MASTER'S THESIS

Mechanistic study of type I ribosome-inactivating protein as anti-influenza and anti-tumour agent

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Mechanistic Study of Type I Ribosome-inactivating Protein as Anti-influenza and Anti-tumour Agent

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Abstract

Ribosome-inactivating proteins (RIPs) such as trichosanthin (TCS) have been used as antiviral and anti-tumour agents. The mechanistic study of antiviral and anti-tumour action of single chain (type 1) RIPs was investigated in this thesis.

An indirect immunotoxin was constructed by conjugating the RIPs, trichosanthin (TCS), with goat immunoglobulin (IgG). The IC_{50} of TCS-IgG immunotoxin (0.042 μM) is 10-fold higher than the native TCS (0.004 μM) in a cell-free protein synthesis inhibition assay. The antiviral activities of TCS-IgG immunotoxin on influenza A/NWS/33 virus infected Madin Darby canine kidney (MDCK) cells were examined. In the absence of immunotoxin, over 90 % of MDCK cells were killed two days after influenza virus infection. However, addition of antiviral hyperimmune serum and TCS-IgG immunotoxin (11.3 μM) resulted in a significant reduction of the cytopathic effect of the influenza virus on the infected MDCK cells. Protective effect was not observed when monoclonal anti-matrix protein antibody was used as the primary antibody. The failure on the reduction of cytopathic effect was related to the localization of matrix protein which was presented only in the cytoplasm but not in the membrane of virus infected cells. These results suggest that the indirect TCS-IgG immunotoxin may be used as an antiviral agent. Neotrichosanthin (NTCS), an isoform of TCS, and its site-directed muteins were used for immunotoxin construction. The NTCS muteins, Y70A mutein (the tyrosine residue at position 70 being replaced by alanine) has a reduced N-glycosidase activity while R163H (arginine residue at

position 163 being replaced by histidine) has an increased DNase activity. The antiviral activity of NTCS-IgG immunotoxin and R163H-IgG immunotoxin were similar to TCS-IgG immunotoxin. But the antiviral activity of Y70A-IgG immunotoxin was the lowest because of the reduced N-glycosidase activity. Results indicates that N-glycosidase activity was responsible for the antiviral activity of the immunotoxins constructed by RIPs.

Anti-tumour activity of RIPs such as TCS has previously been reported. Results indicated that PU-5 cells (a mouse macrophage cell line) were more sensitive towards TCS than L929 cells (a mouse fibroblast cell line). To study the mechanistic actions of TCS or related RIPs on tumour cells, the uptake rate of TCS was examined by confocal laser scanning microscopy and spectrofluorometry. The uptake rate of TCS towards both PU-5 and L929 cells has no significance differences. Within 30 minutes, TCS was found to rapidly internalize into both PU-5 and L929 cells and localized in the cytoplasm. To further characterize the contribution of its enzymatic activity towards the cytotoxic effect, neotrichosanthin (NTCS), an isoform of TCS, and its site-directed muteins were used. The NTCS muteins, Y70A mutein (the tyrosine residue at position 70 being replaced by alanine) has a reduced N-glycosidase activity while R163H (arginine residue at position 163 being replaced by histidine) has an increased DNase activity. Although, R163H mutein possess higher DNase activity, there is no significant differences between the cytotoxicity of R163H mutein and NTCS. However, the reduced N-glycosidase activity in Y70A mutein leads to lower cytotoxicity of Y70A mutein. As a result, the differential cytotoxicity of RIPs towards different tumour cell lines is independent of the uptake rate. And N-glycosidase activity is responsible for the anti-viral and anti-tumour activities of type 1 RIPs.

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