• Brief Report •

Effect of brimonidine tartrate on basophil activation in glaucoma patients

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Abstract

● **AIM:** To evaluate the mechanism of which brimonidine tartrate 0.15% causes clinical hypersensitivity.

● **METHODS:** A prospective case-control study comparing 8 glaucoma patients with clinical hypersensitivity to brimonidine to a control group consisting 13 healthy volunteers. Blood samples were stimulated with brimonidine 0.15%, timolol 0.5% or brimonidine tartrate/timolol maleate 0.2%/0.5%. Premixed antibodies (CD63/FITC and aIgE/PE) were added for direct staining and whole-blood samples were lysed, fixed and analyzed by a flow cytometer. The basophil population was defined by high IgE cell expression. Degranulation was identified by the expression of the activation molecule CD63.

● **RESULTS:** Basophil activation was not significant when comparing percent of activated basophils of patients and healthy controls after exposure to brimonidine (2.58%, 2.45%, respectively, *P*=0.72). There was a significant suppression of basophil activation when a combination of brimonidine-timolol (0.87%) was compared to timolol (2.27%; *P*=0.012) and to brimonidine alone (2.58%; *P*=0.017).

● **CONCLUSION:** The results of our study do not support the hypothesis that brimonidine induces an immediate allergic reaction. Basophil activation was suppressed by the presence of β-blockers in patients hypersensitive to brimonidine and in healthy individuals. This finding indicates that timolol suppress brimonidine drug reaction by a different mechanism.

● **KEYWORDS:** glaucoma; allergy; hypersensitivity; brimonidine; timolol; basophils

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INTRODUCTION

The selective α2 adrenergic agonist, brimonidine tartrate 0.15%, is used for lowering intraocular pressure (IOP) in patients with glaucoma or ocular hypertension. It has been shown to cause hypersensitive reactions such as pruritis, chemosis, conjunctival hyperemia followed by allergic conjunctivitis[1-11]. Allergic reaction occurs in 4.2%-25.7% of patients treated with brimonidine eye drops and is seen within a few days to up to nine months after the initiation of treatment. The disappearance of these symptoms after the discontinuation of brimonidine confirmed the diagnosis.

Few studies have compared the hypersensitivity reaction caused by brimonidine monotherapy versus fixed combination of brimonidine and timolol (non-selective β-adrenergic antagonist). All studies showed that the combination was associated with a lower incidence of ocular hypersensitivity compared to brimonidine monotherapy alone[4-6].

Immediate drug hypersensitivity reactions (DHRs) resemble typical immunoglobulin E (IgE)-mediated symptoms. Key characteristic of allergic effector cells in immediate-type allergy is allergen-specific IgE bound to the high affinity IgE receptor, *i.e.*, FcεRI, on the cell surface. Capturing of allergens by surface IgE results in FcεRI crosslinking and elicits the acute phase of the allergic response involving the sudden release of vasoactive mediators into the tissue and/or circulation. Mast cells and basophils both share these key characteristics. Basophils are peripheral blood circulating granulocytes, well-known effector cells in allergic reactions with a membrane protein receptor that binds IgE[12], and a well-established surrogate for DHR diagnosis[13]. Basophil activation can be measured using the basophil activation test (BAT) by flow

cytometry[13-14]. The test is based upon the expression of CD63 and IgE present on basophil intracellular granules which are exported to the membrane upon activation and degranulation. The BAT is a validated test in the diagnosis of immediate hypersensitivity reaction due to drugs, venom, and food as well as to other allergies. The aim of our study is to both investigate the mechanism of brimonidine-DHR by identifying the underlying reaction and to evaluate the immunological suppression of this reaction by timolol.

SUBJECTS AND METHODS

Ethical Approval This prospective case control study included patients with primary open angle glaucoma, who were diagnosed and treated in the Tel Aviv Medical Center Glaucoma Clinic, which have experienced a clinical hypersensitivity reaction to brimonidine; and a control group, comprised of healthy volunteers without known glaucoma or allergic reactions to medication. Approval and informed consent were obtained from all patients and all included patients were 18 years of age or older. All data for the study were collected and analyzed in accordance with the policies and procedures of the Institutional Review Board (IRB) of the Tel Aviv Medical Center and the tenets set forth in the Declaration of Helsinki.

Design The study was conducted in two phases. The first phase compared between patients with a clinical hypersensitivity reaction to brimonidine in the past to a control group comprised of healthy volunteers who are not treated with any ocular or systemic medication.

Flow cytometry quantitative determinations of basophilic degranulation were carried out by the Basotest (Glycotope Heidelberg, Germany)[15]. Sample preparations were made according to the manufacturer's instructions. Peripheral blood was taken from all patients and collected in sodium heparin tubes which were stored at room temperature and processed within 24h of sampling. Blood samples were stimulated for 10min at 37℃ with brimonidine 0.15% (Alphagan® P, Allergan Inc., Irvine, CA, USA), timolol 0.5% (Vitamed LTD, timolol maleate 0.5%), or the combination drug (Combigan®, Allergan Inc., Irvine, CA, USA, brimonidine tartrate/timolol maleate ophthalmic solution 0.2%/0.5%; all the drugs were purchased from BD Biosciences Pharmingen). In addition, for positive control, one sample from each patient was activated by N-Formil-MetLeu-Phe (fMLP) and for negative control washing solution inset of the activators was used.

Degranulation was stopped by transferring the sample tubes to an ice bath for 5min. Twenty microliters of the premixed antibodies (CD63/FITC and aIgE/PE) were added for direct staining in the dark for 20min on ice. The whole-blood samples were lysed and fixed with 2 mL lysing solution for 10min at room temperature. After washing, each cell pellet was stored in 200 μL of washing solution in an ice bath for up to 2h before analysis. The samples were analyzed by a flow cytometer FACSCalibur (BD Biosciences, San Jose, CA, USA). At least 100 000 basophils were acquired for each sample. The basophil population was defined by high IgE cell expression. Degranulation was identified by the expression of the activation molecule CD63 in those high IgE cells (CD63+IgE high+).

The second phase included a third group of healthy controls. To this group we added the Basotest kit positive control (fMLP) to all three drugs samples (timolol, brimonidine, and the combination drug). The rationale behind this was to activate the basophils from all samples to a maximum level and then to evaluate the suppressive effect of each drug on basophil activation.

Statistical Analysis Data were recorded in Microsoft Excel (2010)™ and analyzed using SPSS version 23 (SPSS Inc., Chicago, IL, USA). Statistical analysis was made by the Wilcoxon signed rank test which was applied to evaluate the significance in CD63 activation between the different drugs tested. Binary variables were compared between subjects using the Fisher's exact test. The threshold for statistical significance was defined as $P<0.05$.

RESULTS

The first phase included eight patients in the study group (Group 1) and six healthy volunteers in the control group (Group 2). The second phase included seven healthy volunteers (Group 3).

Basophil activation was not significant when comparing the percent of activated basophils of the patients (Group 1) nor healthy controls (Group 2) after exposure to brimonidine (2.58%, 2.45%, respectively, $P=0.72$), and when compared to the blood samples of Groups 1 and 2 with the kit's negative control (2.41%, 6.12%, respectively, $P=0.12$). Similar results of basophils activation were seen when comparing both groups upon exposure to timolol alone (2.27%, 1.84%, respectively, $P=0.36$) and compared with the kits negative control in Groups 1 and 2 (2.41%, 6.12%, respectively, $P=0.12$; Figure 1).

A comparison of the percentage of CD63 activation between the three drug regimens in the study group (Group 1) yielded statistically significant difference between the combination drug activation (0.87%) and timolol activation (2.27%; $P=0.012$), as well as the combination drug activation (0.87%) and brimonidine activation (2.58%; $P=0.017$). In the control group (Group 2), a statistically significant difference was found in the percentage of CD63 activation between the combination drug (0.81%) and timolol (1.84%; $P=0.043$), and a trend towards significance between the combination drug (0.81%) and brimonidine (2.45%; $P=0.068$). In both groups, the lowest percentage of CD63 activation was found in the combination drug, higher for timolol and highest for brimonidine.

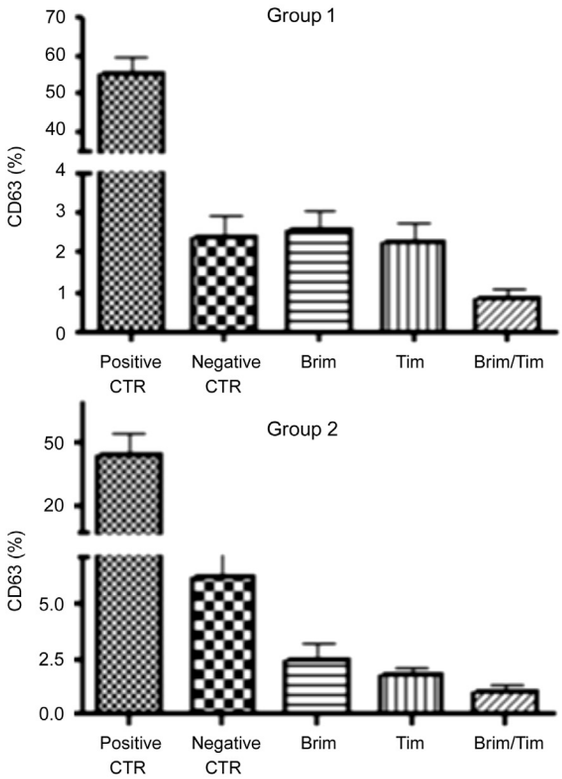

Figure 1 Percentage of CD63 activation Positive CTR: Positive control; Negative CTR: Negative control; Brim: Brimonidine; Tim: Timolol; Brim/Tim: Combination brimonidine and timolol.

Group 3 results were similar to those of the first stage. There was a lower CD63 activation in the combination drug (25.78%) compared to brimonidine (28.79%) or timolol (28.78%). Using the same statistical analysis method, significance was found only when the combination drug was compared to timolol (P=0.018; Figure 2).

DISCUSSION

In this study, we investigated the mechanism of brimonidine-DHR and evaluated the immunological suppression of this reaction by timolol. This current study results demonstrated that the basophils obtained from peripheral blood samples of glaucoma patients with proven hypersensitivity to brimonidine were not found to be activated following exposure *in vitro* to brimonidine. The percentage of basophils activated by brimonidine and timolol was similar to those of the BAT kit's negative control and even lower in the combination drug. These results do not support an immediate hypersensitivity, but rather suggest that the mentioned adverse reaction is induced by a different mechanism.

There are several known hypersensitivity reactions to brimonidine[1-11]. Previous studies reported that adding timolol to brimonidine reduces significantly the rate of hypersensitivity reaction by approximately 50%[4-6]. Butler *et al*[16] have suggested that adrenergic agents may reduce the volume of conjunctival cells, thereby widening the intercellular spaces through which potential allergens may reach the sub-epithelial

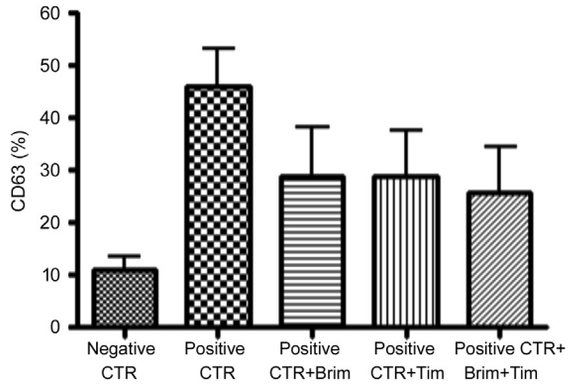

Figure 2 Suppression of activated basophils Positive CTR: Positive control; Negative CTR: Negative control; Brim: Brimonidine; Tim: Timolol; Brim/Tim: Combination brimonidine and timolol.

tissues, causing the adverse reaction. This is supported by earlier findings by Alvarado *et al*[17] in a study which demonstrated that adrenergic agents decrease the cell volume of cultured human trabecular meshwork and Schlemm's canal endothelial cells. This, in turn, results in an increased fluid flow through a widened paracellular route. As these effects were completely blocked by simultaneously administered timolol, they suggested that the cellular changes and the increase in fluid flow are mediated by a beta-2 receptor. Osborne *et al*[18] supported these finding by showing that brimonidine promotes the likelihood of allergy to a subsequently used preparation, but that timolol used prior to brimonidine seems to confer some protection against brimonidine allergy. Their data failed to determine, with statistical significance, whether the preservative used in an ophthalmic preparation has any effect on the likelihood of allergy to that preparation.

To the best of our knowledge, this is the first study to investigate the mechanism that induces this hypersensitivity reaction. A few possibilities may be considered such as toxic, pharmacological or immunological. Among the immune mechanisms, type 1 hypersensitivity was evaluated in our *in vitro* study. An i*n vitro* study was possible in this case, since brimonidine is not a pro drug rather the active ingredient. As such, the molecule of brimonidine does not convert when applied topically to the ocular surface.

Basophils and mast cells are key effector cells in immediate-type allergic reactions, and the clinical impact of BAT is due to the unique ability of these cells to degranulate upon cross-linking of the specific IgE bound on membrane-bound high-affinity IgE receptor by allergen exposure.

It is possible that the adverse events induced by the topical drug is not an IgE-mediated reaction allergic response but rather a delayed type, or due to complement-mediated reaction or direct activation[19]. Another possibility is that the patients developed a toxic reaction to the preservative in the preparation, rather than an allergy to the active ingredient[20].

Both the allergic and non-allergic subjects did, however, show profound inhibition of basophil basal activation when β-blockers were added to activated basophils. The results of Group 3, in which we activated the basophils by adding the positive control fMLP of the Basotest, and analyzed the effect of the drugs added afterwards, supported this phenomenon. In this group, suppression of activated basophils was detected with the addition of all three drug samples; most suppression was found in the addition of the combination drug. These results support the idea that the effect of timolol is most likely pharmacological.

The limitations of this study are small sample size for all groups, the evaluation of basophiles activity in the peripheral blood and not in the tear film and usage of commercial drugs with preservative substances.

In conclusion, the *in vitro* results of our study do not support our initial hypothesis that brimonidine induces an immediate allergic reaction. Basophil activation was found to be suppressed by the presence of β-blockers in patients with proven hypersensitive to brimonidine and in healthy individuals, as described previously by the literature. This finding indicates that timolol suppress brimonidine drug reaction by different mechanism.

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Conflicts of Interest: Rosenfled E, None; **Barequet D,** None; **Rabina G,** None; **Langier S,** None; **Lazar M,** None; **Shemesh G,** None; **Kurtz S,** None; **Kivity S,** None.

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