Cancer Chemother Pharmacol (1986) 17: 80–84

Cancer Chemotherapy and Pharmacology

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*

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Summary. Polysorbate 80 (Tween 80) is present in the IV pharmaceutical preparation of VP-16-213 marketed as VePesid (Bristol-Myers) (etoposide 100 mg, benzylalcohol 150 mg, polyethylene glycol 300 3250 mg, citric acid 10 mg, Tween 80 400 mg and absolute alcohol to 5 ml per 100 mg ampule of VP16), to increase its miscibility with blood. We have examined the effects of 400 mg/m^2 Tween 80 IV and 100 mg/m^2 VP16 on the pharmacokinetics of Adriamycin (ADR, 30 or 40 mg/m^2). ADR and metabolite concentrations were measured by HPLC. ADR plasma profiles were best fitted to a bi-exponential decay and a two-compartment open model. Tween 80 did not alter the values of the two ADR half-lives, nor did it affect metabolite kinetics of their urinary excretion. However, in a similar manner and consistently in all patients, both Tween 80 and VP16 increased the volume of distribution of the central compartment for ADR up to 3-fold, decreased the AUC of ADR up to 2-fold and increased its clearance by exactly the same amount. These effects were due to reduced plasma ADR concentrations during the early phase of its kinetics. Urinary excretion of ADR was also increased. In conclusion, VP16 is likely to affect the kinetics of drugs administered with it: early plasma concentrations will fall due to a general physiological effect of Tween 80 on the apparent volume of circulation.

Introduction

VP-16-213 (VP16) has become one of the most useful anticancer agents of recent years. It possesses significant activity against several tumours, including high-grade non-Hodgkin's lymphoma, small cell carcinoma of the bronchus and teratoma of the testes [9]. The drug is only sparingly soluble in aqueous solutions, and in order to improve its miscibility with blood organic solvents are included in its formulation. Tween 80, the non-ionic surfactant, is one component of the commercially available pharmaceutical preparation of VP16 which is administered IV to patients (VePesid, Bristol-Myers, 400 mg Tween 80 per 100 mg ampule of VP16). Tween 80 can alter the disposition of drugs (including methotrexate) it is co-administered with [1]. Therefore, drugs given in combination with VP16 may have their pharmacokinetics modified as a consequence of Tween 80. Adriamycin (ADR) is commonly administered with VP16, and in this report the influence of Tween 80 on its pharmacokinetics is described.

Materials and methods

Patients. Five subjects each receiving combination chemotherapy for advanced malignant disease were recruited. None had severe liver or kidney dysfunction or was a heavy drinker of alcohol. With the exception of patient MK (who had received one previous course of ADR, VP16 and cyclophosphamide [cyclo] 3 weeks prior to the study), all patients were previously untreated with the drugs under investigation. More details on each patient are to be found in Table 1. Drugs were administered IV at the following doses: ADR, 30 or 40 mg/m^2; cyclo, 750 mg/m^2; VP16, 100 mg/m^2 and Tween 80, 400 mg/m^2 (the dose equivalent to the amount of surfactant in 100 mg/m^2 VP16).

Design of study. Approval was obtained from the local ethical committee. In order to distinguish possible longitudinal effects of repeated drug exposure from Tween 80 effects on ADR pharmacokinetics, drug combinations were administered in a fixed sequence: first ADR, cyclo and VP16, followed 3 weeks later by ADR and cyclo only and finally followed 3 weeks later by ADR, cyclo and Tween 80. In total, the kinetics of ADR were followed three times in each patient: once in the presence of VP16, once in the presence of Tween 80 and once in the absence of both.

Pharmacokinetic studies. Drug combinations were administered in the following order: cyclo, VP16 or Tween, then ADR. Blood samples (10-ml aliquots) were withdrawn through indwelling catheters positioned in the arm opposite to that used for drug injections before ADR treatment and at 5 min, 10 min, 15 min, 30 min, 60 min, 90 min, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h and 24 h after ADR administration. Exact times of blood sampling were recorded. After blood samples were withdrawn they were centrifuged immediately, and plasma samples for drug analysis were separated and stored at −20 °C. Urine was collected in four 6-hourly aliquots for the 24 h period following drug therapy.

* Presented, in part, as an abstract at the 26th AGM of the British Association of Cancer Research (BACR), Birmingham UK., March 1985

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Table 1. The patient group studied

Patient	Age	Sex	Weight kg	Primary tumour	Prior exposure to Adriamycin mg/m²	Dose mg/m²	Liver function			Kidney function	
							Bilirubin 3–18 mol/l[a]	Albumin 36–50 g/l[a]	ALT 10–50 u/l[a]	Urea 2–8 mmol/l[a]	Creatine 60–110 mol/l[a]
AB	78	M	67	Lymphoma	nil	30	15	30	31	4.2	110
AA	33	F	53	S C Lung	nil	40	8	38	11	5.7	75
LMcG	29	F	46	S C Lung	nil	40	7	41	20	4.7	55
WG	68	F	74	Lymphoma	nil	30	16	29	17	NM[b]	125
MK	58	F	75	Lymphoma	30	30	7	35	25	3.9	80

[a] Normal range of values
[b] Not measured

Drug analysis techniques. ADR and metabolites were identified in plasma and quantitated by two high-performance liquid chromatography assays, both with fluorescence detection. HPLC equipment and the separations of ADR and metabolites have already been described in detail [2, 3]. Two methods were employed, in order to identify ADR aglycone metabolites which do not resolve well by HPLC [3]. Both methods used μ-Bondapak C18 (Waters Associates, Northwich, England) as the stationary phase (packed in 250 mm × 4.6 mm ID stainless steel columns). In the first method the mobile phase was 5 m*M* phosphoric acid (final concentration) (6.25%), methanol (15.0%), acetonitrile (15.0%) and propan-2-ol (7.5%), pH 3.2, eluting isocratically at a flow rate of 2 ml/min. In the second method the mobile phase was 5 m*M* phosphoric acid (final concentration) (74%) in propan-2-ol (26%), pH 3.2, eluting isocratically at a flow rate of 1.2 ml/min. All solvents and chemicals were of the highest quality available commercially. Chromatographically pure ADR-HCl, adriamycinol-HCl, ADR 7-deoxyaglycone and ADR 7-hydroxyaglycone were gifts from Dr S Penco (Farmitalia, Milan, Italy): ADR 7-hydroxyaglycone and adriamycinol 7-deoxyaglycone and adriamycinol 7-deoxyaglycone were synthesised by catalytic hydrogenation using a poisoned palladium catalyst [3]. The synthesised aglycones were characterised as previously reported [3].

Rapid extraction of ADR and metabolites from plasma (and urine). Prior to extraction, plasma samples were thawed at room temperature. Daunorubicin was added as an internal standard, and samples were extracted by vortexing for 30 min with 5 vol of chloroform : propan-2-ol (2 : 1). Samples were then centrifuged at 2000 *g* for 15 min to separate two phases. The upper aqueous phase was discarded by aspiration, the lower organic phase was evaporated to dryness at less than 50 °C, and the dry residues were reconstituted in either 50 or 100 μl or methanol and 20 μl was injected onto the HPLC column.

Pharmacokinetic analysis. Pharmacokinetic parameters were derived from an extended least-squares computer fit (ELSFIT, version 3.0, obtained from Dr. Lewis B. Sheiner, Division of Clinical Pharmacology, University of California, San Francisco, USA) to the experimentally determined plasma concentrations of ADR using a Hewlett-Packard HP 85B computer (Hewlett-Packard, South Queensferry, Scotland). Although a tri-exponential elimination has been frequently reported to describe the kinetics of ADR, our data for all five patients were best fitted by a bi-exponential decay where: plasma concentration (c) $= Ae^{-t\alpha} + Be^{-t\beta}$. A and B are defined as the theoretical zero time concentrations (c_o, ng/ml) in a central and peripheral compartment respectively and α and β are defined as apparent elimination rate constants (k_{el}). Other parameters were defined as follows:)

$$\text{Half-life } (t_{l/2}) = \frac{0.693}{k_{el}}$$

$$\text{Area under the curve (AUC)} = \frac{A}{\alpha} + \frac{B}{\beta}$$

$$\text{Clearance (Cl)} = \frac{\text{Dose}}{\text{AUC}}$$

$$\text{Volume of distribution (of central compartment) } (V_{Dc}) = \frac{\text{Dose}}{A}$$

The AUC of ADR metabolites was determined by the trapezoidal rule and their half-lives were estimated by non-linear regression data fitting to the terminal portion of their plasma log concentration time profiles. No attempt was made to model the kinetics of the metabolites.

Results

Influence of Tween 80 on the pharmacokinetics of Adriamycin

Pharmacokinetic parameters of ADR in all five patients after the three different drug combination are contained in Table 2. Apparent half-lives $t_{½\alpha}$ and $t_{½\beta}$ were not significantly changed and therefore did not seem to be affected by Tween 80, either administered alone or present in VP16. However, a consistent effect was noticed on the values of the parameters V_{Dc}, AUC and Cl. V_{Dc}, which had a narrow range of baseline values in all five patients after administration of ADR and cyclo (11.041 ± 2.9 SD), increased substantially in all five patients, in some cases 3-fold, after administration of Tween 80, either alone or present in VP16 (Table 2). The values of AUC decreased substantially, up to 2-fold, and the values of Cl increased by exactly the same degree in all five patients after admin-

Table 2. Influence of 400 mg/m² Tween 80 IV and 100 mg/m² VP16 on the pharmacokinetics of Adriamycin in cancer patients

Patient	Treatment	A		B		$t^1/_2$ (h)	$t^1/_2$ (h)	V_{Dc} (l)	AUC (ng/ml × h)	Cl (l/h)
AB	ADR + cyclo	3463 ± 145	10.1	27.5	0.19	0.07	3.6	8.6	488	61
	ADR + cyclo + Tween 80	837 ± 62	11.8	24.8	0.13	0.06	5.3	37.5	257	140
	ADR + cyclo + VP16	1109 ± 41	6.5	17.7	0.20	0.11	3.5	27.2	259	139
AA	ADR + cyclo	5262 ± 231	11.2	31.5	0.06	0.06	11.0	11.9	969	64
	ADR + cyclo + Tween 80	4662 ± 240	9.2	54.2	0.21	0.08	3.3	13.5	765	81
	ADR + cyclo + VP16	1627 ± 118	5.0	19.9	0.09	0.14	7.9	38.8	546	113
LMcG	ADR + cyclo	3845 ± 301	8.0	31.1	0.09	0.09	8.2	15.0	857	65
	ADR + cyclo + Tween 80	1521 ± 132	5.4	26.2	0.10	0.13	7.1	38.0	551	101
	ADR + cyclo + VP16	2505 ± 225	10.2	18.8	0.06	0.07	10.8	22.8	540	103
WG	ADR + cyclo	4200 ± 344	13.7	13.3	0.08	0.05	8.7	11.9	472	105
	ADR + cyclo + Tween 80	3736 ± 209	12.2	52.1	0.07	0.06	9.9	13.5	406	123
	ADR + cyclo + VP16	2566 ± 187	16.9	16.9	0.08	0.06	8.7	19.4	422	118
MK	ADR + cyclo	9431 ± 1244	15.7	49.4	0.19	0.04	3.6	7.8	860	58
	ADR + cyclo + Tween 80	4751 ± 493	10.9	37.1	0.18	0.06	3.9	10.6	642	78
	ADR + cyclo + VP16	2698 ± 187	9.9	28.7	0.19	0.07	3.6	18.5	423	118

istration of tween, either alone or present in VP16 (Table 2). Inspection of the computer-calculated parameters in Table 2 shows that the most pronounced effect of Tween 80 was to decrease the value of parameter A. A is related to the values of ADR plasma concentrations during the early phase of its kinetics. Thus, the main influence of Tween 80 on the pharmacokinetics of ADR was to reduce early peak plasma concentrations of the drug.

Influence of Tween 80 on the kinetics of Adriamycin metabolites

All metabolite kinetic data are contained in Table 3. Values of the AUC and apparent half-life of adriamycinol, the major metabolite of ADR, which was detected in all five patients, did not seem to be altered by Tween 80. Indeed, the kinetics of the metabolite were similar after the three different combinations of drugs (Table 3). Two other metabolites were identified: these were adriamycin 7-deoxyaglycone (ADR-DONE) and adriamycinol 7-deoxyaglycone (AOL-DONE). The latter metabolite was only detected in one patient (WG), at low levels and for only a short period of time. No meaningful evaluation of the effects of Tween 80 on its kinetics was, therefore, possible. ADR-DONE was detected in all five patients, but in three cases was only detectabel when VP16 was administered together with ADR (Table 3). It is, thus, conceivable that VP16 itself accelerates the biotransformation of ADR, pos-

Table 3. Influence of 400 mg/m² Tween 80 IV and 100 mg/² VP16 on the kinetics of Adriamycin metabolites in cancer patients

Patient	Treatment	Adriamycinol (AOL)		AOL-DONE		ADR-DONE	
		AUC (ng/ml × h)	$t^1/_2$ (h)	AUC (ng/ml × h)	$t^1/_2$ (h)	AUC (ng/ml × h)	$t^1/_2$ (h)
AB	ADR + cyclo	115	15.9	ND	–	ND	–
	ADR + cyclo + Tween 80	114	10.6	ND	–	ND	–
	ADR + cyclo + VP16	230	13.8	ND	–	14	0.2
AA	ADR + cyclo	185	10.6	ND	–	5	0.2
	ADR + cyclo + Tween 80	183	14.8	ND	–	7	0.1
	ADR + cyclo + VP16	121	13.0	ND	–	6	0.2
LMcG	ADR + cyclo	99	17.5	ND	–	ND	–
	ADR + cyclo + Tween 80	150	21.0	ND	–	ND	–
	ADR + cyclo + VP16	234	25.3	ND	–	9	0.4
WG	ADR + cyclo	121	22.2	3	0.1	6	0.1
	ADR + cyclo + Tween 80	150	15.1	2	0.1	6	0.1
	ADR + cyclo + VP16	228	19.5	3	0.1	11	0.1
MK	ADR + cyclo	229	31.1	ND	–	ND	–
	ADR + cyclo + tween 80	150	21.1	ND	–	ND	–
	ADR + cyclo + VP16	228	35.5	ND	–	28	0.3

ND, Not detected

Table 4. Influence of 400 mg/m² Tween 80 on the 24 h cumulative urinary excretion of Adriamycin and driamycinol in cancer patients

Patient	Treatment	Dose (mg)	Adriamycin (mg)	Adriamycinol (mg)
AB	ADR + cyclo	30	0.3	0.01
	ADR + cyclo + Tween 80	30	1.9	0.10
AA	ADR + cyclo	62	0.6	0.14
	ADR + cyclo + Tween 80	62	0.9	0.01
LMcG	ADR + cyclo	57	1.8	0.10
	ADR + cyclo + Tween 80	57	2.1	0.14
WG	ADR + cyclo	50	0.3	0.04
	ADR + cyclo + Tween 80	50	0.6	0.02

sibly by enhancing the flow of reducing equivalents from NADPH and/or NADH-dependent flavoenzymes [12]. In the other two patients Tween 80 did not appear to affect the kinetics of ADR-DONE. Thus, overall, Tween 80 did not influence the kinetics of ADR metabolites.

Influence of Tween 80 on the 24 h cumulative urinary excretion of Adriamycin and metabolites

Urinary data were available only after the second and third courses of drug administration, i.e. after ADR + cyclo and ADR + cyclo + Tween 80. Only trace amounts of 7-deoxyaglycones were detected in urine, and Table 4 therefore only contains data relating to ADR and adriamycinol. In all four patients urinary excretion of ADR increased although this does not appear to be a marked effect. However, only a small percentage of the total administered dose of ADR was excreted in the urine (Table 4). Therefore, in some patients excretion actually increased by a large factor. Excretion of adriamycinol was not affected by Tween 80.

Discussion

We have examined the influence of the dose of Tween 80 present in commercially available VP16 on the pharmacokinetics of ADR. Three kinetic studies were performed on each patient; Tween 80 was administered twice, once as an IV bolus and once as VP16. The reason for designing the study in this manner was to determine whether Tween 80 itself had any effect and then verify whether this effect(s) would be produced by VP16. The results confirmed that both Tween 80 and VP16 influenced the kinetics of ADR in the same way. The most pronounced effects were those on early peak plasma concentrations and the volume of distribution of the central compartment. One must, however, be cautious when interpreting results from a longitudinal study of ADR kinetics. Previous reports indicate that prior exposure to ADR results in decreased plasma levels and a reduction in AUC, similar to our results with Tween 80 [5, 13]. Recently, it was demonstrated that this is not a general rule regarding the kinetics of ADR in man [6]. Here, in three out of five patients after three successive doses of 30 mg/m² ADR AUCs actually increased. Similar findings were also reported in an earlier study [10]. In order to separate longitudinal effects of repeated ADR dosage from those of Tween 80 we decided that the baseline pharmacokinetic study, i.e. ADR + cyclo, would be performed not after the first exposure to ADR, but after the second. Our results show that plasma concentrations and AUCs were consistently lowered in all five patients only when Tween 80 was present. i.e. after the first and third administrations of ADR. These results, therefore, strongly suggest a true effect due to Tween 80 itself. Tipping and co-workers [13] followed the kinetics of ADR and cyclo after four successive courses of treatment in eight patients. Whilst ADR AUCs fell as expected, cyclo AUCs remained constant. Our observation (unpublished) of cyclo AUCs in the five patients studied, after the three different drug combinations, was that they behaved in a fashion similar to those for ADR, falling after the first and third courses of treatment. This indicates that Tween 80 has a more general effect. Tween 80 is a vasodilator [8]; it can also increase the absorption of drugs across the GI tract and the blood-brain barrier in mice [1] by increasing membrane permeability due to its surfactant properties. It can even alter plasma volume significantly in mice after oral administration [7]. Thus, the main effects of Tween 80, are on the circulation and plasma volume. Our findings of reduced drug levels and an increase in the volume of the central compartment are consistent with the known pharmacological and physiological properties of Tween 80, although the effect of other constituents in the commercial preparation of VP-16-213 cannot be discounted.

Tween 80 affected principally the early phase of ADR kinetics. This may explain why metabolite AUCs were not greatly changed, as they are not introduced directly into the circulation but are formed in a peripheral compartment and then equilibrate with blood. In fact, the levels of 7-deoxyaglycones were lower than might have been expected, probably due to concomitant administration of cyclo, which has already been reported to reduce the AUC of ADR aglycones in man [11] and inhibit their formation in vitro by microsomal enzymes [4].

Tween 80 has also been reported to increase the urinary excretion of methotrexate in mice [1]. We now report that Tween 80 can also increase urinary excretion of ADR in man.

In conclusion, it is possible that the observation of reduced peak levels of ADR may translate to changes in its efficacy and toxicity, especially as peak levels have been implicated, strongly, in ADR-induced cardiotoxicity [14]. Studies in which the drugs are given together should be reviewed in the light of these data.

Acknowledgement. We would like to thank Miss Deborah Kinghorn for secretarial assistance.

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Received August 6, 1985/Accepted November 25, 1985