Introduction

Doxorubicin is approved, either alone or in combination with other drugs, for treatment of numerous cancers [1,2]. Unfortunately, the benefits of doxorubicin-based regimens are diminished by dose-dependent, drug-related toxicities, particularly cardiotoxicity [3,4]. Early research suggested that the cardiotoxicity of doxorubicin could be attributable to metabolism of doxorubicin to doxorubicinol and the subsequent action of doxorubicinol on cardiac tissue [5,6]. As a result, dose-intensive or long-term treatment with doxorubicin exceeding a cumulative dose of 550 mg/m² is not recommended.

Aldoxorubicin is a prodrug of doxorubicin that is derivatized at its C-13-keto position and is conjugated to a linker consisting of an acid-sensitive hydrazine moiety, a 6-carbon spacer, and a thiol-binding maleimide (6-maleimidocaprylic acid hydrazide) [7]. When aldoxorubicin enters the bloodstream, this linker molecule binds rapidly (within 5 min) and covalently to the cysteine-34 amino acid of endogenous albumin [7]. Due to the enhanced vascular permeability and poor lymphatic drainage that are characteristic of tumors (enhanced permeability and retention) [8], albumin-bound doxorubicin accumulates in the tumor and is then released in the acidic environment of the tumor or inside tumor cells via cleavage of the acid-sensitive hydrazine bond between the drug and the linker [7].
In a preliminary phase 1 study of aldoxorubicin in patients with advanced solid tumors, dose-limiting toxicities of mucositis and neutropenia were observed at 460 mg/m² (equivalent in drug load to doxorubicin 340 mg/m²) [9]. The dose recommended for further study was 350 mg/m² (equivalent to doxorubicin 260 mg/m²) [9]. No clinical signs of cardiotoxicity were observed on study or during the follow-up period, even among patients treated at the higher dose levels and/or for extended periods [9]. The biological basis for the lack of cardiotoxicity associated with aldoxorubicin treatment is unknown. The amount of doxorubicinol generated after aldoxorubicin administration has not previously been measured.

Recently, a new formulation of aldoxorubicin was developed that eliminates or reduces the amount of inactive ingredients, improves reconstitution, and allows for drug delivery in a short time period (30 min). The phase 1 pharmacokinetic study reported herein was conducted to fully evaluate the pharmacokinetic profile in both serum and urine of the new formulation of aldoxorubicin with additional assessment of free doxorubicin and its metabolite, doxorubicinol, in patients with advanced solid tumors.

Methods

Study design

This was a phase 1, open-label, single-center, pharmacokinetic study of aldoxorubicin 230 or 350 mg/m² (equivalent to doxorubicin 170 or 260 mg/m², respectively) in adult patients with advanced solid tumors that have either relapsed or are refractory to standard therapy (ClinicalTrials.gov registry no. NCT01706835). The primary objective was to evaluate the pharmacokinetic profile of aldoxorubicin in patients with advanced solid tumors. The secondary objective was to evaluate treatment-related toxicities of aldoxorubicin in these patients.

Ethics

The study protocol was approved by the study site’s institutional review board and was conducted in accordance with current US Food and Drug Administration regulations, International Conference on Harmonisation Guideline for Good Clinical Practice, the principles of the Declaration of Helsinki, and other applicable regulations and guidelines. All patients gave signed informed consent before enrollment in the study.

Patients

Eligible patients were 18 years of age or older, had a histologically or cytologically confirmed malignant solid tumor that had relapsed or was refractory to standard therapy, measurable or evaluable disease, Eastern Cooperative Oncology Group (ECOG) performance status of 0–2, and life expectancy of >12 weeks. Prior anthracycline therapy was allowed, with no limitation on cumulative dose. Those who had received prior radiation therapy had stable central nervous system metastasis with no progression of brain metastasis by computed tomography/magnetic resonance imaging scans within 4 weeks of screening. Women of childbearing age were not pregnant or lactating at screening, and used adequate birth control methods during the study.

Patients were excluded if they had palliative surgery, chemotherapy, immunotherapy, and/or radiation treatment within 4 weeks of screening; exposure to any investigational agent within 30 days of screening; laboratory values at screening that indicated impairment of liver function or bone marrow function; clinically evident congestive heart failure (New York Heart Association functional class II or higher); serious, clinically significant cardiac arrhythmia or recent history (within 6 months of screening) or active coronary artery disease with or without angina pectoris; serious myocardial dysfunction; history of human immunodeficiency viral infection or active, clinically significant serious infection requiring medical treatment; or major surgery within 4 weeks of study drug treatment.

Treatments, assessments, technical methods

Aldoxorubicin 230 or 350 mg/m² was administered as a 30-min intravenous infusion on day 1 of each 21-day cycle. The use of granulocyte colony stimulating factor (GCSF) was allowed at the discretion of the investigator and according to the American Society of Clinical Oncology guidelines [10]. Blood samples for pharmacokinetic analyses were taken immediately before the start of the aldoxorubicin infusion, and then at 5, 15, 30, and 60 min, and at 2, 4, 8, 12, 16, 24, 48, and 72 h after the start of the infusion in cycle 1. Blood samples for limited pharmacokinetic analyses were taken before aldoxorubicin administration, and then at 60 min and at 2, 4, and 8 h after the start of the infusion in cycle 3. Serum concentrations of albumin-bound doxorubicin, unbound (or free) doxorubicin, and doxorubicinol were determined by high-performance liquid chromatography tandem mass spectrometry for each sample. Urine samples were taken at 24, 48, and 72 h after the start of the infusion in cycle 1. The amount of doxorubicin and doxorubicinol excreted in urine and renal clearance was determined.

Adverse events were assessed at each scheduled clinic visit, at which time the incidence, severity, duration, causality, seriousness, and types of adverse events, as well as changes in physical examination, vital signs, and clinical laboratory results were documented. Cardiac function was assessed by
echocardiography or multigated acquisition scan on days 28 and 85, at study end or early termination, and every 2 months thereafter.

Sample size

Eighteen patients were enrolled into this study at a single center in the US.

Statistics

All patients who entered active treatment and received at least one dose of aldoxorubicin and had complete pharmacokinetic sampling were included in the pharmacokinetic analyses. Descriptive statistics (mean, minimum, median, maximum, standard deviations, and coefficient of variance) for the plasma concentrations, and plasma and urine pharmacokinetic parameters were calculated for both doses.

All patients who entered active treatment and received at least one dose of aldoxorubicin were included in the safety analyses. Safety data were summarized using descriptive statistics.

Results

Between October 2012 and September 2013, 18 patients were enrolled. All 18 patients were included in the safety analysis and in the pharmacokinetics analyses of cycle 1: 11 patients in the 230 mg/m² cohort and 7 in the 350 mg/m² cohort. Eleven patients were included in the pharmacokinetics analyses of cycle 3: 6 patients in the 230 mg/m² cohort and 5 in the 350 mg/m² cohort.

Baseline patient demographics and disease characteristics are summarized in Table 1. Median age of the study population was 61.7 years. Most patients (83.3 %) were Caucasian, and most (88.9 %) had ECOG performance status of 0 or 1. The most common primary tumor site was the lung, which was diagnosed in 44.4 % of patients. Patients in the 230 mg/m² and the 350 mg/m² cohorts had a median of 2 and 4 prior chemotherapy regimens, respectively.

Results of the cycle 1 pharmacokinetic analyses are summarized in Table 2. Mean plasma concentrations over time after aldoxorubicin infusion for each dose group are shown in Fig. 1. Following a single dose of aldoxorubicin on day 1 of cycle 1, peak plasma concentrations (Cmax) of aldoxorubicin were reached at a median of 0.75 h (230 mg/m²) and 1.00 h (350 mg/m²) after the start of aldoxorubicin infusion (tmax). The tmax of doxorubicin was 0.58 h (230 mg/m²) and 0.68 h (350 mg/m²). The tmax for doxorubicinol was much longer, a median of 36.5 h (230 mg/m²) and 48.5 h (350 mg/m²). The plasma concentration of aldoxorubicin was greater than that of doxorubicin by about 40- to 300-fold, depending on the individual. The plasma concentration of doxorubicinol, the main metabolite of doxorubicin, was very near the limit of detection.

Both Cmax and the area under the plasma doxorubicin concentration-time curve from time 0 to infinity (AUC0–∞) of aldoxorubicin and doxorubicin were higher with the 350 mg/m² dose of aldoxorubicin than the 230 mg/m² dose, but systemic clearance (CL) and volume of distribution at steady state (Vss) of aldoxorubicin, and apparent systemic clearance (CL/F) and apparent volume of distribution (V/F) of doxorubicin were similar between aldoxorubicin doses. Importantly, both the Cmax and AUC0–∞ of free doxorubicin represented a small fraction of the Cmax and AUC0–∞, respectively, of albumin-bound doxorubicin. The Cmax of doxorubicinol was also higher with the 350 mg/m² aldoxorubicin dose, but overall plasma concentration of doxorubicinol, as an absolute quantity and as a fraction of free doxorubicin, was very low. The AUC0–∞ of doxorubicinol could not be calculated.

The harmonic mean ± pseudo-standard deviation half-life (t½) values for aldoxorubicin were similar for the 230 mg/m² and 350 mg/m² doses (20.1±3.3 h vs 21.1±3.4 h). The t½ values for doxorubicin were higher with the 350 mg/m² dose of aldoxorubicin than with the 230 mg/m² dose.

There were no apparent differences in pharmacokinetic parameters between cycle 1 and cycle 3 (Fig. 2). There was no evidence of accumulation of aldoxorubicin over multiple cycles of administration.

Urine levels of doxorubicin and doxorubicinol over time are summarized in Table 3. Overall, urine levels of doxorubicinol were very low, relative to levels of free doxorubicin.

Adverse events occurring on study are summarized in Table 4. Of the 22 grade 3 or 4 adverse events reported, 11 (50.0 %) were deemed possibly or definitely related to study drug. One aldoxorubicin-related serious adverse event, febrile neutropenia, was reported; this patient did not receive GCSF following the occurrence of this event. Two deaths from tumor progression occurred. Although 10 (55.6 %) patients experienced >5 % prolongation of QTc from baseline, none of these events was considered clinically significant. Four (22.2 %) patients, three in the 230-mg/m² dose group and one in the 350-mg/m² dose group, had QTc prolongation ≥470 ms, but none of these events was considered clinically significant. One patient who entered the study with QTc >470 ms had fluctuations in QTc during treatment that stabilized to near baseline value post-treatment. One patient experienced grade 3 tachycardia after 9 cycles; this was not considered a serious adverse event and no specific action was taken. One (5.6 %) patient, in the 230-mg/m² dose group, had a decrease in left ventricular ejection fraction from baseline of at least 10 %, but this was not considered clinically significant. Overall, there
was little evidence of acute cardiotoxicity associated with aldoxorubicin treatment. Six patients had tumor responses or tumor shrinkage on aldoxorubicin during the study. The four patients in the 230-mg/m² group with tumor response had completed a median of 9 cycles of treatment (range, 7–21), and of the two patients in the 350-mg/m² group with tumor response, one completed 5 cycles and the other 9 cycles of treatment. Three patients had partial responses (PR) to treatment, including one diagnosed with thyroid cancer with confirmed PR (230-mg/m² group) who remained on study for over 6 months (9 cycles completed), one diagnosed with mesothelioma with confirmed PR (350-mg/m² group) who was withdrawn from the study after 9 cycles because of paroxysmal atrial fibrillation, and one diagnosed with small-cell lung cancer (SCLC) with unconfirmed PR after 4 cycles (230-mg/m² group) but progressive disease (PD) after 7 cycles. Three patients had stable disease with tumor shrinkage, including one diagnosed with SCLC who remained on study for over 1 year (21 cycles completed; 230-mg/m² group), one diagnosed with a small-cell neuroendocrine tumor who remains on study at the time of this report (1+ year; 230-mg/m² group), and one diagnosed with ovarian cancer with tumor shrinkage after 2 cycles (350-mg/m² group) but PD after 5 cycles.

**Discussion**

The pharmacokinetic analyses described in this report show that aldoxorubicin has a mean circulating t½ of 20.1–21.1 h, a narrow mean volume of distribution of 3.96–4.08 L/m², and a slow mean clearance rate of 0.136–0.152 L/h/m². These characteristics suggest that aldoxorubicin, either bound or unbound to serum albumin, is stable in circulation and does not accumulate to a substantial degree in body compartments outside of the bloodstream. Furthermore, plasma concentrations of free doxorubicin and doxorubicinol were only small fractions of the plasma concentration of albumin-bound doxorubicin. Therefore, almost all doxorubicin in the circulation

| Table 1 Baseline patient characteristics and disease characteristics (N=18) |
|-----------------|-----------------|-----------------|
| Parameter       | Aldoxorubicin dose group |  |
|                 | 230 mg/m² | 350 mg/m² | All patients |
| No. of patients | 11 | 7 | 18 |
| Median age (range), years | 64.0 (34.0–75.9) | 57.9 (37.3–69.4) | 61.7 (34.0–75.9) |
| Male / female, n (%) | 4 / 7 (36.4 / 63.6) | 4 / 3 (57.1 / 42.9) | 8 / 10 (44.4 / 55.6) |
| Race, n (%)      |             |           |               |
| Caucasian       | 8 (72.7) | 7 (100) | 15 (83.3) |
| Black or African American | 1 (9.1) | – | 1 (5.6) |
| Asian           | 2 (18.2) | – | 2 (11.1) |
| ECOG performance status, n (%) | 0–1 | 16 (88.9) |
| Lung            | 9 (81.8) | 7 (100) | 2 (11.1) |
| Lung            | 2 (18.2) | – | 2 (11.1) |
| Tumor primary site |           |           |               |
| Esophagus       |             | 1 (9.1) | 1 (5.6) |
| Lung            |             | 5 (45.5) | 3 (42.9) |
| Ovary           |             | 1 (14.3) | 1 (5.6) |
| Pancreas        |             | 1 (14.3) | 1 (5.6) |
| Soft tissue     |             | 1 (14.3) | 1 (5.6) |
| Testicle        |             | 1 (9.1) | 1 (5.6) |
| Thyroid         |             | 1 (9.1) | 1 (5.6) |
| Urothelium      |             | 1 (9.1) | 1 (5.6) |
| Unknown        |             | 2 (18.2) | 3 (16.7) |
| Median no. of prior chemotherapy regimens (range) | 2 (0–5) | 4 (2–8) | 2 (0–8) |

*ECOG Eastern Cooperative Oncology Group*

*a Includes one diagnosis of metastatic small-cell lung cancer*

*b Includes one diagnosis of mesothelioma of the lung and one diagnosis of late-stage small-cell lung cancer*

*c “Unknown” includes neuroendocrine tumor of unknown or unspecified primary site*
remains bound to albumin via the acid-sensitive linker, and little free doxorubicin is released in the bloodstream. This is an important point that highlights one major advantage of the covalent bond formed between

![Fig. 1](image_url)

**Table 2** Pharmacokinetic parameters after aldoxorubicin infusion on day 1 of cycle 1 (N=18)

| Parameter | Statistic | Albumin-bound doxorubicin |  | Free doxorubicin |  | Doxorubicinol |  |
|-----------|-----------|--------------------------|---|-----------------|---|--------------|---|
|           | 230 mg/m² | 350 mg/m²                |  | 230 mg/m²       | 350 mg/m² | 230 mg/m²   | 350 mg/m² |
| t<sub>max</sub>, h | Median (range) | 0.75 (0.58–1.5) | 1.00 (0.583–2.5) | 0.58 (0.58–1.5) | 0.75 (0.58–0.75) | 36.5<sup>c</sup> (12.5–72.5) | 48.5 (12.5–72.5) |
| C<sub>max</sub>, ng/mL | Mean (SD) | 67,400 (5750) | 105,000 (23,400) | 1,200 (974) | 2,470 (2040) | 4.17 (6.20) | 13.7 (10.4) |
| t<sub>1/2</sub>, h | Harmonic mean (pseudo-SD) | 20.1 (3.28) | 21.1 (3.36) | 11.5 (2.83) | 16.0 (6.67) | NC | NC |
| AUC<sub>α,x</sub>, ng·h/mL | Mean (SD) | 1,420,000 (197,000) | 2,490,000 (623,000) | 6,060 (3320) | 12,000 (5810) | 212 (310) | 661 (581) |
| AUC<sub>α,x</sub>, ng·h/mL | Mean (SD) | 1,550,000 (254,000) | 2,760,000 (724,000) | 8,570 (3880) | 15,700 (7390) | NC | NC |
| CL, L/h/m² | Mean (SD) | 0.152 (0.0249) | 0.136 (0.0395) | NA | NA | NA | NA |
| V<sub>ss</sub>, L/m² | Mean (SD) | 4.08 (0.431) | 3.96 (1.16) | NA | NA | NA | NA |
| CL/F<sup>a</sup>, L/h/m² | Mean (SD) | NA | NA | 23.2 (10.5) | 20.5 (11.2) | NA | NA |
| V<sub>d</sub>F<sup>a</sup>, L/m² | Mean (SD) | NA | NA | 391 (156) | 485 (178) | NA | NA |
| CL/F<sup>b</sup>, L/h/m² | Mean (SD) | NA | NA | 32.4 (14.6) | 27.7 (15.1) | NA | NA |
| V<sub>d</sub>F<sup>b</sup>, L/m² | Mean (SD) | NA | NA | 545 (217) | 653 (239) | NA | NA |

AUC<sub>α,x</sub> area under the concentration-time curve from time 0 to the time of the last measurable plasma concentration, AUC<sub>α-x</sub> area under the concentration-time curve from time 0 to infinity, CL systemic clearance for aldoxorubicin, CL/F apparent clearance for doxorubicin, C<sub>max</sub> peak plasma concentration of aldoxorubicin after dosing, NA not applicable, NC not calculated, SD standard deviation, t<sub>1/2</sub> apparent half-life of aldoxorubicin, t<sub>max</sub> time to reach C<sub>max</sub>, V<sub>d</sub>F apparent volume of distribution of doxorubicin, V<sub>ss</sub> volume of distribution at steady state of aldoxorubicin

<sup>a</sup>Calculated using doxorubicin-equivalent dose; assumes all aldoxorubicin is hydrolyzed to doxorubicin

<sup>b</sup>Calculated using aldoxorubicin dose

<sup>c</sup>The t<sub>max</sub> parameter for doxorubicinol was measured in 4 patients in the 230 mg/m² cohort
aldoxorubicin and serum albumin, that release of the drug is conditional upon transport to an acidic environment such as that found at the tumor site.

Our analyses show that very little doxorubicin is excreted in the urine and it is mostly as unmetabolized drug, reinforcing the conclusion that very little doxorubicin is released into the urine after infusion of aldoxorubicin.

Fig. 2 Comparison of mean plasma concentrations of (a) albumin-bound doxorubicin, (b) free doxorubicin, and (c) doxorubicinol at select time points after infusion of aldoxorubicin on day 1 of cycle 1 (N=18; black bars) and on day 1 of cycle 3 (N=11; gray bars).
Table 3  Urine levels of doxorubicin and doxorubicinol after aldoxorubicin infusion on day 1 of cycle 1 (N=18)

| Aldoxorubicin 230 mg/m² dose group (n=11) |  | Aldoxorubicin 350 mg/m² dose group (n=7) |  |
|------------------------------------------|------------------|------------------------------------------|------------------|
| Time point                               | Free doxorubicin, ng/mL | Doxorubicinol, ng/mL | Time point | Free doxorubicin, ng/mL | Doxorubicinol, ng/mL |
|                                          | Mean (SD) | Median (range) | Mean (SD) | Median (range) | Mean (SD) | Median (range) | Mean (SD) | Median (range) |
| 24 h                                     | 5090 (2903) | 4220 (1250–10,600) | 368 (233) | 363 (57–836) |
| 48 h                                     | 3077 (1527) | 2995 (1350–5420) | 375 (225) | 341 (136–808) |
| 72 h                                     | 1484 (700) | 1585 (591–2360) | 336 (185) | 284 (97–625) |

Table 4  Summary of adverse events, including cardiac events (N=18)

| Aldoxorubicin dose group | Median cycles completed, n (range) | Patients with AEs, n (%) | Total AEs, n | Grade 3 or 4 AEs, n (%) | Serious AEs, n (%) | Deaths, n | Cardiac events, n (%) |
|--------------------------|-----------------------------------|--------------------------|--------------|-------------------------|-------------------|-----------|-----------------------|
|                          | 230 mg/m² (n=11)                  | 350 mg/m² (n=7)          | All patients (N=18) |
| Median cycles completed, n (range) | 3 (2–21) | 3 (1–9) | 3 (1–21) | 11 (100) | 105 | 18 (100) |
| Patients with AEs, n (%) | 165 | 105 | 270 | 8 (4.8) | 14 (13.3) | 22 (8.1) | 3 (1.8) | 3 (2.9) | 6 (2.2) |
| Cardiac events, n (%) | 2 (18.2) | 0 (0) | 2 (0.7) | 1 (9.1) | 0 (0) | 1 (5.6) | 0 (0) | 0 (0) | 0 (0) | 6 (54.5) | 4 (57.1) | 10 (55.6) |
| Patients with >10 % decrease in LVEF | 1 (9.1) | 0 (0) | 1 (5.6) | 0 (0) | 0 (0) | 0 (0) | 6 (54.5) | 4 (57.1) | 10 (55.6) |
| Patients with >5 % prolongation of QTc from baseline | 1 (9.1) | 0 (0) | 1 (5.6) | 0 (0) | 0 (0) | 0 (0) | 6 (54.5) | 4 (57.1) | 10 (55.6) |

AE adverse event, LVEF left ventricular ejection fraction

*Does not include deaths

*Percentage of total AEs
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