

Evaluation of antioxidant, anti-inflammatory and lipoxygenase inhibitory activities of the prenylated coumarin umbelliprenin

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ABSTRACT

Background and objective: Umbelliprenin, the natural prenylated coumarin distributed in the plants of apiaceae family, has shown various biological activities, especially as a cancer chemopreventive agent. In the present study, umbelliprenin, was examined for *in vitro* antioxidant activity, *in vitro* inhibitory activity against lipoxygenase, and *in vivo* anti-inflammatory activity.

Methods: The applied tests were interaction with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, inhibition of lipid peroxidation, inhibition of soybean lipoxygenase and *in vivo* inhibition of the carrageenin-induced rat paw edema.

Results: Umbelliprenin did not show any significant antioxidant activity but exhibited a remarkable and potent inhibition against soybean lipoxygenase ($IC_{50} = 0.0725 \mu M$). This compound, in the *in vivo* anti-inflammatory test, could also inhibit the carrageenin induced paw edema significantly (39 %).

Conclusion: The observed inhibition of lipoxygenase may be a plausible mechanism for the potent cancer chemopreventive activity of umbelliprenin and may pose this compound as a valuable agent for the treatment of inflammatory diseases.

Keywords: Umbelliprenin, Antioxidant, 5-lipoxygenase, Anti-inflammatory activity, Cancer chemoprevention.

INTRODUCTION

Lipoxygenases (LOs) are a family of iron-containing enzymes that catalyse the dioxygenation of polyunsaturated fatty acids in lipids containing a *cis,cis*-1,4-pentadiene structure. They convert arachidonic acid, a component of membrane phospholipids, into pro-inflammatory mediators called leukotrienes (1,2) which are a group of highly potent molecules having diverse biological actions and are increasingly implicated in a variety of disease states including asthma, chronic obstructive pulmonary disease (COPD), cancer, osteoporosis and atherosclerosis (3,4). Recently, there has been considerable interest in the development of LO inhibitors for therapeutic indications (5,6). Although anti-inflammatory drugs are used extensively, prolonged consumption of these medications is usually coupled with numerous side effects (7,8). Therefore, there is a need to explore alternative strategies to lower the formation of inflammatory mediators with the

help of natural dietary products. A group of these natural products are coumarins (known as 1,2-benzopyrone or *O*-hydroxycinnamic acid-8-lactone). Coumarins comprise a very large class of phenolic derivatives found in plants and consist of fused benzene and α -pyrone rings. Up to now, more than 1300 types of coumarins have been identified, chiefly as secondary metabolites in green plants in fungi and bacteria (9,10), possessing anti-inflammatory and cancer chemopreventive properties (11). To date, several natural coumarin derivatives such as esculetin, daphnetin and fraxetin has been recognized as inhibitors of the proinflammatory lipoxygenase and cyclooxygenase pathways of arachidonate metabolism (12).

In the previous paper, promising cancer chemopreventive activity of umbelliprenin was reported (11). In the present study the inhibitory effect of umbelliprenin, as a prenylated coumarin, on soybean lipoxygenase activity to clarify whether this mechanism is responsible for the cancer

chemopreventive activity of the compound is described. In addition, because inflammation is a physiological response in the process of carcinogenesis, *in vivo* anti-inflammatory activity of the tested compound was determined by checking its ability to inhibit the induced carrageenin paw edema (CPE) in rats.

MATERIAL AND METHODS

Chemicals

7-Hydroxycoumarin and DBU (1,8-diazabicyclo [5.4.0]undec-7-ene) were purchased from Merck company. *Trans-trans*-Farnesyl bromide was obtained from Sigma-Aldrich Company. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), caffeic acid (CA), 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH) and nordihydroguaiaretic acid (NDGA) were purchased from the Aldrich Chemical Co. Milwaukee, WI, (USA). Soybean Lipoxigenase, linoleic acid sodium salt and indomethacin were obtained from Sigma Chemical, Co. (St. Louis, MO, USA) and carrageenin, type K, was commercially available. For the *in vivo* experiments, male and female Fischer-344 rats (180–240 g) were used.

Preparation of umbelliprenin

Umbelliprenin was easily synthesized from the reaction of 7-hydroxycoumarin (1M) and *trans-trans*-farnesyl bromide, as described previously (13).

Experiments *in vitro*

In the *in vitro* assays each experiment was performed at least in triplicate and the standard deviation of absorbance was less than 10% of the mean.

Determination of the reducing activity of the stable radical 1,1-diphenyl-picrylhydrazyl (DPPH)
Briefly, a 0.05 mM solution of DPPH in absolute ethanol was prepared. This solution was added to an equal volume of the solution of the tested compound (dissolved in ethanol) to obtain a final concentration of 0.1 mM. Ethanol was used as control solution. After 20 and 60 min at room temperature, the absorbance was recorded at 517 nm and compared with the appropriate standard, namely NDGA (14).

Lipid peroxidation assay

Production of conjugated diene hydroperoxide by oxidation of linoleic acid in an aqueous dispersion was monitored at 234 nm. 2,2'-Azobis

(2-amidinopropane) dihydrochloride (AAPH) was used as a free radical initiator. Ten microliters of the linoleic acid dispersion (16 mM) was added to a UV cuvette containing 0.93 mL of 0.05 M phosphate buffer (pH 7.4) at room temperature under air. Then 50 μ L of 40 mM AAPH solution was added to the previous cuvette. Oxidation was carried out in the presence of umbelliprenin (10 μ L). In the assay without antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37°C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides (15).

Soybean lipoxigenase inhibition study

In vitro study was carried out as reported previously (16). The tested compound was dissolved in ethanol and incubated at room temperature with sodium linoleate (0.1 mM) and 0.2 mL of enzyme solution ($1/9 \times 10^4$ w/v in saline). The conversion of sodium linoleate to 13-hydroperoxylinoleic acid at 234 nm was recorded and compared with caffeic acid as appropriate standard (16).

Inhibition of the carrageenin-induced edema

The animals, which had been bred in our laboratory, were housed under standard conditions and received a diet of commercial food pellets and water *ad libitum* during the maintenance but they were entirely fasted during the experiment period. Studies were in accordance with recognized guidelines on animal experimentation. Both sexes were used and female pregnant rats were excluded. Each group was composed of 6–15 animals. Edema was induced in the right hind paw of Fisher-344 rats (150–200 g) by intradermal injection of 0.1 mL of 2% carrageenin in water. The tested compound (0.01 mmol/kg body weight) was suspended in water, with few drops of Tween 80 and grounded in a mortar before use and was given intraperitoneally simultaneously with the carrageenin injection. The rats were euthanized 3.5 h after carrageenin injection. The difference between the weight of the injected and uninjected paws was calculated for each animal. The change in paw weight was compared with that in control animals (treated with water) and expressed as a percent inhibition of the edema (CPE %). Indomethacin at 0.01 mmol/kg was used for comparison as a reference compound. CPE % values are means from two different experiments with a standard error of the mean less than 10% (17,18).

RESULTS AND DISCUSSION

In this work, *in vitro* and *in vivo* functions of umbelliprenin against inflammation and radical attack was investigated. Many non-steroidal anti-inflammatory drugs have been reported to act either as inhibitors of free radical production or as radical scavengers (19). Consequently, compounds with antioxidant properties could be expected to offer protection against cancer. Therefore, the antioxidant activity of umbelliprenin in comparison to two well known antioxidant agents namely nordihydroguaiaretic acid (NDGA) and

trolox, was investigated.

For the estimation of the antioxidant potential of a compound, different experimental approaches can be used (20). Most of these methods require a spectrophotometric measurement and a certain reaction time in order to obtain reproducible results (21). We used DPPH for the evaluation of radical scavenging activity. AAPH was also used as a free radical initiator to follow oxidative changes of linoleic acid to conjugated diene hydroperoxide. Umbelliprenin presented very low interaction values and no changes were observed after 60 min (Table 1).

Table 1. Antioxidant activity, lipid peroxidation and lipoxygenase inhibitory activities of umbelliprenin, anti-inflammatory activity (inhibition of carrageenin paw edema CPE%) as compared with positive controls.

Name of sample or control	Antioxidant activity		Lipoxygenase inhibition (IC ₅₀)	CPE (0.01 mmol/kg bw)
	DPPH (20 and 60 min)	AAPH		
Umbelliprenin	4 %	18 %	0.0725 µM	39 %
Positive control	81 % ^a	63 % ^b	600 µM ^c	47% ^d

CPE: carrageenin paw edema; ^a NDGA; ^b trolox; ^c caffeic acid; ^d indomethacin;

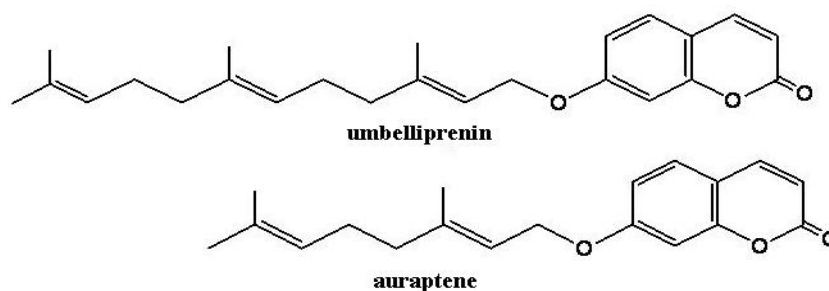


Figure 1. The structures of umbelliprenin and auraptene, two bioactive natural prenylated coumarins.

We also tried to identify the possible inhibitory activity of umbelliprenin on lipid peroxidation. Azo compounds generating free radicals through decomposition are useful for *in vitro* free radical production studies. The water soluble azo compound AAPH has been extensively used as a clean and controllable source of alkylperoxyl free radicals.

Umbelliprenin was evaluated for inhibition of soybean lipoxygenase by the UV absorbance based enzyme assay. Interestingly, umbelliprenin showed a remarkable and significant inhibitory activity against lipoxygenase (IC₅₀ = 0.0725 µM) compared with the reference standard, namely caffeic acid (IC₅₀ = 600 µM, Table 1).

Umbelliprenin was examined *in vivo* for its anti-inflammatory activity using the carrageenin mouse paw edema as a model of inflammation (17). The result was expressed as percentage of inhibition of weight increase at the right hind paw in comparison to the un-injected left hind paw. Carrageenin-induced edema is a non-specific inflammation resulting from a complex of diverse

mediators. Since edema of this type is highly sensitive to NSAIDs, carrageenin has been accepted as a useful agent for studying new anti-inflammatory drugs. In this test, umbelliprenin showed a significant inhibition of inflammation (39 %) whereas indomethacin, as standard reference drug, had 47 % inhibition.

Mounting evidence suggests that lipoxygenase-catalyzed products have a profound influence on the development and progression of human cancers. Compared with normal tissues, significantly elevated levels of lipoxygenase metabolites have been found in lung, prostate, breast, colon, and skin cancer cells, as well as in cells from patients with both acute and chronic leukemias. Lipoxygenase-mediated products elicit diverse biological activities which are required for neoplastic cell growth, influencing growth factor and transcription factor activation, oncogene induction, stimulation of tumor cell adhesion, and regulation of apoptotic cell death. Agents that block lipoxygenase-catalyzed activity may be effective in preventing cancer by interfering with

signaling events needed for tumor growth (22,23).

Umbelliprenin is synthesized by various *Ferula* species (24-26). It has also been found in various plant species which are consumed as food or used for food preparation such as in celery, *Angelica archangelica*, *Coriandrum sativum*, *Citrus limon* and *Ferula* species (9). Our findings revealed that umbelliprenin did not possess scavenging activity and anti-lipid peroxidation effect.

As a prenylated coumarin, umbelliprenin has a structure close to that of auraptene, a cancer chemopreventive agent from the genus *Citrus*.

The only difference is the higher chain length of the 7-prenyloxy which contains 15 instead of 10 carbons (Fig. 1). It has been reported that umbelliprenin inhibits the red pigment production in *Serratia marcescens* (24), decreases MMP activity (26), exhibits antileishmanial activity

against promastigotes (25) and induces apoptosis in human M4Beu metastatic pigmented melanoma cells (27). In a recent study we have shown that umbelliprenin is a potent cancer chemopreventive agent (11). Findings of the present study, shows that observed cancer chemopreventive activity from umbelliprenin could be probably attributed to the potent inhibitory effect of this compound on 5-lipoxygenase activity which reduces the formation of lipoxygenase-carcinogenic products. Previously, a few prenylated phytochemicals have been reported to possess inhibitory effect on lipoxygenase and cyclooxygenase (28,329). The activity of umbelliprenin against inflammation and edema is concordant with previous reports about several naturally occurring coumarins (30), proposing its potential application as a lead compound for designing potent anti-inflammatory and cancer chemopreventive agents.

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