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Experimental Assessment of the Specific Activity of a New Dosage Form of Angiolin in Cataract Modeling

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Abstract

Age-related cataract is currently the leading cause of incurable blindness in the world and the most common eye disease in people over 60 years. Among all ophthalmic diseases, cataract is about 65%.

The following medicines are used to treat cataracts: Oftan Catachrom (Santen AO, Finland), Taufon (Farmak, OAO, Kiev, Ukraine.), Quinax (Alcon - Couvreur, Belgium), Vita-Iodurol (Excelvision AG for Novartis Pharma AG, France/Switzerland), Potassium iodide (OOO "UNIMED PHARMA", Slovak Republic).

Based on the foregoing, it is clear that the range of drugs for the treatment of cataracts is limited, and mainly consists of imported drugs. All of the above opens up new prospects for the creation of new ophthalmic medicines for expanding the range of domestic drugs, namely eye drops with wound healing, reparative, anti-inflammatory effects.

Therefore, the **aim of our work** is to create a new ophthalmic drug based on the original substance Angiolin, which will exhibit anti-inflammatory, wound healing, reparative properties.

Materials and methods: the employees of the Department of Pharmaceutical Chemistry of Zaporizhzhya State Medical University (ZSMU) together with specialists of the NPO Farmatron under the supervision of Professor Mazur I.A. synthesized a new compound called Angiolin.

The entire study of the effectiveness of 1% Angiolin eye drops under cataract modeling was performed on 15 male Chinchilla rabbits (30 eyes) with an average weight of 2-3 kg and an age of 8-10 months. Normal values of the studied parameters were determined on 5 intact animals (10 eyes). An experimental cataract was reproduced on both eyes of 10 animals (20 eyes) by chemical induction of free radical oxidation of biopolymers of eye tissues according to the D.K. Buyan method, for which 30 μl of a sterile solution of dibromide diquat at a dose of 600 nmol was injected once into the vitreous body. Treatment began from 7 days, when the initial opacity of the lens was formed, and was carried out by instillation of drugs into the conjunctival cavity of the eyes 3 times a day.

Results: the course administration of 1% Angiolin eye drops had a significant therapeutic effect in the conditions of chemical cataracts. The use of Angiolin drops reduced the lens opacity of the eye of experimental animals by 43,5%.

Findings: 1% Angiolin eye drops ((S) -2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate) as an active ingredient, exhibit significant anti-inflammatory, wound healing, reparative properties, and also have a high therapeutic effect in the treatment of cataracts.

Keywords: (S)-2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5- thioacetate, thiotriazolin, eye drops, cataract

Introduction

Age-related cataract is currently the leading cause of incurable blindness in the world and the most common eye disease in people over 60 years. Among all ophthalmic diseases, cataract is about 65%. In 2017, more than 20 million people worldwide suffer from bilateral blindness. Among all causes of blindness, cataracts occupy about 20%. The number of cataract patients is growing not only among the population of retirement age, but among the able-bodied population, which is not only a medical, but also a social problem. Surgical treatment is the most common for cataracts. However, the question on the agenda is the need to develop effective medical methods of prevention and treatment based on the results of an in-depth study of the etiology and pathogenesis of lens opacities [5].

There are numerous studies on the role of protein metabolism disorders, lipid peroxidation, energy deficiency, as well as calcium homeostasis disorders in the pathogenesis of cataracts. Convincing evidence has been obtained that a deficiency of endogenous antioxidants - α-tocopherol, glutathione, carnosine and deprivation of Se-glutathione peroxidase leads to uncontrolled growth of reactive oxygen species (ROS) and oxidative protein modification. It is the oxidative modification of the protein molecules of the lens, the appearance of carbonyl groups in their structure that leads to opacity. These studies justified the use of antioxidants in the prevention and treatment of cataracts. There is compelling evidence of the clinical efficacy of selenase, emoxipin, β-carotenes, ionol in the treatment of cataracts. The use of the Thiotriazolin antioxidant in the treatment of cataract in the form of 1% eye drops is known [10].

The following medicines are used to treat cataracts: Oftan Catachrom (Santen AO, Finland), Taufon (Farmak, OAO, Kiev, Ukraine.), Quinax (Alcon –

Couvreur, Belgium), Vita-Iodurol (Excelvision AG for Novartis Pharma AG, France/Switzerland), Potassium iodide (OOO "UNIMED PHARMA", Slovak Republic) [7].

Based on the foregoing, it is clear that the range of drugs for the treatment of cataracts is limited, and mainly consists of imported drugs. Despite the scientific achievements of recent years, an extremely important task is the creation of new ophthalmic medicines, namely: eye drops, which continue to be the most common and widely used in practice dosage form (DF) due to the tradition of production and ease of administration.

Therefore, the **aim of our work** is to create a new ophthalmic drug based on the original substance Angiolin, which will exhibit anti-inflammatory, wound healing, reparative properties.

Materials and methods

The employees of the Department of Pharmaceutical Chemistry of Zaporozhye State Medical University (ZSMU) together with the specialists of the SPA "Farmatron" led by Professor Mazur I.A. synthesized a new compound called Angiolin ((S)-2,6-diaminohexanoic acid 3-methyl-1,2,4- triazolyl-5- thioacetate) [1].

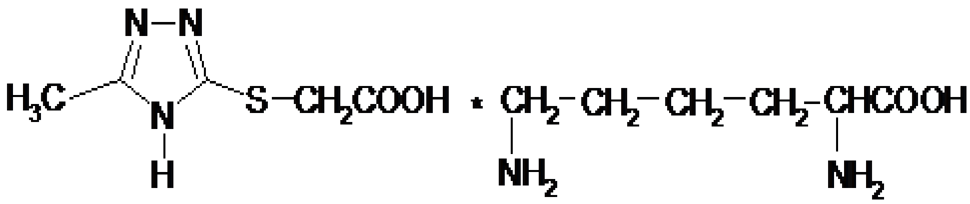

Fig. 1.1 Structural formula of Angiolin ((S) -2,6-aminohexanoic acid 3-methyl-1,2,4- triazolyl-5- thioacetate)

Angiolin exhibits high endothelioprotective, neuroprotective, cardioprotective, anti-ischemic and anti-apoptotic properties [2,3].

In previous studies, the effect of Angiolin eye drops in various concentrations (0,5%, 1%, 1,5%, 2% and 2,5%) for the treatment of chemical eye burns was studied. The experiment was carried out while modeling chemical eye burns in chinchilla rabbits.

In the course of work in the laboratory for standardization and technology of medicines of the Department of Pharmaceutical Chemistry of ZSMU, 1% Angiolin eye drops were prepared (used series: Angiolin, experimental series 9, produced by the State Enterprise "Chemical Reagents Plant" of the Scientific and Technical Complex "Institute of Single Crystals" of the National Academy of Sciences of Ukraine)

During experimental studies, it was found that the most effective were 1% Angiolin eye drops. The results are an experimental justification for the further study of 1% Angiolin eye drops for the treatment of cataracts [4,8,9].

The entire study of the effectiveness of 1% Angiolin eye drops under cataract modeling was performed on 15 male Chinchilla rabbits (30 eyes) with an average weight of 2-3 kg and an age of 8-10 months. Studies were carried out on a sufficient number of experimental animals. All manipulations were carried out in accordance with the provision on the use of animals in biomedical experiments, which are consistent with the provisions of the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes”.

Normal values of the studied parameters were determined on 5 intact animals (10 eyes). An experimental cataract was reproduced on both eyes of 10 animals (20 eyes) by chemical induction of free radical oxidation of biopolymers of eye tissues according to D.K. Buyan method, for which 30 μl of a sterile solution of dibromide diquat at a dose of 600 nmol was injected once into the vitreous body. Treatment began from 7 days, when the initial opacity of the lens was formed, and was carried out by instillation of drugs into the conjunctival cavity of the eyes 3 times a day. Animals were divided into three groups. The first group consisted of intact animals - 5 animals (10 eyes); the second group of control animals — with and without cataract — 5 animals (10 eyes), the third group — animals with cataract, which received a course of a 1% drop of “Angiolin” - 5 animals (10 eyes). The course of treatment was 28 days after 7 days of diquat dibromide administration. At the end of the treatment course (35th day of the experiment), the animals were anesthetized with sodium thiopental (40 mg/kg) and eyes were enucleated and the lens was isolated in the cold.

The lens was homogenized in a Potter homogenizer in a 10-fold volume of medium at (2°C) containing (in mmoles): sucrose - 250, Tris-HCl buffer - 20, EDTA-1 (pH 7,4). At a temperature of +4°C, a cytosolic fraction was isolated by differential centrifugation on a Sigma 3-30k refrigerated centrifuge (Germany). In the cytosol of the crystalline lens, the intermediate diol-sulfide system supporting oxidative/antioxidant crystalline lens homeostasis was determined — glutathione peroxidase (GPR) and glutathione reductase (GR) activity, total SH groups. Markers of oxidative modification of the protein-aldehydephenylhydrazone (APH) and carboxyphenylhydrazone (CPH), as well as nitrotyrosine, were also determined. GR activity was determined by spectrophotometric method in the test with oxidized glutathione and NADPH. GPR activity was determined spectrophotometrically in a test with tert-butyl hydroperoxide. The content of reduced glutathione was determined fluorometrically by reaction with o-phthalic anhydride. Indicators of oxidative protein modification (OPM) were determined spectrophotometrically by B. Halliwell method by the interaction of oxidized amino acid residues with 2,4-dinitrophenylhydrazine (2,4-DNPH) and the formation of aldehyde phenylhydrazones (APH) and carboxylphenylhydrazone (CPH) having an absorption spectrum at 274 nm 363 nm, respectively. The content of total SH groups was determined spectrophotometrically by reaction with 5,5-dithio-bis-7-nitrobenzoic acid. Nitrotyrosine was determined in the cytosolic fraction of cardiac homogenate by solid-phase immunosorbent sandwich ELISA, ELISA Kit (Cat. No. HK 501-02) by Hycult Biotech and expressed in nm/g of tissue. The optical density index of the cytosolic fraction of the lens, which characterized the degree of turbidity, was also determined. This indicator was determined spectrophotometrically at 440 nm. The results of the study were calculated using the standard statistical package of the licensed program STATISTICA® for Windows 6.0 (Stat SoftInc., No. AXXR712D833214FAN5), as well as SPSS 16.0, Microsoft Office Excell 2003. Normalcy of distribution was assessed by the Shapiro-Wilk test. Data are presented as mean values. The statistical significance between the mean values was determined by the Student's test with a normal distribution. In case of a non-normal distribution or analysis of ordinal variables, the U Mann-Whitney test was used. For comparison of independent variables in more than two samples, analysis of variance (ANOVA) with normal distribution or the Kruskal-Wallis test for a distribution other than normal were used. For all types of analysis, differences $p < 0.05$ (95%) were considered statistically significant [10,11].

Results and discussions

As can be seen from the data presented in tables 1 and 2, a single injection of diquat dibromide into the vitreous of the rabbit eye leads to cataract formation after 28 days. So, for these experimental periods in the animals of the control group, lentiform opacity was observed, as evidenced by an increase in the optical density of the cytosolic fraction of the lens by 142%. The lentiform opacities occurred during activation of oxidative stress and inhibition of the antioxidant system of the lens (tabl. 1 and 2).

Table 1

Effect of 1% Angiolin eye drops on oxidative stress markers in the lens of the rabbit eye under 7-day cataract formation and following 28-day treatment

Animal group	Oxidative protein modification products, U/g protein		Nitrotyrosine, nmol/g tissue
	АФГ	КФГ	
Intact ,n=5 (10 eyes)	0,34±0,02	0,15±0,01	12,3±1,00
Control (cataract), n=5 (10 eyes)	0,62±0,04	0,34±0,02	37,7±2,5
Angiolin, n=5 (10 eyes)	0,34±0,02*	0,20±0,010*	17,2±1,0*

Note: *– p≤0,05 relative to the control

According to many researchers, the lens opacity is based on the oxidative modification of proteins and the appearance of carbonyl groups in its structure. In addition, N-; S-; O-nitrosylation of the structural fragments of the lens proteins under the influence of highly reactive NO derivatives - peroxynitrite, nitrosonium ion, also leads to turbidity. Thus, in the cytosolic fraction of the lens of animals of the control group, an increase in the markers of oxidative modification of the protein — APH by 82,3% and CPH by 133,4, as well as an increase in the concentration of nitrotyrosine by 206,5% were observed. A similar activation of oxidative stress reactions occurred against a background of significant inhibition of the antioxidant function of the lens thiol disulfide system. In the cytosol of the lens of animals of the control group, a decrease in the level of reduced glutathione by 44,3% and the content of the total restored thiol groups by 73,1% were recorded [12,13,14].

Table 2

The effect of 1% Angiolin eye drops on the indicators of the thiol disulfide system in the lens of the rabbit's eye under 7-day cataract formation and the following 28-day therapy

Animal groups	GR µmol/mg protein /min	GPR µmol/mg protein /min	Glutathione restored µmol/g tissue	SH-group µmol/g tissue	Optical density in 440 nm
Intact ,n=5 (10 eyes)	21,5 ± 1,5	64,8 ± 2,2	4,7 ± 0,20	57,3 ±2,5	0,19±0,005
Control (cataract), n=5 (10 eyes)	10,2 ± 0,9	40,2 ± 3,0	2,62 ± 0,05	15,4 ± 1,2	0,46±0,01
Angiolin, n=5 (10 eyes)	18,2 ± 1,1*	57,5 ± 3,5*	3,57 ± 0,15*	47,5 ± 2,2*	0,26±0,007*

*Note: * - p <0,05 relative to the control group*

Discussions

The course administration of 1% Angiolin eye drops had a significant therapeutic effect in the conditions of chemical cataracts. The use of Angiolin drops reduced the opacity of the lens of the eye of experimental animals by 43.5%. The use of Angiolin drops also inhibited oxidative modification — a decrease in APH by 45,1% and CPH by 41,1%, as well as a decrease in nitrosating stress reactions — a decrease in nitrotyrosine by 54,4%. Angiolin also had a normalizing effect on the main indicators of the thiol disulfide system of the lens during cataract modeling. So, in the crystalline lens of the eyes of experimental animals receiving Angiolin drops, an increase in the activity of GPR by 43,1% and GR by 78,4% was recorded. Angiolin increased the level of reduced glutathione by 36,2% and the content of total reduced thiols by 208,4% in the cytosol of the lens of experimental animals. The anti-cataract mechanism of 1% Angiolin eye drops is based on its antioxidant properties. Angiolin is a scavenger of reactive oxygen species and NO. Angiolin also positively affects the glutathione link of the thiol disulfide system, increasing the level of its reduced equivalents and increasing the activity of GSH enzymes. The protective effect of

Angiolin in cataracts is also realized by increasing the expression of heat shock protein – HSP_{70}, which, as a chaperone, is involved in the stabilization of intermediate conformations in the process of maturation of proteins in the context of rapid cell adaptation, in assisting the assembly of oligomeric complexes, as well as in preventing lethal non-specific protein association during oxidative stress. HSP_{70}, in addition to directly protection of cellular proteins from denaturation and oxidation, also blocks apoptosis activation pathways and stabilizes cellular structures using various mechanisms [15].

Findings: 1% Angiolin eye drops ((S) -2,6-diaminohexanoic acid 3-methyl-1,2,4-triazolyl-5-thioacetate) as an active ingredient, exhibit significant anti-inflammatory, wound healing, reparative properties, and also have a high therapeutic effect in the treatment of cataracts.

All of the above opens up new prospects for the creation of new ophthalmic medicines for expanding the range of domestic drugs, namely eye drops for the treatment of cataracts and its manifestations.

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