Effects of a potent antioxidant randaiol and its derivatives on ROS-induced cellular damage

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ROS 誘発性細胞障害に対する強力な抗酸化ポリフェノール randaiol 及びその誘導体の作用

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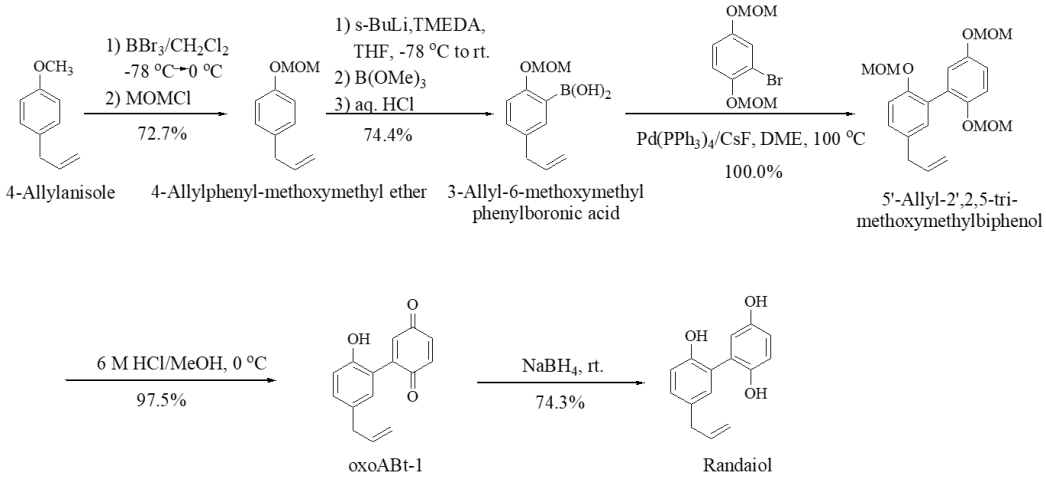
Background:

Reactive oxygen species (ROS) are formed as inevitable byproduct of the normal metabolism of oxygen. The homeostasis of ROS is extremely important for health. Moderate ROS levels are required for many vital physiological processes. However, high ROS levels are detrimental to life. When the balance between production and degradation of ROS cannot maintain, large amounts of ROS are accumulated. These overproduced ROS are highly reactive, and can cause lipid peroxidation, protein oxidation, and DNA damage, finally leading to cell dysfunction and death, which are widely considered to be the main causes of various diseases including diabetic complications and neurodegenerative diseases.[1, 2] Though it is still unclear how natural defense mechanisms against ROS lose effectiveness, current studies showed that antioxidants can demonstrate huge beneficial effects on ROS-involved damage both in vitro and in vivo.[3] Due to the potential therapeutic uses of antioxidant in ROS-induced diseases, the antioxidant with more efficient function are expected to be found. Medicinal herbs are important resources for discovery of natural antioxidant products. *Magnolia officinalis*, a *Magnolia* species plant, has showed antioxidant effect in malondialdehyde (MDA) scavenging activity-guided screening. In addition, it has also been known to have antitumor action and neuroprotective activities, and show protective effects on the heart and the liver.[4] Here, randaiol, a powerful antioxidant, was isolated from *M. officinalis* by DPPH activity-guided separation. Randaiol is a minor lignin of *M. officinalis*, and its anti-hepatitis B activity, NO production and superoxide anion scavenging action have been reported in recent years.[5] However, the effects of randaiol on ROS-related injury are quit limited so far. In this study, the effects of randaiol on ROS-induced vascular injury and inflammatory response in microglial function were investigated. Furthermore, structure-activity relationship and the mechanisms of randaiol activity were compared using its derivatives.

Methods:

The synthesis of randaiol and its derivatives: Commercially available 4-allylanisole was used to produce randaiol. First, the methyl group of 4-allylanisole was converted to methoxymethyl ether (MOM) group to give 4-allylphenyl-methoxymethyl ether. Methyl group was removed by using BBr_3 in CH_2Cl_2 solvent. Then the C2 position was hydroborated to obtain 3-allyl-6-methoxymethyl phenylboronic acid by using s-BuLi. This reaction proceeds at temperatures of -78 °C. 5'-allyl-2', 2, 5-

trimethoxymethylbiphenol was synthesized by Suzuki coupling 3-allyl-6-methoxymethyl phenylboronic acid and MOM-protected bromohydroquinone, followed by deprotection of the hydroxyl groups. Finally, the quinone group was reduced to obtain synthetic randaiol (Scheme 1).



Scheme 1 Synthesis of randaiol

And ten species derivatives were also synthesized from 4-allylanisole for observing structure-activity relationship. They are A ring derivatives (MABt-1, PBt-1), mono-hydroxyl derivatives (ABm-1), di-hydroxyl derivatives (ABd-1, ABd-2, ABd-3) tri-hydroxyl derivatives (ABt-2, ABt-3), and quinone (oxoABt-1) (Fig. 1).

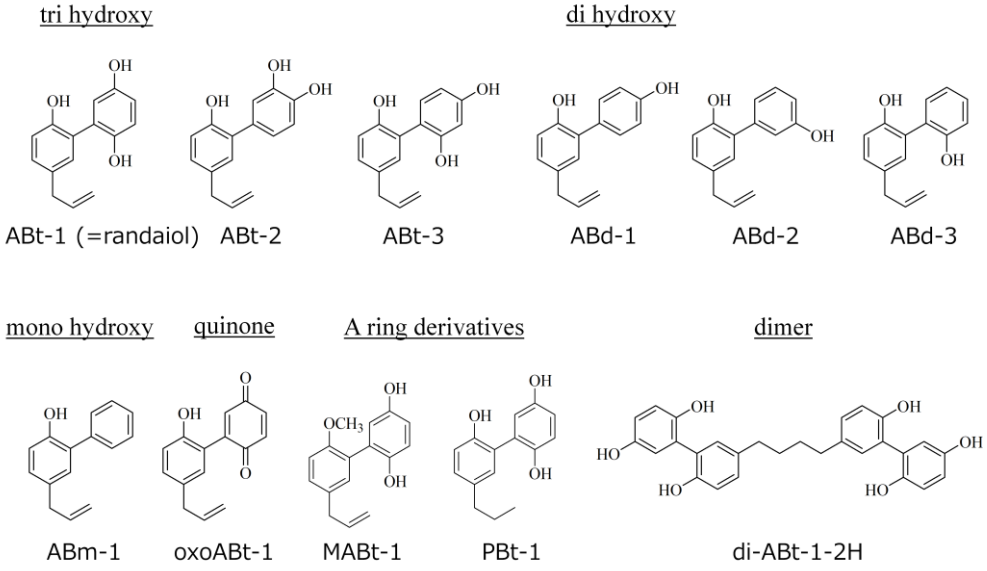


Fig. 1 Structures of randaiol's derivatives

Free-radical scavenging capacity and anti-apoptosis/anti-inflammatory activities: DPPH and Galvinoxyl assay was used. For functional analyses, Hela cells, imoHUVEC cells, microglial cell line, MG6, or primary cultured microglial cells were used. Quantitation of cytokine mRNA was examined by real time RT-PCR method. Cell viability was determined by MTS assay. The levels of protein were observed by immunofluorescence assay.

Results:

In DPPH assay, free-radical scavenging capacity of randaiol and its derivatives decreased in the following order; di-ABt-1-2H > ABt-2 > ABt-1 (randaiol) > ABt-3 > MABt-1 > PBt-1 > oxoABt-1 > ABd-1, ABd-2, ABd-3, ABm-1. In Galvinoxyl assay, the strength of antioxidant capacity of each compound is similar to DPPH radical scavenging activity.

Firstly, the effects of randaiol and its derivatives on methylglyoxal-induced cell damage were detected. As a result, ABt-1 and its A ring derivatives, tri-hydroxyl derivatives and dimer showed direct strong free radical scavenge abilities. ABt-1, catechol derivative (ABt-2), and resorcinol type derivative (ABt-3) inhibited t-BHP-induced apoptosis in Hela cells. However, only ABt-3 could attenuate the detrimental effect of methylglyoxal, a ROS inducer, on cell viability. Moreover, ABt-3 upregulated survivin protein level in methylglyoxal-treated Hela cells. In addition, this protective effect was not observed in imoHUVEC, an immortalized human umbilical vein endothelial cell line.

Secondly, the effects of randaiol and its tri-hydroxyl derivatives on lipopolysaccharide (LPS)-induced microglial activation were observed. All ABt-1, ABt-2 and ABt-3 showed antioxidant effects and increased expression of HO-1 and NOQ1 in LPS-treated microglial cells. Besides anti-oxidative effects, ABt-1 and ABt-2 potently inhibited expression of TNF-α and IL-1β mRNA in LPS-stimulated MG6 cells in a dose-dependent manner. Furthermore, ABt-1 and ABt-2 upregulated Hsp72 gene expression. ABt-1 could decrease IL-1β and enhance Hsp72 level, while expression of TNF-α was increased by treatment of ABt-1 in LPS-treated primary cultured microglia. On the other hand, ABt-3 inhibited neuronal cell death induced by LPS-treated microglia, though it did not affect expression levels of inflammatory cytokine mRNAs

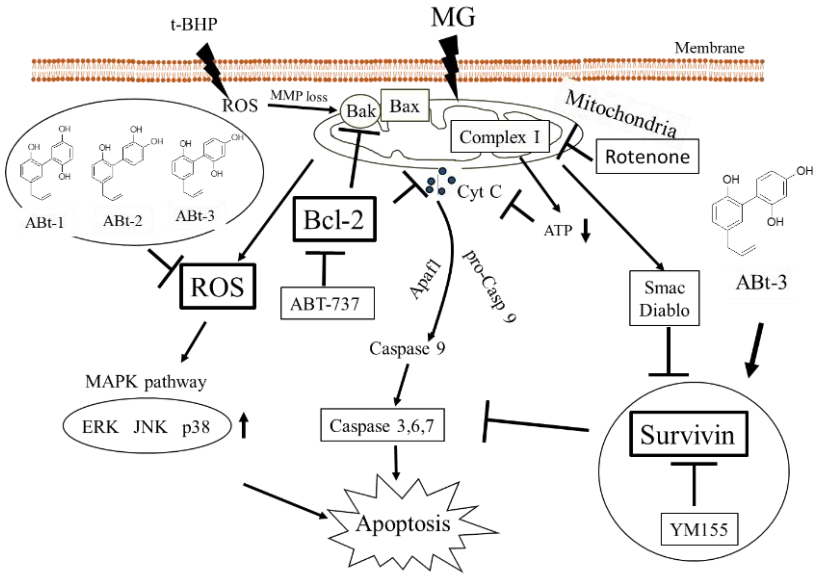


Fig. 2 Effects and mechanisms of ABt-1, ABt-2, ABt-3 on methylglyoxal (MG)-induced cell injury

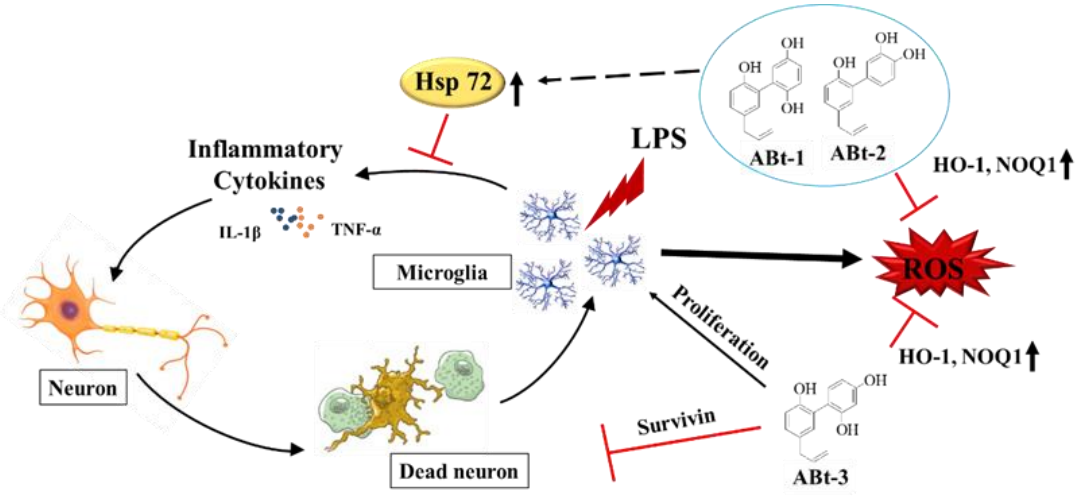


Fig. 3 Effects and mechanisms of ABt-1, ABt-2, ABt-3 on LPS-induced microglial neurotoxicity

Conclusion:

According to DPPH and Galvinoxyl radical-scavenging capacity assay, it seems that antioxidant potential is not only in direct correlation with the number of free hydroxyl groups, but also relative to the position of hydroxyl groups.

In addition, they show different activities with similar antioxidant properties, suggesting that anti-apoptotic and anti-inflammatory effects of randaiol and its derivatives do not correlate with their antioxidant properties. Anti-apoptotic effect of ABt-3 in Hela cells is mediated by upregulating survivin protein level (Fig. 2). Antioxidant effects of ABt-1, ABt-2 and ABt-3 in microglia cells are mediated by increasing expression of HO-1 and NOQ1. Furthermore, ABt-1 and ABt-2 may attenuate inflammation by upregulating Hsp72 in microglia (Fig. 3).

Finally, for using antioxidants against ROS-related disease therapy effectively and safely, further study is needed to clarify the mechanism of their action.

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