Cancer Chemother Pharmacol (1991) 27: 413–415

034457049100032D

Cancer Chemotherapy and Pharmacology

Short communication

Effect of verapamil on daunorubicin accumulation in lymphocytes isolated from patients undergoing chemotherapy*

A. T. McGown[1], D. Murphy[2], D. Crowther[2], and B. W. Fox[1]

[1] CRC Department of Experimental Chemotherapy, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, U. K.
[2] CRC Department of Medical Oncology, Christie Hospital and Holt Radium Institute, Wilmslow Road, Manchester M20 9BX, U. K.

Summary. Verapamil was shown to be capable of increasing intracellular daunorubicin levels in normal lymphocytes isolated from patients undergoing chemotherapy for epithelial ovarian cancer. The extent of the increase in daunorubicin accumulation was variable, occurring in the range of 0–123% as compared with intracellular daunorubicin levels attained in the absence of verapamil. No similar effect was seen in lymphocytes isolated from healthy volunteers. A tentative explanation of these data may be the induction of multidrug resistance (mdr)-like characteristics in normal lymphocytes following cytotoxic chemotherapy.

Introduction

The phenomenon of multidrug resistance (mdr) has been demonstrated in many cell types [3, 4, 11]. However, the clinical importance of mdr in human cancers has yet to be established, although attempts are being made to circumvent the process by the use of agents that either compete with or block the mdr-associated drug efflux process [11]. Verapamil has been shown to be an effective inhibitor of drug efflux in mdr-positive cells [8, 11–13]. This leads to an increased retention of cytotoxic drug within the cell, with consequent restoration of drug sensitivity. The ability of verapamil to elevate intracellular drug levels in cells exhibiting the mdr phenotype was used in the present study as a functional assay for mdr. This report describes the effect of verapamil on daunorubicin retention in normal lymphocytes isolated from patients undergoing chemotherapy for epithelial ovarian cancer. The results are compared with those obtained for lymphocytes obtained from healthy volunteers.

Patients and methods

Isolation of lymphocytes. Lymphocytes were isolated using Ficoll-Paque (Pharmacia, Uppsala, Sweden) according to the manufacturer's instructions.

Measurement of intracellular daunorubicin. Intracellular daunorubicin concentrations were determined using flow cytometry as previously described [7]. Briefly, isolated lymphocytes were resuspended in serum-free medium (Fischer's) and daunorubicin was added (final concentration, 10 μM). The sample was then split and verapamil was added to one aliquot (final concentration, 6 μM). Intracellular fluorescence was measured following incubation (37°C, 2 h). Under the conditions used in these studies, verapamil shows no fluorescent properties. A 2-h incubation was chosen, as this gives maximal daunorubicin uptake (Fig. 1). The

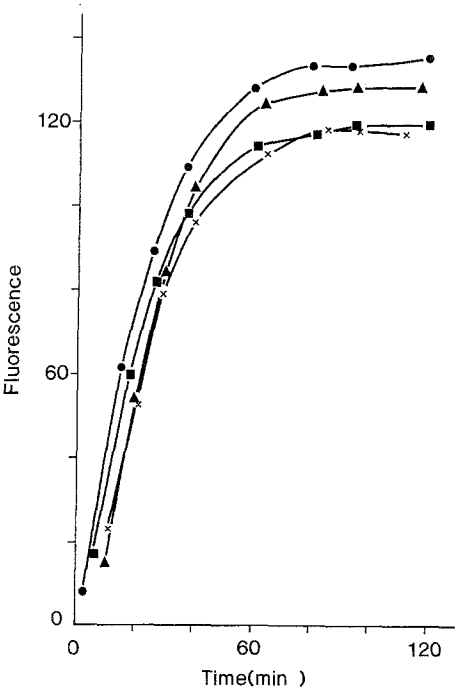

Fig. 1. Time course for the uptake of daunorubicin (fluorescence) in isolated human lymphocytes. ●——●, Patient 1 (+ verapamil); ▲——▲, patient 1 (no verapamil); ■——■, patient 2 (+ verapamil); ×——×, patient 2 (no verapamil)

* This work was supported by the Cancer Research Campaign

Offprint requests to: A. T. McGown

Table 1. Characteristics and response of patients evaluated in the present study

Patient	Chemo-therapy regimen	Disease stage	Previous chemotherapy cycles (*n*)	Response	% Change in fluorescence on verapamil addition
BH	2	3	8		+25
			9	PD	
DH	1	3	1		-6
			3	PD	+123
CH	1	1C	4		+13
			5	CR	+11
VC	1	2C	4		+6
			5	CR	+10
SH	1	4	5		-4
			6	PR	+5
LP	1	4	1		+8
			2		+12
			4	PD	+11
MQ	2	3	3		-3
			6	PD	+3
RW	2	3	2	PR	+13
PJ	1	3	3		+12
			4		-2
			5	CR	+5
IS	3	3	0	PD	+1
EB	2	4	4		-3
			5	PD	+3
MM	2	3	4		-2
			5	PR	+16
DB	2	3	1	PR	+1
MW	1	3	5	CR	+7
NH	4	3	0	PD	+2
JC	2	4	5	PD	+2
			6		+15
JW	2	3	2	PR	-6
EW	3	4	1	PD	+4
SR	4	3	2	CR	+11
MA	1	3	6	PD	+88
NF	1	3	6	PR	+22
NH	1	3	6	PR	-2
SS	1	3	0	–	-1
PL	1	3	0	–	0
MD	1	3	0	–	3
DM	1	3	0	–	0
AM	1	3	0	–	4

CR, Complete response; PR, partial response; PD, progressive disease

concentration of daunorubicin used is comparable with that previously attained in the plasma of patients undergoing chemotherapy [9] and has been shown to be capable of distinguishing between cells with altered anthracycline transport [7, 8]. The concentration of verapamil used has been shown to be attainable in plasma and can reverse mdr-related drug-transport alterations [11].

Patients. The patients evaluated in the present study were selected from a population undergoing chemotherapy for epithelial ovarian cancer. A summary of their details is shown in Table 1. Oral informed consent was obtained. The cytotoxic regimens used were as follows:

1. Regimen 1 consisted of 600 mg/m^2 cyclophosphamide and 300 mg/m^2 carboplatin alternating with 5 g/m^2 ifosfamide and 50 mg/m^2 Adriamycin, with six cycles being given intravenously at 4-week intervals.

2. Regimen 2 comprised 300 mg/m^2 cyclophosphamide and 150 mg/m^2 carboplatin alternating with 2.5 g/m^2 ifosfamide and 25 mg/m^2 Adriamycin, with 12 cycles being given intravenously at 4-week intervals.

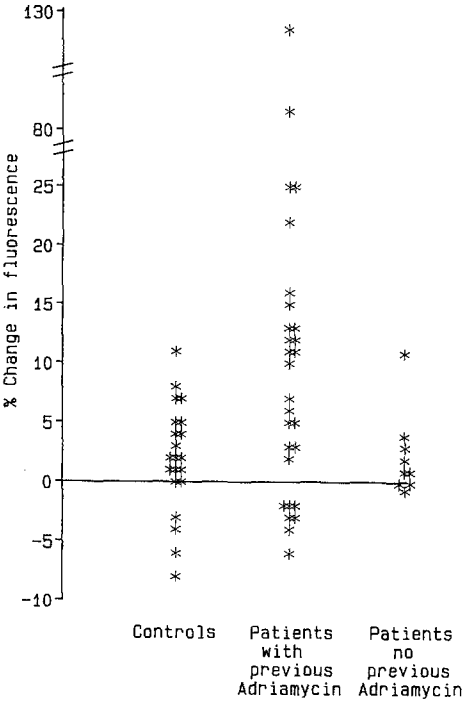

Fig. 2. Effect of verapamil on the uptake of daunorubicin in isolated lymphocytes. The percentage of change in fluorescence represents that caused by the addition of verapamil

3. Regimen 3 consisted of six cycles of 5 g/m^2 ifosfamide and 50 mg/m^2 Adriamycin given intravenously at 4-week intervals.

4. Regimen 4 involved the oral administration of six cycles of 10 mg melphalan per day for 5 days at 5-week intervals.

Venous blood samples were taken at random times during the chemotherapy regimens. Volunteers not receiving chemotherapy were also sampled.

Statistical analysis. Statistical analysis was performed on a Microvax 3600 computer using the Minitab statistical package (Minitab Inc., University Park, Pa., USA).

Results

The effect of verapamil on daunorubicin accumulation is shown in Fig. 2. Initial data analysis comparing two groups [those who had previously received Adriamycin (ovarian tumour patients only) and those who had not received any Adriamycin (ovarian patients + healthy controls)] showed a highly statistically significant difference (Kruskal-Wallis test, $P = 0.008$; Mann-Whitney test, $P = 0.008$). A more detailed study subdividing the data into three groups (patients previously treated with Adriamycin, those who had not received Adriamycin, and healthy volunteers) also showed a statistically significant difference between the groups ($P = 0.028$, Kruskal-Wallis test). Further analysis showed that this was due to the difference between the group who had received Adriamycin and the group of healthy volunteers ($P = 0.012$, Mann-Whitney test). A similar comparison between patients with ovarian tumours who had previously received Adriamycin and those who had not undergone treatment with this drug did not reach significance ($P = 0.07$). However, the group of untreated patients was relatively limited in number ($n = 9$).

Discussion

Verapamil is one of a growing number of agents that can restore sensitivity in mdr-positive cell types [3, 12]. Verapamil interferes with the mdr-associated efflux process, resulting in increased intracellular concentrations of cytotoxic drugs, leading in turn to increased cytotoxicity. The assay applied in the present study uses the intrinsic fluorescence of the anthracyclines to determine drug levels in individual lymphocytes [7, 10] as measured by flow cytometry techniques. The main advantages of this technique are its speed and its ability to measure a statistically valid number of cells ($>10^4$/sample). A major drawback is that the technique measures total cellular fluorescence, which may arise from either the parent drug or its metabolites; this criticism also applies to methods using radiolabelled drug. Although the flow cytofluorimetric assay requires relatively high concentrations of anthracycline, it has successfully been used to measure decreased drug accumulation in leukemic cells in vitro [7, 10].

The assay used in the present study relies on the measurement of the difference in intracellular fluorescence caused by the addition of verapamil. This assay does not attempt the absolute quantitation of fluorescence, as this would require the complex and difficult standardisation of the flow cytometers.

Our results show that the lymphocytes from patients who had previously received Adriamycin showed a response to verapamil that was statistically different from that shown by lymphocytes isolated from patients and healthy volunteers who had not received the drug. Of the drugs used in the regimens tested in the present study, Adriamycin is the only one that is associated with the decreased cellular sensitivity seen in mdr cells. The mechanism by which this increased sensitivity to verapamil occurs is as yet unknown. It is tempting to ascribe these changes to alterations in the level of expression of the mdr gene in normal lymphocytes following exposure to Adriamycin. The time scale on which these changes occur is not known. A study using sequential samples obtained during the monthly cycles of chemotherapy would give information as to the origins of the change. Such information is not available from this work. However, the limited data shown (patients DH and LP, Table 1) indicate that a more detailed study may prove to be enlightening.

If the observations described in this work were caused by a change in mdr-related processes, then a detailed study of patient response and, if possible, of the mdr status of the tumour during the course of cytotoxic chemotherapy would indicate whether this lymphocyte test has any potential use as a monitor of therapy. Moreover, these studies would best be performed on a disease more easily biopsied.

The major gene product gP170 is also expressed in some normal tissues. This expression can be very heterogeneous, even within highly expressing tissues [3]. With few exceptions [5], previous studies on P-glycoprotein expression in clinical material have been limited to small numbers of tumour samples [1, 2, 4, 6]. The present investigation indicates that changes may occur in normal tissue as well as tumour. The wider availability of the P-glycoprotein-specific antibodies and gene probes should prove whether the effects described in this report are related to classic gP170 expression. The functional assay used in this study, although strongly indicative of mdr involvement, should be further investigated using these probes so as to demonstrate definitively the role of gP170.

References

1. Bell DR, Gerlach JH, Kartner N, Buick RN, Ling V (1985) Detection of P-glycoprotein in ovarian cancer: a molecular marker associated with multidrug resistance. J Clin Oncol 3: 311
2. Courhis J, Goldstein LJ, Riou G, Pastan I, Gottesman MM, Benard J (1989) Expression of a human multidrug resistance gene in ovarian carcinomas. Cancer Res 49: 5062
3. Endicott JA, Ling V (1989) The biochemistry of P-glycoprotein-mediated drug resistance. Annu Rev Biochem 58: 137
4. Fojo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, Pastan I (1987) Expression of a multi-drug resistance gene in human tumours and tissues. Proc Natl Acad Sci USA 84: 265
5. Goldstein LJ, Galski H, Fojo A, Wallingham M, Lai S, Gazdar A, Pirker R, Green A, Crist W, Brodeur GM, Sieber M, Crossman J, Gottesman MM, Pastan I (1989) Expression of a multidrug resistance gene in human cancers. J Natl Cancer Inst 81: 116
6. Ma DD, Davey RA, Harman DH, Isbister JP, Scurr RD, Mackertich SM, Dowden G, Bell DR (1987) Detection of multidrug resistance phenotype in non-lymphocytic leukemia. Lancet I: 135
7. McGown AT, Ward TH, Fox BW (1983) Comparative studies of the uptake of daunorubicin in sensitive and resistant cell lines by flow cytometry and biochemical extraction procedures. Cancer Chemother Pharmacol 11: 113
8. McGown A, Poppitt DG, Fox BW (1987) Comparison of daunorubicin and anthrapyrazolone sensitivity and transport in resistant cell lines. Br J Cancer 56: 752
9. Seeber S, Loth H (1981) Individual nuclear uptake patterns for Adriamycin and daunomycin in human leukemia and lymphoma cells. Blut 42: 355
10. Sonneveld P, Engh GJ van den (1981) Differences in uptake of Adriamycin and daunomycin by normal BM cells and acute leukemia cells determined by flow cytometry. Leukemia Res 5: 251
11. Stewart DJ, Evans WK (1989) Non chemotherapeutic agents that potentiate chemotherapeutic efficiency. Cancer Treat Rev 16: 1
12. Tsuruo T, Iida H, Tsukagoshi S, Sakurai Y (1983) Potentiation of vincristine and Adriamycin effects in human hemopoietic tumour cell lines by calcium antagonists and calmodulin inhibitors. Cancer Res 43: 2267
13. Yanovich S, Preston L (1984) Effects of verapamil on daunomycin cellular retention and cytotoxicity in P388 leukemia cells. Cancer Res 44: 1743