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Research paper

Effect of *Arctium lappa* (burdock) extract on canine dermal fibroblasts

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ABSTRACT

Although the biological activities of *Arctium lappa* (burdock) have been already investigated in human and other species, data evaluating the molecular mechanisms have not been reported in the dog. In this study we analyzed for the first time the effect of a root extract of burdock on molecular responses in canine dermal fibroblasts with H_2O_2 stimulation (H group), with burdock treatment (B group) and with H_2O_2 stimulation and burdock treatment (BH group), using RNAseq technology. Differentially expressed genes ($P < 0.05$) of H, B and BH groups in comparison to the untreated sample (negative control, C group) were identified with MeV software and were functional annotated and monitored for signaling pathways and candidate biomarkers using the Ingenuity Pathways Analysis (IPA). The expression profile of canine dermal fibroblasts treated with burdock extract with or without H_2O_2 stimulation, showed an up-regulation of mitochondrial superoxide dismutase (SOD2), disheveled 3 (DVL3) and chondroitin sulfate N-acetylgalactosaminyltransferase 2 (CSGALNACT2). The data suggested that burdock has implications in cell adhesion and gene expression with the modulation of Wnt/β catenin signaling and Chondroitin Sulphate Biosynthesis that are particularly important for the wound healing process.

1. Introduction

The skin forms an effective barrier between the organism and the environment preventing invasion of pathogens and fending off chemical and physical assaults, as well as the unregulated loss of water and solutes (Proksch et al., 2008). However, the mammalian skin can manifest multifarious disorders resulting from complex interactions between genetic and environmental factors (Rizzato et al., 2011). The skin is a major target of toxic insult by a broad spectrum of physical (*e.g.* UV radiation) and chemical (xenobiotic) agents and many environmental pollutants that are either themselves oxidants or catalyze the production of reactive oxygen species (ROS) directly or indirectly (Bickers and Athar, 2006). Previous studies have demonstrated that antioxidants attenuate the damaging effects of ROS and can impair and/or reverse many of the events that contribute to epidermal toxicity and disease (Trouba et al., 2002). Numerous studies have established a close similarity between human and canine atopic dermatitis at the pathogenesis, clinical, epidemiological and therapeutic levels (Olivry, 2012). However, investigations of molecular mechanisms with a full transcriptome analysis in dog are still lacking.

Most promising topical treatments of skin dermatitis include plants and herbal extracts which have been widely accepted also to scavenge free radicals from skin cells and to restore skin health (Tapsell et al., 2006; Iriti et al., 2010; Mukherjee et al., 2011). It has been shown that great varieties of natural compounds such as *Aloe vera*, *Calendula officinalis*, *Centella asiatica* and *Curcuma longa* scavenge free radicals and prevent skin disorders (Kim et al., 2010; Mukherjee et al., 2011). Botanicals are excellent sources for components such as polyphenols which exhibit antioxidant, anticarcinogenic, antimutagenic and anti-inflammatory effects (Farinacci et al., 2008; Pandey

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and Rizvi, 2009; Colitti et al., 2012; Sgorlon et al., 2012). *Arctium lappa*, commonly known as burdock, is a popular vegetable cultivated in many countries (Lin et al., 2002; Chen et al., 2004). Burdock is traditionally used to treat sore throat, rashes, boils and various skin problems (Chan et al., 2011). Previous studies have reported that burdock extract has anti-inflammatory (Sohn et al., 2011), free radical scavenging (Lin et al., 1996), antioxidant (Chan et al., 2011) and liver protective (Lin et al., 2002) effects. Although burdock has these biological activities, the molecular mechanisms involved on skin are not reported yet.

The aim of the research was to investigate the molecular activity of burdock extract on canine dermal fibroblasts, in order to investigate the signaling pathways modulated by burdock treatment. In this study, canine dermal fibroblasts were pre-stimulated with hydrogen peroxide (H_2O_2) to mimic oxidative conditions and then treated with burdock root extract. Viability, adhesion activity and transcriptome profile of fibroblasts were analyzed.

2. Material and methods

2.1. Preparation of A. lappa extract

The extract of *A. lappa* was from whole root and provided by Bayer S.p.a. (Milan, Italy). The extract is in aqueous extraction using maltodextrin as carrier. For the concentrations of 1, 10, 50 and 100 μg/ml of burdock, 50 mg of extract were dissolved in 1 ml of 100% DMSO, while for the concentrations of 200 and 500 μg/ml the extract was dissolved in a water solution of 10% DMSO. The solutions were filtered with 0.22 μm pore size (Millipore, Milan, Italy) and kept at −20 °C until further analysis.

2.2. Cell culture

Canine immortalized dermal fibroblast-hTERT (ABM, Vancouver, Canada) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. All reagents were purchased from Euroclone (Pero, Milan, Italy). Cells were maintained in humidified air with 5% CO_2 at 37 °C. In all experiments, cells were grown to approximately 70–90% confluence and serum free DMEM was used for cell treatment with H_2O_2 and/or burdock extract.

2.3. H_2O_2 cell treatment

To ascertain cell viability at different H_2O_2 concentrations, a preliminary assay was performed by treating dermal fibroblasts with different final concentrations (1, 10, 50, 100, 200 and 500 μM) of H_2O_2 diluted in phosphate-buffered saline (PBS) and further diluted in serum free DMEM. This assay was finalized to identify the concentration of H_2O_2 to induce a defined oxidative stress in the subsequent experiments as described in Table 1. Cell viability was measured either after 1 h of incubation with H_2O_2 or after 1 h of incubation with H_2O_2, subsequent washing with PBS and 24 h of incubation (H) in serum free DMEM. The control cells (C) were incubated 24 h with the same amount of 0.2% DMSO in serum free DMEM.

2.4. Burdock (B) and burdock with H_2O_2 (BH) cell treatments

To test the effect of burdock extract (B) on cell viability, fibroblasts were washed with PBS and treated with burdock extract (1, 10, 50, 100, 200 and 500 μg/ml) in 0.2% DMSO for 24 h in serum free DMEM. The combined effect of burdock and H_2O_2 (BH) was assayed incubating cells for 1 h with H_2O_2 (200 μM) and, after washing with PBS, with 1, 10, 50, 100, 200 and 500 μg/ml of burdock extract in 0.2% DMSO for additional 24 h in serum free DMEM. The control cells (C) were incubated 24 h with the same amount of 0.2% DMSO in serum free DMEM.

2.5. Cell viability assay

Viability of dermal fibroblasts was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay (Pruett and Loftis, 1990; Farinacci et al., 2009). For the assays, confluent dermal fibroblasts (1×10^4 cells/well) were seeded on 96 well tissue culture plate and left to grow for 24 h. Then, cell viability was measured after incubation with H_2O_2 after 1 h and after incubation with each different treatment (H, B, BH and C) after 24 h. For this, 10 μl of MTT reagent per well was added and the mixture was further incubated for 3 h. Next, the mixture in each well was removed, and formazan crystals formed were dissolved in 100 μl of DMSO. Optical density of the mixture was measured (12 observations for each treatment) in a microplate reader at 550 nm of wavelengths.

Table 1
Experimental design used to investigate the effect of 200 μM of H_2O_2 and burdock extract on cell adhesion and molecular response of canine dermal fibroblasts.

Sample	First incubation (1 h)	Second incubation (24 h)	
	200 μM H_2O_2	0.2% DMSO	10 μg/ml burdock + 0.2% DMSO
C	–	+	–
H	+	+	–
B	–	–	+
BH	+	–	+

Confluent dermal fibroblasts were treated with: C: 0.2% of DMSO for 24 h; H: 200 μM of H_2O_2 for 1 h and, after washing, 0.2% of DMSO for 24 h; B: 10 μg/ml (0.2% of DMSO) of burdock extract for 24 h; BH: 200 μM of H_2O_2 for 1 h and, after washing, 10 μg/ml (0.2% of DMSO) of burdock extract for 24 h. + means treatment performed; – means treatment not performed.