Short Communication

Enhancing effect of bromovinyldeoxyuridine on antitumour activity of 5-fluorouracil in mice bearing MOPC-315 plasmacytomas

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It has recently been shown (Desgranges *et al.*, 1986) that bromovinyluracil [BVU: (E)-5-(2-bromo-vinyl)uracil] increases the antitumour effect of 5-fluorouracil (5-FU) in DBA/2 mice inoculated with P388 leukaemia cells, most likely by inhibiting 5-FU degradation in vivo. The inhibitory effect is apparently due to the effect of BVU on dihydro-thymine dehydrogenase, the rate-limiting enzyme in the catabolism of pyrimidines. It has also been reported that bromovinyldeoxyuridine [BVDU; (E)-5-(2-bromovinyl)-2'-deoxyuridine] is rapidly converted to BVU *in vivo* (Desgranges *et al.*, 1984) and induces the same synergistic antitumour effect with 5-FU as observed upon injection of BVU (Desgranges *et al.*, 1986). BVDU itself is an anti-viral compound with great promise for the treatment of herpes simplex virus (type 1) and varicella-zoster virus infections (de Clercq & Walker, 1984).

We have shown (Ben-Efraim *et al.*, 1986) that treatment of MOPC-315 plasmacytoma tumour cells for 1 h with a minimal dose of $0.1 \mu g$ 5-FU/10^7 cells caused marked reduction in incorporation of (methyl)^{3}H thymidine (up to 68% of the degree of thymidine incorporation in control untreated cells). No attempts were made to determine the effect of extending the length of the 5-FU treatment interval on tumour cells. In view of results obtained with other tumour lines (Drewinko & Yang, 1985), it might be that extending the length of 5-FU treatment of tumour cells will result in more marked inhibition of thymidine incorporation and possibly in reduction of the minimal effective dose of 5-FU.

The data indicating tumouricidal effect *in vitro* of 5-FU on MOPC-315 tumour cells prompted us to evaluate the effect of 5-FU therapy in mice bearing MOPC-315 plasmacytoma tumours. We found

(Ben-Efraim *et al.*, 1986) that a single injection of 5-FU at $200 \, \mathrm{mg \, kg^{-1}}$ in BALB/c mice bearing large MOPC-315 plasmacytomas induced transient regression of the tumours. At doses $< 200 \, \mathrm{mg \, kg^{-1}}$, 5-FU was without effect (Ben-Efraim *et al.*, 1986). In view of the inhibitory effect of BVDU (via BVU) on the degradation of 5-FU (Desgranges *et al.*, 1986), it appeared of interest to determine whether low doses of 5-FU which did not affect development of estab-lished MOPC-315 plasmacytoma tumours alone, could be more effective when administered in combination with BVDU.

The MOPC-315 myeloma cell line employed in this study grows preferentially in the mouse BALB/c strain and is characterized by its ability to secrete anti-TNP trinitrophenyl) $IgA\lambda_2$ immuno-globulin (Eisen *et al.*, 1968). Subcutaneous inoculation of 1×10^5 MOPC-315 tumour cells invariably induces formation of local tumour which reach a size of $\sim 15.0 \, \mathrm{mm}$ on the 11th day after inoculation and death within 20 days. An *in vitro* line of MOPC-315 tumour cells, adapted to growth in culture (Yaniv *et al.*, 1978), was maintained in RPMI 1640 medium (GIBCO, NY, USA), supple-mented with $100 \, \mathrm{U \, ml^{-1}}$ penicillin, $100 \, \mu g \, ml^{-1}$ streptomycin, $2 \, \mathrm{mmol \, ml^{-1}}$ L-glutamine, and 10% foetal calf serum. The MOPC-315 cell line was used for evaluating the *in vitro* effects of BVDU and 5-FU on (methyl)^{3}H thymidine (Nuclear Research Centre, Negev, Israel; $1.0 \, \mu Ci/50 \, \mu l$/culture) incor-poration in tumour cells. BVDU was synthesized at the Rega Institute for Medical Research Leuven, Belgium following a described procedure (Jones *et al.*, 1979). 5-FU was kindly donated by Abic, Ramat-Gan, Israel. The effect of BVDU and 5-FU on MOPC-315 tumour cells *in vitro* were monitored in incubating 1×10^7 cells with varying compound concentrations for 1 h at 37°C, washing the cells with serum-free medium and further incubating the cell cultures as described (Bocian *et al.*, 1984). For the experiments *in vivo*, groups of ten BALB/c mice

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(8–12 weeks old) were inoculated s.c. with 1×10^5 viable MOPC-315 tumour cells. BVDU (200 μmol – 69.5 mg kg^{-1}) was injected i.p. in a volume of 0.5 ml on the 11th day after tumour cell inoculation, followed 1 h later by a single i.p. injection of varying doses of 5-FU in 0.5 ml. Untreated inoculated mice and mice treated with either BVDU or 5-FU alone served as controls. The mice were observed for 60 days for tumour development and death.

The significance of differences between groups treated with 5-FU alone and corresponding groups treated with 5-FU plus BVDU was calculated by the double tailed Mann-Whitney U test. The rate of tumour development in various groups as indicated by measurements of tumour sizes was compared till the 19th day after inoculation. Until this day, all of the inoculated mice were still alive in the compared groups. Differences were considered significant when $P < 0.05$. The therapeutic index (TI) was calculated as ratio of maximum tolerated dose (MLD) to minimum effective dose (MED). According to our previously reported data (Ben-Efraim *et al.*, 1986) the MLD for 5-FU was 250 mg kg^{-1} body weight. The MED was taken as the lowest quantity of 5-FU which induced significant transient regression of large MOPC-315 tumours. Injection of 5-FU, 100 mg kg^{-1} body wt plus BVDU (69.5 mg kg^{-1}) in normal, noninoculated mice did not affect the survival of animals and did not cause appreciable loss of weight.

Exposure of MOPC-315 tumour cells *in vitro* to different concentrations of BVDU (0.5 to 100 μg 10^{-7} cells) for 1 h did not affect the rate of thymidine incorporation into the tumour cells. In keeping with previous results (Ben-Efraim *et al.*, 1986), treatment of MOPC-315 tumour cells with 5-FU markedly reduced the extent of thymidine incorporation into the cells (Table I). Treatment with BVDU alone (69.5 mg kg^{-1}) or 5-FU alone (12.5 to 100 mg kg^{-1}), did not affect the growth of MOPC-315 tumour *in vivo*. If however, 5-FU treatment was combined with BVDU treatment, 5-FU at doses ranging from 25 to 100 mg kg^{-1}, caused a transient regression of MOPC-315 tumours and prolongation of survival time. The kinetics of tumour development are presented in Figures 1a, b and the rates of survival are shown in Figures 2a, b. The therapeutic index (TI) was 2.5 for mice treated with 5-FU alone (250 mg kg^{-1} MLD/100 mg kg^{-1} MED) and 20.0 for mice treated with a combination of 5-FU plus BVDU (250 mg kg^{-1} MLD/12.5 mg kg^{-1} MED).

Our findings indicate that BVDU has a potentiating effect on the antitumour activity of 5-FU in mice bearing large progressively growing MOPC-315 tumours. These results are in agreement with previously reported data (Desgranges *et al.*, 1986)

Table I Effect of bromovinyldeoxyuridine (BVDU) and 5-fluorouracil (5-FU) on [methyl-H]thymidine incorporation in MOPC-315 tumour cells *in vitro*.

Treatment[a]		[methyl-^{3}H]thymidine incorporation[b]	
Compound	μg 10^{-7} cells	cpm ± s.e.	% of control
None	——	220,064 ± 12,994	——
BVDU	0.5	291,128 ± 5,030	132.2
	5.0	219,924 ± 3,126	99.9
	15.0	217,218 ± 3,604	98.7
	30.0	196,562 ± 3,394	89.3
	100.0	229,560 ± 5,943	104.3
None	——	178,044 ± 3,933	——
5-FU	3.0	79,837 ± 15,360	44.7
	10.0	42,312 ± 5,596	23.7
	30.0	57,829 ± 3,936	32.4

[a]MOPC-315 tumour cells were incubated for 1 h with the compound at 37°C and washed 3 times before culturing; tumour cells incubated in medium alone were used as controls; [b]Tumour cells were cultured for 72 h; [methyl-^{3}H]thymidine (1 μCi/culture) was added for the last 6 h of incubation; 2×10^4 tumour cells/culture; cpm represent means (±s.e.) for 16 parallel samples; % of thymidine incorporation is expressed as function of the corresponding control.

on the synergistic antitumour activity of BVDU and 5-FU in DBA/2 mice inoculated with P388 leukaemia cells. BVDU alone does not affect MOPC-315 tumour cells either *in vitro* or *in vivo*. Since BVDU is readily converted *in vivo* to BVU (Desgranges *et al.*, 1984) and since BVU is capable of inhibiting the degradation of 5-FU *in vivo* (Desgranges *et al.*, 1986), one may postulate that the increased antitumour activity of combined BVDU and 5-FU therapy in the MOPC-315 model is due to a decrease in the degradation of 5-FU, and hence greater bio-availability of the compound.

Under the experimental conditions used (i.e. single doses of both BVDU and 5-FU), only a transient regression of MOPC-315 tumours was observed. It would seem imperative to examine whether a more intensive treatment regimen, i.e. higher doses of BVDU and/or repeated administrations of BVDU and 5-FU, may yield a more definitive prolonged or even definitive regression of MOPC-315 tumours. In any case, the combination of 5-FU and BVDU is an interesting lead that should be further pursued as a therapeutic modality in the treatment of cancer. Improvement in effectiveness of 5-FU therapy may be of importance in view of the doubts expressed (Drewinko & Yang, 1985) on the efficacy of therapy with this drug alone.
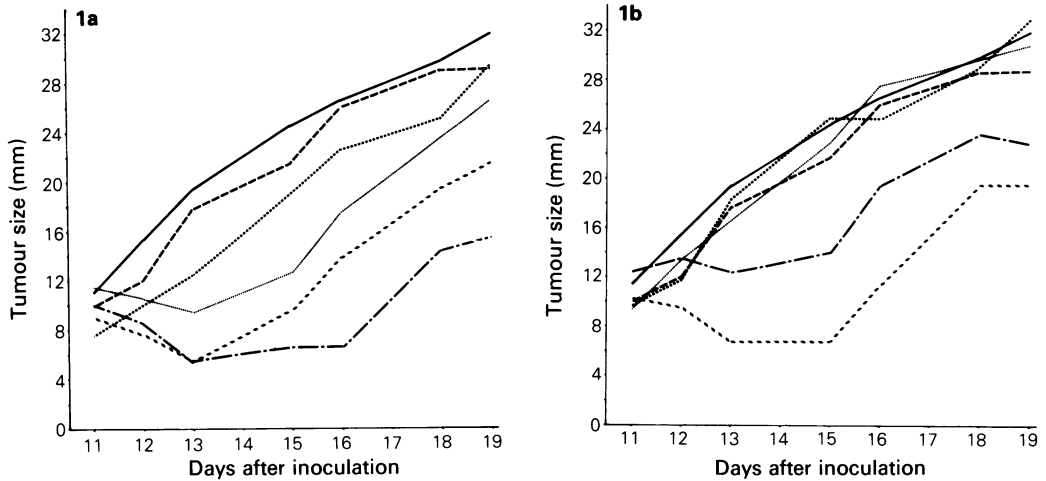
Figure 1 (a) Effect of treatment with bromovinyldeoxyuridine (BVDU) and 5-fluorouracil (5-FU) on the development of MOPC-315 tumours.

1 (– – –) inoculated, untreated mice.
2 (··········) inoculated, 5-FU 100 $(mg\,kg^{-1})$.
3 (- - - -) inoculated, 5-FU 100 $(mg\,kg^{-1})$+BVDU 69.5 $(mg\,kg^{-1})$.
4 (------) inoculated, 5-FU 50 $(mg\,kg^{-1})$.
5 (—–—) inoculated, 5-FU 50 $(mg\,kg^{-1})$+BVDU 69.5 $(mg\,kg^{-1})$.
6 (——) inoculated, BVDU 69.5 $(mg\,kg^{-1})$.

Significant differences ($P<0.05$) were found between groups 4 and 5 on days 13–19 after inoculation.

(b) Effect of treatment with bromovinyldeoxyuridine (BVDU) and 5-fluorouracil (5-FU) on the development of MOPC-315 tumours.

1 (– – –) inoculated, untreated mice.
2 (··········) inoculated, 5-FU 25 $(mg\,kg^{-1})$.
3 (- - - -) inoculated, 5-FU 25 $(mg\,kg^{-1})$+BVDU 69.5 $(mg\,kg^{-1})$.
4 (------) inoculated, 5-FU 12.5 $(mg\,kg^{-1})$.
5 (—–—) inoculated, 5-FU 12.5 $(mg\,kg^{-1})$+BVDU 69.5 $(mg\,kg^{-1})$.

Significant differences ($P<0.05$) were found between groups: 2 *vs.* 3: days 13–19 after inoculation; 4 *vs.* 5: days 13–16 after inoculation.
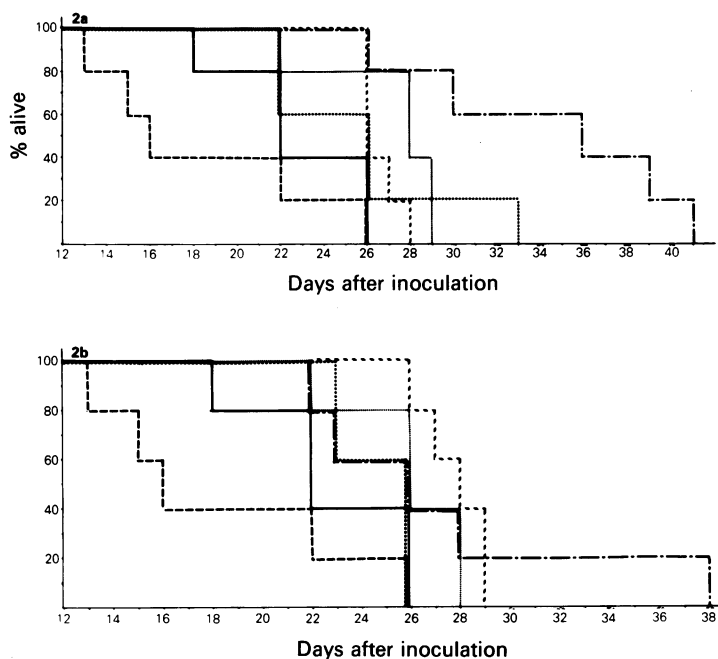
Figure 2 (a) Survival times after inoculation with MOPC-315 tumour cells and treatment with bromovinyl-deoxyuridine (BVDU) and 5-fluorouracil (5-FU).

1 (– – –) inoculated, untreated mice.
2 (··········) inoculated, 5-FU 100 (mg kg^{-1}).
3 (- - - -) inoculated, 5-FU 100 (mg kg^{-1})+BVDU 69.5 (mg kg^{-1}).
4 (------) inoculated, 5-FU 50 (mg kg^{-1}).
5 (—--—) inoculated, 5-FU 50 (mg kg^{-1})+BVDU 69.5 (mg kg^{-1}).
6 (———) inoculated, BVDU 69.5 (mg kg^{-1}).

(b) Survival times after inoculation with MOPC-315 tumour cells and treatment with bromovinyldeoxy-uridine (BVDU) and 5-fluorouracil (5-FU).

1 (– – –) inoculated, untreated mice.
2 (··········) inoculated, 5-FU 25 (mg kg^{-1}).
3 (- - - -) inoculated, 5-FU 25 (mg kg^{-1})+BVDU 69.5 (mg kg^{-1}).
4 (------) inoculated, 5-FU 12.5 (mg kg^{-1}).
5 (—--—) inoculated, 5-FU 12.5 (mg kg^{-1})+BVDU 69.5 (mg kg^{-1}).

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