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Oral Administration of Forskolin and Rutin Contributes to Intraocular Pressure Control in Primary Open Angle Glaucoma Patients Under Maximum Tolerated Medical Therapy

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Abstract

Background: Tight control of intraocular pressure (IOP) is still the only therapeutic approach available for the treatment of primary open angle glaucoma (POAG). However, some patients do not respond adequately to hypotonising drugs, and despite multiple drug combinations they cannot reach their target IOP. Forskolin is a natural compound that has already shown efficacy in IOP reduction following topical application.
Purpose: The aim of this study was to evaluate the effects on the IOP of a food supplement containing forskolin and rutin when administered to POAG patients under maximum tolerated medical therapy (MTMT) and on a waiting list for filtrating surgery to further decrease their IOP.
Methods: The design of the study was open and case-controlled. Ninety-seven (52 in the treatment group, and 45 in the reference group) patients were enrolled in 8 different glaucoma centers in Italy, all under MTMT and with IOP enrollment values above their target pressure. During the 30 days before surgery, patients in the treatment group were prescribed 2 tablets per day of a food supplement containing rutin and forskolin in addition to their usual topical drug treatment. Their IOP values were measured at 3 time points during the day, at enrollment and once a week until surgery. Control patients continued only with their normal topical therapy.
Results: All patients in the treatment group, independently of the combination drug therapy that they were taking, showed a further 10% decrease ($P < 0.01$) of their IOP, starting from 1 week after introduction of the oral supplement and lasting until the last evaluation before surgery. This decrease was more evident (15% of the enrollment value; $P < 0.01$) in those subjects with high (IOP $\geq$ 21 mmHg) enrollment values rather than in those with low (IOP $<$ 21) enrollment values (9%; $P < 0.01$). On the contrary, IOP values in the control group remained stable from the beginning to the end of the observation period, independently of their enrollment values.
Conclusions: Forskolin and rutin given as oral treatment appear to contribute to a better control and a further small reduction of IOP in patients who were poorly responsive to multitherapy treatment.

Introduction

Primary open angle glaucoma (POAG) is no longer considered a disease primarily due to elevated intraocular pressure (IOP).[1] However, elevated IOP remains the main risk factor and is currently the only target with proven therapeutic efficacy.[2] New concepts are emerging in the management of glaucomatous patients that also take into account the neurodegenerative nature of the disease,[3] thus suggesting the need to address neuroprotection in the treatment schedule of such patients.[4,5] The first therapeutic approach still relies on the administration of hypotensive eye

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drops, with the intent of lowering IOP to a target value generally 20%–30% lower than the presenting value.[6] However, some patients may either initially be, or become, unresponsive to some of these drugs so that different combinations of them are required to maintain IOP at the desired value.[7,8]

Forskolin is a naturally occurring compound, a labdane diterpene that is extracted from the roots of the Indian Coleus plant (*Coleus forskohlii*).[9] It is a receptor-independent adenylate-cyclase activator, so that the concentration of the second messenger cAMP rapidly increases in forskolin-stimulated cells.[10] Human nonpigmented ciliary epithelial cells have been shown to contain adenylate-cyclase (subtypes II and IV), the activity of which contributes to the regulation of aqueous humor (AH) dynamics.[11] Forskolin treatment results in a reduced rate of aqueous secretion from the ciliary epithelium in response to activation of adenyl cyclase[12,13] that may explain the hypotensive effect of topical administration of forskolin, as shown in humans[12] and experimental animals.[14,15] Moreover, forskolin-mediated activation of cAMP may contribute to neuronal cell survival and growth.[16]

Rutin is a natural bioflavonoid that has been shown to improve ocular blood flow and electroretinogram recovery in experimental glaucoma models.[17,18] It has a free radical scavenging activity and may thus protect sensitive cells from ischemia/reperfusion damage,[19] as may happen during retinal vein occlusion, or suddenly elevated IOP. No effects have been reported of rutin on IOP, either in humans or in experimental model systems.

To date, no clinical data are available in the literature concerning the effects on the IOP of forskolin given in association with rutin through the oral route. On the Italian market, a food supplement containing an association of forskolin and rutin is available (Kronek®). Such a food supplement delivers 15 mg of forskolin in each tablet. The activity of such a dose can be inferred by published data on oral treatment of asthmatic children with forskolin at 10 mg/day and showing efficacy in preventing asthma attacks.[20]

The purpose of the present study was to evaluate if the addition of 2 tablets per day of this food supplement to POAG patients under maximum tolerated medical therapy (MTMT) could facilitate the control of their IOP values, based on the hypothesis that the mechanisms of action of forskolin appear to be complementary to those exerted by other currently used ocular hypotensive drugs.

Methods

Eight different glaucoma centers participated in this open, prospective, and randomized case–control trial. A total of 97 POAG patients of both sexes, aged between 50 and 75 years, with IOP values ranging between 10 and 41 mmHg, were included. All patients were on a waiting list for scleral flap trabeculectomy. They were enrolled if their IOP value, despite multitherapy treatment, was above the desired target pressure (30% below baseline, as defined by the European Glaucoma Society[21]). Therefore, patients were enrolled even if their IOP pressure was below 21 mmHg, if the ophthalmologist considered the pressure above the desired target for that specific patient. Patients with other concomitant ophthalmic pathologies, those who had already undergone eye surgery (cataract excluded), or with gastrointestinal diseases preventing them from taking the food supplement, were excluded. A description of the patients and their therapies is shown in Tables 1 and 2. Prostaglandins included bimatoprost, travoprost, or latanoprost, all given once a day. Beta blockers included timolol, carteolol, or levobunolol, all given twice per day. The alpha-agonist used was brimonidine, twice per day. Carbonic anhydrase inhibitor (CAI) was dorzolamide hydrochloride, twice per day. All enrolled patients had been followed at their respective centers for several months, and their IOP values appeared to be stably above the target pressure under their given multitherapy. At enrollment and at each further time point (after 1, 2, and 3 weeks), the IOP of all patients (treated and controls) was measured by Goldmann applanation tonometry, and calculated as the average value of 3 repeated measurements during the day (at 8:00, 12:00, and 16:00). Measurements at each time point were repeated 3 times, and the median value was reported for that time point. The 52 patients in the treatment group were given (besides their usual drug treatment) 2 tablets/day (1 in the morning and 1 in the evening, after meals) of a food supplement containing: *Coleus forskolii* extract (150 mg: 15 mg of forskolin), rutin (200 mg), vitamin

Table 1. Demographic Characteristics of Treated and Control Patients

Total number of patients	*M*	*F*	*Average age*
(A) Treated			
52	25	27	63.5 ± 7.62
(B) Control			
45	23	22	67.4 ± 6.37

Table 2. Maximum Tolerated Medical Therapies Given to Treated and Control Patients

Therapy	*Number of patients*
(A) Treated	
Prostaglandins + alpha-agonist	1
Prostaglandins + CAI	1
Prostaglandins + CAI + alpha-agonist	1
Beta-blockers + CAI + alpha-agonist	1
Prostaglandins + beta-blockers	11
Prostaglandins + beta-blockers + CAI	21
Prostaglandins + beta-blockers + alpha-agonist	8
Prostaglandins + beta-blockers + CAI + alpha-agonist	7
Parasimpathomimetic	1
(B) Control	
Prostaglandins + alpha-agonist	1
Prostaglandins + CAI	1
Prostaglandins + CAI + alpha-agonist	1
Beta-blockers + CAI + alpha-agonist	1
Prostaglandins + beta-blockers	7
Prostaglandins + beta-blockers + CAI	12
Prostaglandins + beta-blockers + alpha-agonist	11
Prostaglandins + beta-blockers + CAI + alpha-agonist	11
Parasimpathomimetic	0

CAI, carbonic anhydrase inhibitor.

B1 (0.7 mg), and vitamin B2 (0.8 mg) (Kronek®). IOP was then measured every week for 3 weeks. A group of 45 patients with similar characteristics, who did not take any supplement during the 30 days before surgery, served as the control group.

Statistics

The Shapiro–Wilk test was used to evaluate the Normality assumption. The analysis of variance (ANOVA) for repeated measures was used to compare values over time, separately for each treatment group. The Huynt–Feldt epsilon correction was used to take into account a possible violation of sphericity assumptions. The Dunnet's method was applied for the *post hoc* test comparing follow-up measurements (T1, T2, and T3) with basal values. The Student's *t*-test for paired data was used to compare values at T3 with values at T0 in the control group. A *p*-value <0.05 was considered statistically significant. Stata 12.0 was used for the analysis. The sample size of the study was calculated to find a difference of 2 mmHg between the 2 groups, given a standard deviation of 3 mmHg, a statistical power of 80% at a significance level of 0.05. With these assumptions, a total of 76 patients were required, considering a 10%–15% rate of lost to follow-up.

This study was performed with informed consent and following the tenets of the Declaration of Helsinki.

Results

Figure 1 shows the average overall response in both eyes of the 52 treated patients to the addition of the food supplement to their current therapy. The data analysis by repeated measures ANOVA indicates that there was a clear progressive decrease of IOP, already at significant values ($P<0.01$) after the first week of treatment. After 3 weeks, the average decrease was 1.95 mmHg (9.5% less than the initial average value; $P<0.01$).

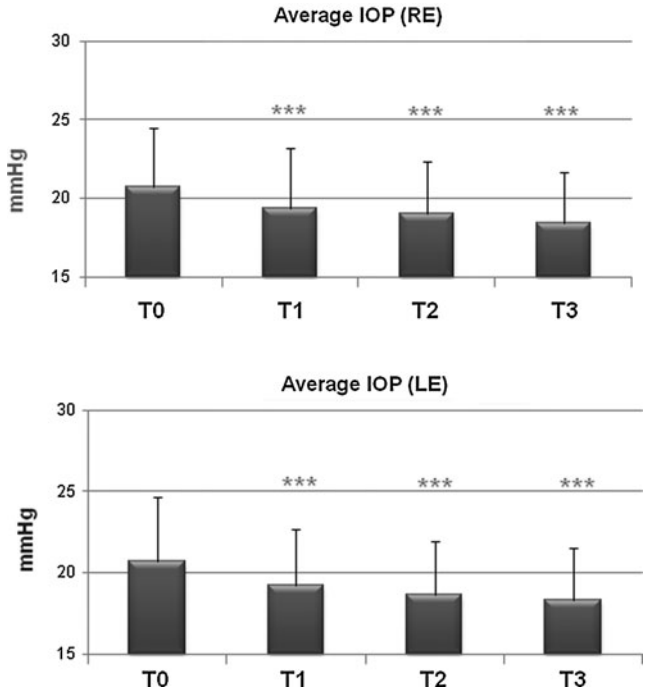

FIG. 1. Averaged IOP values (±SD) for both eyes (RE, right eye; LE, left eye) of all 52 patients measured at enrollment (T0) and for 3 successive weeks (T1–T3). ***$P<0.001$. IOP, intraocular pressure; SD, standard deviation.

A separate analysis by repeated measures ANOVA with *post-hoc* Dunnet's correction on the 2 subgroups of patients treated with the forskolin/rutin association with enrollment IOP values higher or lower than 21 mmHg revealed that the observed decrease in treated patients was a function of the initial IOP values. In fact, results reported in Fig. 2 (right eyes; left eyes showed the same behavior) show that patients with enrollment values <21 mmHg (Fig. 2A) showed a trend, though not significant, toward a slight progressive decrease of IOP, which at the end of treatment amounted to 1.50 mmHg, corresponding to 9% of the initial average enrollment value. On the other hand, a more pronounced and progressive decrease was observed for the subgroup of patients with IOP values >21 mmHg (Fig. 2B, about 3 mmHg at the end of the third week, corresponding to 15% of the initial average enrollment value; $P<0.001$). Figure 2C shows the progressive average decrease achieved in the 2 subgroups in comparison with the average decrease in the total number of patients. Values are expressed as percent of the initial average value at enrollment to make a direct comparison possible. The curves describing the results of the groups in which enrollment IOP was less or more than 21 mmHg remain respectively above and below the curve of the general average, indicating that the additional treatment was more efficient in patients with higher enrollment values.

In contrast, IOP values in the untreated control group measured at the time when surgery was decided (T0), and 30 days later (just before the intervention: T3), appeared to remain stable independently of their enrollment values. In fact, Fig. 3A shows the analysis of 35 control patients with enrollment values higher than 21 mmHg, and Fig. 3B shows those of control patients with enrollment values below 21 mmHg. In both cases, a stability of IOP values was noticed throughout the observation period (the Student's *t*-test for paired data: $P>0.2$ for both Fig. 3A and B).

A further analysis was carried out concerning the effects of the food supplement on patients treated with the forskolin/rutin association on the basis of the combination therapy they were taking (Table 2). Figure 4 shows the data for right eyes (left eyes showed the same behavior) arranged according to this criterion. Only combination therapies given to more than 1 patient were considered in this analysis. The data analysis by repeated measures ANOVA with *post-hoc* Dunnet's correction indicates that in each case, independently of the combination therapy given, there is a consistent and significant IOP decrease ($P<0.05$) after 3 weeks of treatment with the forskolin/rutin association. Such a decrease was often observed at the first week, and was still evident at the end of treatment (Fig. 4B–D).

Discussion

Results obtained in this study indicate that oral administration of forskolin and rutin may allow a further significant IOP decrease in POAG patients in which combination therapies have already reached their maximal effect, even if suboptimal.

Ocular hypertension is due to an imbalance between production and drainage of AH, which is most likely due to a decreased trabecular outflow, or an increased episcleral

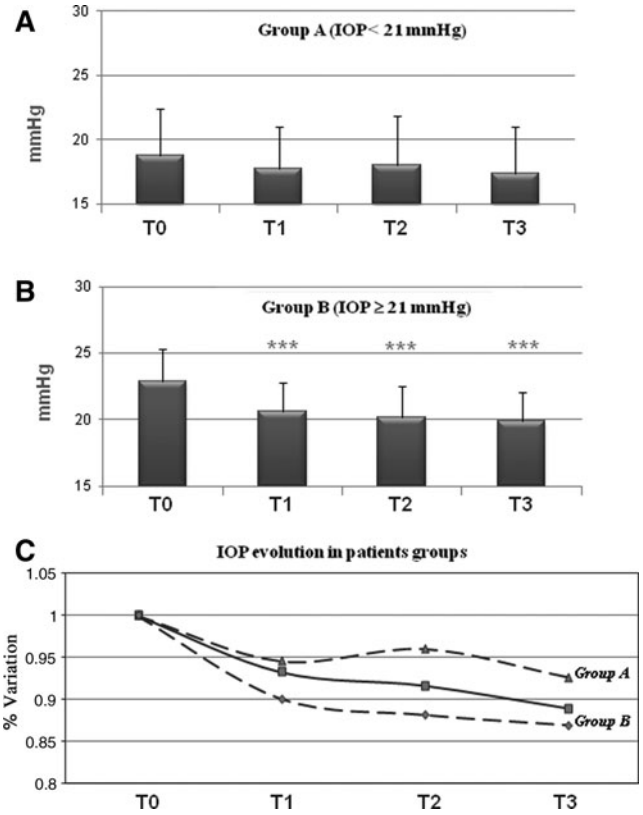

FIG. 2. Averaged IOP values (±SD) for the right eye (left eye behavior was the same) of patients treated with the forskolin/rutin association with **(A)** IOP enrollment values <21 mmHg and **(B)** IOP enrollment values >21 mmHg. In **(C),** a comparison is shown between each subset (A and B) and the average trend of the total number of enrolled patients; data are expressed as percentage variations to make the comparison clear. ***$P < 0.001$.

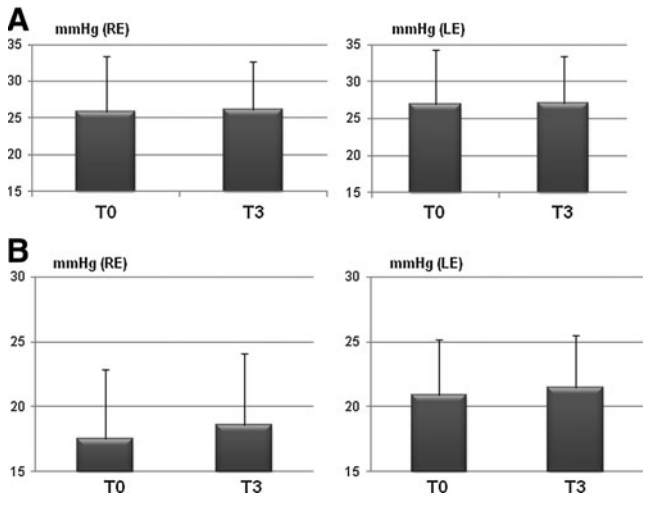

FIG. 3. **(A)** Averaged IOP values (±SD) for both eyes (RE: right eye; LE: left eye) of the 35 untreated control patients with enrollment values >21 mmHg measured at the time when surgery was decided (T0) and 30 days later, just before surgery (T3). **(B)** Averaged IOP values for both eyes of 10 control patients with enrollment values <21 mmHg measured at the time when surgery was decided (T0) and 30 days later, just before surgery (T3).

vein pressure. AH then accumulates in the posterior and anterior chambers of the eye, causing a tension at the level of the *lamina cribrosa*. This event may alter the axoplasmatic flow in retinal ganglion cells, causing neurotrophin deprivation of the cell bodies, thus leading to apoptotic cell death.[22,23] Therefore, the primary approach to glaucoma treatment is still aimed at decreasing IOP. Strategies to achieve such a reduction are directed at reducing AH production (beta-blockers and carbonic anhydrase inhibitors), or increasing its outflow through the uveoscleral path and/or the trabecular meshwork (prostaglandins, cholinergic agonists, and simpathomimetics).[24]

The possible effect shown in this article of the food supplement on ocular tension is most likely due to the presence of forskolin, which has already shown its hypotonising effect after topical application.[12] No evidence of direct rutin effects on IOP have ever been reported or hypothesized, although a contribution of this flavonoid to a better capillary diffusion of forskolin in the eye cannot be ruled out. We did not observe any significant variation of IOP values in the control group (Fig. 3), both at IOP values higher than 21 mmHg (Fig. 3A), when the forskolin effect was maximal in treated patients, and at IOP values below 21 mmHg (Fig. 3B). This indicates that the effect observed in treated patients is not likely to be due to a better compliance with hypotonising drugs of the individuals as a consequence of the knowledge that his/her pathology is progressing, so that surgery will be necessary.

Therefore, our results suggest that after oral systemic treatment enough forskolin reaches the ocular district where it can affect AH balance, resulting in a further IOP decrease. This effect could be explained by the particular mechanism of action of forskolin, mediated by a receptor-independent increase of cAMP,[10] which may decrease IOP[12,13] in a way that is partially independent from, and complementary to, the mechanisms of the other hypotonising drugs.[24]

In fact, it has been postulated[12,13] that the decrease of AH secretion may follow the activation by cAMP of a reverse flow of secretion by the nonpigmented ciliary epithelium, counteracting the normal flow of secretion, and resulting in a decreased net inflow of AH into the eye chambers. Moreover, it has been shown that activation of the protein kinase A (PKA) by the increased cAMP concentration may inhibit the protein Rho-A.[25] This protein is involved in the organization and distribution of the actin cytoskeleton, cellular adhesion, cytokinesis, contraction, morphology, and cell motility.[26] Rho-A coordinates these events through its downstream effectors, such as ROCK.[26] The decreased Rho-A activity in trabecular meshwork cells may cause the actin cytoskeleton to disassemble, increasing the permeability of the tissue to the AH outflow, thus decreasing IOP.[27,28] A similar effect has indeed been reported by the direct action of latrunculin on the trabecular meshwork structure and permeability in monkeys.[29] In fact, according to these findings, molecular inhibitors of ROCK are being developed as potential drugs to decrease IOP in glaucomatous patients.[15,30] Therefore, forskolin itself, by activating PKA,[31] might induce the same cascade of events, resulting in an improved outflow through the trabecular meshwork and a decrease of IOP.

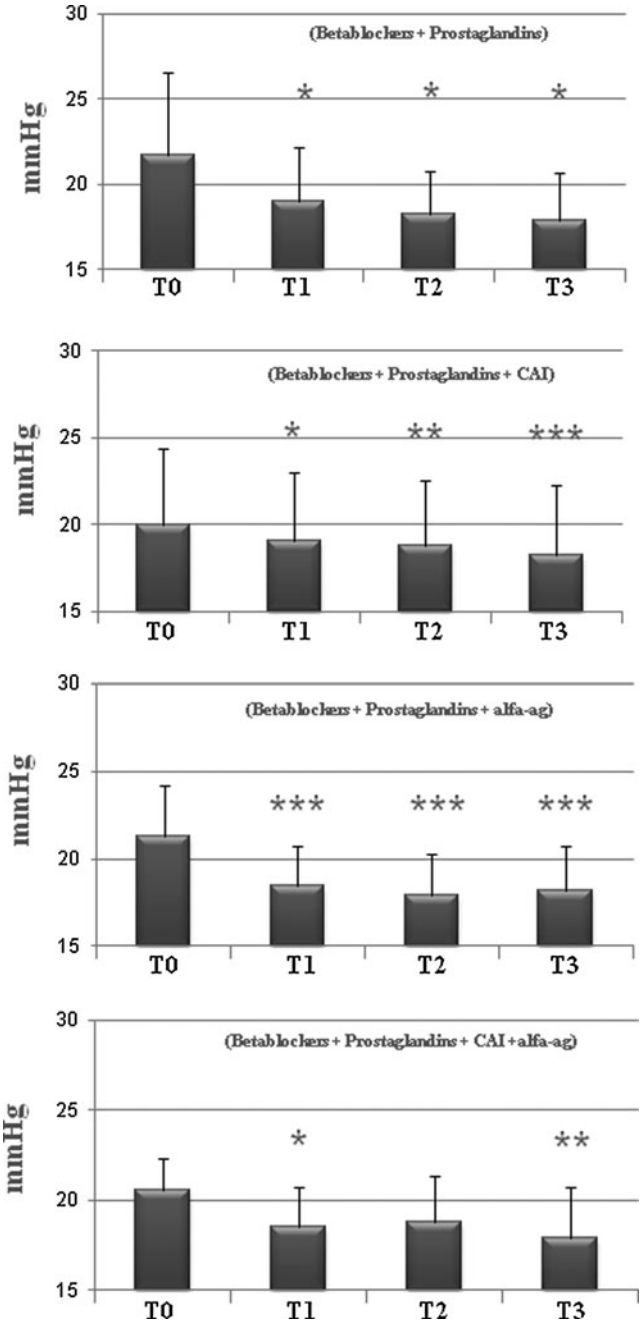

FIG. 4. Averaged IOP values (±SD) of patients treated with the forskolin/rutin association considered on the basis of the multitherapy they were taking during the 3 weeks of observation, as indicated in each graph. Data for the right eye are shown. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Results showing an additional hypotonising effect of oral forskolin treatment have recently been published on POAG patients under pharmacological treatment with either beta-blockers or prostaglandins.[32] Moreover, oral treatment with forskolin of POAG patients taking multiple therapies has confirmed the additional hypotonising effect, which is reversible upon suspension of the treatment.[33] Therefore, all these data, including those presented in this article, show that forskolin may indeed contribute—in a reversible way[33]—to a higher efficiency of hypotonising drugs in glaucoma patients.

The patients recruited in this study were on a waiting list for filtrating surgery, and their treatment with the association of forskolin and rutin was interrupted on the day of the operation. Filtrating surgery resulted in a relevant decrease of IOP and patients were thus not further followed-up. It is, however, interesting to notice that, in a different study, Argon laser trabeculoplasty was more efficient in patients who had been pretreated with the forskolin/rutin association (M. Ciancaglini, pers. comm.).

In conclusion, we have shown in this work that oral administration of a food supplement containing forskolin and rutin to POAG patients under treatment with multiple topical drugs could allow a further reduction of their IOP values. Even though decreasing IOP is considered a necessary, though not sufficient, strategy to slow down glaucoma progression,[34,35] the Early Manifest Glaucoma Trial reported that the hazard ratio for glaucoma progression increases by 11.1% for every 1 mmHg or higher IOP.[36] Therefore, improving the efficacy of pharmacological treatments by forskolin/rutin oral administration might contribute to a slower progression of the disease.

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Disclaimer

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Author Disclosure Statement

No competing financial interests exist.

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