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Development of the Optimal Composition and the Technology of New Combined Injectable Drug with Prolonged Action Based on Disulfiram and Naltrexone

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ABSTRACT

New injectable combined prolonged drug "Naltetlong" containing disulfiram and naltrexone was developed. An optimal pharmaceutical form and polymers-prolongers with a certain molecular weight were chosen based on experimental research. Conditions of interaction of the polymer-carrier of active substances for saving molecules structures of drugs components and formation of particles in the nanospheres form with a uniform size distribution were proposed. Uniform distribution size provides the prolongation of drug activity. The predictable product performance was identified and was equal to 30 days. Preclinical studies of toxicity and specific activity of the combined preparation "Naltetlong" indicate a pronounced pharmacological activity and moderate toxicity. Preliminary data on the use of "Naltetlong" in the treatment of alcohol and drug addiction have shown the prospects of its application for the treatment and rehabilitation of patients.

Keywords: alcohol and drug dependence syndrome, injectable prolonged (injectable long-acting drug), naltrexone, disulfiram, polymers-prolongers

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INTRODUCTION

An urgent task of the alcohol and drug dependence therapy is the relapse prevention. Quality of the course of anti-relapse therapy involves the systematic daily intake of prescribed doses of the medicinal agent for a long time. However, due to personality changes that develop as a result of drug addiction and alcoholism, patients have no interest in the treatment process. This requires the development of new pharmacological approaches [1]. In the absence of clear schemes and protocols of pharmacotherapy, the treatment of addictions is characterized by polypragmasia, which leads to high treatment cost, frequent side effects and reduction of the treatment effectiveness. The application of the prolonged forms provides the continuity of the treatment process and prevention of early recurrences. Intramuscular injections and implants are used of the creation of drug depots in the subcutaneous tissue or muscle tissue. Injectable long-acting drugs have many advantages, they are easier to use and less traumatic for the patient.

One way to improve the traditional medicinal agents is the creation of combined drugs, which can affect different chains of disease pathogenesis and provide a therapeutic effect, covering all groups of patients.

Our marketing research of the drugs which induce abhorrence and reduce the craving for drugs and alcohol, allowed to identify drugs - leaders: naltrexone and disulfiram.

Disulfiram (teturam) is known sensibilizing medicinal agent which creates a biochemical barrier and makes the alcohol consumption impossible. Naltrexone is pharmacologically "clean" opioid receptor antagonist, completely eliminates the side effects of analgesics and is an effective antidote. The main purpose of naltrexone application is the preventive treatment of the opioid addiction and, in part, one of its clinical variants - heroin addiction. According to the Cleveland Clinic Journal of Medicine naltrexone and disulfiram with acamprosate are approved in the U.S. for the treatment of alcohol dependence [2]. These drugs are also licensed in most European countries, Canada, Australia and India.

Disulfiram has demonstrated an efficacy in randomized placebo-controlled clinical trials of drug treatment, including cocaine addiction. Clinical studies have shown that naltrexone reduces the pleasurable sensations associated with the alcohol intake in patients with alcohol dependence. The possibility of the combined use of disulfiram and naltrexone as a treatment for mono dependencies and concomitant pathologies were investigated [3-6]. Blind, randomized, placebo-controlled trials have shown the effectiveness of naltrexone and disulfiram admission separately or in joint application [7]. This allowed us to select these substances as the main objects of study. Therefore, the aim of our work was to develop a combined long-acting drugs based on disulfiram and naltrexone using biodegradable polymers for the use in the complex treatment of alcoholism and opiate addiction.

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MATERIALS AND METHODS

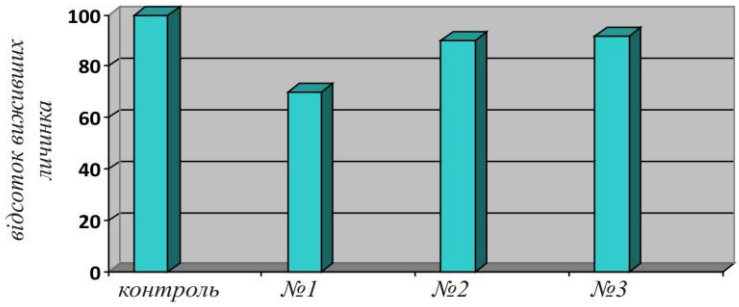
When developing the combined preparation, we proceeded with the recommended therapeutic doses of active substances: disulfiram - 250 mg, naltrexone - 200 mg. We have determined that regardless of the dosage form the annual naltrexone treatment course makes 3200-3700 mg. Drugs based on disulfiram as implants and injections with different term extension contain 2500-3200 mg of the active substance for annual treatment.

When choosing a rational dosage form physical-chemical properties of drugs were taken into account. The possibility of the development of a combined injection long-acting drug in the form of aqueous solution, emulsions of oil/water type and a non-aqueous solutions was considered. The long-acting disulfiram drug "Tetlong-250" for intramuscular injections containing 25% solution in a special solvent, was developed in Ukraine in 1983. This drug is used for the treatment and rehabilitation of alcoholics in various conditions - ambulatory, stationary, penitentiary [8]. As a result of patent search and experimental studies, we have chosen a rational dosage form and well known allowed excipients, namely: solvent – dimethyl sulfoxide (DMSO) [9], and biodegradable polymers – polyvinyl alcohol with molecular weight 30,000 and polyvinylpyrrolidone with molecular weight 10,000.

The technological conditions for the production of long-acting medicinal agent, in which the polymer carriers would be associated with active substances by intermolecular interactions, were studied [10].

The study of the dynamics of the naltrexone release by the dialysis method was performed. As a result, the predicted effect of the long-acting agent – 30 days – was determined.

The biocompatibility of excipients was assessed. The eggs and embryos of loach (*Misgurnus fossilis* L.) during early embryogenesis treated and inseminated by the Neifach method were used as a biological model in the experiment (Fig. 1).



*** Fig. 1. Chart survival of embryos in the test solution:**

Number 1 - 5 ml DMSO 95 ml H20,
Number 2 - 2.5 ml of 2% PVP district in DMSO + 2.5 ml DMSO + 95 ml H20,

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Number 3 - 2.5 ml of 2% district PVA in DMSO + 2.5 ml DMSO + 95 ml H20;

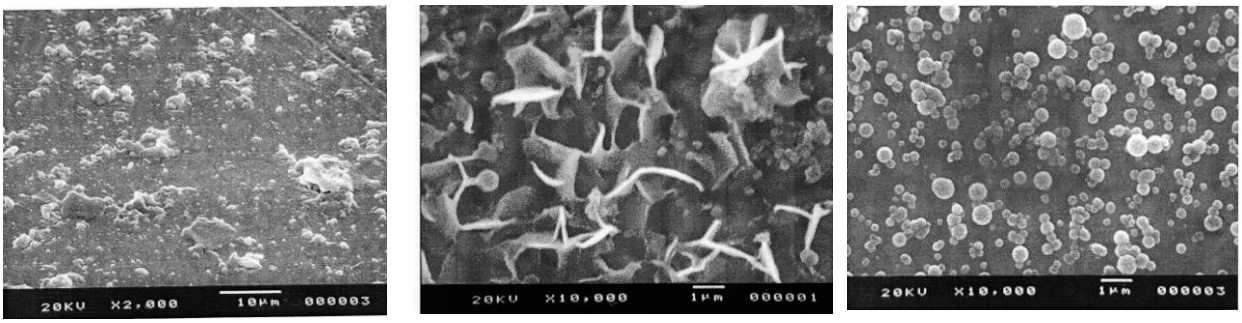
* solution diluted with saline to a concentration by the Holtfreter substances order of 10.6 mg / ml

The results have shown that the number of hatched embryos in the test DMSO solution has increased when adding polymers. Thus, the pathological effect of the solvent in the joint application of excipients was reduced.

To reduce the toxicity of naltrexone and disulfiram the glycosylation of these substances was attempted. However, unlike other cases [11-13], glycosylation led to significant changes in the toxicity levels of the above ingredients.

As a result of investigations the composition of the preparation "Naltetlong" and technology of its production were developed, the main quality indicators were determined, the techniques for the identification and the quantification of the active substance were elaborated and tested in an industrial scale. The composition and the technology of long-acting injection drug are protected by patents [14, 15].

The injection solution was suspended in water's medium. The shape and size of particles of the injection solution in this medium was determined by scanning electron microscopy (SEM) (instrument JEOL-T220A, JEOL Ltd**,** Japan). The sediment in form of separate spherical particles ranging in size from 100 nm to 1 micron with uniform size distribution which provided prolonged action was observed in microphotographs (Fig. 2).



а. б. в.

Fig 2: Microphotographs of dry sediments obtained by SEM: a) dimexide b) 25 % disulfiram solution in DMSO c) "Naltetlong."

The experimental studies of "Naltetlong" stability have shown that the sterilization in the final stages using autoclave under standard conditions is impossible. The sterilization method chosen is sterile filtration, carried out in two stages sequentially through membrane filters with pore size 0.45 µg and 0.22 µg.

Organoleptic indices of the injectable solution meet the requirements for storage in a temperature range from 5 to 15°C. The preservation of the structure of the components of the product in the proposed temperature range was confirmed by high-performance liquid

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chromatography (Fig. 3a-b), infrared spectroscopy, proton magnetic resonance spectroscopy, and differential thermal analysis.

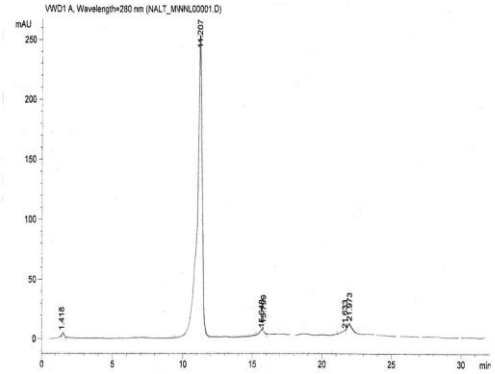

Fig 3a: Chromatogram of naltrexone hydrochloride in the control sample

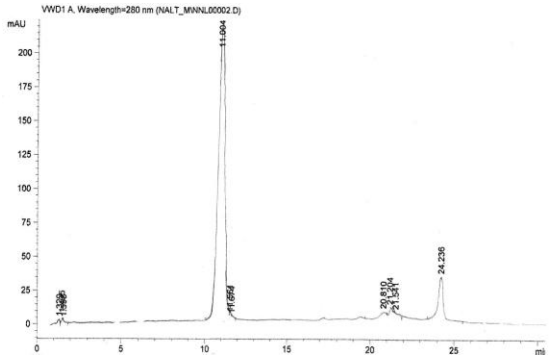

Fig 3b: Chromatogram of naltrexone hydrochloride in the sample of "Naltetlong" after storage at 15 °C

PMR-sectors of drug after storage during 24 months: sample № 1 – at 5°C; sample № 2 – at 25°C shown in the table 1.

Table 1*:* PMR-sectors of drug samples after storage

№ зр.	δ_H (300MHz, DMSO-d_6), ppm
№1	9,507 (1H, s); 9,034 (1H, s); 6,974 (1H, s); 6,634 (2H, dd); 5,018 (1H, s); 4,502 (1H, m); 3,971 (15H, t); 3,065 (6H, m); 2,112 (4H, m); 1,391 (12H, t); 1,189 (10H, t); 0,603-0,401(3H, m)
№2	9,501 (1H, c); 9,040 (1H, d); 6,935 (1H, s); 6,623 (2H, t); 5,009 (1H, s); 4,489 (1H, m);3, 968 (15H, t), 3,061 (6H, m); 2,102 (4H, m);1,389 (12H, t); 1,186 (10H, t); 0,585-0,419 (3H, m)

Based on stability studies storage temperature from 5 to 15 °C was selected.

The pre-clinical studies of a specific activity and toxicity of "Naltetlong" were conducted. The registered changes in quantitative measures of voluntary oral consumption of morphine hydrochloride solution during the test in two drinkers have shown that the use of "Naltetlong" in rats with mental dependence reduces the craving for the drug. The level of reduction of the final advantage index to the initial rate was 2.3 times. Thus, in experiments in vivo in male Wistar line rats with experimental morphine dependence an expressed anti-addictive activity of "Naltetlong" was found which is similar to the effect of the reference drug - naltrexone.

These data indicate that "Naltetlong" more than disulfiram reduces ethanol consumption in rats with experimental alcoholism under psychological dependence. Under the influence of disulfiram, this figure decreased by 1,7 times, while "Naltetlong" caused its reduction in 4 times.

According to the research of the intramuscular introduction of "Naltetlong" LD_{50} was 58.0 mg/kg. The administration of "Naltetlong" to rats for 28 days at a dose of 1.0 mg / kg did

not adversely impact the function of the central nervous and cardiovascular systems, did not cause changes in the morphological composition of peripheral blood and biochemical parameters that characterize the main types of metabolism in laboratory animals. With the introduction of drugs in doses of 10 mg / kg to the test animals the violations of electrolyte metabolism, increased absolute and relative weight of spleen and liver in females, decreased adrenal weight and increased blood glucose levels in males was observed indicating dose-related toxic effects of drugs.

RESULTS AND DISCUSSION

The preclinical studies of specific activity and toxicity "Naltetlong" have shown its pronounced pharmacological activity and moderate toxicity as well as the feasibility of its clinical trial as means of therapy in patients with opioid addiction and chronic alcoholism.

Based on the preclinical research conclusions the clinical trial of an experimental batch of medicinal agent was an initiated. The application of "Naltetlong" allowed the reduction of the term of the improvement of psychophysical condition in most (89.3%) patients. It accelerated the reduction of affective, neurosis, psychopathic, autonomic disturbances and helped reducing the severity of craving for the substances, the formation of stable critical attitude to psychoactive substances and the disease and improvement of the compliance. The use of the combined pharmacological, psychopharmaceutical and social approach in the treatment of alcohol and drug dependencies allowed faster achievement of the complete remission and maintenance of its stability for a long time (up to 2 years, 71.4%) [16].

The introduction of the development in the medical practice has prospects for various rehabilitation and prevention programs.

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