Phytochemistry 117 (2015) 340–350

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Natural lignans from *Arctium lappa* as antiaging agents in *Caenorhabditis elegans*

Shan Su, Michael Wink *

Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany

ARTICLE INFO

Article history:
Received 27 January 2015
Received in revised form 15 June 2015
Accepted 22 June 2015

Keywords:
Arctium lappa
Lignans
Caenorhabditis elegans
Antiaging agent
Free radical scavenger
DAF-16

ABSTRACT

Arctium lappa is a well-known traditional medicinal plant in China (TCM) and Europe that has been used for thousands of years to treat arthritis, baldness or cancer. The plant produces lignans as secondary metabolites, which have a wide range of bioactivities. Yet, their antiaging potential has not been explored. In this study, we isolated six lignans from *A. lappa* seeds, namely arctigenin, matairesinol, arctiin, (iso)lappaol A, lappaol C, and lappaol F. The antioxidant and antiaging properties of the isolated lignans were studied using *Caenorhabditis elegans* as a relevant animal model. All lignans at concentrations of 10 and 100 μM significantly extended the mean life span of *C. elegans*. The strongest effect was observed with matairesinol, which at a concentration of 100 μM extended the life span of worms by 25%. Additionally, we observed that five lignans are strong free radical-scavengers *in vitro* and *in vivo* and all lignans can improve survival of *C. elegans* under oxidative stress. Furthermore, the lignans can induce the nuclear translocation of the transcription factor DAF-16 and up-regulate its expression, suggesting that a possible underlying mechanism of the observed longevity-promoting activity of lignans depends on DAF-16 mediated signaling pathway. All lignans up-regulated the expression of *jnk-1*, indicating that lignans may promote the *C. elegans* longevity and stress resistance through a JNK-1-DAF-16 cascade. Our study reports new antiaging activities of lignans, which might be candidates for developing antiaging agents.

1. Introduction

Aging is an inevitable biological process, which can be defined as a progressive decline in physiological capacities accompanied by an increased vulnerability to environmental challenges and aging-related diseases (Holliday, 2004). Although the exact biological and cellular mechanisms of the aging process are not well understood, a large body of evidence indicates that free radical-induced oxidative damage of cellular components plays a key role in aging and aging-related diseases (Beckman and Ames, 1998; Cadenas and Davies, 2000; Varadarajan et al., 2000). Production of free radicals is an unavoidable process in the course of cellular metabolism. For example, reactive oxygen species (ROS) are well-known byproducts of ATP production via mitochondrial respiration (Getoff, 2007). Cells need a certain level of ROS and their concentrations are usually fine-tuned *in vivo*, However, overproduction and overaccumulation of ROS can lead to DNA damage (mutations), lipid peroxidation and protein oxidation, which are commonly implicated in aging and aging-related diseases, such as cancer, neurodegenerative and circulation disorders (Bergamini et al., 2004). As such, the scavenging of detrimental free radicals by antioxidants may alleviate oxidative damage of cells, therefore promoting longevity and preventing aging-related disorders.

Medicinal plants provide a high diversity of natural products, which can be exploited for potential antiaging agents. Previous studies have successfully identified several natural antioxidants that have promising antiaging capacities, such as resveratrol (Chen et al., 2013b), epigallocatechin gallate (Abbas and Wink, 2009) and quercetin (Kampkotter et al., 2008). *Arctium lappa*, commonly known as burdock, is an important medicinal plant in China (TCM) and Europe that has been used to treat sore throat, skin infections and to alleviate rheumatic pain and fever for thousands

Abbreviations: DCF, 2′,7′-dichlorofluorescein; DMSO, dimethyl sulfoxide; DPPH·, 2,2-diphenyl-1-picrylhydrazyl; EGCG, epigallocatechin gallate; FOXO, Forkhead box O; GFP, green fluorescent protein; H_2DCF, 2,7-dichlorodihydrofluorescein; HPLC, high performance liquid chromatography; IGF-1, insulin/insulin-like growth factor; IIS, insulin/insulin-like growth factor signaling; MS, mass spectrometry; NGM, nematode growth medium; NMR, nuclear magnetic resonance; PBS, phosphate buffered saline; ROS, reactive oxygen species; TCM, Traditional Chinese Medicine.

* Corresponding author.
E-mail address: wink@uni-heidelberg.de (M. Wink).

http://dx.doi.org/10.1016/j.phytochem.2015.06.021
0031-9422/

of years (Van Wyk and Wink, 2004). Nowadays it continues to serve as a valuable source for secondary metabolites that can be explored for new biological and pharmacological applications. Particularly, *A. lappa* is a rich source of bioactive lignans. At least 24 lignans have been isolated and identified from its seed and fruit extracts (Umehara et al., 1993). Lignans comprise a large group of natural compounds that mostly consists of two phenylpropanoid moieties connected via C8-C8′at their side chain or by additional ether, lactone, or carbon bonds (Laurence and Davin, 2003). A large body of research has focused on the pharmacological and biological properties of natural lignans in *A. lappa*. Emerging evidence has shown that lignans have anticancer, anti-inflammatory, antidiabetic, antimicrobial and antiviral properties. For example, arctigenin, one of the major bioactive lignans in *A. lappa*, has strong antiproliferative and apoptotic effects against different cancer cell lines (Ryu et al., 1995). Besides, arctigenin and arctiin have potent anti-inflammatory effects via inhibiting lipopolysaccharide-induced nitric oxide (NO) production and the release of pro-inflammatory cytokines in murine macrophages (Kou et al., 2011; Lee et al., 2011). However, a possible antiaging potential of the bioactive lignans from *A. lappa* has not been reported.

The nematode *Caenorhabditis elegans* is an important model organism to study aging and oxidative stress-related diseases, because it has a rapid reproduction rate and a short life span, and most importantly, the major signaling pathways that regulate longevity and stress resistance in mammals are well conserved in *C. elegans* (Pinero Gonzalez et al., 2009). The evolutionary conserved insulin/insulin-like growth factor (IGF-1) signaling (IIS) pathway is one of the well-understood longevity-regulating pathways in animals. In this pathway, the Forkhead box O (FOXO) transcription factors are key players, which are under control of IGF-1 receptors. In *C. elegans*, similar to other systems, activation of DAF-2, the worm homolog of IGF-1 receptor, recruits and activates the phosphoinositide 3-kinase/protein kinase B signaling cascade, which in turn results in phosphorylation of DAF-16, the single FOXO transcription factor in the worms. Being phosphorylated, DAF-16 is prevented from its nuclear translocation and the induction of gene expression of downstream longevity-promoting genes (Accili and Arden, 2004). On the contrary, inactivation of IIS pathway by using genetic and pharmacological approaches has been shown to significantly increase the life span of *C. elegans* and elevate the worm resistance to different stressors (Accili and Arden, 2004). Besides the IIS pathway, the Jun-N-terminal kinase-1 (JNK-1) signaling pathway is another important upstream pathway that can regulate the DAF-16 nuclear localization by alternating the phosphorylation status of DAF-16 (Oh et al., 2005). Once in the nucleus, several proteins cooperate with DAF-16 to modulate downstream gene transcription. Among these co-regulators, the heat-shock-factor-1 (HSF-1) acts together with DAF-16 to activate expression of specific genes, such as genes encoding small heat-shock proteins, which enhance stress resistance and longevity in *C. elegans* (Hsu et al., 2003). Additionally, the p38 mitogen-activated protein kinase (PMK-1), a parallel pathway to DAF-16, also contributes to longevity of *C. elegans* (Troemel et al., 2006).

In this study, six natural lignans were isolated and identified from the seed extracts of *A. lappa*, namely arctigenin, matairesinol, arctiin, (iso)lappaol A, lappaol C and lappaol F. We individually investigated their antiaging potential using *C. elegans*. We report here for the first time that the major lignans from *A. lappa* can significantly extend the life span of *C. elegans* under normal conditions and promote the survival of the worms under oxidative stress. The possible mechanism of longevity-promoting effects of the lignans on *C. elegans* has also been investigated. This study reports novel antiaging capabilities of natural lignans isolated from *A. lappa* and suggests a novel bioactivity for this medicinally important plant.

2. Results

2.1. The isolated lignans from A. lappa extended the mean life span of wild type C. elegans

We have successfully isolated six lignans from the extract of *A. lappa* seeds, namely as arctigenin, matairesinol, arctiin, (iso)lappaol A, lappaol C and lappaol F (Fig. 1). Their structures were identified by ^{1}H-NMR, ^{13}C-NMR, EI-MS and ESI-MS analyses (Supplementary data). To study whether the isolated lignans have potential antiaging effects, we investigated their potential to extend life span of the wild type *C. elegans* under normal conditions. As shown in Table 1 and Fig. 2, all lignans significantly increased the mean life span of the N2 worms in a concentration-dependent manner compared with the control group. Among these lignans, matairesinol exerted the strongest life span-prolonging activity. At 10 μM and 100 μM concentrations, matairesinol significantly prolonged the mean life span of the worms by 14.0% and 25.0%, respectively ($P < 0.001$). At a moderate concentration of 100 μM, arctigenin, arctiin and (iso)lappaol A, lappaol C, lappaol F increased the mean life span of the worms by 13.7%, 15.3%, 11.2%, 11.5% and 12.5%, respectively ($P < 0.05$) (Table 1). We observed that the bacteria mass on top of the agar plates remained the same when we performed the lifespan experiments. Additionally, the minimal inhibitory concentration (MIC) values of all lignans are higher than 2000 μM, both after treatment of 24 h and 48 h at 20 °C. However the possibility that the living *Escherichia coli* may have an effect on the lifespan of *C. elegans* was not completely ruled out.

2.2. Antioxidant activities of the isolated lignans in vitro and in vivo

Because a large body of research has clearly shown that the longevity-promoting capabilities of many natural products are related to their free radical-scavenging activities (Harrington and Harley, 1988), we were interested to know whether these natural lignans are also active in scavenging free radicals. For this purpose, we examined their free radical-scavenging activities by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay, in which quenching DPPH free radical is used as an indicator for the free radical-scavenging capabilities of the lignans (Eklund et al., 2005). As a result, five lignans (except arctiin) were found to have strong activities in scavenging DPPH free radicals with low IC_{50} values (Fig. 5A). The data indicate that all the lignans except arctiin are powerful *in vitro* free radical scavengers.

To assess whether the isolated lignans exhibit *in vivo* antioxidant activities, we measured endogenous ROS levels in the lignans-treated worms by using H_2DCF-DA as an indicator for intracellular ROS. Pre-treatment with arctigenin, matairesinol, arctiin, (iso)lappaol A and lappaol F (100 μM each) significantly attenuated ROS levels in the worms by 19.20% ($P < 0.0001$), 21.33% ($P = 0.0093$), 18.58% ($P = 0.0261$), 11.03% ($P = 0.0038$) and 13.97% ($P = 0.0047$), respectively. Additionally, arctigenin, arctiin, (iso)lappaol A and lappaol C of 10 μM markedly decreased ROS level in the worms by 12.53% ($P = 0.0217$), 14.35% ($P = 0.0040$), 12.13% ($P = 0.0114$) and 16.71% ($P = 0.0026$), respectively (Fig. 5B). However the highest concentration of lignans (200 μM) failed to further decrease the ROS level in the worms, possibly due to their toxicity to the worms. These results indicate that the isolated lignans at moderate concentrations have *in vivo* antioxidant activities by decreasing intracellular ROS level in *C. elegans*.

2.3. Lignans increase the resistance of nematodes to oxidative stress

To test whether the antioxidant lignans protect the worms against oxidative stress, we further assessed the effects of the