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A First-in-Human Phase 1 Randomized Single and Multiple Ascending Dose Study of RPh201 in Healthy Volunteers

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Zadik Hazan[1], Konstantin Adamsky[1], Andre Lucassen[1], and Leonard A. Levin[2]

Abstract

RPh201 is a drug extracted from gum mastic that has been studied for its anti-inflammatory and antibacterial properties. Preclinical studies of RPh201 demonstrated neuroprotective and neuroenhancing effects. Toxicology studies in animals did not reveal safety concerns or genotoxic effects. This single-center, phase 1, randomized, placebo-controlled, double-masked study in healthy volunteers assessed the safety and tolerability of RPh201, and determined the highest tolerated dose. There were 2 parts: a single ascending dose (SAD) stage, followed by a multiple ascending dose (MAD) stage. Three dosing arms were included in each stage (5 mg, 10 mg, and 20 mg). Safety data in the lower dosing arms were evaluated before higher doses were initiated. Eighteen participants were randomized in the SAD stage: 12 to RPh201 (4 at each dose) and 4 to placebo. Twenty-one participants were randomized in the MAD stage, of which 13 received RPh201. All 18 participants in the SAD stage completed treatment. Sixteen of the 21 participants in the MAD stage completed treatment. The most frequently reported adverse events were local injection site pain and erythema. No deaths or adverse events related to changes in vital signs or electrocardiograms were reported. No occurrences of suicidal behavior or ideation were reported.

Keywords

gum mastic, neuroprotection, phase 1, RPh201

The pursuit of new drug entities derived from plants and plant products for various therapeutic applications has its origins in antiquity and continues to the present. One such source is mastic, also known as gum mastic or mastic gum, which is the resin exudate from the *Pistacia lentiscus* tree, which grows on the southern side of the Aegean island of Chios, Greece.[1,2] Its uniqueness has been acknowledged by the European Union and the United Nations, as Chios mastiha has been identified as a protected designation of origin product, and the cultivation methods of mastic in Chios have been included on the Representative List of the Intangible Cultural Heritage of Humanity by the United Nations Educational, Scientific and Cultural Organization.[1]

Mastic and extracts of gum mastic have been used in traditional medicine for more than 5000 years as a dietary extract for the treatment of digestive disorders,[3] and its properties have been further examined in recent years. In vitro and experimental in vivo studies demonstrated that mastic and its extract have antitumor properties;[4–6] antibacterial, antifungal, and antiviral activities;[7–14] antioxidant activity;[15–20] analgesic activity;[21] and anti-inflammatory activities.[22–25] Based on this large number of potentially beneficial effects, it is currently in use in health care products and in the food industry.[26] Although gum mastic extract and powder have been used orally for many years as a food supplement, they are now being evaluated for several clinical indications, including peptic ulcers,[27] *Helicobacter pylori* treatment,[8,28] reduction of bacteria in the mouth that account for dental caries,[9] functional dyspepsia,[29] and possibly Crohn disease.[30]

RPh201 is a novel botanical drug candidate obtained from gum mastic. RPh drug substance is extracted from gum mastic obtained via a 2-step solvent

[1]Regenera Pharma Ltd., Ness Ziona, Israel
[2]Departments of Ophthalmology & Visual Sciences and Neurology & Neurosurgery, McGill University, Montreal, Canada

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Corresponding Author: Leonard A. Levin, MD, PhD, Department of Ophthalmology and Visual Sciences, 5252 Boul de Maisonneuve West, Montreal, QC H4A 3S5, Canada
(e-mail: leonard.levin@mcgill.ca)

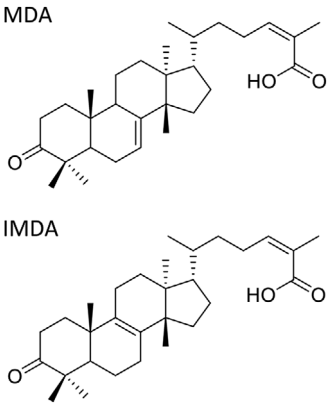

Figure 1. Molecular structures of masticadienonic acid (MDA) and isomasticadienonic acid (IMDA), 2 of the main constituents of gum mastic.

extraction process. It is insoluble in aqueous solutions but soluble in certain organic solvents and oils. RPh201 is a 5% (w/w) formulation of the drug substance in National Formulary–grade cottonseed oil stabilized with butylated hydroxytoluene, and was developed for administration by subcutaneous injection. Two of the main constituents of RPh201 (Figure 1) are masticadienonic acid (MDA) and isomasticadienonic acid (IMDA), known to be present in gum mastic.[31,32]

A biotransformation study in human hepatocytes revealed 7 metabolites of MDA. Four of these biotransformations appeared specific to human hepatocytes because they were not detected with rat and minipig hepatocytes. Conversely, 4 metabolites detected with rat hepatocytes and 3 metabolites detected with minipig hepatocytes were also observed with human hepatocytes. Based on high-resolution mass spectrometry, the biotransformations involved hydroxylation, hydrogenation, glucuronidation, sulfation, or a combination of the last 3 biotransformations.

For IMDA, 9 metabolites were identified, involving the same 4 biotransformation reactions found for MDA. Three of these 9 biotransformations appeared specific for human hepatocytes. Seven metabolites detected with rat hepatocytes and an overlapping set of 7 metabolites detected with minipig hepatocytes were also observed with human hepatocytes. In general, MDA and IMDA were found to be more stable with human hepatocytes than with rat and minipig hepatocytes.

In vitro and in vivo animal toxicology studies of RPh201 dosed for up to 39 weeks revealed no genotoxic effects, local dermal irritation, corrosion, sensitization, adverse clinical or behavioral signs, or effects on the respiratory and central nervous systems.[33] Abscesses >30 mm in size, graded as marked severity, were confined to the high-dose group and were considered as adverse.[34] Local injection site reactions were observed in all animals, with comparable severity and frequency in the placebo and high-dose groups. However, given the relative increase in tissue reaction in the high-dose group, these changes were attributed to RPh201 administration.[33]

Recent in vitro studies of RPh201 demonstrated transdifferentiation of the human retina epithelium cell line ARPE-19 into neuronal cells. In in vivo models, RPh201 promoted neurogenesis and synaptogenesis, and enhanced functional recovery of cognition, memory, and sensorimotor deficits in vascular dementia and stroke (middle cerebral artery occlusion) rat models (unpublished data). Because of these neuroprotective, neuroregenerative, and neuroenhancing results in animals, we initiated a clinical development program to study RPh201 in human diseases of the nervous system. We now report the results of the first-in-human randomized phase 1 study of RPh201 in healthy volunteers.

Methods

Study Design

This phase 1 study was a prospective, single-center, double-masked, placebo-controlled, randomized trial. It was conducted at the INC Research Center in Toronto, Canada (ClinicalTrial.gov identifier NCT01513967). The study had 2 stages: The first was a single ascending dose (SAD) and the second a multiple ascending dose (MAD) involving 4 weeks of treatment.

The study was conducted in compliance with the ethical principles of the Declaration of Helsinki (1964) and Good Clinical Practice, as outlined in the International Conference on Harmonisation (1997). Approval for the study (including protocol and consent forms) was granted by Health Canada and the Ontario Institutional Review Board. Written informed consent was obtained from each participant before any study procedures were conducted. The study protocol is included in Supplement 1. A minor administrative protocol amendment to clarify inconsistencies was implemented during the study.

Participants

Enrolled participants were healthy volunteers, aged 18 to 65 years, with a body mass index of 18 to 33 kg/m^2, and weighing ≥50 kg. Exclusion criteria were allergy to cottonseed oil, mastic, or related drugs; use of a nonprescription drug within 7 days before the first drug administration; use of any prescription medications, recreational drugs, or natural health products (except vitamin or mineral supplements, acceptable forms of birth control, and hormone replacement) within 14 days; positivity for hepatitis B, hepatitis C, or HIV;

treatment with any investigational drug within 30 days before the first drug administration in the treatment phase; drug/alcohol dependence; current or previous enrollment in a drug rehabilitation program; current smoking or within 3 months before screening; or clinically significant abnormalities (as judged by the investigator) on physical examination, 12-lead electrocardiogram (ECG), vital signs, laboratory values, or previous medical history.

Pregnant or nursing females were not eligible for inclusion to the study. Female participants of childbearing age and male participants with a partner(s) of childbearing potential were required to use effective contraception method(s) during the study.

Active Drug

RPh201 is a 5% (w/w) formulation of an extract obtained from gum mastic, the resin exudate of the *Pistacia lentiscus* tree. Gum mastic is extracted in a 2-step procedure using ethanol and hexane. The obtained extract is analyzed using high-performance liquid chromatography. The concentrations of MDA and IMDA are 5 to 6 mg/g of RPh201, which represents approximately 22% of the drug substance. The potency of the formulated RPh201 is tested with a cell-based assay where ARPE cells stop proliferating and differentiate into neuronal cells when incubated with RPh201.

Randomization and Treatment

Randomization codes were generated by the study statistician at INC Research Inc. and provided to the study site in sealed envelopes. The pharmacist at the site sequentially assigned the codes from the envelopes to allocate each participant to a treatment. During the study, only the pharmacist was aware of the treatment allocated to each participant.

Participants were randomized to receive RPh201 in cottonseed oil or placebo (cottonseed oil only) at a ratio of 2:1 and in 3 sequential dosing cohorts (5 mg, 10 mg, and 20 mg). In the SAD stage, participants received a single dose of treatment. During the MAD stage, participants received treatment twice weekly for 4 weeks. Treatment was administered subcutaneously in the clinics by the investigator or designated study personnel.

A higher-dose cohort for each stage was not initiated until the safety data from all participants in a lower-dose cohort were assessed. The MAD stage was not initiated until the safety data from the SAD group had been submitted to Health Canada.

Study Objectives

The aim of the study was to assess the safety and tolerability of RPh201 following SAD and MAD and to evaluate the highest tolerated dose.

Safety Assessments

During the SAD stage, participants stayed at the research site from the day before dosing (day 0) until after the 24-hour postdose procedures had been completed and when the investigator confirmed it was safe to discharge the participant. In the MAD stage, participants returned to the research site twice weekly (days 1 and 4) for outpatient visits and study drug administration. Participants in both the SAD and MAD stages of the study returned for a follow-up visit within 5 to 7 days after the last dose of study medication.

Adverse events (AEs), vital signs (including oxygen saturation), 12-lead ECG, and laboratory values were checked at set time points during the 24-hour postdose period in the SAD group. Urine drug screens and alcohol breath tests were also conducted at screening, day 0, and day 2. These assessments were performed at each of the dosing visits during the MAD stage.

Injection site observations were conducted before dosing and 30 minutes and 2, 8, and 24 hours after dosing during the SAD stage. These injection site observations were conducted before dosing and 30 minutes and 1 hour after dosing during the MAD stage.

The study design included administration of a suicide questionnaire (Columbia-Suicide Severity Rating Scale). The questionnaire was completed by participants at baseline, 8 hours after dosing, and at the follow-up visit during the SAD stage. During the MAD stage, the questionnaire was completed at baseline, then once a week, and at the follow-up visit.

Statistical Analysis

Descriptive statistical analysis was performed using SAS software (SAS Institute, Cary, North Carolina). No formal sample-size calculations for efficacy were performed for the SAD or MAD stages because this was a first-in-human safety study.

Results

Patient Disposition and Baseline Characteristics

Thirty-nine participants were included in the study, 18 in the SAD stage and 21 in the MAD stage. The first participant was enrolled into the SAD stage on January 17, 2012, and the last participant's last visit in the MAD stage was conducted September 10, 2012. The mean age (± standard deviation) of participants in the SAD stage was 45.3 ± 10.2 years, ranging from 27 to 65 years (Table 1). For the MAD stage the mean age was 42.8 ± 11.1 years, ranging from 22 to 63 years (Table 2). Across the 2 stages the majority of participants were white (74%). There were more males in the SAD group than in the MAD group (67% vs 38%, respectively).

Table 1. Demographics for Single Ascending Dose (SAD) Stage

	Placebo N = 6 (%)	RPh201 5 mg, N = 4 (%)	RPh201 10 mg, N = 4 (%)	RPh201 20 mg, N = 4 (%)	All Subjects N = 18 (%)
Sex					
Male	4 (66.7)	1 (25.0)	3 (75.0)	4 (100)	12 (66.7)
Female	2 (33.3)	3 (75.0)	1 (25.0)	0	6 (33.3)
Age (y)					
n	6	4	4	4	18
Mean	43.0	39.8	48.8	50.8	45.3
SD	7.27	13.60	11.59	8.85	10.23
Minimum	32	27	38	38	27
Median	42.0	40.0	46.0	53.5	45.5
Maximum	54	52	65	58	65
Race					
n	6 (100)	4 (100)	4 (100)	4 (100)	18 (100)
Asian	1 (16.7)	0	0	0	1 (5.6)
Black or African American	0	2 (50.0)	0	1 (25.0)	3 (16.7)
Caucasian	5 (83.3)	2 (50.0)	4 (100)	3 (75.0)	14 (77.8)
Disposition					
Early withdrawal	...	...	...	...	...
Completed	6 (100)	4 (100)	4 (100)	4 (100)	18 (100)

SD, standard deviation.

Table 2. Demographics for Multiple Ascending Dose (MAD) Stage

	Placebo N = 8 (%)	RPh201 5 mg, N = 4 (%)	RPh201 10 mg, N = 4 (%)	RPh201 20 mg, N = 5 (%)	All Subjects N = 21 (%)
Sex					
Male	2 (25.0)	2 (50.0)	1 (25.0)	3 (60.0)	8 (38.1)
Female	6 (75.0)	2 (50.0)	3 (75.0)	2 (40.0)	13 (61.9)
Age (y)					
n	8	4	4	5	21
Mean	44.1	42.5	43.0	40.8	42.8
SD	12.46	11.09	15.34	8.04	11.08
Minimum	22	34	23	30	22
Median	47.0	39.0	44.5	43.0	43.0
Maximum	63	58	60	51	63
Race					
n	8 (100)	4 (100)	4 (100)	5 (100)	21 (100)
Asian	1 (12.5)	0	1 (25.0)	0	2 (9.5)
Black or African American	2 (25.0)	0	1 (25.0)	1 (20.0)	4 (19.0)
White	5 (62.5)	4 (100)	2 (50.0)	4 (80.0)	15 (71.4)
Disposition					
Early discontinuation	2 (25.0)	0	1 (25.0)	2 (40.0)	5 (23.8)
Completed all visits	6 (75.0)	4 (100)	3 (75.0)	3 (60.0)	16 (76.2)

SD, standard deviation.

All 18 participants completed the SAD stage. Five participants did not complete the MAD stage. Two participants in the placebo group withdrew due to a positive urine drug screen. Three participants in the RPh201 group withdrew, 1 in the 10-mg group at the discretion of his/her physician, 1 in the 20-mg group because of pregnancy, and 1 in the 20 mg-group who missed a study visit and therefore was considered as not having completed the study.

Treatment Exposure

Of the 18 participants randomized in the SAD stage, 12 received RPh201 (4 for each of the 3 dose levels) and 6 received placebo. None of the participants in this stage withdrew prematurely.

In the MAD stage, 13 participants received RPh201 (4 in the RPh201 5-mg arm, 4 in the RPh201 10-mg arm, and 5 in the RPh201 20-mg arm), and 8 received placebo (Figure 2). Sixteen (76.2%) of the 21

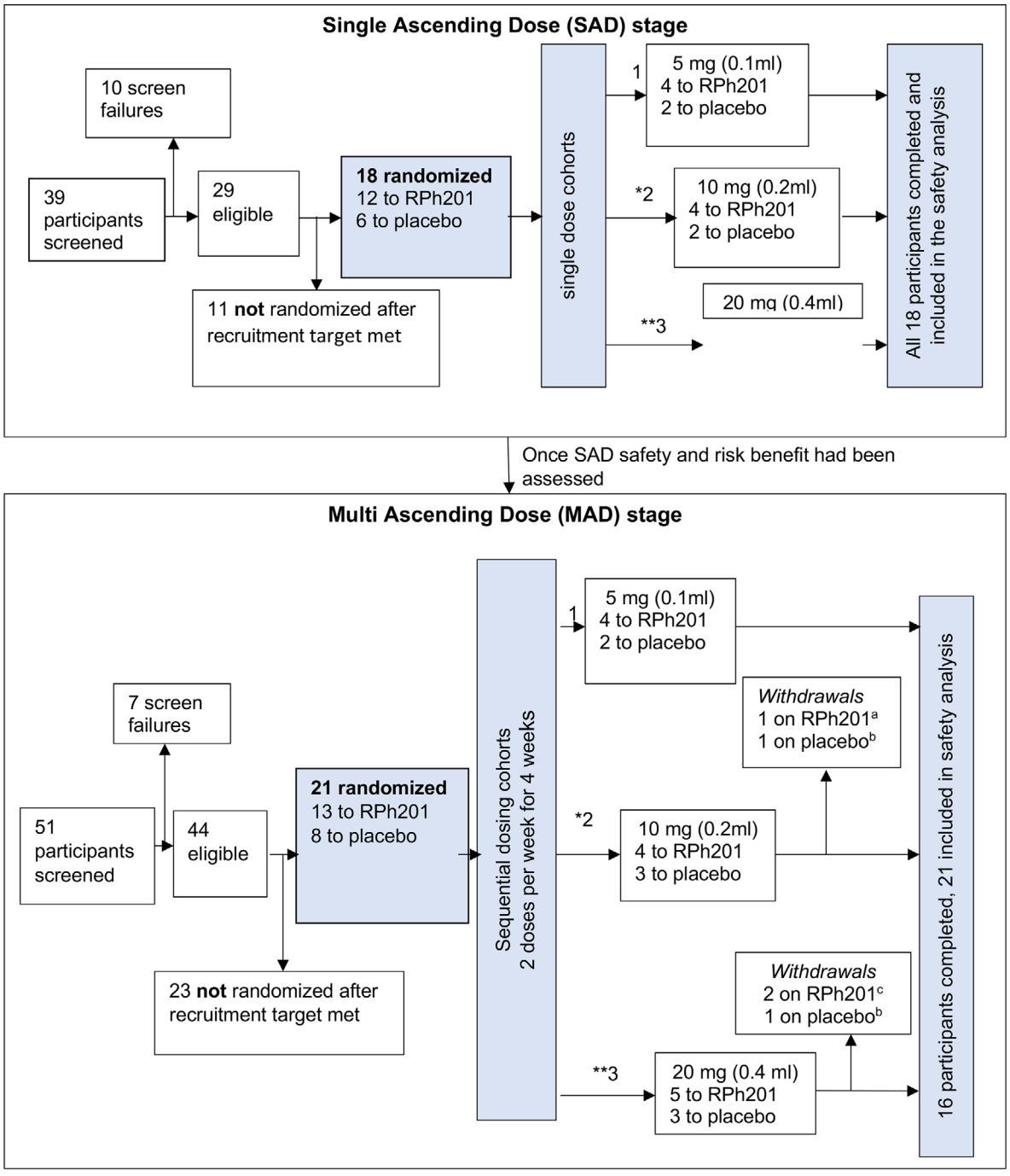

Figure 2. Flowchart displaying details of participation in the single ascending dose (SAD) and multiple ascending dose (MAD) stages of the study.

Table 3. Summary of Adverse Events Considered Related to Study Procedure/Treatment in the Single Ascending Dose (SAD) Stage

		Placebo		RPh201 5 mg		RPh201 10 mg		RPh201 20 mg		All	
System Organ Class	Preferred Term (PT)	N (%)	E	N (%)	E	N (%)	E	N (%)	E	N (%)	E
No. of participants		6		4		4		4		18	
AEs considered related		2 (33.3)	2	1 (25)	1	2 (50)	3	1 (25)	4	6 (33.3)	10
Gastrointestinal disorders		...	...	...	...	...	...	1 (25)	1	1 (5.6)	1
	Paresthesia oral	...	...	...	...	...	...	1 (25)	1	1 (5.6)	1
General disorders and administration site conditions		...	...	1 (25)	1	2 (50)	3	...	...	3 (16.7)	4
	Injection site erythema	...	...	1 (25)	1	...	...	...	...	1 (5.6)	1
	Injection site pain	...	...	...	...	2 (50)	2	...	...	2 (11.1)	2
	Injection site pruritus/itching	...	...	...	...	1 (25)	1	...	...	1 (5.6)	1
Nervous system disorders		2 (33.3)	2	...	...	...	...	1 (25)	3	3 (16.7)	5
	Headache	2 (33.3)	2	...	...	...	...	...	...	2 (11.1)	2
	Paresthesia	...	...	...	...	...	...	1 (25)	3	1 (5.6)	3

AEs, adverse events; E, number of events; N, number of participants.

participants received all 8 doses of study medication. Five withdrew prematurely in the MAD stage: 2 from the placebo group (1 received 1 dose, the other received 6 doses), 2 from the RPh201 20-mg group (1 received 3 doses, the other received 7 doses), and 1 from the RPh201 10-mg group (received 6 doses).

Safety

There were no deaths in the SAD or MAD stage of the study. Of the 99 AEs reported in the study, 97 were rated mild in severity and 2 were rated moderate. The 2 moderate AEs in the study were reported during the MAD stage; 1 was a hordeolum (assessed as unlikely related to the study procedure/treatment) and the other erythema (assessed as possibly related to the study procedure/treatment).

Twelve AEs in 7 participants were reported in the SAD group, with 2 events in 2 participants in the placebo group and 10 events in 5 participants in the RPh201 groups. Of the 12 AEs, 10 were considered related to study procedures/treatment, 2 in the placebo group and 8 in the RPh201 treatment group (Table 3).

In the MAD group, 87 AEs were reported in 14 participants, with 11 events in 4 participants in the placebo group and 76 events in 11 participants in the RPh groups. Of the 87 AEs, 60 were considered related to study procedures/treatment, 4 events in 4 participants in the placebo group and 56 in 10 participants in the RPh201 groups (Table 4).

Across the whole study (MAD and SAD stages combined), 68% of the AEs that were considered to be related to the study procedure/treatment were associated with the administration of the injections, including injection site erythema (17 events in 8 participants) and local site pain (11 events in 10 participants). None of the injection site reactions were dose limiting but were more frequent in the MAD group. No serious AEs were reported in the SAD group. For the MAD group, 1 participant on placebo had a serious AE (pregnancy). Another participant in the MAD RPh201 10-mg group experienced 2 significant AEs (erythema and injection site erythema), which took an unusually longer time to recover. Both participants withdrew prematurely.

No AEs were related to changes in vital signs or ECG. The mean heart rates for the RPh201 groups remained within the normal range. There were no clinically significant changes in laboratory values from baseline during the study.

The Columbia-Suicide Severity Rating Scale suicidal assessment showed no occurrences of suicidal behavior or ideation during the study.

Discussion

Treatment of healthy human volunteers with doses of RPh201 from 5 to 20 mg, either as a single dose or as multiple doses administered twice weekly, was safe and well tolerated. The majority of the AEs considered to be related to the study/treatment were associated with general administration and injection site reactions (68%). RPh201 was administered subcutaneously, a route that is frequently associated with localized injection site reactions.

As expected, more AEs were observed in the MAD stage of the study (12 AEs in SAD, 87 in MAD) because treatment was administered twice weekly and for a longer period. Treatment with RPh201 did not raise any safety findings in terms of clinically significant changes in vital signs, ECG, laboratory values, or assessment of suicidal behavior or ideation.

Table 4. Summary of Adverse Events (AEs) considered related to study procedure/treatment in the MAD stage

System Organ Class	Preferred Term	Placebo		RPh201 5 mg		RPh201 10 mg		RPh201 20 mg		All	
		N (%)	E	N (%)	E	N (%)	E	N (%)	E	N (%)	E
N		8 (100)		4 (100)		4 (100)		5 (100)		21 (100)	
AEs considered related		4 (50)	4	2 (50)	12	4 (100)	31	4 (80)	13	14 (66.7)	60
Gastrointestinal disorders		1 (12.5)	1	...	...	1 (25)	2	...	...	2 (9.5)	3
	Dry mouth	1 (12.5)	1	...	...	1 (25)	2	...	...	2 (9.5)	3
General disorders and administration site conditions		2 (25)	2	1 (25)	1	4 (100)	22	4 (80)	12	11 (52.%)	37
	Injection site erythema	2 (25)	2	...	...	4 (100)	11	1 (20)	3	7 (33.3)	16
	Injection site anesthesia	...	...	...	...	1 (25)	1	...	...	1 (4.8)	1
	Injection site pruritus	...	...	...	...	2 (50)	3	...	...	2 (9.5)	3
	Injection site reaction	...	...	...	...	...	...	1 (20)	1	1 (4.8)	1
	Injection site pain	...	...	1 (25)	1	3 (75)	4	4 (80)	4	8 (38.1)	9
	Injection site induration	...	...	...	...	1 (25)	2	1 (20)	1	2 (9.5)	3
	Injection site hematoma	...	...	...	...	1 (25)	1	1 (20)	1	2 (9.5)	2
	Pain	...	...	...	...	...	...	2 (40)	2	2 (9.5)	2
Musculoskeletal and connective tissue disorders		...	...	...	...	1 (25)	1	1 (20)	1	2 (9.5)	2
	Pain in extremity	...	...	...	...	1 (25)	1	1 (20)	1	2 (9.5)	2
Nervous system disorders		1 (12.5)	1	1 (25)	7	...	...	...	...	2 (9.5)	8
	Hypoaesthesia	1 (12.5)	1	...	...	...	...	...	...	1 (4.8)	1
	Dizziness	...	...	1 (25)	1	...	...	...	...	1 (4.8)	1
	Headache	...	...	1 (25)	6	...	...	...	...	1 (4.8)	6
Skin and subcutaneous tissue disorders		...	...	1 (25)	4	3 (75)	6	...	...	4 (19.0)	10
	Erythema	...	...	1 (25)	4	2 (50)	2	...	...	3 (14.3)	6
	Pruritus	...	...	...	...	1 (25)	1	...	...	1 (4.8)	1
	Rash	...	...	...	...	1 (25)	1	...	...	1 (4.8)	1
	Ecchymosis	...	...	...	...	1 (25)	2	...	...	1 (4.8)	2

AEs, adverse events; E, number of events; N, number of participants

Systemic pharmacokinetic (PK) studies were not required by the Food and Drug Administration in the early stages of development of RPh201, primarily because they were aware of the technical challenges involved with determining standard pharmacokinetic measurements for botanical drug products that often consist of more than 1 chemical constituent. At the time of phase 1, the company had a limited understanding and knowledge of the RPh201 composition and therefore was unable to integrate PK study in phase 1. PK of the 2 major ingredients, MDA and IMDA, has been demonstrated in our recent preclinical toxicology studies,[33,34] and a human PK study is planned.

Therapies proven to exhibit neuroprotection (prevention of neuronal loss), neuroregeneration (reversal of preexisting structural loss to neurons), or neuroenhancement (improvement of neuronal function irrespective of structural changes)[35,36] would have a broad and far-reaching effect in the treatment of neurological disease. The safety data observed in the present study are encouraging, and should allow further development of RPh201 as a neuroprotective, neuroregenerative, and neuroenhancing therapy, at a dose of 20 mg administered twice weekly via the subcutaneous route.

Based on the data from this phase 1 study, a phase 2a randomized, placebo-control study in participants with previous nonarteritic anterior ischemic optic neuropathy, a neurodegenerative disease resulting in vision loss, involving RPh201 was initiated (NCT02045212). Participants received RPh201 at a dose of 20 mg twice weekly for up to 26 weeks. Results from the Phase 2a study were consistent with improvement in visual acuity in the RPh201 arm compared to placebo.[37] A phase 3 clinical trial is ongoing (NCT03547206).

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Declaration of Conflicting Interests

Z.H., K.A., and A.L. are employees of Regenera Pharma Limited, the company that funded these studies. L.A.L. is a consultant to Regenera, and has served as consultant on neuroprotection to Aerie, Allergan, Evevensys, Galimedix, Prilenia, and Quark. He is a recipient of funding from the Canadian Research Chairs program, National Institutes of Health (R21 EY025074), Canada Institutes for Health Research (PJT-162396), and Research to Prevent Blindness.

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Availability of Data and Material

All data generated or analyzed during this study are included in this published article and its supplementary information file.

Author Contributions

Z.H. invented the molecule. A.L. performed the pharmacologic studies and analyses. K.A., Z.H., and INC (the CRO) designed the trial. INC and K.A. analyzed the data. L.A.L. and K.A. wrote and edited the manuscript. All authors read and approved the final manuscript.

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