EXPERIMENTAL STUDY

Rabbit’s intraocular pressure after instillation of timolol and aminoacid lysine, arginine, glycine or taurine mixture

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Abstract

Aim: Presented experimental work was aimed to examine a pharmacokinetic efficiency of 0.5 % Timolol mixtures with 4 free amino acids, present in conjunctival sac: lysine, arginine, glycine or taurine on the IOP physiological values in rabbits.

Methods: The experimental work was performed on 5 female rabbits of the New Zealand White species. After instillation at 8.00 a.m. into the left conjunctival sac: a) the 10 % L-lysine.HCl.2H2O in 0.5 % Timolol; b) the 10 % L-arginine.HCl in 0.5 % Timolol; c) the 10 % L-glycine.HCl in 0.5 % Timolol; d) the 10 % L-taurine.HCl in 0.5 % Timolol, the IOP was measured before and in 5th, 15th, 30th, 60th, 120th, 180th and 240th min and in 24 hours. The right eye of the same rabbit was used as control with the instillation of both 0.5 % Timolol and amino acids alone into the conjunctival sac.

Results: a) The IOP has decreased after mixture of 10 % lysine in 0.5 % Timolol with two spikes – not significant decrease up to 60th min and a high significant decrease between 120th to 240th min; b) The mixture of 10 % arginine in 0.5 % Timolol decreased the IOP values with a high significance (from 2.1 to 4 torr); c) The 10 % glycine in 0.5 % Timolol showed a significant IOP decrease (excluding measurement in 5th min) in all measured times, the biggest IOP decrease was observed in 60th min (mean value 8.5 torr), the decrease was 3.3 torr also in 24 hours; d) The 10 % taurine in 0.5 % Timolol showed also a significant IOP decrease (excluding measurement in 5th min) in all measured times with maximum in 180th min (with a decrease to 6.7 torr) and the decrease showed a significantly low level – 3.3 torr – also in 24 hours.

Conclusions: The results proved that the mixture of antiglaucomatic 0.5 % Timolol and amino acid (lysine, arginine, glycine or taurine) contains a new biologically active substance, the „specific bioantiglaucomatic“ created by interaction. Compared to the substances alone, mixture of amino acid in the antiglaucomatic decreased the physiologic IOP in rabbits with a high significance. The effect on IOP based on the interaction of the mixture of amino acid with antiglaucomatic is specific and its efficacy together with time was changing depending on the type of amino acid. Our in vitro produced bioantiglaucomatic fulfilled the physiological criteria for the IOP reduction (Fig. 4, Ref. 12). Full Text (Free, PDF) www.bmj.sk.

Key words: intraocular pressure, efficiency of 10 % amino acids (Lysine, Arginine, Glycine or Taurine) in 0.5 % Timoptol mixture on rabbit’s IOP, in vitro prepared “bio-antiglaucomatic”.

From a pharmacokinetic view, antiglaucomatics in the conjunctival sac acts as “prodrug”. To penetrate the tissue barriers they should integrate with the free amino acids present in tears. In this work we are modelling a condition that should occur after an antiglaucomatic alone is administered into the conjunctival sac. It is based on the interaction of the in vitro prepared mixtures of antiglaucomatic Timolol with the 4 amino acids present in conjunctival sac: lysine, arginine, glycine, or taurine. Effect of these amino acids on the physiologic IOP values in experiment on rabbits was evaluated.

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This study was partially supported by Grant from VEGA, No. 1/0522/03. Part of this paper were accepted as oral presentation on the 104th DOG Annual Meeting in Berlin (BRD), September 23th, 2006.
Material and methods

Experimental evaluations were performed on 5 female rabbits of the New Zealand White species. Rabbits were treated in accordance with the EU Guidelines for accommodation and care of laboratory animals in Slovak centre of the agriculture research – breeding of rabbits, Nitra, No. reg.: Sk-Ch-29004 (Slovenské centrum poľnohospodárskeho výskumu – chov králíkov, Nitra, č. akred.: SK-Ch-29004).

Into the left conjunctival sac of the rabbit, always at 8.00 a.m. two drops of these substances were instilled.

Group 0 – 0.5 % Timolol;
Group 1 – added 10 % L-Lysine.HCl2H2O in 0.5 % Timolol;
Group 2 – added 10 % L-Arginine.HCl in 0.5 % Timolol;
Group 3 – added 10 % L-Glycine.HCl in 0.5 % Timolol;
Group 4 – added 10 % L-Taurine.HCl in 0.5 % Timolol,

Right eye of the same rabbit was used as control with instillation of each amino acid alone in 10 % concentration.

The IOP values were measured at baseline and in 5th, 15th, 30th, 60th, 120th, 180th, 240th min and in 24 hours after the instillation. The measurements in the groups were performed in regular one week intervals.

Results

Group 0: Antiglaucomatous 0.5 % Timolol alone did not significantly decrease a physiologic IOP value compared to the control eye. The maximal decrease to 2.9 torr was recorded in 120th min after instillation. In the 30th min, the IOP of the control and experimental eye were equal. The average IOP decrease reached by the antiglaucomatous was 0.7 torr (3.6 %).

Group 1: Mixture of the amino acid 10 % L-Lysine.HCl2H2O in 0.5 % Timolol (Fig. 1) affects the IOP values during first 4 hours in two phases. The first phase of not significant changes starts after first 60 minutes. During this phase the IOP values of control and experimental eye were equal. In the 120th min, a second phase started expressed as sudden decrease of IOP to 3.3 torr compared to the control eye. From this moment, the IOP value remains on constant level up to 4th hour.

The control eye – amino acid 10 % L-Lysine.HCl2H2O alone had no effect on IOP (remained on 23.6 torr).

Group 2: Mixture of amino acid 10 % L-Arginine.HCl in 0.5 % Timolol (Fig. 2) during 4 hours significantly decreased the physiologic IOP level ranging from 4.0 torr (in 60th min) to 2.1 torr (in 240th min) compared with the control eye. The IOP
decreased from 3.2 torr (in 30th min) to 3.8 torr (in 180th min).

Summary, this mixture decreased IOP to 3.3 torr (16.3 %).

The control eye – amino acid 10 % L-Arginine.HCl alone except for measurements in the 60th min and 180th min achieved only not significant decrease of the IOP values. In 60th min, IOP was decreased to 1.9 torr and in the 180th min, to 1.2 torr compared to mixture. An average IOP decrease was for 2.9 torr.

Group 3: Mixture of amino acid 10 % L-Glycine.HCl in 0.5 % Timolol (Fig. 3) decreased physiologic IOP with a high significance in all time intervals (excluding measurement in 5th min). The biggest decrease to 8.5 torr (33 %) was observed in the 60th min. In 24 hours, still significant decrease to 3.3 torr was recorded. An average IOP decrease was 5.6 torr (21.9 %). This mixture decreased the IOP level also in the control eye (in 180th min to 3.7 torr and in 24 hours to 3.0 torr).

The control eye – amino acid 10 % L-Glycine.HCl alone did not significantly decrease the IOP level in all time intervals.

Group 4: Mixture of amino acid 10 % L-Taurine.HCl in 0.5 % Timolol (Fig. 4) significantly decreased the physiologic IOP leveling all time intervals except for the 5th min. The biggest decrease to 6.7 torr was recorded in the 180th min. In 24 hours, the IOP level was still significantly lower (to 3.3 torr). On average, this mixture decreased the IOP value to 4.5 torr (18.9 %).

The control eye – amino acid L-Taurine.HCl alone in 5th and 60th min did not significantly decreased the IOP. In all other time intervals not significant decrease of the IOP was observed.

Discussion

Glaucoma disease is frequently treated insufficiently with antiglaucomatics in monotherapy, therefore their use in combinations (double or triple) is increasing. However, literary data (1, 2, 4, 5, 7, 8) cannot clearly explain their mode of action. Insufficient attention is dedicated especially to their relations with proteins.

Our series of experimental evaluations is highlighting the fact that the interaction of antiglaucomatic with amino acids is essential for its function (9, 10, 11, 12). As described in literature (3), as many as 11 amino acids are present in the conjunctival sac. In our previously published experimental works we showed that even in in vitro conditions the interaction of amino acids with antiglaucomatic results in a new metabolite – a substance with ability to influence the IOP. We assumed that after application of antiglaucomatics into the conjunctival sac, the pattern would be the same as in case of amino acids interaction with the antiglaucomatic (present in tears) before penetration into
the target region. Our results with Ninhydrine agent proved assumption that in the mixture of in vitro 10 % amino acid L-Lysine 2HCl 2H2O, 10 % L-arginine.HCl, 10 % L-Glycine.HCl or 10 % L-Taurine.HCl in the antiglaucomatous 0.5 % Timolol a new metabolite is formed. We named the new bioactive substance as a „bio-antiglaucomatous“. Based on our observations we can state that not each antiglaucomatous mixture with a particular amino acid decreases the IOP values significantly and for the same time. Our observations proved a clear interaction specificity of an individual antiglaucomatous with a particular amino acid only in in vivo and in vitro conditions, as was stressed by Veselovský et al (12) and Oláh et al (6). At the same time, the IOP decrease achieved by various antiglaucomatics with the same amino acid is different in significance and duration.

When a prepared mixture is administered (thus already completed metabolite), the interaction between antiglaucomatics and amino acid is much more vigorous. This metabolite instilled into the rabbit’s conjunctival sac influences the IOP more intensively already in physiological conditions. The new “bio-antiglaucomatic” has longer effect caused by probably longer metabolic half-life and by stable bound on specific receptors in the ciliary body. At the same time, the addition of amino acid (which is the main part of the tears in the conjunctival sac) to the antiglaucomatic influenced the improvement of antiglaucomatous penetration into the intraocular structures.

Our previously performed experiments were confirmed by current efficiency results with the mixtures of 10 % amino acids lysine, arginine, glycine or taurine in 0.5 % Timolol. At the same time we observed that the effect of in vitro prepared “bio-antiglaucomatic” is several times higher than its individual components.

We suppose that this mode of action – bioactivation resulting into formation of the new substance – bioregulator acting as “bio-antiglaucomatic” is responsible for the effect of antiglaucomatics. This mechanism probably controls the decrease of production or increase of outflow of the aqueous humour through the uveoscleral vascular system resulting in decreased IOP level.

**Conclusion**

1) The effect of antiglaucomatics is based on the interaction with free amino acids present in the tissue structures and fluids of the eye. During interaction, a new physiologically active substance is formed. We named this new bio-active substance a “bio-antiglaucomatic”.

2) The effect of in vitro prepared “bio-antiglaucomatic” on physiologic IOP value after instillation is several times higher than the effect of its components alone. The influence on IOP is specific for each “bio-antiglaucomatic” regarding the interaction of the specific antiglaucomatous with a particular free amino acid.

3) Our in vitro prepared bio-antiglaucomatous complies with physiologic conditions – this fact was proved by the achieved IOP decrease.

**References**


Received April 16, 2007.
Accepted June 9, 2007.