Antitumor Activity of New Drug UKRAIN and Macrophage Stimulation

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Alkaloids are known to be among the most potent compounds found in nature with respect to biological activity in other organisms. Ukrain (Nowicky Pharma, Austria) was found to have some outstanding properties concerning biological activity with simultaneous low toxicity. Ukrain, exerting malignotoxic properties in vitro and in vivo, was used a effective drug for treatment of some malignancies in experimental animals and humans, but the mechanism of its action is still unclear. The aim of investigation was to establish the effectiveness of Ukrain treatment as macrophage stimulator in mice with special attention to cysteine proteinases as markers of tumor growth and metastasizing. To elucidate the role of macrophage stimulation in antitumor effect of Ukrain we used macrophage stimulator β-1, 3- carboxymethylglucan (Institute of Chemistry, SAN, Slovakia) and gadolinium chloride, induced macrophage depression in vivo.

Methods. HA1 murine hepatoma was transplanted in A/Sn mice (Institute of Cytology and Genetics, Novosibirsk) after i. p. tumor cell injection. Mice were treated by Ukrain in a dose of 0.5 mg per mouse i. p. at 3^{rd} day and were killed at 10^{th} day after tumor transplantation. In the next experiment mice were treated repeatedly every 48 h by Ukrain in the same doses and animals were killed at 12^{th} day of tumor transplantation. Cysteine proteinases activity was determined against fluorogenic substrates with specific inhibitors (CA-074 for cathepsin B). In the next experiment Ukrain was injected to CBA mice i. m. with Krebs-2 carcinoma cells (10^5) in the mixture with tumor cells i. m. or contralateral.

Results. Positive antitumor effect of Ukrain was shown during its injection simultaneously in mixture with Krebs-2 carcinoma cells as a result of attraction of macrophages. In HA-1 tumor Ukrain repeated administration resulted in reprodusible and significant retardation of tumor growth as consequence of the corresponding prolongation of life-span; repeated Ukrain administration diminished ascite formation and decreased mass of ascitic cells compared to untreated tumor mice. Ukrain induced elevation of a number of macrophages in ascites. (and the number of monocytes in periferal blood decreased). According to this finding activity of β-N-acetylhexosaminidase - a marker enzyme for macrophages - was decreased in serum too. Obviously, under the influence of Ukrain periferal blood pool of macrophages was targetting to tumor. HA-1; tumor development was followed by decreased specific activity of cathepsin B in liver, ascitic fluid and serum at the 10^{th} day, while that of β-N-acetylhexosaminidase increased. There is a overexpression of cysteine proteinase mRNAs in many tumors, but there are some of them, where activity of cysteine proteinases are decreased. HA-1 hepatoma in mice is believed to be one of them. Ukrain had a tendency to normalize these indexes (as in intact mice). At the same time the single Ukrain increased pro-cathepsin B activity in ascitic fluid at 10^{th} day of tumor development. Secretion of unmature forms of cathepsins B and L is a characteristic features of tumor development and metastasis. In this case it isn't clear the source of procathepsin B. Macrophages influx to ascites under Ukrain treatment may resulted in such elevation of procathepsin B. In acid tumor environment inactive proforms of cathepsins undertake autocatalitical activation and become able to digest tumour cells. The activity of α1-proteinase inhibitor in ascitic fluid was low, and Ukrain increased this index. Macrophages are the main source of secretion of α1-PI, but it might be the tumor cells also. Macrophage stimulator β-1,3-carboxymethylglucan was shown effective as antitumor drug. We can **conclude** that Ukrain possessed macrophage stimulating activity important in antitumor effect of this drug.