Eur J Cancer, Vol. 26, No. 3, pp. 321–325, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Combination Therapy of ACNU and Ifosfamide in Tumor Bearing Mice with M2661 Breast Cancer, B16 Malignant Melanoma or C38 Colon Cancer

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Abstract—*Three murine tumor lines, B16 melanoma, C38 colon cancer and M2661 breast cancer, were used to evaluate the therapeutic efficacy of the drug combination ACNU and ifosfamide. For each tumor type five treatment groups of 12 mice each were studied, which respectively received* 16.5 mg/kg *ACNU*, 150 mg/kg *ifosfamide*, 33 mg/kg *ACNU*, 300 mg/kg *ifosfamide or* 16.5 mg/kg *ACNU* + 150 mg/kg *ifosfamide. One group served as controls. Growth delay was measured as the endpoint. In the B16 and C38 tumor lines both drugs were active and showed additive antitumor effects. However, no synergism was observed. Neither ACNU or ifosfamide or the combination of both had any activity against the M2661 tumor line.*

INTRODUCTION

NIMUSTINE (3-[(4-amino-2-methyl-5 pyrimidyl)-methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride) (ACNU) is a lipid- and water-soluble nitrosourea derivative. The antitumor activity of this compound is exerted through alkylation and cross-linking of DNA [1, 2]. Because of its water solubility ACNU can be administered intravenously without the local irritation induced by alcohol-soluble nitrosoureas. ACNU has shown marked activity in several experimental murine tumor systems [3–8]. In man it is proven to be active in malignant glioma [9], while some activity in colorectal cancer, malignant melanoma and gastric cancer has also been reported [10, 11]. Ifosfamide (3-(2-chloroethyl) (2-chloroethyl-amino) tetrahydro-(1,3,2-oxazophosphorine-2-oxide) is an alkylating oxazophosphorine requiring metabolic activation in the liver. It has been shown to be active against a broad range of experimental tumors [12, 13]. The clinical antitumor activity of ifosfamide includes soft tissue sarcomas, testicular cancer, small cell lung cancer and pancreatic cancer [14].

Accepted 20 November 1989.
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This work was supported by a grant from ASTA Pharma AG, Frankfurt, F.R.G.

Both for ACNU and for ifosfamide *in vivo* synergism with other drugs have been reported. Ifosfamide has been found therapeutically synergistic with cisplatin, 5-fluorouracil, Ara-C and VP-16 [12, 15]. ACNU has shown synergism with thioguanine and 5-FU in L1210 leukemia [2]. Indications for synergism between ACNU and ifosfamide have been reported in Lewis lung cancer [16]. In view of preliminary data on clinical activity of both ACNU and ifosfamide, we studied the possibility of *in vivo* synergism between ACNU and ifosfamide in B16 melanoma, C38 colon cancer and M2661 breast cancer in mice.

MATERIALS AND METHODS

Animals and tumors

Tumor fragments 2–3 mm in diameter were implanted subcutaneously in the left flank of 10–12-week-old mice, obtained from our own breeding facilities. B16 melanoma was implanted in C57 Bl/Rij mice, C38 colon cancer in BCBA/F1 mice and M2661 breast cancer in CBA mice. The animals were given water and pelleted food *ad libitum*.

Treatment and evaluation

ACNU and ifosfamide (provided by ASTA Pharma AG) were dissolved in sterile water and administered i.v. through the tail vein at a volume of 0.01 ml/g body wt. The LD_{50} in mice for ACNU

is 54 mg/kg [4] and for ifosfamide 620 mg/kg [12]. Six groups of 12 mice were studied for each tumor type. Group A did not receive any treatment and served as a control group. Group B received ACNU at a dose of 16.5 mg/kg, group C received 150 mg/kg ifosfamide, group D was treated with 33 mg/kg ACNU, group E with 300 mg/kg ifosfamide and group F received 16.5 mg ACNU + 150 mg/kg ifosfamide. Tumors were measured twice a week with a slide caliper. The volume was calculated by the equation length × width × thickness and expressed in cubic millimeters. Each tumor volume was then expressed as relative tumor volume (RV),

$$RV = V_n/V_0$$

where V_n is the tumor volume at day n and V_0 is the initial tumor volume at the time the treatment was given. At the start of treatment tumors had a mean volume of 500–700 mm^3. In order to compare growth delay values between tumor types the growth delay was calculated for each treatment group. Growth delay was defined, according to Steel *et al.* [17], as the difference between the mean values of the time needed by the treated and control tumors to grow to 2 times their initial volume (T'_2 and T_2 respectively), divided by the mean doubling time of the control tumors, using the formula:

$$\text{Specific growth delay (SGD)} = T'_2 - T_2/T_2.$$

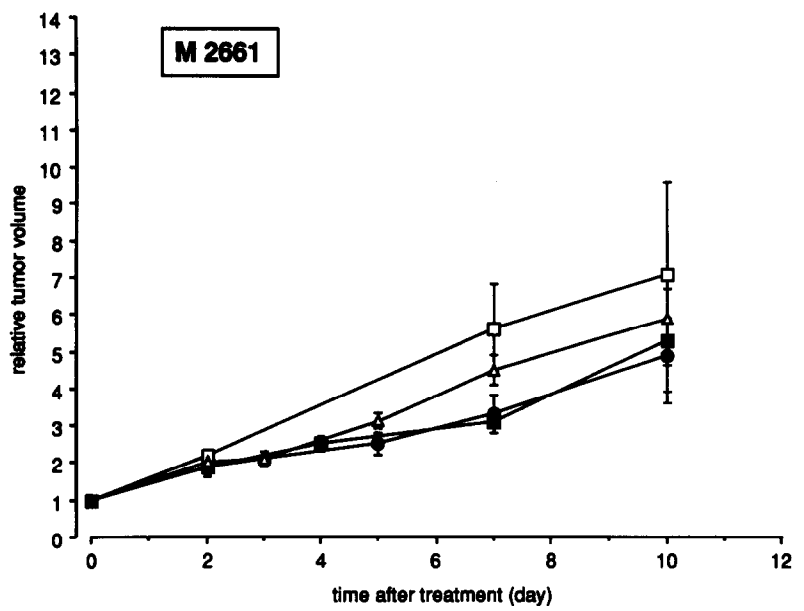

Fig. 1. Growth curves obtained in the M2661 tumor line. The relative tumor volume is the tumor volume at any given day V_n/the volume at the start of treatment. Group A: controls (□); group B: 16.5 mg/kg ACNU (○); group C: 150 mg/kg ifosfamide (▲); group D: 33 mg/kg ACNU (△); group E: 300 mg/kg ifosfamide (■); group F: 16.5 mg/kg ACNU + 150 mg/kg ifosfamide (●). All treatment was given i.v.

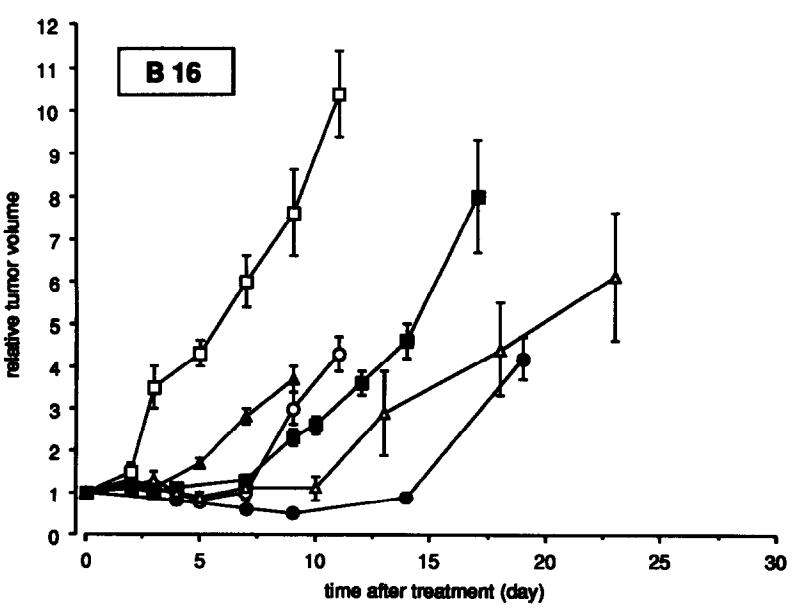

Fig. 2. Growth curves obtained in the B16 tumor line. For explanation of symbols see Fig. 1.

Growth delay is thus expressed in terms of the number of volume doubling times gained by the treatment.

Statistics

The antitumor activity was evaluated with Student's *t* test.

RESULTS

The median growth curves of the control and treatment groups of the different tumor lines are shown in Figs 1–3. The corresponding data for SGD are given in Table 1. The data indicate that both drugs were inactive towards the M2661 transplantable breast cancer (Fig. 1). Therefore the lower dosages of single agent ACNU and single agent ifosfamide were not tested on this tumor. For the B16 melanoma (Fig. 2) and the C38 colon carcinoma (Fig. 3) growth delay was observed in all treated groups in comparison to the control group, however there was no long lasting significant tumor regression. Comparison of the antitumor activity of the various regimens shows that the antitumor effect of the combination of ACNU and ifosfamide was greater than the addition of growth delay due to the separate agents suggesting an additive effect but not synergism. For both drugs a dose–response effect was observed (*cf.* group B with D or C with E). As expected, in view of the dosages used, there were no toxic deaths.

Table 1. Atitumor activity of ACNU and ifosfamide in B16, C38 and M2661 tumor lines

Tumor	Treatment group*	SGD	Toxic deaths
B16	B	2.7†	0/12
	C	1.5†	0/12
	D	4.1†	0/12
	E	2.6†	0/12
	F	5.4†	0/12
C38	B	1.7†	0/12
	C	2.1†	0/12
	D	3.6†	0/12
	E	3.0†	0/12
	F	4.1†	0/12
M2661	B	—	—
	C	—	—
	D	0.0	0/12
	E	0.1	0/12
	F	0.1	0/12

*B: 16.5 mg/kg ACNU; C: 150 mg/kg ifosfamide; D: 33 mg/kg ACNU; E: 300 mg/kg ifosfamide; F: 16.5 mg/kg ACNU + 150 mg/kg ifosfamide.

†Significant difference between treated and control group ($P < 0.05$) evaluated with Student's *t* test.

DISCUSSION

In the present study in murine tumors we have found that the combination of ACNU and ifosfamide in B16 melanoma and C38 colon cancer resulted in an antitumor effect greater than the addition of the growth delay due to the separate agents, but a therapeutic synergism was not observed. In the M2661 breast cancer the drugs were considered to be inactive in the dosages and the schedule used.

In combination chemotherapy regimens the drugs used should not have overlapping mechanisms of action. As ACNU and ifosfamide are both

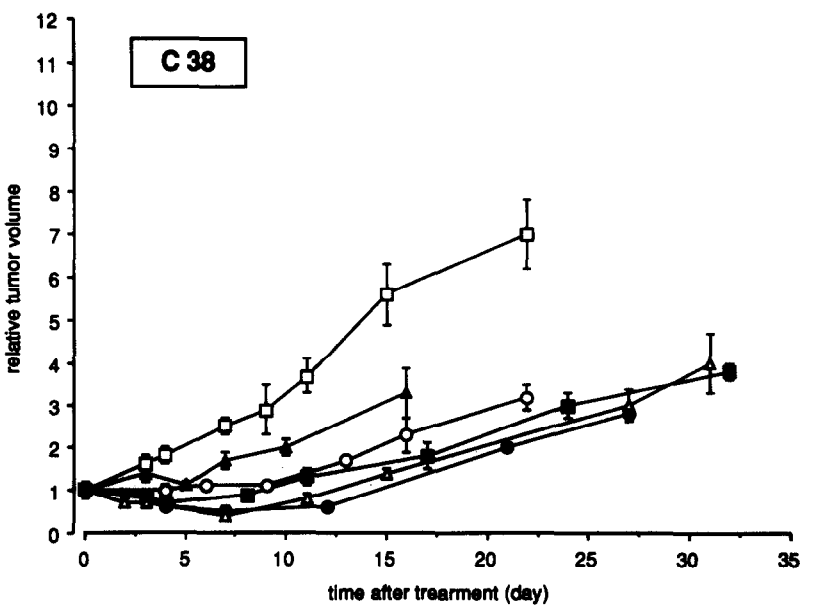

Fig. 3. Growth curves obtained in the C38 tumor line. For explanation of symbols see Fig. 1.

alkylating agents, combining them might seem peculiar. However, there are major differences in mechanism and site of action between different alkylating agents [18]. Besides, cellular resistance to chlorethylnitrosoureas is highly specific and the mechanism of resistance does not allow cross-resistance with other DNA cross-linking agents [19, 20]. Therefore, the use of combinations of alkylating agents [21, 22] cannot be precluded. While both for ACNU and ifosfamide *in vivo* synergism with other drugs has been reported [2, 15], Murakami *et al.* reported synergism between both drugs in Lewis lung cancer [16]. In view of the preliminary clinical data on possible efficacy of ACNU and ifosfamide in malignant melanoma and colon cancer in man [23, 24] and the lack of effective (combination) chemotherapy regimens for these diseases [25], the present study in mice was performed. The reason for including a breast cancer model was the fact that no data on clinical activity of ACNU and only few data on activity of ifosfamide in breast cancer were available [26]. We hoped to find some measurable activity in the model in view of possible clinical studies. Unfortunately, both drugs were inactive in this model. However in the B16 melanoma and the C38 colon cancer there was activity of each drug, with an additive effect in combination. True synergism was not observed. The additive effect, however, warrants further studies on the usefulness of a combination of ACNU and ifosfamide, especially in diseases such as malignant melanoma and colon cancer.

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