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# Inhibition of leukocyte-type 12-lipoxygenase by guava tea leaves prevents development of atherosclerosis

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ABSTRACT

Oxidation of low-density lipoprotein (LDL) is one of the crucial steps for atherosclerosis development, and an essential role of leukocyte-type 12-lipoxygenase expressed in macrophages in this process has been demonstrated. The biochemical mechanism of the oxidation of circulating LDL by leukocyte-type 12-lipoxygenase in macrophages has been proposed. The major ingredients in guava tea leaves which inhibited the catalytic activity of leukocyte-type 12-lipoxygenase were quercetin and ethyl gallate. Administration of extracts from guava tea leaves to apoE-deficient mice significantly attenuated atherogenic lesions in the aorta and aortic sinus. We recently showed that Qing Shan Lu Shui inhibited the catalytic activity of leukocyte-type 12-lipoxygenase. The major components inhibiting the enzyme contained in Qing Shan Lu Shui were identified to be novel monoterpene glycosides. The anti-atherogenic effect of the tea leaves might be attributed to the inhibition of leukocyte-type 12-lipoxygenase by these components.



## 1. Introduction

Lipoxygenase is a dioxygenase producing hydroperoxy fatty acids. The enzyme incorporates molecular oxygen into polyunsaturated fatty acids in regio- and stereo-specific manners. In mammalian tissues, there are 5-, 8-, 12- and 15-lipoxygenases named according to the site of oxygenation in arachidonic acid (Brash, 1999; Funk, 1996, 2001; Kühn & Thiele, 1999; Yoshimoto & Takahashi, 2002). Several isoforms are known in 12-lipoxygenase, namely, leukocyte-type, platelet-type and epidermal-type 12-lipoxygenases producing 12*S*-hydroperoxy-5, 8, 10, 14-eicosatetraenoic acid as well as 12*R*-lipoxygenase producing 12-hydroperoxy fatty acid with *R* configuration (Funk, 2001). The leukocyte-type 12-lipoxygenase is the isoform expressed in a variety of tissues including macrophages. The enzyme shows broad substrate specificity and can directly oxygenate polyunsaturated fatty acids esterified to cholesterol in low-density lipoprotein (LDL) particles (Belkner, Stender, & Kühn, 1998; Kühn, Belkner, Suzuki, & Yamamoto, 1994). LDL oxidation is one of the critical steps for atherosclerosis development (Brown & Goldstein, 1990; Steinberg, 2009). Since macrophages expressing high level of leukocyte-type 12-lipoxygenase are accumulated in atherosclerotic lesions (Ylä-Herttuala et al., 1990, 1991) and capable of causing LDL oxidation (Chisolm, Hazen, Fox, & Cathcart, 1999), the enzyme has been investigated extensively with regard to the contribution to atherogenesis (Takahashi, Zhu, & Yoshimoto, 2005; Zhao & Funk, 2004). It has been proposed that LDL oxidation is initiated by leukocyte-type 12-lipoxygenase by oxygenating linoleate esterified to cholesterol in LDL particles. Subsequent radical chain reactions modify the LDL to its fully oxidized form recognized by scavenger receptors in macrophages that lead to foam cell formation (Belkner et al., 1998; Kühn & Thiele, 1999; Sakashita, Takahashi, Kinoshita, & Yoshimoto, 1999; Takahashi, Zhu, & Yoshimoto, 2005; Zhao & Funk, 2004). Atherosclerosis development as well as LDL oxidation attenuated in leukocyte-type 12-lipoxygenase-deficient mice clearly demonstrating that the enzyme was required for LDL oxidation (Cyrus et al., 1999; George et al., 2001), although the contradictory study suggested that the atherogenesis was suppressed in the homologous enzyme-transgenic rabbit (Funk, 2001; Shen et al., 1996).

We investigated the biochemical mechanism of oxidative modification of extracellular LDL by intracellular leukocyte-type 12-lipoxygenase, and demonstrated that LDL binding to LDL receptor-related protein (LRP), a cell surface receptor expressed on macrophages, but not to LDL receptor was required for the oxidation of LDL (Xu et al., 2001). The LRP mediated the translocation of leukocyte-type 12-lipoxygenase to plasma membranes from cytosol in macrophages which was an essential step for oxidation of LDL (Zhu et al., 2003). Furthermore, LRP contributed to the

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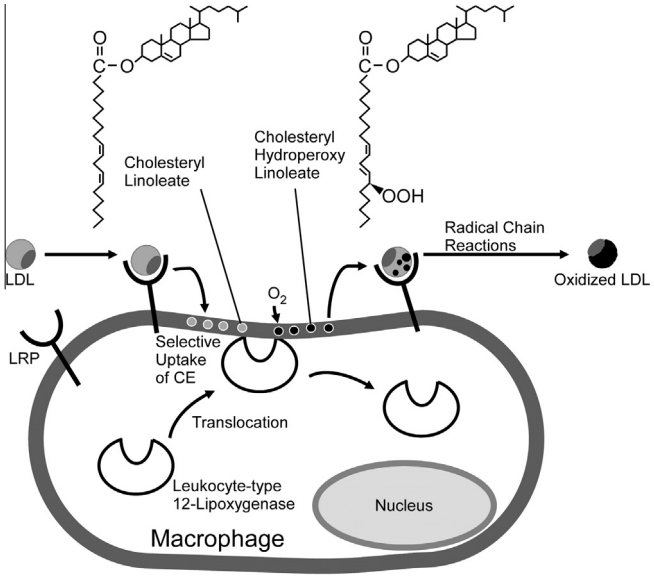


**Fig. 1.** Biochemical mechanism of oxidative modification of extracellular LDL by leukocyte-type 12-lipoxygenase in macrophages. CE, cholesteryl ester.

selective uptake of cholesteryl linoleate from the LDL particle to the cells as well as in the efflux of oxygenated cholesteryl linoleate from the cells to the LDL particles (Takahashi, Zhu, Xu, et al., 2005). The results suggest a critical role of LRP in the process of oxygenation of cholesteryl linoleate in LDL particles by the leukocyte-type 12-lipoxygenase in macrophages (Fig. 1).

## 2. Guava tea leaves inhibit leukocyte-type 12-lipoxygenase activity

Guava tea that is extracted from guava leaves contains a number of polyphenols, and is commonly taken as a dietary supplement. The extracts show a variety of beneficial effects such as antioxidative, anti-inflammatory and antiproliferative activities (Gutiérrez, Mitchell, & Solis, 2008). It is reported that phytochemicals including flavonoids which have these effects modulate the catalytic activities of cyclooxygenases and lipoxygenases (Kawakami et al., 2009; Sadik, Sies, & Schewe, 2003; Yamamoto et al., 2005). We recently showed that quercetin, quercetin glycosides and ellagic acid in guava tea leaves were major ingredients inhibiting the catalytic activity of cyclooxygenase isoforms (Kawakami et al., 2009). We also demonstrated that the catalytic activity of leukocyte-type 12-lipoxygenase as well as LDL oxidation mediated by the enzyme-overexpressing murine macrophage-like J774A.1 cells was inhibited by guava tea leaves (Kawakami et al., 2012). Administration of extracts from guava tea leaves to apoE-deficient mice significantly attenuated atherogenic lesions in the aorta and aortic sinus. The major components showing inhibitory effects on the leukocyte-type 12-lipoxygenase activity were identified as quercetin and ethyl gallate (Fig. 2) which inhibited the enzyme activity with the $IC_{50}$ values of 1.6 and 0.83 μM, respectively (Fig. 3).

## 3. Chinese tea leaves inhibit leukocyte-type 12-lipoxygenase activity

Chinese teas extracted from a variety of Chinese plant leaves are taken for health benefits in South China. Catechins such as epigallocatechin gallate and epicatechin gallate contained in tea leaves are known to show beneficial effects on inflammation and

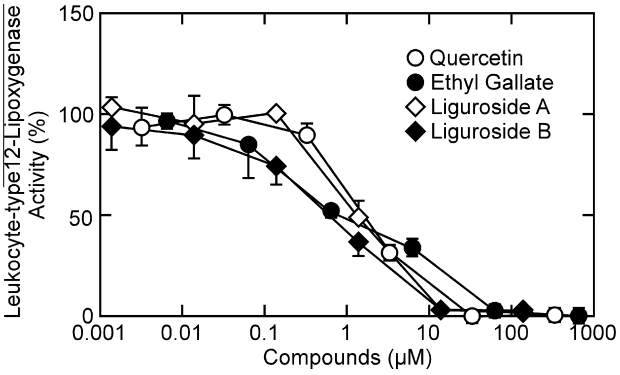


**Fig. 3.** Inhibition of leukocyte-type 12-lipoxygenase activity by quercetin, ethyl gallate and ligurosides A and B. Leukocyte-type 12-lipoxygenase partially purified from mouse macrophage-like J774A.1 cells overexpressing the enzyme (Kawakami et al., 2012) was incubated with 25 μM arachidonic acid at 30 °C for 5 min in 100 mM Tris–HCl buffer (pH 7.4) in the presence of quercetin (open circles), ethyl gallate (closed circles), liguroside A (open lozenges) or liguroside B (closed lozenges) at indicated concentrations. The products reduced with glutathione and glutathione peroxidase were quantified using reverse-phase HPLC monitoring at 235 nm. Relative enzyme activity of leukocyte-type 12-lipoxygenase as compared with the activity without inhibitors are shown. The data represent means ± SD of triplicate experiments.

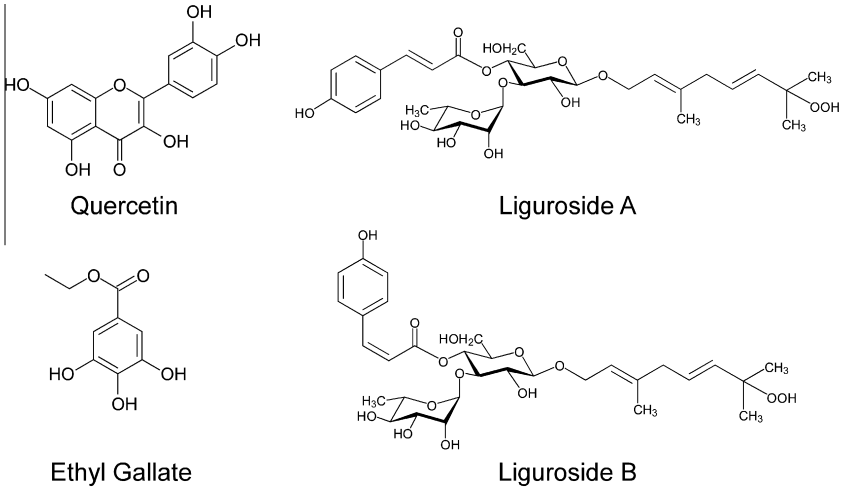


**Fig. 2.** Structures of quercetin, ethyl gallate and ligurosides A and B.

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