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Anti-tumor effect of interferon alpha in combination with cisplatin – animal experiments

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Anti-tumor effect of human interferon-α and cisplatin was studied on B-16 melanoma bearing C57Bl/6 syngeneic mice. When the tumors reached 1 mm in diameter, applications of cisplatin were started at a dosage of 0.001 mg/g of animal's body weight, or human interferon-α was given at a dosage of 5×10^4 IU. A control group of mice received normal saline solution. The treatment was applied every next day, altogether 12 times. Tumor volumes were measured every next day, and their mean values calculated; all 12 measurements confirmed that the mice treated with human interferon-α and cisplatin had smaller mean value than the control group, or the groups receiving either interferon or cisplatin alone, respectively ($p < 0.05$). The mean tumor volumes of cisplatin treated mice were lower than those of the controls or interferon-treated animals ($p < 0.05$). The obtained results indicate that human interferon-α enhances the antitumor effect of cisplatin.

Key words: melanoma, experimental drug therapy; interferon-alpha; cisplatin

Introduction

Melanoma represents less than 5 % of all malignant diseases, though its incidence in the last few decades has been rapidly increasing.[1, 2, 3]

Chemotherapy in melanoma has not been particularly effective. Although several drugs have a low order of antitumor activity, combination chemotherapy has not produced better results that those observed with the use of single agents such as dacarbazine, nitrosoureas, vinca-alkaloids, and cisplatin (CDDP). Moreover, none of these drugs as well as their combinations have been proved to definitely increase the survival of patients with metastatic melanoma.[4, 5]

A number of pre-clinical and clinical trials studying the effects of interferon alpha were consistent in confirming its antitumor activity against melanoma.[6, 7] Even if the mechanism of antitumor effects of interferon are still insufficiently understood, it has become clear that in the majority of the experimental systems investigated, interferons act in a very different way from chemotherapy. Thus, the idea of using interferons in combination with other agents has interested investigators for a long time.[8]

Different experimental data have shown that combined treatment can improve the response rate and prolong the duration of response due

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to different actions of drugs on the tumor, interactions between the drugs and, perhaps, influences on the host immune system.[9, 10]

Therefore, the aim of this study was to investigate the possibility of enhancing the cisplatin antitumor effect by the application of human interferon alpha in suboptimal doses.

Materials and methods

Experimental animals

The animals, 8–10 week old C57Bl/6 mice were obtained from Rudjer Bošković Institute, Zagreb. Mice used in the experiment were of the same sex and age. Animal colonies were maintained in accordance with the recommendations issued by the National Cancer Institute in Bethesda, USA. B-16 melanoma was used as an experimental tumor model. Tumors were implanted to the animals by subcutaneous injection of 5×10^5 viable tumor cells given dorsolaterally. Tumor cell suspension was prepared by mechanical decomposition of viable tumor tissue.

Treatment

Mice were divided into four experimental groups as follows: 1) control group, 2) group treated with CDDP, and 3) group receiving human interferon-α (IFN-α) and 4) group receiving combined CDDP and IFN-α treatment. Each group consisted of 7 animals. Intraperitoneal applications of the cytotoxic agent and/or IFN-α and normal saline solution were started when the tumors reached 1 mm in diameter, or a volume of 0.5 mm^3. The injections of active substances were administered every next day, and the experiment was completed on the 25th day from the beginning of application.

The solution of CDDP (Bristol-Myers Co.) and normal saline was injected at a dose of 0.001 mg/g b.w. or 0.01 ml of the solution per gram of the animal's body weight.

IFN-α (human interferon alpha from the Institute of Immunology, Zagreb, Croatia) dissolved in normal saline was injected at a dose of 5×10^4 IU which equalled to 0.25 ml of the solution per application.

In one group CDDP injections were followed after one hour by IFN-α application; drug dosage was the same as in groups receiving either of the agents alone. Animals in the control group had the same quantity of normal saline solution injected intraperitoneally every next day.

Tumor measurement and statistical analysis

Tumor growth was followed up daily by the evaluation of tumor diameter and thickness. Tumor volume was calculated using the following formula: $0.523 \times a \times b \times c$, where a, b, c were tumor diameters.

Mean volumes, as well as standard deviation and standard error of the mean values were calculated from the results of measurements performed on the same day. The data were statistically analysed by means of CIA software.[11]

Results

Mean values of tumor volumes (MTV) expressed in mm^3, measured every next day during the treatment with normal saline solution, IFN-α, CDDP, or combination of both are presented in Table 1.

In all measurements MTVs of CDDP-treated animals were found to be lower that those of the control group ($p<0.05$), and the values obtained after the 5th measurement were also statistically significantly lower that those of the IFN-α treated animals.

In all 12 measurements MTV of the animals receiving combined CDDP and IFN-α treatment were statistically significantly lower than the relevant values obtained in all other experimental groups ($p < 0.05$).

Figure 1 shows MTV values and 95 % confidence interval resulting from all 12 measurements performed in all experimental groups.

In the groups receiving combined CDDP and IFN-α treatment or CDDP alone 2 animals died immediately after drug application. The

Table 1. B16 melanoma volume in mm^3 in all 4 groups of C57Bl/6 mice.

Measurement	Controls MTV (m^3) 95-CI	IFN-α MTV (mm^3) 95-CI	CDDP MTV (mm^3) 95-CI	IFN-α and CDDP MTV (mm^3) 95-CI
1	0.5	0.5	0.5	0.5
2	3.9	3.1	2.1	1.3
	2.7–4.1	2.7–3.5	1.8–2.4	0.9–1.8
3	13	10.4	9.9	6.2
	11–15	9.6–11.2	9.2–10.7	5–7.4
4	57.7	46.9	49.1	35.2
	55–61	44–50	44.8–53.3	29–42
5	131	122	119	59.3
	127–135	116–128	113–125	48–71
6	181	179	145	91.4
	173–189	170–188	137–153	79–104
7	324	326	240	219
	316–332	314–338	231–249	205–233
8	444	438	369	299
	433–455	428–448	349–389	281–317
9	538	524	417	371
	525–551	510–538	295–439	349–393
10	622	613	498	443
	608–636	600–626	473–523	421–465
11	696	689	602	523
	683–709	676–702	573–631	496–550
12	789	798	711	611
	775–803	784–812	672–750	576–646

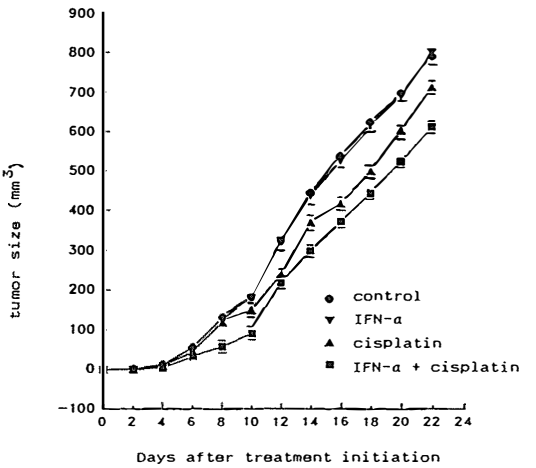

Figure 1. The effect of IFN-α and CDDP on the growth of B-16 melanoma. Mice were treated every next day after the tumors reached 1mm in diameter. IFN-α (5×10^4 IU) and CDDP (0.001mg/g) were injected intraperitoneally. Each experimental group consisted of seven mice. The vertical bars represent 95% CI.

animals in CDDP-treated group had for 1g lower body weight on average, whereas the body weight of animals in other three groups did not differ from that of the control animals.

Discussion

According to the obtained results, IFN-α alone does not exert any direct statistically significant effect on the tumor.

The antiproliferative effect of IFN-α depends on the dose applied, as well as on the mode of application, tumor size and the type of metastases. An indirect, immunomodulatory effect of interferon can be achieved at doses lower than those required for a direct antitumoral effect. In experimental animals human IFN-α does not influence the immune cells such as T-lymphocytes and NK cells.[14]

Balkwill has reported on the interactions between human IFN-α and chemotherapeutic agents in human tumours grown in mice. The efficacy of sub-optimal doses of cyclophosphamide and doxorubicin was greatly increased by interferon in a human breast cancer xenograft growing in nude mice. Even low doses of interferon, which alone had no effect on tumour growth, were able to potentiate the activity of anticancer drugs.[15]

Numerous preclinical and clinical trials have shown synergistic or additive effects between

interferon and at least 20 different cytotoxic agents including doxorubicim, vinca alkaloids, 5-fluorouracil and CDDP.[9, 16]

The most striking synergy was demonstrated when low doses of IFN were used and, and it was associated predominantly with lymphoma cell lines.[17]

However, not all studies have established positive interaction between IFNs and chemotherapy. Antagonistic effects between some cytotoxic drugs and IFN have also been reported.[18]

Little is known about the mode of IFN interaction with CDDP and other cytotoxic drugs. IFNs could potentially alter drug metabolism or act independently of the other agent. The cytochrom P-450 system was inhibited, thus significantly influencing the cell level of glutathione transferase. The decreased levels of cell glutathione result in increased cytotoxic effect of CDDP. IFN slows down the cell cycle by inhibiting the production of nucleic acids in its postmitotic G1 phase. IFN also slows down catabolism and elimination of some cytotoxic agents such as cyclophosphamide and doxorubicin, and influences cell membrane fluidity, i.e. the transport system for cytostatics.[9, 19]

Combined IFN and chemotherapy has resulted in clinical benefit in many patients with solid tumours.[20] In general, however, significantly improved response rates were not observed.

In many regimens, IFN are combined with cytotoxic drugs with different rationale: biochemical modulation, immunopotentiation, immunostimulation or host protection. Each approach is valid; however, the complexity of potential interactions requires close considerations.[9, 22]

Our study showed that IFN, even at a dose insufficient to influence the growth of B-16 melanoma when given as monotherapy, statistically significantly increased the antitumor effect of CDDP (95% CI). Additional prospective clinical trials are of paramount importance for further explanation of the interaction mechanisms involved.

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