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Effect of Korean mistletoe (*Viscum album coloratum*) on the non-specific immune responses in eel (*Anguilla japonica*)

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Introduction

Mistletoe is a semi-parasitic woody perennial commonly found growing on oaks and other deciduous trees. Mistletoe (*V. album*) has been used widely for therapeutic purpose in Europe as well as in Korea. Mistletoe species containing lectins activates macrophages and lymphocytes, leading to secretion leading to secretion of various leading to secretion of various of various kinds of cytokines. The present work was performed to investigate the effect of Korean mistletoe on the non-specific immune responses in eel.

Materials and Methods

In *in vivo* test, three eel in each group were intraperitoneally injected with PBS as control, 0.5mg and 1mg of mistletoe, respectively. At day 2 post-injection, blood and head kidney cells were obtained from individual group. The kidney cells were adjusted at a volum of 1×10^6 cells/well followed by rechallenging with or without mistletoe (0.5mg/ml) in 5%FBS-MEM. And the cells were incubated at 25°C for 24h. For *in vitro* effect of mistletoe, eel kidney cells (1×10^6 cells/well) were suspended in the 5%FBS-MEM containing 0.5, 1, 10, 25, 50, 100, 200, or 500 μ g/ml of mistletoe and cultured at 25°C for 24h. To measure ROI production, cultured cell medium was removed and covered with NBT.

The reduced formazan was solubilized with KOH/DMSO and OD values were read at 620nm in an ELISA reader. Also, supernatants from individual well were collected and assayed for nitrite using the Griess reagent at 550nm in an ELISA reader. Lysozyme activity was based on a microtitre plate version of the *Micrococcus lysodeikticus* (0.15mg/ml of 0.066M PBS) turbidimetric assay and measured at 450nm in a spectrophotometer. To study phagocytosis of eel macrophage, eel

kidney cells previously sensitized by mistletoe were collected and cultured with zymosan in 5%FBS-MEM followed by Wright's stain.

Results and Summary

The level of ROI production was highly augmented in kidney cells from eel injected with 0.5 or 1mg of mistletoe compared with control. At higher concentration of mistletoe, ROI product was much more expressed. *In vitro* effect of mistletoe, kidney cells cultured in 5%FBS-MEM containing 50µg/ml of mistletoe more strongly induced the production of ROI than at other concentrations of mistletoe. NO production, however, was not detected *in vivo* as well as *in vitro*. Interestingly, as kidney cells were rechallenged with 0.5mg/ml of mistletoe, ROI production was suppressed. In eel injected with mistletoe, both lysozyme activity and phagocytosis were significantly higher than the control. In conclusion, the present results indicate that the Korean mistletoe effectively induce the non-specific immune response in eel. Therefore, Korean mistletoe would be utilized as a good adjuvant for cultured fish.

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