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Natural lignans from *Arctium lappa* modulate P-glycoprotein efflux function in multidrug resistant cancer cells

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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 ability to reverse multidrug resistance (MDR) in cancer cells 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 MDR reversal potential of the isolated lignans and the underlying mechanism of action were studied using two MDR cancer cell lines, CaCo2 and CEM/ADR 5000 which overexpress P-gp and other ABC transporters. In two-drug combinations of lignans with the cytotoxic doxorubicin, all lignans exhibited synergistic effects in CaCo2 cells and matairesinol, arctiin, lappaol C and lappaol F display synergistic activity in CEM/ADR 5000 cells. Additionally, in three-drug combinations of lignans with the saponin digitonin and doxorubicin MDR reversal activity was even stronger enhanced. The lignans can increase the retention of the P-gp substrate rhodamine 123 in CEM/ADR 5000 cells, indicating that lignans can inhibit the activity of P-gp. Our study provides a first insight into the potential chemosensitizing activity of a series of natural lignans, which might be candidates for developing novel adjuvant anticancer agents.

Introduction

Cancer cells are capable to develop resistance to a single drug, or a class of anti-cancer drugs. After having obtained resistance to a single drug, cells often also show cross-resistance to many other structurally and functionally unrelated drugs. This phenomenon is called multidrug resistance (MDR), which might explain why therapy that even combine multiple agents with different targets fail to improve therapeutic efficacy (Gottesman et al. 1994). The best known mechanism for MDR is the overexpression of ATP-binding cassette (ABC) transporter proteins in the cell membrane in cancer cells which can mediate the efflux of cytotoxic drugs and thus lower intracellular drug concentrations (Ambudkar et al. 1999). A major member of the ABC transporter family is P-glycoprotein (P-gp), which is involved in the MDR of cancer cells to several anticancer drugs. Thus, targeting P-gp is one approach to overcome and reverse MDR.

Medicinal plants produce a high diversity of secondary metabolites (SM) which include effective and new drugs for cancer treatment. Several attempts have been made to identify natural products with a variety of chemical structures which can inhibit ABC transporters (Wink et al. 2012).

Arctium lappa, commonly known as burdock, is an important medicinal plant in China (TCM) and Europe which has been used for thousands of years (Van Wyk and Wink 2004). *A. lappa* is a rich source of bioactive lignans. Recently, it was discovered that the natural lignans in *A. lappa* have promising anticancer potential. They can induce apoptosis in cancer cells and suppress tumor growth via decreasing tumor tolerance to glucose starvation (Marian et al. 2003). However, to our knowledge, no research on the potential of lignans from *A. lappa* to reverse MDR in cancer cells to chemotherapeutic drugs like doxorubicin has been published. As we had isolated a series of lignans from *A. lappa* seeds, we were interested to combine non-toxic lignans from *A. lappa* with clinically used chemotherapeutic agent to test their potential to reverse MDR of cancer cells.

CaCo2 grow as an adherent cell monolayer, which is a well-established *in vitro* model of the intestinal epithelium that highly expresses P-gp on its apical surface (Hunter et al. 1993). This cell line has been widely used to study a number of substrates and inhibitors of P-gp. CEM/ADR 5000 is a doxorubicin-resistant human T-lymphoblastic leukemia cell line derived from its parental doxorubicin-sensitive cell line, CCRF-CEM. CEM/ADR 5000 also overexpresses P-gp (Efferth et al. 2002). In the present study, we investigated the multidrug-resistant reversal potency of six lignans from

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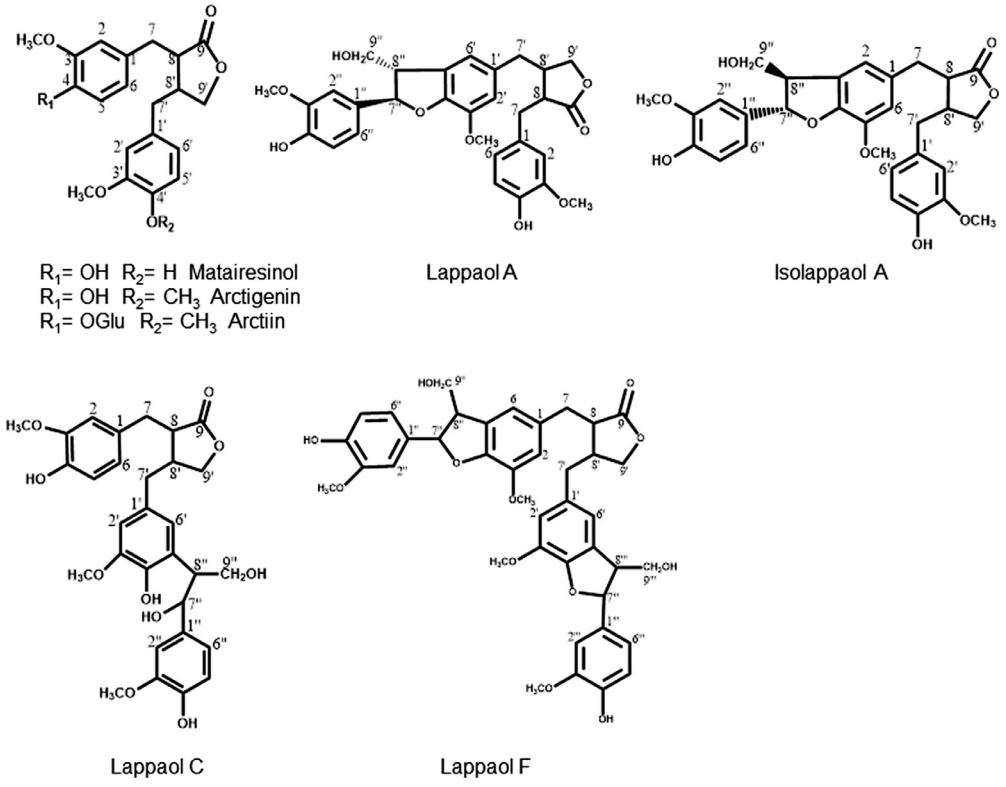

Fig. 1. Chemical structures of the lignans isolated from *A. lappa*.

A. lappa seeds, namely arctigenin, matairesinol, arctiin, (iso)lappaol A, lappaol C and lappaol F using CEM/ADR 5000 and CaCo2 cell lines.

Materials and methods

Plant material

Seeds of *Arctium lappa* were collected from the Neuenheimer Feld, Heidelberg, Germany in July 2011. A voucher specimen of the plant was deposited in the Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Heidelberg, Germany. The voucher number is P8243.

Extraction and isolation of lignans from Arctium lappa

The extraction and isolation of lignans from *A. lappa* extract were performed as described in our article: natural lignans from *Arctium lappa* as novel antiaging agents in *Caenorhabditis elegans* (submitted to *Phytochemistry*). Their structures have been identified as arctigenin, matairesinol, arctiin, a mixture of two isomers containing lappaol A and isolappaol A (we call it (iso)lappaol A), lappaol C and lappaol F (Fig. 1) by analysis of ^{1}H-NMR, ^{13}C-NMR, EI-MS and ESI-MS and comparison with literature data (Han et al. 1994; Liu et al. 2003; Umehara et al. 1993). The isolated lignans were then dissolved in DMSO to prepare stock solutions of 10 mM, 20 mM and 100 mM for further tests.

Cell culture

CaCo2 and human T-cell lymphoma CEM/ADR 5000 were used in this study. Culture conditions were identical to those described previously (El-Readi et al. 2010).

Cytotoxicity assay

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method was carried out as established in our laboratory (El-Readi et al. 2010).

Two-drug combinations of doxorubicin with lignans

Three different concentrations (IC_{10}, IC_{20}, IC_{30}) of lignans were combined with the cytotoxic doxorubicin (Sigma– Aldrich, GmbH, Germany) to measure whether lignans can increase the sensitivity of MDR cancer cells to doxorubicin. Briefly, CaCo2 and CEM/ADR 5000 cells were seeded in 96 well-plates and incubated with serial dilutions of doxorubicin as well as each lignans of different concentrations as mentioned above. MTT assay was carried out to test the cytotoxicity of doxorubicin alone or in combination against CaCo2 and CEM/ADR 5000 cells.

Three-drug combinations of doxorubicin with lignans plus digitonin

In addition to two-drug combination assays, three-drug combinations of doxorubicin with lignans of different concentration (IC_{10}, IC_{20}, IC_{30}) plus the saponin digitonin (Sigma– Aldrich, GmbH, Germany) were also performed. Briefly, CaCo2 and CEM/ADR 5000 cells were seeded in 96 well-plates and incubated with serial dilutions of doxorubicin and each lignan with different concentrations plus digitonin in a non-toxic concentration (IC_{10}) as mentioned above. The corresponding cytotoxicity was determined with the MTT assay.

Analysis of combination effects

For the analysis of synergism or antagonism of drug combinations the combination index (CI) method was employed. The ranges of CI and the symbols were described by Chou (2006) in which CI <1, $=1$,