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Biopharmaceutical evaluation of new semi-solid preparation with thiotriazoline and chloramphenicol

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ABSTRACT

Introduction. Periodontitis numbers among the most widespread diseases. The prevalence of the periodontal inflammation does not tend to decrease, but long-term history of the disease, the complexity in treatment significantly reduce the quality of life in patients, cause the tooth loss. An important principle for the treatment of mentioned disorder is use of drug products in the lesion location. The preference is given to semi-solid preparations with a polyvalent pharmacological action which can eliminate the causative agents and affect the different elements in the pathogenesis.

Aim. Aim of the study was to evaluate the influence of excipients, including the base-forming agents, on the thiotriazoline release from semi-solid preparation and based on the results of investigations to develop the composition of a new drug product for treatment of periodontal inflammation.

Material and methods. Thiotriazoline and chloramphenicol were selected as active pharmaceutical ingredients for the development of semi-solid preparation. Methylcellulose, carboxymethylcellulose sodium and polymethylsiloxane (base-forming agents); glycerol and propylene glycol (plasticizers); methylparaben and propylparaben (preservative agents); peppermint oil (flavouring agent) were also used in the investigations on the pharmaceutical development.

In order to select an optimal composition of the base for dental semi-solid preparation with thiotriazoline and chloramphenicol, six samples with different content were prepared. The appearance of samples was evaluated visually. Measurement of pH value was performed using pH-Meter-240 CORNING. Thiotriazoline release from the test samples was studied *in vitro* by agar diffusion method.

Results and conclusions. The results of investigations showed that excipients largely affected thiotriazoline release from the drug product. According to the results of physical, physical-chemical and biopharmaceutical investigations the following excipients were chosen for the development of dental semi-solid preparation: methylcellulose (base-forming agent), glycerol (plasticizer), methylparaben and propylparaben (preservative agents), peppermint oil (flavouring agent with antiseptic action).

Developed preparation is homogeneous mass with a thick consistency, white color and pleasant fresh smell. The drug product is characterized by stable physical-chemical and rheological properties, and has a neutral pH.

Keywords: thiotriazoline, chloramphenicol, biopharmaceutical evaluation, periodontitis.

Introduction

Periodontitis numbers among the most widespread diseases. About 75% of adults suffer from this disease [1]. The prevalence of the periodontal inflammation does not tend to decrease, but long-term history of the disease, the complexity in treatment significantly reduce the quality of life in patients, cause the tooth loss. Considering this, pharmacotherapy of periodontitis is a topical subject.

An important principle for the treatment of mentioned disorder is use of drug products in the lesion location. The preference is given to semi-solid prepara-

tions with a polyvalent pharmacological action which can eliminate the causative agents and affect the different elements in the pathogenesis.

Since commonly known drug products not always provide the desired therapeutic effect, investigations on development of composition of new combined preparation in the semi-solid dosage form were performed.

Development of the dental semi-solid preparations is justified because they are chemically more stable than liquids and they may prolong the contact time between the drug and affected tissues. Those preparations provide local and uniform release of active ingredients, creating a high drug concentration in the contact site without a significant effect on the drug level in the systemic circulation.

One of the well-known techniques which are used to enhance the therapeutic efficacy and so to shorten the treatment duration is scientifically and experimentally justified combination of several active pharmaceutical ingredients in a single dosage form [2]. Furthermore, such approach is cost-effective as it allows developing of practically new and more effective drug products based on the existing assortment of active pharmaceutical ingredients. Reduction in the number of drug products in the therapeutic scheme will undoubtedly improve the patient compliance. Taking into consideration the above mentioned aspects, thiotriazoline and chloramphenicol were selected as active pharmaceutical ingredients for development of the dental semi-solid preparation.

Thiotriazoline (morpholinium; 3-methyl-4H-[1,2,4] triazol-5-ylsulfanyl)-acetate (**Figure 1**) is a new Ukrainian agent that demonstrates antioxidant, membrane stabilizing, anti-ischemic, anti-inflammatory, immunestimulatory, antiviral activity and stimulates the tissue regeneration.

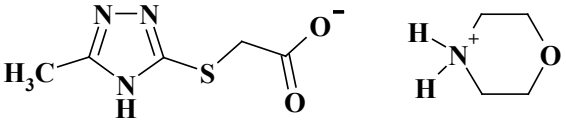

Figure 1. Chemical structure of thiotriazoline

Pharmacological effect of thiotriazoline is provided by activating the enzymatic antioxidant system and inhibition of lipid peroxidation in the ischemic tissues, normalization of the tropism of nervous system, increasing in the intensity and rate of reconstructive processes, declining the tissue inflammation, and improvement of blood microcirculation [3]. Drug products with thiotriazoline have already been used in modern medicine, particularly in cardiology, surgery, ophthalmology, hepatology, stomatology [4]. We have found that an effective concentration of thiotriazoline in drug products for topical use is 2% [5].

Since thiotriazoline does not possess the antibacterial activity, but pathogenic microorganisms are the basic etiologic factors of marginal and apical periodontal inflammations, the possibility of chloramphenicol introduction into the composition of dosage form was studied [6, 7].

Chloramphenicol (2,2-dichloro-N-[2-hydroxy-1-(hydroxymethyl)-2-(4-nitrophenyl)ethyl] acetamide (**Figure 2**) is a broad spectrum antibiotic with activity against gram-positive and gram-negative bacteria, rickettsia, spirochetes.

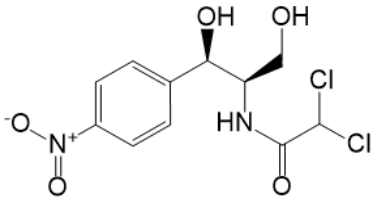

Figure 2. Chemical structure of chloramphenicol

It is the only one natural antibiotic, molecule of which contains residue of nitrobenzene that is toxic against bacteria cells. It is important to emphasize that chloramphenicol is one of the few chemotherapeutic agents that exhibit activity against anaerobic gram-negative bacteria. Minimal inhibitory concentration of chloramphenicol in dosage form for topical use is 0.5% [8].

Material and methods

Thiotriazoline (purity 99%) was generously gifted from the Department of Pharmaceutical Chemistry of Zaporizhzhya State Medical University, Ukraine, where this substance was first synthesized by prof. I.A. Mazur and coauthors.

Chloramphenicol (purity ⩾ 98%) was purchased from Sigma-Aldrich (USA) and used without further purification.

An essential stage in the development of new drug product is the right choice of excipients which serve as a carrier for active pharmaceutical ingredients. The pharmacokinetic and pharmacodynamic parameters of drug product can be controlled by selection of excipients and their concentration.

For development of the composition of dental semi-solid preparation with thiotriazoline and chloramphenі-

col the following excipients were selected: methylcellulose (A4M Pharm), carboxymethylcellulose sodium (medium viscosity) and polymethylsiloxane as base-forming agents; glycerol and propylene glycol – plasticizers; methylparaben and propylparaben – preservative agents; peppermint oil – flavouring agent. All ingredients were purchased from Ashland Inc. (USA) and Sigma-Aldrich (USA), except of polymethylsiloxane (Pharmaceutical company "Ecologoprotective firm company "KREOMA-FARM", Ukraine) and peppermint oil ("Pharmaceutical factory", State Municipal Enterprise, Ukraine).

Selected base-forming agents are hydrostable, thermostable, and allow uniform drug release in the affected areas, providing a longer therapeutic effect in comparison with liquid preparations.

The plasticizers glycerol and propylene glycol ensure the plastic, thixotropic consistency of preparation, and possess the moistening and penetrating action [9].

To prevent microbial contamination and to destroy bacteria and fungi, as well as to extend the shelf life of the drug product, preservatives methylparaben and propylparaben were introduced into the composition of semi-solid preparation [10].

Peppermint oil was chosen as a flavouring agent. It is well-known that peppermint oil is used as antiseptic, anti-inflammatory and analgesic preparation [8].

In order to select an optimal composition of the base, 6 samples with different content were prepared. The amount of thiotriazoline in all sampled was 2%, the amount of chloramphenicol – 0.5%. Composition of the test samples is described in **Table 1**.

An important characteristic of the drug products is appearance which covers such parameters as consistency, color and smell. To prevent irritating action of dental semi-solid preparation on the oral mucous membrane, the pH level of the developed product must be within the allowable range for the mouth cavity (6.3–8.0).

Therefore, all samples were controlled on the mentioned quality parameters in the moment of production and after 3 and 6 months of storage. The appearance of the samples was evaluated visually. Measurement of pH value was performed according to the requirements of State Pharmacopoeia of Ukraine using pH-Meter-240 CORNING [11].

Therapeutic effect of drug product depends mainly on the rate and extent of drug release. Thiotriazoline release from the test bases was studied *in vitro* by agar diffusion method [12]. As indicator was used Reinecke salt that in reaction with thiotriazoline produced pink color. Test samples were distributed into the wells in the agar gel. Prepared system was incubated in a thermostat TS-80-M-2 at 37°C (± 1°C) for one day, and then diameter of colored areas around each well with the drug product sample was measured using beam compass.

Result and discussion

All samples had the look of uniform gelatinous mass of a whitish color and with a distinctive smell of peppermint. Visual characteristics of the ointments with polymethylsiloxane (samples №5 and №6) were worse than ointments with methylcellulose (samples №1 and №2) and sodium carboxymethylcellulose (samples №3 and №4). The pH level of all samples was within normal range.

Introduction of propylene glycol into the composition of bases (samples №2, №4 and №6) led to the deterioration of rheological properties.

Considering the above mentioned, in order to select the most optimal base for the development of a new preparation, the biopharmaceutical evaluation was conducted of the test bases №1 and №3.

Results of the study of base influence on the rate and extent of thiotriazoline (active pharmaceutical ingredient) release are shown in **Figure 3**.

Table 1. Composition of the tested bases

Excipients	№ of the base					
	1	2	3	4	5	6
Methylcellulose	+	+	–	–	–	–
Carboxymethylcellulose sodium	–	–	+	+	–	–
Polymethylsiloxane	–	–	–	–	+	+
Glycerol	+	+	+	+	+	+
Propylen glycol	–	+	–	+	–	+
Methylparaben	+	+	+	+	+	+
Propylparaben	+	+	+	+	+	+
Peppermint oil	+	+	+	+	+	+

(+) – presence of ingredient in the base; (–) – absence of ingredient in the base

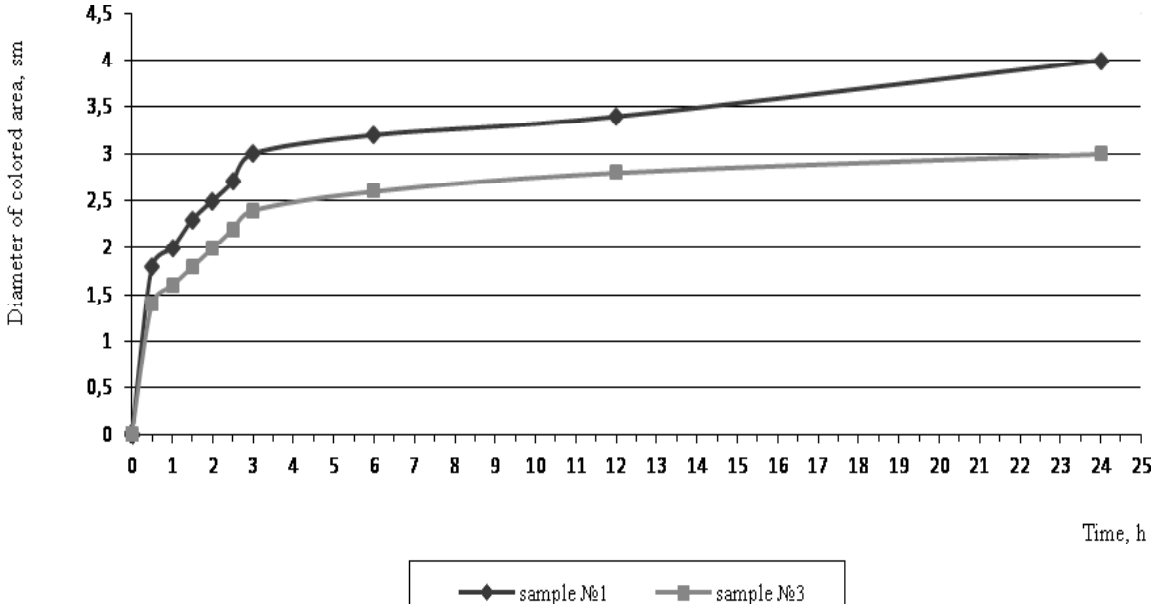

Figure 3. Influence of the nature and composition of the base on the thiotriazoline release from semi-solid preparations

As shown in **Figure 3**, thiotriazoline release from semi-solid preparations was observed within 24 h that indicated the prolonged action of test samples. But drug release from preparations was different. It was found that after an hour of the experiment the diameter of coloured area of the sample №1 was larger than the sample №3. After 90 min from the beginning of the experiment the drug release from the sample №1 was also more complete than from the sample №3 (diameter of coloured area was larger on 27%). Such a tendency was noted throughout the experiment. After 24 h the diameter of coloured area of the sample №1 was larger by 33% that indicated better thiotriazoline release from the methylcellulose based drug product.

The results of biopharmaceutical study proved that drug release from semi-solid preparations depended on the nature and amount of excipients, including the base-forming agents. An optimal base for the dental semi-solid preparation with thiotriazoline and chloramphenicol is base №1 that contains methylcellulose.

Conclusions

Combination of thiotriazoline and chloramphenicol (active pharmaceutical ingredients) in the semi-solid preparation intended for treatment of periodontitis will provide a complex therapeutic effect on the different pathogenetic links.

Performed investigations on substantiation of the composition of dental semi-solid preparation with thiotriazoline and chloramphenicol showed that excipients largely affected drug release from the drug product. According to the results of physical, physical-chemical and biopharmaceutical investigations the following excipients were chosen for the development of dental semi-solid preparation: methylcellulose (base-forming agent), glycerol (plasticizer), methylparaben and propylparaben (preservative agents), peppermint oil (flavoring agent with antiseptic action).

Developed drug product is homogeneous mass with a thick consistency, white color and pleasant fresh smell. It is characterized by stable physical-chemical and rheological properties, and has a neutral pH. The preparation adhered well to the marginal periodontium and can be easily inserted into the root canal.

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Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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