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Research Article

Lipoxygenase Inhibitory Activities of Some Plant Extracts and Chemical Compounds

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Abstract

Lipoxygenases (LOXs, linoleate: oxygen reductase, E.C. 1.13.11.12) are a family of non-heme iron-containing dioxygenases. LOXs are associated with several inflammation-related diseases such as arthritis, asthma, cardiovascular, kidney, skin and allergic diseases, neurodegenerative disorders, cancer and metabolic syndrome. In this study, the inhibitory effects of ethanolic extracts prepared from 12 different plants and 12 different chemical compounds were investigated on the activity of LOX which has an important value in the health area. All the plant extracts and chemical substances used in our study showed LOX inhibitory effect. The enzyme inhibitory activities of the extracts and chemical compounds were increasing in a dose-dependent manner. The results obtained in the present study indicate that plant extracts and chemical compounds examined can be a potential source of novel antiinflammatory therapeutics.

Keywords: Lipoxygenase inhibitory activity, plant extracts, chemical compounds.
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Bazı Bitki Ekstreleri ve Kimyasal Bileşiklerin Lipoksijenaz İnhibitör Aktiviteleri

Özet

Lipoksijenazlar (LOXs, linoleat: oksijen redüktaz, E.C.1.13.11.12) hem içermeyen, demir içeren dioksijenaz ailesidir. Lipoksijenazlar, artrit, astım, kalp damar, böbrek, deri ve alerjik hastalıklar, nörodejeneratif hastalıklar, kanser ve metabolik sendrom gibi çeşitli inflamasyon ile ilişkili hastalıklar ile ilgilidir. Bu çalışmada, farklı bitkilerden hazırlanan etanollü ekstrelerin ve bazı kimyasal bileşiklerin sağlık alanında önemli bir alana sahip olan LOX aktivitesi üzerine inhibe edici etkileri incelenmiştir. Çalışmada kullanılan tüm bitki ekstreleri ve kimyasal maddelerin LOX inhibitör etkisine sahip olduğu belirlenmiştir. Bitki ekstreleri ve kimyasal bileşiklerin enzim inhibitör aktiviteleri, doza bağlı olarak artmaktadır. Bu çalışmadan elde edilen sonuçlara göre, incelenen bitki ekstreleri ve bazı kimyasal bileşikler antiinflamatuar tedavide yeni bir potansiyel kaynak olabilirler.

Anahtar kelimeler: Lipoksijenaz inhibitör aktivite, bitki ekstreleri, kimyasal bileşikler.

Introduction

Lipoxygenases (LOXs, linoleate: oxygen reductase, E.C. 1.13.11.12) are a large monomeric protein family of non-sulphur non-heme, and iron-containing dioxygenases (Brash 1999). LOXs are widely distributed in plants, animals and fungi.

LOXs are associated with several inflammation-related diseases such as arthritis, allergic asthma, psoriasis, inflammatory bowel disease, cardiovascular, kidney, metabolic syndrome, skin diseases (Iversen and Kragbella 2000) and neurodegenerative disorders such as Alzheimer's disease (Khanna et al. 2003; Moreno 2009; Dobrian et al. 2011). In addition, LOXs and their metabolites are involved in many human cancer types such as prostate, lung, breast, colon and other cancer cell lines (Samuelsson 1987). It is very important to find new LOX inhibitors for the therapy of human diseases such as cancer, cardiovascular and neurodegenerative disorders (Kelavkar et al. 2000). According to the site of oxygen insertion within arachidonic acid, LOXs are classified as 5-, 8-, 11-, 12- and 15-LOX. These 5-, 8-, 12-, and 15-LOX isoenzymes are important for mammalian organisms. Also, 5- and 15- LOXs are important for plants. The substrate for mammalian LOXs are arachidonic acid while, for plants LOXs are linoleic and α-linoleic acids (Aparoy et al. 2008). The arachidonic acid pathway is a source of powerful bioregulators such as prostaglandins, thromboxanes, leukotrienes and lipoxins (Parker 1987; Samuelsson et al. 1987; Funk 1996; Yamamato et al. 1997). This pathway is initiated by mammalian LOXs. The lipoxygenase pathway of arachidonic acid metabolism occur reactive oxygen species (ROS). Other arachidonic acid metabolites formed in this way and ROS might play a role in tumour formation and inflammation. (Juntachote and Berghofer 2005). There are many LOX inhibitors for therapeutic application available in pharmacies such as Zileuton and Minocyclines (Misra et al. 2013). Due to their side effects, these inhibitors have been forbidden or limited (Charlier and Michaux 2003). Due to such side effects, developing of new LOX inhibitor drug with minimum side effects is important (Tomy et al. 2014).

In this study, *in vitro* enzyme inhibition potential of ethanolic extracts prepared from different plants and some chemical compounds for LOX were investigated.

Materials and methods

Plant material and chemical compounds

All the plants were purchased from an Istanbul Local Market. Chemical compounds were obtained from Merck, Sigma–Aldrich and were of analytical grade.

Preparation of the plant extracts

The plant extracts were prepared by refluxing 10 g of the dried leaves with 100 ml 70% ethanol for four hours using a Soxhlet apparatus and then filtering through linen cloth at room temperature. The filtrates were evaporated to dryness in a rotary evaporator. The extracts were kept in - 20˚C until use. All the extracts were dissolved in 70% ethanol before use. Chemical compounds were also dissolved in water.

In vitro lipoxygenase-inhibitory assay

The inhibition of lipoxygenase activity was determined by a spectrophotometric method reported by Yawer et al. (2007). The reaction mixture, containing test compound solution (inhibition solution), lipoxygenase solution in 0.1 M phosphate buffer (pH 8.0) was incubated for 10 min at 25 ˚C. Then, the reaction was initiated by addition of a solution substrate. After 6 min, absorbans value was measured at 234 nm. Quercetin was used as standard inhibitor.

The percent inhibition of lipoxygenase activity was calculated as:

$$\text{Inhibition (\%)} = (1\text{-}A/B)x100$$

Where A is the enzyme activity without inhibitor, B is the activity in presence of inhibitor.

The IC_{50} was determined as the concentration of plant extracts and chemical drugs required to inhibit lipoxygenase activity by 50%.

Results

The effect of the ethanolic extracts of plants on lipoxygenase activity was shown in Table 1. The enzyme inhibitory activities of the extracts were increasing in a dose-dependent manner. Higher inhibitory activity is associated with a lower IC_{50} value. Persimmon was found to have the highest LOX inhibitor activity. IC_{50} value was found 1.2 ± 0.7 ng/mL. On the other hand, grape was found to have the lowest LOX inhibitor activity (102.0 ± 9.4 ng/mL) (Table 1). Quercetin was used as standard. IC_{50} value was found 7.9± 0.5 ng/mL. This plant extract was the most active compound against the enzyme and even better than the standard inhibitor. The lipoxygenase inhibitory activity have decreased in order of persimmon > lavender > ginger > green lentil > camomile = artichoke > broad bean > thyme > chick pea > onion > quercetin > garlic > grape (Table 1).

The effect of some chemical compounds on lipoxygenase activity is shown in Table 2. Atorvastatine, ascorbic acid, phloridzine and vanadium sulphate were found to have the highest LOX inhibitory activity. IC_{50} value was found 0.2 ± 0.1 ng/mL. Benzoic acid was found to have the lowest LOX inhibitor activity (9.4 ± 0.8 ng/mL) (Table 2). Lipoxygenase inhibitory activity of chemical compounds and standard compound decreased in the order of atorvastatin = ascorbic acid = phloridzin = vanadium sulphate > nicotinic acid > DL-α-tocopherol > manganase sulphate > chromium (III) picolinate > chromium (III) chloride > fish oil > quercetin > benzoic acid (Table 2). In our study, in comparison to plant extracts, LOX has been inhibited at higher rates by chemicals that have lipid lowering properties (Table 2).

Discussion

LOXs have been postulated to play an important role in the pathophysiology of several inflammatory and allergic diseases. Reactive oxygen radicals are well known to be produced during the inflammatory process. ROS have been implicated in the process of inflammation (Trouillas et al. 2003). Antioxidants (such as polyphenolics, flavonoids) are known to inhibit plant lipoxygenase. Polyphenols are widely distributed in nature and some studies have revealed that polyphenols constitute rich inhibitors of LOX product synthesis (Werz 2007). Middleton et al. (2000) and Arts and Hollman (2005) have shown the role of

Table 1. Lipoxygenase inhibitory activity of plant extracts.

Plant Extract	IC_{50} (ng/mL)*
Persimmon	1.2 ± 0.7
Lavender	1.5 ± 1.2
Ginger	1.7 ± 1.0
Green lentil	2.2 ± 1.1
Artichoke	2.7 ± 2.3
Camomile	2.7 ± 1.4
Broad bean	3.2 ± 1.0
Thyme	3.3 ± 1.6
Chick pea	3.4 ± 1.7
Onion	3.5 ± 1.9
Quercetin	7.9 ± 0.5
Garlic	52.7 ± 3.9
Grape	102.0 ± 9.4

*Mean ± SD

Table 2. Lipoxygenase inhibitory activity of some chemical compounds.

Chemical Compounds	IC_{50} (ng/mL)*
Ascorbic acid	0.2 ± 0.1
Atorvastatin	0.2 ± 0.1
Phloridzin	0.2 ± 0.1
Vanadium sulfate	0.2 ± 0.1
Nicotinic acid	1.1 ± 0.3
DL-α-Tocopherol	1.8 ± 0.1
Manganase sulfate	3.0 ± 0.3
Chromium (III) picolinate	3.1 ± 0.1
Chromium (III) chloride	3.3 ± 0.3
Fish oil	4.0 ± 1.3
Quercetin	7.9 ± 0.5
Benzoic acid	9.4 ± 0.8

*Mean ± SD

antioxidants in the inhibition of inflammatory enzymes such as LOX enzymes (Middleton et al. 2000; Arts and Hollman 2005). The antiinflammatory (anti-lipoxygenase) activities of plants extracts could be explained by the potent inhibitory effects of their phenolic compounds on arachidonic acid metabolism through the lipoxygenase pathway. In this study, all the plant extracts show strong LOX inhibitory activity (Table 1). Plant extracts, except garlicand grape extracts have inhibited LOX at a higher rate than quercetin (Table 1). Celik Onar et al. (2012) have demonstrated that *Epilobium angustofolium* extract inhibited the activity of lipoxygenase (Celik Onar et al. 2012). IC_{50} value was found as 0.57± 0.06µg/ml. Our results have shown a higher inhibition value than these results. The results suggest that plant extracts have potentially high anti-inflammatory effect (antilipoxygenase activity), which might be related to polyphenolic content and other antioxidant substances. Phenolic compounds and antioxidants such as flavonoids, saponin etc may block the arachidonic acid pathway by inhibiting LOX activity and thus may serve as scavengers of ROS which are produced during arachidonic acid metabolism.

Many synthetic and natural compounds are known to inhibit LOX. For this reason, some chemical compounds have been selected in this study and their effects on LOX were examined (Table 2). These compounds showed the highest LOX inhibition activity (Table 2). In the literature, chromium compounds, nicotinic acid, atorvastatin, phloridzin and fish oil were reported to have lipid lowering properties (Bolkent et al. 2004; Atac et al. 2006; Suren Castillo et al. 2008; Tu et al. 2014, Najafian et al. 2014; Tillander et al. 2014; Ras et al. 2014). Some experimental animal studies reported that vanadium had beneficial effects on blood lipids in diabetic rats (Tunali and Yanardag 2006). Some studies have reported an inhibitory effect of hydroxylated benzoic acid derivatives on LOX (Blake 2015). Tocopherol was reported to inhibit LOX activity (Jiang 2014). Our results indicate compliance with these results. Chen et al. (2000) have suggested that manganese has protective effect against lipid peroxidation (Chen et al. 2000). In our study, except benzoic acid, all the chemical compounds have inhibited LOX at a higher rate than quercetin which has been used as a standard (Table 2). In addition, chemicals that have lipid lowering properties have been found to have higher LOX inhibition rates than plant extracts (Table 2).

Conclusions

In the present study, all plants extracts and chemical compounds have good LOX inhibitor activity, indicating that they could be useful in the treatment of several inflammation related diseases such as cancer, allergic diasease, asthma, aging and atherosclerosis.

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