Anticancer effect of protocatechuic aldehyde

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Introduction

Protocatechuic aldehyde (3,4-Dihydroxybenzaldehyde; DHBZ) is a major compound found in the root of *Salvia miltiorrhiza* (Figure 1) which is a herbal plant widely used in Traditional Chinese Medicine for treatment of cardiovascular and liver diseases (1). The structure of protocatechuic aldehyde is similar to vanillin which has been shown to inhibit metastasis of mouse breast cancer cells (4T1 cells) *in vivo* (2). In this study, we demonstrate anticancer effect of protocatechuic aldehyde and structurally-related compounds including vanillin, vanillic acid, and protocatechuic acid, and also investigated mechanism underlying apoptotic inducing effect of the compounds in 4T1 cells.

Figure 1. Morphology of *Salvia miltiorrhiza* and structure of protocatechuic aldehyde.

Methods

Chemicals: **Protocatechuic aldehyde, protocatechuic acid, vanillin, and vanillic acid were dissolved in dimethyl sulfoxide (DMSO) and kept at −20 °C as stock solution.**

Cell culture: **4T1 mouse mammary adenocarcinoma cell line was obtained from the American Type Culture Collection (ATCC), and cultured in DMEM supplemented with 10% fetal calf serum and antibiotics. The culture was maintained at 37 °C in a humidified atmosphere of 5% CO_2.**

Cell viability assay: **Cells were cultured and treated with the test compounds for 2 days in 96-well plates. Cell viability after treatment was determined using a WST-1 Assay kit.**

Western blot analysis: **Cell lysates were separated by 7.5% SDS-PAGE and transferred to PVDF membranes. Membranes were incubated with the primary antibodies at 4 °C overnight, followed by secondary antibodies conjugated with horseradish peroxidase for 1 h at room temperature. Bands were visualized by chemiluminescence.**

Results and Discussion

Protocatechuic aldehyde differs from vanillin at a functional group at C-3 position (Figure 2), IC_{50} values for protocatechuic aldehyde (DHBZ), protocatechuic acid (DHBA), vanillin (VNL), and vanillic acid (VNA) were 21.3 µM, 1.4 mM, 4.0 mM, and >8.0 mM, respectively (Figure 3). The IC_{50} value of protocatechuic aldehyde was lower than IC_{50} of vanillin ~187 fold, indicating that mechanisms underlying anti-proliferative effect of both compounds are difference.

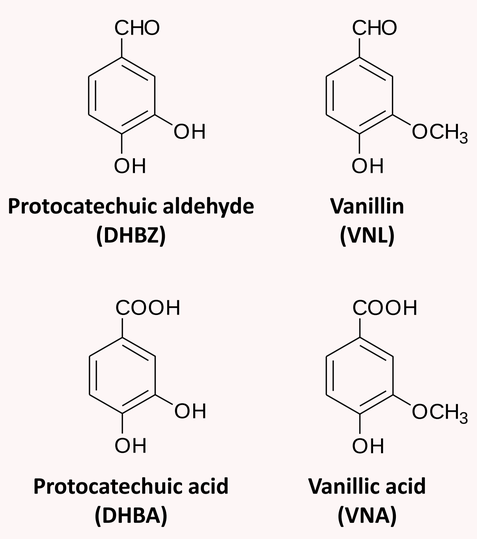

Figure 2. Structure of protocatechuic aldehyde and related compounds.

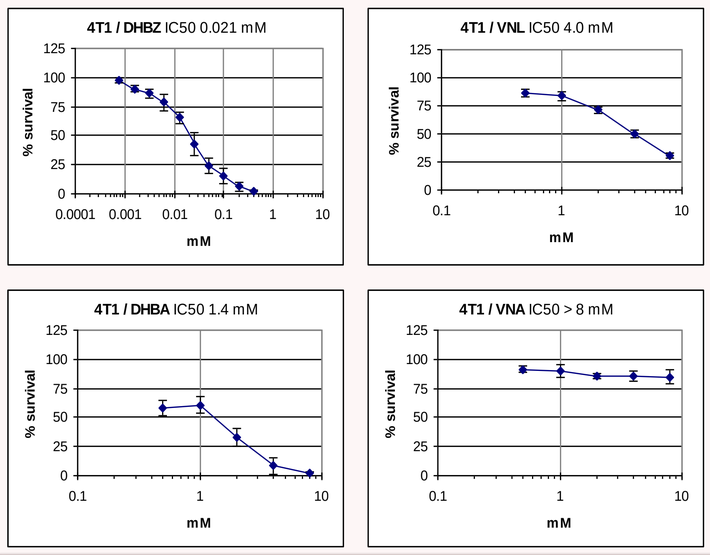

Figure 3. Anti-proliferative effect of the compounds against 4T1 cells were evaluated by WST-1 assay.

Caspase-3 activation and PARP cleavage are key events in apoptosis. The caspase-3 activation and PARP cleavage were observed in 4T1 cells treated with protocatechuic aldehyde or vanillin, and the effects could be detected after 12 h treatment (Figure 4).

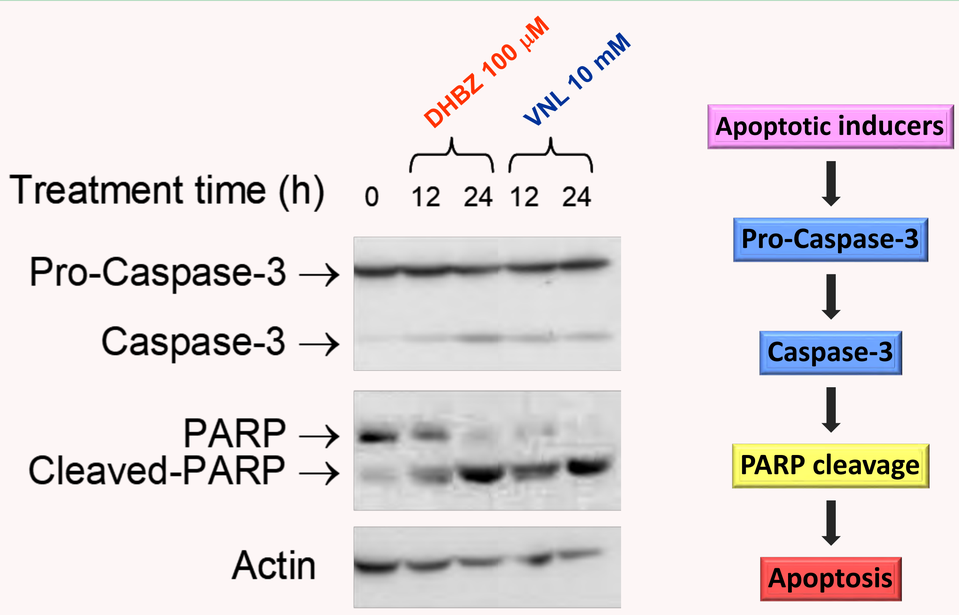

Figure 4. Apoptosis-inducing effect of protocatechuic aldehyde and vanillin.

MAP kinases and PI3K/Akt signaling pathways are involved in apoptosis regulation. Activation of JNK and p38 are participated in induction of apoptosis, whereas activation of ERK and PI3K/Akt promote cell survival and suppress apoptosis, cell fate is depended on the balance between these signaling pathways (Figure 5).

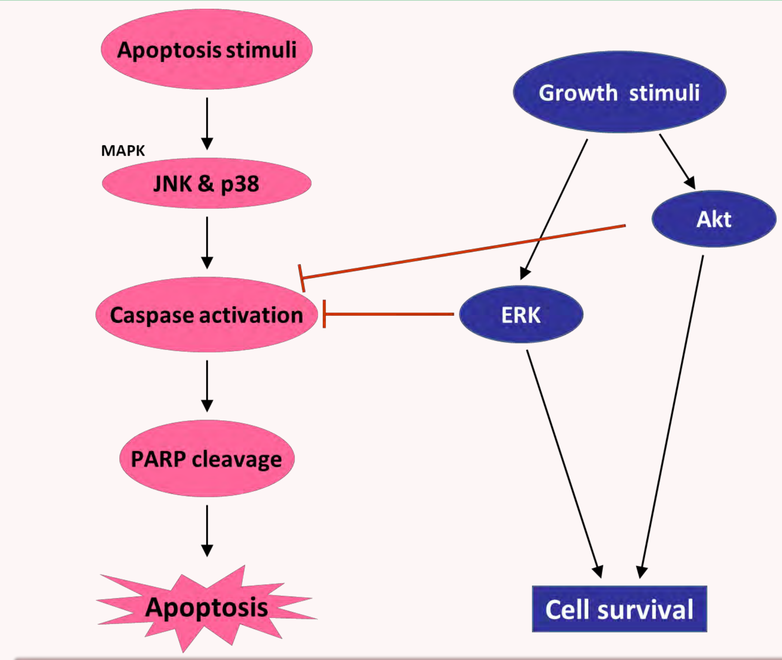

Figure 5. Regulation of apoptosis by Akt and MAPK signaling.

Effects of protocatechuic aldehyde and vanillin on the signaling pathways were determined by western blot analysis. Both protocatechuic aldehyde and vanillin inhibited Akt phosphorylation, but had no effects on p38 phosphorylation. The difference between these two compounds was the effects on ERK and JNK phosphorylation. Protocatechuic aldehyde inhibited ERK phosphorylation and activated JNK phosphorylation (Figure 6). In contrast, vanillin did not inhibit ERK phosphorylation and did not activate JNK phosphorylation.

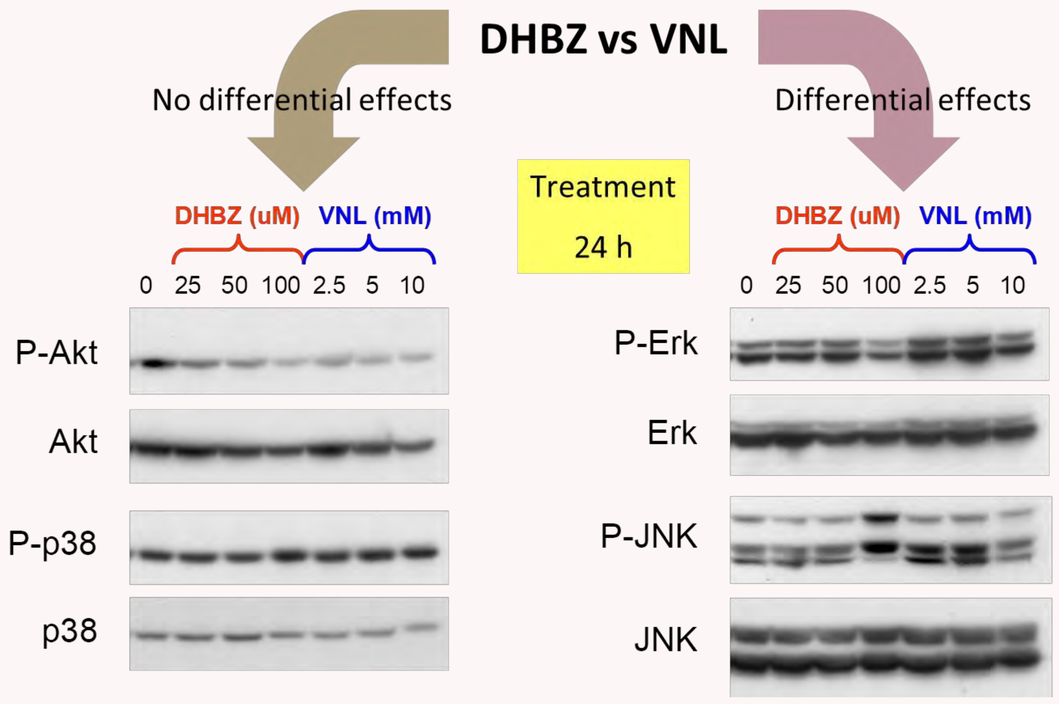

Figure 6. Effects of protocatechuic aldehyde and vanillin on Akt and MAPK signaling in 4T1 cells.

Conclusions

1. **Protocatechuic aldehyde possessed a strong anticancer activity.**
2. **The caspase-3 activation and PARP cleavage were observed in 4T1 cells treated with the protocatechuic aldehyde or vanillin, indicating cells undergo apoptosis.**
3. **The anti-proliferative effect of protocatechuic aldehyde and vanillin was due to induction of apoptosis, by interfering on MAP kinases and PI3K/Akt signaling pathways.**
4. **Protocatechuic aldehyde inhibited ERK phosphorylation and activated JNK phosphorylation, whereas vanillin did not have such effects. These might explain the large fold difference (~187 fold) between IC_{50} values of the two compounds.**

References

1. **Ding, M., Ye, T.X., Zhao, G.R., Yuan, Y.J., Guo, Z.X., 2005. Aqueous extract of *Salvia miltiorrhiza* attenuates increased endothelial permeability induced by tumor necrosis factor-α. Int. Immunopharmacol. 5, 1641-1651.**
2. **Lirdprapamongkol, K., Sakurai, H., Kawasaki, N., Choo, M.K., Saitoh, Y., Aozuka, Y., Singhirunnusorn, P., Ruchirawat, S., Svasti, J., Saiki, I., 2005. Vanillin suppresses in vitro invasion and in vivo metastasis of mouse breast cancer cells. Eur. J. Pharm. Sci. 25, 57-65.**

Acknowledgements

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