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Compatibility and stability of the novel anticancer agent ES-285 · HCl formulated with 2-hydroxypropyl-β-cyclodextrin in infusion devices

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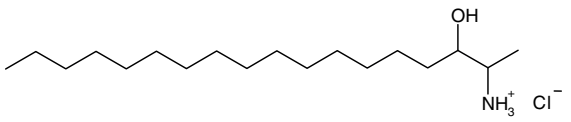
ES-285 · HCl is a novel marine-derived anticancer agent isolated from the clam *Spisula polynyma*. The compound is pharmaceutically formulated as a lyophilised product containing 25 or 50 mg ES-285 · HCl and 500 or 1000 mg 2-hydroxypropyl-β-cyclodextrin per dosage unit and requires reconstitution with sterile water for injection before intravenous administration. The aim of this study was to determine the stability and compatibility of ES-285 · HCl in infusion devices. ES-285 · HCl was shown to be stable at concentrations of 10–1400 μg/ml after dilution in 5% dextrose in water and compatible with PE infusion containers and PE and silicone tubing. No sorption on- or into the administration set was observed at concentrations equal to or above 20 μg/ml. In conclusion, ES-285 · HCl infusion solutions can be administered without stability or sorption problems using a PE infusion container and PE or silicone tubing in concentrations equal or above 20 μg/ml in 3-hour or 24-hour infusion administration schedules.

1. Introduction

ES-285 · HCl (spisulosine; anti-β-amino alcohol; (2*S*,3*R*)-2-aminooctadecan-3-ol hydrochloride) is a novel marine-derived anticancer agent isolated from the clam *Spisula polynyma* and has shown *in vitro* and *in vivo* cytotoxic activity against various tumour cell lines with selectivity for certain solid tumours (i.e. hepatocellular, prostate and renal) (Jimeno et al. 1999). ES-285 · HCl has a novel mechanism



Chemical structure of ES-285 · HCl ($C_{18}H_{40}ClNO$, Mw = 321)

of action different from other anticancer agents; it disrupts the cytoskeleton of cancer cells (Cuadros et al. 2000). Molecular studies suggest that ES-285 · HCl may achieve this by targeting the activity of the GTP-binding protein Rho, a crucial factor for the formation of cytoskeletal fibres, cell migration and tumour cell proliferation (Chrzanowska-Wodnicka and Burridge 1996; Cuadros et al. 2000). The drug is formulated as a lyophilised solid for intravenous use containing 25 or 50 mg ES-285 · HCl and 500 or 1000 mg of 2-hydroxypropyl-β-cyclodextrin (HPβCD) and is currently evaluated in Phase I clinical trials. Before the start of the trial, the stability of ES-285 · HCl in infusion solutions was examined in order to determine the preparation and administration parameters. The potential of ES-285 · HCl infusions to adsorb to contact surfaces was investigated *in vitro* by mimicking the passage of infusion solutions at different concentrations through infusion devices.

2. Investigations, results and discussion

2.1. Preliminary studies

Preliminary studies were performed in order to select the infusion fluid and infusion container most suitable to administer ES-285 · HCl with during Phase I clinical trials. When solubilising drugs in concentrations above their aqueous solubility with the use of solvent systems, dilution in infusion fluids could result in precipitation of the solubilised drug. ES-285 · HCl is formulated as lyophilised product containing 25 or 50 mg ES-285 · HCl with 500 and 1000 mg HPβCD, respectively. Reconstitution with 5 ml sterile water for injection results in ES-285 · HCl and HPβCD concentrations of 8.71 mg/ml (27 mM) and 17.4% (w/w) of HPβCD (124 mM). The shape of the phase-solubility diagram for the ES-285 · HCl/HPβCD system illustrates that upon dilution of the reconstituted product with water no precipitation of ES-285 · HCl is expected (Fig.).
The complexation efficacy of cyclodextrins, however, can be lowered or enhanced as a result of the addition of a third component like dextrose or sodium chloride. High salt (i.e. sodium chloride) concentrations can lower the intrinsic solubility of the drug through the salting-out effect, which will decrease complexation efficacy (Loftsson et al. 1999). In contrast, the presence of carbohydrates have been shown to enhance the drug intrinsic solubility by the so called sugaring-in effect (Loftsson et al. 1999).

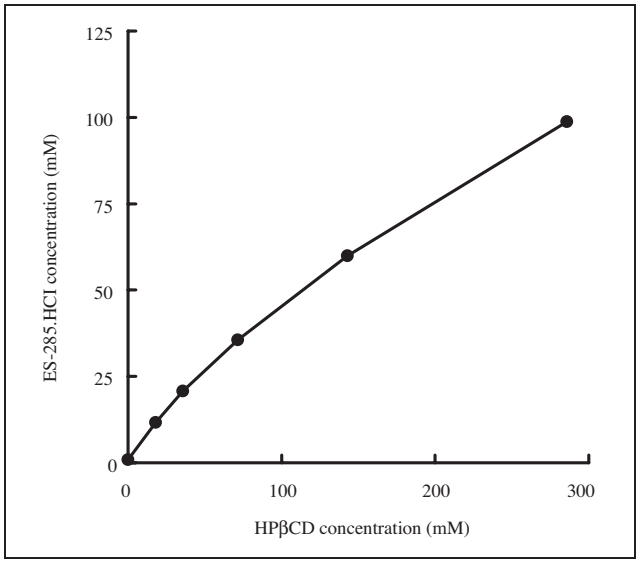


Fig.: Phase solubility diagram for the ES-285 · HCl/HPβCD system in water

No visual precipitation or decrease in ES-285 · HCl concentration was observed upon dilution of ES-285 · HCl 50 mg/vial reconstituted product (reconstituted with 5.0 ml of water) to concentrations of 4.4 mg/ml, 0.87 mg/ml, or 0.17 mg/ml in normal saline of D_5W during 1 h of storage at ambient light and temperature (+20−25 °C). Though, when a solution containing 10 mg/ml ES-285 · HCl in 20% HPβCD (without prior lyophilisation) was diluted in normal saline, precipitation of ES-285 · HCl was observed with 58.0% and 73.0% of the nominal ES-285 · HCl content recovered after dilution to 0.20 mg/ml and 1.0 mg/ml, respectively. Recoveries after dilution in D_5W were 94.8% and 96.7%, respectively. On the basis of these results, D_5W was selected as infusion fluid for ES-285 · HCl.

Several materials are used to construct infusion containers available for intravenously administered drugs, including glass, LD-PE and PVC. PVC infusion systems are known for leaching of the plasticizer DEHP under influence of solutions containing an organic solvent or surfactant (Pearson and Trissel 1993; Nuijen et al. 2001; Jenke 2001). The excipient HPβCD present in ES-285 · HCl infusion solutions has the potential to solubilise and/or stabilise lipophilic drugs via (partial) inclusion in its lipophilic cavity, and was earlier shown to extract the extraneous compound 2-phenylphenol from silicone tubing (den Brok et al. 2004). Indeed, when storing blank infusion solutions containing 3% (w/v) HPβCD in normal saline for 24 h in a PVC container, we found concentrations of 2.8 μg/ml of DEHP. The same solution stored in PVC lining contained 3.3 μg/ml DEHP after 24 h. Lower DEHP concentrations were recovered after incubation with 3% (w/v) HPβCD in D_5W (0.5 μg/ml for the container and 0.8 μg/ml for the lining). The addition of a competing drug for HPβCD complexation was earlier shown to decrease the amounts of 2-phenylphenol extracted from silicone tubing (den Brok et al. 2004). No decrease in the amounts of DEHP extracted from the PVC material, however, was observed when ES-285 · HCl was present in a concentration of 1.5 mg/ml in the 3% (w/v) HPβCD solution. The toxic and carcinogenic effects of DEHP have been well established in experimental animals, but the ability of this compound to produce adverse effects in humans is controversial (Center for Devices and Radiological Health of the US Food and Drug Administration 2001). However, until unambiguously established as non-toxic, the exposure of patients to DEHP should be minimised or avoided. Therefore, ES-285 · HCl infusion solutions should be administered in clinical practice using a non-PVC administration set. LD-PE infusion containers were selected for the infusion simulation experiments as this container is flexible, chip-proof and less likely to produce particle contamination than a glass container. Moreover, in contrast to PVC, LD-PE possesses an intrinsic flexibility which makes the use of plasticizers as DEHP unnecessary.

ES-285 · HCl infusion solutions at concentrations of 70 μg/ml and 1.6 mg/ml in D_5W proved to be stable for at least 72 h and 48 h in PE infusion containers, respectively, with 97.6 ± 5.2% and 103.4 ± 4.0% of the initial concentration remaining.

2.2. Infusion simulation experiments

The stability and compatibility of ES-285 · HCl infusion solutions with infusion devices were tested using infusion simulation experiments which mimic the clinical setting. Infusion solutions were prepared at four concentration levels, based on the total doses expected in Phase I clinical trials of 2.5–350 mg. ES-285 · HCl infusion solutions at concentrations of 20.0 μg/ml and 1400 μg/ml were selected for the 3 h administration and concentrations of 10.0 μg/ml and 700 μg/ml for the 24 h administration schedule.

ES-285 · HCl was stable at each dosage level during the entire infusion simulation with no degradation peaks observed in any of the chromatograms. In the Table, the concentrations of ES-285 · HCl during the infusion simulation experiment and the absolute and relative amount of ES-285 · HCl released using a LD-PE infusion container and PE or silicone lining are given. It can be seen that the total amount of ES-285 · HCl released during the infusion simulation depends on the drug concentration in the infusion solution. Significant sorption was observed only at the lowest concentration tested. The absence of degradation peaks indicates that the decrease in concentration is most likely due to sorption processes. These sorption processes might consist of a combination of adsorption of ES-285 · HCl onto the surface of the administration set material and absorption into the administration set matrix resulting in loss of drug substance. The decrease in solution concentration was already apparent at the first sample point (1 h) and no further decrease was observed during the remaining of the infusion simulation, indicating that ES-285 · HCl sorption was a fast and quickly saturated process. No significant sorption was observed for the infusion solutions at ES-285 · HCl concentrations of 20 μg/ml and higher. The percentage of ES-285 · HCl which was lost during the 24 h infusion period of a 10 μg/ml infusion concentration was found reproducible and limited to approximately 12%. The concentration of 10 μg/ml simulated a "worst case" approach corresponding to a patient with a body surface area of 1.25 m^2 in the lowest administration level of the Phase I clinical trial. Nonetheless, when dosing patients at concentrations below 20 μg/ml, the possibility for sorption should be taken into account.

2.3. Conclusion

The infusion solutions of ES-285 · HCl displayed sufficient stability for the use in 3 h and 24 h infusion schedules when prepared at ES-285 · HCl concentrations ranging from 10–1400 μg/ml in D_5W. Infusion solutions

Table: ES-285 · HCl concentrations (μg/mL) of infusion solutions for the 3-hours and 24-hours infusion simulation study

Time (h)	Initial ES-285 · HCl concentration (μg/mL)					
	PE tubing (ALARIS) 24-hours infusion		PE tubing (ALARIS) 3-hours infusion		Silicone tubing (Maxxim) 3-hours infusion	
	9.50	691	19.4	1427	19.5	1437
0.25	N/A	N/A	18.1 (1.3)	1393 (0.5)	18.6 (0.7)	1423 (2)
0.5	N/A	N/A	19.0 (0.0)	1370 (1.5)	19.1 (0.3)	1424 (26)
1	8.43 (0.03)	682 (7)	19.2 (0.4)	1396 (22)	19.3 (0.1)	1412 (8)
1.5	N/A	N/A	18.9 (0.1)	1428 (8)	19.0 (0.0)	1421 (12)
2	7.74 (0.45)	681 (6)	19.0 (0.4)	1411 (7)	20.0 (0.2)	1412 (7)
2.5	N/A	N/A	18.7 (0.2)	1397 (2)	19.3 (0.2)	1411 (16)
3	8.26 (0.66)	694 (8)	19.0 (0.1)	1401 (7)	19.8 (0.1)	1410 (4)
5	8.64 (0.10)	688 (1)	N/A	N/A	N/A	N/A
8	8.49 (0.30)	698 (6)	N/A	N/A	N/A	N/A
24	8.26 (0.01)	682 (1)	N/A	N/A	N/A	N/A
Total amount of ES-285 · HCl released (mg)	4.14 (0.84)	346 (1)	4.72 (0.01)	350 (1)	4.84 (0.06)	354 (1)
% of the nominal ES-285 · HCl dose	88.4 (1.8)	99.9 (0.3)	97.2 (0.2)	98.3 (0.3)	99.5 (0.2)	98.5 (0.3)

Samples were taken from the outlet of the infusion system. Initial concentrations were determined before introducing the infusion solution into the infusion container. Mean values of two experiments are given with the standard deviation within parenthesis. N/A: not analysed

should be administered using a PE infusion container and PE or silicone tubing. To prevent sorption of ES-285 · HCl to the administration set, infusions should be prepared at concentrations equal or above 20 μg/ml.

3. Experimental

3.1. Chemicals

ES-285 · HCl was provided by PharmaMar Sociedad Unipersonal (Colmenar Viejo, Spain). ES-285 · HCl 25 mg/vial and 50 mg/vial lyophilised products were manufactured in-house (Department of Pharmacy & Pharmacology, Slotervaart Hospital, Amsterdam, The Netherlands). 5% Dextrose in water (D_5W), 0.9% sodium chloride (normal saline) and water for injection (WfI) were obtained from B. Braun (Melsungen, Germany). Diethylhexyl phthalate (DEHP) was obtained from Sigma Aldrich Chemie (Zwijndrecht, The Netherlands) and acetonitrile from Biosolve Ltd. (Amsterdam, The Netherlands).

3.2. Liquid chromatography

ES-285 · HCl content was determined after derivatisation with phenylisothiocyanate using a validated, stability-indicating liquid chromatography (LC)-ultraviolet (UV) absorbance detection method (den Brok et al. 2003).

DEHP was assayed using a series 1100 Agilent HPLC system equipped with a diode array detector (DAD) (Agilent Technologies, Waldbronn, Germany). Separation was achieved using a Zorbax SB-C_{18} analytical column (150 mm · 4.6 mm I.D., particle size 3.5 μm, Rockland Technologies Inc., Newport, DE, USA) with the column temperature kept at +40 °C. A linear gradient of acetonitrile and water (50–100% acetonitrile in 15 min) was used as mobile phase and was pomped at a flow rate of 0.8 ml/min. UV-detection was performed at 228 nm. Samples were injected in a volume of 25 μL and a run time of 20 min was employed. A series of standard solutions of DEHP in acetonitrile in the concentration range of 0.1–300 μg/ml were prepared from a stock solution of 1 mg/ml DEHP in acetonitrile. Least-squares regression analysis was used to calculate the slope and intercept of the standard calibration curves from measured peak areas versus standard concentration. From the obtained peak areas, sample concentrations were calculated using the regression equations.

3.3. Preliminary studies

3.3.1. Phase solubility diagram

A phase solubility diagram of ES-285 · HCl in HPβCD solutions was generated according to the method of Higuchi and Connors (Higuchi and Connors 1965). An excess amount of ES-285 · HCl was suspended in 1.0 ml of solutions containing 0, 5, 10, 20 and 40% w/v HPβCD in water in glass screw-capped test tubes and subsequently shaken at room temperature until equilibrium. The resulting suspensions were centrifuged (4100 · g, 5 min), the supernatant filtered through a 0.45 μm membrane filter (Durapore syringe filter, Millipore, The Netherlands) and analysed for ES-285 · HCl content. Experiments were conducted in duplicate.

3.3.2. Stability upon dilution

ES-285 · HCl 50 mg/vial lyophilised product was reconstituted with 5.0 ml WfI resulting in a ES-285 · HCl concentration of 8.71 mg/ml (total volume of 5.74 ml due to volume displacement by the cake) and further diluted with normal saline or D_5W in glass containers to ES-285 · HCl concentrations of 4.4 mg/ml, 0.87 mg/ml, and 0.17 mg/ml, respectively. Solutions were left to stand at ambient light and temperature (+20–25 °C) for 1 h. The solutions were visually examined for precipitation and LC-UV analysis was used to determine ES-285 · HCl content.

3.3.3. Stability and compatibility in infusion containers

The reconstituted product was diluted with normal saline or D_5W to an ES-285 · HCl concentration of 1.5 mg/ml and incubated for 24 h in glass, low density polyethylene (LD-PE), or polyvinylchloride (PVC) infusion sets. ES-285 · HCl content and purity and DEHP content were determined after 24 h of storage at ambient light and temperature (+20–25 °C).

3.4. Infusion simulation experiments

ES-285 · HCl infusion solutions were prepared at concentrations of 10.0 μg/ml, 20.0 μg/ml, 700 μg/ml, and 1400 μg/ml. The infusion solutions were prepared by dilution of reconstituted solution with D_5W in volumetric flasks. Total volumes of 250 ml (20.0 and 1400 μg/ml) or 500 ml (10.0 and 700 μg/ml) of infusion solution were injected in emptied infusion containers with a polypropylene syringe. Initial ES-285 · HCl concentrations were determined in the final infusion solutions prior to injection into the infusion container. The infusion container used was a multi-layer, co-extruded polyolefin based material with a polyethylene (PE) layer in contact with the solution (Viaflo®, Baxter, Melsungen Germany). Two tubing sets were tested for compatibility with ES-285 · HCl infusion solution: a PE tubing set (ALARIS, San Diego, USA, reference number 2260–0006) and a silicone tubing set (Maxxim Medical Europe, Oss, The Netherlands).

The infusion systems were connected to an infusion pomp (Gemini PC2 infusion pump, ALARIS), which was programmed to deliver the complete contents of the infusion container during a 3 h or 24 h infusion duration. Samples were taken from the needle outlet after 15 min, 30 min, 1 h, 1.5 h, 2.5 h and at the end of infusion for the 3 h infusion duration and after 1 h, 2 h, 3 h, 5 h, 8 h and at the end of infusion for the 24 h infusion duration. Experiments were performed in duplicate at room temperature (+20–25 °C) and ambient light. The total amount of ES-285 · HCl delivered by the infusion system was calculated from the infusion rate and the area under the concentration-time curves (AUCs) eq. 1. The AUCs were calculated using the linear trapezoidal rule.

$$\text{Total amount of ES-285 · HCl delivered (mg)} = \text{AUC (mg/ml · min)} \cdot \text{infusion rate (ml/min)} \quad (1)$$

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