The cytogenetic efficiency of the antitumor agents bleomycin and peplomycin is enhanced by the heart drug verapamil (isoptin)

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Summary. The induction of 2-break chromosome aberrations (dicentrics and ring chromosomes) in human lymphocytes by the antitumor agents bleomycin and peplomycin is strongly enhanced when those agents are applied together with the heart drug verapamil (isoptin).

Bleomycin (BLM), a widely used antitumor agent, is a basic glycopeptide consisting of bleomycinic acid and a terminal amine[2-4].

Peplomycin (PEP) is a new bleomycin derivate with promising clinical qualities, which has already been successfully used for cancer therapy[2]. Whereas the BLM preparations used for cancer therapy are mixtures of several BLM forms, differing from each other by their amine, PEP is a monosubstance[2-4].

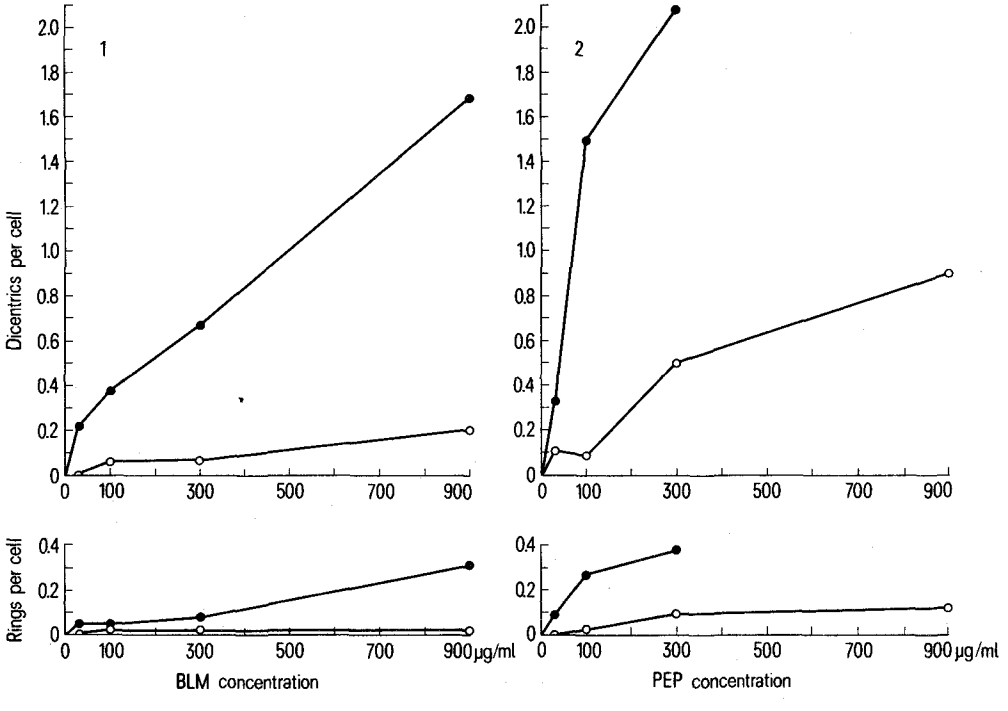
Verapamil (VERA) – other designation: isoptin – is a common antiarrhythmic and vasodilatory drug. PEP combined with VERA kills HeLa cells and mouse FM3A cells more efficiently than PEP applied alone[5]. The mechanism of the cell-killing induced by antitumor agents like BLM and PEP, i.e. by radiomimetic substances, can be assumed to be partly based on the production of chromosome aberrations[6]. If so, besides the cytotoxic efficiency (cell killing) the cytogenetic efficiency (production of chromosome aberrations) of BLM and PEP should also be enhanced by VERA. The results of the following in vitro experiments carried out with human lymphocytes satisfy this expectation. Dicentrics and ring chromosomes were scored in our experiments; these are the main types of chromosome aberrations induced in G_0.

To study the influence of VERA on the cytogenetic efficiency of BLM and PEP, human peripheral blood of a healthy adult female donor was incubated either with BLM or PEP (both from Mack, Illertissen, FRG) alone or in combination with VERA (verapamil hydrochloride = isoptin, Knoll AG, Ludwigshafen, FRG). 2 ml of heparinized blood (10 units sodium heparin/ml) were mixed with 8 ml prewarmed medium McCoy 5a (37 °C) containing BLM (PEP) at concentrations of 30, 100, 300 and 900 µg/ml or BLM (PEP) at the same concentrations plus 0.15 mM of VERA. The relatively high BLM (PEP) concentration of 900 µg/ml was chosen to compensate for the relatively short incubation time of 1 h. This BLM (PEP) dose is not identical with the ED_{50}.

Table 1. The enhancement of the frequency of BLM or PEP induced chromosome aberrations by VERA

BLM concentration (µg/ml)	Dicentrics/cell Without VERA	With VERA	Rings/cell* Without VERA	With VERA
0	0.001**	0.00 (0/100)	0.00**	0.00 (0/100)
30	0.01 (1/109)	0.22 (23/103)	0.00 (0/109)	0.05 (5/103)
100	0.06 (7/112)	0.38 (39/104)	0.02 (2/112)	0.05 (5/104)
300	0.07 (7/107)	0.67 (68/101)	0.02 (2/107)	0.08 (8/101)
900	0.20 (22/109)	1.68 (193/115)	0.02 (2/109)	0.31 (36/115)
PEP concentration (µg/ml)	**Dicentrics/cell Without VERA**	**With VERA**	**Rings/cell Without VERA**	**With VERA**
0	0.001**	0.00 (0/100)	0.00**	0.00 (0/100)
30	0.11 (11/102)	0.33 (33/101)	0.00 (0/102)	0.09 (9/101)
100	0.09 (9/102)	1.49 (164/110)	0.03 (3/102)	0.27 (30/110)
300	0.50 (53/107)	2.07 (60/29)	0.10 (11/107)	0.38 (11/29)
900	0.89 (92/103)	–***	0.14 (14/103)	–***

* Sum of centric plus acentric rings.
** Historical control value, based on a critical literature study[8].
*** No cells reached the metaphase.



Figures 1 and 2. Dependence of the number of dicentrics or ring chromosomes per cell on the concentration of BLM (fig. 1) or PEP (fig. 2) in the absence (○) or presence (●) of VERA (0.15 mM).

Table 2. Intercellular distribution of dicentrics after treatment with BLM alone (experiments 1–4) or with BLM plus verapamil at a concentration of 0.15 mM (experiments 5–8)

Experiment	BLM concentration (μg/ml)	Dicentrics per cell															
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	30	108	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	100	109	2	–	–	–	1	–	–	–	–	–	–	–	–	–	–
3	300	101	5	1	–	–	–	–	–	–	–	–	–	–	–	–	–
4	900	92	13	3	1	–	–	–	–	–	–	–	–	–	–	–	–
5	30	93	7	–	1	–	1	–	–	1	–	–	–	–	–	–	–
6	100	81	13	7	2	–	–	1	–	–	–	–	–	–	–	–	–
7	300	63	24	7	4	–	1	1	1	–	–	–	–	–	–	–	–
8	900	50	23	13	10	4	5	6	2	1	–	–	–	–	–	–	1

Table 3. Intercellular distribution of dicentrics after treatment with PEP alone (experiments 1–4) or with PEP plus verapamil at a concentration of 0.15 mM (experiments 5–8)

Experiment	PEP concentration (μg/ml)	Dicentrics per cell																		
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	30	93	7	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
2	100	93	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	300	77	18	8	1	1	1	–	1	–	–	–	–	–	–	–	–	–	–	–
4	900	58	21	12	9	1	1	–	–	–	–	–	1	–	–	–	–	–	–	–
5	30	82	12	4	2	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–
6	100	52	23	15	10	4	–	1	1	–	1	–	–	1	–	–	1	1	–	–
7	300	11	7	3	5	–	–	1	–	1	–	–	–	–	–	–	–	–	–	1
8	900*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

*No cells survived.

The cells were incubated in sterile tubes at 37 °C in the dark. They were then washed in prewarmed medium (McCoy 5a) and centrifuged for 10 min at 100 × g. This procedure was repeated 3 times. After the supernatant had been removed with a pipette the remaining cell pellet (about 1 ml) was divided into 2 parts. To each part 5 ml culture medium was added. The culture medium consisted of medium McCoy 5a, 20% foetal calf serum, 5 units/ml sodium heparin, 0.1 mg/ml streptomycin, 100 units/ml penicillin, and 0.15 ml phytohemagglutinin M. The cultures were incubated for 48 h at 37 °C in the dark. After 45 h colcemid (0.33 μg/ml) was added. Slide preparations were made by standard methods. Each metaphase was analysed by 2 observers for the presence of dicentrics, polycentrics, and ring chromosomes. Only metaphases with 46 centromeres were used. Polycentrics were scored as dicentrics (number of centromeres −1 = number of dicentrics).

Our main finding is that the cytogenetic efficiency both of BLM and PEP is strongly enhanced when these agents are applied together with VERA (table 1, figs 1 and 2).

After treatment with PEP more dicentrics and rings were observed than after treatment with BLM, both in the absence and presence of VERA. This does not necessarily indicate that PEP is a stronger mutagen than BLM, but may result from some loss of activity of the BLM sample used by us. The intercellular distribution of the dicentrics observed is 'overdispersed' for most of the BLM and PEP concentrations tested (tables 2 and 3). This means that, compared with the Poisson distribution, there are too many cells without dicentrics and with high numbers of dicentrics (e.g., 4 dicentrics per cell), and – 'for compensation' – too few cells with low numbers of dicentrics, especially with 1 dicentric. The difference between the Poisson distribution and the observed distribution is highly significant in most cases (χ^2-test). This agrees with the overdispersion found for BLM induced dicentrics[7]. The intercellular distribution of the rings induced by BLM or PEP in our experiments was not tested statistically, because the absolute numbers of rings were too small.

The following conclusions can be drawn from our results. 1. The agreement between our cytogenetic observations and the cytotoxicity results[5] supports the idea that chromosome aberrations play a causal role in cell killing induced by antitumor agents like BLM or PEP. 2. VERA is a comutagen. 3. In view of both the cytogenetics and the cytotoxicity results it seems possible that a combined therapy with BLM (PEP) and VERA potentiates the antitumor efficiency and some of the toxic side effects of these cytostatics.

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