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Short communication

The 5-HT_3 antagonist, BRL 43694, does not compromise the efficacy of cisplatin in tumour-bearing mice

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Summary. Binary combinations of cisplatin and the 5-HT_3 antagonist, BRL 43694, have been used to treat both conventional mice bearing either L1210 leukaemia or ADJ/PC6 plasmacytoma and nude mice xenografted with a human ovarian carcinoma (HX/110). In no case was there evidence for antagonism by the antiemetic of the antitumour properties of cisplatin.

Introduction

BRL 43694 [endo-*N*-(9-methyl-9-azabicyclo(3,3,1)non-3-yl)-1-methyl-1H-indazole-3-carboxamide] is a selective 5-HT_3 receptor antagonist that has exhibited potent antiemetic properties in patients receiving cisplatin [2, 3]. The purpose of the present investigation was to determine whether BRL 43694 influences the antitumour properties of cisplatin. Three separate tumour models were exploited for this purpose: ADJ/PC6 plasmacytoma, L1210 leukaemia (both of which have featured prominently in the discovery and development of platinum-based anticancer drugs) [4] and a human ovarian papillary cystadenocarcinoma, HX/110, xenografted in the nude mouse.

Materials and methods

Rodent tumour models. BDF_1 mice weighing 20–25 g were inoculated intraperitoneally (i.p.) with 10^5 L1210/0 cells on day 0. Treatment was given on days 1, 5 and 9 post-implantation. Cisplatin was given in saline by i. p. injection at 2, 4 and 8 mg/kg, respectively, to groups of five mice per dose level, either alone or concurrently with BRL 43694 injected intravenously (i. v.) at a dose of 0.5 mg/kg in water. This dose was chosen since it invariably ablates cisplatin-induced emesis in the ferret [1]. The antiemetic was also given alone i. v. to five tumour-bearing mice on days 1, 5 and 9. A group of ten untreated, tumour-bearing mice was included as a control. The experiment was repeated, exchanging the routes of administration of both cisplatin and the antiemetic, to obviate any possibility of route dependency for the latter.

The ADJ/PC6 plasmacytoma was transplanted into female BALB/c mice (weighing 20 g) by subcutaneous (s. c.) implantation of a 1-mm^3 fragment. At 20 days post-implantation, mice carrying tumours of comparable size were randomised into groups of three mice per dose level, including ten untreated controls. Cisplatin was given by single i. p. injection in saline on day 20 at 0.25, 0.5, 1, 2, 4, 8 and 16 mg/kg, either alone or concurrently with the antiemetic, which was given as a single i. v. injection of 0.5 mg/kg in water. Three tumour-bearing mice received the antiemetic alone at the same i. v. dose. The experiments were terminated on day 30, when the tumour weights of control and treated groups were measured.

Xenograft studies. The HX/110 tumour, which we had previously found to be sensitive to cisplatin (data not shown) was implanted s. c. as 2-mm^3 fragments into the right flank of female Charles River nude mice (weight range, 25–30 g). The mice were housed in a negative-pressure, flexible film isolator and maintained *ad lib* on autoclaved tap water and Labsure ERD diet, sterilised at 2.5 Mrad. At 53 days post-implant, mice bearing tumours of comparable size were randomised into seven treatment groups of six mice each and a control group containing ten mice. Cisplatin was given i. p. at the maximum tolerated dose (MTD), $^1/_2$MTD and $^1/_4$MTD (i. e. 8, 4 and 2 mg/kg, respectively) both alone and in combination with BRL 43694 at 0.5 mg/kg i. v. One group received i. v. BRL 43694 alone at 0.5 mg/kg. When the antiemetic and cisplatin were given in combination, the former was injected i. v. immediately prior to i. p. cisplatin. Treatment was given weekly for 4 weeks. The longest tumour diameter (a) and that at right angles (b) were measured by calipers and volumes calculated from the formula $V = \pi/6 \times a \times b^2$ [5]. These data are expressed as relative tumour volumes (RTV) = V_t (volume at time t)/Vo (volume at first treatment) $\times 100$.

Results and discussion

Table 1 lists the results of two experiments with L1210 leukaemia. It is apparent that the antiemetic alone had no effect on tumour growth. Furthermore, when given in binary combination with an optimal dose of cisplatin (4 mg/kg), antitumour activity was apparently enhanced, although this effect was not statistically significant ($P > 0.2$). There was no evidence of route dependency with either drug. The enhanced effect of cisplatin given i. p. was significant ($P < 0.05$), reflecting coincidence of the location of the tumour and the route of administration.

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Table 1. Antitumour activity against L1210 leukaemia (days 1, 5, 9)

Cisplatin dose (mg/kg)	Cisplatin i.p. ± BRL 43694 i.v.				Cisplatin i.v. ± BRL 43694 i.p.			
	−Antiemetic		+Antiemetic		−Antiemetic		+Antiemetic	
	T/C	% ILS	T/C	% ILS	T/C	% ILS	T/C	% ILS
2	1.42 (0.09)	39	1.73 (0.11)	73	1.20 (0.03)	20	1.36 (0.08)	36
4	1.84 (0.20)	84	2.13 (0.15)	113	1.51 (0.05)	51	1.76 (0.14)	76
8	Toxic				Toxic			
BRL 43694 alone (0.5 mg/kg)			1.02 (0.05)	−2			1.01 (0.04)	1

BDF1 mice ($n = 5$; control = 10) were injected with 10^5 L1210/0 cells, obtained from Mason Research Institute, on day 0
% ILS (increase in life span) was calculated with respect to untreated control values; average survival for control animals (killed by cervical dislocation when moribund) = 9 days. % ILS of all treatment groups receiving 2 or 4 mg/kg cisplatin was significantly different from that of controls ($P < 0.01$)
T/C = mean survival of treated animals/mean survival of controls (days)
Numbers in parentheses represent the standard deviation

Table 2. Antitumour activity against ADJ/PC6 plasmacytoma

Cisplatin dose (mg/kg)	Experiment 1:				Experiment 2:			
	−Antiemetic		+Antiemetic		−Antiemetic		+Antiemetic	
	T/C	% TGI	T/C	% TGI	T/C	% TGI	T/C	% TGI
0.25	0.19 (0.12)	81	0.24 (0.06)	76	0.80 (0.26)	20	0.43 (0.09)	57
0.5	0.52 (0.50)	48	0.03 (0.01)	97	0.5 (0.09)	50	0.25 (0.17)	75
1.0	0.01 (0.01)	99	0.04 (0.02)	96	0.07 (0.02)	93	0.04 (0.01)	96
2.0	0.10 (0.07)	90	0.04 (0.01)	96	0.03 (0.01)	97	0.08 (0.04)	92
4.0	0.0	100	0.03 (0.01)	97	0.03 (0.01)	97	0.02 (0.01)	98
8.0	0.0	100	0.0	100	0.01 (0.00)	99	0.02 (0.01)	98
16.0	Toxic				Toxic			
BRL 43694 alone (0.5 mg/kg)			1.00 (0.25)	−2.5				

Summary of therapeutic parameters:

	Experiment 1:			Experiment 2:		
	LD_{50} mg/kg	ED_{90} mg/kg	T. I.	LD_{50} mg/kg	ED_{90} mg/kg	T. I.
Cisplatin	9.4	0.9	11	9.6	0.94	10
+ BRL 43694	11.3	0.4	29	11.3	0.8	14

Numbers in parentheses represent the standard deviation
Control, 10 animals/group; treated, 3 animals/group
Cisplatin, single dose injected i.p.; BRL 43694, single dose injected i.v.
ED_{90} dose to cause 90% regression of tumour mass; T.I. = LD_{50}/ED_{90}; TGI, tumour growth inhibition; T/C, mean weight of tumours from treated animals/mean weight of tumours from control animals

Table 2 lists the results derived from two replicate experiments in mice bearing ADJ/PC6 plasmacytoma. Both sets of data are closely comparable. The apparent reduction by BRL 43694 in the ED_{90} of cisplatin in the first experiment, thus apparently doubling the T.I. value achieved in the second experiment, was of no significance. It is clear that the antiemetic did not reduce the antitumour activity of cisplatin in ADJ/PC6 plasmacytoma.

The results obtained in studies with the HX/110 human ovarian tumour xenograft are shown in Table 3, which lists T/C values obtained 28 days after the commencement of treatment; the corresponding growth curves are displayed in Fig. 1. The therapeutic parameters at each dose were closely comparable for cisplatin given both alone and in combination with BRL 43694, whereas the corresponding growth curves essentially overlap.

Table 3. Summary of 28-day T/C values for the HX/110 tumour

Cisplatin dose (mg/kg)	T/C	
	Cisplatin	+ BRL 43694
0	–	0.67 (0.18)
2	0.34 (0.09)	0.33 (0.10)
4	0.15 (0.05)	0.20 (0.08)
8	0.08 (0.02)	0.09 (0.02)

Numbers in parentheses represent the standard deviation

BRL 43694 alone was devoid of antitumour activity in this model.

We conclude that in the three tumour models studied, there is no evidence of therapeutic antagonism between cisplatin and BRL 43694, the latter drug showing no evidence of antitumour activity. Thus, it would be predicted from this work that BRL 43694 is unlikely to influence the antitumour properties of cisplatin in the clinical setting.

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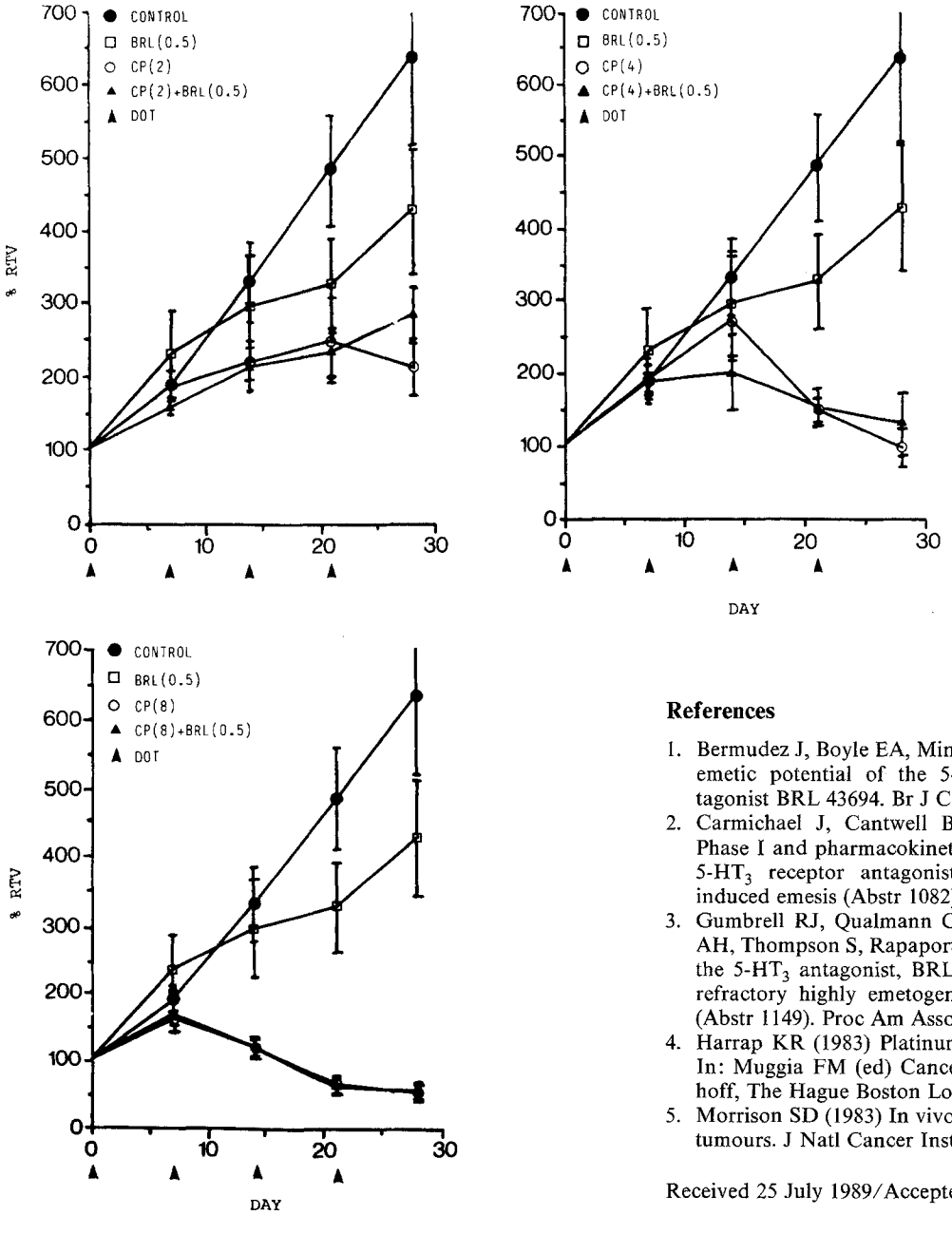

Fig. 1. HX/110-bearing animals treated weekly with i.p. cisplatin ± i.v. BRL 43694 for 4 weeks. Numbers in parentheses represent doses (mg/kg). *BRL*, BRL 43694; *CP*, cisplatin; *RTV*, relative tumour volume; *DAY*, days after initiation of treatment (on day 0); *DOT*, day of treatment

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