antibiotics (penicillin, 1000 IU/ml; streptomycin, 100 μg/ml) and the antimycotic (0.25 μg/ml) to prevent microbial contamination.

SV samples were allocated as "Normal" conditions without treatment and "Inflammatory" induced conditions treated with (IL-1b, 100 ng/ml), lipopolysaccharide (LPS, 100 μg/ml) (Foudi et al., 2009), in the presence or absence of ATG (10 μM or 100 μM). After these steps, the plates were set immediately in a culture incubator for 18 h in a humidified chamber at 37 °C gassed with 95% O_2 and 5% CO_2. All related supernatants from the samples were stored at − 80 °C for further ELISA experiments for the measurement of proatherogenic and inflammatory mediators IL-1β (Interleukine 1 beta), MCP-1 (Monocyte Chemoattractant Protein-1), TNF-α (Tumor Necrosis Factor Alpha), Interleukine-6 (IL-6) Prostaglandin E2 (PGE2) and IL-8 (Interleukine-8) in the culture media by enzyme immunoassay kit.