EXPERIEMT

Contribution Focused on Clarifying the Correlation between Mixtures of Free Amino Acids with Antiglaucomatics in Influencing IOP in Rabbits after Application into the Conjunctival Sac

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SUMMARY

Purpose: Correlation between the amino acid L-arginine.HCl and 3 antiglaucomatics (Timolol, Xalatan or Trusopt) mixture in their effect on the physiological IOP values in rabbits
Methods: The experimental works were performed on 5 female rabbits of the New Zealand White species. After instillation of the 2 drops of in vitro prepared 10% solution of the amino acid L-arginine. HCl in 0.5% Timolol maleat (Timoptol, Zentiva), 0.005% Lanatosid (Xalatan, Pharmacia&Upjohn) or 2% Trusopt (Dorsolamid, Merc&Co.) at 8, 00 am. into the left conjunctival sac the IOP was measured before and in 15th, 30th, 60th, 120th, 180th, 240th min. and 24 hours after instillation. The right eye was used as control.
Results: The amino acid 10% L-arginine.HCl solution applicated separately decreased the physiologic IOP in rabbit's in confrontation with the control eyes for – 2.9 torr. The antiglaucomatics applicated separately reduced the IOP values for: 0.5% Timoptol -0,69 torr, 0.005% Xalatan -2.1 torr and 2% Trusopt -2.45 torr . The 10% L-arginine. HCl in combination with the antiglaucomatics decreased the physiologic IOP by these values: in mixture with 0.5% Timolol by -3.32 torr (16.3% compared with control, but 4.8 times lower in confrontation with 0,5% Timolol); with 0.005% Xalatan by -2.91 torr (exactly the same decrease as measured after the 0.005% Xalatan separate application); with 2% Trusopt by – 4.45 torr (23.8% compared with control, but only 1.99 times lower compared with 2% Trusopt).
Conclusions: In our experiments we applicated into the conjunctival sac in fact the already ready in vitro prepared metabolite („bio antiglaucomatic") that can immediately penetrate the hemato-ocular barrier and enter into the target area. These experiments proved different relation between the amino acid L-arginine HCL and various antiglaucomatics. The 10% L-arginine HCL mixture with 0.5% Timoptol decreased the IOP values for more than 5x compared with Timoptol alone and mixture of the same substance with 2% Trusopt showed almost two-fold decrease (-1.99 torr, also compared with Timoptol). Comparing the L-arginine HCL mixture with 0.005% Xalatan with Xalatan alone no increased effectivity was constated. These differences in results are proving the individual specificity in influencing the IOP in rabbits by interaction of the specific amino acid in mixture with antiglaucomatics applicated into the conjunctival sac

Key words: correlation between mixtures of the free amino acids with antiglaucomatics in influencing the IOP in rabbits

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INTRODUCTION

In the series of our experimental works (Veselovsky et al., 1998a, 1998b, 2002; Oláh et al., 2003), we determined that the mechanism of effect of antiglaucomatics in general is bound to their interaction with free amino acids present in the conjunctival sac. In this process a new metabolite (a bioactive substance) forms, which may penetrate the barriers to the target tissue structure and reduce physiological IOP in rabbits. The interaction of free amino acids with antiglaucomatics in the conjunctival sac has a specific, graded character, which we explain in this contribution through the part of our results using the amino acid L-arginine hydrochloride in combination with certain standard clinically applied antiglaucomatics (Timoptol, Xalatan or Trusopt).

MATERIAL AND METHOD

The experiments were always conducted on a series of five adult female rabbits – of the New Zealand White pedigree, from standard rearing of the Research Institute for Animal Production in Nitra (reg. No. Sk – Ch – 29004, Nitra, Slovakia), reared under standard conditions.
Out of the essential amino acids present in the conjunctival sac we selec-

ted a dicarbon amino acid L-arginine. HCl (prepared in 10% solution) and the 3 most frequently used antiglaucomatics (0.5% Timololi maleas [0.5% Timoptol, Zentiva company], 0.005% Latanoprost [0.005% Xalatan, Pharmacia & Upjohn company] and 2% Dorsolamid.Hcl [2% Trusopt, Merck & Co.]), non-toxic for animals and humans.

First of all we determined the influence of the 10% amino acid solution or antiglaucomatics themselves on physiological IOP of rabbits. We then evaluated the effect of 10% amino acid solution in a mixture with the above-stated 3 antiglaucomatics. The amino acid L-arginine.HCl was mixed with the selected antiglaucomatic always in such a manner as to ensure that the concentration of amino acid was 10 %, and the original concentration of the antiglaucomatic and the pH remained unchanged. The solutions were instilled into the conjunctival sac of the left eye always at 08:00 a.m.. The right eye was left without treatment as a control.

We measured IOP before instillation and at a time of 15, 30, 60, 120, 180 and 240 minutes and 24 hours after instillation. We evaluated the determined IOP values by means of a Student t-test.

RESULTS

a) Application of 10% L-arginine.HCl alone into the conjuctival sac during the entire period of observation significantly reduced the level of IOP with an average reduction of -2.9 torr (as against 16.3 % in the control eye).

b) Application of a mixture of 10% L-arginine.HCl in 0.5% Timoptol (Timolol maleas) over the course of the full 24 hours of observation highly significantly reduced the level of IOP by -3.32 torr. The determined average reduction as against the control eye was 16.3 %. In comparison with the effect of 0.5% Timoptol alone, with a reduction of -0.69 (only 3.6 % as against the control eye), a pronounced, 4.8-fold (i.e. almost fivefold) reduction of IOP was achieved after application of the mixture in the eye.

c) Instillation of the mixture of 10% L-arginine.HCl in 0.005% Xalatan (Latanoprost) reduced IOP in comparison with the control eye over the course of 24 hours by -2.1 torr (reduction of 9.60 % in comparison with the control eyes). The value of the IOP reduction was identical in comparison with the data on the reduction of IOP determined following the application of 0.005% Xalatan -2.1 torr (9.50 %). From this it ensues that L-arginine. HCl in combination with Xalatan does not increase the effectiveness of the mixture on IOP.

d) Instillation of a mixture of 10% L-arginine.HCl in 2% Trusopt (Dorsolamid.HCl) at all time intervals during 24 hours significantly reduced the physiological level of IOP. The average reduction of IOP of -4.85 torr was significant and was as much as 23.8% higher in comparison with the control. Compared to the effect of 2% Trusopt alone with -2.43 torr effectiveness on IOP (with a reduction by 12.6 % in comparison with the control eyes) this represented a 1.99 times (i.e. virtually twofold) reduction.

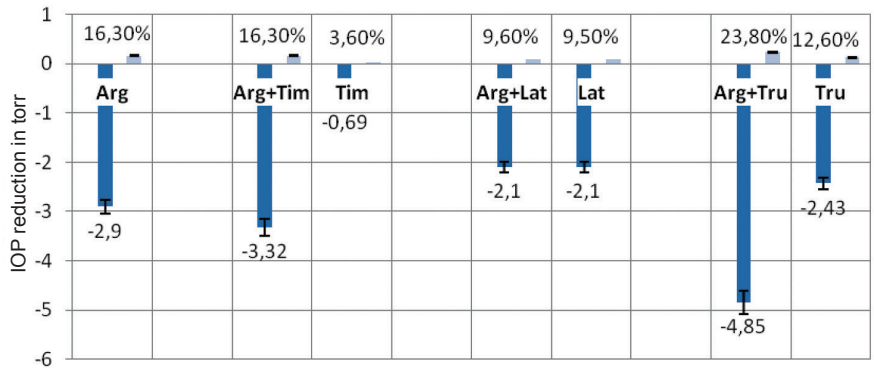
SUMMARY

Upon the summary comparison of the effectiveness of application of a mixture of 10% solution of the amino acid L-arginine.HCl with the selected 3 antiglaucomatics, we found out that the amino acid L-arginine.HCl had markedly higher activity in reducing physiological IOP in rabbits in a mixture with the antiglaucomatic Timoptol (almost 5 times) than Timoptol alone, and almost doubled activity in comparison with a mixture of the amino acid with Trusopt compared to Trusopt alone. However, the mixture of L-arginine.HCl in combination with Xalatan did not influence IOP, the determined value was identical to the result after application of Xalatan alone. The illustration and comparison of the reduction of IOP after the application of 10% L-arginine.HCl solution and its combination in a mixture with the selected 3 antiglaucomatics is presented in summary in graph 1.

DISCUSSION

The comparisons of our experimental results confirm our repeatedly recorded mechanism of the effect of antiglaucomatics on reducing IOP levels in rabbits. In our previous works (Veselovský et al., 1998a, 1998b, 2002; Oláh et al., 2003) we determined that the mechanism of the effect of antiglaucomatics generally lies in their interaction with free amino acids as early as in the conjunctival sac, with formation of a new bioactive substance (metabolite). This new metabolite reduces physiological intraocular pressure (IOP) in rabbits. We learned that the interaction of free amino acids with antiglaucomatics in the conjunctival sac has a specific character (Veselovský et al, 2003a, 2003b, 2004a, 2004b).

The antiglaucomatics topically applied into the conjuctival sac act as a “pro-drug”, and must bioactivate through interaction with free amino acids in order to reduce IOP significantly (Bito et al., 1983; Chuman et al., 2000). Kahán (1982) demonstrated the presence of 11 free amino acids in the conjunctival sac, where they may specifically interact with antiglacomatics applied into the conjunctival sac in vivo. In this, the fact of local synthesis of the arginine in the cornea is accentuated, which evidently plays a significant role as a basis for interaction with antiglaucomatics. Only after the interaction with the free amino acids in the tears can the antiglaucomatic penetrate the barriers and act within the tissue structures of the eye. The new bioactive substance obtained through the interaction of the specific free amino acid with the



Graph 1 Arg = 10% L-arginine.HCl; Tim = 0.5% Timolol; Arg&Tim = 10% L-arginine.HCl & 0.5% Timolol; Xal = 0.005% Xalatan; Arg&Xal = 10% L-arginine.HCl & 0.005% Xalatan; Tru = 2% Trusopt; Arg&Tru = 10% L-arginine.HCl & Trusopt. The percentage reduction in comparison with the control is summarily stated in the first horizontal column. The dash lines represent the dispersal.

relevant antiglaucomatic has a greater effect on the reduction of the physiological level of IOP in rabbits than antiglaucomatics applied separately (Oláh et al., 2004). We also determined that this interaction is highly specific (Veselovský et al., 2004a).

The results of our experiments demonstrate that for the effect of antiglaucomatics on IOP of rabbits to take place it is necessary for them to interact with a free, specific amino acid present in the conjunctival sac (Oláh and Veselovský, 2009; Oláh, 2011; Oláh and Veselovský, 2012). In our experiment an interaction of the selected amino acid L-arginine.HCl with the relevant antiglaucomatic took place in vitro. In the interaction, the newly formed metabolite ("bio-antiglaucomatic") enabled (or even accelerated) penetration of the antiglaucomatic into the target area (ciliary muscle) after application into the conjunctival sac, and subsequently caused a reduction of physiological IOP. The formed new metabolite could be indicated as a physiological IOP regulator.

The interaction between the relevant amino acid and the antiglaucomatic is specific and selective, which is confirmed by our findings that only a certain antiglaucomatic with a certain amino acid provides a highly significant and also long-term reduction of physiological IOP in rabbits (Oláh and Veselovský, 2007). Therefore only a certain amino acid together with a certain antiglaucomatic forms the required bioactive substance which influences IOP. The new bioactive substance formed upon interaction of the amino acid and antiglaucomatic gave a colour-positive reaction with Ninhydrin reagent (blue-purple colouring) characteristic of peptide substances. This finding confirms that the interaction of the amino acid L-arginine.HCl with the antiglaucomatics (Timoptol, Xalatan and Trusopt) in vitro generates a new metabolite, which represents an actually finished product, causing a decrease in production of chamber fluid by the ciliary muscle. It ensues from the above that in the case of an insufficient level of free amino acids in the conjunctival sac after the application of antiglaucomatics the new bioactive substance is formed only in an insufficient quantity, and as a result the antiglaucomatic does not influence or influences only slightly the physiological values of IOP. Evidently, as a result it is sometimes necessary to implement a double to triple combination of antiglaucomatics in clinical practice.

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