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Vincristine-Methotrexate Combination Chemotherapy and the Influence of Weight Loss on Experimental Tumour Growth

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Summary. *According to pharmacokinetic reports, vincristine administration should precede methotrexate therapy. Our sequential treatment of L1210 leukaemic mice, in which vincristine was administered before methotrexate therapy, was as effective as treatment with the two drugs given simultaneously. In solid tumour experiments we were unable to show any increase in the antitumour effect of methotrexate when vincristine was injected before methotrexate administration. Consequently, we advocate the re-evaluation of the practice of vincristine → methotrexate therapy as used in many clinical protocols for the treatment of patients with osteosarcoma.*

Pretreatment with vincristine resulted in methotrexate-induced weight loss and sometimes in toxic death of the mice. Since the growth of tumours can be modified by regulation of the caloric intake of the host, this aspect was investigated in more detail. The effect of starvation, which was comparable to the effect of drug-induced weight loss, had a retarding effect on tumour growth. The growth rates of smaller tumour volumes were less severely affected than were those of large tumour masses.

Introduction

Methotrexate (MTX) is the most widely used antifolate drug. Its mode of action and pharmacokinetic properties, and the therapeutic implications of preclinical research have been extensively reviewed. The number of compounds altering the activity of MTX range from commonly used barbiturates and tranquilisers to citrovorum factor, used for the well-known rescue technique.

Zager et al. investigated the effects of several clinically useful antibiotics and antineoplastic agents on the intracellular accumulation of MTX and on its antitumour effects in L1210 leukaemia-diseased mice [18]. Vincristine (VCR) resulted in an increase of MTX uptake and potentiated the antitumour effect of MTX in vivo. The positive experimental results obtained by this group had a great impact on the drug schedule design of treatment protocols. A basic factor influencing the antitumour efficacy of combination chemotherapy is the limitation imposed by toxicity for the host [11–13; Mulder and Van Putten, submitted for publication]. Therefore, an enhanced effect of VCR and MTX on tumour cells should not be accompanied by a marked increase in toxicity to critical normal tissues.

The effect of combination chemotherapy with VCR and MTX was investigated in three experimental tumour lines and in critical normal tissue: the L1210 leukaemia, the mouse C22LR osteosarcoma, the Lewis lung carcinoma, and normal haemopoietic bone marrow stem cells. Pretreatment with VCR resulted in MTX-induced weight loss and sometimes in toxic death of the mice. Since the growth of tumours can be modified by regulation of the host's caloric intake [3, 16], we extended our investigations to the effect of starvation of the animals on tumour growth.

Materials and Methods

Materials

(Balb/C × DBA2)F1 hybrids (hereafter called CD2 F1) were used for the L1210 leukaemia experiments. (C57BL/Ry × CBA/Ry)F1 mice (hereafter called BCBA F1) were used for the Lewis lung carcinoma and C22LR osteosarcoma investigations [12]. Vincristine sulphate was obtained from Eli Lilly & Co and MTX was a gift from Lederle. The two drugs were given either simultaneously or sequentially. In the sequential treatment schedule, the time interval between VCR and midday MTX administration was 24 h unless otherwise stated. VCR and single-dose treatment with MTX were administered IP. When MTX injections were repeated, they were administered SC

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at 10 a.m., 1 p.m., and 4 p.m. Plasticized carbon microspheres (15 ± 5 μm) were obtained from 3 M Company, Saint Paul, Minnesota, USA.

Methods

From an exponentially growing L1210 in vitro culture, 10^5 cells were injected IP to CD2 F1 mice. The end point of treatment was the mean survival time (MST), which was expressed in days. Single cell suspensions were made from the two solid tumour lines [12]. Cells were inoculated SC or IM to mice and the volume of the tumours was determined twice weekly. The end point of treatment was growth delay, expressed in days, i.e., the displacement in time between the growth curve of the control group and the growth curve of the tumours recurring after treatment. If no original tumour volume was known (e.g., for tumour inocula exposed to treatment before a tumour was palpable), the delay, in comparison with the control group, before an arbitrary volume between 400–800 mm^3 was reached was estimated. The Lewis lung carcinoma inoculated SC or IM gives rise to spontaneous lung metastases. To enhance the formation of these metastases, 10^6 microspheres were injected IV at day 1 after tumour cell inoculation. The Lewis lung tumour-bearing mice were sacrificed at a specified time after tumour inoculation and, after fixation of the lungs in Bouin's fluid, the number of metastases was counted. The C22LR osteosarcoma does not give rise to spontaneous lung metastases. Therefore, to assess the effect of treatment on microscopic disease, osteosarcoma cells were injected IV into one of the tail veins. Approximately 2 weeks later, the number of artificially induced macroscopic tumour colonies on the surface of the lung was determined. The spleen colony technique of Till and McCulloch was used to assess the effect of treatment on normal resting haematopoietic stem cells. The effect of treatment on recruited, rapidly proliferating normal bone marrow stem cells was also investigated [12]. The survival of resting and proliferating colony-forming units (CFUs) was calculated as the ratio of the mean number of CFUs per donor femur and per spleen, respectively, of treated and simultaneously assayed control mice.

In the starvation experiments, the animals were intermittently deprived of their standard diet, but not water, for 2 or 3 days. Other housing conditions, such as cage size, 12-h light-dark regimen, temperature of 25° C, and 65% humidity, were kept constant. For the assessment of drug-induced toxicity, animal survival was checked daily. Drug-induced early toxic death is defined as death within 10 days after the end of treatment unless clearly caused by tumour growth. The Student's *t*-test was used for the evaluation of the results of treatment with the different sequential combinations.

Results

The effect of VCR-MTX administration on L1210 leukaemia is shown in Table 1. Two different dosages of VCR were used in combination with a fixed dose of MTX. A synergistic effect on L1210 leukaemia is evident when the MST after combined treatment is greater than the algebraic sum of the values for MST determined for the two agents separately minus that of controls. The observed MST with combination chemotherapy is what would be the expected MST calculated from the results determined for the two agents separately. Only an additive effect was observed, and no sign of drug schedule dependency. The effect of shorter time intervals of 12 and 6 h between VCR and MTX administration was also investigated (Expt. no. II). The results (not shown in Table 1), were never significantly different from those obtained for simultaneous treatment. In Experiments III and IV, the dosages and time intervals between VCR and MTX administration were chosen according to the schedule described by Zager et al. [18]. VCR pretreatment did not potentiate the effect of MTX therapy. Since single-dose MTX administration (30 $mg \cdot kg^{-1}$ IP) in mice inoculated with either Lewis lung carcinoma or osteosarcoma did not result in a significant tumour response, we repeated the MTX injection SC three times at 3-h intervals. Some tumour volume reduction was observed with the 3 × 7.5 mg/kg SC schedule in the Lewis lung tumour experiments; however, the treatment was very toxic to the tumour-bearing host. As shown in Table 2, a total of 13 out of 15 mice died within 10 days after treatment when VCR preceded

Table 1. The effect of vincristine and methotrexate treatment on the mean survival of L1210 diseased mice

Agents and treatment schedule[a]	Expt. no.	I	II	III	IV
	Days of treatment	1, 2	1, 2	2, 6, 10, 14	2, 6, 10, 14
	Dose of VCR (mg)	0.075	0.5	0.5	0.5
	Dose of MTX (mg)	40	40	25	25
	Time interval (h)	24	24	0.5	0.5
Control		9.2 ± 0.1	8.2 ± 0.2	8.8 ± 0.6	8.7 ± 0.2
VCR		10.3 ± 0.3	10.6 ± 0.4	11.9 ± 0.4	11.3 ± 0.8
MTX		15.9 ± 0.6	13.5 ± 0.3	15.8 ± 0.7	16.3 ± 0.3
VCR → MTX		17.2 ± 1.2	13.5 ± 0.3	18.5 ± 1.8	16.9 ± 0.3
VCR + MTX		17.7 ± 0.5	13.0 ± 0.5		
MTX → VCR		16.9 ± 0.4	14.9 ± 0.4		
Calculated effect of combination		17.0	15.9	18.9	18.9

[a] Groups of 10 CD2 F1 mice were injected IP with 10^5 L1210 cells on day 0. Dosages are expressed in $mg \cdot kg^{-1}$ IP

[b] Dosages and treatment schedule according to Zager's experiment [18]

MTX administration (VCR $\xrightarrow{24\,h}$ MTX), in contrast to none out of 15 when the reverse sequence was applied. The observed maximal growth delay in the Lewis lung experiments was approximately 1.5 days. Since this antitumour effect was accompanied by the toxic effects of the VCR $\xrightarrow{24\,h}$ MTX treatment, it was decided to discontinue the Lewis lung tumour experiments.

In contrast to the Lewis lung carcinoma, the osteosarcoma was sensitive to repeatedly given MTX injections. Table 3 shows growth delay data, and the effect of treatment on artificially induced lung colonies is given in Table 4. The tentative conclusion from the data is that there seems to be no synergism and no effect of drug scheduling on tumour response. As with the Lewis lung tumour experiments, the results were influenced by the VCR $\xrightarrow{24\,h}$ MTX-induced toxicity of the treatment. To determine whether a concomitantly increased toxicity of VCR $\xrightarrow{24\,h}$ MTX treatment for the bone marrow stem cells could be demonstrated, spleen colony assays were

Table 2. Early toxic death as a result of sequential administration of vincristine and methotrexate

Agents and treatment schedule[a]	Expt. no. Dose of VCR Dose of MTX	I 1 3 × 10	II 1 3 × 7.5	III 1 3 × 6
VCR		0	0	0
MTX		0	0	0
VCR $\xrightarrow{24\,h}$ MTX		5	5	3
VCR + MTX		3	1	0
MTX $\xrightarrow{24\,h}$ VCR		0	0	0

[a] Groups of 5 Lewis lung tumour-bearing mice were treated with VCR (mg · kg^{-1} IP) and MTX (mg · kg^{-1} SC every 3 h) as indicated

Table 3. The effect of sequential treatment on tumour volume in C22LR osteosarcoma[a]

Treatment schedule	Mean growth delay and standard errors (days)
Control	0 ± 0.4
VCR (1 mg/kg IP)	1.9 ± 0.5
MTX (3 × 7.5 mg/kg SC)	4.2 ± 0.6
VCR $\xrightarrow{24\,h}$ MTX	4.0 ± 0.5
VCR + MTX	3.6 ± 0.4
MTX $\xrightarrow{24\,h}$ VCR	3.1 ± 0.4

[a] Groups of 5 BCBA F1 mice were inoculated bilaterally SC with 10^6 osteosarcoma cells on day 0. MTX was administered every 3 h on day 3. Although marked weight loss was observed, no mice died as a result of VCR → MTX treatment

Table 4. The effect of vincristine and methotrexate administration in mice intravenously injected with osteosarcoma cells[a]

Treatment schedule	Number of lung colonies per mouse; median (range)	
	Expt. I	Expt. II
Control	210 (188–242)	*[c]
VCR	72 (63–250)	266 (230–*)
MTX	34 (26–175)	60 (26–*)
VCR $\xrightarrow{24\,h}$ MTX	All mice died of toxicity	27 (1–77) [1][b]
VCR + MTX	7 [3][b]	60 (3–94)
MTX $\xrightarrow{24\,h}$ VCR	14 (13–64)	31 (10–45)

[a] One million osteosarcoma cells mixed with 10^6 microspheres in 0.5 ml Hank's balanced salt solution were injected into one of the tail veins of groups of 5 BCBA F1 mice on day 0. VCR 1 mg · kg^{-1} IP was given on day 3. MTX was given in a dosage of 3 × 10 mg · kg^{-1} SC every 3 h in Expt. I and of 3 × 7.5 mg · kg^{-1} SC every 3 h in Expt. II

[b] The figures in square brackets show the numbers of deaths due to drug toxicity. Lung colonies were counted on day 18

[c] An asterisk indicates that the lung colonies were too numerous to be counted accurately

Table 5. Percentage of surviving normal haemopoietic bone marrow stem cells per femur and repopulating spleen in comparison with untreated control mice

Treatment schedule	Resting stem cells[a]			Proliferating stem cells[b]		
	Expt. I	Expt. II	Expt. III	Expt. I	Expt. II	Expt. III
VCR	101.5	79.7	n.d.[c]	60.1	45.9	n.d.
MTX	114.8	78.2	n.d.	70.1	78.0	n.d.
VCR $\xrightarrow{24\,h}$ MTX	100.1	80.5	74.3	57.0	30.4	35.8
VCR + MTX	91.8	59.2	71.1	65.3	43.7	33.0
MTX $\xrightarrow{24\,h}$ VCR	126.3	101.8	61.4	56.0	23.7	42.4

[a] In the resting stem cell experiments, the dosage of VCR was 0.5 mg · kg^{-1} IP and that of MTX, 30 mg · kg^{-1} SC

[b] In the proliferating stem cell experiments, the dosage of VCR was 1.0 mg · kg^{-1} IP and that of MTX was 30 mg · kg^{-1} SC in Expt. I, 3 × 7.5 mg · kg^{-1} SC every 3 h in Expt. II, and 3 × 10 mg · kg^{-1} SC every 3 h in Expt. III

[c] n.d., indicates the investigation was not done

performed. It is concluded from Table 5 that all the therapy schedules resulted in an additive response.

To distinguish between a direct effect on tumour growth as a result of the drug treatment and an indirect effect related to the toxic effect on the host, starvation experiments were designed. To calculate the weight loss resulting from VCR $\xrightarrow{24\,h}$ MTX administration, nontumour-bearing animals were treated as indicated in Figure 1. Pretreatment with VCR induced a maximum weight loss of approximately 4.5 g per mouse. By depriving the animals of food for 2 days, it was possible to imitate the VCR $\xrightarrow{24\,h}$ MTX-induced weight loss. During the fast, a 15% loss of body weight was routinely observed. To determine whether weight loss could have made any significant contribution to an antitumour effect, we repeatedly deprived the mice of food. The effect of intermittent starvation on the weight of the mice and on tumour growth is shown in Figure 2. A marked decrease in the growth rate of the primary tumour was observed. Note, however, that the effect on the number of lung metastases was negligible. This differential effect of fasting on a large tumour mass and on small tumour volumes was also observed in an osteosarcoma experiment, as shown in Table 6. Finally, we submitted mice inoculated SC with Lewis lung tumour or osteosarcoma

Table 6. Comparison of tumour volume and number of lung metastases or lung colonies in control and intermittently starved mice

Tumor type and regimen	Tumor volume after starvation (mm^3)	Number of lung deposits per mouse at day 30
Lewis lung carcinoma[a]		
Control	1,649 ± 82 ($n = 14$)	79 ± 3 ($n = 11$)
Starved	1,144 ± 52 ($n = 11$)	86 ± 8 ($n = 8$)
C22LR osteosarcoma		
Control	1,500 ± 134 ($n = 10$)	49 ± 9 ($n = 9$)
Starved	796 ± 84 ($n = 10$)	65 ± 10 ($n = 10$)

[a] For details of the Lewis lung carcinoma experiment, see Figure 2

[b] Osteosarcoma cells (10^6 per 0.1 ml) were injected to BCBA F1 mice either SC or IV, resulting in primary tumour growth and lung colonies, respectively. Animals were deprived of food three times in succession from day 9 onward over a total of 140 h. Data are expressed as mean ± SE and n is the number of animals

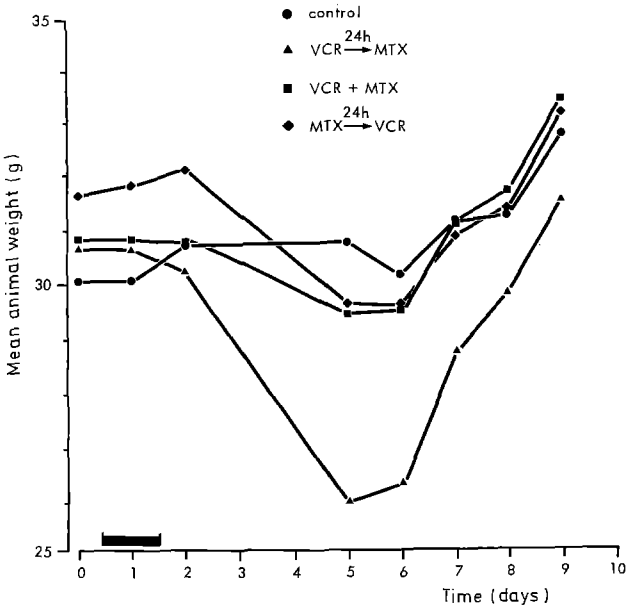

Fig. 1. Schedule-dependent weight loss in nontumour-bearing animals. Groups of five nontumour-bearing C57BL mice were injected with VCR at a dosage of 1 mg · kg^{-1} IP in combination with MTX at a dosage of 3 × 10 mg · kg^{-1} SC every 3 h as indicated. The maximum standard error of the animal weights was ± 1.1 g. ●, controls; ▲, VCR $\xrightarrow{24\,h}$ MTX; ■, VCR + MTX; ♦, MTX $\xrightarrow{24\,h}$ VCR

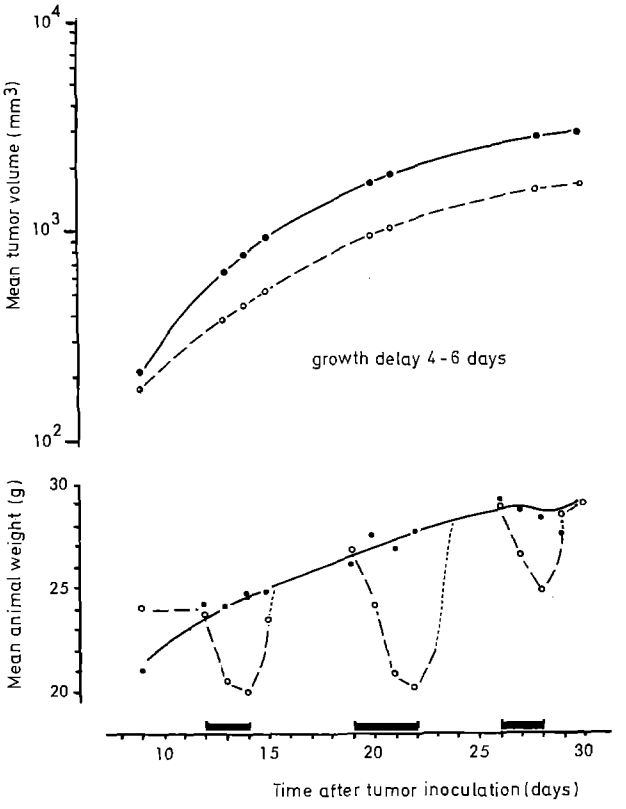

Fig. 2. Animal weight and tumour growth in control and intermittently starved mice. Groups of 15 BCBA F1 mice were inoculated IM with 10^6 Lewis lung tumour cells on day 0. To enhance the formation of spontaneous lung metastases, 10^6 microspheres were injected into one of the tail veins on day 1. One group was submitted to intermittent starvation as indicated. The maximum standard error of the tumour volume measurements was ± 200 mm^3 and that of the animal weights ± 1.2 g. The animals were sacrificed on day 30 and the number of macroscopic lung metastases counted. During the end stage of the disease, a total of 11 mice died. The number of lung metastases per mouse in the control group (●——●) was 79 ± 3 (n = 11) and the number in the intermittently starved mice (○——○), 86 ± 8 (n = 8)

cells to the fasting procedure, starting either very early on day 3, after tumour inoculation, or late on day 10, at a tumour volume for both tumour cell lines of approximately 200 mm^3. As with the results obtained in small lung deposits, shown in Table 6, the effect of fasting on early tumour disease was far less pronounced than the effect on large tumour volumes (data not given).

Discussion

In 1973, Zager et al. described the effects of antibiotics and cancer chemotherapeutic agents on the cellular transport of MTX in L1210 leukemia [18]. They applied the enhancing effect of VCR on MTX transport in L1210 survival studies. Sequential treatment (VCR → MTX) with a time interval of 30 min was significantly more effective than treatment in which no VCR was given prior to MTX administration. In vitro studies performed by Goldman et al. [8] showed an augmented net MTX accumulation in Ehrlich ascites tumour cells when VCR was given in combination with MTX. Fussner et al. recently reported on the effect of VCR administration on the half-life of MTX in dogs [7]. Their results suggested an augmented intracellular accumulation of MTX. The effect of VCR on MTX transport at various VCR and MTX dosages has been extensively investigated by the group of Bender at the NCI, Bethesda, Md., USA [17]. Although VCR sometimes raised intracellular levels of MTX, presumably through inhibition of the efflux process, this did not always correlate with the antitumour effect of MTX as determined by the capacity of MTX to suppress the incorporation of deoxyuridine into DNA. They suggest further studies with a variety of tumours. Very recently, Bender et al. showed a lack of therapeutic synergism between VCR and MTX in L1210 in vivo. In fact, they showed increased toxicity when high dosages of MTX were used [2]. In marked contrast to the findings of Zager et al., Chello et al. presented data indicating that the administration of VCR at an interval after MTX can result in a marked synergistic effect [5]. Our results in L1210 leukaemia do not suggest a significant synergistic effect, nor was a marked schedule dependency observed. The lack of agreement in the results of the various investigators may be the result of differences in L1210 cell lines.

The data obtained from the solid tumour experiments are inconclusive. Although we intended to investigate the antitumour effect of VCR-MTX combination chemotherapy and the effect of drug scheduling, we decided to focus more attention on the marked side effects of the treatment: weight loss and early toxic death. These toxic effects were highly dependent on the drug schedule. The sequence VCR $\xrightarrow{24\,h}$ MTX induced weight loss as a result of gastrointestinal toxicity, frequently leading ultimately to the death of the animals. Increased MTX accumulation inside intestinal cells could be an attractive explanation for the observed VCR $\xrightarrow{24\,h}$ MTX-induced toxicity. According to Chello, however, VCR does not alter the steady-state level of MTX in mouse intestinal epithelium [4]. Alternatively, VCR administration could lead to a rapid recruitment of resting intestinal epithelial cells into cycle, resulting in an increased toxic effect of MTX treatment. As we have no data on recruitment in gastrointestinal tissues after either VCR or MTX administration, no definite conclusions on the mechanism of action of VCR $\xrightarrow{24\,h}$ MTX induced toxicity are possible. Early toxic death as a consequence of bone marrow failure seems unlikely. No adverse effect of VCR $\xrightarrow{24\,h}$ MTX administration on haematopoietic stem cells could be demonstrated.

Drug-induced weight loss and fasting had a slowing effect on tumour growth. The growth rates of smaller tumour volumes were less affected than were those of large tumour masses. This nonspecific antitumour effect may have consequences, for instance, for drug screening studies. The administration of any new anticancer agent that causes a marked weight loss in the animal can eventually lead to false-positive results. Regular monitoring of the animals, weight will prevent misinterpretation of growth delay data. Sandor investigated the influence of fasting on tumour growth and, although he observed an effect on tumour weight, no explanation of the mechanism of action could be given [15]. Metabolic changes in growing tumours resulting in central tumour necrosis may possibly be influenced by the nutritional status of the animal. Since we showed a correlation between the effect of fasting and the starting volume of the tumour and tumour volume response, this volume-dependent effect may be a fruitful area for further investigations.

Experimental results can have a great impact on the clinical management of cancer patients. When Zager et al. showed a therapeutic gain with VCR administration followed by MTX therapy in L1210 mice, their findings stimulated the clinical practice of VCR administration preceding MTX therapy. Neither our observations nor those of other investigators discussed specifically discourage VCR → MTX treatment. However, we question whether the preclinical research data have been properly applied in clinical practice. Clinical investigators should re-evaluate the role of VCR pretreatment in many of the MTX combination chemotherapy protocols for patients with osteosarcoma. Firstly, VCR is not a very effective drug in the treatment of patients with advanced osteosarcoma [10], and therefore the arguments for including VCR in the therapy seem questionable. Secondly, at the extracellular levels achieved by the high-dose MTX regimens, membrane transport of MTX probably occurs by passive diffusion and VCR would have little effect [1]. Lastly, in nearly all the registered protocols, the time

interval between VCR injection and MTX therapy varies from 24 h to 30 min [6, 9, 14]. The gastrointestinal mucosa is one of the sensitive target organs of MTX-induced toxicity. Therefore, we would suggest not including VCR pretreatment routinely in any MTX treatment schedule unless experimental data proves VCR → MTX to have a higher therapeutic index than simultaneous treatment. The efficacy of VCR in this setting should be demonstrated more conclusively, not in the clinical trial context but in relevant animal studies.

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References

1. Bender, R. A., Drake, J. C., Fisher, R. I., Chabner, B. A.: Letter to the Editor. N. Engl. J. Med. **292,** 432 (1975)
2. Bender, R. A., Nichols, A. P., Norton, L., Simon, R. M.: Lack of therapeutic synergism between vincristine and methotrexate in L1210 murine leukemia in vivo. Cancer Treat. Rep. **62,** 997 (1978)
3. Brennan, M. F.: Uncomplicated starvation versus cancer cachexia. Cancer Res. **37,** 2359 (1977)
4. Chello, P. L., Dorick, D. M., Sirotnak, F. M.: Different effects of vincristine on methotrexate uptake by L1210 cells and mouse intestinal epithelia in vitro and in vivo. Proc. Am. Assoc. Cancer Res. **18,** 121 (1977)
5. Chello, P. L., Sirotnak, F. M., Dorick, D. M., Moccio, D. M., Gura, J.: Schedule-dependent synergism of methotrexate and vincristine against L1210 leukemia. Proc. Am. Assoc. Cancer Res. **19,** 113 (1978)
6. EORTC and SIOP: Controlled clinical trial on adjuvant therapy in the treatment of osteosarcoma of the limbs (03 protocol). 1978
7. Fusner, J. E., Ramu, A., Gratwohl, A.: Effect of vincristine (VCR) on methotrexate (MTX) pharmacokinetics in vivo. Proc. Am. Assoc. Cancer Res. **19,** 235 (1978)
8. Goldman, D., Gupta, V., White, J. C., Loftfield, S.: Exchangeable intracellular methotrexate levels in the presence and absence of vincristine at extracellular drug concentrations relevant to those achieved in high dose methotrexate-folinic acid "rescue" protocols. Cancer Res. **36,** 276 (1976)
9. Jaffé, N., Frei, E., III, Watts, H., Traggis, D.: High-dose methotrexate in ostogenic sarcoma: a 5-year experience. Cancer Treat. Rep. **62,** 259 (1978)
10. Livingston, R. B., Carter, S. K.: Single agents in cancer chemotherapy. New York: Ifl/Plenum 1970
11. Mulder, J. H., Van Putten, L. M.: Lack of vincristine/cyclophosphamide potentiation in different experimental tumour lines. Eur. J. Cancer (in press, 1979)
12. Mulder, J. H., Smink, T., Van Putten, L. M.: Schedule-dependent effectiveness of CCNU and 5-Fluorouracil in experimental chemotherapy. Eur. J. Cancer **13,** 1123 (1977)
13. Mulder, J. H., Edelstein, M. B., Lelieveld, P., Van Putten, L. M.: Synergism and schedule dependent cytotoxicity of cyclophosphamide and CCNU in experimental cancer chemotherapy. Eur. J. Cancer **14,** 537 (1978)
14. National Cancer Institute: Compilation of clinical protocol summaries. Bethesda: U.S. Department of Health, Education and Welfare 1977
15. Sandor, R. S.: Brief communication: Effects of fasting on growth and glycolysis on the Ehrlich ascites tumor. J. Natl. Cancer Inst. **56,** 427 (1976)
16. Strogand, J. J., Braunschweiger, P. G., Pollice, A. A., Schiffer, L. M.: Cell kinetic alterations in murine mammary tumours following fasting and refeeding. Eur. J. Cancer **15,** 281 (1979)
17. Warren, R. D., Nichols, A. P., Bender, R. A.: The effect of vincristine on methotrexate uptake and inhibition of DNA synthesis by human lymphoblastoid cells. Cancer Res. **37,** 2993 (1977)
18. Zager, R. F., Frisby, S. A., Oliverio, V. T.: The effects of antibiotics and cancer chemotherapeutic agents on the cellular transport and antitumor activity of methotrexate in L1210 murine leukemia. Cancer Res. **33,** 1670 (1973)

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