European Review for Medical and Pharmacological Sciences 2017; 21: 4720-4725

Mirtogenol® supplementation in association with dorzolamide-timolol or latanoprost improves the retinal microcirculation in asymptomatic patients with increased ocular pressure

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Abstract. – OBJECTIVE: Supplementation with Mirtogenol® improves the retinal microcirculation and reduces intraocular pressure (IOP) in ocular hypertension, when administrated either alone or in association with an ophthalmic solution (latanoprost). In this study, microcirculatory parameters (perfusion of the circle of Zinn-Haller and retinal circulation) and oxidative stress were tested to assess the effects of Mirtogenol® plus traditional antihypertensive drugs in patients with elevated IOP.

PATIENTS AND METHODS: 88 otherwise healthy patients with increased IOP were followed-up in a supplement registry for 12 weeks. Three groups received; (a) dorzolamide-timolol plus Mirtogenol®; (b) latanoprost drops plus Mirtogenol® or (c) latanoprost only. Oral supplementation consisted of two tablets/day of Mirtogenol® (80 mg of bilberry extract, Mirtoselect® plus 40 mg of Pycnogenol®). IOP, retinal blood flow, perfusion of the circle of Zinn-Haller, and oxidative stress were measured during the registry period.

RESULTS: The three study groups were comparable; IOP and ocular blood flow velocity at inclusion were also comparable. Over the study period the decrease in IOP and the improvements in retinal microcirculation were statistically significant for all management groups, with a marginally more evident benefit in Mirtogenol®+latanosprost-treated patients. At 12 weeks, the altered perfusion at the circle of Zinn-Haller was improved in all groups; patients using Mirtogenol® showed a better perfusional pattern compared with subjects using only latanoprost. A reduction in oxidative stress was observed in supplemented subjects at the end of the study period; no significant change was seen in non-supplemented patients. All managements were well-tolerated without side effects.

CONCLUSIONS: Supplementation with Mirtogenol®, in addition to local ophthalmic treatments, is safe and may contribute as a supplementary management to reach a normal IOP and ocular microcirculatory parameters.

Key Words:
Intraocular pressure, Latanoprost, Dorzolamide-timolol, Mirtogenol, Mirtoselect, Pycnogenol, Bilberry extract.

Introduction

By 2020 around 80 million people will develop glaucoma worldwide, and this condition will represent the second leading cause of blindness, with 11.1 million people bilaterally blind for primary glaucoma[1]. The Ocular Hypertension Treatment Study (OHTS), which included patients with intraocular pressure (IOP) between 21 mmHg and 32 mmHg and no glaucomatous damage, revealed that central corneal thickness, as well as age, vertical and horizontal cup-to-disc ratio, pattern standard deviation, and IOP, are good predictors for the development of glaucoma[2,3]. Also, the probability of developing glaucoma in 5 years was considerably reduced in treated eyes with lower IOP, compared with untreated eyes (4.4% *vs.* 9.5%)[2,3]. These results suggest that patients with ocular hypertension and at moderate-high risk to develop glaucoma should receive adequate management in order to control the elevated IOP. Among standard topical antihypertensive agents, prostaglandin-analogs such as latanoprost are the most commonly used first-line treatment. Prostaglandins promote ciliary muscle relaxation and increase the aqueous humor outflow through the suprachoroidal space[4]. Beta-blockers and car-

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bonic anhydrase inhibitors can be used alone or in combination as IOP-lowering medications. A dorzolamide-timolol formulation combining these two different pharmacologic classes of ocular hypotensive drugs decreases the secretion of aqueous humor through multiple pathways[5]. Improvement in microcirculation could represent an additional approach that may complement traditional ocular antihypertensive drugs[6]. Flavonoids are widely distributed in plants and include anthocyanins[7]. Supplementation with bilberry (*Vaccinium myrtillus* L, standardized in 36% anthocyanins) has been shown to improve eye health and microcirculation[8]. Mirtogenol® (Farmigea, Pisa, Italy; including a standardized bilberry extract and a pine bark), has been successfully used as preventive intervention[9] when used as supplementation in association with standard management (latanoprost[10]) for asymptomatic intraocular hypertension. In this registry study, we assessed the effects of the pharma-standard (PS) supplement Mirtogenol® as supplementary management to antihypertensive drug treatment in asymptomatic subjects with high IOP. The registry evaluated IOP, ocular blood flow velocity and perfusion, perfusion of the circle of Zinn-Haller and oxidative stress during an observational period of 12 weeks.

Patients and Methods

Patients

Subjects available for this registry, supplement study, were recruited among subjects with ocular hypertension (> 28 mmHg) and no sign of glaucomatous optic neuropathy. Subjects with any degenerative eye disorder, diabetes mellitus or microangiopathy were excluded. Also, patients with any cardiovascular diseases requiring medical treatment, and those who had undergone surgery, radiotherapy or chemotherapy in the last three months were excluded. No hypertensive patients were included. Pregnant or breastfeeding women and women planning conception were excluded as well. Inclusion criteria were:

– Elevated IOP without glaucoma;
– Decreased central retinal artery systolic flow velocity;
– Decreased central retinal artery diastolic flow velocity;
– Altered perfusion of the circle of Zinn-Haller;
– No significant pressure or flow asymmetry;
– Absence of other diseases or clinical risk conditions.

PS supplement studies define the field of activity of PS supplements and their possible preventive, pre-clinical or protective applications. "Supplement studies" produce supplementary data (in registries) to be compared with results from the best available management plans. These types of studies are performed with PS products with high level of safety and with high pharmaceutical standards and planned as non-interventional trials[11-13]. In this registry a group of 90 patients was pre-selected for their characteristics: 88 patients could be followed for the 12 weeks of the registry. All patients had complete *ophthalmic evaluation*, showing no signs of primary open-angle glaucoma, clinically-significant cataract or previously implanted intraocular lens. Their cup-to-disk ratio was lower than 0.5, they had a central corneal thickness greater than 555 μm, and no significant visual field defects. Registry patients were given the choice of three management approaches: dorzolamide-timolol fixed-formulation eye drops associated with the oral supplement Mirtogenol®; latanoprost ophthalmic solution in association with Mirtogenol® oral supplementation; latanoprost eye drops only. Mirtogenol® supplementation consisted of two tablets/day: one tablet in the morning and one in the evening. The tablet includes as active ingredients: 80 mg of the standardized bilberry extract Mirtoselect® (Indena, Milan, Italy) and 40 mg of the French maritime pine bark extract Pycnogenol® (Horphag Research, Geneva, Switzerland). Latanoprost (Xalatan®, Pfizer Italia srl, Rome, Italy) was taken at the dosage of *1 drop* (equivalent to 1.5 μg latanoprost) per eye, once daily in the evening. Dorzolamide-timolol fixed-formulation (Cosopt®, Merck Sharp and Dohme SpA, Kenilworth, NJ, USA) was used at the dosage of 2 drops/day: 1 in the morning and 1 in the evening. None of the enrolled ocular hypertensive patients had been treated with latanoprost, dorzolamide-timolol fixed-formulation or other ophthalmic solutions in the 3 months before inclusion. Clinical assessments and laboratory exams were performed at inclusion, at 6 weeks and the end of the observational period (12 weeks) and included:

IOP: measurements were performed in the morning between 9 and 10 a.m.. At each visit, IOP was measured in duplicate with 5-minute intermission between measurements, and the mean values were recorded.

Table I. Demographic details of the study population.

	Dorzolamide-timolol + Mirtogenol®	Latanoprost + Mirtogenol®	Latanoprost only
Subjects (female)	28 (14)	31 (15)	29 (15)
Age, years (mean ± SD)	48.6 ± 3.2	48.7 ± 4.0	49.0 ± 5.5

High-resolution transpalpebral color Duplex imaging (Preirus, Hitachi, Japan) with an elastosonography software (Hitachy) were used to measure peak systolic flow velocity and diastolic flow velocity at the level of the central retinal artery. All measurements were made through the closed eyelids with minimal probe pressure.

Perfusion of the circle of Zinn-Haller evaluated by an arbitrary analogue scale (1 = not visible, 2 = just visible, 3 = normal, 4 = dilated).

Plasma free radical: oxidative stress was evaluated by the FRAS Analyzer measuring reactive oxygen metabolites (d-ROMs) in a drop of blood (FRAS4 system, H&D, Parma, Italy).

Each ultrasound test was carried out in both eyes and no difference between eyes was observed. Blood flow velocity values and IOP values were reported as the mean value of measurements at both eyes.

Statistical Analysis

All recorded measurements were considered non-parametric. One-way analysis of the variance (ANOVA) for repeated measurements followed by post-hoc Bonferroni's correction was used for the intragroup comparisons. A value of $p < 0.05$ was considered statistically-significant.

Results

The three study groups resulted comparable for their distribution (Table I) and for their basal IOP values (Figure 1). During the study period (after 6 and 12 weeks), the decrease in IOP was statistically significant for all management groups (Figure 1). The treatment with latanoprost plus the Mirtogenol® supplementation was associated with a more evident decrease in IOP. In addition, at 12 weeks, 18 out of 31 (58.1%) subjects treated with latanoprost plus Mirtogenol® reached an IOP value < 20 mmHg. The number of subjects reaching the IOP < 2 0 mmHg was 12/29 (41.4%) in latanoprost group and 8/28 (28.6%) in subjects receiving dorzolamide-timolol plus Mirtogenol®. All registry patients responded to treatments. At baseline, flow velocities were similar in the 3 management groups (Table II); systolic blood flow velocity was lower in comparison with normal values. Improvements in systolic and diastolic flow velocities were observed during the study in all treatment groups (Table II). The best improvements at 6 and 12 weeks were observed with the combination Latanoprost+Mirtogenol®. The perfusion at the level of the circle of Zinn-Haller (Table III) – the terminal, distal part of the optic nerve – was comparably reduced in the 3 groups at inclusion, with a few vascular branches just visible at high-resolution duplex scanning and significant perfusional asymmetries in more than 60% of the subjects. The disturbed, altered perfusion at this level tends to be associated to important vasospasm. At 12 weeks, all perfusional patterns appeared to be improved and the asymmetries of perfusion were reduced to less than 15% of the subjects. Patients supplemented with Mirtogenol® showed a better perfusional

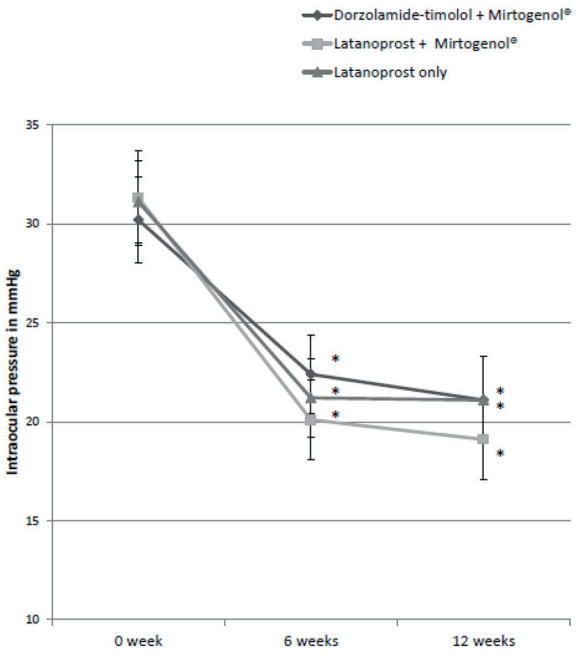

Figure 1. Intraocular pressure (IOP) in the three study groups over the observational period of 12 weeks. *$p < 0.05$.

pattern (evaluated by the analogue scale) in comparison with those using only latanoprost (Table III). The d-ROMs test – which measures oxidative stress in peripheral (finger) blood - revealed a significant decrease in the level of plasma free radicals in supplemented subjects at the end of the study period (with an average of 322 ± 12 CARR units and 320 ± 14 units, in dorzolamide-timolol + Mirtogenol® and latanoprost + Mirtogenol®, respectively) in comparison with baseline values (388 ± 23 CARR units and 386 ± 19, respectively) (Figure 2). No significant change was observed in patients not using the supplement (latanoprost only). All management plans were well-tolerated without side effects; there were no dropouts. The compliance to the supplement was optimal with > 95% of the cases in which the supplement was timely and properly used.

Discussion

This supplement registry study provides indications on the beneficial effects of Mirtogenol® as an oral supplementation in otherwise healthy, asymptomatic patients with ocular hypertension. Previous studies showed that supplementation with Mirtogenol® alone or in association with traditional antihypertensive ophthalmic solution, such as latanoprost, is effective in reducing IOP and improving ocular blood flow[9,10]. In the study by Steigerwalt et al[9] a decrease in IOP as well as improvements in ocular blood flow was achieved after 3 months of supplementation. The dosage of Mirtogenol® was 2 tablets per day[9]. In our

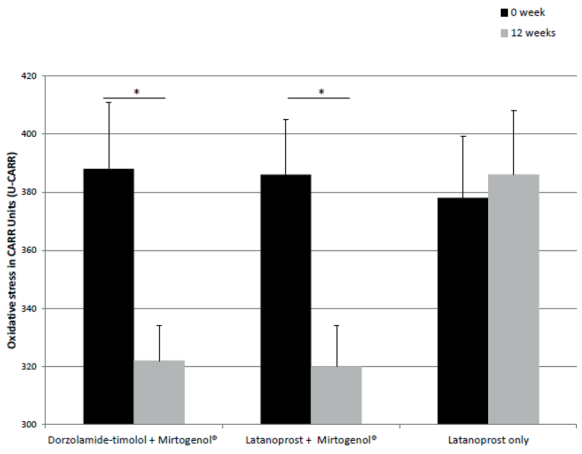

Figure 2. Oxidative stress as plasma free radicals in the three study groups over the observational period of 12 weeks. *$p < 0.05$.

Table II. Ultrasound systolic and diastolic blood flow velocity in response to treatment. Measurements were obtained using high-resolution color duplex ultrasonography (velocity of the central retinal artery in cm/s).

	Dorzolamide-timolol + Mirtogenol®			Latanoprost + Mirtogenol®			Latanoprost only		
	Inclusion	6 weeks	12 weeks	Inclusion	6 weeks	12 weeks	Inclusion	6 weeks	12 weeks
Systolic blood flow velocity (cm/s)	16.3 ± 2.2	18.5 ± 2.3*	21.6 ± 2.5*	15.8 ± 2.0	19.7 ± 3.2*	23.7 ± 2.6*	16.0 ± 1.5	17 ± 2.4*	20.1 ± 1.2*
Diastolic blood flow velocity (cm/s)	2.4 ± 0.3	4.6 ± 0.4*	4.8 ± 0.4*	2.6 ± 0.5	5.1 ± 0.4*	5.8 ± 0.3*	2.5 ± 0.6	3.1 ± 0.6*	4.0 ± 0.4*

Values are expressed as mean ± standard deviation. *$p < 0.05$ *vs.* inclusion.

Table III. Assessment of the perfusion of peripapillary circle of Zinn-Haller.

	Dorzolamide-timolol + Mirtogenol®	Latanoprost + Mirtogenol®	Latanoprost only
Inclusion	1.2 ± 0.3	1.2 ± 0.3	1.3 ± 0.2
12 weeks	2.3 ± 0.2*	2.7 ± 0.4*	1.8 ± 0.3*

Values are expressed as mean ± standard deviation. *p <0.05 *vs*. inclusion.

work a clinical positive effect was observed at 6 weeks. Similar results were described in another study by Steigerwalt et al[10]: retinal systolic blood flow and IOP improved with 6 weeks of Mirtogenol® plus latanoprost treatment, in patients with ocular hypertension. These results confirm that supplementation with Mirtogenol® in addition to an ocular antihypertensive product produces a faster and greater effect, compared to the single managements. We also observed a significant improvement in perfusional patterns at the Zinn-Haller circle in all treated groups, with a better improvement in patients using supplementation plus latanoprost. Supplementation with Mirtogenol® also reduced plasma free radicals, possibly producing a systemic protective action against the increase in oxidative stress and releasing the underlying vasospasm. Both constituents of Mirtogenol® (Bilberry[14-16] and Pycnogenol)[17-19] are powerful anti-oxidants. Recent studies have shown that Mirtoselect® improves several retinal microcirculatory and perfusional parameters[8,20,21]. Pycnogenol® also increases endothelium-dependent vasodilation[22] and marginally decreases systolic blood pressure (releasing vasospasm when present) in mildly hypertensive patients[23] confirming that Pycnogenol® has an important effect on endothelial dysfunction. Initial data – obtained by contact laser Doppler perfusional measurements - indicate that even conjunctival perfusion may be altered in patients with increased IOP and associated retinal circulation vasospasm. The effect on the microcirculation may be apparent in days and studies are in progress[24]. No side effects have been reported for Mirtogenol® - or any of the single products -supplementation.

Conclusions

Supplementation with Mirtogenol® – in addition to the standard management using local ophthalmic treatments – is safe, and may further contribute to the lowering of IOP to normal levels with an improvement in ocular microcirculatory parameters. More studies, in line with some recently-reported data[25,26], are needed to evaluate retinal perfusional patterns in patients with glaucoma and the potential preventive impact of supplements in this delicate field of ophthalmology and microcirculation.

Acknowledgements

We thank Sara Parodi, Ph.D, who provided medical writing services, supported by internal funds. We are very grateful to the Royal Society of Medicine, Wimpole St, London.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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