Technology of Liposomal Tiosens, Cifelin and Lysomustin for Industrial Purposes

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Abstract. This work is devoted to the development of national antineoplastic drug (Tiosens, Cifelin, Lysomustin) liposomal dosage form (LDF) circuit technology and their manufacturing technology. In modern oncology liposomes, which are hollow phospholipid vesicles, are used as delivery systems protected drugs from biodegradation, and healthy cells from the toxic effect of chemotherapeutic agents. The technology of their production is stretching and multistage. It is also necessary to give consideration a lot of factors that influence on the finished product quality.

1. Introduction

Tiosens (tetra-3-phenylthiophthalocyanin aluminum hydroxide) refers to the photosensitizer of the second generation and is designed to treat tumors by photodynamic therapy. Tiosens LDF was proposed to increase the selectivity effects on tumor tissue and improve the solubility of the substance. This LDF showed a high antineoplastic activity at the preliminary stages of the study and has been recommended for batch production.

Lysomustin is nitrosoalkylurea derivative based on aminoacid L-homocitrulline. Biological studies have shown the effectiveness of this drug in small cell lung cancer and melanoma. Lysomustin is unstable both in alkaline and in acidic solutions, having the maximum stability of its solutions in a narrow range of pH values, and undergo degradation under influence of visible light at room temperature in aqueous solution. In this connection, to enhance clinical trials a novel lysomustin LDF, provided drug stability during storage, high selectivity for tumor cells and low toxicity were proposed for intravenous administration.

Cifelin is less toxic alkylating agents represented chloraethylamin peptide derivative with such aminoacids as L',D'-phenylalanine and L',D'-valine, synthesized in VONTS USSR AMS. Clinical trials of Cifelin tablets have been revealed significant antineoplastic activity of the drug in several tumors monotherapy, but because of the substance insolubility in the water is needed to apply high daily doses causing a lot of side effects. Cifelin incorporation into liposomal vesicles was one of the possible way to increase its solubilization and bioavailability. During investigation of developed

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dosage form specific activity in a number of transplantable tumors in mice by intraperitoneal injection was showed that the dosage form multiply exceeds substance antitumor activity. 1.

2. Materials and methods
Preparation of large multilayer liposomes (LML) was performed using the lipid film hydration. Exact samples of phosphatidylcholine (LIPOID E PC S, Germany), cholesterol (SIGMA, Germany), polyethyleneglycol-2000-distearoylphosphatidylethanolamine (LIPOID, Germany), Tiosens substance (SRC "NIOPIK") or Cifelin substance (RCRC n.a. N.N. Blokhin) were dissolved in 200 ml of chloroform (chemically pure, Himmed, Russia) and transferred to a round bottom flask with a capacity of 20 liters. The chloroform solution was evaporated on a rotary evaporator (Heidolph Laborota 20 control, Germany) at water bath of 35±2 °C to form a lipid thin film, followed by redrying for 2 hours under vacuum before residual solvent have been completely removed. After this, the lipid film was hydrated by water for injection or an aqueous lysomustin solution (for liposomes containing Lysomustin) to complete washing out of the flask walls. The resulting LML emulsion was filtered through membrane filters with pore diameters of 1.2 and 0.45 µm (Pall, Germany), and reduce to a small size in a homogenizer Microfluidizer M-110S (Microfluidics, USA) at a pressure of 40 mm Hg to prepare monolayer vesicles. The resulting liposomes were sterilized by filtration through a polycarbonate membrane filters with pore diameter of 0.22 µm and mixed with sterilized 40% sucrose solution in a 3:1 ratio. Prepared Tiosens, Cifelin and Lysomustin liposomal emulsion were dosed by 6 ml in 25 ml vials and subjected to freeze-dried using a set Edwards Minifast DO.2 "(Ero Electronic SpA, UK). Lyophilization was performed on previously setted mode of cooling before -55ºC and heating shelves to +20ºC.

For studying the chemical and pharmaceutical properties of freeze-dried LDF vial content was rehydrated in 5.8 ml of deionized water to produce homogeneous emulsion. At all stages of manufacturing liposome size was measured by Nicomp 380 Submicron Particle Sizer (U.S.A.), pH value of freeze-dried LDF was potentiometrically determined.

3. Results and discussion
In the course of investigation the formulations were selected and circuit preparation Tiosens, Lysomustin, Cifelin LDF based on making of the lipid film, production of large lipid vesicles, followed by homogenization, sterilizing filtration and freeze-drying of product were developed. All series met the declared quality parameters: visual environment (dry porous mass), identification (the absorption maximum), the average mass of vial content, pH value, weight loss after drying (not more than 3%), particle size (not more than 200 nm) and active substance quantity (mg/vial).

Sufficiently high antitumor activity of Tosens liposomes were shown during a PDT session in transplanted mice tumors by calculating the inhibition of tumor growth values (ITG%). ITG for Ehrlich’s tumor was 70 - 76%, for lymphocytic leukemia P-388 was 70%, and for epidermoid Lewis lung carcinoma was 60-64%.

Also a high antitumor effect of Lysomustin nanostructured dosage form was shown on lymphoblastic leukemia L-1210 in all tested doses (125-250 mg / kg) and on the Lewis lung carcinoma maximum Lysomustin antitumor effect occured at doses 225 and 250 mg / kg, curing 71% and 86% of the animals, respectively.

The third studing drug was effective against a tumor model of breast adenocarcinoma (ITG = 84%) and a model of cervical cancer (ITG = 83%). The study of liposomal Cifelin in outbred rats with transplanted Walker carcinoma caused 89% of animal treatment when administered orally, and 100% was for intravenous administration.

It is necessary to ensure liposome manufacturing in order to proceed to the next stages of preclinical research consisted in investigation of Tiosens, Lysomustin and Cifelin nanostructured formulation toxicity, pharmacokinetics, safety assessment and preparing for clinical trials. The manufacturing technology allows to prepare minimum 800 ml of liposomal dispersion to produce a series including at least 100 vials.
4. Conclusions
The manufacturing technology for production of stable and effective Tiosens, Lysomustin, Cifelin LDF was developed and studied its critical points. Obtained drugs can be directed for in-depth pre-clinical and clinical trials.

5. Acknowledgements
The work was supported by the grant of the President of the Russian Federation for state support of young Russian scientific candidates of science.

Work is executed according to the scientific and technical program «Creation and practical development in public healthcare of new methods and means of preventive maintenance, diagnostics and treatment of oncological, infectious and other dangerous diseases» at financial support of the Government of Moscow.