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Clofilium induces apoptosis of human promyelocytic leukemia (HL-60) cells via BCl-2 insensitive activation of caspase-3 protease

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Purpose: Clofilium, potassium channel blocker, which used as an antiarrhythmic drug has been reported that it has exerted antiproliferative action and apoptotic effect to the human leukemias and lymphomas. The purpose of this study is to verify these effects of clofilium on human promyelocytic leukemia (HL-60) cells and to clarify the action of mechanism on clofilium-inducing apoptosis.

Methods: Untreated and treated HL-60 cells were evaluated for antiproliferative effect and apoptosis using cell viability test, DNA fragmentation, comet assay, flow cytometry with annexin V and propidium iodide (PI), and light and electromicroscopic examination. To clarify the action of mechanism, we also performed immunoblotting assay for Bcl-2 and Bax proteins and assessed the activity of caspase-3 protease using flourometric immunoabsorbent enzyme assay (FINEA).

Results: HL-60 cells treated with clofilium led to suppression of viability and proliferation in both time and concentration-dependent manners (approximately 50% suppression in 2.5 uM of clofilium by 72 hr). The morphologic examinations with light and electromicroscopies revealed typical morphologic features of apoptosis in cells treated with clofilium which was further verified in DNA fragmentation on cells treated with 20 uM of clofilium for 24 hr. Flow cytometry analysis with annexin V and PI revealed that cells treated with 20 uM of clofilium shifted to early and late apopotic zone at 16 and 24 hr, respectively. Comet assay also demonstrated that the percentage of apoptotic cells increased approximately up to 30% in cells treated with 20 uM of clofilium for 24 hr. Furthermore, FINEA assay for activity of caspase-3 protease showed 10 times increase of activity in cells treated with 20 uM of clofilium for 2 hr compared with basal level of its activity in untreated cells. However, there was no significant change of Bcl-2 and Bax proteins using immunoblotting analysis.

Conclusions: These results indicate that clofilium exerts antiproliferative and apoptosis -inducing activity in HL-60 cells which mediate via bcl-2 insensitive activation of caspase-3 protease and might have a potent activity of cancer chemotherapy in human leukemias.

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Expression of lung resistance protein/human major vault potein (LRP/hMVP) is inducible by therapyassociated stress factors such as cytostatic drugs and heat in human colon carcinoma cells

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Purpose: Cancer therapy-associated stress factors may induce the expression of multidrug resistance (MDR)-associated genes which could result in the acquirement/enhancement of the MDR phenotype. Therefore, we investigated the influence of cytostatic drugs and of heat on the expression of the MDR-associated protein LRP/hMVP.

Methods: In order to mimic the clinical situation of the treatment of colon cancer with chemotherapy or hyperthermia two colon carcinoma cell lines (highly resistant HCT15, moderate resistant HCT116) were treated with cytostatics (adriamycin or vincristine) or heat (43°C 2 h). Expression of LRP/hMVP was monitored up to 72 h or 120 h, respectively, by immuno flow cytometry using the monoclonal antibodies LRP-56 and LMR-5. Expression levels were evaluated by two control cell systems: SW1573 and SW1573/2R120 as well as GLC-4 and GLC-4/ADR.

Results: Expression of LRP/hMVP was found to be induced in both colon carcinoma lines by both therapy-associated factors with highest induction rates after 72 h by adriamycin (4 fold) and vincristine (3 fold). Heat-induced LRP/hMVP expression was up to 6 fold enhanced 120 h post heat shock.

Conclusion: The expression of the MDR-associated protein LRP/hMVP is inducible by cancer therapy-associated factors such as drugs and heat in a time-dependent manner.

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Analysis of antitumor mechanism of 1-OHP (Oxaliplatin) against established human gastric cancer cell lines

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Purpose: We analyzed of antitumor mechanism of l-OHP (Oxaliplatin) against established human gastric cancer cell lines.

Method: We used 5 human gastric cancer cell lines: MKN45, KATO-III, OKAJIMA, MKN28, MKN74. For them, in vitro chemosensitivity to l-OHP were studied with MTS assay. Induction of apoptosis to gastric cancer cells was analyzed by DNA ladder formation, DNA fragmentation and actin cleavage. Activation of MAPKs (ERK, SAPK, p38) were studies with specific antibodies to each active form.

Results: 1) All gastric cancer cell lines were sensitive to l-OHP. 2) Poorly differentiated ones were more sensitive than the others. 3) DNA ladder formation and/or DNAfragmentation were detected in all gastric cancer cell lines. 4) Actin cleavage was not detected in any gastric cancer cells. 5) Activation of SAPK was detected in all gastric cancer cell lines.

Conclusion: 1) l-OHP had anti-cancer efficacy for human gastric cancer cell lines especially for poorly differentiated adenocarcinoma, which means l-OHP would be effective for patients with poorly differentiated gastric cancer. 2) l-OHP induced the apoptosis to gastric cells, but actin cleavage was not detected. 3) SAPK was activated prior to cascade family protease activation. So, it was supposed that other pathway besides caspase cascade might be relate to apoptosis for gastric cancer cells treated by l-OHP.

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Molecular effects of cisplatin (CDDP) downstream of the formation of CDDP-adducts in the process of induction of apoptosis in human testicular carcinoma cell line 2102Ep

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CDDP is one of the most widely used anticancer agents with considerable acitivity in testicular and ovarian cancer. It is generally accepted that CDDP exerts it's cytotoxic effects by the formation of cisplatin-DNA-adducts, particularly GpG. However, it was recently reported that due to it's interaction with DNA CDDP induces apoptosis. Several lines of evidence indicate a general association between susceptibility towards apoptosis and response to chemotherapy. Using the 2102Ep human testicular carcinoma cell line as a model system we investigated the induction of apoptosis and molecular effects of CDDP downstream of the formation of CDDP-DNA-adducts. 2102Ep cells were treated for 2 h with an IC90 (30 μM) CDDP which was determined using the sulforhodamine B assay. Initial formation of CDDP-adducts by treatment of cells with 30 μM CDDP at 0 h was assessed using an CDDP-DNA-adduct-ELISA-assay. For measurement of apoptosis cells were harvested by trypsinization at 0 h, 4 h, 8 h, 24 h and 48 h and mixed with floating cells. Induction of apoptosis was detected using methylene blue staining, DNA-gelelectrophoresis and flowcytometric detection of apoptosis by the novel Apo2.7 antibody. Overexpression of p53 protein was detected by western blot. Induction of apoptosis became significantly detectable by all methodologies at 24 h post drug treatment. This was accompanied by a strong appearance of floating cells in the growth media. By separating floating from adherent cells the apoptotic signal could be ascribed to the floating cell population. Overexpression of p53 protein was detectable immediately after 2 h drug treatment with further increase up to 24 h post drug treatment. At 24 h post drug treatment a significant induction of caspase-3 catalytic activity the main effector of apoptotic proteolytic process was detectable. These results were summarized in a model illustrating the molecular downstream effects of CDDP treatment subsequent to induction of CDDP-DNA-adducts. In conclusion, the formation of CDDP-DNA-adducts seems to function as a trigger of downstream molecular events, subsequently leading to the induction of apoptosis and cell death.