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

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論文題名　：**Effects of a potent antioxidant randaiol and its derivatives on ROS-induced cellular damage**　（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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論　文　内　容　の　要　旨

Background: The homeostasis of ROS is extremely important for health. 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, DNA damage, finally lead to cell dysfunction and death which are widely considered to be the main reasons of various diseases.[1, 2] Though, to date, 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 and lower molecular weight are expected to be found. Here, we focused on a powerful antioxidant, randaiol, isolated from *Magnolia officinalis* by TLC-DPPH activity-guided separation. In this study, we investigated the effects of randaiol on ROS-induced vascular injury and microglia-neuron interaction. Furthermore, structure-activity relationship and mechanisms of randaiol's properties was observed using its derivatives.

Material & Methods: The synthesis of randaiol and its derivatives begins from commercially available material 4-Allylanisole. Quantitation of cytokine mRNA was examined by Real time RT-PCR method. The cell viability was determined by MTS assay. The level of protein was observed by immunofluorescence assay.

Results: All of randaiol (ABt-1) and its A ring derivatives, tri-hydroxyl derivatives and dimer shown direct strong free radical scavenge abilities, and ABt-1, catechol derivative (ABt-2), resorcinol type derivative (ABt-3) can inhibit t-BHP-induced apoptosis in Hela cells. However, only ABt-3 can 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 wasn't observed in imoHUVEC. All of ABt-1, ABt-2 and ABt-3 showed antioxidant effects in primary microglia cells and increased expression of HO-1 and NOQ1 in LPS-treated microglia 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, they were observed to upregulate Hsp72 gene expression. ABt-1 and ABt-2 can decrease IL-1β and enhance Hsp72 level in primary microglia cells, however expression of TNF-α was increased by treatment of ABt-1 and ABt-2. On the other hand, though ABt-3 showed no effect on the expression levels of cytokine mRNAs, it inhibited neuronal cell death induced by LPS-activated microglia.

Conclusion: These results demonstrated that ABt-3's anti-apoptotic effect in Hela cells is mediated by upregulating Survivin protein level. Antioxidant effects of ABt-1, ABt-2 and ABt-3 in primary 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 MG6 microglia cells. Because they show different activities with similar antioxidant properties, it suggests that anti-apoptotic and anti-inflammatory effects of randaiol and its derivatives do not correlate with their antioxidant properties. Finally, due to the complicated results in normal cells and primary cells, the antioxidants should be used with caution.

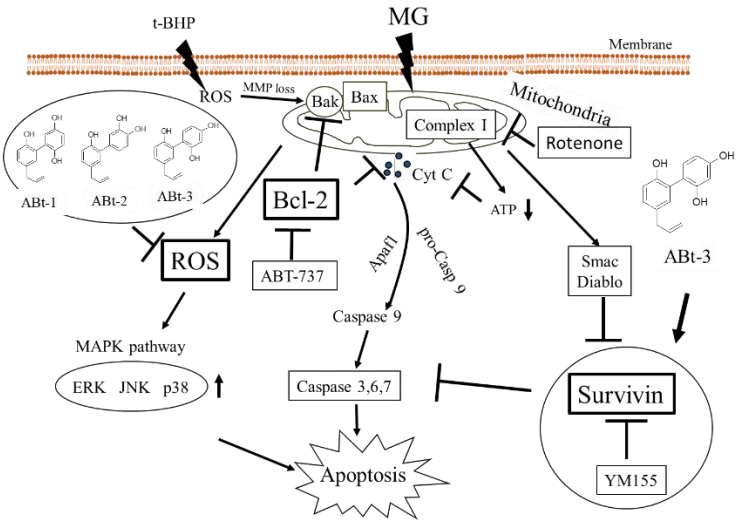

Fig. 1 Effects and mechanisms of ABt-1, ABt-2, ABt-3 on methylglyoxal-induced cell injury

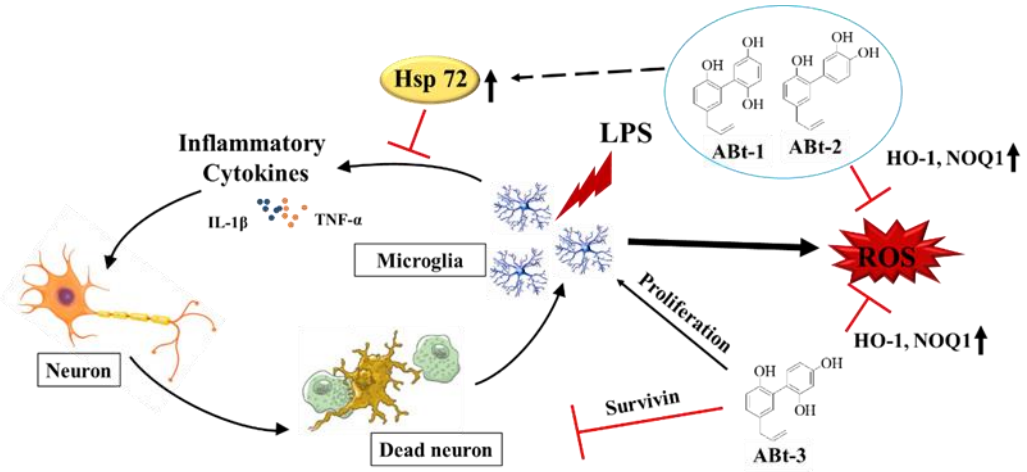

Fig. 2 Effects and mechanisms of ABt-1, ABt-2, ABt-3 on LPS-induced microglia-neuron interaction

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