Evaluation of toxicity and single-dose pharmacokinetics of intravenous ursolic acid liposomes in healthy adult volunteers and patients with advanced solid tumors

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Objective: The purpose of this study was to investigate the maximum tolerated dose (MTD), dose-limiting toxicity (DLT), and pharmacokinetics of ursolic acid liposomes (UAL), as a new drug, in healthy adult volunteers and patients with advanced solid tumors.

Methods: All subjects received a single-dose of UAL (11, 22, 37, 56, 74, 98, and 130 mg/m²) administered as a 4-h intravenous infusion. Toxicity was assessed and plasma samples were analyzed using validated ultra-performance liquid chromatograph/tandem mass spectroscopy method.

Results: A total of 63 subjects including 4 patients and 35 healthy adult volunteers for toxicity study and 24 healthy adult volunteers for pharmacokinetic study were enrolled in this trial. The DLT was encountered at 74, 98, and 130 mg/m², and consisted of hepatotoxicity and diarrhea. Other adverse events included grade 1 nausea, grade 2 abdominal distention, grade 1 microscopic hematuria, grade 2 elevated serum sodium, grade 1 vascular stimulation, and grade 1 skin rash. The MTD was 98 mg/m². The single-dose pharmacokinetic parameters revealed a linear relationship between Cmax, AUC0-24 h, or AUC0-∞, and escalated doses.

Conclusions: The clinical data reported for the first time that UAL had manageable toxicities with MTD of 98 mg/m². The DLT were hepatotoxicity and diarrhea. Meanwhile, UAL had a linear pharmacokinetic profile. The registration number of this trial is ChiCTR-ONC-12002385.

Keywords: dose-limiting toxicity, maximum tolerated dose, pharmacokinetics, Phase I, ursolic acid liposome

1. Introduction

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid, UA), as a naturally pentacyclic triterpene acid, has been isolated and identified from various vegetarian foods, medicinal herbs, and plants including Eriobotrya japonica, Rosmarinus officinalis, and Glechoma hederaceae [1,2], and its structure is shown in Figure 1. It has been reported to have strong pharmacological effects including antibacterial, hepatoprotective, immunomodulatory, and antiproliferative activities [3]. However, application of
UA is greatly restricted due to its poor water solubility. Previous studies have shown that liposomes are biodegradable drug delivery systems and it can overcome effectively drug’s poor solubility, increase therapeutic efficiency, and reduce adverse events [4,5]. Up-to-date, several liposomal formulations have been approved by Food and Drug Administration including Caelyx, DOXIL, and so on. Now, ursolic acid liposomes (UAL) have been also developed successfully to overcome poor water solubility, meanwhile, it has been approved by State Food and Drug Administration (SFDA) of China to enter clinical trials (NO. 2009L00634).

It has been reported that UA could inhibit invasion and metastasis of tumor by downregulating the expression of MMP-9 [6]. It could also inhibit skin tumorigenesis [7], inhibit angiogenesis [8], induce tumor cell differentiation [9] and induce tumor cell apoptosis [10,11]. Therefore, UA has long been considered as a potentially valuable drug. However, UAL has more advantages compared to UA due to its features including favorable water solubility and bioavailability, passive targeting characteristics, and low toxicity. Thus, UAL is immediately approved by SFDA. But studies on toxicity and single-dose pharmacokinetics of UAL in Phase I trial have not been reported until now.

In this trial, our aim is to investigate the maximum tolerated dose (MTD), dose-limiting toxicity (DLT), other toxicities, and pharmacokinetics of UAL for the first time. The single doses of UAL were designed as 11, 22, 37, 56, 74, 98, and 130 mg/m². Each cohort consisted of eight eligible healthy adult volunteers. Randomly recruited into three dosing cohorts including 37, 56, and 74 mg/m². Each cohort consisted of at least three subjects. The starting dose was selected as 11 mg/m², corresponding to 10% of the lethal dose observed in preclinical models [12]. Then, doses were escalated according to designed times (100% increment, 67% increment, 50% increment) until DLT or grade 2 toxicity (the Common Terminology Criteria for Adverse Events, CTCAE v 3.0) appeared at first. Next, dose was escalated by ≤ 50% of the preceding dose. If a first DLT was observed in one of three (1/3) subjects, dose escalation proceeded with a maximum 35% increment. If 1/3 subjects experienced DLT, up to three additional subjects were enrolled at the same dose level. If none of the additional three subjects experienced DLT, dose escalation was continued. On the contrary, if one of the additional three subjects experienced a DLT (therefore, two or more out of the total of six subjects experienced DLT), further enrolment into this cohort would be ceased; meanwhile, the MTD was also exceeded. The DLT was defined as grade 4 thrombocytopenia, grade 4 neutropenia lasting for ≥ 7 days, febrile neutropenia, grade 4 anemia, and grade 3 or grade 4 non-hematological toxicity. The MTD was defined as the dose level at which 0/3 or 1/6 patients experienced a DLT with the next higher dose having ≥ 2/3 or ≥ 2/6 patients experiencing a DLT.

In the single-dose pharmacokinetics study, 24 subjects were randomly recruited into three dosing cohorts including 37, 74, and 98 mg/m². Each cohort consisted of eight eligible healthy adult volunteers.
2.3 Safety and tolerability assessments
Toxicity was assessed according to the CTCAE v 3.0. Medical history and complete physical examination, ECOG PS, body weight, height, Karnofsky score, hematology, blood biochemistry, a pregnancy (if applicable), signs and symptoms, vital signs, urine routine, stool routine, and electrocardiogram were examined at screening, on study day 0 (baseline) and specified time points. The follow-up was performed at least 3 days for non-adverse events subjects. If adverse events appeared, the follow-up would continue until recovery.

2.4 Pharmacokinetic sampling and data analysis
For pharmacokinetic analysis, adequate blood samples were collected into tubes containing heparin sodium at designated time points of 30 min before the infusion (time point 0), 0.5, 1, 2, and 4 h during the infusion and then 5, 15, 30, 45 min, 1, 1.5, 2, 4, 6, 8, 12, and 24 h after the infusion, respectively. All samples were centrifuged immediately and supernatants were stored at −20°C until analysis. Plasma concentrations of UA were analyzed using validated ultra-performance liquid chromatograph/tandem mass spectroscopy (UPLC/MS/MS) method described previously [13]. In brief, chromatographic separation was carried out on a Waters Acquity UPLC™ BEH C8 column (100 × 2.1 mm, 1.7 µm) using mobile phase consisting of acetonitrile and 10 mM ammonium formate (9:1, v/v) at a flow rate of 0.2 mL/min. The elution time was 3 min. The multiple reaction monitoring was performed at m/z 455.1 → 455.0 for UA and m/z 469.3 → 425.2 for glycyrhetinic acid (internal standard, IS) in negative ion mode with electrospray ionization source. All pharmacokinetic parameters were calculated using the Drug and Statistics (DAS) software, version 2.1.1, edited and published by the Mathematical Pharmacology Professional Committee of China.

2.5 Statistical analysis
Descriptive statistics were used to evaluate toxicities. Pharmacokinetic parameters were determined from individual plasma concentration-time data using two-compartmental method (DAS version 2.1.1). Statistical comparisons were made with the one-factor analysis of variance (ANOVA) and Student’s t test. The statistical significance level was established at p < 0.05. All statistical analyses were performed using SPSS 16.0 software.

3. Results
3.1 Subject characteristics and disposition
Between August 2009 and March 2012, four patients with advanced solid tumors and 35 healthy adult volunteers were enrolled in dose-escalation study and 24 healthy adult volunteers were recruited in pharmacokinetic study in Tianjin Medical University Cancer Hospital in China. Subject baseline characteristics are listed in Table 1. Subjects were enrolled into seven cohorts (11, 22, 37, 56, 74, 98, and 130 mg/m²) for the dose-escalation study (the dose level scheme is summarized in Table 2) or three cohorts (37, 74, and 98 mg/m²) for pharmacokinetic study. Four subjects were treated with the starting dose of 11 mg/m², and they were patients with non-Hodgkin lymphoma, Hodgkin lymphoma, renal carcinoma, or hepatoma.

3.2 Toxicity
All subjects enrolled in single dose-escalation study were evaluable for toxicity. The vital signs were monitored at baseline, 1, 2, 4, 6, 8, 12, 24, and 72 h after infusion (no baseline for heart rate). The data of vital signs including body temperature, heart rate, respiration, systolic pressure, and diastolic pressure are summarized in Figure 2. The results showed that the values of each vital sign fluctuated within normal range at various time points. And the values of respiration rose slightly along with dose escalation, especially at the dose of 98 and 130 mg/m² at the same time. This phenomenon was not observed in other vital signs. The physical examinations were performed in screening period, trial period, and follow-up period. The physical examinations consisted of craniofacial organs, skin/lymph nodes, sclera/pupil, car/nose/throat, respiratory system, cardiovascular system, liver, spleen, urinary system, musculoskeletal, and central nervous system. The results revealed that only one patient treated with the dose of 11 mg/m² experienced grade 1 skin rash and two patients treated with the dose of 98 mg/m² experienced grade 1 vascular stimulation (see Table 3). And the subjects occurring skin rash recovered without any treatments after 3 days. The hematological tests including red blood cell (RBC), hemoglobin, WBC, ANC, lymphocyte, and platelet were also examined in screening period, trial period, and follow-up period. The data are shown in Figure 3. The results revealed that no abnormal changes were observed.

The urine routine (urinary protein, glucose, erythrocyte, leukocyte, and urinary casts) and stool routine (fecal erythrocyte and fecal leukocyte) were also examined in screening period, trial period, and follow-up period. Grade 1 microscopic hematuria was observed in three subjects (7.7%), who received a dose of 11 (patient with hepatoma), 74, and 130 mg/m², respectively. However, this adverse event disappeared after 3 days without any treatments.

The blood biochemistry tests were also examined during screening period, trial period, and follow-up period. And the results suggested that there were no abnormalities in subjects received the doses of 11, 22, 37, and 56 mg/m². Unfortunately, the increasing levels of AST, ALT, GGT, DBIL, and TBIL were observed in some subjects received the dose of 74, 98, and 130 mg/m² (see Table 3). The DLTs were hepatotoxicities: two (5.1%) subjects experienced grade 3 AST increment, four subjects (10.3%) experienced grade 3 ALT increment, one subject (2.6%) experienced grade 3 GGT increment, and one subject (2.6%) experienced grade 3 DBIL increment. The subjects occurring hepatotoxicities received the treatment with ademetionine, 4-Butanedisulfonate, polyene phosphatidylcholine, or tiopronin [14,15], then they recovered after 1 week.
Otherwise, diarrhea (2.6%) was another DLT. Other drug-related adverse events included one (2.6%) grade 1 nausea, one (2.6%) grade 2 abdominal distension, two (5.1%) grade 1 vascular stimulation, and three (7.7%) grade 1 triglyceride (TG) increase. Other nondrug-related adverse events included one (2.6%) grade 1 skin rash and one (2.6%) grade 2 elevated serum sodium. The subject with diarrhea recovered after anti-diarrhea treatment with Smecta in the next day. However, the subject occurring diarrhea experienced also grade 1 nausea, grade 2 abdominal distention, and grade 3 ALT/AST increment after administration. Therefore, there was a correlation between diarrhea and UAL.

3.3 Maximum tolerated dose
At the dose of 74 mg/m², 1/6 subject experienced DLT (grade 3 non-hematological toxicity including grade 3 AST/ALT increment and grade 3 diarrhea). At the next higher dose of 98 mg/m², only 1 of 11 subjects experienced DLT (grade 3 non-hematological toxicity including grade 3 ALT/GGT increment). However, 2/3 subjects experienced DLT at the subsequently higher dose of 130 mg/m², the DLT included grade 3 ALT increment, grade 3 AST increment, and grade 3 DBIL increment. Therefore, the MTD was confirmed to be 98 mg/m². The doses of multiple-dose administration trial of UAL were recommended as 56, 74, and 98 mg/m².

3.4 Pharmacokinetics
Following 37, 74, and 98 mg/m² infusion in 24 healthy adult volunteers, the plasma concentrations of UA decreased rapidly with the plasma clearance (CL) of 8.65 ± 1.09, 10.2 ± 1.46, and 9.94 ± 1.13 l/(h·m²), respectively. The elimination half-lives (t1/2) were, respectively, 4.59 ± 2.44, 4.46 ± 1.41, and 3.90 ± 2.08 h. The mean residence time (MRT0-24h) was, respectively, 3.69 ± 0.36, 3.93 ± 0.37, and 3.84 ± 0.34 h. The MRT0-∞ was, respectively, 4.28 ± 0.91, 4.56 ± 0.88, and 4.41 ± 0.95 h. The maximum plasma concentration (Cmax) was, respectively, 1835 ± 438, 2865 ± 868, and 3457 ± 856 ng/mL. The time to peak plasma concentration (tmax) was, respectively, 4.03 ± 0.04, 4.02 ± 0.04, and 4.0 ± 0.00 h. The area under the plasma concentration time curve (AUC0-24h) was, respectively, 4213 ± 606, 7175 ± 999, and 9696 ± 1134 ng·h/mL. And the AUC0-∞ was, respectively, 4339 ± 574, 7418 ± 1057 and 9971 ± 1144 ng·h/mL. The plasma concentration profiles of UAL were suitable for two-compartmental pharmacokinetic model. There were no significant difference among the three dose groups about CL, t1/2, MRT0-24h, MRT0-∞, and tmax (p > 0.05). However, some linear increments in the Cmax, AUC0-24h, and AUC0-∞ were observed along with dose increment. The linear correlation coefficients (r) of Cmax, AUC0-24h and AUC0-∞ were 0.9881, 0.9989, and 0.9986.

4. Discussion
UAL was approved by SFDA of China for clinical development based on its potent anti-inflammatory [16-18], inducing tumor...
differentiation [9], inhibiting tumor cell proliferation [19], and inducing tumor cell apoptosis [11,20–22] activities in preclinical experiments. We performed the first-in-human study of UAL on its toxicity and pharmacokinetics. Because UAL was administered to human for the first time, some toxicity could not be predicted. Therefore, the subjects of starting dose (11 mg/m²) were selected as patients with advanced solid tumors instead of healthy volunteers. The results revealed that the starting dose was safe and only one patient with hepatic carcinoma experienced slight adverse events including grade 1 skin rash.

Table 2. Dose levels by cohort assignment during the dose-escalation study.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose levels (mg/m²)</td>
<td>11</td>
<td>22</td>
<td>37</td>
<td>56</td>
<td>74</td>
<td>98</td>
<td>130</td>
</tr>
<tr>
<td>Escalated rate (%)</td>
<td>–</td>
<td>+100</td>
<td>+67</td>
<td>+50</td>
<td>+33</td>
<td>+33</td>
<td>+33</td>
</tr>
<tr>
<td>N</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Type of subjects</td>
<td>Patients</td>
<td>Healthy volunteers</td>
<td>Healthy volunteers</td>
<td>Healthy volunteers</td>
<td>Healthy volunteers</td>
<td>Healthy volunteers</td>
<td>Healthy volunteers</td>
</tr>
</tbody>
</table>

Figure 2. The vital signs data of seven cohorts at baseline, 1, 2, 4, 6, 8, 12, 24, and 72 h after the end of infusion: body temperature, heart rate, respiration, systolic pressure, and diastolic pressure (mean ± SD).
Table 3. Number of subjects with adverse events.

<table>
<thead>
<tr>
<th>AE, n</th>
<th>UAL</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>11 (n = 4)</td>
</tr>
<tr>
<td></td>
<td>I (%)</td>
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<tr>
<td>Hepatotoxicity</td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>-</td>
</tr>
<tr>
<td>ALT</td>
<td>-</td>
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<tr>
<td>GGT</td>
<td>-</td>
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<tr>
<td>DBIL</td>
<td>-</td>
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<tr>
<td>TBIL</td>
<td>-</td>
</tr>
<tr>
<td>TG</td>
<td>-</td>
</tr>
<tr>
<td>Digestive reaction</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>-</td>
</tr>
<tr>
<td>Abdominal distention</td>
<td>-</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>-</td>
</tr>
<tr>
<td>Microscopic hematuria</td>
<td>1</td>
</tr>
<tr>
<td>Elevated serum sodium</td>
<td>-</td>
</tr>
<tr>
<td>Vascular stimulation</td>
<td>-</td>
</tr>
<tr>
<td>Skin rash</td>
<td>-</td>
</tr>
<tr>
<td>Cardiovascular toxicity</td>
<td>-</td>
</tr>
<tr>
<td>Neurotoxicity</td>
<td>-</td>
</tr>
<tr>
<td>Hematologic toxicity</td>
<td>-</td>
</tr>
</tbody>
</table>

All AEs do not have grade 4 toxicity.

AE: Adverse event; I: Grade 1; II: Grade 2; III: Grade 3; -: No occurrence.
Figure 3. The hematological data of seven cohorts in screening period, trial period, and follow-up period: RBC, hemoglobin, WBC, ANC, lymphocyte, and platelet (mean + SD).

and grade 1 microscopic hematuria. Moreover, the slight adverse events disappeared without any treatments. Consistent with animal toxicology studies, the mainly DLT of UAL was also hepatotoxicity including transient AST, ALT, GGT, and DBIL increment. However, the adverse events quickly disappeared when the patients were treated with ademetionine, 4-Butanedisulfonate, polyene phosphatidylcholine, or tiopronin. Therefore, the hepatotoxicity should be monitored intensively in the future clinical trial. Fortunately, all subjects did not experience hematological toxicity, which might be significant difference between UAL and chemotherapeutic drugs. The potential reasons were presumed as followings: First, UA was isolated from medicinal herbs/plants and its toxicity was low itself. Second, UAL was a liposome system and UA had been encapsulated in liposomes to further decrease its toxicity. However, routine chemotherapeutic drugs usually resulted in severely hematological toxicity. In this study, the DLTs consisted of hepatotoxicities and diarrhea. There were no subjects experiencing DLT within the dose range of 11 – 56 mg/m². When the dose was escalated as 74 mg/m², the DLTs began to appear, but only 1/6 subject
experienced DLT. Meanwhile, when the dose continued to be escalated as 98 mg/m², only 1/11 subject experienced DLT. However, when the dose was finally escalated as 130 mg/m², 2/3 subjects experienced DLT. Thus, the dose-escalation was ceased. Therefore, the MTD was confirmed to be 98 mg/m² instead of 74 mg/m² or 130 mg/m².

5. Conclusions

This study demonstrated UAL, as a new drug, was safety for subjects as a 4-h intravenous infusion. The MTD was 98 mg/m² and the DLT were hepatotoxicity and diarrhea. The doses of multiple-dose administration trial of UAL could be recommended as 56, 74, and 98 mg/m². There was a linear relationship between Cmax or AUC0–24h or AUC0–∞ and escalated doses of UAL, suggesting that UAL had a linear pharmacokinetic profile.

Bibliography

Papers of special note have been highlighted as either of interest (●) or of considerable interest (●●) to readers.

5. This article revealed the characters and merits of liposomes.
8. This article reported the tumorogenesis-inhibiting activity of UA.
10. This article reported the differentiation-inducing activity of UA.

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X-H Wang, S-Y Zhou, and Z-Z Qian equally contributed to this paper.

Declaration of interest

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- This article reported the apoptosis-inducing activity and its mechanism of UA.


- This article reported the apoptosis-inducing activity in vivo and its mechanism of UA.


- This article reported the sensitizing activity and its mechanism of UA.

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