DEVELOPMENT OF A TECHNOLOGY FOR PRODUCING A STABLE INJECTABLE DOSAGE FORM OF A HYDROPHOBIC INDOLOCARBAZOLE DERIVATIVE

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ABSTRACT

Objective: Development of a technology for the production of a stable injectable dosage form (IDF) of indolocarbazole derivative LHS-1269.

Methods: LHS-1269 is an active pharmaceutical ingredient that was synthesized in the N. N. Blokhin National Medical Research Center of Oncology of the Ministry of Health of the Russian Federation. The IDF includes dimethyl sulfoxide (DMSO), 95% ethanol, Kollidon® 17PF and water for injection. Magnetic stirrer and overhead stirrer with a propeller stirring element were used to prepare the model solution of the IDF of LHS-1269. Sterilizing filtration of the solution was performed with 0.20-0.22 μm polycarbonate, cellulose, polyvinylidene fluoride, polyethersulfone and nylon membrane filters. The aqueous solution of LHS-1269 was lyophilized in Edwards Minifast D0.2 freeze dryer. Assay of LHS-1269 was performed by spectrophotometry at 320±3 nm. Potentiometry was used to measure pH, a viscosimetry method was used to measure the viscosity of the solutions.

Results: 0.5% aqueous solution of LHS-1269 was produced by mixing the solution of the active substance in DMSO and ethanol with an aqueous solution of polylvinyllpyrrolidone gradually at the ratio of LHS-1269/DMSO/ethanol/Kollidon® of 1/1/11/32/40 by weight. The aqueous solution of the study substance cannot be lyophilized, so a sequence of technological operations was presented to produce an anhydrous concentrate “LHS-1269, concentrate for solution for injection and infusion 25 mg”.

Conclusion: A technology was developed to produce a stable IDF of a hydrophobic indolocarbazole derivative LHS-1269, a high-potential antitumor drug.

Keywords: Hydrophobic indolocarbazole derivative, Dimethyl sulfoxide, Solubilization, Dosage form, Freeze drying, Concentrate

INTRODUCTION

Indolocarbazoles are natural and synthetic compounds that have attracted considerable attention due to their distinctive structural features and therapeutic potential. The class of indolocarbazoles includes 5 subclasses with different structures of the planar aromatic ring. The subclasses correspond to 5 isomers of the polycycle structure: indolo[2,3-a]carbazole, indolo[3,2-c]carbazole, indolo[2,3-c]carbazole, indolo[3,2-a]carbazole and indolo[3,2-b]carbazole. The widest, most biologically significant, and well-known is the indolo[2,3-a]carbazole subclass that includes mostly substances with a structural basis of indolo[2,3-a]pyrrolo[3,4-c]carbazole ring, where 2 indole fragments are connected to amide or imide group via benzene ring. The indole fragments are connected to the carbohydrate fragment via 1 link (rebeccamycin group) or 2 links (staurosorine and its derivatives) [1].

Clinical studies of various indolocarbazole derivatives demonstrated a wide range of potential therapeutic indications and good compatibility with the known drugs. Indolo[2,3-a]carbazoles have the most pronounced antitumor effect, in line with the antibacterial, antiparasitic and other types of biological activity. The antitumor effect is based on various mechanisms of action, including intercalation in DNA, inhibition of topoisomerases I and II, protein kinases C and A, cyclin-dependent kinases [2, 3].

The chemical synthesis laboratory of N. N. Blokhin National Medical Research Center of Oncology of the Ministry of Health of the Russian Federation developed an original technology to produce indolo[2,3-a]pyrrolo[3,4-c]carbazole-5,7-dione N-glycosides (fig 1) with a cytotoxic and antitumor activity [4]. Up to date, this method has been used to synthesize and study more than 300 indolocarbazole derivatives. A few active compounds with different carbohydrate residues, including LHS-1269, were selected as antitumor drug candidates for the next phases of development.
LHS-1269 substance (N. N. Blokhin National Medical Research Center of Oncology of the Ministry of Health of Russian Federation), dimethyl sulfoxide (DMSO) (Klimmed, Russia); 95% ethanol (Flora Kavkaza OSIC, Russia), Kollidon® 17PF (BASF The Chemical Company, Germany), water for injections and purified water. Nylon membrane filters with a diameter of 25 mm and a pore size of 0.22 um ( Pall Eurasia LLC, Russia); polycarbonate membrane filters Nuclepore with a diameter of 25 mm and a pore size of 0.2 um (Whatman, Great Britain); cellulose ester filter with a diameter of 25 mm and a pore size of 0.22 um (Merck Millipore, Ireland); polyvinylidene fluoride membranes Durapore with a diameter of 25 mm and a pore size of 0.22 um (Millipore, Ireland); Express Plus® polyethersulfone membrane filters with a diameter of 25 mm and a pore size of 0.22 um (Merck Millipore Ltd., Ireland); Stericup® GF Millipore Express® Plus filtration system with polyethersulfone filters with a pore size of 0.22 um (Merck KGaA, Germany).

**Materials and Methods**

**Equipment**

Laboratory scales DI-120 (AND, Japan), mechanical dispenser Proline Prospenser 1–10 ml (Sartorius, Germany), magnetic stirrer IKA® C-MAG HS 4 (IKA -Werke GmbH and Co KG, Germany), overhead mechanical stirrer RZR 2021 Heidolph with propeller stirring element PR 30 Heidolph (Heidolph, Germany), water bath Büchi Heating Bath B-490 (Büchi Labortechnik AG, Switzerland), Lipex™ extruder (Northern Lipids, Canada), Millipore WP6122050 vacuum pressure pump (Merck, Billerica, MA, USA), water for injection system УВОИ-Ф/1812 (MedianaFilter, Russia), freeze dryer Edwards Minifast D0.2 (Bro Electronic SpA, Italy), semiautomatic device ПФР-34-ВПЛС-МЕД for vial capping (VIPS-MED LLC, Russia), spectrophotometer Cary 100 (Agilent Technologies, Australia), vibrating viscometer Vibro Viscometer Y-10 (AND Company Limited, Japan), pH meter HANNA HI 2211 (Hanna Instruments, Romania).

**Methods**

**Production of an injectable dosage form of LHS-1269**

The solubilization, dissolution, filtration and freeze-drying steps were performed after preparing model solutions of the studied indolocarbazole derivative.

**Quantitative analysis of LHS-1269 in the model solutions**

Assay of the active substance was performed using spectrophotometry with a working reference standard (RS) at 320±3 nm.

**Analysis of pH of LHS-1269 solution**

The pH value of the samples of LHS-1269 dosage form was determined at 21 to 25 °C. Procedure: Add 8 ml of purified water to the concentrate and mix to obtain a homogenous solution and measure the pH value.

**Analysis of viscosity of LHS-1269 solution**

The dynamic viscosity of the samples of LHS-1269 dosage form was determined at 21 to 25 °C. Procedure: Add 2.9 ml of purified water to the concentrate and mix to obtain a homogenous solution with a 5 mg/ml concentration of the active substance. Mix the obtained solutions from 2 vials and measure the viscosity.

**Estimation of an average weight of the vial contents**

Ten filled vials with concentrate were sampled. The labels were removed, the vials were weighed individually. The contents were quantitatively removed from each vial, the vials were thoroughly rinsed inside. Each vial was dried and weighed again. The weight of the contents was calculated as the difference between the weight of the filled vial and the empty vial.

**RESULTS AND DISCUSSION**

While developing a water-soluble LHS-1269 dosage form, we considered a few pharmaceutical technological approaches that comply with the physical and chemical properties of the active substance. The approaches included solubilization/complex formation, production of solid dispersion, change of the pH/salt formation and inclusion into biocompatible phospholipid vesicles, i.e., liposomes. A comparative study of the produced models of the injectable dosage form was conducted. The most effective in vivo and the most technologically acceptable composition included DMSO and ethanol as co-solvents of the hydrophobic substance, solubilizer/complexing agent Kollidon® 17PF with 1/11/32/40 ratio of LHS-1269/DMSO/ethanol/Kollidon® by weight. Concentration of the active substance in the aqueous solution was 5 mg/ml.

Procedure: To prepare an aqueous solution of LHS-1269, add DMSO to the portion of the active substance in 1/11 ratio by weight and mix to obtain a clear red and orange solution. Add a respective volume of ethanol to the solution of LHS-1269 in DMSO. Color of the solution will intensify. Dissolve Kollidon® 17PF in water for injection to obtain a clear colorless solution. Heat the mixture to 50–60°C on a water bath to speed up the dissolution process. Then add the aqueous solution of Kollidon® portion-wise to the organic solution of LHS-1269. Note that the mixture will become opaque if the LHS-1269 solution is added to the Kollidon® solution. Filter the resulting solution of LHS-1269 through a 0.22 um membrane filter to sterilize it.

Permeability of membranes during filtration of LHS-1269 aqueous solution was evaluated for 5 types of filters: nylon (N), polyethersulfone (PES), polyvinylidene fluoride (PVDF), cellulose ether (CE) and polycarbonate (PC) (Table 1).

**Table 1: Content of LHS-1269 in the solution depending on the type of filter**

| Content of LHS-1269 in the solution, % | Before filtration | After filtration, type of filter |
|--------------------------------------|-------------------|----------------------------------|
|                                      | N                 | PES                                |
|                                      | 100               | 98.0±0.53                         |
|                                      | 97.7±0.50         | 97.2±0.69                         |
|                                      | 97.4±0.51         | 97.2±0.42                         |

Note. The data are given as mean±SD, n=3

The data analysis showed that the content of LHS-1269 in the solution is almost independent of the type of filter. The loss of the active substance was minimal with the use of nylon and polyethersulfone filters.
The solution of LHS-1269 becomes opaque and forms a precipitate after 3 d of storage at +4 °C. Since the model dosage form has low stability, the potential improvement of the stability by freeze drying was considered. Freeze drying of the solution of LHS-1269 was performed by long-term incubation at -45-47 °C because the solution contains organic solvents with low eutectic points [12]. The sterile aqueous solution of LHS-1269 was dispensed in the portions of 3 ml into 20 ml vials, placed at the shelves of the freeze dryer and frozen at -47 °C. Then the drug product was incubated for around 12 h at this temperature. The vacuum pump was turned on afterward. In 3.5 h after equilibration of the vacuum, the temperature of the shelves was raised to -45 °C, the drug product was incubated at this temperature for 3.5 h. Then the shelf temperature was raised to -43 °C. At this point, a dome-shaped film appeared at the surface of the frozen mass. When the temperature of the drug product reached -35 °C, the mass began to break down. As a result, lyophilization was discontinued.

The high content of ethanol probably caused the difficulties with lyophilization of the studied dosage form of LHS-1269. According to the literature [13], alcohol affects the freeze drying of solutions significantly. Duration of the drying is prolonged if the solutions contain 10% of ethanol. The solutions with 20% ethanol develop an abnormal shape/structure during freeze-drying, the process is elongated considerably as a result. Various combinations of excipients of different origins (Kollidon® 17PF, Macrogol 400, and Poloxamer 188) were used as solubilizers to decrease ethanol concentration in the studied composition. This decrease, together with the increase of pH to slightly alkaline value, lowered solubility of LHS-1269 or therapeutic efficacy of the drug.

Freeze drying of the aqueous solution of LHS-1269 is not possible, so we propose producing a stable injectable dosage form of LHS-1269 as a concentrated mixture of drug substance, DMSO, 95% ethanol, and Kollidon® 17PF. The concentrate is dissolved in water for injection immediately before administration. Production of the dosage form LHS-1269, concentrate for solution for injection and infusion 25 mg, includes the following technological stages:

I. Preparation of the solution of LHS-1269. Add the required volume of DMSO to a weighed amount of the active substance, mix with a magnetic stirrer or a propeller stirrer at 200-250 rpm until the complete dissolution of LHS-1269.

II. Preparation of the solution of Kollidon® 17PF. Place a calculated volume of ethanol on a magnetic stirrer in a tightly closed container to prevent evaporation of the solvent. Divide the weighed amount of Kollidon® into approximately 810 equal portions. Add these portions one by one to the liquid during constant stirring with the average rate of magnet stirrer of 400 rpm. Add each subsequent portion of the solubilizer to the solution only after the previous one dissolves. Polyvinylpyrrolidone agglutinates and forms lumps if added excessively, which elongates and complicates the process considerably. A clear alcohol solution of Kollidon® 17PF is obtained.

III. Sterilization filtration and dispensing the concentrate. Mix the solutions of LHS-1269 and polyvinylpyrrolidone during constant stirring with the average rate of magnet stirrer of 400 rpm. Add each subsequent portion of the solubilizer to the solution only after the previous one dissolves. Polyvinylpyrrolidone agglutinates and forms lumps if added excessively, which elongates and complicates the process considerably. A clear alcohol solution of Kollidon® 17PF is obtained.

CONCLUSION

The study led to development of a technology to produce a stable injectable dosage form of an original hydrophobic derivative of indolocarbazole LHS-1269. The aqueous solution of the studied indolocarbazole derivative has low stability and cannot be lyophilized, so an anhydrous concentrate of LHS-1269 was produced that is dissolved in water for injection immediately before administration. LHS-1269 is a high-potential antitumor drug. Our study showed that it could be formulated in a stable injectable dosage form convenient for clinical practice.

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Nil

AUTHORS CONTRIBUTIONS

Maria Dmitrieva, Alexander Kolpakski: Research design, obtaining and analyzing data, writing the text of the manuscript. Olga Orlova, Ivan Krasnyuk: Research design, data analysis, verification of the final version of the manuscript. Elena Ignatyeva: Obtaining and analyzing data. Anna Lantsova, Ludmila Nikolaeva: Review of publications, translation.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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Table 2: Composition of the drug product LHS-1269, concentrate for solution for injection and infusion 25 mg, per vial

| Component of the dosage form | Weigh of the component, g | Content of the component, % |
|-----------------------------|--------------------------|----------------------------|
| LHS-1269                    | 0.025                    | 1.2                        |
| DMSO                        | 0.275                    | 13.1                       |
| Ethanol 95%                 | 0.800                    | 38.1                       |
| Kollidon® 17PF              | 1.000                    | 47.6                       |

Table 3: Quality characteristics of the experimental batches of LHS-1269, concentrate for solution for injection and infusion 25 mg

| Characteristics | Batch          | 011119 | 010220 | 010121 |
|-----------------|----------------|--------|--------|--------|
| Appearance      | Viscous clear liquid of a vivid yellow color |        |        |        |
| pH of the solution | 2.08±0.01 | 2.08±0.01 | 2.07±0.02 |
| Average weight of contents of the vial, g | 3.9±0.15 | 3.9±0.13 | 3.9±0.13 |
| Average content of LHS-1269 in the vial, mg | 25.4±0.21 | 25.7±0.26 | 23.8±0.18 |
| Viscosity*, mPa·s | 8.4±0.43 | 8.5±0.33 | 7.8±0.79 |

Note: Viscosity of the 5 mg/ml solution of LHS-1269 prepared by dissolving the concentrate in water for injection. The data are given as mean±SD, n=3.

CONCLUSION

The study led to development of a technology to produce a stable injectable dosage form of an original hydrophobic derivative of indolocarbazole LHS-1269. The aqueous solution of the studied indolocarbazole derivative has low stability and cannot be lyophilized, so an anhydrous concentrate of LHS-1269 was produced that is dissolved in water for injection immediately before administration. LHS-1269 is a high-potential antitumor drug. Our study showed that it could be formulated in a stable injectable dosage form convenient for clinical practice.

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