Comparative plasma and tissue distribution of Sun Pharma’s generic doxorubicin HCl liposome injection versus Caelyx® (doxorubicin HCl liposome injection) in syngeneic fibrosarcoma-bearing BALB/c mice and Sprague–Dawley rats

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Abstract

Purpose The liposomal formulation of doxorubicin [doxorubicin (DXR) hydrochloride (HCl) liposome injection, Caelyx®] alters the tissue distribution of DXR as compared with nonliposomal DXR, resulting in an improved benefit-risk profile. We conducted studies in murine models to compare the plasma and tissue distribution of a proposed generic DXR HCl liposome injection developed by Sun Pharmaceuticals Industries Limited (SPIL DXR HCl liposome injection) with Caelyx®.

Methods The plasma and tissue distributions of the SPIL and reference DXR HCl liposome injections were compared in syngeneic fibrosarcoma-bearing BALB/c mice and Sprague–Dawley rats. Different batches and different lots of the same batch of the reference product were also compared with each other.

Results The SPIL and reference DXR HCl liposome injections exhibited generally comparable plasma and tissue distribution profiles in both models. While minor differences were observed between the two products in some tissues, different batches and lots of the reference product also showed some differences in the distribution of various analytes in some tissues. The ratios of estimated free to encapsulated DXR for plasma and tissue were generally comparable between the SPIL and reference DXR HCl liposome injections in both models, indicating similar extents of absorption into the tissues and similar rates of drug release from liposomes.

Conclusions The plasma and tissue distribution profiles of the SPIL and reference DXR HCl liposome injections were shown to be generally comparable. Inconsistencies between the products observed in some tissues were thought to be due to biological variation.

Keywords Anthracycline · Doxorubicin HCl liposome injection · Pharmacokinetics · Plasma distribution · Preclinical · Tissue distribution

Introduction

The use of doxorubicin (DXR), a potent chemotherapeutic agent, is limited in the clinical setting by its toxicity [1]. The cardiotoxicity caused by DXR is of special concern. Doxorubicin hydrochloride (HCl) liposome injection is a liposomal formulation of DXR that alters the plasma and tissue distribution of DXR, leading to an at least comparable efficacy and an improved toxicological profile over non-liposomal DXR [2–4].

Doxorubicin HCl liposome injection received approval from the US Food and Drug Administration (FDA) in 1995.
for the treatment of AIDS-related Kaposi’s sarcoma. Since then, it has received worldwide approval for the treatment of multiple myeloma (combination therapy with bortezomib) and ovarian carcinoma. In the European Union, it is additionally approved for patients with breast cancer who are at increased risk of DXR-associated cardiotoxicity [2, 5]. Janssen is currently marketing DXR HCl liposome injection as Doxil® in the US and Japan and as Caelyx® elsewhere.

Sun Pharmaceutical Industries Limited (SPIL) has developed a generic DXR HCl liposome injection (SPIL DXR HCl liposome injection). In February 2012, the FDA temporarily allowed the importation of Sun Pharma’s domestic liposomal DXR to cope with a Doxil® drug shortage, and because there were no approved generic alternatives [6]. One year later, SPIL DXR HCl liposome injection was formally approved by the FDA.

This study was one of a program of studies, conducted in line with European Medicines Agency (EMA) guidance, to demonstrate similarity between the SPIL DXR HCl liposome injection and Caelyx®. The program included physicochemical equivalence studies (structure, content and stability of liposomes in vitro and in vivo), which confirmed that the two liposomal forms are similar (SPIL data on file).

Caelyx® alters the plasma and tissue distribution of DXR, resulting in an improved benefit-risk profile as compared with nonliposomal DXR. Therefore, SPIL DXR HCl liposome injection must achieve comparable plasma and tissue distribution in humans to be considered truly comparable to Caelyx® [2, 7].

Previously, we demonstrated that the preclinical antitumour efficacy and toxicity profile and the extent of total DXR absorption of the SPIL DXR HCl liposome injection were comparable to Caelyx® in relevant mouse models of cancer (Burade et al. Paper accepted by BMC Cancer subject to revision).

The objectives of the studies presented in this paper were to compare the plasma and tissue distribution of the SPIL DXR HCl liposome injection with the reference product (Caelyx®) following single intravenous injection in syngeneic fibrosarcoma-bearing BALB/c mice and Sprague–Dawley rats. The plasma and tissue distribution of different batches and different lots of the same batch of the reference product were also compared with each other in Sprague–Dawley rats.

Materials and methods

Drug administration

The SPIL DXR HCl liposome injection (SPIL, Halol, India) and reference DXR HCl liposome injection (Caelyx®, Janssen-Cilag International NV, Beerse, Belgium) were stored at 2–8°C. The SPIL and reference DXR HCl liposome injections contained 2 mg/mL of the active ingredient, DXR HCl. The SPIL and reference DXR HCl liposome injections also contained N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycerol-3-phosphoethanolamine (mPEG-DSPE), hydrogenated soy phosphatidylcholine (HSPC), cholesterol, ammonium sulphate, l-histidine as a buffer, hydrochloric acid and/or sodium hydroxide for pH control, sucrose to maintain isotonicity and water for injection. All products were either used at 2 mg/mL, or diluted to the desired concentration in sterile 5% glucose solution.

Animals

The project proposal for the study was approved by the Institutional Animal Ethics Committee (IAEC), and their recommendations regarding animal care and handling were followed. Male BALB/c mice and male Sprague–Dawley rats were supplied by Laboratory Animal Resources (LAR), Sun Pharma Advanced Research Company Limited (SPARC Ltd.). The BALB/c mice were 6–10 weeks of age at the time of receipt and weighed 25±5 g. The Sprague–Dawley rats were 5–8 weeks of age and weighed 150–180 g at receipt. For BALB/c mice, a veterinary health check was performed before tumour propagation to select healthy animals. For Sprague–Dawley rats, a veterinary health check was performed on day 0 of the study. The BALB/c mice and Sprague–Dawley rats used in the studies were housed in individually ventilated polystyrene cages. The BALB/c mice were housed individually, while Sprague–Dawley rats were housed two to three per cage. Cages were maintained under constant temperature (18–26°C), humidity (30–70%) and lighting conditions (12 h light and 12 h dark). Animals received reverse osmosis (RO) water supplied by LAR, and Harlan Teklad Rodent Diet 2018 ad libitum.

Cell lines

WEHI 164 cells suspended in phosphate buffer saline (1–2×10⁷ viable cells per mL) were used for the induction of solid tumours. Briefly, male BALB/c mice were shaved with the help of a shaver and hair-removing cream, and inoculated intradermally on the shaved portion of dorsal skin with tumour cells. Calibrated digital Vernier calipers were used to measure tumour diameter in three perpendicular planes, after the tumours became palpable. Tumour volume was calculated using the formula for an ellipse, \( V = \pi D_1/2 \times D_2/2 \times D_3 \) mm³, where \( D_1/D_2/D_3 \) is the diameter (mm) in each of the three different planes.
**Study design**

The respective designs of the plasma and tissue distribution studies are shown in Table 1. Fibrosarcoma-bearing male BALB/c mice were screened and selected for randomisation into study groups on the basis of body weight and tumour volume. Selected animals weighed 22.2–30.5 g and had a tumour volume 150 ± 50 mm³ at randomisation. Male Sprague–Dawley rats were screened and selected for randomisation into study groups on the basis of body weight, and weighed 190.1–258.0 g at randomisation. The doses of SPIL and reference DXR HCl liposome injections used in these studies (BALB/c mice, 2.4 or 6 mg/kg; Sprague–Dawley rats, 4 or 10 mg/kg) were calculated to be equivalent to 20 or 50 mg/m², respectively, in humans. Intravenous injections were administered on day 0. Blood samples for plasma distribution analysis were collected at 1, 4, 24, 48, 96, 168, 240, 336, or 672 h postinjection (Table 1). Animals were euthanised at this time in order to harvest the heart, skin, liver, kidney, spleen, lung, bone marrow and tumour (BALB/c mice only) for tissue distribution analysis. Plasma was separated from blood samples by centrifugation. For BALB/c mice, plasma samples were pooled from four animals serially, providing three pooled samples for each time point. The tumour (BALB/c mice only), heart, skin, liver, kidney, spleen and lung were dissected out from all animals. Both hind legs were dissected out for bone marrow preparation, which was scraped and collected after cutting bones longitudinally using scalpel blades. All tissues were blotted on Whatman filter paper No. 1 to remove the blood. For BALB/c mice, samples of each tissue from four animals were pooled, providing three pooled samples per tissue for each time point (except bone marrow, where samples from all 12 animals were pooled for each time point). Tissue homogenates were prepared using a homogenizer. A 20% weight/volume (w/v) tissue homogenate was prepared for tumour (BALB/c mice only), heart, liver, kidney, spleen, lung and bone marrow. For skin, either a 10 or 20% w/v tissue homogenate was prepared (2.4 mg/kg mouse study: a 10% w/v skin homogenate was prepared for all time points except at 96 h; 6 mg/kg mouse study: a 20% w/v skin tissue homogenate was prepared; 4 mg/kg rat study: a 10% w/v skin homogenate was prepared for all time points except at 1 and 4 h; 10 mg/kg rat study: a 20% w/v skin tissue homogenate was prepared). Heart and skin tissues were minced before homogenization.

**Experimental outcomes**

Plasma drug levels were measured from the plasma samples for each time point by liquid chromatography–tandem mass spectrometry (LC-MS/MS), and reported as micrograms per millilitre (mcg/mL). Tissue drug levels were measured from tissue samples for each time point by LC-MS/MS, and reported as micrograms of DXR per gram of tissue (mcg/g of tissue). Plasma and tissue data were used to calculate the mean peak concentration (Cmax) and mean area under the curve from zero (0) hours to time (t) (AUC0–t) of total DXR, encapsulated DXR, free DXR, and doxorubicinol. The ratio of estimated free to encapsulated DXR was defined as the estimated mean Cmax (or AUC0–t) of free DXR divided by the mean Cmax (or AUC0–t) of encapsulated DXR.

**Statistical methods**

The mean AUCs and 95% confidence intervals of the difference between every two groups were calculated by the Bailer–Satterthwaite method. A difference in AUCs was considered to be statistically significant when abs(tobs) ≥ tcrit. For mean Cmax, the 95% confidence intervals of the differences between every two groups were calculated by unpaired t-test. A difference in Cmax was considered to be statistically significant when p ≤ 0.05. Zero-hour concentration values were reported (as zero) for the purpose of calculating AUC only. A statistical analysis of bone marrow derived from syngeneic fibrosarcoma-bearing BALB/c mice was not performed, as only 1 value was available for each group (sample was pooled from 12 animals for concentration analysis owing to limited quantity).

**Results**

**Plasma distribution of DXR in syngeneic fibrosarcoma-bearing mice**

Mean plasma Cmax and AUC0–t values for total DXR and doxorubicinol, following a single intravenous injection with either the SPIL or reference DXR HCl liposome injection in syngeneic fibrosarcoma-bearing BALB/c mice, are shown in Fig. 1.

**Maximum concentration**

In plasma, the mean Cmax values of total DXR (Fig. 1a), free and encapsulated DXR (data not shown) and doxorubicinol (Fig. 1c) were comparable after 2.4 mg/kg SPIL compared with 2.4 mg/kg reference DXR HCl liposome injection. The mean plasma Cmax values of total DXR (Fig. 1a), free DXR (data not shown) and doxorubicinol (Fig. 1c) were also comparable after 6 mg/kg SPIL and reference DXR HCl liposome injections. However, the mean plasma Cmax of encapsulated DXR was significantly lower after 6 mg/kg SPIL DXR HCl liposome injection compared with
| Group no. | Dose groups | Dose (mg/kg) | Concentration (mg/mL) | Dose volume (mL/kg) | Time point (h) | No. of animals per time point |
|-----------|-------------|--------------|-----------------------|---------------------|---------------|-----------------------------|
| (a) Study groups for the syngeneic fibrosarcoma-bearing BALB/c mouse study (2.4 mg/kg dose) | | | | | | |
| 1 | SPIL DXR HCl liposome injection | 2.4 | 0.6 | 4 | 1 | 12 |
| | | | | | | |
| | | | | | | 4 |
| | | | | | | 12 |
| | | | | | | 24 |
| | | | | | | 12 |
| | | | | | | 48 |
| | | | | | | 12 |
| | | | | | | 96 |
| | | | | | | 12 |
| | | | | | | 168 |
| | | | | | | 12 |
| | | | | | | 240 |
| | | | | | | 12 |
| | | | | | | 336 |
| | | | | | | 12 |
| | | | | | | 672 |
| | | | | | | 12 |
| 2 | Reference DXR HCl liposome injection | 2.4 | 0.6 | 4 | 1 | 12 |
| | | | | | | |
| | | | | | | 4 |
| | | | | | | 12 |
| | | | | | | 24 |
| | | | | | | 12 |
| | | | | | | 48 |
| | | | | | | 12 |
| | | | | | | 96 |
| | | | | | | 12 |
| | | | | | | 168 |
| | | | | | | 12 |
| | | | | | | 240 |
| | | | | | | 12 |
| | | | | | | 336 |
| | | | | | | 12 |
| | | | | | | 672 |
| | | | | | | 12 |
| Total number | | | | | | 216 |
| (b) Study groups for the syngeneic fibrosarcoma-bearing BALB/c mouse study (6 mg/kg dose) | | | | | | |
| 1 | SPIL DXR HCl liposome injection | 6 | 2 | 3 | 1 | 12 |
| | | | | | | |
| | | | | | | 4 |
| | | | | | | 12 |
| | | | | | | 24 |
| | | | | | | 12 |
| | | | | | | 48 |
| | | | | | | 12 |
| | | | | | | 96 |
| | | | | | | 12 |
| | | | | | | 168 |
| | | | | | | 12 |
| | | | | | | 240 |
| | | | | | | 12 |
| | | | | | | 336 |
| | | | | | | 12 |
| | | | | | | 672 |
| | | | | | | 12 |
| 2 | Reference DXR HCl liposome injection | 6 | 2 | 3 | 1 | 12 |
| | | | | | | |
| | | | | | | 4 |
| | | | | | | 12 |
| | | | | | | 24 |
| | | | | | | 12 |
| | | | | | | 48 |
| | | | | | | 12 |
| | | | | | | 96 |
| | | | | | | 12 |
| | | | | | | 168 |
| | | | | | | 12 |
| | | | | | | 240 |
| | | | | | | 12 |
| | | | | | | 336 |
| | | | | | | 12 |
| | | | | | | 672 |
| | | | | | | 12 |
| Total number | | | | | | 216 |
### Table 1 (continued)

| Group no. | Dose groups                                      | Dose (mg/kg) | Concentration (mg/mL) | Dose volume (mL/kg) | Time point (h) | No. of animals per time point |
|-----------|--------------------------------------------------|--------------|-----------------------|--------------------|----------------|------------------------------|
|           | Study groups for the Sprague–Dawley rat study (4 mg/kg dose) |              |                       |                    |                |                              |
| 1         | SPIL DXR HCl liposome injection                   | 4            | 2                     | 2                  | 1              | 10                           |
|           |                                                   |              |                       |                    | 4              | 10                           |
|           |                                                   |              |                       |                    | 24             | 10                           |
|           |                                                   |              |                       |                    | 48             | 10                           |
|           |                                                   |              |                       |                    | 96             | 10                           |
|           |                                                   |              |                       |                    | 168            | 10                           |
|           |                                                   |              |                       |                    | 240            | 10                           |
|           |                                                   |              |                       |                    | 336            | 10                           |
|           |                                                   |              |                       |                    | 672            | 10                           |
|           |                                                   |              |                       |                    | 24a            | 10                           |
|           |                                                   |              |                       |                    | 48a            | 10                           |
|           |                                                   |              |                       |                    | 96a            | 10                           |
| 2         | Reference DXR HCl liposome injection (batch 1)    | 4            | 2                     | 2                  | 1              | 10                           |
|           |                                                   |              |                       |                    | 4              | 10                           |
|           |                                                   |              |                       |                    | 24             | 10                           |
|           |                                                   |              |                       |                    | 48             | 10                           |
|           |                                                   |              |                       |                    | 96             | 10                           |
|           |                                                   |              |                       |                    | 168            | 10                           |
|           |                                                   |              |                       |                    | 240            | 10                           |
|           |                                                   |              |                       |                    | 336            | 10                           |
|           |                                                   |              |                       |                    | 672            | 10                           |
|           |                                                   |              |                       |                    | 24a            | 10                           |
|           |                                                   |              |                       |                    | 48a            | 10                           |
|           |                                                   |              |                       |                    | 96a            | 10                           |
| 3         | Reference DXR HCl liposome injection (batch 2)    | 4            | 2                     | 2                  | 1              | 10                           |
|           |                                                   |              |                       |                    | 4              | 10                           |
|           |                                                   |              |                       |                    | 24             | 10                           |
|           |                                                   |              |                       |                    | 48             | 10                           |
|           |                                                   |              |                       |                    | 96             | 10                           |
|           |                                                   |              |                       |                    | 168            | 10                           |
|           |                                                   |              |                       |                    | 240            | 10                           |
|           |                                                   |              |                       |                    | 336            | 10                           |
|           |                                                   |              |                       |                    | 672            | 10                           |
|           |                                                   |              |                       |                    | 24a            | 10                           |
|           |                                                   |              |                       |                    | 48a            | 10                           |
|           |                                                   |              |                       |                    | 96a            | 10                           |

| Total number | 360 |

| Study groups for the Sprague–Dawley rat study (10 mg/kg dose) |
|---------------------------------------------------------------|
| 1 | SPIL DXR HCl liposome injection | 10 | 2 | 5 | 1 | 10 |
|   |                                 |    |   |   | 4 | 10 |
|   |                                 |    |   |   | 24 | 10 |
|   |                                 |    |   |   | 48 | 10 |
|   |                                 |    |   |   | 96 | 10 |
|   |                                 |    |   |   | 168 | 10 |
|   |                                 |    |   |   | 240 | 10 |
|   |                                 |    |   |   | 336 | 10 |
|   |                                 |    |   |   | 672 | 10 |
the reference DXR HCl liposome injection \((p=0.0351\); data not shown).

**Area under the curve**

The mean plasma \(AUC_{0\rightarrow t}\) values of total DXR (Fig. 1b) and free and encapsulated DXR (data not shown) after 2.4 and 6 mg/kg were comparable between the SPIL and reference DXR HCl liposome injections. However, the mean plasma \(AUC_{0\rightarrow t}\) of doxorubicinol was significantly lower after 2.4 mg/kg SPIL DXR HCl liposome injection compared with the reference DXR HCl liposome injection \((t_{\text{crit}} = 2.4469 \text{ and } t_{\text{obs}} = -5.5357; \text{ Fig. 1d)}\). This was not the case after 6 mg/kg, where the mean plasma \(AUC_{0\rightarrow t}\) of doxorubicinol (Fig. 1d) was comparable between the SPIL and reference DXR HCl liposome injections.

**Tissue distribution of DXR in syngeneic fibrosarcoma-bearing mice**

Mean tissue \(C_{\text{max}}\) and \(AUC_{0\rightarrow t}\) values of total DXR and doxorubicinol (liver and heart) for syngeneic fibrosarcoma-bearing BALB/c mice, following a single intravenous injection with either the SPIL or reference DXR HCl liposome injection, are also shown in Fig. 1.

### Table 1 (continued)

| Group no. | Dose groups | Dose (mg/kg) | Concentration (mg/mL) | Dose volume (mL/kg) | Time point (h) | No. of animals per time point |
|-----------|-------------|--------------|-----------------------|--------------------|----------------|-----------------------------|
| 2         | Reference DXR HCl liposome injection (lot 1) | 10 | 2 | 5 | 1 | 10 |
|           |             |              |                       |                    | 4 | 10 |
|           |             |              |                       |                    | 24 | 10 |
|           |             |              |                       |                    | 48 | 10 |
|           |             |              |                       |                    | 96 | 10 |
|           |             |              |                       |                    | 168 | 10 |
|           |             |              |                       |                    | 240 | 10 |
|           |             |              |                       |                    | 336 | 10 |
|           |             |              |                       |                    | 672 | 10 |
| 3         | Reference DXR HCl liposome injection (lot 2) | 10 | 2 | 5 | 1 | 10 |
|           |             |              |                       |                    | 4 | 10 |
|           |             |              |                       |                    | 24 | 10 |
|           |             |              |                       |                    | 48 | 10 |
|           |             |              |                       |                    | 96 | 10 |
|           |             |              |                       |                    | 168 | 10 |
|           |             |              |                       |                    | 240 | 10 |
|           |             |              |                       |                    | 336 | 10 |
|           |             |              |                       |                    | 672 | 10 |

Total number 270

*DXR* doxorubicin, *HCl* hydrochloride, *SPIL* Sun Pharmaceutical Industries Limited

*Repeat time points*
Maximum concentration: doxorubicinol

The mean $C_{\text{max}}$ of doxorubicinol was comparable between the SPIL and reference DXR HCl liposome injections in heart tissue, after 2.4 mg/kg. However, the mean liver $C_{\text{max}}$ was significantly lower for the SPIL compared with the reference DXR HCl liposome injection ($p=0.0005$; Fig. 1c). The mean $C_{\text{max}}$ of doxorubicinol was comparable between the SPIL and reference DXR HCl liposome injections in heart and liver tissue, after 6 mg/kg.
Area under the curve: doxorubicinol

The mean heart AUC\textsubscript{0–t} values were comparable for the SPIL and reference DXR HCl liposome injections after either 2.4 or 6 mg/kg (Fig. 1d). The mean liver AUC\textsubscript{0–t} values were comparable for 6 mg/kg SPIL and reference DXR HCl liposome injections, but a significantly lower liver AUC\textsubscript{0–t} value was observed after 2.4 mg/kg SPIL compared with 2.4 mg/kg reference DXR HCl liposome injection (t\textsubscript{crit} = 2.7765 and t\textsubscript{obs} = −4.0255). This reflects the same trend observed in plasma.
Plasma distribution of DXR in Sprague–Dawley rats

Mean plasma \( C_{\text{max}} \) and \( \text{AUC}_{0-t} \) values for Sprague–Dawley rats, following a single intravenous injection with either the SPIL or reference DXR HCl liposome injection, are shown in Fig. 2. At 4 mg/kg, the plasma distribution of the SPIL DXR HCl liposome injection was compared with 2 different batches of the reference product [batch 1 (B1) and batch 2 (B2)] and, at 10 mg/kg, with 2 different lots of the reference product [lot 1 (L1) and lot 2 (L2)]. The different batches and lots of the reference product were also compared with each other.

Maximum concentration

In plasma, the mean \( C_{\text{max}} \) values of total DXR were comparable between all three groups [SPIL and both batches (B1 and B2) of the reference product] at 4 mg/kg. The mean plasma \( C_{\text{max}} \) of encapsulated DXR was significantly lower for the SPIL DXR HCl liposome injection compared with B1 and B2 of the reference product \((p = 0.0126 \text{ and } p = 0.0001)\); however, the \( C_{\text{max}} \) values of B1 and B2 were also significantly different when compared with each other \((p = 0.0240)\). The mean plasma \( C_{\text{max}} \) of free DXR was significantly higher for the SPIL

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**Fig. 2** Plasma and tissue distribution of total DXR and doxorubicinol in Sprague–Dawley rats. a \( C_{\text{max}} \) and b \( \text{AUC}_{0-t} \) of total DXR after dosing with the SPIL or reference DXR HCl liposome injection. c \( C_{\text{max}} \) and d \( \text{AUC}_{0-t} \) of doxorubicinol after dosing with the SPIL or reference DXR HCl liposome injection. Ten animals were analysed per time point. Differences in \( C_{\text{max}} \) were analysed by unpaired \( t \)-test. \( P \) values \( \leq 0.05 \) were considered significant. Differences in \( \text{AUC}_{0-t} \) were analysed by the Bailer–Satterthwaite method, and a difference was considered to be statistically significant when \( \text{abs}(t_{\text{obs}}) \geq t_{\text{crit}} \). AUC area under the concentration–time curve, \( C_{\text{max}} \) mean peak concentration, B1/batch 1, B2/batch 2, DXR doxorubicin, L1/lot 1, L2/lot 2, ns not significant, SPIL Sun Pharmaceutical Industries Limited
DXR HCl liposome injection than for reference DXR HCl liposome injection B2 at 4 mg/kg ($p=0.0354$). Also at 4 mg/kg, the mean plasma $C_{\text{max}}$ of doxorubicinol was significantly lower for the SPIL DXR HCl liposome injection compared with reference DXR HCl liposome injection B2 ($p=0.0217$; Fig. 2c). The mean plasma $C_{\text{max}}$ values of all four analytes (total, free and encapsulated DXR, and doxorubicinol) were comparable between the SPIL and both lots (L1 and L2) of the reference DXR HCl liposome injection, at 10 mg/kg (Fig. 2a).

**Area under the curve**

The mean plasma AUC$_{0-t}$ values of total DXR were comparable between the SPIL and reference DXR HCl liposome injection B1 and B2 at 4 mg/kg. However, the AUC$_{0-t}$ values of total DXR for the SPIL DXR HCl liposome injection were significantly lower compared with reference DXR HCl liposome injection L1 at 10 mg/kg ($t_{\text{crit}} = 1.9955$ and $t_{\text{obs}} = -2.7108$; Fig. 2b). The mean plasma AUC$_{0-t}$ values for doxorubicinol for the SPIL DXR
HCl liposome injection were significantly lower compared with reference product B2 at 4 mg/kg (t_\text{crit} = 2.00030 and t_\text{obs} = -3.82304) and reference product L2 at 10 mg/kg (t_\text{crit} = 1.9908 and t_\text{obs} = -2.3110). However, this was not the case for the SPIL DXR HCl liposome injection compared with reference product B1 at 4 mg/kg and reference product L1 at 10 mg/kg, where plasma AUC\textsubscript{0–}\text{t} values were comparable (Fig. 2d). Mean AUC\textsubscript{0–}\text{t} for free DXR was significantly higher for SPIL compared with reference DXR HCl liposome injection B2 at 4 mg/kg and reference product L2, at 10 mg/kg (p = 0.0351 and p = 0.0137, respectively). The mean AUC\textsubscript{0–}\text{t} in the kidney were significantly lower for the SPIL DXR HCl liposome injection compared with either reference DXR HCl liposome injection B1 or B2, at 4 mg/kg. These findings suggest that at this dose, there may be a difference between the SPIL and reference DXR HCl liposome injections in plasma distribution of encapsulated versus free forms of DXR.

**Tissue distribution of DXR in Sprague–Dawley rats**

**Maximum concentration: total DXR**

Overall, the C_{\text{max}} values of total DXR for most tissues were comparable between SPIL and each batch and lot of the reference DXR HCl liposome injection (Fig. 2a). The mean C_{\text{max}} values of total DXR were significantly lower in the heart for the SPIL DXR HCl liposome injection compared with reference product B2, at 4 mg/kg, and reference product L2, at 10 mg/kg (p = 0.0351 and p = 0.0137, respectively). The mean C_{\text{max}} values of total DXR in the kidney were significantly lower for the SPIL DXR HCl liposome injection compared with reference product B2, at 4 mg/kg, (p = 0.0089), and with reference product L1 and L2, at 10 mg/kg (p = 0.0044 and p = 0.0005). In lung tissue, mean C_{\text{max}} values of total
DXR were also significantly lower for the SPIL than for the reference DXR HCl liposome injection L1 at 10 mg/kg ($p = 0.0019$); however, the $C_{\text{max}}$ values of L1 and L2 were also significantly different in the lung ($p = 0.0002$). The mean $C_{\text{max}}$ value of total DXR in the spleen was significantly higher for SPIL compared with one of the reference DXR HCl liposome injection lots (L1; $p = 0.0155$).

### Area under the curve: total DXR

Overall exposure to total DXR in the heart and kidney was similarly lower for SPIL compared with either of the reference DXR HCl liposome injections at 4 and 10 mg/kg (Fig. 2b). This was also true in the liver at 4 mg/kg, with significantly lower $AUC_{0-\infty}$ for SPIL compared with reference DXR HCl liposome injection B1 and B2; however,
at 10 mg/kg, the SPIL DXR HCl liposome injection was comparable with reference product L1 and L2, in the liver. There was some evidence of differences between the AUC_{0-t} values for SPIL compared with those for one of the batches and lots of the DXR HCl liposome injection in some of the other tissues, but there was no obvious trend to this, and for some tissues, there was a significant difference between mean AUC_{0-t} values of reference product B1 and B2, and between mean AUC_{0-t} values of reference product L1 and L2.

Maximum concentration: doxorubicinol

The mean C_{max} of doxorubicinol was comparable between the SPIL and reference DXR HCl liposome injections in liver tissue, after 4 mg/kg. However, the mean liver C_{max} was significantly lower for 10 mg/kg SPIL compared with reference DXR HCl liposome injection L1 and L2 (p = 0.0274 and p = 0.0315). The mean heart C_{max} of doxorubicinol was significantly lower for SPIL compared with reference product B1 and B2 at 4 mg/kg, and compared with reference product L1 and L2 at 10 mg/kg (Fig. 1c).

Area under the curve: doxorubicinol

The mean liver AUC_{0-t} values of doxorubicinol were comparable for the SPIL and reference DXR HCl liposome injections after either 4 or 10 mg/kg (Fig. 2d). A significantly lower heart AUC_{0-t} value was observed for SPIL compared with reference product B1 and B2 at 4 mg/kg, and compared with reference product L1 and L2 at 10 mg/kg.

Ratios of free to encapsulated DXR

The ratios of estimated free to encapsulated DXR for syngeneic fibrosarcoma-bearing mice and Sprague–Dawley rats, following a single intravenous injection with either the SPIL or reference DXR HCl liposome injection, are shown in Table 2. In syngeneic fibrosarcoma-bearing mice, the ratios of estimated free to encapsulated DXR for all the tissues were comparable between the two products at 2.4 mg/kg (Table 2a). At 6 mg/kg, the ratios of estimated free to encapsulated DXR were also comparable for all the tissues between the two products, except for minor differences in bone marrow, tumour and skin (Table 2b).

In Sprague–Dawley rats, the ratios of estimated free to encapsulated DXR for plasma and all tissues are comparable between the SPIL and reference DXR HCl liposome injection in murine models. To compare the two products in a tumour background, a syngeneic fibrosarcoma mouse model was used.

In syngeneic fibrosarcoma-bearing BALB/c mice, the ratios of estimated free to encapsulated DXR for all the tissues were comparable between the two products, except for minor differences in bone marrow, tumour, and skin at 6 mg/kg. This indicates that the SPIL and reference formulations have similar extents of absorption into the tissues and similar rates of drug release from liposomes. Despite some evidence of biological variations (especially in the liver and kidney), at 2.4 mg/kg, the SPIL and reference products showed generally comparable plasma and tissue distribution profiles in syngeneic fibrosarcoma-bearing BALB/c mice.

Discussion

The availability of a generic DXR HCl liposome injection could potentially improve access to an important anticancer agent. Because the improved benefit-risk profile of Caelyx® results from its altered tissue distribution, a generic liposomal formulation of DXR must demonstrate a comparable plasma and tissue distribution [7]. As it is not possible to study tissue distribution in humans, in vivo nonclinical data are crucial to the regulatory decision of whether to approve a generic DXR liposomal product [7].

The objectives of the studies presented in this paper were to compare the plasma and tissue distribution of the SPIL DXR HCl liposome injection with the reference DXR HCl liposome injection following single intravenous injection in murine models. To compare the two products in a tumour background, a syngeneic fibrosarcoma mouse model was used.

In Sprague–Dawley rats, the ratios of estimated free to encapsulated DXR for plasma and all tissues are comparable between the SPIL and reference DXR HCl liposome injection and the two batches and lots of the reference product, indicating that the test and reference formulations have similar extents of absorption into the tissues and similar rates of drug release from liposomes. The minor differences observed between the SPIL and reference products in some tissues may be attributed to inherent biological variations of the test system, since two different batches of the reference DXR HCl liposome injection also showed some differences. The SPIL and reference products exhibited generally comparable plasma and tissue distribution profiles in Sprague–Dawley rats. Even two different batches and lots of the reference DXR HCl liposome injection failed to show comparable distribution of all the analytes in all the tissues, suggesting any variations of tissue distribution may be caused by the inherent biological fluctuation of the test system.

Based on C_{max} and mean AUC_{0-t} values, there was evidence that the SPIL DXR HCl liposome injection may lead to lower exposure of the cardiotoxic metabolite doxorubicinol in the heart in rats, and also some suggestion of the...
Table 2 Ratios of free to encapsulated DXR

| Tissues             | Free drug/encapsulated drug $C_{max}$ | Free drug/encapsulated drug AUC$_{0-t}$ |
|---------------------|--------------------------------------|----------------------------------------|
|                     | SPIL DXR HCl liposome injection       | Reference DXR HCl liposome injection   | SPIL DXR HCl liposome injection       | Reference DXR HCl liposome injection   |
|                     |                                       |                                        |                                       |
| (a) Ratios of free to encapsulated DXR in syngeneic fibrosarcoma-bearing mice (2.4 mg/kg dose) |                                       |                                        |                                       |
| Plasma              | 0.03                                 | 0.03                                   | 0.04                                 | 0.03                                   |
| Bone marrow         | 1.4                                  | 1.5                                    | 5.3                                  | 4.4                                    |
| Tumour              | 0.1                                  | 0.1                                    | 0.2                                  | 0.4                                    |
| Skin                | 0.7                                  | 1.2                                    | 1.3                                  | 1.2                                    |
| Kidney              | 0.04                                 | 0.04                                   | 0.1                                  | 0.1                                    |
| Heart               | 0.2                                  | 0.1                                    | 0.1                                  | 0.1                                    |
| Spleen              | 0.08                                 | 0.1                                    | 0.1                                  | 0.1                                    |
| Lung                | 0.1                                  | 0.08                                   | 0.1                                  | 0.4                                    |
| Liver               | 0.08                                 | 0.06                                   | 0.2                                  | 0.1                                    |

(b) Ratios of free to encapsulated DXR in syngeneic fibrosarcoma-bearing mice (6 mg/kg dose)

| Tissues             | Free drug/encapsulated drug $C_{max}$ | Free drug/encapsulated drug AUC$_{0-t}$ |
|---------------------|--------------------------------------|----------------------------------------|
|                     | SPIL DXR HCl liposome injection       | Reference DXR HCl liposome injection   | SPIL DXR HCl liposome injection       | Reference DXR HCl liposome injection   |
|                     |                                       |                                        |                                       |
| Plasma              | 0.02                                 | 0.02                                   | 0.03                                 | 0.03                                   |
| Bone marrow         | 1.6                                  | 0.5                                    | 3.9                                  | 1.9                                    |
| Tumour              | 0.3                                  | 0.1                                    | 0.4                                  | 0.2                                    |
| Skin                | 2.2                                  | 0.8                                    | 1.8                                  | 0.9                                    |
| Kidney              | 0.09                                 | 0.09                                   | 0.1                                  | 0.09                                   |
| Heart               | 0.3                                  | 0.3                                    | 0.5                                  | 0.4                                    |
| Spleen              | 0.5                                  | 0.4                                    | 0.8                                  | 0.8                                    |
| Lung                | 0.2                                  | 0.1                                    | 0.2                                  | 0.1                                    |
| Liver               | 0.2                                  | 0.2                                    | 0.5                                  | 0.4                                    |

(c) Ratios of free to encapsulated DXR in Sprague–Dawley rats (4 mg/kg dose)

| Tissues             | Free drug/encapsulated drug $C_{max}$ | Free drug/encapsulated drug AUC$_{0-t}$ |
|---------------------|--------------------------------------|----------------------------------------|
|                     | SPIL DXR HCl liposome injection       | Reference DXR HCl liposome injection   | Reference DXR HCl liposome injection   | Reference DXR HCl liposome injection   |
|                     |                                       |                                        |                                       |
| Plasma              | 0.02                                 | 0.01                                   | 0.02                                 | 0.02                                   |
| Bone marrow         | 1.6                                  | 2.3                                    | 1.3                                  | 1.9                                    |
| Skin                | 1.2                                  | 1.5                                    | 3.3                                  | 2.0                                    |
| Kidney              | 0.7                                  | 0.3                                    | 1.4                                  | 0.7                                    |
| Heart               | 0.5                                  | 0.4                                    | 1.1                                  | 1.1                                    |
| Spleen              | 0.7                                  | 0.3                                    | 1.3                                  | 0.9                                    |
| Lung                | 0.2                                  | 0.3                                    | 0.2                                  | 0.1                                    |
| Liver               | 0.3                                  | 0.2                                    | 0.8                                  | 0.5                                    |

(d) Ratios of free to encapsulated DXR in Sprague–Dawley rats (10 mg/kg dose)

| Tissues             | Free drug/encapsulated drug $C_{max}$ | Free drug/encapsulated drug AUC$_{0-t}$ |
|---------------------|--------------------------------------|----------------------------------------|
|                     | SPIL DXR HCl liposome injection       | Reference DXR HCl liposome injection   | Reference DXR HCl liposome injection   | Reference DXR HCl liposome injection   |
|                     |                                       |                                        |                                       |
| Plasma              | 0.01                                 | 0.01                                   | 0.02                                 | 0.02                                   |
| Bone marrow         | 2.9                                  | 5.0                                    | 3.5                                  | 4.4                                    |
| Skin                | 9.9                                  | 8.4                                    | 14.1                                 | 10.1                                   |
| Kidney              | 0.4                                  | 0.2                                    | 1.5                                  | 0.8                                    |
| Heart               | 0.7                                  | 0.6                                    | 1.3                                  | 1.2                                    |
| Spleen              | 0.7                                  | 0.4                                    | 2.4                                  | 1.9                                    |
| Lung                | 0.3                                  | 0.2                                    | 0.3                                  | 0.3                                    |
same in plasma and the liver, compared with the reference DXR HCl liposome injection. Doxorubicinol is thought to be responsible for the cardiotoxicity associated with DXR. The SPIL DXR HCl liposome injection may therefore be less cardiotoxic in rats. However, we acknowledge that this observation may not carry over to humans, and may be the result of inherent biological variation within the test system. There was also some evidence that the SPIL DXR HCl liposome injection leads to lower exposure of the cardiotoxic metabolite doxorubicinol in the liver (where DXR is metabolised) and potentially in plasma of mice, compared with the reference DXR HCl liposome injection. Because DXR is metabolised in the liver, lower levels of doxorubicinol in this tissue suggests that doxorubicinol should be lower in other tissues as well. This was not the case, so the doxorubicinol differences may be the result of inherent biological variation within the test system.

In conclusion, the plasma and tissue distribution profiles of the SPIL and reference DXR HCl liposome injections were shown to be generally comparable. Further studies, comparing the toxicology of SPIL DXR HCl liposome injection with Caelyx® in murine models, will be published in the future.

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Compliance with ethical standards

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Conflict of interest All authors were paid employees of Sun Pharmaceutical Industries Ltd. at the time the study was conducted.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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