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VALPROIC ACID ENHANCES CISPLATIN CYTOTOXICITY IN MELANOMA CELLS

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Abstract: In recent years, there has been a growing interest in anticancer potential of valproic acid (VPA) resulting from inhibition of histone deacetylase activity. The aim of our study was to evaluate the influence of valproic acid and cisplatin (CPT) on the growth rate of human melanoma cell lines: A375 (melanotic) and C32 (amelanotic). Both tested drugs decreased cell proliferation in a dose-dependent manner. VPA used alone significantly inhibited the growth of both cell lines at concentrations of 3 and 10 mM. Cisplatin significantly decreased cell proliferation at concentration = 0.3 μM. However, VPA enhanced the cytostatic action of CPT since simultaneous exposure of cells to 1 mM VPA and 0.1 μM CPT resulted in a significant reduction of cell growth. It can be concluded that VPA increases the sensitivity of melanoma cells to chemotherapeutic agent – cisplatin.

Keywords: human melanoma cell lines, proliferation, valproic acid, cisplatin

Malignant melanoma *(melanoma malignum)* is a malignant tumor derived from melanocytes – melanin-producing cells of the skin (1–3).

An early diagnosis, in the initial phase of development of *melanoma malignum,* increases the chance of complete recovery, whereas the advanced stage of melanoma is almost fully resistant to all available therapies. A surgical removal of the lesion is the primary method of melanoma treatment, whereas radio-, chemo- and immunotherapy are a supplementary treatments (4–8).

From among platinum-based chemotherapeutic drugs, cisplatin (*cis*-diamminedichloroplatinum, CPT) was the first used in the treatment of melanoma. Binding of cisplatin to nucleophilic N7-sites of purine bases in DNA, in the cell nucleus is largely responsible for its antitumor activity. The damage induced by cisplatin (DNA adducts) may interfere with DNA replication and/or transcription mechanism. Consequently, these alterations in DNA structure can trigger off cytotoxic processes that lead to death of the cancer cells (9, 10). CPT has a low efficacy in therapy of melanoma, which is associated with cell resistance to this drug, and high toxicity. For this reason, novel therapeutic strategies are sought (analogues of cisplatin) (11) or combined therapies with carboplatin are applied (12–14).

A new promising group of compounds used in the therapy of melanoma are histone deacetylase (HDAC) inhibitor**s (15)**. One of them is valproic acid (2-propylpentanoic acid, VPA) which undergoes phase I and II clinical trials (16–18). VPA as a short-chain fatty acid has shown antitumor activity in a variety of *in vitro* and *in vivo* models, by altering the expression of numerous genes (16, 19).

The aim of our study was to evaluate the influence of valproic acid and cisplatin on the growth rate of human melanoma cell lines: A375 (melanotic) and C32 (amelanotic).

EXPERIMENTAL

Cell culture

The human malignant melanoma cell lines A375 and C32 were obtained from LGC Promochem (Łomianki, Poland). Cells were grown in Minimum Essential Medium Eagle (MEM, Sigma-Aldrich), supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin (Sigma-Aldrich) and 10 mM

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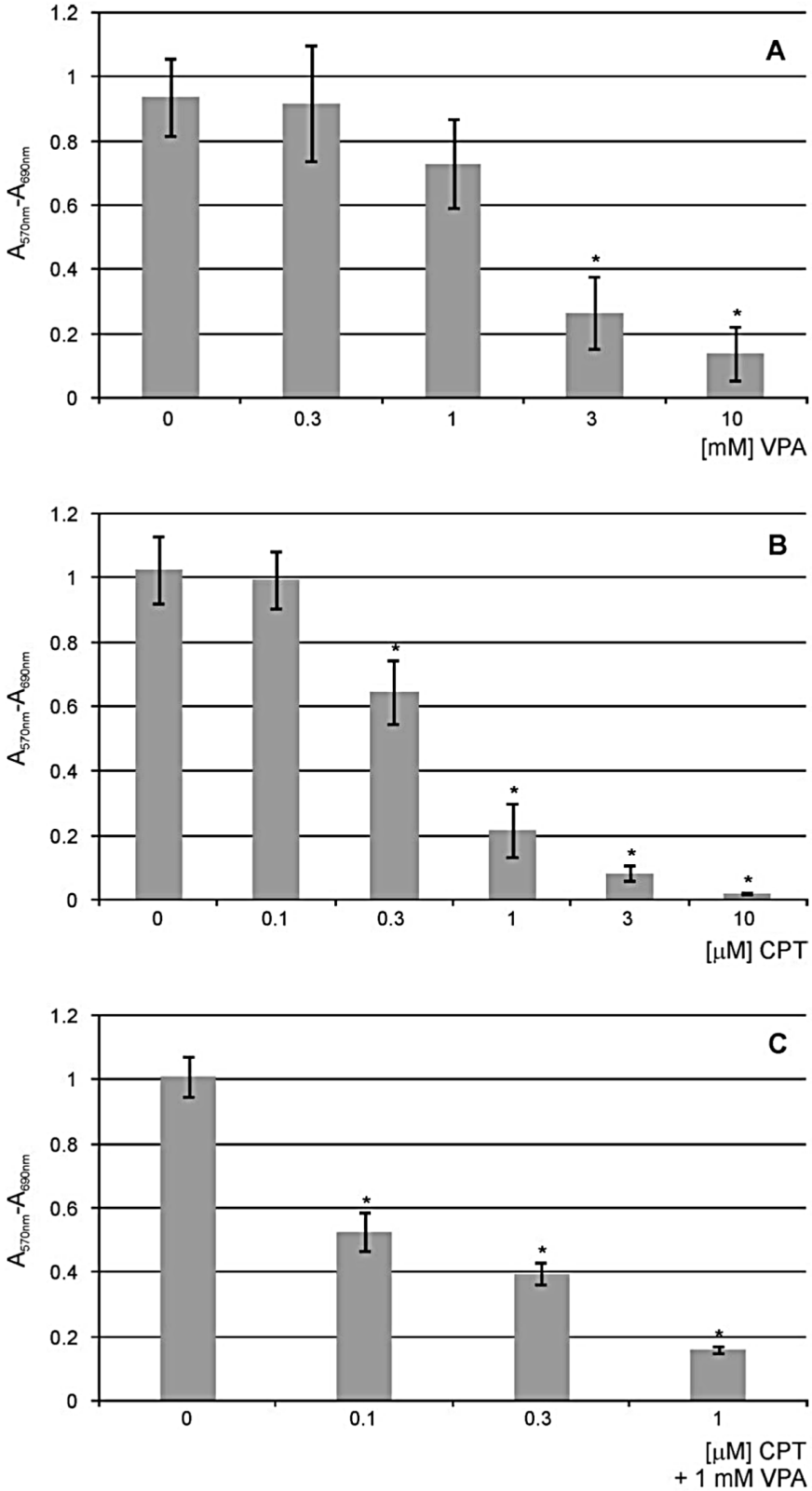

Figure 1. Growth of A375 cells cultured in the presence of various concentrations of VPA (**A**), CPT (**B**) and combination of VPA and CPT (**C**). Each bar represents the mean ± SD; *$p < 0.05$

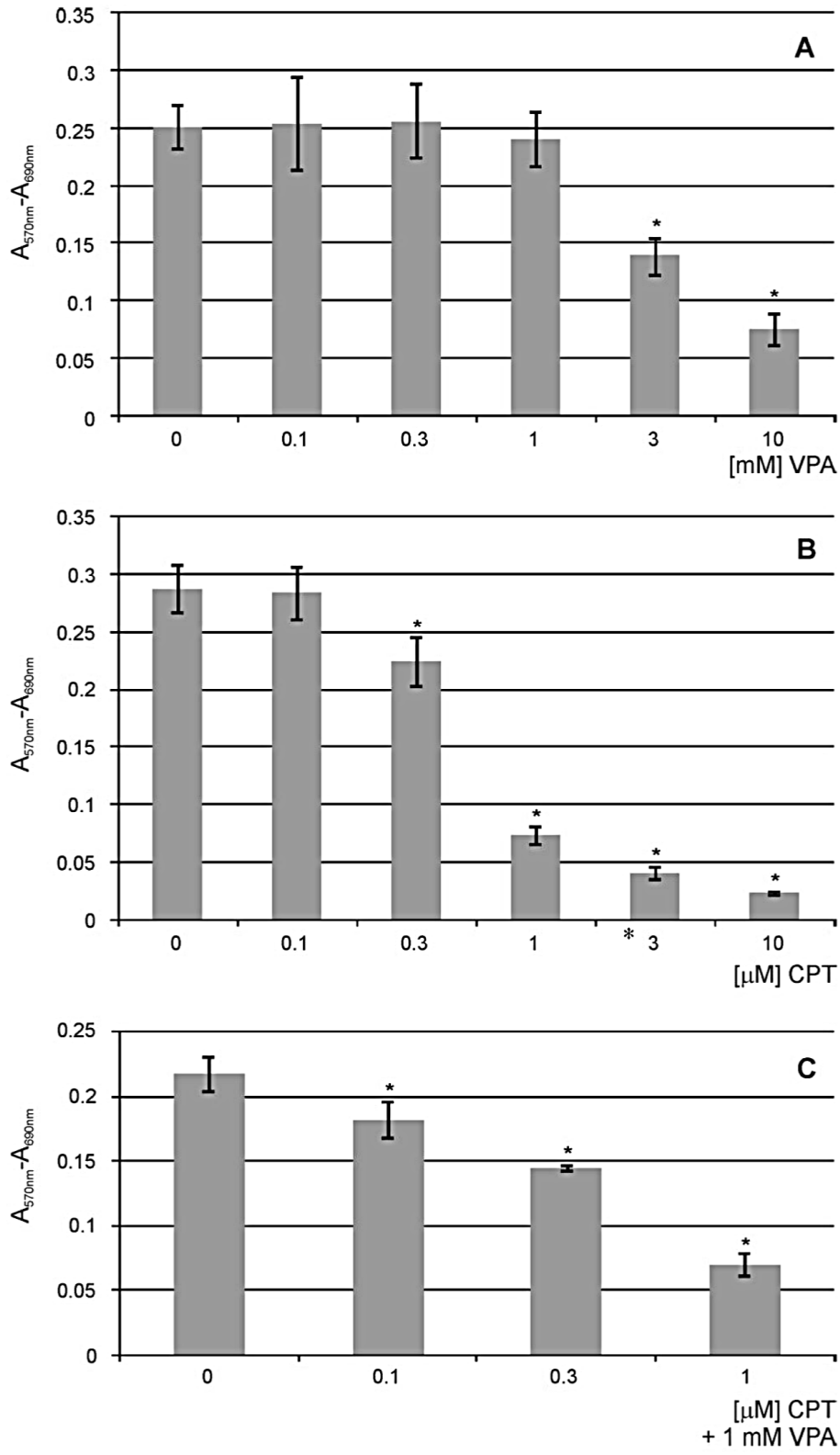

Figure 2. Growth of C32 cells cultured in the presence of various concentrations of VPA (**A**), CPT (**B**) and combination of VPA and CPT (**C**). Each bar represents the mean ± SD; *$p < 0.05$

HEPES (Sigma-Aldrich). The cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

Sulforhodamine assay

Cell proliferation was measured using sulforhodamine B (*In Vitro* Toxicology Assay Kit, Sulforhodamine B based; Sigma-Aldrich), a dye binding to cellular proteins. Cells were plated in 96-well plates (initial density 10^3 cells/well) and cultured for 24 h. Subsequently, the cells were treated with: VPA (concentration range: 0.1–10 mM), CPT (concentration range: 0.1–10 mM) and combination of 1 mM VPA and CPT (at concentration of 0.1, 0.3 or 1 mM) for 72 h. Finally, cells were fixed using 10% trichloroacetic acid (TCA) for 1 h at 4°C. Next, the cells were incubated with sulforhodamine B for 30 min and washed with 1% acetic acid. The bound dye was solubilized in 200 µL of 10 mM Tris base solution and the absorbance was measured at λ = 570 nm and λ = 690 nm (reference wavelength) using the MRX Revelation plate reader (Dynex Technologies).

RESULTS AND DISCUSSION

As an experimental *in vitro* model of melanoma we used A375 and C32 cell lines. These cell lines originate from different melanoma types: melanotic (A375) and amelanotic (C32). The cells were treated with valproic acid, cisplatin or their combination. Valproic acid and cisplatin suppressed proliferation of both A375 (Fig. 1) and C32 (Fig. 2) cells in a concentration-dependent manner. VPA, used alone caused a strong inhibition of cell proliferation at concentrations of 3 and 10 mM. There was no significant change in cell proliferation at low concentrations of VPA (0.1, 0.3 and 1 mM) in comparison to control culture (Fig. 1A; Fig. 2A). Many studies have shown that valproic acid modulates the biology of various tumor cells *in vitro* by the inhibition of cell proliferation and induction of apoptosis (19, 20). We also observed a dose dependent inhibition of cell growth following the treatment with cisplatin. A significant growth suppression of both cell lines was observed after treatment with CPT at concentrations of 0.3, 1, 3 and 10 µM (Fig. 1B; Fig. 2B). On the other hand, the inhibitory effect was not noticeable at the lowest drug concentration (0.1 µM). Platinum-based drugs, and in particular cisplatin, are used for the treatment of wide variety of solid malignancies, including testicular, ovarian, head and neck, cervical, bladder and lung cancer as well as metastatic melanoma. Cisplatin can be used either alone or in combination with other chemotherapeutic agents (21, 22). In our study, we demonstrated that valproic acid enhances the cisplatin-induced inhibition of cell growth. In A375 cell line, the contemporary administration of 1 mM VPA and 0.1 µM CPT (Fig. 1C) reduced cell number to 50% of that found in cultures treated with cisplatin alone. A simultaneous exposure of cells to 1 mM VPA and cisplatin at concentrations of 0.3 and 1 µM suppressed cell growth to 35 and 25%, respectively. The treatment of C32 cells with the same combination of VPA and CPT decreased cell growth to 37, 34 and 15% respectively (Fig. 2C). Similarly to our observations, Valentini et al. (23) found that VPA and CPT synergistically inhibited proliferation of M-14 melanoma cell line. VPA-mediated cell cycle arrest of M-14 cells was associated with up-regulation of $p16^{INK4A}$ and $p21^{waf1/cip1}$ proteins (cell cycle inhibitors) (23). Recently, Erlich et al. (24) revealed that valproic acid potentiates the cytotoxic effect of cisplatin in head and neck squamous cell carcinomas. All these data suggest that valproic acid therapy may enhance the chemosensitivity of melanoma cells.

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