Molecular Vision 2005; 11:811-5 <http://www.molvis.org/molvis/v11/a96/>
Received 31 January 2005 | Accepted 21 September 2005 | Published 23 September 2005

Polymorphism of the β-2 adrenoceptor and IOP lowering potency of topical timolol in healthy subjects

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Purpose: β-2 Adrenoceptor antagonists such as timolol have been used in the treatment of glaucoma for more than 30 years. Several functionally important polymorphisms for the β-2 receptor have been described. In the present study we hypothesized that a relation between the intraocular pressure (IOP) lowering effect of timolol and β-2 adrenoceptor polymorphisms may exist.
Methods: A total of 270 healthy nonsmoking subjects were screened and individuals homozygous for the wild β-2 Adrenoceptor (Arg16/Gln27) and two polymorphisms (Gly16/Gln27 or Gly16/Glu27) were included. In these subjects the IOP lowering effect of timolol was compared.
Results: Twenty-four subjects were included in the group Arg16/Gln27, 18 subjects in the group Gly16/Gln27, and 47 subjects in the group Gly16/Glu27. The ocular hypotensive effect of timolol was between 40 and 45% in all groups, but not significantly different between the three study groups (p=0.979).
Conclusions: The present study indicates that the β-2 adrenoceptor polymorphism does not influence the ocular hypotensive effects of topical β adrenoceptor antagonists. Accordingly, other factors appear to be responsible for the intersubject variability seen with timolol in glaucoma subjects.

The treatment of glaucoma is currently focused on the reduction of intraocular pressure (IOP), which is the most important identified risk factor for the disease. This may either be achieved pharmacologically or surgically. In recent years a number of topical ocular hypotensive drugs were introduced, which have considerably facilitated IOP control in most patients. The introduction of β adrenoceptor antagonists to lower intraocular pressure (IOP) in patients with POAG in the early 1970s has considerably improved the management of these patients in clinical routine. Since then β adrenoceptor antagonists have been considered a first line therapy in newly diagnosed primary open angle glaucoma (POAG) patients. The mechanism of action of β adrenoceptor antagonists in lowering IOP is not completely understood. It has been shown that d-isomers of propranolol, which have no β blocking activity, do not lower IOP [1], indicating a role of β adrenoceptor blockade in the ocular hypotensive effect. In addition, there is evidence that the β-2 adrenoceptor subtype is predominating over the β-1 adrenoceptor subtype in regulating aqueous humor dynamics in a variety of species, including humans [2].

Although timolol has considerable IOP lowering efficacy (on the order of 20-25%), adequate IOP control can not be achieved in all glaucoma patients [3]. In addition, some interindividual differences in the responses to timolol have been described [3]. Such differences in the IOP lowering efficacy of timolol may obviously be related to interindividual differences in pharmacokinetic properties, but drug distribution to select compartments is hitherto not measurable in humans. Alternatively, β adrenoceptor polymorphisms may account for these differences. Since the β-2 adrenoceptor is the predominant adrenoceptor subtype in the iris-ciliary body [4], polymorphisms of this subtype may help to elucidate interindividual variability in IOP control.

β-2 adrenoceptors are cell surface receptors activating adenylyl cyclase via coupling to the stimulatory G-protein Gs. The pharmacogenetics of β-2 adrenoceptors has recently been reviewed [5,6]. Several functionally important polymorphic loci have been described. At nucleotide 46, A or G can be found resulting in Arg or Gly being encoded at amino acid 16 having an allele frequency of approximately 60% in caucasians. At nucleotide 79, a C or G results in Gln or Glu at amino acid 27 with an allele frequency of approximately 43% in caucasians.

In the present study we investigated the relation between the IOP lowering effect of timolol and β-2 adrenoceptor polymorphisms in healthy volunteers. This was done in an effort to elucidate whether the above mentioned β-2 adrenoceptor polymorphism is of functional importance with regard to glaucoma therapy with β adrenoceptor antagonists.

METHODS

Subjects: The present study was performed in adherence to the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines. After approval of the study protocol by the

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Ethics Committee of the Vienna University School of Medicine and after written informed consent was obtained, 270 healthy nonsmoking Caucasian male subjects were screened (age: 26.3±3.2 years, mean±SD). All subjects were drug free for at least 3 weeks prior to inclusion, had no history of β blocker intake and passed a prestudy screening that included medical history and physical examination, 12-lead electrocardiogram, blood pressure and pulse rate measurement, and an ophthalmic examination. Subjects were excluded if any abnormality was found as part of the pretreatment screening unless the investigators considered an abnormality to be clinically irrelevant. Subjects with ametropia of more than 3 D, IOP <12 mm Hg, IOP >16 mm Hg, or any evidence of eye disease that might interfere with the purpose of the present trial were excluded.

Study design and description of study days: In those subjects who passed the pre-study screening (n=270) a blood sample was drawn for β-2 adrenoceptor genotyping. The result of the β-2 adrenoceptor genotyping was the basis for scheduling a study day with timolol administration. In order to allow for clear interpretation of the results, three groups of subjects were selected. These groups consisted of individuals homozygous for Arg16/Gln27 (wild-type, group 1), Gly16/Gln27 (group 2) or Gly16/Glu27 (group 3). All subjects who did not fall within these three groups were not included in the timolol experiments.

On the trial day subjects arrived between 7:30 and 12:00 after a light breakfast and sleep for at least 7-8 h. After a 20 min resting period baseline IOP and systemic hemodynamic variables were measured. Four and 8 h after administration of one drop of topical timolol measurements of IOP and systemic hemodynamics were repeated. The investigators who measured IOP and systemic hemodynamic parameters were masked with respect to the results of the β-2 adrenoceptor genotyping.

Systemic hemodynamics: Systolic, diastolic, and mean arterial pressure (SBP, DBP, MAP) were measured on the upper arm by an automated oscillometric device. Pulse rate (PR) was automatically recorded from a finger pulse-oxymetric device (HP-CMS patient monitor; Hewlett Packard, Palo Alto, CA).

Applanation tonometry and ocular perfusion pressure: Intraocular pressure (IOP) was measured with a Perkins applanation tonometer in the sitting position (Clement Clarke, Edinburgh, United Kingdom). Oxybuprocainhydrochloride was used to anesthetize the cornea.

Genotyping: β adrenoceptor genotyping was performed by a combination of allele-specific real-time PCR for differentiation as described previously [7]. This new automatic screening technique allows for detecting common codon 16 and 27 polymorphisms of the β-2 adrenoceptor. This single step β-2 adrenoceptor codon 16/27 genotyping on chromosomal DNA is based on a combination of allele-specific codon 16 amplification primers in a real time PCR combined with allele-differentiating fluorescent probes. Use of this assay allows for cost effective and unequivocal identification of coding genotypes within a short period of time.

Data analysis: All statistical analyses were done using the Statistica® software package (Release 4.5; StatSoft Inc., Tulsa, OK). All outcome variables were calculated for each subject individually and then averaged. Comparison of the effects of timolol in the three groups was done using a repeated measures ANOVA model as the interaction between time and treatment. The effects of timolol on the outcome parameters were assessed as the time effect. For data description values are expressed as percent change from baseline. Data are presented as means±SD. An α level of 0.05 was chosen; two tail statistical tests were used.

RESULTS

The number of individuals included in the study day with timolol administration was n=24 (8.9%) for the Arg16/Gln27 group, n=18 (6.7%) for the Gly16/Gln27 group, and n=47

Table 1. Blood pressure, pulse rate, and intraocular pressure in the three groups

Measurement	Arg16/Gln27	Gly16/Gln27	Gly16/Glu27
Systolic blood pressure (mm Hg)	113 ± 7	115 ± 8	116 ± 9
Diastolic blood pressure (mm Hg)	73 ± 8	75 ± 9	75 ± 10
Pulse rate (bpm)	67 ± 10	68 ± 12	70 ± 11
Intraocular pressure (mm Hg)	13.9 ± 1.9	12.6 ± 1.1	13.1 ± 1.4
Age (years)	26.7 ± 2.7	26.1 ± 3.2	26.5 ± 3.0

The three groups included in the present study did not differ with respect to baseline systemic hemodynamic measurement, intraocular pressure or age. Data are presented as means±SD.

Table 2. Effects of timolol on blood pressure and pulse rate in the three groups

Measurement	Arg16/Gln27	Gly16/Gln27	Gly16/Glu27
2 h after administration			
Systolic blood pressure	-1.0 ± 6.9	-1.1 ± 6.1	0.3 ± 7.2
Diastolic blood pressure	-2.2 ± 7.7	-1.9 ± 7.1	-2.7 ± 7.5
Pulse rate	-3.1 ± 10.2	-1.1 ± 9.2	-2.2 ± 10.9
4 h after administration			
Systolic blood pressure	-3.2 ± 7.4	-1.4 ± 5.6	-1.9 ± 6.9
Diastolic blood pressure	-4.4 ± 7.0	-3.8 ± 6.1	-4.9 ± 8.0
Pulse rate	-5.7 ± 9.7	-5.3 ± 10.4	-5.0 ± 9.0

Administration of timolol had no significant effect on blood pressure or pulse rate in either of the three groups. Data are presented as percent change from baseline (means±SD).

(17.4%) for the Gly16/Glu27 group. The three groups were comparable with respect to age, baseline blood pressure, pulse rate and IOP (Table 1). The effects of topical timolol on systemic blood pressure and pulse rate in the three groups are summarized in Table 2. Timolol had no effect on DBP in either of the three groups (p=0.721 between groups). There was a tendency towards a reduction in SBP from baseline with timolol administration (p=0.056), which was not significantly different between groups (p=0.489). A tendency was also seen towards a decrease in PR, but this effect was again not significantly different from the baseline (p=0.066). The effect of timolol on PR was not different between groups (p=0.813).

As expected, timolol reduced IOP from baseline in all groups (Figure 1; p<0.001). The ocular hypotensive effects of timolol were comparable between the three study groups (p=0.979). Eight hours after administration of timolol the decrease in IOP was -42.1±8.3%, -40.6±8.3%, and -45.3±10.1% in the Arg16/Gln27 group, the Gly16/Gln27 group, and the Gly16/Glu27 group, respectively.

DISCUSSION

The present study does not provide evidence for a dependence of timolol-induced ocular hypotensive effects on genotyping of the β-2 adrenoceptor. Functional differences of the β-2 adrenoceptor polymorphism have been shown by several investigators in various tissues [5]. In cell cultures differences in agonist-promoted trafficking in dependence of the genotype were observed [8,9]. Whereas Gly16 adrenoceptors demonstrate enhanced downregulation, Glu27 adrenoceptors are resistant to downregulation. Accordingly, β-2 adrenoceptor genotypes may well be associated with different risks for cardiovascular disease. An association between β-2 adrenoceptor polymorphism and systemic hypertension has been proposed, but the results in different populations are inconsistent [10-18]. Obviously ethnic differences may partially account for the reported differences. In most European studies no link between β-2 adrenoceptor polymorphism and systemic hypertension were observed. Recently, it has been reported that left ventricular function in subjects homozygous for the Gly16 adrenoceptor type is better than in subjects carrying the Arg16Gly or the Arg 16 types [19]. In addition, elderly Glu27 carriers were reported to have a decreased risk of cardiovascular events [20].

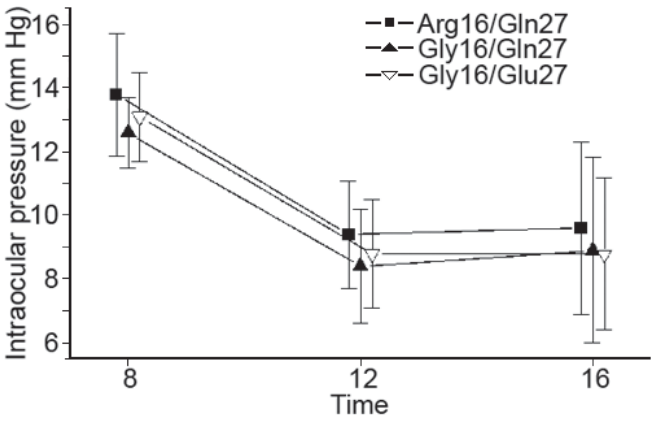

Figure 1. Effects of timolol on intraocular pressure in the three study groups. Administration of timolol reduced intraocular pressure to a comparable degree in all groups. The data points represent the pressures; the error bars represent the standard deviation.

A number of studies also investigated potential pharmacogenomic consequences of the β-2 adrenoceptor polymorphism. There is consistency that in patients with asthma treated with β-2 adrenoceptor agonists, the greatest bronchodilation is achieved in Arg16 carriers [21,22]. The situation is less clear with respect to the vasodilator response to β-2 agonists where the largest response has been reported in subjects homozygous for the Arg16 type after systemic administration, whereas the largest response after local administration has been observed in subjects homozygous for the Gly16 type [17,23-25]. One needs to consider that discrepancies in these studies may either be related to counter-regulatory systems after systemic administration or to the time span of the studies opposing acute effects to long term desensitization [24].

It has been pointed out that a factor that may well limit the relevance of many pharmacogenomic studies investigating the role of β-2 adrenoceptor polymorphisms in drug responsiveness is the failure to consider haplotype [26]. In a study in which, as in the present study, only subjects with homozygous haplotypes were studied, subjects with Arg16/Gln7 and Gly16/Gln27 haplotypes had a reduced vasodilator response to β-2 agonists as compared to Gly16/Glu27 carriers. On the other hand subjects homozygous for Arg16/Gln27 showed a greater desensitization. Accordingly, we enrolled homozygous haplotypes only to allow for clear interpretation of the results.

Only one study has previously investigated a potential role of the β-2 adrenoceptor polymorphism in the eye [27]. In this study in healthy subjects the post-exercise reductions in IOP were shown to depend on genotyping of the β-2 adrenoceptor. More specifically, the authors observed that the return to baseline IOP after exercise was longer in subjects with the homozygote mutant Gly16Gly than in subjects with the heterozygote mutant Arg16Gly. The interpretation of the results of this study is hampered by the very small sample size (n=19 in total, 8 in the Gly16Gly group and 8 in the Arg16Gly group). In addition, the role of the β-2 adrenoceptor in exercise-induced changes in IOP is not well established. How could the negative results of the present study be interpreted in view of the results of the previous studies in other tissues and the eye? One possibility is of course that the β-2 adrenoceptor does not play a major role in the ocular hypotensive effect of timolol. Other mechanisms including involvement of the dopaminergic system have been implicated in this response [28], but a proof of the contribution of other non β adrenergic receptors is lacking. A more likely explanation for the findings of the present study is that other factors including pharmacokinetic properties or drug distribution are responsible for

the intra-individual variability in the response to timolol, which mask a potential subtle effect related to β-2 adrenergic polymorphism.

The present study was performed in healthy subjects rather than in patients with POAG or ocular hypertension. This was done in an effort to overcome some problems associated with a study in patients to test the hypothesis of the present study: (1) only newly diagnosed patients may be used, because long term influence of previous antiglaucoma medication on timolol-induced hypotensive effects can not be excluded even after short term washout, (2) heterogeneity in baseline untreated IOP, and (3) potential influence of medications that are used for reasons other than glaucoma.

In conclusion, the present study indicates that β-2 adrenoceptor polymorphism does not influence the ocular hypotensive effects of topical β adrenoceptor antagonists. Accordingly, other factors appear to be responsible for the variable results obtained with these drugs in glaucoma subjects.

REFERENCES

1. Vale J, Phillips CI. Effect of DL- and D-propranolol on oscular tension in rabbits and patients. Exp Eye Res 1970; 9:82-90.
2. Lotti VJ, Le Douarec JC, Stone CA. Autonomic nervous system: adrenergic antagonists. In: Sears ML, editors. Pharmacology of the Eye. Berlin: Springer-Verlag; 1984. p. 249-277.
3. Zimmerman TJ, Boger WP 3rd. The beta-adrenergic blocking agents and the treatment of glaucoma. Surv Ophthalmol 1979; 23:347-62.
4. Wax MB, Molinoff PB. Distribution and properties of beta-adrenergic receptors in human iris-ciliary body. Invest Ophthalmol Vis Sci 1987; 28:420-30.
5. Liggett SB. Pharmacogenetics of beta-1- and beta-2-adrenergic receptors. Pharmacology 2000; 61:167-73.
6. Small KM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. Annu Rev Pharmacol Toxicol 2003; 43:381-411.
7. Lucas T, Losert D, Allen M, Halaschek-Wiener J, Pratscher B, Friedrich C, Wolschek M, Fuchsjager-Mayrl G, Schmetterer L, Pehamberger H, Muller M, Wacheck V. Combination allele-specific real-time PCR for differentiation of beta 2-adrenergic receptor coding single-nucleotide polymorphisms. Clin Chem 2004; 50:769-72.
8. Green SA, Turki J, Hall IP, Liggett SB. Implications of genetic variability of human beta 2-adrenergic receptor structure. Pulm Pharmacol 1995; 8:1-10.
9. Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human beta 2-adrenergic receptor impart distinct agonist-promoted regulatory properties. Biochemistry 1994; 33:9414-9. Erratum in: Biochemistry 1994; 33:14368.
10. Herrmann SM, Nicaud V, Tiret L, Evans A, Kee F, Ruidavets JB, Arveiler D, Luc G, Morrison C, Hoehe MR, Paul M, Cambien F. Polymorphisms of the beta2-adrenoceptor (ADRB2) gene and essential hypertension: the ECTIM and PEGASE studies. J Hypertens 2002; 20:229-35.
11. Jia H, Sharma P, Hopper R, Dickerson C, Lloyd DD, Brown MJ. beta2-adrenoceptor gene polymorphisms and blood pressure variations in East Anglian Caucasians. J Hypertens 2000; 18:687-93.
12. Tomaszewski M, Brain NJ, Charchar FJ, Wang WY, Lacka B, Padmanabahn S, Clark JS, Anderson NH, Edwards HV, Zukowska-Szczechowska E, Grzeszczak W, Dominiczak AF. Essential hypertension and beta2-adrenergic receptor gene: linkage and association analysis. Hypertension 2002; 40:286-91.
13. Bengtsson K, Orho-Melander M, Melander O, Lindblad U, Ranstam J, Rastam L, Groop L. Beta(2)-adrenergic receptor gene variation and hypertension in subjects with type 2 diabetes. Hypertension 2001; 37:1303-8.
14. Kato N, Sugiyama T, Morita H, Kurihara H, Sato T, Yamori Y, Yazaki Y. Association analysis of beta(2)-adrenergic receptor polymorphisms with hypertension in Japanese. Hypertension 2001; 37:286-92.
15. Busjahn A, Li GH, Faulhaber HD, Rosenthal M, Becker A, Jeschke E, Schuster H, Timmermann B, Hoehe MR, Luft FC. beta-2 adrenergic receptor gene variations, blood pressure, and heart size in normal twins. Hypertension 2000; 35:555-60.
16. Bray MS, Boerwinkle E. The role of beta(2)-adrenergic receptor variation in human hypertension. Curr Hypertens Rep 2000; 2:39-43.
17. Gratze G, Fortin J, Labugger R, Binder A, Kotanko P, Timmermann B, Luft FC, Hoehe MR, Skrabal F. beta-2 Adrenergic receptor variants affect resting blood pressure and agonist-induced vasodilation in young adult Caucasians. Hypertension 1999; 33:1425-30.
18. Kotanko P, Binder A, Tasker J, DeFreitas P, Kamdar S, Clark AJ, Skrabal F, Caulfield M. Essential hypertension in African Caribbeans associates with a variant of the beta2-adrenoceptor. Hypertension 1997; 30:773-6.
19. Tang W, Devereux RB, Kitzman DW, Province MA, Leppert M, Oberman A, Hopkins PN, Arnett DK. The Arg16Gly polymorphism of the beta2-adrenergic receptor and left ventricular systolic function. Am J Hypertens 2003; 16:945-51.
20. Heckbert SR, Hindorff LA, Edwards KL, Psaty BM, Lumley T, Siscovick DS, Tang Z, Durda JP, Kronmal RA, Tracy RP. Beta2-adrenergic receptor polymorphisms and risk of incident cardiovascular events in the elderly. Circulation 2003; 107:2021-4.
21. Lima JJ, Thomason DB, Mohamed MH, Eberle LV, Self TH, Johnson JA. Impact of genetic polymorphisms of the beta2-adrenergic receptor on albuterol bronchodilator pharmacodynamics. Clin Pharmacol Ther 1999; 65:519-25.
22. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. J Clin Invest 1997; 100:3184-8.
23. Cockcroft JR, Gazis AG, Cross DJ, Wheatley A, Dewar J, Hall IP, Noon JP. Beta(2)-adrenoceptor polymorphism determines vascular reactivity in humans. Hypertension 2000; 36:371-5.
24. Dishy V, Sofowora GG, Xie HG, Kim RB, Byrne DW, Stein CM, Wood AJ. The effect of common polymorphisms of the beta2-adrenergic receptor on agonist-mediated vascular desensitization. N Engl J Med 2001; 345:1030-5.
25. Hoit BD, Suresh DP, Craft L, Walsh RA, Liggett SB. beta2-adrenergic receptor polymorphisms at amino acid 16 differentially influence agonist-stimulated blood pressure and peripheral blood flow in normal individuals. Am Heart J 2000; 139:537-42.
26. Johnson JA, Lima JJ. Drug receptor/effector polymorphisms and pharmacogenetics: current status and challenges. Pharmacogenetics 2003; 13:525-34.
27. Gungor K, Beydagi H, Bekir N, Arslan C, Suer C, Erbagci I, Ergenoglu T, Aynacioglu AS. The impact of acute dynamic ex-

ercise on intraocular pressure: role of the beta 2-adrenergic receptor polymorphism. J Int Med Res 2002; 30:26-33.

28. Chiou GC, Watanabe K, McLaughlin MA, Liu HK. Are beta-adrenergic mechanisms involved in ocular hypotensive actions of adrenergic drugs? Ophthalmic Res 1985; 17:49-53.

The print version of this article was created on 23 Sep 2005. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.