Cancer Chemother Pharmacol (1997) 39: 557–560

SHORT COMMUNICATION

Lorraine K. Webster · Martha E. Linsenmeyer
Danny Rischin · Maureen E. Urch
David M. Woodcock · Michael J. Millward

Plasma concentrations of polysorbate 80 measured in patients following administration of docetaxel or etoposide

Received: 21 July 1996 / Accepted: 4 November 1996

Abstract Docetaxel (Taxotere, Rhone-Poulenc Rorer) and etoposide are water-insoluble drugs formulated with polysorbate 80 for intravenous administration. We have previously reported that surfactants, including polysorbate 80 and Cremophor EL, can reverse the multidrug resistance (MDR) phenotype in an experimental system and that plasma Cremophor EL concentrations measured following a 3-h infusion of paclitaxel were ≥ 1 μl/ml, sufficient to modulate MDR in vitro. The purpose of this study was to measure polysorbate 80 plasma concentrations in patients following intravenous administration of etoposide or docetaxel using a bioassay in which MDR-expressing cells are incubated with daunorubicin (DNR) plus 50/50 growth medium/plasma and equilibrium intracellular DNR fluorescence is measured by flow cytometry. In vitro experiments show maximal reversal of MDR at concentrations of 1.0–2.0 μl/ml and 50% reversal at 0.2–0.3 μl/ml. Patients received docetaxel at 75 mg/m^2 (five patients) or 100 mg/m^2 (four patients) (total dose 125–178 mg, containing 3.12–4.45 ml polysorbate 80) over 60 min. The median end-infusion polysorbate 80 concentration was 0.1 μl/ml (range 0.07–0.41 μl/ml). Only one patient had a level of >0.2 μl/ml. Five patients received intravenous etoposide at 120 mg/m^2 over 45–120 min (total dose 180–250 mg, containing 0.67–0.93 ml polysorbate 80). In the end-infusion plasma sample, polysorbate 80 was not detectable (<0.06 μl/ml) in any patient. Plasma polysorbate 80 levels following an intravenous infusion of 120 mg/m^2 etoposide or of docetaxel at doses used in Phase II trials, are insufficient to show modulation of MDR in vitro.

Key words Polysorbate 80 · Multidrug resistance · Docetaxel · Etoposide

L.K. Webster (✉) · M.E. Linsenmeyer · D.M. Woodcock
Division of Research, Peter MacCallum Cancer Institute,
Locked Bag No. 1, A'Beckett Street, Melbourne, VIC, Australia 3000
Tel. +61-3-9656-1275; Fax +61-3-9656-1411;
E-mail lwebster@petermac.unimelb.edu.au

D. Rischin · M.E. Urch · M.J. Millward
Division of Haematology and Medical Oncology,
Peter MacCallum Cancer Institute, St. Andrews Place,
East Melbourne, Victoria, Australia 3002

Introduction

Drugs with low aqueous solubility require the addition of solubilizing agents to allow intravenous administration. Non-ionic detergents such as Cremophor EL, a polyethoxylated castor oil derivative, and polysorbate 80 (Tween 80), an oleate ester of sorbitol, are used to solubilize several anticancer drugs. Paclitaxel is currently formulated at 6 mg/ml in 50% Cremophor EL and 50% ethanol, and teniposide is prepared with 2.5 g Cremophor EL/50 mg. Docetaxel is supplied at 40 mg/ml formulated in 100% polysorbate 80, and the intravenous formulation of etoposide includes 400 mg polysorbate 80/100 mg etoposide.

Although surfactants are usually considered to be pharmacologically inert, we and other investigators have shown that both Cremophor EL and polysorbate 80 can modulate multidrug resistance (MDR) [7, 15, 18, 19]. These agents are believed to interfere with the function of the transmembrane drug-export pump P-glycoprotein (Pgp) either directly or through membrane perturbations [4, 5, 7]. We have developed an assay to measure Cremophor EL in plasma on the basis of its ability to reverse MDR and have previously demonstrated that plasma concentrations of Cremophor EL measured in patients following short infusions (3–6 h) of paclitaxel are sufficient potentially to modulate MDR [14, 16]. Cross-resistance to docetaxel has been observed in some, but not all, cell lines expressing Pgp-related MDR; however, when

present, this resistance can be modulated by verapamil [11]. Thus, polysorbate 80 may be relevant to some of the activity observed for docetaxel in resistant tumours. The presence of potentially active levels of polysorbate 80 may also affect the antitumour activity of other cytotoxics where resistance is mediated by MDR, such as doxorubicin, when these drugs are combined with docetaxel. In this paper we report the first measurements of polysorbate 80 using a methodology similar to that used to measure Cremophor EL [16] in patients receiving either etoposide or docetaxel.

Patients and methods

Patients

Patients with advanced solid tumours receiving chemotherapy with docetaxel or etoposide were eligible for this study. Docetaxel (Taxotere, Rhone-Poulenc Rorer) was given intravenously over 1 h at a dose of 100 or 75 mg/m^2. Etoposide was given intravenously and all patients received 120 mg/m^2. If other cytotoxic drugs were given, the docetaxel or etoposide was given first and blood sampling was completed before other cytotoxics were given. From each patient, 20-ml blood samples were collected into lithium heparin tubes pre-dosing and at the end of the docetaxel or etoposide infusion. Plasma was immediately separated by centrifugation and stored at $-70\,^{\circ}$C until assayed. Written informed consent was obtained for the blood sampling.

Polysorbate 80 assay

The method for measurement of polysorbate 80 in plasma was a modification of our previously published Cremophor EL bioassay [16]. The principle of the bioassay is that MDR cells incubated with daunorubicin will have low equilibrium fluorescence and that the presence of MDR modulators, whether added to the sample or present in a patient's plasma, will increase this fluorescence. MDR VLB_{100} cells (derived from the human CCRF-CEM T-cell leukemia) were maintained in Eagle's α-MEM supplemented with 10% newborn calf serum (Cytosystems, Castle Hill, Australia) and vinblastine at 100 ng/ml (David Bull Laboratories, Melbourne). Approximately 5×10^5 cells in logarithmic growth were incubated at 37 °C for 1 h with daunorubicin at 2 μg/ml (David Bull Laboratories) in 0.5 ml serum-free HL-1 medium (Ventrex Laboratories, Portland, Me.) and 0.5 ml plasma. Equilibrium intracellular daunorubicin fluorescence was measured on a FACStar Plus cell sorter (Becton Dickinson, Mountain View, Calif.). As was found with the Cremophor EL assay, the variability in daunorubicin fluorescence from a given concentration of polysorbate 80 added to plasma from different patients was high ($>20\%$). Therefore, a standard curve was constructed for each patient by addition of polysorbate 80 (Sigma Chemical Company, St. Louis, Mo.) to pre-treatment plasma to achieve six final concentrations ranging from 0.06 to 1.0 μl/ml. The amount of polysorbate 80 in a given end-infusion sample was calculated from that patient's standard curve. All measurements were done in triplicate, and intra-assay variability was normally $<15\%$. The lower limit of quantitation was 0.06 μl/ml.

Results

A comparison of Cremophor EL and polysorbate 80 showed that at a given concentration, polysorbate 80 was more active than Cremophor EL at increasing daunorubicin fluorescence (Fig. 1). In most plasma samples the concentration of polysorbate 80 required

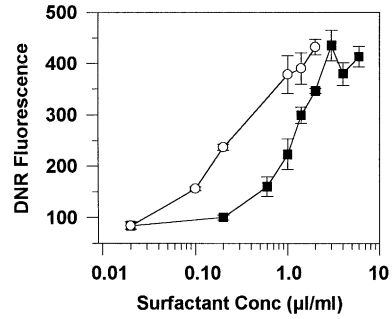

Fig. 1 Comparison of equilibrium intracellular daunorubicin fluorescence detected in cells expressing the MDR phenotype (CCRF-CEM VLB_{100} cells) following incubation with increasing concentrations of either polysorbate 80 (○) or Cremophor EL (■) added to plasma. Data represent mean values ±SD for 3 replicates; where no error bar is apparent, the SD was less than the size of the point

Table 1 Patients' characteristics and polysorbate 80 plasma concentrations measured following treatment with docetaxel

Patient	Age (years)	Sex[a]	Docetaxel[b] dose (mg)	Polysorbate 80 dose (ml)	Infusion time (min)	End-infusion polysorbate 80 concentration (μl/ml)
1	57	M	140	3.50	55	0.19
2	62	M	125	3.12	58	0.10
3	57	M	125	3.12	60	0.08
4	63	M	148	3.70	62	0.10
5	46	M	135	3.38	60	0.10
6	34	M	164	4.10	60	0.41
7	50	F	178	4.45	60	0.10
8	51	M	160	4.00	65	0.07
9	46	F	160	4.00	75	0.13

[a] Men had non-small-cell lung cancer and women had breast cancer
[b] Patients 1–5 received 75 mg/m^2 docetaxel, and patients 6–9 received 100 mg/m^2

to modulate MDR by 50% ranged between 0.2 and 0.3 μl/ml versus 1 μl/ml for Cremophor EL. The highest daunorubicin fluorescence occurred at a polysorbate 80 level of between 1 and 2 μl/ml and at a Cremophor EL concentration of between 1.5 and 3 μl/ml. The standard curves generated for polysorbate 80 were log-linear between 0.06 and 1.0 μl/ml.

Nine patients received a 1-h infusion of docetaxel, with the total dose ranging from 125 to 178 mg (Table 1). The total amount of polysorbate 80 delivered was 3.12–4.45 ml. The median end-infusion polysorbate 80 plasma concentration measured following docetaxel administration was 0.10 μl/ml (range 0.07–0.41). There was no correlation ($r = 0.3$, $P = 0.43$) between end-infusion polysorbate 80 levels and the total dose of docetaxel (and, thus, to the total amount of polysorbate 80 given); however, the narrow range of doses means that a relationship cannot be excluded.

Five patients (three women, and two men, aged 48–72 years) received intravenous etoposide at total doses of 180–250 mg (containing 0.67–0.93 ml polysorbate 80) over 45–120 min. None of the post-infusion plasma samples had measurable activity in the bioassay, indicating that following these doses of etoposide, polysorbate 80 concentrations were less than 0.06 μl/ml.

Discussion

In the present study we used a bioassay to determine polysorbate 80 concentrations in human plasma. Thus, the measurements reflect the MDR-reversing ability of polysorbate 80 rather than a direct quantitation of the total amount of this surfactant. Complete reversal of MDR in vitro occurs at polysorbate 80 concentrations ranging between 1.0 and 2.0 μl/ml, whereas 50% inhibition occurs at levels of between 0.2 and 0.3 μl/ml. The plasma concentrations of polysorbate 80 measured following docetaxel infusion were lower than that required to reverse MDR completely in vitro, although most patients had levels that showed some activity. The degree of reversal necessary to induce a response in resistant tumours in humans is unknown. However, our results suggest that the potential for induction of MDR reversal by polysorbate 80 during docetaxel infusion is lower than that for paclitaxel, where, at least during short infusions, the amount of Cremophor EL was sufficient to cause significant reversal in vitro [14, 16].

Cremophor EL has been demonstrated to change the pharmacokinetics of doxorubicin in mice [17] and of etoposide in an isolated perfused rat-liver model [6], and initial results suggest that it alters doxorubicin pharmacokinetics in humans [13]. The presence of Cremophor EL in the paclitaxel formulation may also be responsible for the pharmacokinetic and pharmacodynamic interactions seen in some clinical trials when paclitaxel and doxorubicin are combined [8, 9, 12]. Polysorbate 80 can also influence the pharmacokinetics of other drugs. In the isolated perfused rat liver it decreases the clearance and volume of distribution of etoposide [6], whereas in mice it increases plasma levels of doxorubicin by decreasing the plasma volume [10] and increases the renal and biliary excretion of methotrexate [1]. In patients who received the same amount of polysorbate 80 that was present in 100 mg/m^2 of intravenous etoposide, both the volume of distribution and the clearance of doxorubicin were increased [3]. The effect of docetaxel on doxorubicin pharmacokinetics has not been reported, but initial results of a phase I trial of this combination do not indicate a greater than expected doxorubicin toxicity, suggesting that there is no major pharmacokinetic or pharmacodynamic interaction [2].

At a nominal concentration of 1 μl/ml in perfusate in the isolated perfused rat-liver model, polysorbate 80 caused both haemolysis and cholestasis [6], and haemolysis has been noted in blood samples obtained from patients immediately following docetaxel administration (unpublished observations). In addition, cellular integrity studies performed with the cytofluorograph indicate that polysorbate 80 begins to lyse cells at a concentration of 0.1 μl/ml following a 1-h period of exposure [19]. It is therefore possible that polysorbate 80 may contribute to some of the toxicities of docetaxel. Cumulative fluid retention is a side effect of docetaxel that can be dose-limiting, and its aetiology is not understood. Polysorbate 80 is known to alter membrane fluidity, leading to increased membrane permeability [4], and this may potentially result in fluid retention. Further investigation of polysorbate 80 levels in patients receiving docetaxel may indicate a correlation between differences in polysorbate 80 pharmacokinetics and the subsequent development of fluid retention.

References

1. Azmin MN, Stuart JFB, Florence AT (1985) The distribution and elimination of methotrexate in mouse blood and brain after concurrent administration of polysorbate 80. Cancer Chemother Pharmacol 14:238–242
2. Bourgeois H, Gruia G, Dieras V, Kalla S, Giaccetti S, Cvitkovic E, Aussel JP, Azli N, Riva A, Poullart P, Misser JL (1996) Docetaxel in combination with doxorubicin as first line chemotherapy of metastatic breast cancer: a phase I dose finding study. Proc Am Assoc Clin Oncol 15:148
3. Cummings J, Forrest GJ, Cunningham D, Gilchrist NL, Soukop M (1986) Influence of polysorbate 80 (Tween 80) and etoposide (VP-16-213) on the pharmacokinetics and urinary excretion of Adriamycin and its metabolites in cancer patients. Cancer Chemother Pharmacol 17:80–84
4. Drori S, Eytan GD, Assaraf YG (1995) Potentiation of anticancer-drug cytotoxicity by multidrug-resistance chemosensitizers involves alterations in membrane fluidity leading to increased membrane permeability. Eur J Biochem 228: 1020–1029

5. Dudeja PK, Anderson KM, Harris JS, Buckingham L, Coon JS (1995) Reversal of multidrug resistance phenotype by surfactants: relationship to membrane lipid fluidity. Arch Biochem Biophys 319 : 309–315
6. Ellis AG, Crinis NA, Webster LK (1996) Inhibition of etoposide elimination in the isolated perfused rat liver by Cremophor EL and Tween 80. Cancer Chemother Pharmacol 38 : 81–87
7. Friche E, Jensen PB, Sehested M, Demant EJF, Nissen NN (1990) The solvents Cremophor EL and Tween 80 modulate daunorubicin resistance in the multidrug resistant Ehrlich ascites tumor. Cancer Commun 2 : 297–303
8. Gianni L, Locatelli A, Vigano L, Capri G, Giani A, Munzone E, Tarenzi E, Fulfaro F, Bonadonna G (1995) Order of administration and pharmacokinetics of paclitaxel by 3 h infusion and doxorubicin by iv bolus. Proc Am Soc Clin Oncol 14 : 169
9. Gianni L, Munzone E, Capri G, Fulfaro F, Tarenzi E, Villani F, Spreafico C, Laffranchi A, Caraceni A, Martini C, Stefanelli M, Valagussa P, Bonadonna G (1995) Paclitaxel by 3-hour infusion in combination with bolus doxorubicin in women with untreated metastatic breast cancer: high antitumor efficacy and cardiac effects in a dose-finding and sequence-finding study. J Clin Oncol 13 : 2688–2699
10. Harrison SD, Cusic AM, McAfee SM (1984) Tween 80 increases plasma adriamycin concentrations in mice by an apparent reduction of plasma volume. Eur J Cancer 17 : 387–389
11. Hill BT, Whelan RDH, Shellard SA, McLean S, Hosking LK (1994) Differential cytotoxic effects of docetaxel in a range of mammalian tumor cell lines and certain drug resistant sublines in vitro. Invest New Drugs 12 : 169–182
12. Holmes FA, Madden T, Newman RA, Valero V, Theriault RL, Fraschini G, Walters RS, Booser DJ, Buzdar AU, Willey J, Hortobagyi GN (1996) Sequence-dependent alteration of doxorubicin pharmacokinetics by paclitaxel in a phase I study of paclitaxel and doxorubicin in patients with metastatic breast cancer. J Clin Oncol 14 : 2713–2721
13. Rischin D, Millward M, Webster L, Tinnelly K, Toner G, Bishop J, Linsenmeyer M, Woodcock D (1994) Phase I trial of Cremophor EL and doxorubicin in patients with advanced cancer. Anticancer Drugs 5 [Suppl 1] : 43
14. Rischin D, Webster LK, Millward MJ, Linahan BM, Toner GC, Woollett AM, Morton CG, Bishop JF (1996) Cremophor pharmacokinetics in patients receiving 3, 6 and 24 hour infusions of paclitaxel. J Natl Cancer Inst 88 : 1297–1301
15. Schuurhuis GJ, Broxterman HJ, Pinedo HM, Heijningen HM van, Kalken CK van, Vermorken JB, Spoelstra EC, Lankelma J (1990) The polyoxyethylene castor oil Cremophor EL modifies multidrug resistance. Br J Cancer 62 : 591–594
16. Webster L, Linsenmeyer M, Millward M, Morton C, Bishop J, Woodcock D (1993) Measurement of Cremophor EL following Taxol: plasma levels sufficient to reverse drug exclusion mediated by the multidrug-resistance phenotype. J Natl Cancer Inst 85 : 1685–1690
17. Webster LK, Cosson EJ, Stokes KH, Millward MJ (1996) Effect of the paclitaxel vehicle, Cremophor EL, on the pharmacokinetics of doxorubicin and doxorubicinol in mice. Br J Cancer 73 : 522–524
18. Woodcock DM, Jefferson S, Linsenmeyer ME, Crowther PJ, Chojnowski GM, Williams B, Bertoncello I (1990) Reversal of the multidrug resistance phenotype with Cremophor EL, a common vehicle for water-insoluble vitamins and drugs. Cancer Res 50 : 4199–4203
19. Woodcock DM, Linsenmeyer ME, Chojnowski G, Kriegler AB, Nink V, Webster LK, Sawyer WH (1992) Reversal of multidrug resistance by surfactants. Br J Cancer 66 : 62–68