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Vinblastine increases antitumor effectiveness of bleomycin

Maja Čemažar, Marija Auersperg and Gregor Serša

Institute of Oncology, Ljubljana, Slovenia

In our previous study, vinblastine (VELBE) was shown to increase the plasma membrane fluidity. This effect of VELBE might be exploited for better transport of drugs through the plasma membrane. Bleomycin (BLM) is a highly cytotoxic drug when present inside the cells but has a hampered transport through the plasma membrane. The aim of the present study was to determine whether pretreatment with VELBE can increase the effect of BLM on intraperitoneal SA-1 tumors in mice. BLM and VELBE were used as single agents or in various combinations, i.e. VELBE and BLM injected simultaneously, BLM injected 24 h before VELBE or VELBE injected 24 h before BLM. Mice survival was the end-point used for determining the effect of this combined treatments. VELBE and BLM as a single treatment significantly prolonged median survival time of study animals compared to controls. Furthermore, when VELBE and BLM were combined, all three tested combinations were more effective than VELBE or BLM as single treatments. The effect on animal survival was equal when VELBE was given 24 h after or simultaneously with BLM. The longest survival, however, was obtained when VELBE was injected 24 h before BLM. From these results we can conclude that the underlying mechanisms for more than additive effect of VELBE and BLM when VELBE was given 24 h before BLM could be attributed to an increased membrane fluidity, possibly in combination with a cell kinetic effect.

Key words: sarcoma experimental-drug therapy; vinblastine; bleomycin; antineoplastic aagents, combined

Introduction

Vinblastine (VELBE) is an antimitotic alkaloid isolated from periwinkle plant Catharanthus roseus G. Don (Vinca rosea L.). It exerts cytotoxic activity against various tumors and is presently used mainly in combined chemotherapeutic schedules for treatment of testis tumors, Hodgkin's and non-Hodgkin's lymphomas, breast carcinoma, gastric carcinomas, squamous cell carcinoma, thyroid carcinomas, sarcomas and many others.[1-5]

Most presently used combined chemotherapeutic schedules are designed empirically. However, the increasing knowledge of mechanisms of action of cytotoxic drugs forms the basis for rational planning of clinical chemotherapy. In our previous study we have demonstrated that VELBE increases the plasma membrane fluidity and consequently its permeability.[6] Therefore, the rationale of the use of VELBE in combined chemotherapeutic schedules would be that VELBE is administered before an agent which has a hampered transport though the plasma membrane. One of such agents is bleomycin (BLM), a highly cytotoxic drug when present inside the cells.[7] It was shown that as little as several thousand molecules of BLM present inside the cell induce cell death.[8] Based on these properties of VELBE and BLM we conducted a study with the aim to determine the effect of BLM on i.p. SA-1 tumors pretreated with VELBE. Animal survival was the end-point used for determining the effect of this combined treatment.

Correspondence to: Maja Čemažar, M.Sc., Department of Tumor Biology, Institute of Oncology, Zaloška 2, SI-1105 Ljubljana, Slovenia. Tel.: +386 61 323 063 ext. 29-33, Fax.: +386 61 131 41 80, E-mail: mcemazar@onko-i.si

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Materials and methods

Drug formulation

VELBE (Vinblastine sulphate, Lilly France S.A.) was dissolved in 0.9% NaCl solution at a concentration 2.5 μg/ml. BLM (Mack, Germany) was dissolved in 0.9 % NaCl solution at a concentration 500 μg/ml. The drugs were injected intraperitoneally in 0.5 ml. According to Freireich *et al.,* the corresponding doses for VELBE in humans would be 0.2 mg/m^2 (0.005 mg/kg) and for BLM 37 mg/m^2 (1.0 mg/kg).[9]

Animals

Inbred A/J mice were purchased from the Rudjer Boskovic Institute (Croatia). Mice were maintained at a stable room temperature (24°C) and natural day/night light cycle in a conventional animal colony. Before experiments, mice were subjected to an adaptation period of at least 10 days. Mice of both sexes in good condition, weighing 20-22 g, without signs of infection, 10-12 weeks old, were included in the experiment.

Tumor model

Intraperitoneal (i.p.) SA-1 fibrosarcoma syngeneic to A/J mice was used in the study. The tumor was maintained i.p. as ascites by serial transplantation once a week. For induction of i.p. tumors, tumor cells from the donor mouse were harvested by peritoneal lavage with 4 ml of 0.9 % NaCl solution, washed and resuspended at a concentration of 6 x 10^5 cells/ml. Tumors were induced by i.p. injection of 3 x 10^5 viable SA-1 cells in 0.5 ml 0.9 % NaCl solution. Cell viability, determined by dye exclusion test, was over 95 %.

Study design

To determine the effect of BLM on i.p. SA-1 tumor pretreated with VELBE, mice were treated with VELBE (2.5 μg/mouse) and 24 hours later with BLM (250 μg/mouse). The control groups were as follows: a group without treatment (same procedure; injected with 0.9 % NaCl solution instead of VELBE or BLM), groups treated with VELBE or BLM as single treatment, a group treated with VELBE and immediately afterward with BLM (simultaneously), and a group treated with BLM 24 h before VELBE. Each experimental group consisted of 10 mice.

Statistical analysis

Survival curves were plotted by the Kaplan-Meier method and the differences between the survival curves were determined using the log-rank test.

Results

The effect of BLM and VELBE injected in different combinations on SA-1 tumors was evaluated by animal survival. VELBE and BLM as single treatment significantly prolonged median survival time of animals compared to controls (Table 1. 2., Figure 1.). Furthermore, when VELBE and BLM were combined, all three tested combinations were more effective than VELBE or BLM as single treatments. The effect on animal survival was equal when

Table 1. Antitumor effect of VELBE and BLM treatment on SA-1 i.p. tumors.

Group	n	median survival time (days)
control	10	8.0
VELBE	10	9.5
BLM	10	13.5
BLM-24h-VELBE[1]	10	15.0
BLM-0h-VELBE[2]	10	14.5
VELBE-24h-BLM[3]	10	17.0

[1] BLM injected 24 h before VELBE
[2] BLM and VELBE injected simultaneously
[3] VELBE injected 24 h before BLM

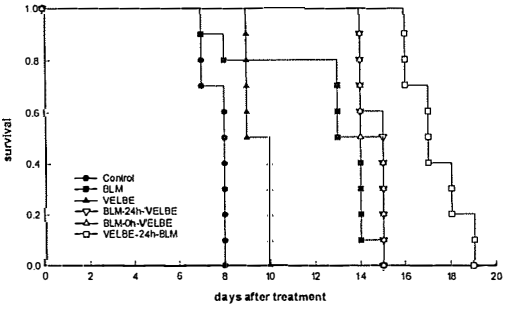

Figure 1. Effect of BLM and VELBE injected in different combinations on survival of mice with i.p. SA-1 tumors. Each experimental group consisted of 10 mice. The survival curves were plotted by the Kaplan-Meier method.

Table 2. Comparison of survival of mice with i.p. SA-1 tumors treated with VELBE and BLM injected in different combinations.

		p compared to			
Group	control	BLM	VELBE	BLM-24h-VELBE	BLM-0h-VELBE
BLM	0.0011				
VELBE	0.0092	0.0042			
BLM-24h-VELBE[1]	<0.0001	<0.0001	<0.0001		
BLM-0h-VELBE[2]	<0.0001	<0.0001	<0.0001	0.6613	
VELBE-24h-BLM[3]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1] BLM injected 24 h before VELBE
[2] BLM and VELBE injected simultaneously
[3] VELBE injected 24 h before BLM

VELBE was given 24 h after or simultaneously with BLM. However, the longest survival was obtained when VELBE was injected 24 h before BLM.

Discussion

Our study showed that VELBE administered 24 h before BLM increased BLM antitumor effectiveness on i.p. SA-1 tumors in mice.

This study was based on the results of our previous study showing that VELBE increases the plasma membrane fluidity of SA-1 tumor cells at low doses which do not affect cell viability.[6] Membrane fluidity was increased already 0.5 h after VELBE treatment with the maximum values at 24 and 48h. Therefore, in the present study aiming to determine the influence of pretreatment with VELBE on BLM effectiveness we have used a schedule with a 24 h time interval between the two drugs. The antitumor effectiveness was evaluated on the same tumor model, i.e. SA-1 fibrosarcoma, as in our previous study.[6]

VELBE and BLM have different mechanisms of action. VELBE interferes with polymerization of tubulin, a protein which is involved in formation of mitotic spindle microtubules and is also an important component of cytoskeleton. In accordance with its effect on mitotic spindle microtubules, VELBE blocks the cells in the metaphase of mitosis and thus acts as a cell synchronizing agent.[10] The effect on cytoskeleton, however, might influence the plasma membrane fluidity. On the other hand, BLM acts directly on DNA inducing single and double strand DNA cleavage produced by a C-4' deoxyribose hydroperoxide, which is the result of radical abstraction.[7] An additive effect was obtained when VELBE and BLM were given simultaneously or when BLM was injected 24 h before VELBE. In contrast, more than additive effect was obtained when VELBE was given 24 h before BLM. This increased effect of chemotherapy could be the result of either an increased plasma membrane fluidity or a cell kinetic effect caused by VELBE or a combination of both effects. In our previous study we found that VELBE increases membrane fluidity of SA-1 tumor cells and thus we assumed that this could be exploited to facilitate BLM uptake into the cells.[6] To prove that increased plasma membrane fluidity facilitates better accumulation of BLM in the cells, a measurement of BLM concentration in the cells after VELBE treatment would be necessary. In order to facilitate the transport across the plasma membrane other methods were used in addition, such as electroporation.[8,11] In the study of Poddevin *et al.*, an increased accumulation of Co labeled BLM was determined after electroporation of plasma membrane.[11] In our previous clinical studies using ^{99m}Tc labeled BLM (Tc-BLM) we showed that an increased accumulation of Tc-BLM was found in the tumors from approximately 24-48 h after infusion of VELBE.[5,12] In addition, a cell kinetic effect of VELBE was proven by DNA single cell measurement.[12] During the period of increased Tc-BLM an accumulation of cells in S and G2/M compartments of the cell cycle was demonstrated. Cell kinetic effect of VELBE seems to be dose dependent. Higher doses prolong the transition of cells through S phase, whereas lower doses stopped the cells in the G2/M compartment.[13] BLM is reported to be the most effective in G2/M and G1 and less in S phase of the cell cycle.[14] Based on these properties of VELBE and BLM, we assumed that in the present study, a combination of increased membrane fluidity and accumulation of cells in BLM-specific phase of cell cycle could be responsible for the best effect of VELBE and BLM combination in which VELBE preceded BLM by 24 h.

In conclusion, understanding of interactions of agents in combined chemotherapeutic schedules could lead to better planning and timing of drugs in clinical chemotherapy.

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References

1. Haskell CM. Drugs used in cancer chemotherapy. In: Haskell CM ed. *Cancer Treatment.* WB Saunders Company. 1990: 69-70.

2. Van Tellingen O, Sips JMH, Beijnen JH, Bult A, Nooijen WJ. Pharmacology, bio-analysis and pharmacokinetics of the Vinca alkaloids and semi-synthetic derivates (review). *Anti-Cancer Res* 1992; **12:** 1699-716.

3. Auersperg M, Šoba E, Vraspir-Porenta O. Intravenous chemotherapy with synchronization in advanced cancer of oral cavity and oropharynx. *Z Krebsfotsch* 1977; **90:** 149-59.

4. Auersperg M, Us-Kraševec M, Lamovec J, Erjavec M, Benulič T, Porenta Vraspir O. Chemotherapy-a new approach to the treatment of verrucous carcinoma. *Radiol Iugosl* 1989; **23:** 387-92.

5. Auersperg M, Šoba E, Porenta O, Erjavec M, Us-Kraševec M, Bergant D, Furlan L. Rational scheduling of multidrug chemotherapy with synchronization in advanced squamous cell carcinoma of the oral cavity and oropharynx. In: Čupar I and Padovan I eds. *Currents concepts of head and neck cancers.* Jugoslavenska Akademija Znanosti i Umjetnosti, 1981: 286-99.

6. Serša G, Čemažar M, Šentjurc M, Us-Kraševec M, Kalebić S, Drašlar K, Auersperg M. Effects of vinblastin on cell membran fluidity and the growth of SA-1 tumor in mice. *Cancer Lett* 1994; **79:** 53-60.

7. Lazo JS, Sebti SM, Schellens JHM. Bleomycin. In: Pinedo HM, Longo DL, Chabner BA, eds. *Cancer chemotherapy and biological response modifiers annual 16.* Elsevier Science B.V., 1996: 39-47.

8. Tounekti O, Pron G, Belehradek J, Mir LM. Bleomycin, anapoptosis-mimetic drug that induced two types of cell death depending on the number of molecules internalized. *Cancer Res* 1993; **53:** 5462-69.

9. Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemother Rep* 1966; **50:** 219-45.

10. Jordan MA, Thower D, Wilson L. Mechanism of inhibition of cell proliferation by *Vinca* alkaloids. *Cancer Res* 1991; **51:** 2212-22.

11. Poddevin B, Orlowski S, Belehradek JJr, Mir LM. Very high cytotoxicity of bleomycin introduced into the cytosol of cells in culturs. *Biochem Pharmacol* 1991; **42(S):** 67-75.

12. Auersperg A, Erjavec M, Us-Kraševec M. Accumulation of ^{99m}Tc-Bleomycin in human squamous cell carcinoma in vivo after synhronisation by vinblastine. *IRCS Medical Science: Cancer; Clinical Pharmacology and Therapeutics; Radiology and Nuclear Medicine* 1975; **3:** 560.

13. Madoc-Jones H, Mauro F. Intrphase action of vinblatine and vincristine: differences in their lethal action through the mitotic cycle of cultured mammalian cells. *J Cell Physiol* 1972; **72:** 185-96.

14. Olive PL, Banath JP. Detection of DNA double strand breaks through the cell cycle after exposure to X-rays, bleomycin, etoposide and 125IdUrd. *Int J Radiat Biol* 1993; **64 (4):** 349-58.