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Collateral sensitivity of a methotrexate-resistant L1210 cell line to the vinca alkaloids

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Summary. *L1210 mouse leukaemia cell lines showing a 20,000-fold differential sensitivity to methotrexate have been shown to exhibit some collateral sensitivity to at least two of the vinca alkaloids, vinblastine and vindesine. Vinblastine is the more cytotoxic for both cell lines. The extent of the collateral sensitivity decreases in the order vindesine > vinblastine > vincristine.*

Total cellular uptake studies with radiolabelled methotrexate showed only a two- to three-fold greater incorporation in the sensitive line. On the other hand, a two-fold greater incorporation of labelled vincristine occurred in the resistant line. No significant difference in the uptake occurred following labelled vinblastine treatment by the two cell lines.

It is unlikely that differences in uptake account for the altered drug responses observed in the two cell lines.

Introduction

The anti-neoplastic agent methotrexate is known to bind rapidly to the enzyme dihydrofolate reductase [5], and resistance to the antifolate has been shown to occur by three distinct mechanisms, viz. (a) elevation of intracellular levels of the target enzymes [2]; (b) production of an altered dihydrofolate reductase with decreased affinity for the drug [1]; and (c) alterations in drug transport resulting in a lowering of intracellular concentrations [24]. The mode of action of the vinca alkaloids is generally considered to be metaphase arrest as a result of inhibition of microtubular assembly [10], although other cytotoxic actions have been postulated [19, 20, 22, 25]. Several interactions between methotrexate and vincristine have been reported in cell culture and in vivo. For example, vincristine has been shown to enhance intracellular levels of methotrexate in L1210 cells [26] and in human leukaemic blast cells [4] in vitro. A time-schedule-dependent synergism of vincristine and methotrexate against murine lymphocytic leukaemia cells has also been observed in vivo [8]. Collateral sensitivity, whereby the acquisition of resistance to one agent results in increased sensitivity to another, has been reported for other different antitumour agents in a number of mammalian cell lines in vitro [12, 13, 16, 17], and in the L1210 cell line in vivo [7, 23].

A methotrexate-resistant L5178Y cell line was found to be as sensitive as the parent line to vincristine and vindesine [15]. No evidence, however, for collateral sensitivity between methotrexate and the vinca alkaloids in an experimental system has yet been reported to our knowledge.

There is, however, some clinical evidence [3, 4] for an increased sensitivity of certain methotrexate-resistant tumours to the vinca alkaloids.

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Materials and methods

Methotrexate was supplied by Lederle Laboratories Division, Cyanamid of Great Britain. The vinca alkaloids were kindly supplied by Eli Lilly and Co Ltd, England. Radiolabelled vincristine and vinblastine sulphates and the methotrexate were obtained from the Amersham International, Amersham, UK.

Cell culture. The cells used in this study were two L1210 lymphoblastoid leukaemia cell lines exhibiting differential sensitivity to the antifolate, methotrexate. The original lines were kindly donated by the Department of Biochemical Pharmacology, Institute of Cancer Research, London, The L1210/R7A line is grown under a constant challenge of methotrexate (10^{-6} *M*). In separate experiments it was shown that resistance could be maintained for at least 3 months in the absence of the drug. Population-doubling times are 15.7 ± 0.6 h and 19.1 ± 0.3 h for the L1210 and L1210/R7A cell lines, respectively. In all experiments and for routine serial dilution the cells are grown in suspension culture in RPMI 1640 (Flow Laboratories, UK) supplemented with 10% horse serum (Gibco, Europe). They were regularly screened by the method of Chen [9] for mycoplasma and found to be free. For assays of cell survival cells were grown in 0.25% agar with a plating efficiency of 80%. Colony-forming ability was measured after a 10-day incubation of cells at 37° C in the presence of the agents.

Uptake studies. Intracellular accumulation at treatment levels corresponding to 75% survival for vincristine was determined using (G-^{3}H) vincristine sulphate (specific activity 7.2 Ci/m*M* (Amersham International, Amersham, UK) at a concentration of 0.5 μCi/ml for each drug studied in the cell suspension (10^5 ml^{-1}) in RPMI 1640 medium supplemented with 10% horse serum. After predetermined time intervals of incubation (7×10^{-9} *M* vincristine) at 37° C, drug uptake was stopped by cooling on ice and by dilution with cold buffer (0.14 *M* sodium chloride, 0.02 *M* potassium phosphate, pH 7.4). After centrifugation (2,000 *g*, 4° C, 5 min) the cells were washed twice with the cold buffer and resuspended in the same buffer solution.

Cell numbers were counted on an electronic cell counter (Coulter Electronics). Radioactivity was assayed using Aquasol (New England Nuclear) on a Packard Tricarb liquid scintillation spectrometer. Uptake was expressed as nmol/10^6 cells. The same procedure was adopted for the uptake of (G-^{3}H) vinblastine sulphate (sp. act. 9.9 Ci/mmol) at 7×10^{-9} *M* concentration and [^{3}H]methotrexate (sp. act. 18.1 Ci/mmol) at 10 μ*M* concentration for up to 22 h.

Chromosome analysis. An exponentially growing cell suspension in RPMI 1640 supplemented with 10% horse serum was treated for 2 h at 37° C with vinblastine (1.23×10^{-7} *M*). The cells were centrifuged (160 *g*, 4° C) and the pellet resuspended

Table 1. The effect of methotrexate and the vinca alkaloids on the colony-forming ability of L1210 murine lymphoma and L1210/R7A (methotrexate-resistant L1210) cells grown in RPMI 1640 supplemented with 10% horse serum

	LD_{50} (M)	
	L1210	L1210/R7A
Methotrexate	3.6×10^{-8}	7.7×10^{-4}
Vincristine	1.0×10^{-8}	7.8×10^{-9}
Vinblastine	2.8×10^{-9}	1.3×10^{-9}
Vindesine	1.1×10^{-8}	2.0×10^{-9}

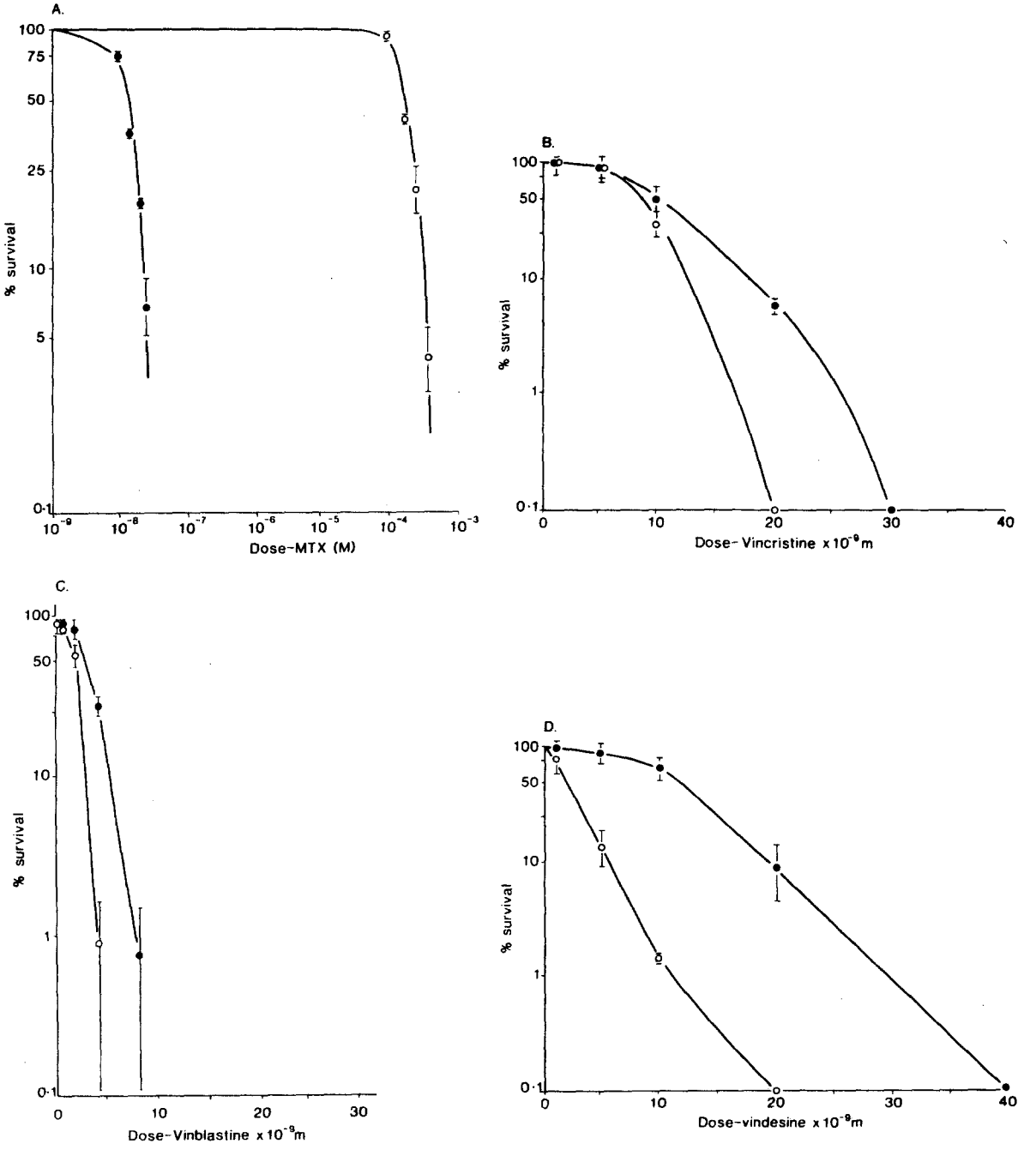

Fig. 1A–D. Colony-forming ability of L1210 (●——● (and L1210/R7A (○——○) cell lines in the presence of methotrexate (**A**), vincristine (**B**), vinblastine (**C**), and vindesine (**D**)

in 0.075 *M* KCl for 10 min at 4° C. The cells were again centrifuged (160 *g*, 4° C) and the resulting cell pellet fixed in methanol : glacial acetic acid (3 : 1), washed a further three times with the fixative, and spread on a microscope slide and air-dried. Following staining with 2% Giemsa in Gurr's buffer (pH 6.8, 8 min), they were washed with buffer and mounted. Fifty metaphases were counted for each cell line.

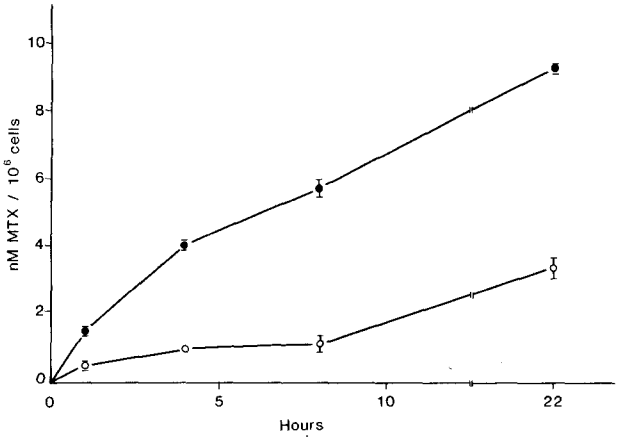

Fig. 2. Time course of [^{3}H]methotrexate uptake to L1210 (●——●) and L1210/R7A (○——○)

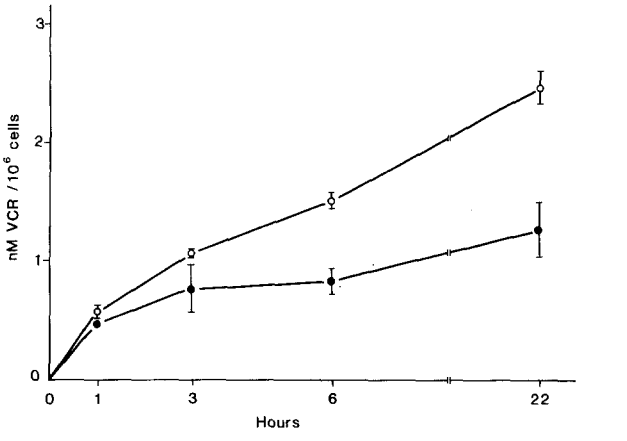

Fig. 3. Time course of [^{3}H]vincristine uptake in L1210 (●——●) and L1210/R7A (○——○) cells

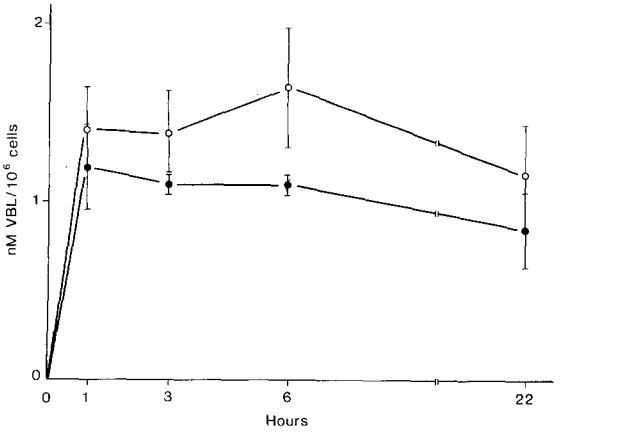

Fig. 4. Time course of [^{3}H]vinblastine uptake in L1210 (●——●) and L1210/R7A (○——○) cells

Results

Cell survival

The effect of continuous exposure of methotrexate and the three vinca alkaloids on the survival of L1210 and L1210/R7A cell lines are shown in Fig. 1. It can be seen from the relative LD_{50} values (Table 1) that the R7A cell line is over 20,000 times more resistant to methotrexate than the sensitive parental cell line, and that these cell lines show collateral sensitivity with their sensitivity to vinblastine and vindesine increased 2.2- and 5.5-fold, respectively. Only marginal collateral sensitivity (ratio 1.3) is seen with vincristine.

Labelled drug uptake

The intracellular uptake of radiolabelled methotrexate, vincristine, and vinblastine is shown in Figs. 2, 3, and 4, respectively. The L1210 parental line accumulated three-fold greater amounts (nmol) of labelled methotrexate than the resistant L1210/R7A cell line.

However, the L1210/R7A line accumulated a two-fold higher level of labelled vincristine than the L1210 line by 22 h (Fig. 3). The labelled vinblastine, however, followed a more rapid incorporation in the first hour, but by 22 h no further incorporation had occurred and the difference was barely significant (Fig. 4). Labelled vindesine was not available for a similar study.

Chromosome counting

Both the L1210 parental line and the L1210/R7A methotrexate-resistant line had similar median chromosome numbers (38.2 ± 1.41 and 37.2 ± 1.48, respectively). There was no evidence of double minutes in the cell lines; however, some evidence for homogenous staining regions has been obtained by Giemsa banding with the L1210/R7A cell line (Ockey, personal communication), which together with the high levels of dihydrofolate reductase [21] suggests that gene amplification is the most probable cause of resistance to methotrexate in this cell line*.

Discussion

A decrease in membrane transport has frequently been reported [6, 11, 24] to be associated with resistance in mammalian cells to antitumour agents. A difference in intracellular accumulation of methotrexate [23] and the vinca alkaloids [6] has been associated with resistance in some cell lines.

In the present study the presence of homogenous staining regions and the lack of double minutes on chromosome analysis strongly suggests that gene amplification may be responsible for the methotrexate resistance. The slightly longer doubling time, i.e., 15.7 ± 0.6 h compared with 19.1 ± 0.3 h, of the resistant cell line may be due to some difficulty being experienced by the resistant cells during their transit of the cell cycle. Such an elongation of the cell-doubling time has previously been reported for other methotrexate-resistant lines. For example, an S180 mouse sarcoma had a cell-doubling time of 18 h and a subline approximately 10,000-fold more resistant to methotrexate (AT 3000) had a cell doubling time of 24 h [2]. In addition, another report by Kaufman et al. [18] used a similar S180 murine line (doubling time 16 h) and a resistant line (AT/300) with a reported doubling time of 23 h.

The resistance of both these cell lines was attributed to gene amplification.

The total uptake of labelled methotrexate in the present studies did not relate to the larger differences observed in the dihydrofolate reductase content in these cell lines [21], which suggests that a considerable proportion of the cellular content of the drug was non-specifically bound. However, the relative uptakes of vincristine and vinblastine do not correlate with the survival data for these two alkaloids.

In conclusion, a collateral sensitivity towards at least two of the three vinca alkaloids has been observed for a methotrexate-resistant line of L1210, which cannot be fully accounted for by differences in the relative uptake of at least two of them, viz. vincristine and vinblastine, into the two cell lines. It is particularly noteworthy that vindesine showed the greatest degree of collateral sensitivity in this pair of cell lines.

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References

1. Albrecht AM, Biedler JL, Hutchison DJ (1972) Two different species of dihydrofolate reductase in mammalian cells differentially resistant to amethopterin and methasquin. Cancer Res 32: 1539
2. Alt FW, Kellems RE, Bertino JR, Schimke RT (1978) Selective multiplication of dihydrofolate reductase genes in methotrexate resistant variants of cultured murine cells. J Biol Chem 253: 1357
3. Bagshawe KD (1969) Vinca alkaloids in the treatment of gestational choriocarcinoma. In: Shedden WI (ed) The vinca alkaloids in the chemotherapy of malignant diseases. Proceedings of the Symposium, London, p 61
4. Bender RA, Bleyer WA, Frisby SA, Oliverio VT (1975) Alteration of methotrexate uptake in human leukaemia cells by other agents. Cancer Res 35: 1305
5. Bertino JR, Booth BA, Bieber AL, Cashmore A, Sartorelli AC (1964) Studies on the inhibition of dihydrofolate reductase by the folate antagonists. J Biol Chem 239: 479
6. Bleyer WA, Frisby SA, Oliverio VT (1975) Uptake and binding of vincristine by murine leukaemia cells. Biochem Pharmacol 24: 633
7. Bradner WT, Hutchison DH (1966) Necarcinostatin (NSC-69856): an antitumor antibiotic effective against ascitic leukaemia L1210 in mice. Cancer Chemother Rep 50: 79
8. Chellow PL, Sirotnak FM, Dorick DM, Moccio DM (1979) Schedule-dependent synergism of methotrexate and vincristine against murine L1210 leukaemia. Cancer Treat Rep 63: 12
9. Chen T (1977) In situ detection of mycoplasma contamination in cell culture by fluorescent Hoechst 33258 stain. Exp Cell Res 104: 255
10. Creasey WA (1975) Vinca alkaloids and colchicine. In: Sartorelli AC, Johns DG (eds) Hdb Exp Pharmacol, vol XXXVIII/2. Springer, Berlin Heidelberg New York, p 670
11. Dano K (1973) Active outward transport of daunomycin in resistant Ehrlich ascites tumour cells. Biochim Biophys Acta 323: 466
12. Fox BW (1977) Collateral sensitivity between methylene dimethanesulphonate and halogenated methotrexate derivatives in the Yoshida sarcoma in vivo and in vitro. J Natl Cancer Inst 58: 955
13. Herman TS, Cress AE, Gerner EW (1979) Collateral sensitivity to methotrexate in cells resistant to adriamycin. Cancer Res 39: 1937
14. Hertz R, Lewis J, Lipsett MB (1961) Five years' experience with the chemotherapy of metastatic choriocarcinoma and related trophoblastic tumours in women. Am J Obstet Gynecol 82: 631
15. Hill BT, Whelan RDH (1981) Comparative cell killing and kinetic effects of vincristine or vindesine in mammalian cell lines. J Natl Cancer Inst. 67: 437
16. Hutchison DJ (1965) Studies on cross resistance and collateral sensitivity (1962–1964) Cancer Res 25: 1581
17. Hutchison DJ, Schmid FA (1973) Cross resistance and collateral sensitivity. In: Mihich E (ed) Drug resistance and selectivity: Biochemical and cellular basis. Academic Press, New York, p 73
18. Kaufmann RJ, Bertino JR, Schimke RT (1979) Quantitation of dihydrofolate reductase in individual parental and methotrexate resistant murine cells. J Biol Chem 253: 5852
19. Madoc-Jones H, Mauro F (1968) Interphase action of vincristine and vinblastine: differences in their lethal action through the mitotic cycle of cultured mammalian cells. J Cell Physiol 72: 185
20. Paintrand M, Dantchev D, Pignot I, Bourut C, Reiss F, Mathé G (1981) Comparative actions of vindesine, vinblastine and vincristine: an electron microscopical study in mice using P388 leukaemia cells. Biol Cell 40: 93
21. Poppitt DG, McGown AT, Fox BW (1984) The use of a fluorescent methotrexate probe to monitor the effects of three vinca alkaloids on a mixed population of parental L1210 and gene-amplified methotrexate resistant cells by flow cytometry. Cancer Chemother Pharmacol 13: 54
22. Rosner F, Hirshaut Y, Grunwald HW, Dieterich M (1975) In vitro combination chemotherapy demonstrating potentiation of vincristine cytotoxicity by prednisone. Cancer Res 35: 700
23. Rutman RJ (1964) Experimental chemotherapy studies. V. The collateral sensitivity to alkylating agents of several antimetabolite-resistant ascites tumours in mice. Cancer 24: 634
24. Sirotnak FM, Kurita A, Hutchison DJ (1968) On the nature of a transport alteration determining resistance to amethopterin in L1210 leukaemia. Cancer Res 28: 75
25. Stryckman PA, Lurie PM, Manaster J, Vameoa G (1973) Mode of action of chemotherapy in vivo on human acute leukaemia. II. Vincristine. Eur J Cancer 9: 613
26. Zager RF, Frisby SA, Oliverio VT (1973) The effects of antibiotics of cancer chemotherapeutic agents on cellular transport and antitumour activity of methotrexate in L1210 murine leukaemia. Cancer Res 33: 1670

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* *Note added in proof.* A study (J. Brennand) of this resistant line using a cDNA probe specific for the DHFR gene, shows an amplification of between 25 and 100 fold of DHFR gene sequences.