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Clinical report

Pharmacokinetic interaction between etoposide and tamoxifen in patients with hepatocellular carcinoma

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The effect of tamoxifen (TAM) on the pharmacokinetics of oral administration of etoposide (VP-16) in patients with non-operable hepatocellular carcinoma was investigated. The pharmacokinetics of VP-16 was studied by using a validated limited sampling strategy. The pharmacokinetic parameters of VP-16, such as area under curve (AUC), free AUC and protein binding, were determined from drug plasma concentrations at 1 and 4 h after VP-16 administration on the first day (day −1) and at the end of the chemotherapy cycle (day −21) for VP-16 alone and VP-16+TAM, respectively. When VP-16 was administered in association with TAM, the median total systemic exposure was not significantly (p=NS) different from that observed when VP-16 was administered alone [33.74 (range 11.19–56.58) versus 32.97 (range 20.23–119.28) mg/l/h]. Moreover, TAM did not affect significantly (p=NS) the levels of protein binding of VP-16 [median 94.6 (range 87.7–98.2) versus median 94.9 (range 91.6–98.0) % for VP-16+TAM and VP-16 alone, respectively] and the systemic exposure of the free drug (free AUC) [1.86 (range 0.21–4.57) versus median 1.78 (range 0.59–3.73) mg/l/h for VP-16+TAM and VP=16 alone, respectively]. These results indicate a lack of pharmacokinetic interaction between VP-16 and TAM, and suggest that the increased hematological toxicity observed when TAM is given in combination with VP-16 could be related to pharmacodynamic interactions. [© 1999 Lippincott Williams & Wilkins.]

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Introduction

Tamoxifen (TAM) is a multifunction antiestrogenic triphenylethylene widely used in the treatment and prevention of breast cancer.[1,2] It is also used in the treatment of other cancers including brain, liver and ovary cancers[3] or in combination with other antiblastic drugs in adjuvant therapy.[4] Among its multifunction activities,[5] TAM is able to reverse the multidrug-resistance (MDR) phenotype, induced by the P-glycoprotein (P-gp) in several carinoma cell lines.[6] This characteristic of TAM has been extensively investigated in different clinical settings where TAM was used in combination with drugs belonging to the MDR phenotype.[7,8] Although the efficacy of such combinations on tumor response in conventional clinical protocols is still controversial, promising results have been reported for low doses of oral etoposide (VP-16) plus TAM chronically administered in patients with non-operable hepatocellular carcinoma.[9] However, in our previous trial, the efficacy of this combination was not evaluable because of a remarkable increase in hematological toxicity as compared to that observed in patients with similar characteristics and the same pathology treated with prolonged oral VP-16 as a single agent. Although the observation of the increase in hematological toxicity for VP-16+TAM combination suffers from the limitations of the comparison between a phase II study and a historical control, it has been previously reported that the clinical use of different MDR modulating agents may increase hematologic toxicity of antiblastic drugs as a consequence of their pharmacokinetic interaction, which leads to an increase of systemic drug exposure.[10,11] So far, little is known about the pharmacokinetic interaction of TAM with antineoplastic drugs.

In an attempt to better clarify this issue, in the present study we investigated the effect of prolonged

administration of TAM on the pharmacokinetics of oral VP-16 in patients with hepatocellular carcinoma receiving the VP-16+TAM oral drug combination.

Materials and methods

Patients

Patients with progressive, histologically documented, multifocal unresectable or metastatic hepatocellular carcinoma were eligible for this study. Entry criteria were those established by the Eastern Cooperative Oncology Group: a Karnofsky performance status >60, life expectancy $\geqslant 12$ weeks, adequate renal function (serum creatinine <2.0 mg/dl) and normal bone marrow function (WBC count $>3400/mm^3$, neutrophil count $>2000/mm^3$ and platelet count $>100\ 000/mm^3$). There was no exclusion of patients or dose modifications on the basis of abnormal liver functions.

Design of the study

Enrolled patients (study group) received 100 mg of oral VP-16 daily for 21 consecutive days as a single dose, according to the protocol by Cheng *et al.*[9] with a few modifications. An oral dose of 40 mg/day of TAM was concomitantly given with VP-16 from day 2 until day 21 of the chemotherapy cycle. At that time, oral VP-16 administration was discontinued for 1 week before starting a new cycle. During this week, patients maintained the daily dose of TAM. Toxicity was evaluated using the WHO criteria and then compared with the toxicity observed in an Institutional retrospective group of patients (control group) with similar characteristics and the same pathology treated with prolonged oral VP-16 as a single agent.

Pharmacokinetic analysis

The pharmacokinetics of VP-16 alone was determined on day 1 of chemotherapy when TAM was excluded, whereas the pharmacokinetics of VP-16 in association with TAM was evaluated on day 21, at the end of the first cycle of VP-16+TAM chemotherapy. To determine the VP-16 area under the curve (AUC) using a small number of blood samples, a validated limited sampling strategy was used. On the basis of this limited sampling model, only two blood samples, drawn at 1 and 4 h after the oral administration of VP-16, were needed to estimate the total plasma AUC with good accuracy. The level of etoposide protein binding and the exposure to the free drug (free AUC) were also determined, as previously reported.[12]

Statistical analysis

Statistical significance was assessed by the non-parametric Mann–Whitney test when two groups of unpaired samples were compared and by the non-parametric Wilcoxon test in the case of two groups of paired samples. $p \leqslant 0.05$ was considered significant.

Results

Patients and toxicity

Table 1 summarizes the characteristics of the patients receiving the oral combination of V-16+TAM and those of the control group receiving the same oral dose of VP-16 as a single agent. Both groups of patients had homogeneous clinico-pathological characteristics and pretreatment. Chronic low-dose administration of VP-16 alone was generally well tolerated in the patients used as controls. However, in patients receiving the same oral dose of VP-16 in conjunction with TAM, a great increase in the hematological toxicity was reported during the first cycle of chemotherapy. Compared to the control group, the patients receiving VP-16+TAM showed an increase in G3 and G4 hematological toxicity (43 versus 26% for the study and control group, respectively) and a significantly (p=0.014) higher percentage of decrease in WBC at nadir [median 69 (range 16.0–95.45) versus median 38 (range 13.13–95.30) % for the study and control group, respectively] (Figure 1). Moreover, the VP-16+TAM treatment was discontinued in three patients on days 14 and 15 because of severe leukopenia and, in two cases, neutropenic fever resulted in toxic death (14%).

Pharmacokinetics

The total drug exposure (AUC) was estimated using a limited sampling model represented by the following equation: AUC$=-1.649+1.082\times$(VP-16 plasma concentration at 1 h)$+11.952\times$(VP-16 plasma concentration at 4 h) obtained by a mutiple linear regression analysis of historical pharmacokinetic data (extensive sampling), according to the procedure reported by Gentili *et al.*[13] The validation of the model was performed using a different set of consistent pharmacokinetic data obtained from 30 patients with different

cancer diseases previously treated in our Institute with the same dose of oral VP-16. The AUC observed and the AUC estimated on the basis of the model were highly correlated (R=0.91 $p<0.0000001$) and the precision, evaluated by calculating the root mean square prediction relative error (% RMSE), was 12.5%.

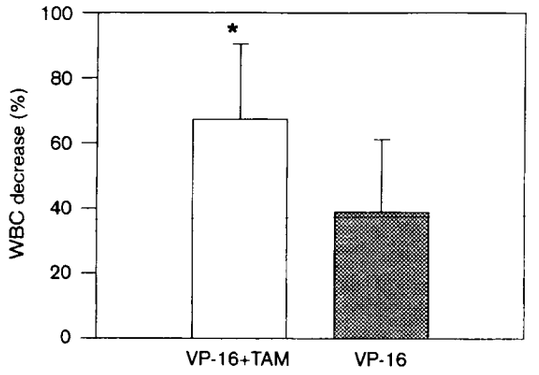

Figure 1. Comparison of decrease in WBC count between patients receiving oral VP-16+TAM (study group) and patients receiving VP-16 as a single agent (control group). Data are expressed as the percentage of decrease in WBC between baseline count and nadir value. Data ± SD. *Significance, $p<0.05$ by the Mann–Whitney test.

The overall systemic exposure to VP-16, expressed as the median value of the estimated AUC when the drug was administered alone and in association with TAM, in both the study and control groups is shown in Table 2. In the study group, the median AUC of VP-16 on the first day of chemotherapy, when TAM was omitted, was 32.97 (range 20.23-119.28) mg/l/h (n=14) and the median drug protein binding and free AUC were 94.9 (range 91.6-98.0) % (n=14) and 1.78 (range 0.59-3.73) mg/l/h (n=14), respectively. The systemic total and free AUCs were not significantly different (p=NS) (by the Mann-Whitney test) from that observed in the group of patients used as control and receiving the same dose of VP-16 as a single agent [31.87 (range 10.20-51.73) mg/l/h (n=17) and 2.07 (range 0.44-4.87) mg/l/h (n=17) for total AUC and free AUC, respectively]. The concurrent chronic administration of 40 mg/day of TAM did not affect substantially the pharmacokinetic parameters of VP-16 evaluated at the end of the chemotherapy cycle of VP-16+TAM. The median total and free AUCs of VP-16 were 33.74 (range 11.19-56.58) mg/l/h (n=11) and 1.86 (range 0.21-4.57) mg/l/h (n=11), respectively, whereas the median level of VP-16 protein binding was 94.6 (range 87.7-98.2) % (n=11) (Table 2). These

Table 1. Clinical and pathological characteristics of the patients

	Study group (VP-16+TAM)		Control group (VP-16)	
	No.	%	No.	%
No. of patients	14	—	17	—
Male/female	11/3	78/22	16/1	94/6
No. of pretreated patients[a]	7	50.0	12	70.6
Karnofsky PS	90		90	
Hepatocellular classification				
child A	12	85.7	9	52.9
child B	2	14.3	6	35.3
child C	—	—	2	11.8
extrahepatic metastasis	3	21.4	—	—
cirrhosis	14	100.0	17	100.0
HCV	4	28.6	5	29.4
Clinical characteristics	Median	Range	Median	Range
Age (years)	65.0	52.0–73.0	65.0	52.0–83.0
Total protein (g/dl)	7.5	6.3–8.4	7.4	6.1–9.2
Albumin (g/dl)	3.8	2.9–4.7	3.6	3.3–4.8
GOT (U/l)	97.0	3.5–322.0	77.0	24.0–700.0
Total bilirubin (mg/dl)	1.2	0.5–3.2	1.2	0.5–5.0
Creatinine (mg/dl)	1.1	0.8–1.5	1.0	0.7–2.5
Creatinine clearance (ml/min)	71.8	13.0–78.0	81.7	30.1–143.0
MEGX (ng/ml)	37.0	52.0–73.0	35.0	14.5–87.0
Alpha-fetoprotein (ng/ml)	853.0	4.5–145000	447.0	20.0–312000
WBC (cell count × 10^3/mm^3)	8.4	3.5–15.0	5.7	2.8–10.1

[a]The patients were previously treated with TACE, PEI, RT and chemotherapy including VP-16, idarubicin and 5-fluorouracil.

Table 2. Etoposide pharmacokinetics

	Study group (VP-16+TAM)							Control group (VP-16)			
	VP-16			VP-16+TAM				VP-16			
	Median	Range	No.	Median	Range	No.	p	Median	Range	No.	p
AUC (mg/l/h)	32.97	20.23–119.28	14	33.74	11.19–56.58	11	NS[a]	31.87	10.20–51.73	17	NS[b]
Free AUC (mg/l/h)	1.78	0.59–3.73	14	1.86	0.21–4.57	11	NS	2.07	0.44–4.87	17	NS
Protein binding (%)	94.9	91.6–98.0	14	94.6	87.7–98.2	11	NS	93.2	84.4–98.1	17	NS

[a]Statistical significance was assessed by the Wilcoxon test by comparing VP-16+TAM and VP-16 data observed in the study group.
[b]Statistical significance was assessed by the Mann–Whitney test by comparing VP-16 data between the study and control group.

values were not significantly different (p=NS) (by the Wilcoxon test) from those observed in the same patients when receiving VP-16 alone.

Discussion

In this study, we assessed the effect of TAM on the pharmacokinetics of VP-16 by comparing total systemic exposure of VP-16 when administered alone and in association with TAM. We observed that, in the presence of TAM, AUC of VP-16 was not significantly different from that observed when VP-16 was administered alone on the first day of chemotherapy. These results indicate that TAM at the dose of 40 mg/day was unable to increase VP-16 exposure by reducing drug body excretion through the inhibition of liver and kidney P-gp activity or by interfering with the drug liver metabolism mediated by the cytochrome P450, as suggested for other MDR modulating agents.[14,15] Moreover, TAM did not displace VP-16 from the plasma protein binding site and consequently did not increase the systemic exposure to free VP-16, which has been reported to be a better indicator of hematological toxicity.[16,17] Even if these findings derive from a limited pharmacokinetic analysis, they clearly indicate a lack of pharmacokinetic interaction between TAM and VP-16. Since no significant differences were observed in the systemic total and free drug exposure between the study and control group (Table 2), it can be suggested that the greater hematological toxicity observed in patients treated with VP-16+TAM may be related to pharmacodynamic interactions. However, it is worth considering that the differences in hematological toxicity observed between the two groups of patients could also be related to differences in patients' characteristics. This may represent an important factor. However, even if we cannot completely exclude this factor, the two groups of patients showed homogenous basal clinical characteristics, site of disease and type of pretreatment, suggesting a similar chemotherapy outcome.

The observation that TAM may enhance the myelotoxicity of VP-16 without altering the systemic exposure of the drug could be a consequence of the relatively high TAM concentration achievable in the bone marrow. Despite the low dose of TAM used in this study, it has been previously reported that TAM and its metabolites can accumulate in the tissues at concentrations substantially higher (about 10-fold) than in the plasma,[18] and are likely to modulate P-gp function and to increase the cytotoxic effect of etoposide in stem cells which normally express high levels of P-gp. Moreover, TAM may have other

functions relevant in enhancing the cytotoxic efficacy of antiblastic drugs, independent of the MDR, such as altering the transmembrane cellular signaling and growth factor networks.[19,20]

In conclusion, our results indicate that the increase of the side effects observed when TAM was given in conjunction with prolonged oral VP-16 cannot be related to the effect of TAM on the pharmacokinetics of VP-16. Further cross-over studies, however, are needed to better define the pharmacodynamic interaction between TAM and VP-16.

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