EXPERIMENTAL STUDY

The free amino acids and the aqueous humor pH after antiglaucomatics in vitro

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Abstract

Authors present comparison of the previous results on the inluence of free amino acids of the aqueous humor in rabbits and human on the pH of the aqueous humor in vitro. Four clinically used antiglaucomatics were added (2% Trusopt – pH 5.33; 0,005% Xalatan – pH 6.4; 0.5% Timoptol – pH 6.80 and 1% Pilocarpine – pH 5.87) into the aquired aqueous humor of rabbits (pH 7.59) and human (pH 7.11 ± 0.11). After their instillation pH of the aqueous humor immediately decreased towards acid levels. The pH changes after Timoptol, Xalatan and Pilocarpine showed a similar time pattern and after return to the baseline values the pH shifted to alkaline levels. In case of Trusopt instillation the pH value reached the baseline of the aqueous humor after 240 min. Almost no pH changes were recorded from 240 min up to 24 hours after all tested antiglaucomatics. From the wiev on the speed of activity and effectivity depending on time required to reach the initial pH value of the aqueous humor we to make this order: Pilocarpine›Timoptol›Xalatan›Trusopt. Comparing our results the effect of antiglaucomatics is connected with different composition and also with shift of the aqueous humor pH towards slightly alkaline level (similar effect in rabbits and human). Results presented in this work are theoretical but also practical contribution to the treatment of glaucoma. *(Fig. 1, Ref. 38.)*

Key words:. free aminoacids and pH of the aqueous humor, aqueous humor and antiglaucomatics, influencing the pH of the aqueous humor in vitro after antiglaucomatics.

Antiglaucomatics influence the production or outflow of the aqueous humor. Some of them are able to influence both the outflow increase and inhibition of the production. In the bovine eye tissue structures we observed (Veselovský et al., 1989) that antiglaucomatics (e.g. pilocarpine, timoptol) bind to amino acids during target penetration. These observations were confirmed using the interaction of the 2 % pilocarpine water solution and 0.25 % timolol maleat (Timoptol) solution with amino acids in vitro. We discovered that the application of antiglaucomatics is able to prevent the unwanted acceleration of the aqueous humor formation by inhibition of increased production of the free amino acids by the ciliary epithelium. These observations are in agreement with the previous data (Sebastiani et al, 1989), which show a high level of all amino acids (except taurine and proline) in the eye with glaucoma.

Recent developments give us the opportunity to examine interactions of the antiglaucomatics in the eye tissue structures. Under the experimental conditions we found that the pH value 7.659 of the aqueous humor was the physiologically optimal environment of the anterior eye chamber (Oláh et al., 1997, Veselovský et al., 2000). In this environment the collagen is not hydrated and maintains the normal morphologic status of the outflow channels in the trabecular tissue and in the Schlemm´s channel. As example we used the antiglaucomatic pilocarpine and proved that the mode of action is based on interaction of pilocarpine with the free aminoacids (Veselovský et al., 1998), creating the bioactive metabolite. The effect of antiglaucomatics

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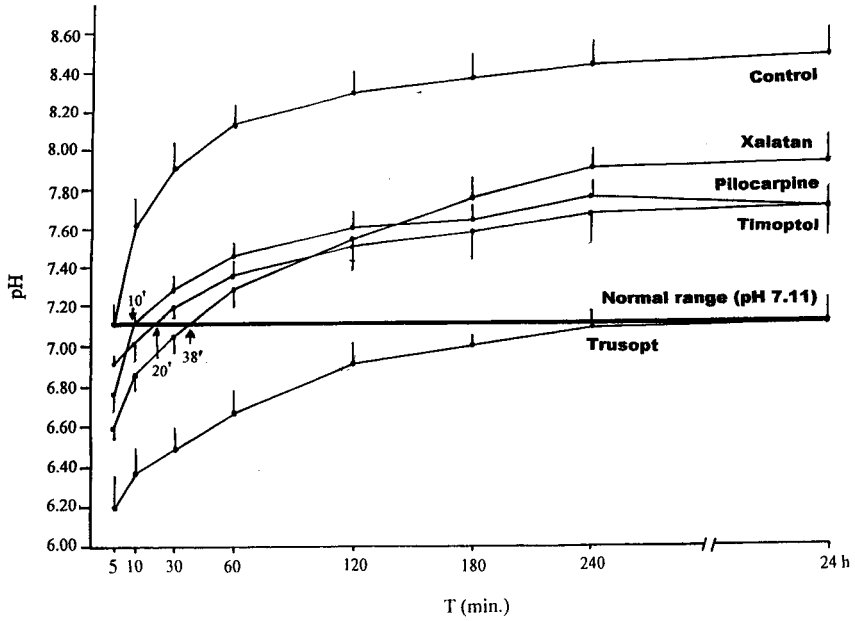

Fig. 1. pH of the human aqueous humor after application of the 2 % Trusopt (pH 5.33), of the 0.005% Xalatan (pH 6.43), of the 0.5 % Timoptol (pH 6.80) and of the 1 % Pilocarpine (pH 5.87).

is connected not only with the different composition, but also with changes of the pH towards alkaline levels in the anterior segment of the eye and mainly in the aqueous humor. Changes in pH are reflected in non-physiologic composition of the aqueous humor, in the hydrodynamics and morphologic structure of the systems connected with the formation and outflow of the aqueous humor. These deviations finally result in the glaucoma with pH of the aqueous humor 6.8 – 7.4 (Duke-Elder, 1968).

These problems are not sufficiently covered by available data. Therefore we focused on detail study of the interactions between antiglaucomatics, tissue structu-res of the anterior eye segment, the pH of the aqueous humor and influence on the IOP. Using our observations we would like to stress importance of the pH of the antiglaucomatic preparations from the view of need to maintain the approximate physiologic pH value together with decrease of the IOP and minimize their side effects.

Materials and Methods

We focused on the four antiglaucomatics used in the clinical practice as standard therapy: the carboanhydrase inhibitor dorsolamid (TRUSOPT), the prostaglandin $F_{2\ alfa}$ analogue latanoprost (XALATAN), the beta blocker TIMOPTOL and the parasympatomimetic PILOCARPINE. All measurements were made on the aqueous humor obtained from 20 eyes of adult rabbits (in average amount 285–297 µl) and from 20 eyes adult persons (female and male 48–85 years old) in average amount 170–260 µl obtained during the cataract operation. The obtained aqueous humor was divided into two sections and thermo stated under 37 °C. Antiglaucomatic was added into the one section, the second was the control as follows:

Group 0 – the aqueous humor – control (without antiglaucomatics);

Group 1 – added carbonic anhydrase inhibitor: 1 drop of the 2% TRUSOPT pH 5.33;

Group 2 – added F_{2alfa} analog: 1 drop of the 0,005% XALATAN pH 6.43;

Group 3 – added for the beta block: 1 drop of the 0,5% TIMOPTOL pH 6.8;

Group 4 – added: 1 drop of the 1% PILOCARPINE pH 5.87.

The pH values of the aqueous humor were measured in vitro, using the micro pH meter SENTRON 2001 and the probe ASEFT (fa ECOMED) immediately after aquiration, after instillation of the antiglaucomatics and in 10th, 30th, 60th, 120th, 180th, 240th min and in 24th hour.

Results were statistically evaluated using the Student´s t-test.

Results

The initial pH values of the eye's aqueous humor control at rabbits were pH 7.59, analogous to the human pH 7.11 ± 0.11. This basic value immediately after aquiration without astiglaucomatics shifted towards alkaline environment and was increased to the 60 min in each case (in average 1.03 units), followed by slight increase to the 240th min. (0.30 units). From 240th min to 24th hour almost no changes in pH were measured.

The 4 examined antiglaucomatics (tab. 1.) significantly decreased pH of the aqueous humor immediately after instillation in vitro parallel in both rabbit and human aqueous humor.

1. pH of the aqueous humor immediately after application carbonic anhydrase inhibitor of the 2% TRUSOPT (with pH 5.33) decreased for 0.91 units. To the 180th min the pH value slowly

shifted increased for 0.74 units. From 240th min. to the 24th hour the pH level was maintained under the pH of the control aqueous humor (pH 7.11).

2. pH of the aqueous humor immediately after F_{2alfa} analog 0,005 % XALATAN (with pH 6.43) application decreased for 0.12 units. After 1 hour we constated a rapid increase (for 0.69 units). From 60th min to 240th min the pH level increased moderately (for 0.62 units). From 240th min to 24th hour after Xalatan application no changes in pH of aqueous humor were measured. We accent our finding that the pH level of aqueous humor in 38th min in human (in 55th min in rabbits) achieved the pH of the control aqueous humor. From 180th min the pH values were the highest compared with the all examined samples of the aqueous humor.

3. pH of the aqueous humor after the beta blocker 0.5 % TIMOPTOL (with pH 6.80) application in 0 min. decreased for 0.21 units in human (0.56 units in rabbits). However, pH of the control group was achieved already in 20th min human eye (in rabbits after 110 min). Within next 4 hours the pH level increased slowly (for 0.77 units in human). From 240th min to 24th hour no changes in pH were measured.

4. pH of the aqueous humor after the parasympaticomimeticum 1% PILOCARPINE (with pH 5.87) application in human decreased for 0.34 units (in rabbits for 0.79 units). But in 10th min pH of the human aqueous humor increased rapidly to the level of the control aqueous humor, but in rabbits this level was achieved only in 240th min. From this time to 240th min the pH value showed slightly increased tendency (for 0.65 units) and in next 20 hours no changes in pH of aqueous humor were measured.

Discussion

Under physiologic conditions, production, outflow and composition of the aqueous humor should maintain homeostatic state indicating the correct physiologic functions of the tissue structures in the anterior uvea and also the stable IOP. However, clinical and experimental results show that neural and also humoral pathways change levels of some biochemical and physiological markers e.g. glutamic acid, free radicals, potassium, neurotrophic factors, NO metabolism, cAMP synthesis (Bartels et al., 1980, Schwartz and Yoles, 1999). Based on these changes, production of the aqueous humor is accelerated and composition is altered resulting in the increased IOP.

Available literary data dealing with application of standard antiglaucomatics in various concentrations are very limited in describing connections between the antiglaucomatic pH and the aqueous humor pH after instillation. David et al. (1978) noted that 4 % pilocarpine with pH 4.1 and 5.8 showed no significant difference in IOP decrease. Anderson et al. (1968) and Birmingham et al. (1976) recorded that the pilocarpine solutions with pH 6.5 and 7.2 are more effective compared with the acid pH 4.0 in patients with glaucoma. Conroy et al. (1995) discovered that in case of pH levels 4.4 – 4.9 the carbonic anhydrase inhibitor showed higher effect on IOP decrease compared with pH 7.1. Studying the penetration of cornea with different pH values of pilocarpine Ramera et al. (1975) observed double decrease when pH was changed from 7.5 to 4.0. Suhonen et al. (1998) contributed and confirmed these findings presenting the permeability of pilocarpine decreased 3x in case of pH change from 7.65 to 5.5 and in case of pH 4.5 the corneal absorption of pilocarpine was reduced 5 x and permeability 7 x. Sieg and Robinson (1977) also proved that absorption and penetration of Pilocarpine is 2-3 times increased in case of pH change from 5 to 8. Ashton et al. (1991) proved in pigmented rabbits the conjunctiva is more permeable for beta blockers (Atenolol, Timoptol, Levobunolol and Betaxolol) than cornea and this penetration is more intensive in case of pH increase to 8.4. Also pH of tears is important factor in penetration of the instilled ophthalmologic preparation. Longwell et al. (1976) measu-red the rabbit tears pH decrease for 1.1 – 1.63 units after instilled pilocarpine with pH 4.4 – 5.3. Authors continue with remarks that pilocarpine reduces pH of tears more than HCL with pH 4 or 2. Gadea et al. (1999) surprisingly discovered that the Müller cells absorb the amino acid glycine depending on pH and the most optimal pH value is 7.4.

We observed that the 4 tested antiglaucomatics immediately after application into the obtained aqueous humor noticeably decreased the pH value. Following changes of the pH in the aqueous humor after application of Timoptol, Xalatan and Pilocarpine showed the same pattern indicating almost the same effect of those antiglaucomatics in decreasing the IOP. The gradual specific pH increase of the aqueous humor towards alkaline levels after instillation of the mentioned antiglaucomatics prove our findings from our previous studies (Kováčik and Veselovský, 1977; Kováčik et al., 1987) that the antiglaucomatics should be from the view of transport previously connected with protein in the process of their specific interaction with the free amino acids resulting in formation of the new metabolite (the protein molecule is probably playing the role of messenger). We suppose this new metabolite causes creation of the alkaline environment in the aqueous humor. The aqueous humor after Xalatan instillation showed already from the 180th min higher pH values compared with instilled Pilocarpine and Timoptol proving the higher effect of the prostaglandin derivate Xalatan on the IOP. These facts are confirmed by literary data, e.g. Alm et al. (1995), Drance et al. (1998), Mastropasqua et al. (1999), Aung et al. (2000), Hedman and Alm (2000) and Orzalesi et al. (2000) presented the Latanaprost (Xalatan) to be more effective than Timolol in decreasing the production of the aqueous humor together with IOP.

The pH shift the control aqueous humor to the alkaline levels during incubation – averaged for 1.03 resp. 1.3 units (discovered both in the human and rabbit samples) can be explained by the fact that it was not influenced by any antiglaucomatic. At the same time, this pH shift proves, (based on our experimental results) that in case of absence of antiglaucomatics no new metabolite with ability to maintain the required physiologic pH range of the aqueous humor was formated

We suppose that from these pH changes we can predict also the additive effect of the tested antiglaucomaticts. This assumption is supported by literary reports about Latanaprost (F_{2alfa} analog) having the additive effect with Pilocarpine (Fristrom and

Nilson, 1993; Hoyng et al., 1997) and also with Timolol (Rulo et al., 1994; Nicolela et al., 1996; Hoyng et al., 1997; Diestelhorst and Almegard, 1998; Emmerich, 2000). Fristrom (1996) commented that Latanoprost is at least as effective as Timoptol. On the contrary, Nordman et al. (2000) presented that Latanoprost is more effective than Timolol combined with pilocarpine. However, Bucci (1999) presented that Latanoprost together with Timolol reduced IOP more than Latanoprost with Pilocarpine.

The carbonic anhydrase inhibitor Trusopt immediately after instillation decreased the pH value of the aqueous humor for 12.52 % – the strongest effect from the group compared with Timoptol (2.95 % decrease), Xalatan (7.31 % decrease) and Pilocarpine (4.92 % decrease). However, this rapid change was followed by only mild increase up to the 240th min, possibly proving formation of the new metabolite by gradual interaction and slight decrease of the aqueous humor production and IOP. This fact is proved by our measurements – the pH values of the control aqueous humor were not exceeded and is according to the results of Maren et al. (1997) and Wu et al., (1998) saying that these carbon anhydrase inhibitors are inhibiting the speed of pH changes caused by the bicarbonate resulting in decrease of volume and speed of the aqueous humor production.

From our previous observations (Veselovský et al, 1989) we constate that the level of free amino acids in the ciliary body epithelium after application of antiglaucomatics is decreased - almost for 40 %. Together with change the aqueous humor pH documented in recent observation towards alkaline environment result increased activity of the adenylcyclase (inhibited by acid environment). This changed environment leads to the increased activity of the cAMP and to the parallel enlargement of the intracellullary "ciliary channels" present between two layers of the ciliary epithelium and in the anterior part of the ciliary processes. These changes facilitate the aqueous humor outflow through the cilary body – uveal structures (cilio-uveal outflow).

Decreased level of free amino acids in the aqueous humor for almost 16 % after antiglaucomatics (Veselovský et al., 1989) proves also that pH of this extra cellular liquid is shifted towards normal values leading to the water release from the collagen tissues and extension of the outflow channels of the trabecular meshwork to the normal diameter. According to Tchumper and Johnson (1990) this condition is connected also with number of trabecular cells and trabecular cellularity. We assume the acid environment in the glaucomatic disease decreases the trabecular cellularity and reduces the number of trabecular cells leading to the decreased capacity of the aqueous humor outflow channels. This can be one of the following factors in patho-physiologic condition of the hypertension of the eye, resp. glaucomatic disease.

Comment

Based on our experiments, the therapeutic effect of antiglaucomatics on IOP is in mutual interaction with their binding on the free amino acids and actual pH of the aqueous humor. From our published results we can constate that in case of glaucomatic disease it is more convenient to use antiglaucomatics with mild acid up to mild alkaline pH. The physiologic pH of the aqueous humor is achieved more rapidly, irritation of the eye is prevented, the tear hyper production is limited and higher amount of the antiglaucomatic drug can reach the anterior chamber. This is connected with production of higher amount of the bioactive metabolite with alkaline pH. In clinical practice we can maintain more intensive and longer decrease of the IOP in therapy of the glaucomatic process using the lower dose of antiglaucomatic preparation. We suppose that in this work presented results are not only theoretical but also practical contribution to the antiglaucomatic therapy.

1. Based on our experimental results we can stress the pH of the aqueous humor to act as one of the main regulators pointing out not only the biochemical and physiological changes in the aqueous humor after application of antiglaucomatics, but also changes in the tissue structures of the eye.

2. With our observations we contribute to the literary data measuring the highest pH values of the aqueous humor with the observed antiglaucomatics and their proposed highest effect from the 4th hour to the 24th hour after instillation. We suppose from these pH changes we can predict also the additive effect of the tested antiglaucomatics.

3. Application of antiglaucomatics through direct interaction change concentration of the free amino acids and pH of the aqueous humor to the physiologic value (pH 7.659). This factor influences the effectivity of antiglaucomatics and should be leading in preparation and control of the medications used for decrease of the IOP.

4. Therefore, pH of the aqueous humor can be added to the determining factors indicating also the development of the glaucoma.

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