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Pharmacokinetics of 4′-O-tetrahydropyranyladriamycin given on a weekly schedule in patients with advanced breast cancer

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Abstract Improved quality of life has gained importance over shortly lasting remissions in yet incurable metastatic breast cancer. Fractionation of drug administration is one of the possible approaches to reduce the concentration-dependent toxicity of anthracyclines. We evaluated the pharmacokinetics of 4′-*O*-tetrahydropyranyladriamycin (THP-ADM) under weekly administration in patients with advanced breast cancer (dose escalation, from 20 to 27 mg/m^2 THP-ADM). The concentration-time curves of THP-ADM in plasma were best described by an open three-compartment model [half-life of the first disposition phase ($t_{1/2\alpha}$), 3.15 min; terminal elimination half-life ($t_{1/2\gamma}$), 13.9 h] with a mean area under the curve (AUC) of 12.2 ng h ml^{-1}mg^{-1} m^{-2}, resulting in a mean plasma clearance of 86.9 l h^{-1} m^{-2}. Metabolism included the formation of Adriamycin (ADM), Adriamycinol (ADM-OH), 13-dihydro-4′-*O*-tetrahydropyranyladriamycin (THP-OH), 7-deoxyadriamycinone (7H-ADn), and 7-deoxy-13-dihydroadriamycinone (7H-ADn-OH), with maximal plasma concentrations ranging from 2.8 to 5.5 ng/ml. The mean total amount of cytotoxic anthracyclines excreted into urine, mainly as the parent drug, was 5% of the delivered dose. ADM and ADM-OH, but not the parent drug, were observed in urine at up to 4 weeks after the last therapeutic cycle. There was a significant correlation between the leukocyte nadir under therapy and the AUC of ADM-OH ($r = 0.800$, $P < 0.05$). Since no shift in the plasma kinetics was observed from the first to the sixth cycle, the favorable ratio of the AUCs of THP-ADM and ADM after fractionation of THP-ADM suggests lower toxic side effects attributable to ADM. This hypothesis was confirmed in a clinical study, where no severe cardiotoxicity and only mild alopecia were observed in 19 patients. Thus, pharmacokinetics studies might be helpful in both individualization of therapy with THP-ADM and optimization of the administration schedule.

Key words THP-ADM · Metabolism · Breast cancer

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Introduction

The semisynthetic derivative 4′-*O*-tetrahydropyranyladriamycin (THP-ADM) is an analogue of Adriamycin (ADM) with a tetrahydropyranyl group added in the C-4′ position. This compound, first synthesized by Umezawa and co-workers [23], exhibited antineoplastic activity in vitro and against several murine tumors, exceeding the potency of the parent compound ADM. In the clonogenic assay or the mouse model in preclinical studies, cytostatic activity was observed against malignancies of the breast, head and neck, ovary, cervix, and lung and against colorectal cancer, hepatoma, lymphoma, and leukemia [8, 22]. In clinical trials, this antineoplastic in vitro activity against several malignant tumors has been confirmed [12, 15, 20].

In the literature, high uptake rates of THP-ADM in leukemic cells [21], accumulation in leukocytes but not in erythrocytes [9], and nearly equal uptake into ADM-resistant cells and into the ADM-sensitive parent cell line have been reported [24]. Fast uptake of THP-ADM into tissue was observed in the spleen, lung, and kidney in mice. Lower levels of THP-ADM were detected in the liver, heart, and thymus. When ADM was compared with THP-ADM after administration to mice, the tissue concentrations of ADM exceeded those of THP-ADM in the kidney, liver, and heart but were lower in the spleen and thymus. Higher enterohepatic circulation was confirmed for ADM as compared with THP-ADM by analysis of bile fluid

[6, 7]. The uptake of THP-ADM into heart tissue was significantly lower than that of ADM. This phenomenon is believed to contribute mainly to the lower cardiotoxicity reported from clinical trials with THP-ADM. In vivo, higher uptake of THP-ADM into malignant versus normal tissue of the stomach was observed [9].

THP-ADM has shown activity in the treatment of metastasized breast cancer when used every 3 weeks. A 70-mg/m^2 dose of THP-ADM, although less toxic than that of ADM, was cardiotoxic and myelosuppressive. Fractionation of drug administration is one of the possible approaches to reduce concentration-dependent side effects of drugs. Thus, we evaluated the pharmacokinetics of THP-ADM in a dose-escalation study starting with 20 mg/m^2 THP-ADM under weekly administration in patients with advanced breast cancer [5]. The metabolism and renal elimination of the parent drug and its metabolites were evaluated under this regimen. In selected patients, these investigations were repeated in cycles 1 and 6 so as to retrace possible shifts in the pharmacokinetic behavior of the compounds.

Patients and methods

Patients

Eight patients with histologically confirmed metastatic breast cancer were entered in a phase I–II study. Only patients with normal liver (bilirubin, <1.5 mg/dl) and renal function (creatinine, <1.5 mg/dl) and a life expectancy of at least 3 months were monitored in the pharmacokinetics study.

The patients' age was in the range of 39–68 years (median, 60 years). The localization of metastases were visceral ($n = 3$), soft tissue ($n = 1$) and multiple ($n = 4$). Prior treatments included surgical therapy ($n = 8$), radiotherapy ($n = 2$), hormonal therapy ($n = 5$), and chemotherapy ($n = 3$). The performance status of all patients was 0 according to Eastern Cooperative Oncology Group (ECOG) criteria.

Administration of THP-ADM

THP-ADM doses ranging from 20 to 27 mg/m^2 were given weekly if leukocyte counts were $>3{,}500$/mm^3 and platelet counts were $>100{,}000$/mm^3. Toxicity was evaluated routinely and documented according to WHO criteria.

The evaluated weekly bolus i.v. doses of THP-ADM (given within 1 min) were 20 ($n = 4$), 24 ($n = 3$), 25 ($n = 2$), and 27 mg/m^2 ($n = 3$). In four patients the pharmacokinetics of the first cycle and in four patients the pharmacokinetics of the first and the sixth cycles (at 20, 24, 25, and 27 mg/m^2, respectively) were evaluated. All patients received the same dose throughout all therapeutic courses. From the second to the fifth cycle a possible shift in pharmacokinetics was monitored by the analysis of a blood sample prior to therapy and at 6 h after administration of the drug (the beginning of the terminal elimination phase) in these patients.

Reagents

Stock solutions from the following reference substances were prepared in acetonitrile:water at a volume ratio of 50:50 (concentration of the stock solutions, between 0.1 and 1 mg/ml): 4′-*O*-tetrahydropyranyladriamycin.HCl (0.11 mg/ml; substance obtained from Sanraku, Japan, with 98.14% purity), Adriamycin.HCl (substance purchased from Sigma, Germany), and daunomycin.HCl (DNM, substance purchased from Sigma, Germany). 13-Dihydroadriamycin, 13-dihydro-4′-*O*-tetrahydropyranyladriamycin.HCl, 7-deoxyadriamycinone, and 7-deoxy-13-dihydroadriamycinone were obtained from Behring (Marburg/Lahn, Germany).

Except for water (Rathburn, Scotland) and acetonitrile (Nanograde from Promochem, Germany) used for chromatographic purposes, all other reagents were purchased from Merck (Germany) and were of analytical grade.

Analysis of plasma and urine samples

Blood samples were drawn prior to therapy and at 5, 10, 20, and 30 min and at 1, 2.5, 4, 6, 9, 12, 24, 48, and 72 h after drug administration into polystyrene tubes containing ethylenediaminetetraacetic acid (EDTA). Plasma was separated from blood cells within 5 min and stored at -20°C until analysis. Urine was collected in polystyrene bottles at 12-h intervals until 72 h after administration of THP-ADM. Urine was stored in a dark, cool place until the end of the sampling period. After determination of the sample volume, an aliquot was stored at -20°C.

DNM was added to 1 ml plasma or urine as an internal standard. The unchanged drug and its metabolites were extracted on conditioned C_{18} columns (Bond-Elut, Analytchem International, Harbor City, Calif. USA), washed with water (plasma samples) or acetonitrile:ammonium chloride buffer, (pH9; 20:80 v/v; urine samples only), "dried" with 1 ml *n*-hexane, and eluted with chloroform:methanol (2:1, v/v). The organic solvent was removed under a stream of nitrogen, and 0.1 ml of acetonitrile:water (30:70, v/v) was added to redissolve the sample for injection onto the high-performance liquid chromatography (HPLC) column.

THP-ADM, THP-ADM-OH, ADM, ADM-OH, the aglycones 7H-ADn and 7H-ADn-OH, and the internal standard DNM were separated by reversed-phase chromatography (Bondapak phenyl, 10 μm; Waters, Milford, Mass. USA) with a mobile phase consisting of acetonitrile:35 m*M* ammonium formate buffer (pH3, 35:65, v/v) containing 1 m*M* Na-EDTA. The fluorometric detector was set to a 478-nm excitation wavelength and a 552-nm emission wavelength [10].

Quality control of the HPLC method

The mean recovery from plasma was determined by assaying five spiked samples at a concentration of 250 ng/ml as described above – THP-ADM (83.4 ± 3.9%), THP-ADM-OH (85.9 ± 2.2%), ADM (89.0 ± 2.2%), ADM-OH (88.8 ± 2.5%), and DNM (86.0 ± 2.5%) – using external standard calibration of an aqueous solution. The mean recovery from urine was determined assaying five spiked samples at a concentration level of 250 ng/ml as described above – THP-ADM (97.5 ± 4.9%), THP-ADM-OH (98.5 ± 2.3%), ADM (96.4 ± 3.8%), ADM-OH (78.3 ± 8.4%), and DNM (98.0 ± 1.2%) – using external standard calibration of an aqueous solution.

To validate the interassay variance of the analytical method, plasma was spiked with THP-ADM and ADM and stored in aliquots at -20°C. Samples were thawed and analyzed on 6 consecutive days according to the procedure described below. The mean coefficient of variation determined for between 2 and 400 ng substance/ml plasma was 6.6% for ADM and 9.1% for THP-ADM. The mean deviation from the target value was 2.6% for ADM and 6.1% for THP-ADM.

Analysis of data

The data obtained for THP-ADM were described by an open three-compartment model by nonlinear estimation using the

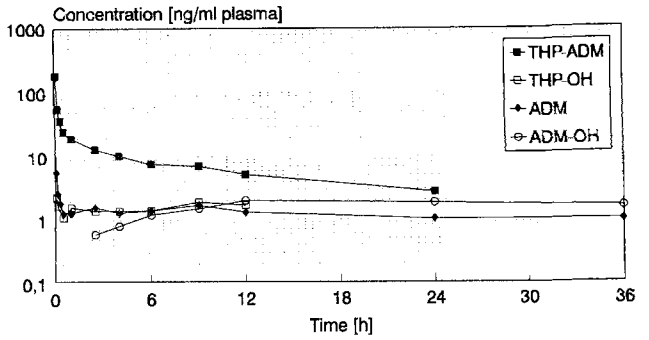

Fig. 1 A representative concentration-time profile obtained for THP-ADM and the metabolites ADM, ADM-OH, and THP-OH after i.v. administration of 25 mg/m^2 THP-ADM (first course of one patient)

equation $c = A \times e^{-\alpha t} + B \times e^{-\beta t} + C \times e^{-\gamma t}$, where c is the plasma concentration of THP-ADM at time t after i.v. administration; A, B, and C are the ordinate intercepts of the exponential terms at time zero; and α, β and γ are the corresponding first-order rate constants. A weight of $1/C(t)^2$ was used to account for the relative measurement error, which showed a constant variance at all concentrations. Graphs of residuals versus calculated concentrations of weighted and unweighted fits supported this approach. The data were computed by the software program HOEREP-PC, which uses a nonlinear Gauss-Newton procedure (modified by Stoer) and Gram-Schmidt orthogonalization [2]. The AUCs for ADM, ADM-OH, and THP-ADM-OH were calculated as areas under the data curve using the linear trapezoidal rule without extrapolation beyond 72 h after bolus injection of THP-ADM.

Clearance values were calculated for the period ranging from 0 to 72 h after i.v. administration by the following equations:

1. Total clearance – $Cl_{tot} = D/AUC$,

where AUC is the area under the concentration-time curve and D is the dose of THP-ADM given

2. Renal clearance – $Cl_{ren} = Ae/AUC$,

where Ae is the cumulative amount excreted into the urine within 72 h and AUC is the area under the concentration-time curve from 0 to 72 h

The relationship between the leukocyte nadir and the AUC was calculated by nonparametric correlation analysis using Kendall's Tau.

Results

The concentration-time curves generated for THP-ADM in plasma were best described by an open three-compartment model (parameters are summarized in Table 1). The plasma half-life of THP-ADM in the first disposition phase was short ($t_{1/2}$, 3.15 min); this was followed by a second disposition phase with a mean half-life of 0.86 h. The onset of an elimination phase was observed at 6 h after the bolus injection at a plasma concentration of approximately 10 ng THP-ADM/ml and was generally terminated within 48 h (detection limit, 2 ng THP-ADM/ml plasma) (Fig. 1). Within this period, the renal elimination of THP-ADM was also terminated. Binding strongly to tissue, THP-ADM shows high volumes of distribution (Vd, 1,273 l/m^2) and mean residence times of 11.6–18.3 h in the body. The mean AUC for THP-ADM was 12.2

Table 1 Pharmacokinetic parameters of THP-ADM after i.v. bolus injection of 20–27 mg/m^2

Parameter	Mean ± SD[a]
AUC (THP-ADM)	284.1 ± 65.8 ng h ml^{-1}
AUC (THP-ADM)	12.2 ± 3.41 ng h ml^{-1} mg^{-1} m^{-2}
$t_{1/2\alpha}$	3.15 ± 1.47 min
$t_{1/2\beta}$	0.86 ± 0.33 h
$t_{1/2\gamma}$	13.9 ± 1.62 h
Cl tot	151.5 ± 24 l/h
Cl tot	86.9 ± 22.1 l h^{-1}m^{-2}

[a] $n = 12$

ng h ml^{-1}m^{-1} m^{-2} (normalized by the delivered dose of THP-ADM), resulting in a mean plasma clearance of 86.9 l h^{-1} m^2 for the parent drug.

The drug formulation of THP-ADM contains impurities of ADM, which was therefore observed in plasma immediately after i.v. injection of the drug (maximal concentration of ADM, 13.4 ng/ml plasma at 5 min after administration). Analysis of drug vials for clinical use revealed a content of 1.04% ADM in the drug formulation. ADM is also derived from metabolism, leading to plasma concentrations of up to 5.5 ng/ml at between 4 and 6 h after administration of THP-ADM. ADM persists over more than 72 h within the systemic circulation at concentrations higher than 1 ng/ml. The ratio of the AUCs for THP-ADM and ADM was calculated to be 2.6:1. Due to their protracted elimination, ADM and ADM-OH were always observed at 72 h after administration of THP-ADM, but we never detected the parent drug itself at this time (mean AUC for ADM, 118.8 ng h ml^{-1}; mean AUC for ADM-OH, 99.2 ng h ml^{-1}). Both compounds were observed in urine for up to 4 weeks after the last therapeutic cycle (ADM range, 0.8–7.1 ng/ml; ADM-OH range, 1.2–2.8 ng/ml).

Metabolism to THP-OH, 7H-ADn, and 7H-ADn-OH was accompanied by maximal plasma concentrations of 2.8, 5.8, and 3.3 ng/ml, respectively, on administration of THP-ADM doses ranging between 20 and 27 mg/m^2. Formation of the aglycones 7H-ADn and 7H-ADn-OH was observed in most patients and was subject to a high interpatient variability (7H-ADn range, 0–5.8 ng/ml; 7-H-ADn-OH range, 0–3.3 ng/ml). In some patients the maximal plasma levels of 7H-ADn or 7H-ADn-OH exceeded those of ADM and ADM-OH. The renal elimination of THP-ADM, ADM, and ADM-OH ranged from 1.6 to 2.7 l h^{-1} m^{-2}, whereas THP-ADM-OH displayed a mean renal clearance of 6.7 l h^{-1} m^2. The mean total amount of cytotoxic anthracyclines excreted into urine, mainly as the parent drug, was 5% of the delivered dose (Table 2).

No shift in plasma kinetics from the first to the sixth cycle was observed in four patients (the data of all patients are summarized in Table 3). The variation of the plasma concentrations of THP-ADM and ADM

Table 2 Urinary excretion and renal clearance of THP-ADM and its metabolites

Compound	Amount excreted[a] (% of the dose)	Renal clearance[a] ($l\,h^{-1}m^{-2}$)
THP-ADM	3.1 ± 0.96	2.7 ± 1.0
ADM	0.83 ± 0.34	1.7 ± 0.87
ADM-OH	0.59 ± 0.38	1.6 ± 0.73
THP-ADM-OH	0.43 ± 0.19	6.7 ± 2.4
Total	4.95	

[a] Mean values ± SD ($n = 12$)

was monitored by drawing a blood sample at the beginning of the elimination phase at each cycle (6 h after the administration of THP-ADM). The mean coefficients of variation calculated for the plasma concentrations throughout cycles 1–6 were 18% for THP-ADM and 23% for ADM. No significant accumulation of the parent drug or the metabolites in the systemic circulation was observed. Prior to treatment at 1-week intervals, the plasma concentration of all monitored substances was below the detection limit of approximately 1 ng/ml.

We observed correlations between the first leukocyte nadir under therapy and the AUC for ADM-OH during the first cycle (coefficient of correlation, 0.800; $P < 0.05$) but not the AUC for THP-ADM (coefficient of correlation, 0.333; $P = 0.348$). The leukocyte depression was calculated as the percentage of decrease from pretherapeutic levels to the first leukocyte nadir, which occurred at between 1 and 4 weeks after the first therapeutic course. The complete blood cell count was monitored weekly. In two patients the leukocyte count dropped below 2,000/mm^3; all other patients suffered only minor, if any, hematotoxicity.

Discussion

For anthracyclines, metabolic clearance is more important than renal excretion as a route of elimination. Notwithstanding this observation, the total plasma clearance of THP-ADM declined to a higher extent in patients with renal dysfunction as compared with patients showing hepatic dysfunction [19]. This is surprising, since THP-ADM is metabolized to a variety of related compounds, including cleavage of the sugar side chain, yielding the corresponding aglycones. The extremely fast distribution in the body ($t_{1/2\alpha}$, 3.15 min) and the residence time of between 11.6 and 18.3 h observed for the parent drug favor metabolism. From a clinical point of view, this is particularly important for the drug's conversion to ADM, well known as a strongly cardiotoxic agent.

The ratio of the AUCs for THP-ADM and ADM was determined to be 2.6, values for the parent drug clearly exceeding levels of the metabolite ADM over at least 24 h in the systemic circulation. A different schedule with the administration of THP-ADM every 3 weeks at doses of up to 70 mg/m^2 was investigated by Miller and Schmidt [11], who reported the inverse relationship between these two compounds. Their data suggest a dose-dependent metabolism, which, in the case of the weekly low-dose regimen, leads to reduced formation of ADM. Thus, one would expect fewer toxic side effects attributable to ADM. This hypothesis was confirmed in our clinical study, where no severe cardiotoxicity was observed in 19 patients [5]. Cardiac dysfunction, assessed by the evaluation of systolic intervals and left ventricular ejection fraction, was mild to moderate. In addition, alopecia was observed in only 6 of 19 patients.

It should be noted that the acute cardiotoxicity of THP-ADM is believed to be higher than that of ADM in the rat [4]. The long persistence of the metabolite ADM in the body is perhaps even more important with regard to cardiotoxicity. This agent is a potent inhibitor of the calcium pump of the sarcoplasmic reticulum, compromising both systolic and diastolic cardiac function [13]. For several membrane-associated ion pumps, ADM-OH is at least 80 times more effective than ADM as an inhibitor [1].

When THP-ADM was given on 3 consecutive days, ADM-OH accumulated in the body [17]. Dosing

Table 3 Comparison of the pharmacokinetic parameters of four patients from the first and the sixth cycle after i.v. administration of THP-ADM[a]

	1st cycle/6th cycle			
Dose (mg/m^2)	20	24	25	27
THP-ADM AUC (ng h ml^{-1})	210/260	275/264	269/238	377/333
Elimination half-life (h)	15.2/14.4	14.2/13.9	13.2/16.4	12.1/12.2
Total clearance (l/h)	143/116	160/167	186/210	130/147
Metabolites:				
ADM AUC (ng h ml^{-1})	76/103	139/162	86/119	115/127
ADM-OH AUC (ng h ml^{-1})	– –	89/86	111/143	93/130
THP-ADM-OH AUC (ng h ml^{-1})	19/8	– –	19/15	39/34

[a] No statistically significant difference between the 1st and the 6th cycle was observed by the Wilcoxon test.

intervals of 1 week, as used in this study, were sufficient to prevent accumulation of ADM and ADM-OH in the systemic circulation. The accumulation of both compounds in tissue, however, became evident from the protracted excretion into urine observed even several weeks after the last therapy with THP-ADM. The AUC for ADM-OH was approximately 5 times higher after administration of ADM than after treatment with the same dose of THP-ADM [3]. Beyond that, the AUC ratio of THP-ADM and ADM overestimates the fraction of ADM generated by metabolism, since the longer elimination half-life of ADM results in an apparently high metabolism rate. Thus, THP-ADM exhibits attributes of an antineoplastic drug but also behaves as a prodrug of ADM.

There was a good correlation between the leukocyte nadir under therapy and the AUC for ADM-OH (coefficient of correlation, 0.800; $P < 0.05$), indicating a relationship between metabolism and myelotoxicity. A similar observation was made by Robert and co-workers [18], who reported a correlation between the granulocyte survival and the AUC for ADM and ADM-OH as well as between the platelet survival and the AUC for THP-ADM and THP-OH. The coefficient of correlation, however, ranged from 0.54 to 0.65, suggesting trends rather than strong relationships. The results of the study of Robert and co-workers, however, were biased by the concomitant administration of 750 mg/m^2 5-fluorouracil daily for 5 days, which per se is not a regimen neutral to bone marrow cells. A weak correlation between the leukocyte nadir and the plasma concentration of ADM-OH has also been reported in a population study after administration of ADM ($r^2 = 0.26$) [16].

In plasma samples, ADM was detected immediately after the administration of THP-ADM. Estimations of the impurity of ADM in the clinical formulation of THP-ADM were done by back-extrapolation of the first distribution phase from the concentration-time curves generated in other pharmacokinetics studies and amounted to 0.87%. Other authors have concluded it to be unlikely to observe metabolism as fast as 5 min after administration of the drug and reported a contamination content of up to 5% ADM [14], raising the question as to whether clinical trials should be performed under these conditions. The magnitude of contamination is sometimes overestimated by the calculation of drug impurities based on plasma levels without back-extrapolation to time zero because of the different velocity of distribution of compounds such as ADM and THP-ADM. We evaluated the content of ADM to be 1.04% by analyzing drug vials of THP-ADM. Metabolism of ADM was observed at a maximum of between 4 and 6 h after administration of THP-ADM, whereas ADM-OH circulated at mean concentrations ranging between 1 and 2 ng/ml plasma until 72 h after THP-ADM administration.

In plasma, no significant shift of the pharmacokinetic parameters from the first to the sixth cycle was observed. This was confirmed in four patients at four dose levels (range, 20–27 mg/m^2 THP-ADM). Although weekly administration implies a continuous exposure of tissue to THP-ADM, no enhanced induction of enzymatic degradation mechanisms was detected. Resistance phenomena, which were observed clinically, have therefore been attributed to primary or secondary tumor resistance and not to changes in the pharmacokinetics.

We conclude that THP-ADM is an agent with characteristics that differ significantly from those of other anthracyclines. Its complex metabolism shows great inter-patient variability and leads to metabolites that differ in their renal excretion by a factor of 3. Redistribution from tissue to the systemic circulation is observed in the form of the metabolites ADM and ADM-OH at even 4 weeks after administration of THP-ADM, indicating high persistence of the latter in tissue. The formation of ADM and ADM-OH is accompanied by toxic side effects, which are known from therapy with ADM, but these are remarkably attenuated by dose-dependent metabolism in comparison with that observed for equivalent regimens containing THP-ADM or ADM. In addition, myelotoxicity can be correlated with the generation of ADM-OH from the parent drug. Thus, pharmacokinetics studies might be helpful in both individualization of therapy with THP-ADM and optimization of the administration schedule.

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