Correlation Between Effectiveness and Antioxidant Activity of Some Anti Cataract Eye Drops

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Cataracts, which are the leading cause of blindness worldwide, are a consequence of oxidative stress and are largely correlated with age. The present study aims to investigate the effectiveness of anti-cataract drops frequently commercialized on the Romanian market and its correlation with some potential mechanisms of action such as the antioxidant activity of certain chemical components. We administered eye drops over the course of three years in three groups of cataract subjects, and we examined status of the lens until the end of the survey period. The lens transparency stood in 90%, 65% and 60% of the treated patients; 10% of the placebo control group remained stable. The progression of cataract occured in 90% of control group, thus certifying effectiveness of medical therapy in cataract, previously doubted. Furthermore, evolution of lens tranparency correlated to antioxidative activity of pharmaceutical products. Antioxidative activity plays a key role in the effectiveness of some cataract eye drops by significantly preserving lens transparency. We anticipate that our findings will motivate new cataract eye drops with improved antioxidant activity. These drops may be effective enough to change public health problem strategy, by offering an alternative to surgery that is cheaper, less risky and less invasive than traditional surgical methods.

Keywords: cataract, eye drops, antioxidant activity, lens

Cataracts represent the leading cause of blindness worldwide [1, 2]. Opacification of the lens is known to be a direct consequence of oxidative stress, and is primary correlated with age [3-10]. These facts motivated us to focus on chemistry and to investigate the effectiveness of cataract eye drops and their correlation with some potential mechanisms of action in order to determine if this therapy truly deserves to be abandoned or if it deserves a second chance of being the subject of additional public scrutiny and pharmaceutical research. Furthermore, given the increasing interest in public health regarding patient adherence and compliance with therapy, solving the dilemma of *eye drops versus surgery* might offer a better balance between risks and benefits.

Cataracts are an important public health problem associated with a significant potential for blindness and high surgical costs [11]; they are mainly considered to be a surgical disease, and their etiopathogeny remains largely unknown [12-20]. The effectiveness of cataract medical therapy has been regarded as uncertain and typically a way of delaying unavoidable surgery. However, registered cases of constant patient status of timelines of years have been documented under such treatment. The trend within the last few decades has been to focus on improving surgical devices and considering cataracts to be an exclusively surgical disease; this viewpoint is associated with high costs for society, costs that represent a heavy burden, especially in developing countries. The ophthalmologists who perform cataract surgery are witness to both the surgical success of restored vision and the extreme fear of some patients facing an eye operation. Enormous improvements in cataract surgery are obvious, but even under such circumstances risks exist and complications still occur [21-23]. Our research shows that the effectiveness of pharmaceutical eye drops recommended in cataracts for preserving vision is real and is correlated with the level of antioxidant activity provided by certain chemical components [24]. We determined the Trolox equivalent antioxidant activity (TEAC) of such drops using a Photochem, Analytik Jena AG apparatus and we recovered different antioxidant capacities in the different eye drops that we evaluated [25-32].

Experimental part

Our study was based on the World Medical Association Declaration of Helsinki and on the principle that well-being of the individual research subjects must take precedence over all other interests. Our primary purpose was to improve therapy in cataract, considering that even the best current interventions must be continuously evaluated through research regarding their effectiveness.

As recommended by the Declaration of Helsinki, each subject in our study has been adequately informed regarding the aims, methods, funding sources, possible conflicts of interest, institutional affiliations of the researchers, anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study.

The Student Test for power analysis was performed, as shown in the Results and discussions part of the manuscript.

Antioxidant activity data

Apparatus used: PHOTOCHEM, Analytik Jena AG, Germany;

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Sample preparations: We took 5 µL (10 µL working volume) from the ophthalmic pharmaceutical products according to the Antioxidative Capacity in Lipid-soluble substances (ACL) procedure of Analytik Jena AG, Germany.

The free radicals (superoxide anion radicals) were produced via optical excitation (irradiation) of a photosensitizer substance. These radicals were partially eliminated from the sample via a reaction with the antioxidants present in the sample. By optical excitation (exposure) of a photosensitizer substance added in standardized volumes to the sample to be measured, we were able to produce radicals (superoxide anion radicals). Residual radicals cause the detector substance Luminol *(5-amino-2,3-dihydro-1,4-phthalazinedione)* to luminesce. The luminescence was then exactly determined in a separate cell using a photomultiplier tube. Measuring the remaining radicals caused the detector substance, Luminol, to luminesce; we thereby determined the antioxidant capacity of the sample. We tracked the measuring signal produced by the luminescence over 120 s, and we recovered various results for the measuring curves. We constructed a calibration curve by measuring a series of standard solutions, namely 0.5, 1.0, 2.0 and 3.0 nmol Trolox corresponding to 5–30 µL Reagent 4 (the working solution) (fig. 1). The standard substance Trolox, a derivative of vitamin E, is Hoffman-LaRoche's trade name for 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. It is an antioxidant, like vitamin E and is used in biological or biochemical applications to reduce oxidative stress or cell damage. The TEAC is a measure of antioxidant strength, and its units are Trolox Equivalents (TE). Due to difficulties measuring individual antioxidant components of a complex mixture, TE are used as a benchmark for the total antioxidant capacity of a mixture compounds and are measured in nmol/sample.

The ACL Kit components are as follows: *Reagent 1*: ACL Diluent: sample solvent; *Reagent 2*: Reaction buffer; *Reagent 3*: PS-1 Stock solution (photosensitizer and detection reagent), 250 µL/vial, dissolved in 750 µL of Reagent 2; *Reagent 4*: Calibration standard for the quantification of lipid soluble antioxidants in TE.

We performed the ACL calibration and measurements according to the standard kit protocol ACL using the volumes shown in the scheme below (table 1).

In each step we placed the samples on a vortex before conducting measurements using Photochem. Was constructed the calibration curve by measuring a series of standard solutions (0.5, 1.0, 1.5, 2.0 and 3.0 nmol Trolox) corresponding to 5–30 µL of the Reagent 4 working solution(X) (fig.1).

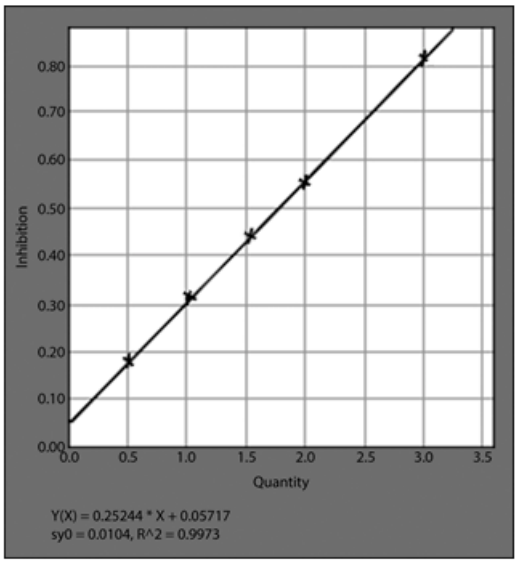

Fig.1. Calibration curve by measuring a series of standard solutions (0.5, 1.0, 1.5, 2.0 and 3.0 nmol Trolox)

Were administered three anti-cataract pharmaceutical products to patients as frequency topic medication (eye drops): *Potassium-U (P.), Quinax (Q.) and Rubjovit (R.),* and also artificial tear eye drops, considered as placebo. *Potassium-U* contains natrium chloride, potassium iodine and natrium thiosulfate, disodium EDTA, chlorhexidine diacetat; *Quinax* contains natrium azapenthacene polisulphonate, potassium chloride, boric acid, natrium tetraborate; *Rubjovit* contains natrium iodine, potassium iodine, rubidium iodine, calcium chloride, boric acid, natrium tetraborate.

These products were administered for 3 years to 60 patients with LOCS III grading stage I cataracts in both eyes (-30 women and 30 men; 60-70 years old); these patients free of comorbidities and they did not exhibit any associated risk factors for cataracts. We divided the patients into three groups consisting of 20 subjects each. The first group received 2 drops daily of *P.* in their right eye and 2 drops of artificial tears in their left eye. The second group received daily 2 drops of *Q.* in their right eye and 2 drops of artificial tears in their left eye. The third group received daily 2 drops of *R.* in their right eye and 2 drops of artificial tears in their left eye. Prior to administration, all of the patients received an ophthalmologic examination that included tests of visual acuity, visual field, a slit lamp examination and an ophthalmoscopy. The transparency of the crystalline lens was particularly considered during the examination. Every 6 months during the survey we repeated the ophthalmologic examination in order to estimate the transparency of the crystalline lens; we were accordingly able to compare the influence of the different administered drops.

Results and discussions

The ophthalmologic examination revealed that 90% of the *R.* treated patients preserved their cataract grading and their visual parameters at the end of the survey period (student test: calculated T=2.519637904, $T_{95\%}$=2.021); in the *P.* treated group, only 65% of the patients maintained their initial lens state. (Student test: calculated T=4.148934818, $T_{95\%}$=2.021). In the cohort of *Q.* patients, we found that 60% of patients were in the same state as they had been in before receiving eye drops (student test: calculated T=3.273323291, $T_{95\%}$=2.021). In the untreated

Reagent	R1	R2	R3	R4	Sample
Blank	2.300	200	25	0	0
Calibration	2.300 – x	200	25	x	0
Sample Measurement	2.300 – y	200	25	0	y

Table 1
PIPETTING SCHEME (ALL VOLUMES IN µL)

Lens transparency evolution	Eyes treated with *Rubjovit* (20)	Eyes treated with *Potassium–U* (20)	Eyes treated with *Quinax* (20)	Eyes treated with *Artificial tears/Placebo* (60)
Stability	18(90%)	13(65%)	12(60%)	6(10%)
Progression	2(10%) • 1→Stage 2 • 1→Stage 3	7(35%) • 3→Stage 2 • 3→Stage 3 • 1→Stage 4	8(40%) • 4→Stage 2 • 3→Stage 3 • 1→Stage 4	54(90%) • 21→Stage 2 • 21→Stage 3 • 12→Stage 4

Table 2
EVOLUTION OF THE LENS TRANSPARENCY IN TREATED VERSUS PLACEBO EYES

No.	Sample type	Volume sample (µL)	Maximum Radicals Inhibition value	Quantity mean (TEAC) (nmol equiv. Trolox/ volume sample)
1.	*Potassium–U*, stock solution	5	0.449	1.327
		10	0.996	11.440
2.	*Quinax*, stock solution	5	0.350	0.912
		10	0.970	9.734
3.	*Rubjovit*, stock solution	5	0.992	10.875
		10	0.998	17.225
4.	*Rubjovit*, dilution 1:10 with reagent R1	5	0.877	6.224

Table 3
THE TOTAL ANTIOXIDATIVE CAPACITY OF THE CONSIDERED OPHTHALMIC PHARMACEUTICAL PRODUCTS

eye of the patients, the crystalline lens opacities progressed toward LOCS III cataract stage 2 in approximately 35% of patients, stage 3 in approximately 35% of patients, and stage 4 in about 20% of the evaluated eyes; only 10% of eyes were stationary. These findings are summarized in table 2.

There were no significant differences in therapy response based on gender.

Our results of the total antioxidative capacity determination of the considered ophthalmic pharmaceutical products are listed in table 3.

At the working solution volume (10 µL) according to the ACL procedures, we observed a high total antioxidative capacity for the ophthalmic pharmaceutical that exceeded the calibration curve. At the working solution volume (5 µL) according to the ACL procedures, we observed an obvious total antioxidative capacity for the considered ophthalmic pharmaceutical products. Our findings are summarized as follows:

- for the ophthalmic product *Rubjovit*, we recorded an increased value of TEAC (10.875 nmol TE/volume sample). This value exceeded the calibration curve of Trolox, and it was necessary for the determination to make a dilution of the stock solution, in a molar ratio of 1:10 with the working reagent (Reagent 1). We recorded a TEAC value of 6.224 nmol TE/volume sample for diluted *Rubjovit*, which was higher than the results for *Potassium –U* and the *Quinax* stock solution.

Our comparative determinations of the total antioxidant capacity activity revealed that the ophthalmic pharmaceutical product *Rubjovit* exhibited the largest increase in TEAC values at the working solution volume (5 µL).

Our goal was to investigate if there was any difference between the administered drops in terms of the effectiveness of preserving the transparency of the crystalline lens. If such a difference was present, we sought to understand how it was correlated with the total antioxidative capacity of the product.

It is known that the cristalline lens contains only a few minerals compared with the plasma and aqueous humor. Some fraction of these minerals are ionized (potassium, natrium, chlorum, calcium and iron), and some of the minerals are incorporated into organic molecules such as phosphorus and sulphur [17]. All of our tested products contained such minerals in similar amounts; this composition might explain some benefits of the crystalline lens maintenance. However, this explanation is not sufficient to explain the differences in effectiveness observed among the three different patient groups.

Even so, it is also known there are some oxidative damages and protective mechanisms acting on the lens level. Free radicals are generated during normal cellular, metabolic activities and by various external agents such as radiant energy [33-36]. These highly reactive free radicals can damage lens fibers. Peroxidation of the lens fiber plasma or lens fiber plasma membrane lipids has been suggested as a factor contributing to lens opacification. In the process of lipid peroxidation, the oxidizing agent removes a hydrogen atom from the polyunsaturated fatty acid, forming a fatty acid radical, which, in turn, attacks molecular oxygen, forming a lipid peroxy radical. This reaction may propagate the chain, leading to the formation of lipid peroxide, which eventually can react further to yield malondialdehyde, a potent cross-linking agent. Malondialdehyde is hypothesized to cross-react with membrane lipids and proteins, rendering them incapable of performing their normal functions [20]. The differences in total antioxidative activity among the three pharmaceutical products used in our study are similar to the ratios among the final states of the lenses in our three groups of patients. Considering these facts, the value of the total antioxidative activity seems to be a key in crystalline lens preservation. Only 10% of the control lens retained the same stage of transparency compared with between 60 and 90% of the treated eyes retaining the same stage of transparency; the effectiveness of eye drops is obvious.

It is already known that patient adherence to therapy depends upon certain factors. Some of these factors are patient-related (e.g., open communication with health providers that results in a good understanding of the treatment), and other factors are therapy-related [37, 38]. All patients have individual preferences about cataract surgery; some patients avoid discussing the hazards, and other patients are interested in being as informed as possible about every potential risk. Some patients prefer a partnership in consenting to therapy with the treating doctor, other patients are more confortable leaving

decision-making to the doctor [39]. Reducing the risks of therapy would certainly provide significantly improved patient compliance.

Conclusions

The antioxidative activity of some pharmaceutical eye drops products seems to confer effectiveness at preserving the transparency of the crystalline lens.

Additional studies with larger cohorts of patients are necessary to develop new eye drops with improved antioxidant activity that might prove effective enough to prevent expensive cataract surgery procedures.

The risks of such topical eye-drop therapy with a very small amount of substances permeating through the circulatory system are nearly zero for the patient, compared with the risks of surgery. This additional option for clinical risk management in cataracts might be a better option for non-adherent patients than traditional surgery.

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