Conclusion: We suggest the small basic penta-hexapeptides as a new class of biological response modifiers which can modulate both the metastatic properties of cancer cells and angiogenesis.

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Pattern of combine activity of 8-CL-cAMP and paclitaxel on the growth of murine melanoma in vitro and in vivo

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Purpose: 8-Cl-cAMP (ICN Pharmaceuticals) is site-selective cyclic AMP analogue that specifically down-regulates type I protein kinase A, involved in cell proliferation and neoplastic transformation. Paclitaxel (P) promotes microtubule assembly and stabilizes the tubulin polymers. Modern chemotherapeutic strategies are based on the combination of substances having different targets within malignant cells. Safety profile of 8-Cl-cAMP is not anticipated to have myeloablative effects, what makes it an ideal candidate for combination therapy with traditional cytotoxic treatment.

Material and methods: B16 cells were seeded in 96 well plates using standard RPMI 1640 medium supplemented with 10% fresh FBS. Cells were left for 24 hours to settle down, when were treated with 8-Cl-cAMP (1, 3 and 10 microM) or P (3, 10 and 30 nM) alone, and both in coincubation for 48, 72 and 96 hours of incubation period. Results were evaluated by SRB assay and expressed as a percent of growth inhibition. In vivo growth inhibition of B16 tumors in C57Black mice was evaluated using 65 and 100 mg/kg of 8-Cl-cAMP i.p., d 1- 14 and P 20 mg/kg, d 1-5, alone and in combination.The antitumour activity was examened by measuring tumor volume on days 5, 8, 11 and 14 and determining life span for each group. The analysis of combination treatments was made using the isobole method (combination index D for in vitro and interaction index I.I. for in vivo).

Results: We demonstrated that 8-Cl-cAMP, in a time- and dose dependent manner, inhibited growth of murine melanoma B16 in vitro, with IC50 value (7.6 microM) obtained after 72 hours incubation period. We further determined the IC50 values of P on B16 cell line (26, 7.83, 6.87 nM for 48, 72 and 96 hours respectively). The D values for low dose P (3 nM) showed no synergistic activity while at higher P concentrations (10 and 30 nM) showed mostly synergistic relations. Analysis of combination treatment on tumor growth in vivo, confirmed synergy between these two compounds.

Conclusion: Results indicate synergy between 8-Cl-cAMP and paclitaxel on murine melanoma growth in vitro and in vivo.

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Modulation of phospholipase d by hexadecylphosphorylcholine: a putative mechanism for its antitumoral activity

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Purpose: Hexadecylphosphorylcholine (HePC) belongs to the new family of alkylphosphocholines with anticancer activity. Its mode of action could be mediated by interference at the level of generation of lipid-derived second messengers or to the inhibition of the corresponding regulated enzymes. We have investigated the effect of HePC on two enzymes recently reported to play a role in cell growth proliferation such as phospholipase D (PLD) and choline kinase (ChoK).

Methods: Assays of ChoK and PLD activity were used. Analysis of protein levels were carried out by Western-blot of Hek 293T cells extracts trasiently transfested with different isoforms of PLD (PLD1 and PLD2).

Results: Treatment with HePC induces a rapid stimulation of PLD. Depending on the cell line investigated, activation of PLD by HePC may be achieved by PKC- dependent or independent mechanisms. PLD1 and PLD2 isoenzymes are sensitive to HePC activation. Furthermore, a chronic exposure of the cells to HePC abrogates the response of PLD to stimulation by either phorbol esters or HePC itself with no effect on total cellular PLD levels. By contrast, no effect was observed by HePC on choline kinase (ChoK), a new target for anticancer drug development.

Conclusion: Many evidences support a role of PLD in signal transduction pathways controlling mitogenesis. The net balance between short activation and desensitisation induced by chronic treatment with HePC on PLD can be important for the final response of the different cells to membrane-active agents. This novel interpretation may be useful for a better understanding of the mechanisms of action of this family of anticancer drugs. Thus, the observed effects on PLD regulation by HePC may be related to its antiproliferative action.

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Growth inhibition of mouse autochthonous skin cancer by oral administration of new serine protease inhibitor ONO-3403

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Purpose: The purpose of this experiment is to present experimental evidence that oral administration of new serine protease inhibitor ONO-3403 is effective inhibiting the development of 3-methylcholanthrene-induced autochthonous skin cancer in mice.

Methods: The experiment was started when the tumors reached a size of 5 mm in diameter. Animals were divided into 2 groups. ONO-3403 was dissolved in sterile distilled water and 6 tumor bearing mice were given 3 times daily at a dosage 10 mg/kg with stomach tube in a 1-ml of volume of ONO-3403 for 9 weeks. While 5 mice of control group were administered saline alone same times daily.

Results: The Oral administration of ONO-3403 inhibited significantly the growth of autochthonous mouse skin cancer ($p < 0.001$) and also prolonged survival time of tumor bearing mice ($p < 0.01$).

Conclusion: Recently we have reported that orally active serine protease inhibitor ONO-3403 obtained by injection inhibited the mouse skin cancer. In the present study, the antitumor effect of oral administration of this material in the same experimental tumor system was confirmed.

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Enzyme inhibitor derivative of targeted nonbiodegradable GnRH analogue conjugate with high anticancer selectivity

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Purpose: To improve the therapeutic efficacy and tumour selectivity target specific gonadotropin hormone releasing analogue (GnRH-III) with selective anti-cancer activity and its antimetabolite enzyme inhibitor derivative were coupled to a carrier molecule poly(N-vinyl pyrrolidone-co-maleic acid).

Methods: The target specificity of the conjugates was determined by investigating their specific binding affinity and receptor-mediated internalization using radionuclide labelled compounds. Effects of conjugates on mitotic signal and cell cycle progression were also studied. The antiproliferative and antitumour activity of the compounds were tested in vitro on GnRH receptor-positive MCF-7 and MDA-MB-231 cell lines as well as in vivo on immunosuppressed MDA-MB-231 xenograft bearing mice (i.p. treated daily).

Results: Conjugation significantly enhanced the stability and receptor-mediated internalization of hormone-receptor complexes. Conjugates exerted retarding effect on the cell division cycle at G2 phase suggesting an inhibitory action in the premitotic stage. As a result of increased stability of GnRH-III the in vivo therapeutic efficacy of the conjugates versus unbound peptide hormone was significantly enhanced. By the end of the 7th week of i.p. administration with GnRH-III conjugate as well as its enzyme inhibitor derivative MDA-MB-231 tumour mass was decreased by 45% and 75% in comparison to the age-matched control.

Conclusion: Our results confirm the role of a properly selected carrier molecule in enhancement of tumour selectivity and anti-cancer activity of covalently bound target specific peptide hormone, or antimetabolic enzyme inhibitor. GnRH analogue conjugates offer new possibilities in the complex therapy of GnRH receptor-positive breast cancer.

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