The Effect of Combination Treatment Of Anticancer Agents with Reactor Produced Radioisotope on Cytotoxicity in-vitro

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INTRODUCTION

The radioisotope, ^{166}Ho was chosen because ^{166}Ho has a 26.8 hour half-life and decays with the emission of β particles with energies of 1.77 MeV (48 %) and 1.85 MeV (51 %), which are suitable for cancer treatment. For the development of new controlled drug delivery systems, the application of a combination therapy using radioisotopes and tumor static agents has drawn great attention. This approach would be very beneficial for cancer treatment especially when a new drug delivery system utilizing biodegradable polymers is developed. Therefore, the present study has been focused on the manifestation of the mechanism for the cellular apoptotic effects when the combination therapy of radiation and anti-cancer agents was carried out.

EXPERIMENTAL METHODS

As chemotherapeutic agent, paclitaxel was applied to achieve synergistic tumoricidal effects. For an *in-vitro* cytotoxicity study, several tumor cell lines were used. The cell lines were MKN45, Hep3B, NIH-ovcar3, Calu6, C6, L929, SNU719 and T98G. An apoptosis study was done by the Tunnel assay. Also for the manifestation, to obtain a precise mechanism of the tumoricidal effects upon a combination therapy, the alterations in the genetic materials were measured through molecular biological method, western blot. A dose response study was also done with drugs from nM to mM concentrations for the cytotoxicity study followed by 48 hrs of incubation. In the apoptosis study, Ho-166 (10μCi /ml) and IC_{20} of each agent were treated followed by a 24 hr incubation to detect early apoptosis. Also the western blotting assay was carried out to obtain the effects on the genetic material such as p53 and Fas gene.

RESULTS AND DISCUSSION

In-vitro cytotixicity showed that paclitaxel had a cytotoxic effect when treated in the concentration range OfnM to mM.

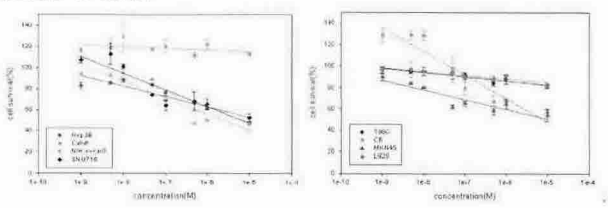

Figure 1. A dose response effect of cytotoxicity of paclitaxel.

In the combination treatment study, a significant synergistic effect on the cell cytotoxicity was obtained in T98G ($p<0.01$) with the treatment of Ho-166 and paclitaxel compared to a single treatment of either Ho-166 or a drug only.

Figure 2. Combination treatment effect on cytotoxicity

In addition, the Tunnel assay was carried out for the early apoptotic modality of drugs and beta-emitters. When a cell was treated with either drugs or holmium-166, no significant cell death was noticed. However, when the tumor cells went into the combination treatment, results showed that more cell death was induced compared to single treatment only. Therefore, the mechanism of the inhibition of cell proliferation of the drugs and radioisotopes was due to the enhancement of the apoptosis in the early cell death, especially in hepatoma and ovary tumor cell lines.

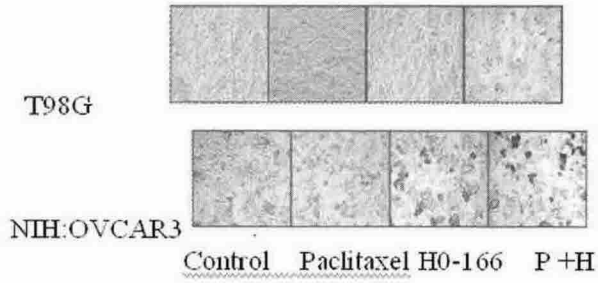

Figure 3. Early apoptotic effect of the combination treatment on T98G and NIH:ovcar3.

According to the results from the western blot, a significant increase was shown in the Fas and p53 gene contents when the cells were treated with Paclitaxel and Holmiun-166 in T98G and NIH:OVCAR3 cells.

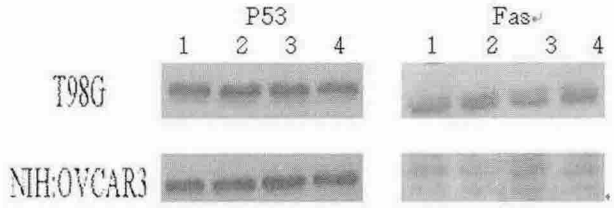

Figure 4. Western bolt analysis showed the responsive expression of Fas and p53 to Paclitaxel treatment. T98G and NIH:OVCAR3 cells were treated with Palitaxel(2), Holmium(3) and Paclitaxel with Holmium(4). The concentration of drugs was IC_{20} (M) and Ho-166 was 10 μ Ci/ml. (1: Control, 2: Paclitaxel, 3: Ho-166, 4: Paclitaxel+Ho-166)

CONCLUSION

Taken altogether, it can be postulated that the combination treatment of radioisotope, β-emitters, with other drugs would produce the synergistic effect in tumor static effects and this synergism would be exerted via inducing on early apoptosis through the activation of a cell death receptor. In conclusion, the combination therapy would be very beneficial to cancer treatment overcoming not only an unnecessary exposure to high a radiation level during radiation therapy but also a drug resistance caused by chemotherapy.

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