Compatibility and stability of Imexon in infusion devices and its *in vitro* biocompatibility

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Imexon is an investigational anti-cancer agent, pharmaceutically formulated as a lyophilized solid for i.v. infusion requiring reconstitution and subsequent dilution in infusion fluid before infusion. Imexon contains a highly reactive aziridine ring in its structure, which limits its stability in aqueous solutions. In the present study, several *in vitro* studies were conducted to determine the administration parameters for use in the forthcoming phase I clinical trial. The stability of Imexon in the reconstituted solution and infusion solutions was investigated, including its tendency to degrade to its main degradation product BM41.209, and to its hydroxy and chloride adducts. The compatibility of the infusion solution with glass, low-density polyethylene and freeflex polyolefin containers, and its potency to cause vascular irritation and hemolysis upon i.v. infusion were investigated. Imexon was found to be stable for 8 h in the reconstituted product and for 2 h after dilution to infusion solution concentrations in normal saline. The infusion solution was compatible with the freeflex polyolefin container and polyvinylchloride infusion lining, showing no sorption of Imexon during a 15-min infusion duration and no release of the plasticizer diethylhexyl phthalate. Furthermore, Imexon infusion solution showed no indication for vascular irritation or hemolysis upon i.v. infusion, as measured with a static *in vitro* model with incubation with whole blood. In conclusion, Imexon should be administered using a freeflex polyolefin infusion container within 2 h after preparation and a 15-min infusion duration. The results obtained with an *in vitro* model show that no vascular irritation or hemolysis is expected upon i.v. infusion. *Anti-Cancer Drugs* 16:727–732 © 2005 Lippincott Williams & Wilkins.

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Introduction

Imexon (4-imino-1,3-diazabicyclo[3,1,0]-hexan-2-one; Fig. 1) is a 2-cyanoaziridine derivative and has been of interest as an immunomodulator and anti-cancer agent since the late 1970s [1]. Recent *in vitro* research demonstrated high cytotoxic activity for lymphoid malignancies, such as malignant lymphomas and multiple myeloma [2]. Imexon is scheduled for phase I clinical trials for which it was pharmaceutically formulated as a lyophilized solid for i.v. infusion containing 100 mg Imexon, 30 mg polyvinylpyrrolidone and 200 µg polysorbate 80 per dosage unit, freeze-dried from a dimethylsulfoxide solution. Before administration, the product is to be reconstituted with 10/90% (v/v) propylene glycol in water for injection and further diluted with 0.9% (w/v) sodium chloride for infusion (normal saline). Before the start of the phase I clinical trials, the stability of Imexon after reconstitution in its primary container and after subsequent dilution in normal saline in infusion containers composed of glass, low-density polyethylene (LD-PE) and freeflex polyolefin was investigated. The administration of Imexon infusion solutions was mimicked *in vitro* by passage of solutions of different, clinically relevant concentrations through a selected infusion system. Additionally, in order to determine any adverse effect upon i.v. administration of Imexon infusion solution as a consequence of the presence of propylene glycol or other formulation components, *in vitro* biocompatibility studies were conducted.

Materials and methods

Chemicals

Imexon active drug substance and BM41.209 were provided by Heidelberg Pharma (Ladenburg, Germany). Imexon 100 mg/vial lyophilized product was manufactured in-house (Department of Pharmacy and Pharmacology, Slotervaart Hospital, Amsterdam, The Netherlands). Water for chromatography, disodium hydrogen phosphate and sodium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany). All chemicals were of analytical grade and were used without further purification.

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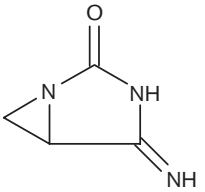
Fig. 1



Chemical structure of Imexon ($C_4H_5N_3O$, MW = 111).

Normal saline and Water for Injection (WfI) were obtained from Braun (Melsungen, Germany). Fresh, citrated blood originated from the local blood bank (Central Laboratory for Blood Transfusion, Amsterdam, The Netherlands).

Chromatography

Imexon was assayed with a validated, stability-indicating reversed-phase high-performance liquid chromatography (HLPC) method. The HPLC system consisted of an 1100 Series binary HPLC pump, model G1312A (Agilent Technologies, Amstelveen, The Netherlands), a Spectra Series AS3000 automatic sample injection device, equipped with a 100-µl sample loop (Thermo Separation Products, Breda, The Netherlands) and a Waters 996 photodiode array detector (Waters Chromatography, Etten-Leur, The Netherlands). Chromatograms were processed using Chromeleon software (Dionex, Sunnyvale, CA). Separation was achieved with a Zorbax Bonus RP analytical column (150 mm × 4.6 mm ID, particle size 3.5 µm; Rockland Technologies, Newport, Delaware, USA), which was protected by a guard column packed with reversed-phase material (3 × 10 mm) (Chrompack, Middelburg, The Netherlands). The mobile phase, pumped at a flow rate of 0.8 ml/min, consisted of phosphate buffer (50 mM; pH 6). UV detection was performed at 230 nm and a run time of 20 min was employed. Samples were diluted to a concentration of 20 µg/ml with phosphate buffer (20 mM; pH 7.4) and analyzed immediately after preparation to prevent degradation after sampling. The injection volume was 10 µl.

Diethylhexyl phthalate (DEHP) release from the polyvinylchloride (PVC) lining used in the infusion simulation studies was analyzed using a reversed-phase HPLC method [3]. Samples were injected undiluted into the system to determine whether any DEHP was present.

Preparation of admixtures

Imexon 100 mg/vial lyophilized product was reconstituted with 10.0 ml of propylene glycol/WfI 10/90% (v/v) resulting in a concentration of 9.92 mg/ml (taking the volume displacement of the lyophilized powder of 80 µl into consideration). Infusion solutions at Imexon concentrations of 0.5 and 5.0 mg/ml were prepared in normal saline. For this, the appropriate volume of reconstituted solution (5 and 50 ml, respectively) was extracted from the vial(s) with a syringe (Luer Lock; Becton Dickinson, Franklin Lakes, New Jersey, USA) and an aluminum needle (1.2 × 38 mm; CODAN, ref. 22.1853) and added to the infusion container, of which an equal volume of normal saline was extracted making up a final volume of 100 ml. As overage volumes of infusion solution are added as standard practice to commercially available infusion containers (of which the precise amount can vary between defined ranges), the nominal Imexon concentrations present in the infusion containers were calculated using the exact volume present in the infusion containers as determined by weighing of the filled and emptied containers. Infusion solutions were prepared at ambient light and temperature (20–25°C) conditions.

Stability after reconstitution and in infusion containers

The reconstituted solution was monitored for solution pH, chromatographic purity and Imexon concentration immediately after reconstitution, and after 1, 3, 5, 8 and 24 h of storage. Stability of Imexon infusion solutions was examined in glass (Braun), LD-PE (Ecoflac; Braun) and freeflex polyolefin (Freeflex; Fresenius Kabi, Bad Homburg, Germany) infusion containers. The infusion containers were stored at ambient light and temperature (20–25°C). Samples from the infusion solutions were taken immediately after preparation, and after 1, 2, 4 and 24 h of storage and analyzed immediately for Imexon concentration and chromatographic purity with HPLC-UV analysis. The pH was measured immediately after infusion preparation, and after 2 and 24 h of storage. Experiments were performed in duplicate.

Infusion simulation experiments

Imexon infusion simulation was conducted using an infusion system consisting of a freeflex polyolefin infusion container containing 100 ml normal saline, PVC lining (Infusomat; Braun) and an aluminum needle (1.2 × 38 mm; CODAN, ref. 22.1853). The 15-min infusions of 100 ml of 0.5 and 5.0 mg/ml Imexon infusion solutions were simulated at ambient light and temperature (20–25°C). Samples were taken from the needle outlet 0, 5, 10 and 15 min after initiating the infusion simulations, and analyzed for Imexon content. The samples were also analyzed for DEHP concentration and solution pH was determined after completion of the infusion simulation. Experiments were performed in duplicate. The total amount of Imexon delivered by the infusion system was calculated from the infusion rate and the area under the Imexon concentration–time curves (AUCs): total amount Imexon delivered (mg) = AUC (mg/ml·min) × infusion rate (ml/min). The AUCs were calculated using the linear trapezoidal rule: total amount Imexon delivered (mg) = AUC (mg/ml·min) × infusion rate (ml/min).

Hemolysis studies

The potential of the Imexon formulation to induce cell lysis upon continuous i.v. infusion was tested using a static *in vitro* model for hemolysis. Fresh, citrated human blood with a labeled hematocrit content of 40% was used. Aliquots of 170 and 300 µl of infusion solution at a concentration of 5 mg/ml Imexon were added to 1 ml of whole blood and slowly whirl-mixed for 5 s. The solution was incubated at 37°C for 15 min before quenching any hemolytic reaction with 100 ml of normal saline. An aliquot of 3.0 ml of the resulting solution was centrifuged (10 min, 3000 r.p.m.) and cell lysis was measured using the absorption (A) of hemoglobin present in the supernatant at 540 nm with a UV-Vis 918 spectrophotometer (GBC Scientific Equipment, Victoria, Australia). The baseline cell lysis level was determined with normal saline as the test solution at both formulation:blood ratios. The 100% cell lysis level was determined by diluting 1 ml of blood with a 100-fold volume of distilled water instead of normal saline. As a positive control, a mixture of 40/10/50% (v/v/v) propylene glycol/ethanol/water (PEW) was used, known to cause extensive hemolysis upon i.v. injection [4]. Experiments were performed in triplicate. The percentage cell lysis induced by the test solutions was calculated using: % hemolysis $= [(A_{\text{test solution}} - A_{\text{normal saline}})/(A_{\text{water}} - A_{\text{normal saline}})] \times 100\%$.

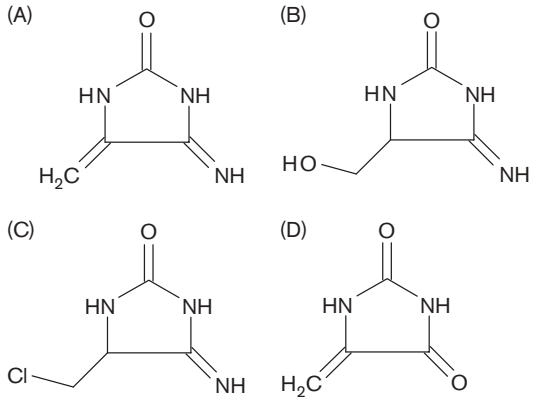
Results and discussion

Stability after reconstitution

Inherent to the reactivity of the aziridine ring present in the molecule, which has been shown to be crucial to its cytotoxic activity [5], Imexon was shown to degrade to several degradation products lacking this ring structure upon accelerated stress testing (Fig. 2) [6]. The proposed degradation mechanism involves a nucleophilic attack on the preferably protonated aziridine ring by water or other nucleophils resulting in the opening of the aziridine ring to release ring strain energy. The main degradation product was BM41.209 (Fig. 2A), which was also the main degradation product observed upon stability testing of the lyophilized product.

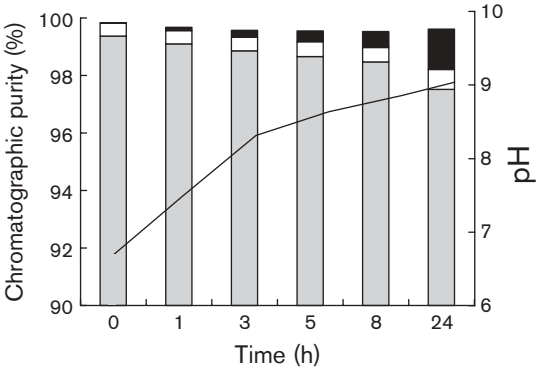
Stable reconstituted solutions were defined as slightly yellow solutions, containing no precipitate, more than 90.0% of the nominal Imexon concentration and a maximum of 0.5% of total peak area of BM41.209 in the HPLC chromatogram. The specification for BM41.209 percentage was based on the low solubility of this compound (the maximum aqueous solubility is approximately 1.5 µg/ml). Incomplete reconstitution upon manual shaking was observed for the lyophilized product when BM41.209 was present in chromatographic percentages above 0.9% of the Imexon peak area, which was found to correspond to a concentration above 0.43 µg/ml BM41.209 (Imexon concentration of 20 µg/ml). Immediate and complete reconstitution was observed when BM41.209 chromatographic percentages of 0.5% or below were present. The reconstituted solution was shown to be stable for 8 h of storage at ambient light and temperature (20–25°C), with 0.5 ± 0.0% of BM41.209 present in the solution and 96.8 ± 0.8% of the initial Imexon concentration remaining. No precipitate was observed in the slightly yellow solution. The chromatographic purity profile and pH values of the reconstituted solution in time are shown in Fig. 3. The most pronounced degradation product after 24 h of storage was the hydroxy derivative of Imexon (Fig. 2B). The upward trend in pH over time indicates acid consumption during degradation.

Fig. 2



Structures for Imexon degradation products with (A) BM41.209, (B) 4-hydroxymethyl-5-imino-imidazolidin-2-one (hydroxy adduct), (C) 4-chloromethyl-5-imino-imidazolidin-2-one (chloro adduct) and (D) BM41.237.

Fig. 3



Chromatographic purity profile and pH values (continuous line) for Imexon reconstituted product during storage at ambient light and temperature (20–25°C) with Imexon (grey), BM41.209 (white) and the hydroxy adduct of Imexon (black).

Stability and compatibility after dilution

Imexon infusion solutions were prepared in normal saline. One of the known degradation products of Imexon is the chloro adduct depicted in Fig. 2(C), which was formed during accelerated stress conditions under acidic conditions in the presence of HCl [6]. ThioTEPA, which contains three aziridine moieties, also showed degradation to its chloro adducts at neutral solution pH (pH 5.5–7.5) upon dilution with normal saline. Unacceptable levels of chloro adducts [determined at 0.6% (chromatographic relative peak area) for ThioTEPA] were observed after 24 h of storage of ThioTEPA diluted with normal saline to a concentration of 2.5 mM at 8 or 25°C [7].

To examine the stability of Imexon when diluted with normal saline, infusion solutions were prepared in different infusion containers at two concentration levels based on the total doses expected in the phase I clinical trial of approximately 50–500 mg and an infusion volume of 100 ml. Results of the stability studies of Imexon in infusion solutions at concentrations of 0.5 and 5.0 mg/ml stored in glass, LD-PE and freeflex polyolefin infusion containers containing a total volume of 100 ml are given in Tables 1 and 2. The degradation of Imexon was shown to be concentration dependent with a slightly increased degradation rate observed at the 0.5 mg/ml concentration level (chromatographic purity of 92 versus 94% after 24 h), with increasing levels of, in particular, BM41.209.

Table 1 Stability of Imexon after dilution in normal saline to approximately 0.5 mg/ml in glass, LD-PE or freeflex polyolefin containers during storage at ambient light and temperature (20–25°C) [values are given as means (SD)]

Container	Nominal concentration (mg/ml)	Item	Initial	1 h	2 h	4 h	24 h
Glass	0.50 mg/ml	A	95.6 (2.6)	94.6 (1.6)	93.9 (1.2)	91.9 (1.1)	81.4 (1.2)
		B	99.5 (0.0)	99.4 (0.0)	99.2 (0.0)	98.5 (0.1)	92.2 (0.1)
		C	0.38 (0.01)	0.40 (0.00)	0.52 (0.01)	1.15 (0.11)	6.43 (0.07)
		D	0.11 (0.03)	0.11 (0.00)	0.17 (0.01)	0.31 (0.04)	1.28 (0.05)
		E	0.00 (0.00)	0.04 (0.00)	0.09 (0.01)	0.09 (0.02)	0.10 (0.02)
		pH	6.7 (0.1)	NA	7.5 (0.1)	NA	7.9 (0.4)
PE	0.44 mg/ml	A	96.7 (1.3)	96.2 (1.3)	94.7 (1.9)	92.9 (1.2)	80.9 (2.2)
		B	99.5 (0.0)	99.3 (0.2)	99.1 (0.1)	98.7 (0.1)	92.1 (0.1)
		C	0.37 (0.01)	0.51 (0.14)	0.65 (0.14)	0.95 (0.10)	6.54 (0.12)
		D	0.12 (0.05)	0.18 (0.08)	0.19 (0.02)	0.27 (0.01)	1.32 (0.03)
		E	0.00 (0.00)	0.01 (0.02)	0.05 (0.03)	0.09 (0.01)	0.07 (0.04)
		pH	6.6 (0.2)	NA	7.4 (0.1)	NA	8.1 (0.1)
Freeflex polyolefin	0.48 mg/ml	A	98.6 (0.4)	98.7 (1.0)	96.9 (0.5)	93.9 (0.1)	83.6 (0.1)
		B	99.5 (0.1)	99.3 (0.0)	99.2 (0.1)	98.7 (0.1)	92.5 (0.1)
		C	0.38 (0.01)	0.46 (0.01)	0.49 (0.03)	0.91 (0.07)	6.13 (0.08)
		D	0.10 (0.04)	0.16 (0.04)	0.17 (0.01)	0.27 (0.00)	1.25 (0.02)
		E	0.00 (0.01)	0.02 (0.01)	0.08 (0.01)	0.09 (0.01)	0.12 (0.00)
		pH	6.0 (0.1)	NA	7.1 (0.3)	NA	8.0 (0.0)

A = percentage of nominal concentration; B = chromatographic purity; C = percentage peak area BM41.209; D = percentage peak area hydroxy adduct; E = percentage peak area chloro adduct. NA = not analyzed.

Table 2 Stability of Imexon after dilution in normal saline to approximately 5 mg/ml in glass, LD-PE or freeflex polyolefin containers during storage at ambient light and temperature (20–25°C) [values are given as means (SD)]

Container	Nominal concentration (mg/ml)	Item	Initial	1 h	2 h	4 h	24 h
Glass	5.0 mg/ml	A	100.5 (3.2)	97.5 (1.3)	97.5 (3.7)	95.2 (0.2)	82.0 (0.6)
		B	99.5 (0.0)	99.4 (0.0)	99.3 (0.0)	98.7 (0.1)	94.0 (0.2)
		C	0.36 (0.02)	0.41 (0.02)	0.50 (0.01)	0.90 (0.06)	4.24 (0.12)
		D	0.13 (0.01)	0.16 (0.01)	0.22 (0.00)	0.36 (0.00)	1.43 (0.03)
		E	0.00 (0.00)	0.01 (0.01)	0.02 (0.03)	0.04 (0.00)	0.02 (0.00)
		pH	6.8 (0.1)	NA	8.3 (0.1)	NA	8.8 (0.0)
PE	4.4 mg/ml	A	99.2 (0.7)	96.7 (0.1)	97.0 (3.2)	93.4 (0.4)	81.5 (0.3)
		B	99.5 (0.0)	99.2 (0.0)	99.1 (0.2)	98.7 (0.0)	94.0 (0.1)
		C	0.39 (0.02)	0.54 (0.07)	0.60 (0.15)	0.92 (0.02)	4.48 (0.05)
		D	0.16 (0.02)	0.25 (0.06)	0.28 (0.05)	0.36 (0.01)	1.39 (0.01)
		E	0.00 (0.00)	0.01 (0.02)	0.02 (0.01)	0.04 (0.00)	0.02 (0.01)
		pH	6.8 (0.2)	NA	8.3 (0.1)	NA	8.7 (0.0)
Freeflex polyolefin	4.8 mg/ml	A	94.6 (1.4)	93.7 (0.8)	93.7 (0.5)	92.6 (0.6)	78.3 (2.1)
		B	99.5 (0.0)	99.4 (0.1)	99.2 (0.1)	98.6 (0.2)	93.9 (0.1)
		C	0.38 (0.01)	0.40 (0.03)	0.50 (0.04)	0.93 (0.17)	4.37 (0.06)
		D	0.13 (0.03)	0.18 (0.00)	0.24 (0.0)	0.38 (0.03)	1.46 (0.01)
		E	0.00 (0.00)	0.01 (0.01)	0.02 (0.00)	0.02 (0.00)	0.00 (0.00)
		pH	6.8 (0.0)	NA	7.8 (0.1)	NA	8.9 (0.0)

A = percentage of nominal concentration; B = chromatographic purity; C = percentage peak area BM41.209; D = percentage peak area hydroxy adduct; E = percentage peak area chloro adduct. NA = not analyzed.

The degradation rate of Imexon to the hydroxy adduct, on the other hand, was increased at the 5.0 mg/ml Imexon concentration level. The chloro adduct of Imexon (with the same absorption maximum as Imexon) was formed in the solutions within 1 h of storage and more pronounced at the 0.5 mg/ml Imexon concentration level. BM41.209 was the main degradation product at both Imexon concentrations.

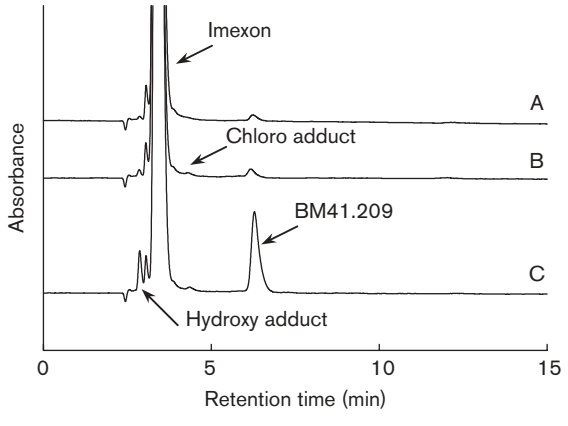
The infusion solutions were stable for 2 h in glass and freeflex polyolefin containers, and for 1 h in LD-PE containers. The maximal percentage of chloro adducts measured in the solutions after these storage periods was $0.09 \pm 0.01\%$. This percentage is far below the percentage of 0.6% defined as acceptable for ThioTEPA. Representative chromatograms of the infusion solutions stored in the freeflex polyolefin containers are shown in Fig. 4.

In conclusion, either glass or freeflex polyolefin containers can be used for the administration of Imexon infusion solutions when administered within 2 h after preparation. For practical reasons (e.g. breakage of the glass container), however, the freeflex polyolefin container was selected for further investigations.

Infusion simulation experiments

In order to set the final administration parameters, infusion simulation experiments were performed with freeflex polyolefin containers containing Imexon infusion solution at concentrations of 0.5 and 5.0 mg/ml in normal saline, a PVC infusion line, and an aluminum needle. The intended infusion duration of 15 min for the phase I clinical trial was used in the *in vitro* infusion simulation study.

Fig. 4



Representative chromatograms of Imexon infusion solution at a concentration of 0.5 mg/ml in a freeflex polyolefin container after (A) 0, (B) 2 and (C) 24 h of storage, under ambient temperature (20–25°C) and light conditions.

Table 3 Imexon concentrations (mg/ml) of infusion solutions in normal saline during the infusion simulation study (samples taken from the outlet of the infusion systems) [values are given as means (SD) of two experiments]

Time (min)	Nominal Imexon concentration (mg/ml)	
	0.475 (0.005)[a]	4.75 (0.04)[a]
0	0.467 (0.004)	4.55 (0.15)
5	0.462 (0.000)	4.68 (0.21)
10	0.469 (0.002)	4.56 (0.08)
15	0.464 (0.000)	4.61 (0.06)
Total amount of Imexon released (mg)	46.6 (0.1)	461 (6)
Percentage with respect to the nominal Imexon dose	98.1 (1.3)	97.0 (2.1)
pH	6.6 (0.2)	8.1 (0.3)

[a]The Imexon concentration was corrected for the exact volume of normal saline present in the infusion container (103–105 ml nominal volume).

Results of the infusion simulation experiments are given in Table 3, including the pH values of the infusion solutions. No lag time was observed in the effluent concentration and thus no sorption of Imexon to the infusion material took place. The final Imexon released after a 15-min infusion duration was 97–98% of the nominal amount.

The PVC material from the infusion lining is known for leaching of the plasticizer DEHP under influence of solutions containing an organic solvent or surfactant [8–10]. The potential of Imexon infusion solutions, which contain a small amount of the surfactant polysorbate 80 (2.5–25 μg/ml), to extract DEHP from the infusion lining was investigated. No DEHP could be detected in any of the samples collected during the infusion simulation (detection limit 0.5 μg/ml).

In conclusion, when using a freeflex polyolefin infusion container connected to a PVC infusion tubing, no loss of Imexon was observed during the 15-min infusion duration and no DEHP was detected in the infusion solution.

In vitro biocompatibility

Adverse effects that can result from the i.v. administration of a pharmaceutical formulation are hemolysis, phlebitis and pain. The i.v. administration of propylene glycol has been associated with all of these adverse effects [11–14]. Concentrations up to 40% propylene glycol are used in marketed products for i.v. injection with precautions (e.g. slow injections, injections into large veins, flushing with normal saline post-injection) frequently included in the administration instructions to reduce vascular irritation [15]. The induced side-effects have been linked to high hypertonicity of propylene glycol containing formulations (iso-osmotic value of 2.1%) [16]. For the Imexon infusion solutions, propylene glycol almost solely accounts for the osmolality resulting in an osmotic value of 2.8 sodium chloride equivalents at an

Imexon infusion concentration of 5 mg/ml (containing 5%(v/v) propylene glycol). This is far less hyperosmotic than reported for some marketed products containing propylene glycol with sodium chloride equivalents up to 35 [16].

The potential of Imexon to cause hemolysis and vascular irritation upon i.v. infusion was tested using *in vitro* test models.

Vascular irritation

During bolus injections with rather short contact times between the venous wall and formulation solution, prolonged contact of precipitated drug with the vein wall cells is believed to be the primarily cause of phlebitis [17]. Precipitation of Imexon upon i.v. administration is not expected as the aqueous solubility of Imexon is sufficiently high (above 50 mg/ml) to prevent precipitation of Imexon upon i.v. infusion at each formulation: blood ratio. Prolonged contact between the components of the infusion solution and the venous wall cells will, however, occur as a result of the administration of Imexon as a 15-min infusion. The potential of Imexon infusion solutions to cause tissue damage was tested using a static *in vitro* model with erythrocytes as surrogate for venous wall cells [17,18] and using a contact time of 15 min.

The formulation:blood ratio used was based on the intended infusion rate of 6.7 ml/min (100 ml, 15-min infusion duration) and the approximate venous blood flow of 40 ml/min, resulting in a formulation:blood ratio of 0.17. An additional formulation:blood ratio of 0.30 was tested.

Except for the reference solution PEW 40/10/50% (v/v/v), which showed 87.0 ± 1.3 and 87.9 ± 0.2 % cell lysis for the formulation:blood ratios of 0.17 and 0.3, respectively, no cell lysis was detected for any of the samples tested. As the Imexon infusion solution did not cause any cell lysis at the highest infusion concentration expected in the phase I clinical trial, also at a formulation:blood ratio corresponding to almost twice the expected infusion volume, no vascular irritation resulting from tissue damage is expected upon administration of Imexon.

Hemolysis

Hemolysis is associated with serious medical conditions such as renal dysfunction, splenomegaly, jaundice and kernicterus [4]. Upon i.v. infusion, the contact time between the formulation and erythrocytes will be as short as 1 s as a result of the immediate formulation dilution in the blood stream [19]. As Imexon infusion solutions were shown not to cause hemolysis upon contact times of 15 min, no hemolysis is expected to occur upon i.v. administration at the expected dosing range of the phase I clinical trial.

Conclusion

The highly reactive compound Imexon displayed sufficient stability after reconstitution of the lyophilized product and subsequent dilution in normal saline for its use in the forthcoming phase I clinical trial. The infusion solution should be administered using a freeflex polyolefin infusion container within 2 h after preparation and a 15-min infusion duration. The results from *in vitro* biocompatibility tests show that no vascular irritation or hemolysis is expected upon i.v. infusion of Imexon.

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