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ORIGINAL ARTICLE

An open-label study to evaluate dose and cycle dependence of the pharmacokinetics of pegylated liposomal doxorubicin

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Abstract
Purpose There are no definitive data in humans on the dose dependence and/or cycle dependence of the pharmacokinetics (PK) of pegylated liposomal doxorubicin (PLD). This study examined the PK of PLD across a twofold dose variation and along 3 cycles.
Methods Fifteen patients received PLD in successive doses of 60, 30, and 45 mg/m^2 (Arm A) and 30, 60, and 45 mg/m^2 (Arm B), every 4 weeks. Twelve patients, six on each arm, completed all three cycles and were fully evaluable. Plasma levels of doxorubicin were analyzed by HPLC and fluorimetry. PK analysis was done by non-compartmental method. Repeated measures ANOVA and paired tests were used for statistical analysis.
Results There was no significant difference in the PK parameters examined when the dose was increased from 30 to 60 mg/m^2. However, when we analyzed the effect of cycle number on the PK, we found a gradual and significant inhibition of clearance ($P < 0.0001$) from the 1st through the 3rd cycle of PLD, with a geometric mean increase of 43% in dose-normalized AUC ($P = 0.0003$). Dose-normalized C_{max} and T½ mean values increased by 17 and 18%, respectively between the 1st and 3rd cycles, but only the increase in T½ was statistically significant ($P = 0.0017$).
Conclusions While the PK of PLD is not dose-dependent within the dose range of 30–60 mg/m^2, there is evidence of a cycle-dependent effect that results in inhibition of clearance when patients receive successive cycles of PLD. These results suggest the need for dose adjustments of PLD upon retreatment to minimize the risk of toxicity.

Keywords Liposome · Doxorubicin · Pegylation · Pharmacokinetics · Open-label clinical study

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Introduction

The pharmacokinetics (PK) of pegylated liposomal doxorubicin[1] (PLD) is characterized by long-circulation time and minimal drug leakage (<5%) from circulating liposomes [14]. The clearance of the liposomal carrier is the primary determinant of the pharmacokinetics of PLD, given the negligible rate of drug leakage [14]. In murine models, treatment with PLD followed one day later by injection of drug-free radio-labeled liposomes or repeated treatment with PLD every 4 days for a total of four injections have been shown to cause a delay in liposome and liposomal drug clearance, indicating damage or saturation of the reticulo-endothelial system (RES) [15]. This temporary inhibition of RES-mediated liposome clearance is caused specifically by PLD, and is not observed with free doxorubicin [15], or with drug-free pegylated liposomes for which clearance is dose-independent over a wide dose range [2]. The results of various PK studies with PLD point to half-lives in the range of 50–55 h for dose levels of 10–20 mg/m^2 in AIDS-related Kaposi's sarcoma patients [4], and

[1] Marketed under the trade names of DOXIL® and CAELYX ®.

around 60–80 h for dose levels of 35–70 mg/m^2 in solid tumor patients [14]. In pediatric patients receiving 40–70 mg/m^2, the half-life is significantly shorter averaging 36 h [25]. One study [4] examined the PK of PLD when the dose is escalated in the same patient population from 10 to 20 mg/m^2, and found not evidence of dose-dependent PK. In a dog study designed to investigate a correlation between dose-schedule of PLD and skin toxicity, the clearance of PLD was reported to decrease with repeated PLD treatment [3]. Yet, no clinical study has addressed the PK effects of a change in dose and repeated treatment with PLD in the dose range of solid tumors (30–60 mg/m^2) with intra-individual comparisons.

PLD has major advantages over doxorubicin and other anthracyclines with regard to important toxicity parameters such as cardiomyopathy [1, 5, 17, 26, 27], myelosuppression, and alopecia (reviewed in 1). However, treatment with PLD is associated with a high incidence of stomatitis and palmar–plantar erythema (PPE, also known as hand-foot syndrome) [1, 22, 29]. Indeed, skin toxicity, in the form of PPE, and stomatitis are the dose-limiting toxicities of PLD [29]. Although not life-threatening, PPE is problematic to control and/or foresee since it usually occurs after cumulative damage to the skin from two or more courses of PLD. Stomatitis is generally correlated with peak dose level [10, 24]. Skin toxicity correlates with dose interval, dose intensity, and T½ (half-life) of PLD [3, 24, 29]. Skin toxicity of PLD tends to manifest after two or more cycles of treatment [22, 29], hinting at a complex PK–PD relationship. In murine studies, repeated treatment with PLD at short intervals has been shown to cause skin toxicity and a correlation of the latter with incomplete clearance of liposomal doxorubicin from the skin has been demonstrated by Charrois and Allen [7].

All the above led us to design a study aimed at examining the dose and cycle dependency of PLD PK. The objectives of this study were to examine the effect of a doubling of dose on the PK of PLD within the same patient population, as well as the effect of three repeated cycles of treatment on the PK of PLD.

Patients and methods

Study design

This study was designed to obtain information on the effect of a twofold change in the dose level as well as on the effect of repeated cyles (1–3) of therapy. As seen in Fig. 1, patients with various solid tumors were randomized to two arms of treatment (A and B) in an open-label study design. To balance the effect of an increasing versus decreasing dose along time on study, patients were randomized into two groups (A, B) with opposing dose sequence. Group A received PLD at 60 mg/m^2 in the 1st cycle, 30 mg/m^2 in the 2nd cycle, and 45 mg/m^2 in the 3rd cycle. Group B received PLD at 30 mg/m^2 in the 1st cycle, 60 mg/m^2 in the 2nd cycle, and 45 mg/m^2 in the 3rd cycle. All cycles were given at 4-week intervals. The aim was to generate cohorts of 12 patients or more from at least 6 patients per arm completing all three cycles. Two patients did not complete the plasma sampling required for PK analysis of the two first cycles, leaving 13 patients evaluable for dose level comparison. One more patient dropped out of the study just before starting the 3rd cycle, thus leaving 12 fully evaluable patients for the three-cycle comparison, out of a total of 15 patients recruited.

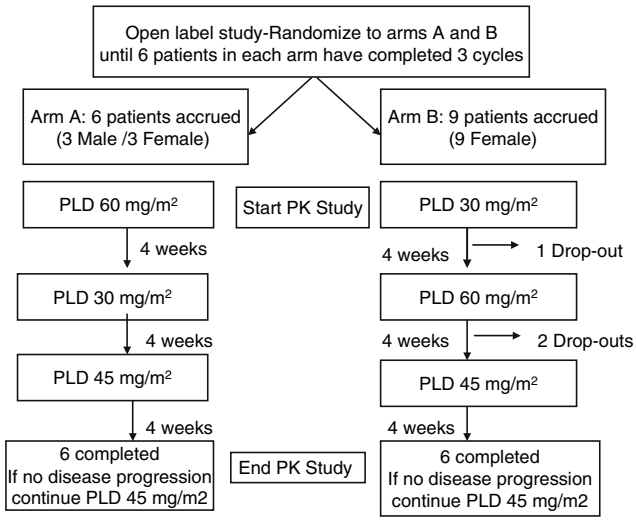

Fig. 1 Diagram of study design

The study protocol was approved by the Institutional Review Board of the Shaare Zedek Medical Center and required signed witnessed consent. For randomization, a total of six A and six B ballots was used as pool. In case of a patient drop-out the corresponding ballot was returned to the pool.

Plasma sampling

PLD was administered i.v. after dilution in 500 ml dextrose 5%. The start drip rate was slow (~0.1–0.2 mg/min) and gradually raised to ~1 mg/min, resulting in an infusion time in the range of 1–2 h in all patients. Blood (3–5 ml) was withdrawn into vacuum-sealed K-EDTA containing tubes at the following time points: Pre-infusion, and 1, 24 h, between 72–96 h, 7 ± 1, 14 ± 1, 21 ± 1, and 28 ± 1 days after end of infusion. Given the slow clearance of PLD, the variation in infusion time (1–2 h) is insignificant and did not mandate any change in the sampling

schedule. Plasma was separated by centrifugation and stored at −20°C until testing.

Measurement of PLD concentration

Plasma levels of doxorubicin were analyzed by HPLC-fluorimetry following the method of Chin et al. [8] with minor modifications. For extraction of doxorubicin, 1 μg daunorubicin was added as internal standard to 200 μl of plasma, followed by 20 μl of 3% (v/v) Triton X-100, and 20 μl of 65% (w/v) 5-sulfosalycilic acid. After each addition, the sample was vortexed for 10 s. Next, the samples were centrifuged for 5 min at 20,000*g*. The supernatants were harvested and 35 μl of 3 M sodium acetate was added to each sample, followed by filtration through 0.22 μm-pore membranes. The filtered samples were injected (100 μl/injection) with an automatic injector into an isocratic HPLC system with a mobile phase consisting of 35% acetonitrile/65% DDW containing 10 mg/l desipramine at pH 2.5, using an Econosphere C8—5 μm column (length 150 mm, internal diameter 4.6 mm), and a flow rate of 2 ml/min, for a total run time of 10 min per sample. Doxorubicin (retention time 2.60 min) and daunorubicin (retention time 3.60 min) were detected with a fluorescence detector at ex:470/em:590 nm wavelength. The concentration of doxorubicin was calculated based on the relative peak areas of doxorubicin and the daunorubicin internal standard. This method was able to detect doxorubicin within a range of 10 ng–5 μg. The drug measured here represents the total amount of drug in plasma including the liposomal fraction, protein-bound fraction, and free fraction. However, in patients receiving PLD, it has been shown that >95% of the doxorubicin measured in plasma is liposome-bound with any released doxorubicin being cleared at a rate several hundred-fold greater than liposomal drug [4, 12, 30]. Therefore, the results presented here can be considered representative of the PK of PLD itself.

PK and statistical analysis

The PK of PLD was analyzed by non-compartmental method using PK Solutions™ software (Summit Research Services, Montrose, CO). The following parameters were obtained: extrapolated peak plasma concentration (C_{max} = *Y*-intercept), terminal half-life (T½ = ln2/slope), area under the curve from zero to infinity (AUC), clearance (CL = dose/AUC), and volume of distribution at steady state (V_{ss}=dose × AUMC/AUC^2, *where AUMC stands for area under the moment curve*). AUC_{0-t} values, where *t* is last sampling time point, were in all cases very close (≥95%) to $AUC_{0-\infty}$ values. For comparison purposes, C_{max} and AUC were dose-normalized (C_{max}/mg dose, AUC/mg dose).

To avoid the confounding factor of inter-patient variability, our statistical approach was based on intra-patient comparison of paired PK data using repeated measures one-way ANOVA (analysis of variance) with the post-test for linear trend when three data groups were analyzed, and the paired Wilcoxon (non-parametric) and *t* tests when two data groups were analyzed statistically (Prism 4, Graphpad Software, San Diego, CA, USA). The data pairing was always verified and found to be significantly effective in all cases.

The dose levels chosen were based on prior clinical experience with PLD in solid tumors to ensure that most patients could complete the study without dose reductions or delays. Our choice of the patient cohort number was based on the assumption that for a clinically relevant difference of a key PK parameter such as dose-normalized AUC or clearance, a change in the mean of ≥20% is required. A 12-patient paired sample in each dose level cohort gives us a power of 95% (two-tailed $\alpha = 0.05$) to detect a 21% difference between the means assuming a coefficient of variation of 35% and a correlation among pairs (*r*) of 0.85 (Statmate 2, Graphpad Software).

Results

Patient characteristics, toxicity, and treatment outcome

Fifteen patients suffering from various malignant solid tumors were accrued to this study (Table 1). Females were over-represented, but this goes along with the clinical use of PLD which is mainly in ovarian and breast cancers. Patient accrual began on October 2004 and was completed within 12 months. Three patients did not complete the three study cycles (Fig. 1), one after 1 cycle because of frank disease progression and two after 2 cycles because of disease progression and toxicity (see below for details).

Treatment was generally well tolerated except for three heavily pretreated patients with advanced disease in whom all the severe toxicities seen in this study were clustered. One patient with recurrent carcinoma of esophagus after chemo-radiotherapy developed mucositis (esophagitis) grade 3 after a first course of PLD at 60 mg/m^2 and was treated with intravenous fluids. A second patient with heavily pretreated metastatic breast cancer developed neutropenic fever and stomatitis grade 3 requiring hospitalization after 2 courses of PLD (30 mg/m^2, followed by 60 mg/m^2). Although she recovered from toxicity within 7–10 days, she was not further treated with PLD given the appearance of obstructive jaundice and evidence of progressive disease. A third patient with pretreated metastatic gastric cancer and severe ascites developed neutropenia grade 4 and stomatitis grade 4 after 2 courses of PLD (30 mg/m^2 followed by 60 mg/m^2). PLD was discontinued as she remained bedrid-

Table 1 Patient characteristics

Total number of patients	15
Sex: male/female	3 /12
Age: median (range)	61 (33–78)
Type of cancer	No. of patients
Soft tissue sarcoma	4
Breast carcinoma	3
Ovarian carcinoma	3
Stomach carcinoma	2
Peritoneal (1ary) carcinoma	1
Prostate carcinoma	1
Thymoma (epithelial)	1
Prior chemotherapy (yes/no)	14/1
For metastatic disease	11
As adjuvant only	3
Prior anthracyclines (yes/no)	6/9
Median ECOG P.S.	1 (0–2)
ECOG-0/1/2	*6/5/4*
Median no of cycles (range)	9 (1–22+)

den and required protracted hospitalization further complicated by evidence of progressive disease. The two latter patients also suffered from PPE grade 3. Other cases of PPE were of lesser severity and did not affect the course of treatment.

There was no evidence of cardiac toxicity, neither clinical nor radio-angiocardiographic (MUGA scan) with the maximal cumulative dose reaching in one of the patients 925 mg/m^2 by October 2006. Moderate to severe hair loss (grade 2) was observed in one patient only. All other patients had none or minimal hair loss.

With a minimal follow-up of 1 year by October 2006, the median time to disease progression is 8 months (range 1–24+). Median survival has not yet been reached (8 alive, 7 dead) and stands at 16+ months (range 1–24+). The median number of cycles given per patient is 9 (range 1–22+). Several durable (>1 y-long) stabilizations with or without objective anti-tumor responses were observed in patients with sarcoma (2), ovarian (1), breast (1), and prostate (1) carcinoma. Two patients with liver metastases of soft tissue sarcoma are still responding and on treatment.

PK Results

Table 2 presents a summary of the mean, coefficient of variation, geometric mean, and confidence interval of PK parameters according to dose and cycle cohorts. When the 30 and 60 mg/m^2 dose levels are compared, there was no significant change in any of the PK parameters analyzed (Table 2, Fig. 2). In contrast, there is a significant increase of dose-normalized AUC values and a correspondingly significant decrease of CL values when comparing the 1st cycle of treatment to the 2nd cycle and more so to the 3rd cycle (Table 2, Fig. 2c, d). A 43% increase in the dose-normalized AUC geometric mean is observed when the 1st and 3rd treatment cycles are compared, pointing to a major potential increase in patient exposure to drug by merely retreating the patient without increasing the dose. The differences in AUC and CL comparing repeated treatment cycles were highly significant when analyzed by the repeated measures one-way ANOVA and the post-test for linear trend (Table 2).[2] Terminal T½ was also significantly prolonged when repeated treatment cycles are compared (Table 2, Fig. 2b), while other parameters (C_{max}, V_{ss}) were affected to a much lesser extent with a non-significant increase (+17%) of dose-normalized C_{max} (Table 2, Fig. 2a), and decrease (−19%) of V_{ss}. (Table 2, Fig. 2e). Finally, the C_{max}/AUC ratio, a parameter sometimes used to check for dose proportionality [11], also points to a significant difference for repeated treatment cycles (Table 2).

To compare the plasma clearance curves for all patients examined according to dose level (30 or 60 mg/m^2) or cycle number (1st vs. 3rd cycle), we transformed the plasma concentration values (μg/l) to % injected dose per liter plasma, and performed regression analysis using the equation, $Concentration = A \times e^{-B \times time}$, where A (*Y*-intercept) is the C_{max} average and B (slope) is the elimination rate constant average of each dose/cycle cohort tested. As seen in Fig. 3, the resulting curves clearly underscore the fact that clearance is not affected within the dose range 30–60 mg/m^2 (Fig. 3a), while a substantial and significant retardation in clearance with a shallower slope (Wilcoxon signed rank test, $P = 0.0049$; Paired *t* test, $P = 0.0040$,) is observed with retreatment when the 1st and 3rd cycles of PLD are compared (Fig. 3b).

Discussion

While free drugs are mainly handled by hepatic and/o renal clearance, nanoparticles such as liposomes are mainly cleared by the RES. Polyethylene-glycol (PEG) coating of liposomes protects liposomes from opsonization and delays their clearance from circulation, preventing the rapid and massive RES uptake seen after injection of non-pegylated liposomes [31]. Prolonged stay in circulation enables liposomes to reach in greater amounts tissues with transient or inherent increase in vascular permeability such as specific skin areas and tumors [13, 16], but ultimately Kupffer cells,

[2] The differences in AUC/mg dose between the 1st and 3rd cycle were also significant even if we would cancel the pairing of the data and compare the means with the unpaired *t* test ($P = 0.0104$; $n = 12$) or simply with the nonparametric Mann–Whitney test ($P = 0.0142$).

Table 2 Summary of PK values by dose level and treatment cycle group

	Dose level group[a]		Treatment cycle group[b]		
PK Parameter	30 mg/m^2	60 mg/m^2	1st Cycle	2nd Cycle	3rd Cycle
C_{max}/mg dose (μg/l)					
Mean (%CV)	413 (23)	413 (28)	406 (26)	420 (31)	475 (29)
G-Mean[c] (95% CI)	391 (339–452)	390 (329–461)	397 (345–457)	407 (343–484)	465 (409–530)
T½ (h)					
Mean (%CV)	76 (22)	83 (30)	73 (25)*	86 (26)*	87 (26)*
G-Mean (95% CI)	73 (64–84)	78 (64–95)	71 (60–85)	83 (69–100)	84 (70–100)
AUC/mg dose (mg × h/L)					
Mean (%CV)	49 (33)	53 (39)	46 (29)*	56 (33)*	66 (32)*
G-Mean (95% CI)	45 (37–55)	48 (37–61)	44 (36–54)	54 (43–66)	63 (52–77)
CL (ml/h)					
Mean (%CV)	22 (35)	21 (43)	24 (33)*	20 (34)*	16 (33)*
G-Mean (95% CI)	22 (18–27)	21 (16–27)	23 (19–28)	19 (15–23)	16 (13–19)
V_{ss} (L)					
Mean (%CV)	2.5 (29)	2.5 (29)	2.5 (24)	2.3 (30)	2.0 (24)
G-Mean (95% CI)	2.4 (2.0–2.8)	2.4 (2.0–2.9)	2.4 (2.1–2.8)	2.2 (1.8–2.6)	2.0 (1.7–2.3)
C_{max}/AUC(%)					
Mean (%CV)	0.90 (24)	0.87 (38)	0.935 (31)*	0.79 (30)*	0.76 (26)*
G-Mean (95% CI)	0.873 (0.76–1.01)	0.820 (0.67–1.01)	0.898 (0.75–1.08)	0.760 (0.64–0.90)	0.737 (0.63–0.86)

[a] $N = 13$. None of the dose level group comparisons of PK data were statistically significant

[b] $N = 12$. Asterisk points to parameters with statistically significant differences. P values of treatment cycle groups by repeated measures one-way ANOVA and the post-test for linear trend: C_{max}, $P = 0.0769$ (n.s.); T½ $P = 0.0017$ (post-test $P = 0.0013$); AUC $P = 0.0003$ (post-test $P<0.0001$); CL $P<0.0001$ (post-test $P<0.0001$); V_{ss}, $P = 0.0700$ (n.s.); % C_{max}/AUC, $P = 0.0010$ (post-test $P = 0.0005$)

[c] *G-Mean* Geometric mean

spleen, and bone marrow macrophages are the major liposome destination [20]. Therefore, RES-mediated clearance plays a major role in determining the PK of formulations such as PLD, and factors affecting RES function will have an impact on liposomal drug clearance. Unfortunately, there are no clinical tests of RES function that could predict the clearance of particulate carriers. However, preclinical findings indicating temporary depression of RES activity after administration of PLD as measured by bacterial clearance [28] or by clearance of an additional dose of radiolabeled liposomes [15]. This prompted us to test effects of a change of dose and of repeated cycles of treatment on the PK of PLD.

The dose range tested 30–60 mg/m^2, is most relevant since it covers the spectrum of dose used in the treatment of patients with solid tumors [1]. By dividing the patients in two groups with reversed order of treatment (30 → 60 and 60 → 30 mg/m^2), we wished to neutralize any variability due to cycle number rather than to dose change. In addition, by adding a 3rd cycle of treatment at the same dose (45 mg/m^2) to all patients, we could obtain reliable information on the PK of PLD along three cycles of treatment with a balanced dose distribution and maximize the value of the study.

We were not able to detect any significant change in clearance rate and other dose-normalized PK parameters of PLD when 30 and 60 mg/m^2 doses were compared, in spite of the fact that the study was 95% powered to reveal a difference of 20% in the paired means, leading to the conclusion that the PK of PLD is dose-independent. This is consistent with results from two previous studies examining the PK of PLD in the range of 35–70 mg/m^2 [24], and 10–20 mg/m^2 [4], in which there was no significant change in clearance and a linear correlation of dose and AUC was observed [24]. This would suggest, in principle, a lack of RES saturation after PLD treatment. However, as a note of caution, when extreme dose levels (10–80 mg/m^2) are compared across different studies, a trend to longer half-lives (∼50 → 80 h) with higher doses is noted [14].

In contrast to the lack of a dose effect, our finding of an inhibition of clearance upon retreatment with PLD indicate that the PK is cycle-dependent and that prior exposure to PLD is likely to be followed by inhibition of RES-mediated liposome clearance. Barring non-RES factors, which are unlikely, this implies some degree of RES toxicity related to PLD which would be envisaged to occur following liposome uptake, liposome processing, intra-cellular release of doxorubicin, and damage to macrophages. Apparently, the

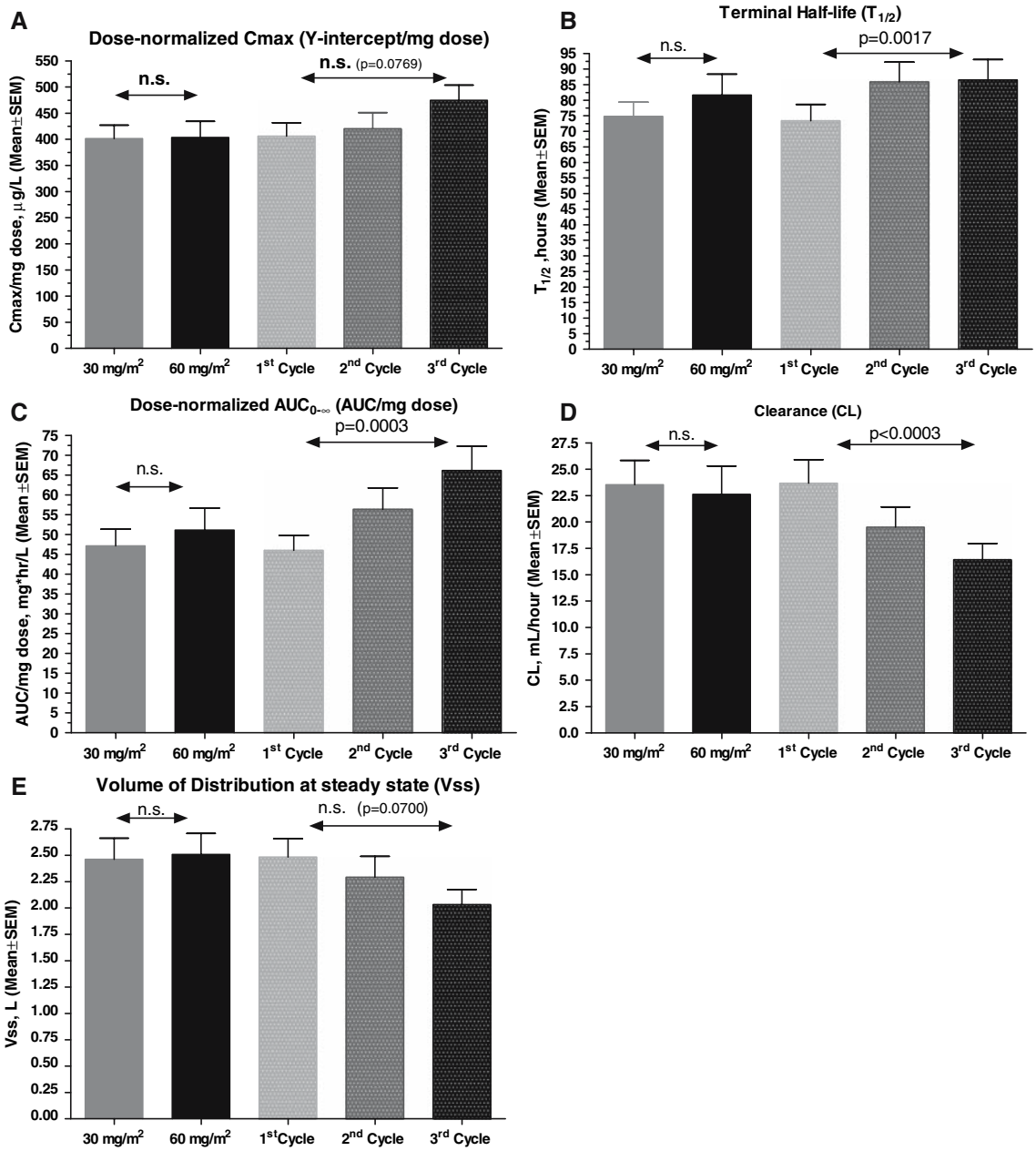

Fig. 2 *Bars* represent mean values and SEM (standard error of the mean) of PK parameters of PLD by dose and cycle. *P* values shown were calculated by repeated measures ANOVA. *n.s.* not significant. **a** C_{max}/mg dose; **b** T½; **c** AUC/mg dose; **d** clearance; **e** V_{ss}

kinetics of this process is not rapid and extensive enough to impact significantly on the clearance of the ongoing PLD infusion. This results in a lag phase between PLD infusion and toxicity manifested by inhibition of RES uptake of a later infusion of liposomes. Thus, dose escalation of PLD within the therapeutic dose range does not cause significant RES saturation upfront, but, nevertheless, results in a delayed damage to the RES which manifests as slower liposome clearance upon subsequent treatments. This effect may account for the delayed skin toxicity of PLD. Since AUC values are well correlated with dose of PLD [24], this would amount to circa 43% increase in patient exposure to drug when going from 1st to 3rd cycle without changing the dose, according to the results of our current study.

To avoid delayed toxicity, clinicians often refrain from using the MTD (60 mg/m^2 q4w) [29] and the recommended dose (50 mg/m^2 q4w) [18] of PLD. In fact, a dose of 40 mg/m^2 q4w has been proposed as a convenient starting dose for treating recurrent ovarian cancer while avoiding skin toxicity [21]. This is so despite evidence in Kaposi's sarcoma [4] and preclinical models [7, 9, 15] for a correlation of average C_{max} and/or peak dose level with therapeutic efficacy.

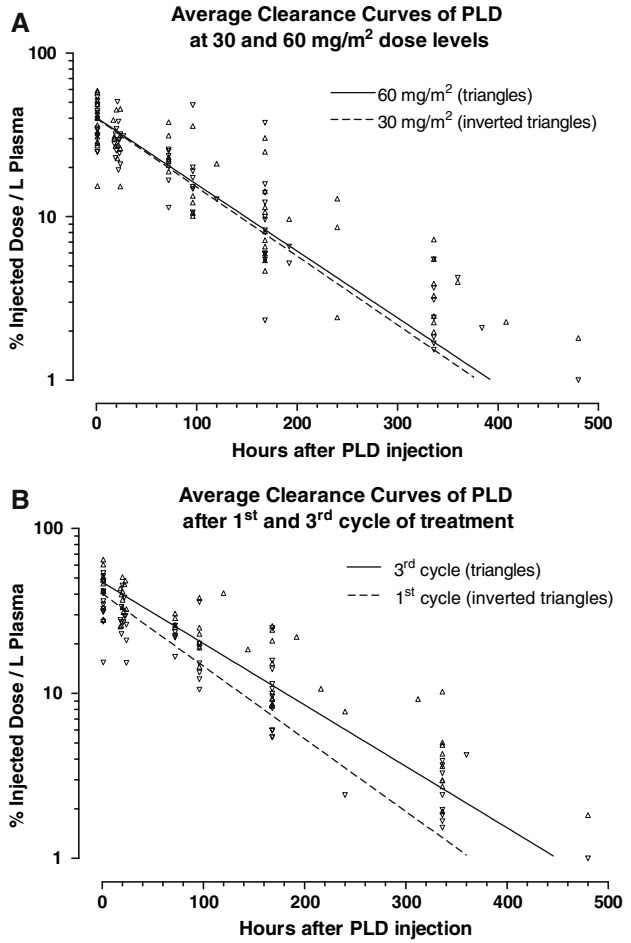

Fig. 3 PLD plasma clearance curve as fraction of injected dose comparing 30 to 60 mg/m^2 dose level (**a**), and 1st to 3rd cycle (**b**). The *curves* were simulated based on the average of the *Y*-intercepts (transformed to % of injected dose) and slopes of each patient cohort. *Discrete points* represent concentration values of individual patients (transformed to % of injected dose). Slope comparison (paired *t* test): **a** not significant ($n = 13$); **b** $P = 0.0040$ ($n = 12$)

An attempt in breast cancer patients to increase the dose level of PLD above 60 mg/m^2 using a long dose interval of 6 weeks failed due to dose-limiting stomatitis at 70 mg/m^2 [19]. To minimize the risk of delayed toxicity and to avoid the unnecessary reduction of the starting dose of PLD, the use of a high loading dose (e.g., 55–60 mg/m^2) in the initial cycle, followed by a lower maintenance dose (e.g., 40–45 mg/m^2) in further cycles at regular 4-week intervals may be considered. This approach will balance the actual dose exposure of patients to PLD when going from 1st to subsequent cycles. It may allow administering an optimal initial dose for anti-tumor response, while preventing toxicity along further cycles.

Obviously, these results are to be interpreted cautiously when PLD is used in combination chemotherapy since the clearance of PLD may be affected by other drugs. Such is the case of cisplatin which accelerates PLD clearance and decreases the rate of PPE [23] and taxanes which retard clearance of PLD and aggravate muco-cutaneous toxicities [6].

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