https://doi.org/10.21516/2072-0076-2019-12-3-51-57

Using ultraviolet corneal crosslinking for the treatment of corneal ulcers and other corneal pathologies in animals

Elena N. Iomdina — Dr. Biol. Sci., Professor, principal researcher[1]
Larisa F. Sotnikova — Dr Vet. Sci., Professor, head of chair[2]
Anna V. Goncharova — Cand. Vet. Sci., assistant professor[2]
Evgenija I. Kabanova — PhD student[2]
Anastasia V. Chechneva — PhD student[2]
Natalia Yu. Sapego — PhD student[2]
Elena V. Jani — Cand. Med. Sci, head, department of viral and allergic eye diseases[1]

[1] Moscow Helmholtz Research Institute of Eye Diseases, 14/19, Sadovaja-Chernogryazskaja St., Moscow, 105062, Russia
[2] K.I. Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology, 23, Skryabin St., Moscow, 109472, Russia
iomdina@mail.ru

Purpose. *To study the effects of corneal collagen cross-linking (CCL) using ultraviolet-A (UVA) irradiation with riboflavin on the eye's anterior segment 0.1% structures in corneal ulcers and other keratopathies in animals.* ***Material and methods.*** *The study involved 26 animals (39 eyes) — 10 cats, 10 dogs, and 6 horses with ulcers, corneal staphyloma, dry and autoimmune keratoconjunctivitis. Examination included slit-lamp biomicroscopy, ophthalmoscopy (in cases of transparent cornea), tear production test, and staining the eye surface with vital dyes. CCL was performed using UVA radiation of LEDs with a fiber optic output (wavelength 370 nm, radiation power 3 mW/cm^2) combined with 0.1% riboflavin instillation according to the protocol we developed, which specifies various numbers and durations depending on the diagnosis and therapeutic effect desired.* ***Results.*** *Restoration of the shape and transparency of the cornea, relief of anterior eye inflammation, and normalization of tear production occurred much sooner than using traditional treatment. The restoration of vision occurred 2 to 3 months earlier in eyes with corneal staphyloma; more than 4 to 5 months earlier in eyes with keratoconjunctivitis; no relapses were noted in eyes with ulcerative processes, and the restoration of the function of stratified squamous epithelium was achieved within 2 or 3 weeks.* ***Conclusions.*** *The inclusion of modified UFA-based CCL in the treatment scheme of corneal ulcers and staphylomas as well as dry and autoimmune keratoconjunctivitis enables a high therapeutic effect of anterior eye structure restoration. In future, the results obtained could serve as basis for using the developed technology in the treatment of similar eye pathologies in humans.*

Keywords: cornea; crosslinking; serpiginous corneal ulce; keratoconjunctivitis; staphyloma

Conflict of interests: The authors declare no conflict of interest.

Financial disclosure: No author has a financial or property interest in any material or method mentioned.

For citation: Iomdina E.N., Sotnikova L.F., Goncharova A.V., Kabanova E.I., Chechneva A.V., Sapego N.Yu., Jani E.V. Using ultraviolet corneal crosslinking for the treatment of corneal ulcers and other corneal pathologies in animals. Russian ophthalmological journal. 2019; 12 (3): 51–7 (In Russian). doi: 10.21516/2072-0076-2019-12-3-51-57

Keratopathies, leading to changes in the structure of the cornea, violations of its trophicity, as well as, a decrease in visual function, are a common problem in ophthalmology, including veterinary [1–3]. This is due to the specific features of anterior eye inflammation, the specific trophicity of respiratory diseases affecting the conjunctiva and cornea of animals, and a decrease in tear production. These effects result in the development of inflammatory and degenerative processes with impaired physiological barriers of the eye, characterized by significant polymorphism and severe course [4, 5]. Duration and low efficiency of traditional treatment, increased risk of complications (ulcerative keratitis, corneal perforation, symblepharon, pigment keratitis, and sub-atrophy of the eye) make it necessary to search for new methods of treating keratopathy in animals.

One of these promising approaches is collagen cross-linking, which is used for biomechanical stabilization of the cornea in keratoconus and other keratectasias in humans. Cross-linking — polymerization of stromal fibrils and an increase in the number of intra- and interfibrillar covalent bonds between collagen molecules — occurs under the influence of photodynamic reactions of the interaction of ultraviolet-A (UVA) and riboflavin solution, and as a result of the active release of oxygen free radicals, cross-linking of collagen structures is induced. As a result of therapeutic photochemical and photophysical effects and the formation of cross-links, the stroma becomes denser and stronger, its proteolytic stability increases, and its transparency improves [6–8]. This non-invasive technology is characterized by its low risk for injuring the deeply located, internal structures of the eye — lens, retina, etc., since almost the entire dose of UVA is absorbed by riboflavin in the stroma of the cornea [9].

Additionally, it was found that UVA cross-linking has a bactericidal and bacteriostatic effect on a wide range of pathogenic microorganisms, which gives grounds for its use in the treatment of keratitis of bacterial etiology [10]. In addition to the biomechanical effect (cornea stiffening), with UVA cross-linking, it has been shown that induced high levels of oxidative stress — intensive formation of reactive oxygen species for a short period of time — irreversibly destroys the DNA and cell membranes of pathogenic microorganisms present in the cornea and stops their replication [11]. In 2014, O. Richotz and F. Hafezi showed that this procedure destroys up to 95% of corneal pathogens in an in vitro study [12]. Recently there have been publications describing the successful use of UVA cross-linking for the treatment of ulcerative lesions of the cornea, including those resistant to antibiotic therapy in humans, as well as, in individual works in animals (cats and dogs) [13–16]. However, the influence of this technology implemented at the same time as other pathologies are affecting the anterior part of the eye, such as keratoconjunctivitis of various etiologies or corneal staphyloma, has not yet been studied.

Considering the present knowledge regarding kerotopathies, it is advisable to develop an algorithm and evaluate the effectiveness of using UVA and riboflavin for the treatment of various keratopathies in animals as the basis for future application of this technology in humans with similar eye pathologies.

The aim of the work was to study the effect of corneal collagen cross-linking, carried out using ultraviolet A-band exposure in combination with 0.1% riboflavin on the structures of the anterior eye in various keratopathies in animals.

MATERIAL AND METHODS

The subjects of the study were 26 animals (39 eyes) with various keratopathies: creeping corneal and secondary ulcers (4 cats and 6 horses), corneal staphyloma (6 cats), developed after complicated keratoconjunctivitis, and dry and autoimmune keratoconjunctivitis (10 dogs). Diagnostic complex included collecting of anamnesis data, general clinical examination of the animal (using standard methods), and examination of pathologic area.

The ophthalmologic examination of the area in which the pathological process occurred before, every 5–7 days during the treatment and after it included examination of the eye in lateral (focal) illumination, slit biomicroscopy, and ophthalmoscopy (provided that the cornea was transparent). During the eye examination, the Heine head magnifier and Heine slit lamp were used to determine the symmetry of the eyes, the shape of the cornea, transparency, moisture, specularity, luster, vascularization, pigmentation, symblepharon, the presence of erosions, ulcers, and corneal perforation. During external examination of the animal's eye, attention was paid to the position and condition of the eyelids, and the frequency of blink movements was evaluated. The number and nature of the conjunctival discharge was visually assessed. The evaluation attended to the absence of lacrimal menisci and gluing of the swollen, hyperemic conjunctiva of the eyelid to the conjunctiva of the sclera, as well as, on the eyelids. With the help of vital dyes, a 1% solution of sodium fluorescein, surface defects of the cornea were determined. Minor epithelial defects and non-viable cells were detected with a 1% solution of rose bengal and 3% lissamine green.

Total tear production was determined using Schirmer's test (Table 1). The data presented in the Table 1 indicate a significant decrease in the total tear production as compared to the norm (15–25 mm) in all examined animals.

To identify the specific causative agent of viral and specific bacterial infections in cats, washings from the

Table 1. Condition of tear production in animals with various forms of keratopathy before treatment (Schirmer test, total tear production), M ± SD

Parameter	Creeping corneal ulcer	Corneal staphyloma	Keratoconjunctivitis
Length of wet area, test-line, mm	13 ± 1	7 ± 1	9 ± 1

conjunctival, nasal, and oral cavities were used to carry out the polymerase chain reaction (PCR-diagnostics).

To identify the specific causative agent of viral and specific bacterial infections in cats, washings from the conjunctival, nasal, and oral cavities were used to carry out the polymerase chain reaction (PCR-diagnostics).

PCR diagnostics in cats with keratoconjunctivitis revealed mycoplasma spp. in 30% and chlamydia in 20%. In 50% of cases, the PCR diagnosis results were negative, which does not exclude a past respiratory infection (mycoplasmosis in 10% of cases and rhinotracheitis in 20% of cases).

The corneal cross-linking technique in animals was performed as described further. Initially, local ophthalmic anesthetic was instilled. Then, a 0.1% solution of riboflavin, 1 drop per minute for 20 minutes, was instilled into the conjunctival cavity. Afterwards, the cornea was exposed to UVA radiation using the LED device, "Impulse Intensity", with a fiber optic output, a wavelength of 370 nm, and the radiation power of 3 mW/cm^2 (Fig. 1) [17, 18]. This device, unlike the stationary device traditionally used in ophthalmic practice, was more convenient to use, since it allows the user to influence the interested pathological areas of the cornea in manual mode and does not require sedation and immobilization of the animal. After exposure, antibacterial drops were instilled, which were used 3 times a day for 21 days after cross-linking.

The cross-linking protocol we used in animals with keratopathies was different from that used in ophthalmic practice for treating patients with keratoconus [19, 20]. This protocol provides for the preliminary removal of the epithelium (which prevents the riboflavin solution from penetrating the cornea stroma). However, since the integrity of the epithelium was violated in animals with keratopathies as a result of the existing pathological process, it was not necessary to remove it. In addition, according to the existing protocol, cross-linking is usually carried out once, while the duration of UVA exposure is 25–30 minutes. Recently, a protocol of one-time accelerated UVA cross-linking was proposed, which provides for more intense exposure for a shorter period (wavelength of 365 nm and exposure power of 9 mW/cm^2 for 10 minutes) [21].

We have developed another cross-linking algorithm implemented using the "Impulse Intensity" LED device with a fiber-optic radiation output, which allows varying the duration and frequency of its implementation depending on the diagnosis (Table 2) [22, 23].

Evaluation of the results of treatment was carried out every 5–7 days on the basis of data from a clinical examination of the anterior eye and functional testing using vital dyes (fluorescein sodium 1%, Bengal pink 1%). With insufficient corneal epithelialization, the procedure of corneal crosslinking was repeated with an interval of 5–10 days, according to the described algorithm (Table 2).

Statistical analysis. All data are expressed as means ± standart deviation (M ± SD). A paired t-test was used to compare two means within a group (before and after treatment). Student's t-test was used. A p-value less than 0.05 was considered statistically significant.

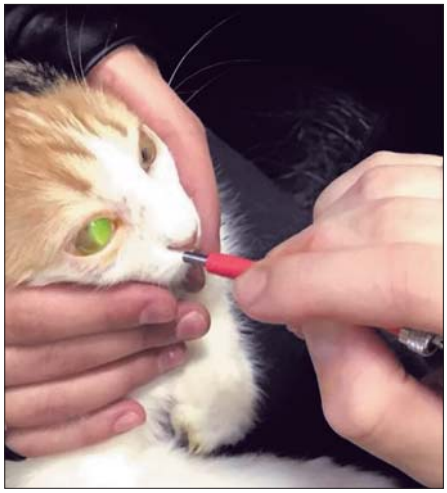

Fig. 1. Cross-linking of the corneal collagen using a LED device "Impulse Intensity" with a fiber-optic output: UVA effect was directed to the cornea, saturated with riboflavin solution

RESULTS AND DISCUSSION

The results of the treatment are presented in Tables 3–7 and Figures 2–4.

Analysis of the results of treating animals (cats and horses) with a creeping corneal ulcer showed that already on the 5th day after the first procedure there was a reduction in the diameter of erosion by 70 ± 20%, its epithelialization, a decrease in blepharospasm and a decrease in tearing (Fig. 2–4). After the end of treatment (after the 3rd procedure), i.e. in 17–21 days after its beginning, full recovery was noted in 100% of cases (4 cats and 6 horses) (Table 3). At the same time, according to our data, when applying the traditional treatment regimen, the relief of ulcerative lesions was achieved over a longer period (30 days in cats and 60 days in horses [24–26].

Evaluation of the results of treatment of animals (cats) with corneal staphyloma using corneal cross-linking

Table 2. The algorithm of corneal cross-linking in various types of keratopathies in animals

Diagnosis	Duration of the UVA radiation	Interval between procedures, days
Creeping corneal ulcers (horses, cats)	The 1st and 2nd – 5 minutes, the 3rd procedure — 5–7 minutes	5–7
Staphyloma (cats)		7–10
Autoimmune keratoconjunctivitis (dogs)		7–10
Dry keratoconjunctivitis (dogs)		7–10

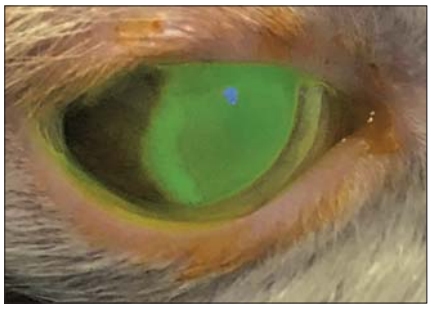

Fig. 2. Anterior segment of the cat's eyeball before treatment: subtotal creeping corneal ulcer (fluorescein staining)

Fig. 3. View of the anterior segment of the cat's eyeball 5 days after corneal cross-linking: a significant decrease in the area of ulcerative lesion

Fig. 4. View of the anterior segment of the cat's eyeball 10 days after corneal cross-linking: no ulcerative lesion of the cornea

Table 3. The results of treatment of animals with creeping corneal ulcers using corneal UVA cross-linking

Diagnosis	Number of procedures/ days of treatment	Decrease in erosion diameter	Epithelialization of erosion	Blepharospasm	Presence of discharge from the conjunctival cavity
Creeping corneal ulcer	3 times for 21 days	By 70 ± 20% on the 5th day after the first procedure	On the 5th day after the first procedure	Decreased by the 5th day after the first procedure	Abundant tearing during the first 2 days, a reduction in tearing on the 5th day after the first procedure

showed that on the 7th day after the first procedure, the size of prolapse decreased by 30 ± 10%, 14 days after the 2nd procedure — by 60 ± 20%, and at the end of treatment (on average on day 21 after it started), the size of staphyloma was reduced by 80 ± 10% ($p \leq 0.05$) (Table 4).

Thus, the treatment made it possible to reduce the time needed to restore the form of the cornea by 2–3 months compared with the known schemes [24].

Evaluation of the results of treating keratoconjunctivitis showed that in autoimmune keratoconjunctivitis in dogs, superficial vascularization decreased by 70 ± 20% on the 10th day after the first procedure, focal corneal pigmentation decreased by 21 days after the first procedure, and corneal luster appeared on the 14th day after the first procedure. In dogs with dry keratoconjunctivitis, superficial vascularization decreased by 70 ± 20% on the 10th day after the first procedure, focal corneal pigmentation decreased by the 18th day after the first procedure, and corneal luster appeared on the 14th day after the first procedure (Table 5).

In secondary corneal ulcer in dogs the decrease in erosion diameter by 70 ± 20% on the 5th day after the first procedure was noted (Table 6).

Analysis of the total tear production assessment (Table 7) and clinical examination showed that as a result of treatment the significant improvement of Schirmer test parameter and the remission of the inflammatory process in the anterior segment of the eye was achieved in the majority of sick animals.

Table 4. The results of treatment of animals with corneal staphyloma using corneal cross-linking

Diagnosis	Number of procedures/ days of treatment	Decrease in staphyloma size	Characteristics of inflammatory reaction in the area of staphyloma
Corneal staphyloma	3 procedures with 7 day interval	by 30 ± 10% on the 7th day after the 1st procedure by 60 ± 20% on the 14th day by 80 ± 10% on the 21st day $p<0.05$	Hyperemia during the first two days after corneal crosslinking as the stage of inflammatory edema

Table 5. The results of treatment of animals with keratoconjunctivitis using corneal cross-linking

Diagnosis	Number of procedures/days of treatment	Superficial vascularization	Focal corneal pigmentation	Blepharospasm	Tear production
Autoimmune Keratoconjunctivitis	3 procedures with 10 day interval	Decrease by 70 ± 20% on the 10th day after the first procedure	Decrease on the 21st day after the first procedure	Decrease on the 5th day after the first procedure	On the 14th day after the first procedure corneal luster was apparent
Dry Keratoconjunctivitis	3 procedures with 10 day interval	Decrease by 70 ± 20% on the 10th day after the first procedure	Decrease on the 18st day after the first procedure	Decrease on the 5th day after the first procedure	On the 14th day after the first procedure corneal luster was apparent

Table 6. The results of treatment of animals with secondary ulcerative keratitis using corneal cross-linking

Diagnosis	Number of procedures/ days of treatment	Decrease in erosion diameter	Epithelialization of erosion	Blepharospasm	Presence of discharge from the conjunctival cavity
Secondary ulcerative keratitis	3 procedures for 15 days	By 70 ± 20% on day 5 after the first procedure	On day 5 after the first procedure	Reduction on day 5 after the first procedure	Abundant tearing during the first 2 days, a reduction of tearing on the 5th day after the first procedure

Table 7. The production of tears in animals with various forms of keratopathy after treatment (Schirmer test), M ± SD

Indicator	Creeping corneal ulcers	Corneal staphyloma	Keratoconjunctivitis in dogs (Shepherds)
Length of wet area, test-line, mm	18 ± 1	13 ± 1	12 ± 1

It is important to note that the stabilization of the inflammatory process was achieved only if the recommended treatment regimens were applied.

CONCLUSIONS

Thus, modified cross-linking when treating the cornea in animals with keratopathies of various origins, demonstrated an excellent therapeutic effect. In corneal ulcerative lesions the disruption in inflammatory processes, corneal epithelialization, significant reduction of diameter and smoothing the edges of ulcers and erosions, reduction of purulent discharge from the conjunctival ulcer and erosions, as well as normal tear production were observed. In eyes with staphyloma and keratoconjunctivitis of various origin it was possible to restore the transparency, luster, and sphericity of the cornea in the short time, and therefore, to increase the visual function of animals. Significant advantages of the proposed therapeutic approach are that the effect is painless, the technique is convenient to use, it does not require general sedation, and the procedure has a short rehabilitation period. It is important to note that the disruption of pathological processes was achieved only when the recommended treatment algorithm and regimens (combination with local antibiotic drops instillations in corneal ulcers) were observed.

The results obtained can be used as a basis for the inclusion in future the developed technology in the complex therapy of similar eye pathologies in humans.

References

1. *Goncharova A.V.* Clinical and diagnostic criteria for keratopathy in animals. Veterinarny Vrach. 2013; 6: 48–51 (In Russian).
2. *Goncharova A.V., Sotnikova L.F.* Secondary form of ulcerative keratitis in horses as a violation of metabolic and microcircular processes in the cornea. Veterinariya, zootekhniya i biotekhnologiya. 2015; 4: 6–10 (In Russian).
3. *Goncharova A.V., Sotnikova L.F.* Treatment of horses with corneal abscess depending on the form of the course. Veterinariya, zootekhniya i biotekhnologiya. 2016; 4: 38–43 (In Russian).
4. *Goncharova A.V., Sotnikova L.F.* Risk factors of emergence and clinical-ophthalmic characteristics of horses' corneal abscess. Russian Journal of Agricultural and Socio-Economic Sciences. 2016; 50(2): 31–8.
5. *Goncharova A.V., Sotnikova L.F.* Clinical and morphological rationale for the classification of ulcerative keratitis in horses. Russian Journal of Agricultural and Socio-Economic Sciences. 2017; 61(1): 309–19.
6. *Bikbov M.M., Khalimov A.R., Usubov E.L.* Ultraviolet Corneal Crosslinking. Annals of the Russian academy of medical sciences. 2016; 71(3): 224–32 (In Russian). https://doi.org/10.15690/vramn562
7. *Wollensak G., Iomdina E.* Long-term biomechanical properties of rabbit cornea after photodynamic collagen crosslinking. Acta Ophthalmologica Scandinavica. 2009: 87(1): 48–51.
8. *Spoerl E., Wollensak G., Seiler T.* Increased resistance of crosslinked cornea against enzymatic digestion. Curr. Eye Res. 2004; 29: 35–40.
9. *Wollensak G. Iomdina E., Dittert D.-D. Herbst H.* Wound Healing in the rabbit cornea after corneal collagen-crosslinking using riboflavin and UVA. Cornea. 2007; 26: 600–5.
10. *Makdoumi K.* UVA-Riboflavin photochemical therapy of bacterial keratitis: a pilot study. Graefs Arch. Clin. Exp. Ophthalmol. 2012; 250: 95–102.
11. *Makdoumi K., Bäckman A.* Photodynamic UVA-riboflavin bacterial elimination in antibiotic resistant bacteria. Clin. Exp. Ophthalmol. 2016; 44(7 Sep.): 582–6. doi: 10.1111/ceo.12723
12. *Richotz O., Hafezi F.* Antibacterial efficacy of accelerated corneal cross-linking with photoactivated chromophore (PACK-CXL). J. Refract Surg. 2014; 30: 850–4.
13. *Alio J.L., Abbouda A., Valle D.D., Del Castillo J.M., Fernandez J.A.* Corneal cross linking and infectious keratitis: a systematic review with a meta-analysis of reported cases. J. Ophthalmic Inflamm. Infect. 2013; 3: 47. doi:10.1186/1869-5760-3-47
14. *Tabibian D., Richoz O., Hafezi F.* PACK-CXL: Corneal cross-linking for treatment of infectious keratitis. J. Ophthalmic Vis. Res. 2015; 10: 77–80.
15. *Pot S.A., Gallhöfer N.S., Matheis F.L., et al.* Corneal collagen cross-linking as treatment for infectious and noninfectious corneal melting in cats and dogs: results of a prospective, nonrandomized, controlled trial. Vet. Ophthalmol. 2014; 17(4 Jul.): 250–60. doi: 10.1111/vop.12090
16. *Chentsova E.V., Verigo E.N., Makarov P.V., Khazamova A.I.* Crosslinking in the complex treatment of corneal ulceration and corneal grafting. Russian Ophthalmological Journal. 2017; 10(3): 93–100 (In Russian). https://doi.org/10.21516/2072-0076-2017-10-3-93-100
17. *Iomdina E.N., Tarutta E.P., Semchishen V.A., et al.* Experimental realization of minimally invasive techniques of scleral collagen cross-linking. Vestnik oftal'mologii. 2016; 6: 49–57. https://www.mediasphera.ru/msph/en/oftal/Vestnik_Oftalmology_2016-6_049_EN.pdf
18. *Iomdina E.N., Tarutta E.P., Semchishen V.A., et al.* Low invasive technology of sclera crosslinking: an experimental implementation. H. Eskola et al. (eds.), EMBEC NBC. 2017; 37 IFMBE Proceedings 65: 382-5. doi: 10.1007/978-981-10-5122-7_96
19. *Wollensak G.* Crosslinking treatment of progressive keratoconus: new hope. Curr. Opin. Ophthalmol. 2006; 17: 356–60. doi:10.1097/01.icu.0000233954.86723.25
20. *Iomdina E.N.* The biomechanical aspects of keratorefractive surgery and corneal cross-linking Russian Pediatric Ophthalmology. 2015; 4: 32–7 (In Russian).
21. *Knyazer B.* Accelerated PACK-CXL for moderate therapy-resistant infectious keratitis. Cornea. 2018; 37: 528-31. doi: 10.1097/ICO.0000000000001498
22. *Chechneva A.V., Sotnikova L.F., Iomdina E.N.* The use of corneal collagen cross linking for the treatment of complications of infectious keratoconjunctivitis of cats. Izvestija MAAO. 2018; 42: 117–22 (In Russian).
23. *Iomdina E.N., Sotnikova L.F., Goncharova A.V., et al.* The method of treatment of viral and bacterial keratoconjunctivitis complications in animals using collagen crosslinking. Application for RF patent #2018147002, 27.12.2018
24. *Stekolnikov A.A., Sotnikova L.F.* Veterinary ophthalmology. Sankt-Petersburg: Prospect Nauki: 2017 (In Russian).
25. *Goncharova A.V., Sotnikova L.F.* Keratophathy in horses: the physiological barriers, clinical and bacteriological monitoring. Veterinaria i kormlenie. 2017; 6: 8–11 (in Russian).
26. *Plummer C., Gelatt K.* Color atlas of veterinary ophthalmology. Second Edition: Wiley Blackwell; 2017.

Received: 30.04.2019
Accepted: 05.06.2019

For contacts: Elena N. Iomdina
E-mail: iomdina@mail.ru