ORIGINAL RESEARCH

Topical Isopropyl Unoprostone for Retinitis Pigmentosa: Microperimetric Results of the Phase 2 Clinical Study

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ABSTRACT

Introduction: The purpose of this study was to determine whether topical 0.15% isopropyl unoprostone (IU), a BK-channel activator, could improve or maintain the central retinal sensitivity in patients with middle- to late-stage retinitis pigmentosa (RP). IU was approved for glaucoma and ocular hypertension in 1994. The

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drug re-profiling strategy is one of the effective ways to develop safe drugs for patients with RP. ***Methods***: A randomized, double-blind, and placebo-controlled phase II safety/efficacy trial was conducted. One hundred and nine patients with middle- to late-stage RP having a visual acuity of ≥ 0.5 were studied at six ophthalmological centers in Japan. The treatments of IU/day were divided into three groups: placebo group; two-drop group; and four-drop group for 24 weeks. The primary outcome measure was changes in the retinal

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sensitivity from baseline in the central 2° determined by MP-1 microperimetry (MP-1, Nidek, Japan). The secondary outcomes were changes in best-correct visual acuity, contrast sensitivity, retinal sensitivity of the central 10° by MP-1, mean deviation (MD) by a Humphrey field analyzer (HFA; Carl Zeiss Meditec, Dublin, CA, USA) 10-2, and the Visual Functioning Questionnaire 25 (VFQ-25) questionnaire scores.

Results: There was a tendency for a dose-dependent responsiveness in retinal sensitivity in the central 2°, MD, and total VFQ-25 score after 24 weeks of IU instillation by a simple linear regression analysis. A stratified analysis showed a significant dose-dependent responsiveness of the 2° central retinal sensitivity in more advanced patients ($P = 0.028$). The number of patients having a ≥ 4 dB decrease in the primary outcome measure was significantly fewer in the four-drop group than in the placebo group ($P = 0.02$). No adverse reactions were observed.

Conclusions: A higher dose of IU can delay progression of the central retinal sensitivity decrease through an improvement of retinal sensitivity.

Keywords: BK-channel activator; Central retinal sensitivity; Clinical trial; Isopropyl unoprostone; MP-1 microperimetry; Multiple topical instillations; Neuroprotection; Retinitis pigmentosa

INTRODUCTION

Retinitis pigmentosa (RP) is a heterogeneous group of inherited retinal degenerative diseases characterized by night blindness and progressive decrease of peripheral vision [1, 2]. At more advanced stages, the central vision is also reduced. The worldwide prevalence of RP is about 1 in 4,000 for a total of more than 1 million affected individuals [1, 2], and over 50 genes have been cloned or mapped for RP. RP typically begins with a degeneration of the rods followed by a progressive and irreversible death of the cones, which accounts for the loss of central vision and complete blindness. It appears that cones are dependent on rods for survival [3] and once the rods die, death of the cones is inevitable. Most patients with RP visit ophthalmologists in the middle to late stages of the diseases where most rods die and cones start deteriorating. Thus, prevention of the secondary degeneration of cones is an important goal for preserving central vision in patients with RP.

The results of clinical trials, including nutritional supplementation trials have shown that photoreceptor degeneration can be slowed [4–8]. In a recent phase I clinical trial, patients with severe RP had encapsulated ciliary neurotrophic factor-secreting cells implanted intraocularly [9], which this led to an improvement in vision. The results of two clinical studies with *RPE65* gene replacement showed that there was improvement of visual acuity and retinal sensitivity in patients with Leber's congenital amaurosis [10, 11]. However, long-term efficacy, immune responses, and complications associated with the use of vectors to deliver the genes need to be considered when gene therapy is used. Although cell or gene replacement therapy may be the ultimate method to rescue photoreceptor cells in patients with RP, it is still reasonable to test small molecule drugs with known neuroprotective properties and good safety profiles. This drug re-profiling strategy could be effective in developing safer drugs for patients with RP. It must be remembered that the drugs will need to be used throughout the patient's lifetime. From this point, four exploratory clinical studies in patients with RP have been conducted: intravitreal implantation of

brimonidine (NCT00661479), oral valproic acid [7] (NCT01233609 and NCT01399515), oral Ca^{2+}-channel blocker [8], and topical 0.12% isopropyl unoprostone (IU; Rescula®, R-tech Ueno, Tokyo, Japan) [12]. These mutation-independent therapies could possibly slow or stop photoreceptor degeneration without directly treating the original abnormality.

IU is a metabolized form of prostaglandin $F_{2\alpha}$, a chemically synthesized docosanoid (22-carbon basic skeleton), and 0.12% IU was approved for clinical use in Japan in 1994, and 0.15% IU was approved in the United States in 2001. IU is used to treat eyes with glaucoma or ocular hypertension [13], and clinical studies showed that topical IU led to an increase in retinal and choroidal blood flow in patients with glaucoma [14, 15]. The mechanism of action of IU is as a BK-channel or maxi-K channel activator [16–18] and not as a prostaglandin F(FP) receptor agonist [17, 18]. BK channels are potassium channels that reach an activation threshold only during depolarization and/or at high intracellular Ca^{2+} concentrations [17, 19]. Laboratory experiments showed that an intravitreal injection of IU in rats protected the photoreceptors against light-induced damage in a dose-dependent manner [20], and retinal ganglion cells against endothelin 1 (ET-1)-induced neuronal injury through ERK phosphorylation [21]. An in vitro study demonstrated that IU had anti-apoptotic activity on retinal neuroglial progenitor R28 cells of rats [22]. More recently, IU was shown to protect mice cone photoreceptors and human retinal pigment epithelium (RPE) cells against oxidative stress and light-induced damage through BK channels [18]. The results of two clinical reports on Japanese patients with RP showed that 0.12% IU may be effective in treating patients with RP [23, 24]. The results of a clinical study of 30 patients with RP

demonstrated that two drops of IU twice daily improved the retinal sensitivity in the central 2° as determined by fundus-related microperimetry, MP-1 after 6 months [12].

To treat patients with RP throughout their lives, IU should be considered because it has a good safety profile [25] and has neuroprotective properties [17–22] (e.g., anti-oxidative, anti-apoptosis, anti-light induced injury, and anti-ET-1-induced neuronal injury properties). In addition, although the primary lesion in RP results in apoptosis of the photoreceptor cells due to a gene mutation, secondary factors are considered to damage photoreceptor cells, in particular cone cells [3]. One of the factors might be reduced choroidal blood flow, which has been reported to be present in patients with RP [26–28]. In a rat model, impairment of choroidal circulation was probably involved in delayed cone death after light toxicity [29]. The authors suggested that improving choroidal circulation might preserve the cones and remaining rods in patients. An increase in the plasma level of ET-1, which is related to a decrease in foveal choroidal blood flow, has been reported in some patients with RP [30, 31]. Multiple instillations of IU were shown to partially block the ET-1-induced vasoconstriction of the human choroidal vessels [32].

In patients with the later stage of RP, preservation of the secondary death of central cones is an important goal to maintain daily lives. The purpose of this study was to determine the safety and efficacy of 0.15% topical IU for the treatment of patients with the middle to late stage of typical RP whose central cones started to deteriorate. The clinical trial was conducted with a study design of a randomized, double-blind, placebo-controlled, parallel assignment, six-center, phase II study of 6 months' duration.

METHODS

Study Population

All of the participants were diagnosed with typical RP by clinical and electrophysiological findings [2]. Eligibility criteria were: (1) aged between 20 and 65 years; (2) middle- to late-stage RP [33]; (3) best-corrected visual acuity (BCVA) ≥0.5 in decimal units; (4) mean sensitivity of central four spots of 6–16 dB by the Micro Perimeter 1 (MP-1, Nidek, Japan); and (5) presence of a scotoma in Humphrey field analyzer (HFA; Carl Zeiss Meditec, Dublin, CA, USA) 10-2. A detailed description of the inclusion and exclusion criteria is shown in Table 1.

Eligibility was determined by the findings at a screening examination at each of the clinics. A clinical evaluation of the baseline data that included; ophthalmic history, measurements of BCVA by the Early Treatment Diabetic Retinopathy Study chart, contrast sensitivity, intraocular pressure, MP-1, HFA 10-2, Visual Functioning Questionnaire 25 (VFQ-25) questionnaire, and slit-lamp biomicroscopy, optical coherence tomography (OCT), and dilated fundus examinations (Table 2).

Study Objectives

The primary endpoint was changes of the mean retinal sensitivity of the central four spots (central 2° in diameter) after 24 weeks of IU. The retinal sensitivity was determined by MP-1. The secondary endpoints were changes of: (1) the mean retinal sensitivity of the central 24 spots (central 10° in diameter) by MP-1; (2) the MD of HFA 10-2; (3) BCVA; (4) contrast sensitivity; and (5) the VFQ-25 score.

Study Design

This was a phase II randomized, double-masked, parallel three-arm comparative study of 0.15% IU eye drops in patients with RP conducted at six ophthalmological centers in Japan (JapicCTI-090748). The study protocol and informed consent forms were reviewed and approved by each institutional review board. The study adhered to tenets of the Declaration of Helsinki. This clinical study was performed in accordance with Article 14-3 and Article 80-2, the Pharmaceutical Affairs Law, and Good Clinical Practice. The performance of the clinical study was noticed to the Pharmaceuticals and Medical Devices Agency in Japan.

The three arms of the dose-dependency study were as follows: arm 1, the placebo group, received two drops of 0% IU at 5-min intervals in both eyes, morning and night; arm 2, the two-drop group, received one drop of 0% IU and one drop of 0.15% IU at 5-min intervals in both eyes, morning and night; and arm 3, the four-drop group, received two drops of 0.15% IU at 5-min intervals in both eyes, morning and night (Table 3). Participants were instructed to place four drops of IU two times, morning and night daily for 24 weeks. In terms of dose-dependency, although all participants received the same number of drops, four drops daily, the placebo group, two-drop group, and four-drop group received 0, 2, and 4 drops of IU, respectively. If only one eye satisfied the inclusion criteria, the efficacy of IU was evaluated for that eye. If both eyes satisfied all of the criteria, efficacy was evaluated for the eye with the better visual acuity. If the BCVA was equal in both eyes, the right eye was used for efficacy evaluation. Participants were examined in one of six clinics in Japan every month.

Table 1 Inclusion and exclusion criteria

Eligibility

1. Age: 20 years or more but not exceeding 65 years

2. Decimal visual acuity of 0.5 or more

3. Results of a Goldmann visual field test show a visual field abnormality of a moderate to severe stage

4. The difference in mean retinal intensity of the four retinal points is up to 2 dB between two measurements conducted within a month using an MP-1, and any of the value ranges from 6 to 16 dB. If the criteria are not met with two measurements, a third measurement is made within a month after the second measurement, and either of the two values with a difference of up to 2 dB is within the range 6–16 dB

5. The difference in the MD is up to 3 dB in two reliable measurements[a] of retinal sensitivity made within a month using a Humphrey visual field analyzer (10-2, SITA-Standard), and visual field abnormality is recognized in these two measurements. If the criterion is not met with the two measurements, a third measurement is made within a month after the second measurement. If the third measurement is reliable, the difference of the value from the first or second measurement is up to 3 dB, and visual field abnormality is recognized in the two measurements

Ocular exclusion criteria (applies to the eye evaluated)

1. Under treatment for glaucoma or ocular hypertension

2. Either eye enucleated or eviscerated

3. Cone-rod dystrophy with primarily impaired cone function

4. History of other ocular disease that can confound the outcome of the study (e.g., diabetic retinopathy, uveitis)

5. Complicated by remarkable cystoid macular edema, epimacular membrane, or macular traction syndrome, diagnosed by OCT

6. Complicated by a nuclear cataract of moderate grade (grade 3, Emery classification), an anterior subcapsular cataract, or a posterior subcapsular cataract, that may seriously affect vision

7. 0.12% isopropyl unoprostone used within the past 1 year

8. Any of the following drugs used within 1 month before informed consent is obtained. Steroids, drugs that dilate the blood vessels, and dark adaptation improving drugs (Xantofyl palmitato[b])

[a] The poor function is 20% or less. The false positive rate is 33% or less. The false negative rate is 33% or less
[b] Brand name 'Adaptinol®' manufactured by Bayer Yakuhin, Japan. Adaptinol® is an approved drug for retinitis pigmentosa in Japan since August 1956

The scheduling of clinical evaluations is shown in Table 2. A review of the ocular and systemic symptoms or adverse events was determined at each visit.

Laboratory evaluations included serum chemistry, hematology, urinalysis, and urine chemistry. They were performed at baseline and weeks 12 and 24. The concentrations of IU and its metabolite M1, an active form of IU, were determined on week 20. Blood samples were collected in tubes containing sodium heparin at 15 min after the second instillation the morning administration on week 20. The plasma samples were stored at −20 °C until analysis. The samples were analyzed in 76 patients with RP who received two or four drops/time of IU.

Table 2 Investigation and testing schedule

Test item	Pre-observation period (baseline)	Treatment period							At the time of discontinuation
		After 2 weeks	After 4 weeks	After 8 weeks	After 12 weeks	After 16 weeks	After 20 weeks	After 24 weeks	
MP-1 microperimetry Humphrey perimetry	●●		●	●		●		●	●
logMAR Contrast sensitivity Slit-lamp microscopy IOP	●	●	●	●	●	●	●	●	●
VFQ-25 OCT Ocular fundus exam	●							●	●

IOP intraocular pressure, *OCT* optical coherence tomography

Table 3 Three-arm dose-dependency study of patients with retinitis pigmentosa

Arms		Morning		Night	
		First drop	Second drop	First drop	Second drop
1	Placebo group	Placebo	Placebo	Placebo	Placebo
2	Two-drop group	Placebo	Unoprostone	Placebo	Unoprostone
3	Four-drop group	Unoprostone	Unoprostone	Unoprostone	Unoprostone

Isopropyl unoprostone (IU) 0.15% and 0% (placebo) eye drops were administered. Arm 1, the placebo group, received two drops of 0% IU; arm 2, the two-drop group, received one drop of placebo and one drop of IU; arm 3, the four-drop group, received two drops of IU

Examination Procedures

The CSV-1000E (Vector Vision Co, Greenville, OH, USA) was used to determine BCVA by the Early Treatment Diabetic Retinopathy Study method and the spatial contrast sensitivity. Spatial frequencies of 3, 6, 12, and 18 cycles/degree were studied; each spatial frequency included eight different levels of contrast. From the data obtained by CSV-1000E, the area under the log contrast sensitivity function (AULCSF) was calculated by the method of Applegate and associates [34].

Microperimetric measurements were made with an automatic fundus-related perimeter, the MP-1. Measurements were made on all subjects after the pupils were dilated with 0.5% tropicamide eye drops. Patients were not dark-adapted before the measurements. Patients were allowed to practice before the first test to eliminate the effects of learning. The following parameters were used on the MP-1: a white background of 1.27 cd/m^2; Goldman III stimulus size; projection time of 200 ms; a rectangular $3° \times 3°$ test grid with 24 stimulus locations covering the central 10° centered on the fovea. A 4-2 double-staircase method was used. The stimulus was projected on to a predefined retinal position by an automatic eye-tracker that compensated for eye movements. The mean retinal sensitivity within the central 2° was determined from the central four points, and the mean retinal sensitivity within the central 10° was

determined from all 24 points. The follow-up system was used to examine the same retinal locations. The central 10° of the visual field was tested using a HFA using the 10-2 SITA standard software program.

All of the patients answered the Japanese version of the NEI-VFQ-25 by themselves following the protocol by Suzukamo et al. [35] who developed the Japanese version of the questionnaire. All of the patients were able to read and complete the questionnaire without an interviewer because all had good central vision and understood the questions. The NEI-VFQ-25 is made up of 25 questions composed of 12 aspects of daily living: General Health, Ocular Pain, Peripheral Vision, Driving, General Vision, Near Vision, Distance Vision, Role Limitation, Dependency, Social Function, Mental Health, and Color Vision; the authors excluded the first four aspects. In this trial, the VFQ-25 is made up of 22 questions composed of eight vision-targeted themes. The answer to each of the VFQ questions was converted to a 100-point scale in which 100 represents the best possible score and 0 represents the worst score. The scores from the eight subtopics were averaged to yield a composite score.

Statistical Analysis

A one-way analysis of variance was used for continuous data obtained at the baseline, and Fisher's exact probability test was used for nominal data to examine whether there were any imbalances in the distribution between the three groups. The two-tailed significance level was set to 15% as a guide. A simple linear regression analysis was done to determine whether the changes in the values of the endpoints at the end of the clinical study were significantly correlated with the three doses of IU as explanatory variables. The dose-dependent responsiveness was examined by a test with 0 as a slope with a significance level <0.05. If any imbalance is identified in the background factors of the groups, a statistical adjustment was made for the analysis of the primary endpoint according to the protocol of this trial. In addition, the Williams' test was performed for the comparison between the placebo group and the four-drop group. The one-tailed significance level was <0.25. Fisher's exact probability test was used to examine the frequency of exacerbated patients among the three groups or improved patients. The significance level was <0.05.

RESULTS

A signed informed consent form to participate in the clinical study was obtained from 180 patients with RP. Sixty-eight patients did not to meet the inclusion criteria during the screening stage, and 112 patients were studied. These 112 patients were randomized into three groups: 35 in the placebo group; 39 in the two-drop group; and 38 in the four-drop group. The eye drops were discontinued in four patients during the course of the study; three from the placebo group, and one from the two-drop group, and 108 patients completed the study. The reasons for discontinuation of the eye drops were patients' request in one of the placebo group, and the onset of adverse events in three patients; two in the placebo group, and one in the two-drop group.

The efficacy of IU was determined from 109 patients; 108 who completed the 24-week protocol and one from the placebo group who was examined at week 16 but was not able to complete the study due to adverse events. In the end, data from 33 patients in the placebo group, 38 patients in the two-drop group, and 38 patients in the four-drop group were

statistically analyzed. The demographics of patients in the three groups are shown in Table 4. There was a tendency of a background difference in the MD values of the HFA among the three groups, including patients with more advanced RP in the four-drop group (*P* = 0.10). The distribution in the number of the patients with moderate cystoid macular edema in the OCT images was also different although not significant (*P* = 0.08).

The mean changes in retinal sensitivity of the central 2° determined by MP-1 during the 24 weeks are shown in Fig. 1. There were greater improvements of the retinal sensitivity in the two-drop group and the four-drop group than the placebo group after week 4 of the ocular instillation. A simple linear regression analysis showed that although the retinal sensitivity was related to the dose, the dose-dependent responsiveness was not significant (*P* = 0.09; Fig. 2). However, an adjusted multilinear regression analysis based on the MD value at screening (week 0) showed a significant dose-dependent responsiveness (*P* = 0.038). A scatter plot of the mean retinal sensitivities for the central 2° for the three groups (0 W) in patients with low to high values is shown in Fig. 3. Among the 82 patients with middle- to late-

Table 4 Baseline demographic and ocular data for participants

	Placebo group	One-drop group	Two-drop group	*P* value
Analysis subjects	33	38	38	
Sex (male/female)	19:4	16.22	21:17	0.384[d]
Age (years)	43.3 ± 12.6[a]	46.0 ± 14.0	45.4 ± 13.0	0.673[e]
Inheritance pattern				0.825[d]
Autosomal dominant	8 (24.2%)[b]	5 (13.2)	6 (15.8)	
Autosomal recessive	4 (12.1)	5 (13.2)	4 (10.5)	
X-linked recessive	0 (0.0)	1 (2.6)	0 (0.0)	
Sporadic	21 (63.6)	27 (71.1)	28 (73.7)	
logMAR visual acuity	0.24 ± 0.14	0.25 ± 0.14	0.26 ± 0.15	0.81[e]
Contrast sensitivity (AULCSF)	0.95 ± 0.24	0.92 ± 0.92	0.91 ± 0.21	0.81[e]
MP-1 retinal sensitivity of central 4 points (dB)	11.4 ± 3.7	12.5 ± 3.4	11.1 ± 3.3	0.71[e]
MP-1 retinal sensitivity of central 24 points (dB)	6.8 ± 5.3	8.3 ± 5.1	6.5 ± 4.8	0.22[e]
HFA10-2 MD (dB)	−15.0 ± 9.9	−14.1 ± 8.8	−18.4 ± 8.1	0.10[e]
VFQ-25 total score	66.4 ± 17.3	69.2 ± 16.0	66.2 ± 15.2	0.67[e]
Cystoid macula edema (OCT)[c]	1 (3.0)[b]	4 (10.5)	8 (21.1)	0.08[d]
IS/OS line (+) (OCT)	24 (72.7)[b]	26 (68.4)	24 (63.2)	0.70[d]

OCT optical coherence tomography, *VFQ-25* Visual Functioning Questionnaire 25

[a] Mean ± SD

[b] Subjects (%)

[c] Excluded severe CME with/without EMR or macula traction

[d] Fisher's exact probability test
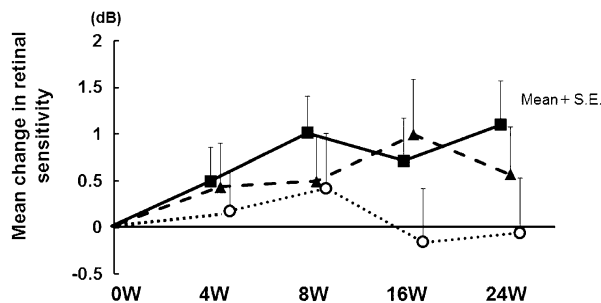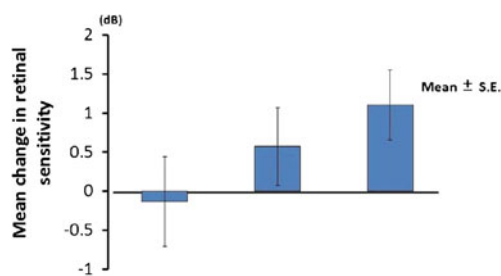
[e] One-way analysis of variance

Fig. 1 Mean change in retinal sensitivity of central 2° from baseline determined by MP-1 microperimetry during the 24-weeks after beginning topical 0.15% isopropyl unoprostone. *Filled squares* four-drop group; *filled triangles* two-drop group; *open circles* placebo group

	Placebo group $n = 33$	Two-drop group $n = 38$	Four-drop group $n = 38$
Retinal sensitivity			
(mean ± SD)			
0W	11.4 ± 3.7	12.5 ± 3.4	11.1 ± 3.3
24W	11.3 ± 5.4	13.1 ± 5.2	12.2 ± 4.5
Change	−0.13 ± 3.3	0.57 ± 3.1	1.11 ± 2.7
LogMAR visual acuity			
(mean ± SD)			
0W	0.24 ± 0.14	0.25 ± 0.14	0.26 ± 0.15
24W	0.23 ± 0.14	0.24 ± 0.15	0.25 ± 0.15
Change	−0.02 ± 0.08	−0.01 ± 0.08	−0.01 ± 0.11

Fig. 2 Dose–response change in mean retinal sensitivity of the central 2° determined by MP-1 at 24 weeks or at the time of discontinuation in the three groups

stage RP with a BCVA <0.5 logMAR, the standard deviations of the retinal sensitivity of 4° were reported to range from 3.1 to 3.8 dB [36]. Thus, a change in the retinal sensitivity of ≥4 dB was regarded as an improvement. On the other hand, a change of ≤−4 dB was taken as a worsening. There were seven patients with a worsening of the retinal sensitivity of ≤−4 dB

(21.2%) in the placebo group (33 patients), six (15.8%) in the two-drop group (38 patients), and one (2.6%) in the four-drop group (38 patients). The number in the four-drop group was significantly fewer than in the placebo group ($P = 0.02$, Fisher's exact probability test). Five patients (15.1%) showed an improvement of ≥4 dB in the placebo group, three (7.9%) in the two-drop group, and seven (18.4%) in the four-drop group. The differences in the numbers were not significant. Among the eight patients having a retinal sensitivity of ≥4 dB in the placebo group and the two-drop group before the beginning of IU, seven had a mean retina sensitivity that was higher than the median value of 12.5 dB at 24 weeks. On the other hand, the retinal sensitivity in the seven patients in the four-drop group ranged from 6 to 16 dB before the beginning of IU. These observations suggested that improvements in the placebo group were natural fluctuations and associated with better retina sensitivity before the beginning of IU. A stratified analysis was made on 109 patients by dividing them into two groups with a cutoff value of 12.5 dB, the median sensitivity, before beginning the IU (Fig. 4). A dose-dependent responsiveness was found in the three groups with the mean retina sensitivity of less than 12.5 dB ($P = 0.028$, simple linear regression analysis) (Fig. 4b). An intergroup comparison showed that the value of the improvement in the four-drop group was significantly greater than that in the placebo group ($P = 0.019$, Williams' test). The four-drop group had a mean improvement of 2.1 (1.2 + 0.9) dB over the placebo group. With a mean retina sensitivity of 12.5 dB or more all groups did not have a significant increase in their retinal sensitivities (Fig. 4a).

The changes in secondary endpoints in the three groups as a function of the dose of IU are
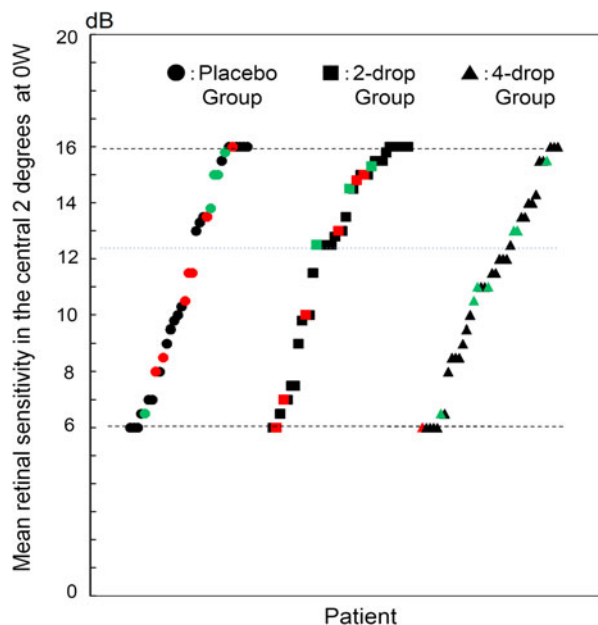
Fig. 3 Distribution of individual values of the mean retinal sensitivity of the central 2° for the three groups. Changes of ≥4 dB increase in retinal sensitivity were regarded as improved and indicated in *green*. Changes of ≥4 dB decrease in retinal sensitivity were regarded as worsened and indicated in *red*, and other changes were indicated in *black*

shown in Table 5. There were no significant changes other than a slight tendency for a decrease in MD ($P = 0.108$) and an increase in the composite score of VFQ-25 to be associated with the use of IU ($P = 0.12$). A significant dose-dependent responsiveness was found in the changes in the social life functions due to vision, one of the eight components of VQF-25 ($P = 0.001$). The blood concentrations of IU and its metabolite M1, an active form of IU, were determined at week 20 after the beginning of the topical IU (Table 6). Fifteen minutes after the ocular instillation, the M1 form was predominant in the blood and only a very small amount of IU was present. The concentration of M1 was about 1.6 times greater with four drops than two drops indicating a dose-dependency of the drug in the serum.

Adverse reactions to the drug observed during this clinical trial are shown in Table 7. A dose-dependent reaction was not evident in any of the items except eye irritation. However, there is no significant difference between the two-drop and the four-drop groups. The most frequently observed adverse event was eye irritation. The highest incidence was observed in the two-drop group (53.8%), while it was 11.4% in the placebo group and 44.7% in the four-drop group. The next most frequent adverse event was punctate keratitis, which was observed most frequently in the placebo group (17%), and the incidence was not significantly different between the two-drop group and the four-drop group (10%).

DISCUSSION

Most patients with RP visit ophthalmologists in the middle to late stages of the disease process when the constricted visual field and decreased visual acuity affect their everyday visual functioning. As to visual acuity, in general, a visual acuity of ≥0.5 (20/40) is sufficient for a good QOL [37, 38]. The authors studied patients with RP in the middle to late stages of the disease process who had constricted visual fields but with a decimal visual acuity of ≥0.5. The authors selected this visual acuity because the fixation was stable enough for perimetry testing. The number of patients with vision of ≥0.5 was reduced when the central visual field was ≤30° using Goldmann perimetry, and only 30% of the patients had ≥0.5 vision when the central visual field was ≤10° [39]. Thus, the central cone function, including the foveal cone is probably impaired even in patients with relatively good vision. However, psychophysical studies of the central retina have shown that the photoreceptors are functioning and the retinal morphology is

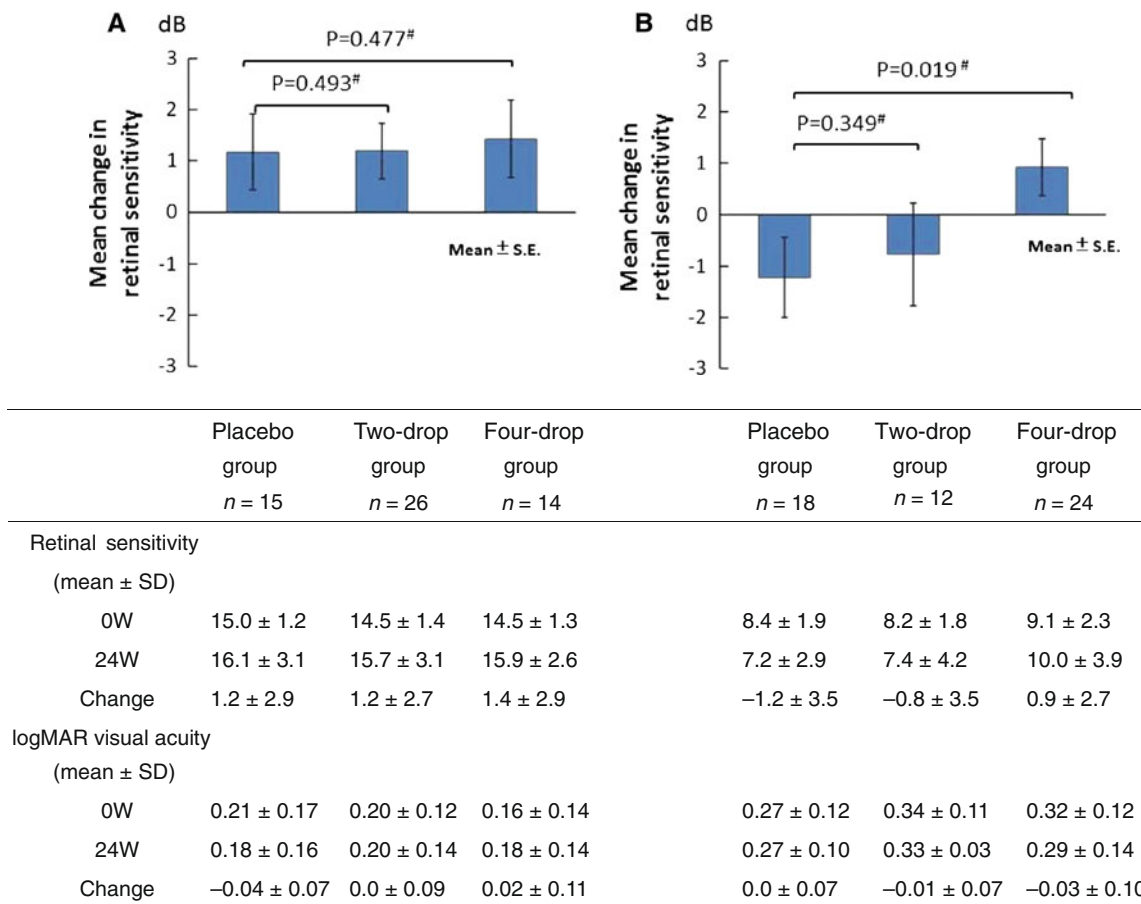|	Placebo group n = 15	Two-drop group n = 26	Four-drop group n = 14		Placebo group n = 18	Two-drop group n = 12	Four-drop group n = 24
Retinal sensitivity (mean ± SD)							
0W	15.0 ± 1.2	14.5 ± 1.4	14.5 ± 1.3		8.4 ± 1.9	8.2 ± 1.8	9.1 ± 2.3
24W	16.1 ± 3.1	15.7 ± 3.1	15.9 ± 2.6		7.2 ± 2.9	7.4 ± 4.2	10.0 ± 3.9
Change	1.2 ± 2.9	1.2 ± 2.7	1.4 ± 2.9		−1.2 ± 3.5	−0.8 ± 3.5	0.9 ± 2.7
logMAR visual acuity (mean ± SD)							
0W	0.21 ± 0.17	0.20 ± 0.12	0.16 ± 0.14		0.27 ± 0.12	0.34 ± 0.11	0.32 ± 0.12
24W	0.18 ± 0.16	0.20 ± 0.14	0.18 ± 0.14		0.27 ± 0.10	0.33 ± 0.03	0.29 ± 0.14
Change	−0.04 ± 0.07	0.0 ± 0.09	0.02 ± 0.11		0.0 ± 0.07	−0.01 ± 0.07	−0.03 ± 0.10

Fig. 4 Dose–response changes in mean retinal sensitivity by stratified analysis

normal in patients with RP even in advanced stages of RP [40]. Thus, these photoreceptor cells should be the ones targeted to be saved by the treatments. To examine the natural course of RP after the middle stage, it is recommended that changes in retinal sensitivity within the central 10° be followed by static perimetry instead of Goldmann perimetry [41]. This would correspond to the change in the univariate linear regression of MD obtained by HFA 10-2 in the middle to late stages of PR [5, 8, 41]. A recent cross-sectional study reported a higher correlation between visual acuity and retinal sensitivity in the central 2° ($r = -0.788$) in HFA 10-2 than with the MD ($r = -0.533$) in 123 patients with RP [42].

MP-1 microperimetry has been used in patients with macular diseases, including RP, to test the retina while directly observing the retina [36, 43–45]. The results of a recent study showed there was a significant improvement in the mean retinal sensitivity in the central 2° by MP-1 microperimeter after 0.12% IU in 30 Japanese patients with RP [12]. The MP-1 microperimeter is able to measure approximately the same retinal locations by the automated retina-tracking function with an acceptable reliability [45–47], although its background luminance is low to evaluate cone function sufficiently and the range of measurements is narrow (0–20 dB) compared with those of the HFA. The authors confirmed

Table 5 Results of clinical endpoints and dose-dependency of isopropyl unoprostone treatment

Clinical endpoints	Dose-dependency (linear regression analysis)
Retinal sensitivity of MP-1 central 2° (4 points)[a]	$P = 0.09$ ($P = 0.038$[b])
Retinal sensitivity of MP-1 central 10° (24 points)	$P = 0.738$
logMAR visual acuity	$P = 0.804$
Contrast sensitivity	$P = 0.958$
Humphrey perimetry 10-2 MD	$P = 0.108$
VFQ-25 (Comp. 8) composite score	$P = 0.120$

[a] Primary endpoint
[b] Adjusted for baseline due to imbalance among the three groups in MD

Table 6 Concentration of UF-021 and M1 in serum

	Mean ± SD	
	UF-201 (ng/mL)	M1[a] (ng/mL)
2-drop group ($n = 38$)	0.15 ± 0.15	1.42 ± 0.86
4-drop group ($n = 38$)	0.24 ± 0.25	2.24 ± 1.94

Concentration is measured 15 min after instillation
[a] M1 is an active form of UF-021

that the reproducibility of data obtained by either an MP-1 or HFA by making two to three measurements within a month for the baseline data.

In this clinical trial, the authors validated changes in the mean retinal sensitivity of the 2° by a MP-1 as the primary endpoint from three aspects: the results of the previous pilot study [12]; the central 2° area reportedly having the best reproducibility and statistical significance in a MP-1 [48]; and a higher correlation between visual acuity and retinal sensitivity in the central 2° in HFA 10-2 [42]. The authors

selected a range of mean retinal sensitivity in the central 2° from 6 to 16 dB as an inclusion criterion. The central retinal sensitivity was reported to be 19.7 ± 0.8 dB ranging 16–20 dB in normal subjects [49], and 18.6 ± 1.5 dB in normal elderly individuals [48]. Accordingly, the authors set the upper limit to 16 dB as the inclusion criteria for this study because of the lower limit of normal value to 16 dB. On the other hand, a previous pilot study with 0.12% IU performed on 30 patients with RP suggested that it would be difficult to make improvements in patients whose mean retinal sensitivity was ≤5 dB [12]. Thus, the lower limit was set to 6 dB in this clinical trial.

The results of the dose-dependent responsiveness of 0.15% IU showed a tendency for an improvement of retinal sensitivity ($P = 0.09$), which was confirmed by an adjusted analysis of the dose-dependency ($P = 0.038$). Furthermore, the blood concentration of M1, the active form of IU, also showed a dose-dependent association 15 min after an instillation of one or two drops of IU. Thus, an increase in the serum M1 by multiple instillations of IU is thought to be related to that in central retinal sensitivity in patients with RP. As the responsiveness to the drug varied according to the different retinal sensitivities before treatment, the authors divided the 109 patients into two groups by the median value of 12.5 dB for the stratified analysis. The authors then found a significant dose-dependent responsiveness in the patients with a baseline retinal sensitivity <12.5 dB ($P = 0.028$). The mean retinal sensitivity in patients with <12.5 dB ranged from 8.2 to 9.1 dB in the three groups, and the mean BCVA ranged from 0.27 to 0.32 logMAR units, while in patients with ≥12.5 dB, the mean retinal sensitivity ranged from 14.5 to 15.0 dB and the BCVA from 0.16 to 0.21 logMAR units.

Table 7 Lists of adverse drug reactions

Symptoms	Incidence (%)			Dose-responsiveness (Cochrane–Armitage test) P value
	P group ($n = 35$)	Two-drop group ($n = 39$)	Four-drop group ($n = 38$)	
All adverse reactions	12 (34.3)	28 (71.8)	21 (55.3)	0.101
Abnormal sensation	0 (0.0)	1 (2.6)	1 (2.6)	1.000
Eye dryness	1 (2.9)	1 (2.6)	0 (0.0)	1.000
Eye irritation	4 (11.4)	21 (53.8)	17 (44.7)	0.0052
Eye swelling	0 (0.0)	1 (2.6)	0 (0.0)	–
Macular edema	0 (0.0)	1 (2.6)	0 (0.0)	–
Ocular hyperemia	0 (0.0)	1 (2.6)	1 (2.6)	1.000
Punctate keratitis	6 (17.1)	4 (10.3)	4 (10.5)	0.483
Macular hole	1 (2.9)	0 (0.0)	0 (0.0)	–
Eye pruritus	0 (0.0)	1 (2.6)	1 (2.6)	1.000
Hypertrichosis	0 (0.0)	0 (0.0)	1 (2.6)	–

These findings suggested that IU was more effective in patients with RP whose central retinal sensitivities were more decreased, namely those at late- or advanced-stage RP.

The four-drop group showed an average improvement of 1.24 (0.13 + 1.11) dB over the placebo group. This is a relatively small change because all the values were averaged. When looking at the value of each patient with RP in Fig. 3, however, the number of patients who had a worsening of −4 dB or more in retinal sensitivity of the central 2° was significantly fewer in the four-drop group than in the placebo group ($P = 0.02$). Clinically, this degree of change should indicate that there was a slowing in the progress of the disease process in the 24-week observation period.

What might be the mechanism for the improvement of mean retinal sensitivity in the central 2° after 24 weeks of topical IU? The route of IU to the posterior ocular tissues is by a direct diffusion through the eye [50] or through the ocular blood circulation through the nasal and conjunctival vascular systems. The human choroidal blood flow improved after ocular instillation of three and four drops of 0.12% IU/time after the blood flow was decreased by the injection of ET-1 intravenously [32]. In this study, the ocular instillation of four drops of 0.15% IU/day in both eyes, in total eight drops/day, should be sufficient to increase the choroidal blood flow. Although we did not measure the foveal choroidal blood flow, an improvement of choroidal blood flow after IU might be suggested. This is important because a decrease in choroidal blood flow has been reported to be present in some patients with RP and improving the choroidal circulation could help preserve the cone photoreceptors [26–29].

This clinical study was conducted by less than 40 patients in each arm in a 24-month observation. To confirm the long-term efficacy and safety of the drug, the phase III clinical trial is now planned as a long-term observation with a large number of patients.

CONCLUSION

Isopropyl unoprostone is approved for glaucoma treatment with no serious adverse drug reactions reported and has neuroprotective properties. Our results showed that four drops/day of IU could delay the decrease in the central retinal sensitivity in patients with RP through an improvement of retinal sensitivity without directly treating the original abnormality. A greater improvement of central retinal sensitivity was found in patients with RP with more advanced stages of RP. This drug re-profiling strategy could be effective in developing safer drugs for patients with RP.

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