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A First-In-Human Phase 1 Study to Evaluate the Safety and Tolerability of LEM-S401, a Novel siRNA-DegradaBALL Drug Targeting CTGF in Healthy Adults

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ABSTRACT

This study evaluated the safety, tolerability, and pharmacokinetics of LEM-S401, a novel siRNA therapeutic with DegradaBALL, a mesoporous silica nanoparticle-based delivery system. LEM-S401 is designed to deliver siRNA targeting connective tissue growth factor (CTGF) to fibroblasts for treating hypertrophic scars and keloids, both of which result from abnormal collagen proliferation. LEM-S401, containing unmodified siRNA LEM-17234 encapsulated in DegradaBALL nanoparticles, was administered subcutaneously to healthy adults in a randomized, double-blind, placebo-controlled, single-ascending dose study. Safety and tolerability assessments included vital signs, adverse events (AEs), laboratory tests, and cytokine levels. Pharmacokinetic analysis of LEM-17234 and silicon (Si), the primary component of DegradaBALL, was performed using blood samples collected at specified time points. LEM-S401 demonstrated a favorable safety and tolerability profile with only mild, self-resolving injection site reactions including pain and erythema. No systemic AEs were observed, and cytokine levels showed no significant changes between the treatment and placebo groups. Pharmacokinetic analysis revealed that LEM-17234 was below the plasma detection limit, indicating no notable systemic exposure of siRNA, while Si showed no dose-dependent systemic exposure, suggesting minimal systemic circulation of the mesoporous silica nanoparticles. These findings suggest DegradaBALL effectively encapsulates and delivers siRNA locally without significant systemic exposure. The novel DegradaBALL delivery system enables the stable and targeted delivery of siRNA, which presumably overcomes challenges related to siRNA instability and off-target effects. LEM-S401 has the potential to advance the treatment of fibrotic skin diseases such as keloids and hypertrophic scars by delivering siRNA directly to fibroblasts, thereby inhibiting excessive collagen production.

Trial Registration: ClinicalTrials.gov identifier: NCT04707131. https://clinicaltrials.gov/study/NCT04707131?cond=NCT04707131&rank=1

Ha-Yeon Kim and Jaeso Cho contributed equally to this manuscript.

Summary

- What is the current knowledge on the topic?
  - siRNA therapeutics targeting fibrosis-related pathways are emerging as promising treatments for chronic conditions.
  - CTGF is a key mediator in fibrosis, and siRNA drugs targeting CTGF could potentially manage fibrotic diseases more effectively.
- What question did this study address?
  - This study evaluated the safety, tolerability, and pharmacokinetics of LEM-S401, a CTGF-targeted siRNA drug, in healthy adults.
  - It aimed to provide foundational safety and tolerability data to guide appropriate dosing and future trials for fibrotic conditions.
- What does this study add to our knowledge?
  - LEM-S401 was generally safe and well-tolerated in healthy adults, with predictable pharmacokinetics and mild, transient side effects.
  - This first-in-human data supports LEM-S401's potential as a safe siRNA option for treating fibrosis.
- How might this change clinical pharmacology or translational science?
  - LEM-S401 advances siRNA therapy for fibrotic diseases, showing promise as a targeted and safer option.
  - It provides a clinical foundation for future siRNA developments and potential applications across broader therapeutic areas.

1 | Introduction

Wound healing is a complex, dynamic process involving multiple regulatory pathways [1]. This process in the skin occurs in three overlapping stages: inflammation, proliferation, and remodeling [2, 3]. The inflammatory stage involves hemostasis, cellular debris clearance, and phagocytosis by immune cells [3]. In the proliferative stage, tissue contracts, and a new epithelial barrier forms [2]. The final remodeling phase focuses on restoring tensile strength and achieving normal tissue structure [3]. However, in cases of excessive wound healing, abnormal scars, such as keloids and hypertrophic scars, can develop [4]. These scars are characterized by excessive collagen production, which leads to cosmetic and functional impairments, making them a clinical challenge [4]. Although traditional therapies such as compression and steroids aim to reduce collagen synthesis, there remains an unmet need for more effective treatments [4–6].

In recent years, siRNA (small interfering RNA) technology has emerged as a promising therapeutic strategy for conditions involving abnormal collagen production, including keloids and hypertrophic scars [6]. By effectively silencing target genes through messenger RNA degradation, siRNA provides an ideal approach for modulating gene expression. In particular, the silencing of connective tissue growth factor (CTGF), a key regulator of fibrosis whose overexpression leads to aberrant fibrosis and scar formation, represents a novel and more specific therapeutic strategy [7, 8]. Unlike broader targets such as TGF-β, which are associated with extensive physiological roles and potential side effects, CTGF inhibition via siRNA offers a focused approach to reduce pathological fibrosis and promote normal wound healing [9]. However, the clinical application of siRNA faces significant challenges due to its inherent instability and susceptibility to degradation by ribonucleases (RNases), as well as the difficulty in delivering it effectively to target cells outside the liver [7, 8].

To address these challenges, DegradaBALL, a novel drug delivery system using mesoporous silica nanoparticles (MSNs), was developed [10, 11]. DegradaBALL for LEM-S401 is specifically designed to encapsulate and protect siRNA molecules, such as LEM-17234 (siRNA specific for CTGF), from degradation in the body, enabling their efficient delivery to target tissues [12–14]. DegradaBALL offers several advantages over traditional drug delivery systems (DDS), including its ability to bypass RNase-mediated degradation, protect siRNA within its porous structure, and sustain release at the cellular level [15]. Furthermore, DegradaBALL can potentially facilitate endosomal escape, ensuring that the siRNA payload reaches the cytoplasm, where it can exert its gene-silencing effects [7, 8]. DegradaBALL provides a high surface area and large pore size, enabling stable siRNA encapsulation and sustained release, thereby maintaining gene silencing effects for over 96 h. Additionally, it enhances siRNA retention at the injection site, prolonging localized RNA interference (RNAi) effects while minimizing systemic side effects. These characteristics make DegradaBALL a promising RNAi delivery system for the treatment of diseases such as fibrosis [8].

LEM-S401, a formulation incorporating DegradaBALL as a carrier for the siRNA LEM-17234, was developed to inhibit CTGF expression, thereby reducing excessive collagen deposition in hypertrophic scars and keloids [4–6]. In preclinical studies, LEM-S401 demonstrated potent suppression of CTGF mRNA in fibroblast cells and significant therapeutic efficacy in animal models of wound healing [1, 2]. Importantly, repeated subcutaneous administration of LEM-S401 for 4 weeks in mice and monkeys showed good tolerability, without significant local or systemic adverse effects [8]. Moreover, its low systemic exposure minimized off-target side effects, and transient inflammatory responses at the injection site resolved during the recovery period without additional medication. These favorable safety and efficacy profiles further underscore the potential of DegradaBALL as an advanced drug delivery platform technology, capable of overcoming the barriers to siRNA stability, targeting, and extra-hepatic delivery, thereby revolutionizing the field of RNA therapeutics (Figure 1) [7, 8].

This first-in-human (FIH) study aims to evaluate the safety, tolerability, and pharmacokinetic profile of LEM-S401, delivered subcutaneously using DegradaBALL, in healthy human volunteers. The results will provide valuable insights into the clinical potential of DegradaBALL as an innovative drug delivery system for siRNA therapies.

2 | Participants and Methods

This study included healthy adult volunteers aged 19–65 years. Participant eligibility was assessed based on vital signs, physical examination (PE), 12-lead electrocardiogram (ECG), clinical laboratory tests (blood hematological, biochemical, and urinalysis), and alcohol breath tests. The study protocol was approved by the Korea Ministry of Food and Drug Safety and the Institutional Review Board of the ChungBuk National University Hospital, Cheongju, Republic of Korea. This study was conducted in accordance with the Declaration of Helsinki and the regulations of Korean Good Clinical Practice and was registered with the Clinical Trials Registry before participant recruitment (ClinicalTrials.gov identifier: NCT04707131).

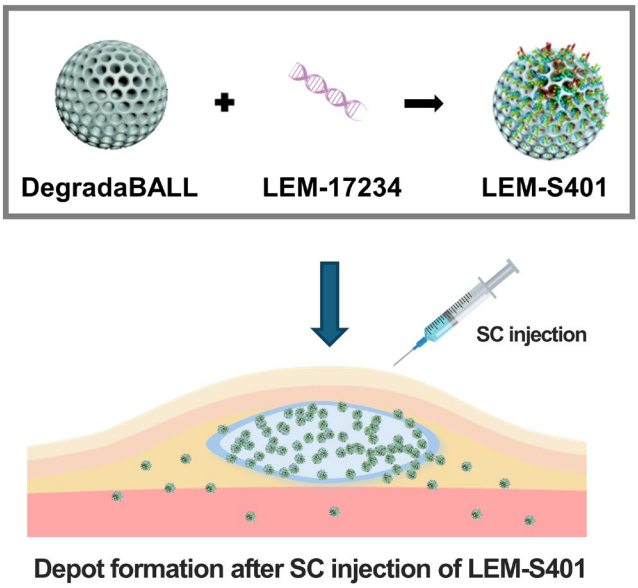

FIGURE 1 | Schematic Representation of LEM-S401 Structure and Components.

2.1 | Study Design

This was a randomized, double-blind, placebo-controlled, single subcutaneous administration ascending dose study. In this study, different doses of LEM-S401 (Lemonex Inc., Seoul, Republic of Korea) and 0.9% normal saline (JW Pharmaceutical Corporation, Seoul, Republic of Korea) were used for each cohort, with the administration volume of both the experimental and placebo groups set at 0.2 mL. In Cohort 1, 40 μg LEM-S401 (a mixture of 40 μg of LEM-17234 and 200 μg of DegradaBALL) was used, while in Cohort 2, 100 μg LEM-S401 (a mixture of 100 μg of LEM-17234 and 500 μg of DegradaBALL) was used, and in Cohort 3, 200 μg LEM-S401 (a mixture of 200 μg of LEM-17234 and 1000 μg of DegradaBALL) was used.

Each cohort consisted of a sentinel group (one participant for both the test and placebo groups) and a remaining group (three participants for the test group and one participant for the placebo group). As this study was an exploratory study designed to assess tolerability rather than for hypothesis testing, six participants were enrolled in each cohort. In the absence of significant adverse events (AEs) in the sentinel group, the remaining group was treated (Figure 2). Dose escalation was determined after a review of the safety and tolerability results in at least five participants in each cohort, considering the possibility of participant dropout. However, as no dropouts occurred in Cohort 1 and 2 during the actual study, dose-escalation evaluations were conducted with six participants.

Screening was performed 28–1 day prior to the first dose and included only volunteers who weighed at least 50.0 kg and had a body mass index (BMI) in the range of 18.0–30.0 kg/m^2. In

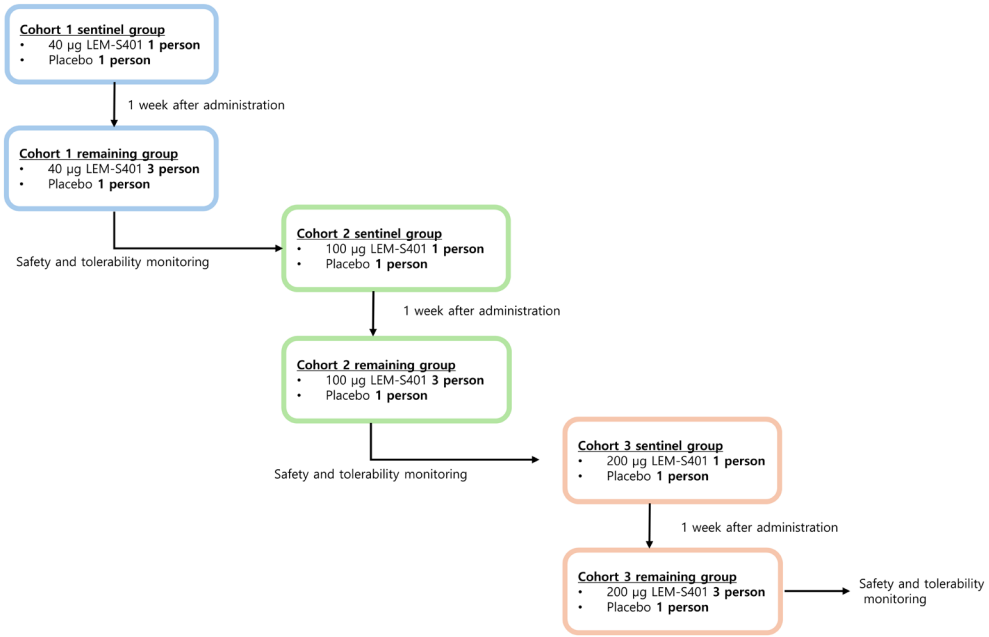

FIGURE 2 | Study design.

addition, only those without clinically significant medical conditions and deemed suitable for study enrollment by the investigator were included.

In this study, LEM-S401 or placebo was administered subcutaneously to evaluate the safety, tolerability, and PKs following a single dose of LEM-S401. Participants were assigned to each treatment using a block randomization method to ensure a 2:1 ratio of test to placebo. The dosing interval was maintained at 30 min or more for the sentinel group and 15 min or more for the remaining group.

2.2 | Safety and Tolerability

To evaluate safety and tolerability after investigational product administration, vital signs were monitored continuously from admission to discharge, and physical examination (PE), local tolerability tests, AEs, and concomitant medications (CMs), as well as 12-lead electrocardiography (ECG) and clinical laboratory tests were performed at defined time points. In addition, cytokine tests were performed at set time points, and the levels of TGF-β1, TGF-β3, IFN-γ, TNF-α, IL-2, IL-1β, IL-4, IL-6, IL-8, IL-10, and monocyte chemoattractant protein (MCP)-1 were analyzed.

Safety and tolerability analyses were performed using SAS Analytics Pro Version 9.4 (SAS Institute Inc., Cary, NC, USA). Cytokines from human plasma samples were processed and analyzed using the most current version of multiplex assay methods. Cytokine concentrations were calculated using Discovery Workbench (Meso Scale Discovery Inc., MD, USA) with the low and high ends of the ranges defining the lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ), respectively. The determination coefficient (r [2]) was >0.98, and the precision estimate was $\leq 9.7\%$, with an accuracy in the range of 86.2% ~ 130.0%. The precision of the quality control specimen was $\leq 30.1\%$, and the accuracy values were in the range of 80.8% ~ 119.1%.

2.3 | Pharmacokinetics

Blood was collected at 0, 0.167, 0.33, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 168 h post-dose and at the post study visit (PSV) to evaluate the pharmacokinetics of LEM-17234 and silicon (Si), the main component of DegradaBALL, in the blood. For pharmacokinetic analysis of LEM-17234, 10 mL of blood was collected and centrifuged at 4°C and 1100 relative centrifugal force (RCF) for 10 min. The plasma was then transferred to protein LoBind tubes and stored frozen at −70°C or below before being transported to the analytical laboratory. For the pharmacokinetic analysis of Si, 6 mL of blood was collected and centrifuged at 4°C and 1100 RCF for 10 min. Whole blood was transferred to a 5 mL Eppendorf DNA LoBind tube, frozen in a freezer below −70°C, and transported to the analytical laboratory. The frozen samples were thawed at room temperature before analysis.

The concentration of LEM-17234 in human plasma using liquid chromatography-fluorescence detection (LC-FD) (Waters Corporation, MA, USA) was performed based on an absolute calibration curve method. A regression equation with a weighting factor of $1/x^2$ was applied, and the analytical validation confirmed the accuracy, precision, and reproducibility of this method. Human plasma samples (study samples) were frozen at Kobe Laboratory, CMIC Pharma Science Co. Ltd., in Tokyo, Japan. The calibration curve range was 1–1000 ng/mL, with a correlation coefficient of ≥ 0.998535. The accuracy of the quality control specimens ranged from 86.6% to 109.1%.

The concentration of Si in human whole blood was determined by an inductively coupled plasma–optical emission spectrometry (ICP–OES) system (Thermo Scientific Inc., MA, USA). The linear regression data for the ICP–OES were obtained from Qtegra Intelligent Scientific Data Solution Software (Thermo Scientific Inc., MA, USA). The LLOQ for Si was 200 ng/mL, the coefficient of correlation was ≥ 0.9969, and the accuracy of the quality control specimen was in the range of 84%–115%. The quality control specimens showed an accuracy ranging from 84% to 115%. All analyses were performed in accordance with the Bioanalytical Method Validation Guidance for Industry (US FDA, 2018.05).

Pharmacokinetic analyses were performed by noncompartmental methods using Phoenix WinNonlin Version 8.3 (Certara, NJ, USA). Pharmacokinetic analysis of Si was performed at the original concentrations as well as at baseline-corrected concentrations. The PK parameters of interest were as follows: area under the concentration-time curve from the point of administration to the last time point of blood sampling (AUC_{0-t}); area under the concentration-time curve from the point of administration to infinity (AUC_{inf}); maximum concentration of drug in plasma (C_{max}); time of peak plasma concentration (T_{max}); terminal half-life ($t_{1/2}$); apparent clearance (CL/F); and apparent volume of distribution (Vd/F).

The means and standard deviations of the pharmacokinetic endpoints were calculated, and the PK parameters were calculated using the linear up log down method.

3 | Results

3.1 | Demographics

A total of 46 volunteers were screened after being informed about the study and providing written consent. Eighteen participants passed the screening, were enrolled in the study, and completed the entire study schedule. The 18 participants in the study had an average age of 26.5 years, height of 176.47 cm, weight of 72.93 kg, and BMI of 23.42 kg/m^2 (Table 1). There were no statistically significant differences in variables such as height, weight, or BMI between participants assigned to each treatment group, except for age, and there were no significant differences between treatment groups in terms of alcohol, caffeine, smoking, or allergy status.

3.2 | Safety and Tolerability

Safety and local tolerability were evaluated in 18 participants administered LEM-S401, with 14 AEs occurring in 7 volunteers

TABLE 1 | Summary of demographic and baseline characteristics.

Variable	40 μg LEM-S401 ($n=4$)	100 μg LEM-S401 ($n=4$)	200 μg LEM-S401 ($n=4$)	Placebo ($n=6$)
Gender, *n* (%)				
Male	4 (100.0)	4 (100.0)	4 (100.0)	6 (100.0)
Age (year)	30.0 ± 6.0	27.0 ± 5.7	32.4 ± 8.3	26.5 ± 3.7
Height (cm)	174.3 ± 7.0	172.7 ± 8.8	176.8 ± 6.2	176.5 ± 4.7
Weight (kg)	66.5 ± 8.8	66.1 ± 11.9	71.2 ± 12.6	72.9 ± 9.4
BMI (kg/m^2)	21.8 ± 1.8	22.1 ± 3.1	22.7 ± 3.4	23.4 ± 2.7

Note: The values are presented as the means ± SDs.
Abbreviations: BMI, body mass index; SD, standard deviation.

(Table 2 and Table S1). All AEs occurring during the study were associated with the injection site, and their causal relationship with the investigational product was judged to be either certain or probable. After the administration of 40 μg LEM-S401, one adverse drug reaction (ADR) was reported, which consisted of injection site pain graded as mild in severity. This pain occurred the day after administration and resolved after lasting for 1 day. ADRs occurring after 100 μg LEM-S401 included five cases of injection site pain and one case of injection site erythema, all of which were rated as mild in severity. Injection site pain occurred 12 h after administration and resolved within 2 days on average, while injection site erythema occurred 40 min after administration and resolved within 1.5 h. The ADRs that occurred after the administration of 200 μg LEM-S401 included five cases of injection site pain and one case of injection site erythema, with one case of injection site pain rated as moderate in severity and all other ADRs rated as mild. Injection site pain occurred as early as 9 h post-dose and resolved within 37 h on average, and injection site erythema occurred 20 min post-dose and lasted 40 min before resolving (Table 2). All ADRs resolved naturally without further intervention, and no significant changes from baseline were observed in safety and tolerability assessments other than symptoms collected as adverse events.

In the cytokine analysis, TGF-β3, IL-2, and IL-10 were undetectable, and TGF-β1, IFN-γ, TNF-α, IL-1β, IL-4, IL-6, IL-8, and MCP-1 levels were not significantly different between the treatment groups (Table S2). Moreover, no clinically significant findings in terms of vital signs, PE, local tolerability tests, CMs, 12-lead ECG, or clinical laboratory tests were observed.

3.3 | Pharmacokinetics

A pharmacokinetic evaluation was performed for LEM-17234 and Si, the major component of DegradaBALL, following the subcutaneous administration of LEM-S401 in 18 volunteers who completed pharmacokinetic blood sampling. LEM-17234 had concentrations below the LLOQ (< 1.00 ng/mL) at all time points, and pharmacokinetic parameters could not be calculated. Si was detected in all participants; however, quantifiable concentrations corresponding to the elimination phase were measured in only three participants from the placebo group, one participant from the 40 μg group, two participants from the 100 μg group, and one participant from the 200 μg group. Consequently, only some pharmacokinetic parameters were derived, and no dose-dependent trends were observed. Given that Si concentrations were detected at 0 h blood sampling in more than half of the participants, the analysis was performed on the baseline uncorrected and corrected concentrations (Table 3). The change in Si concentration over time before and after baseline correction is presented (Figures 3 and 4).

4 | Discussion

This clinical study was conducted to evaluate the safety, tolerability, and pharmacokinetic characteristics of LEM-S401 following a single subcutaneous injection in healthy adult volunteers. The investigational product, LEM-S401, is a chemically unmodified siRNA, LEM-17234, encapsulated in DegradaBALL.

siRNAs are highly unstable in the body, and developing systems to safely and effectively deliver siRNAs into cells is a major challenge in siRNA research [13]. To date, various delivery systems, such as cationic polymers, lipid nanoparticles (LNPs), viral vectors, and nanomaterials, have been developed for siRNA delivery [10]. Cationic polymers and LNPs show efficient cytoplasmic delivery but require complex procedures for siRNA loading [11]. Furthermore, in clinical practice, cationic polymers and LNPs are cautiously applied because of their potential toxicity or in vivo structural instability [14]. Viral vectors are advantageous in terms of efficiency due to their high efficiency of intracellular nucleic acid delivery, but other delivery vehicles are considered due to biosafety concerns, such as the risk of mutagenesis [13]. The DegradaBALL used as siRNA carriers in this study are advantageous as carriers because they can facilitate cellular uptake of the loaded siRNA while protecting the cargo and maintaining its physical and chemical stability. It has also been reported that DegradaBALLs are stable and able to escape endosomes and sustainably release their cargo into the cytoplasm [8]. Thus, it is shown that DegradaBALL protects siRNA from degradation by external environments and RNases, thereby maintaining a relatively prolonged duration of action and high delivery efficiency while reducing the required siRNA dosage. Additionally, it is expected to provide a new therapeutic option that will effectively overcome the inherent instability of siRNA, providing a stable and efficient delivery system [8].

The ADRs measured during the study were all related to injection site reactions, included 12 cases of injection site pain and two cases of injection site erythema. This suggests that LEM-S401

TABLE 2 | Drug-related adverse events: incidence and duration.

Adverse event		40 μg LEM-S401 ($n=4$)		100 μg LEM-S401 ($n=4$)		200 μg LEM-S401 ($n=4$)	
System of class	**Preferred term**	**Incidence**	**Duration (h)**	**Incidence**	**Duration (h)**	**Incidence**	**Duration (h)**
General disorders and administration site conditions	Injection site pain	1 (1 [25.0])	24.00	5 (3 [75.0])	47.97 (35.00–60.92)	6 (3 [75.0])	36.96 (12.50–62.00)
	Injection site erythema	—	—	1 (1 [25.0])	1.33	1 (1 [25.0])	0.60

Note: Incidence is presented as the number of events (number of participants [% of participants]), and duration is presented as mean (minimum-maximum).

TABLE 3 | Pharmacokinetic parameters of Si after a single administration of LEM-S401.

	Parameters	40 μg LEM-S401 ($n=4$)	100 μg LEM-S401 ($n=4$)	200 μg LEM-S401 ($n=4$)	Placebo ($n=6$)
Baseline-uncorrected	T_{max} (h)	15.00 (6.00–24.00)	0.75 (0.17–2.00)	3.25 (0.33–96.00)	0.50 (0.17–2.00)
	C_{max} (ng/mL)	365.75 (109.96)	505.25 (31.55)	517.25 (195.94)	664.25 (423.86)
	AUC_{0-t} (ngh/mL)	6429.78 (3399.10)	37236.51 (24781.91)	53238.43 (47934.64)	43672.59 (54676.95)
	$t_{1/2}$ (h)	1037.63[a]	199.53 (15.86)	433.56[a]	492.14 (499.85)
Baseline-corrected	T_{max} (h)	15.00 (6.00–24.00)	0.75 (0.17–2.00)	3.25 (0.33–96.00)	0.50 (0.17–2.00)
	C_{max} (ng/mL)	365.75 (109.96)	222.75 (38.72)	243.25 (330.38)	403.75 (382.55)
	AUC_{0-t} (ngh/mL)	5713.02 (3396.80)	346.64 (328.91)	2472.79 (4266.69)	1985.18 (3318.75)
	$t_{1/2}$ (h)	1037.63[a]	—	—	61.01[a]

Note: Data are provided as the mean (SD), except for T_{max} which is presented as the median (minimum-maximum).
[a]The values for $t_{1/2}$ represent data from a single participant.

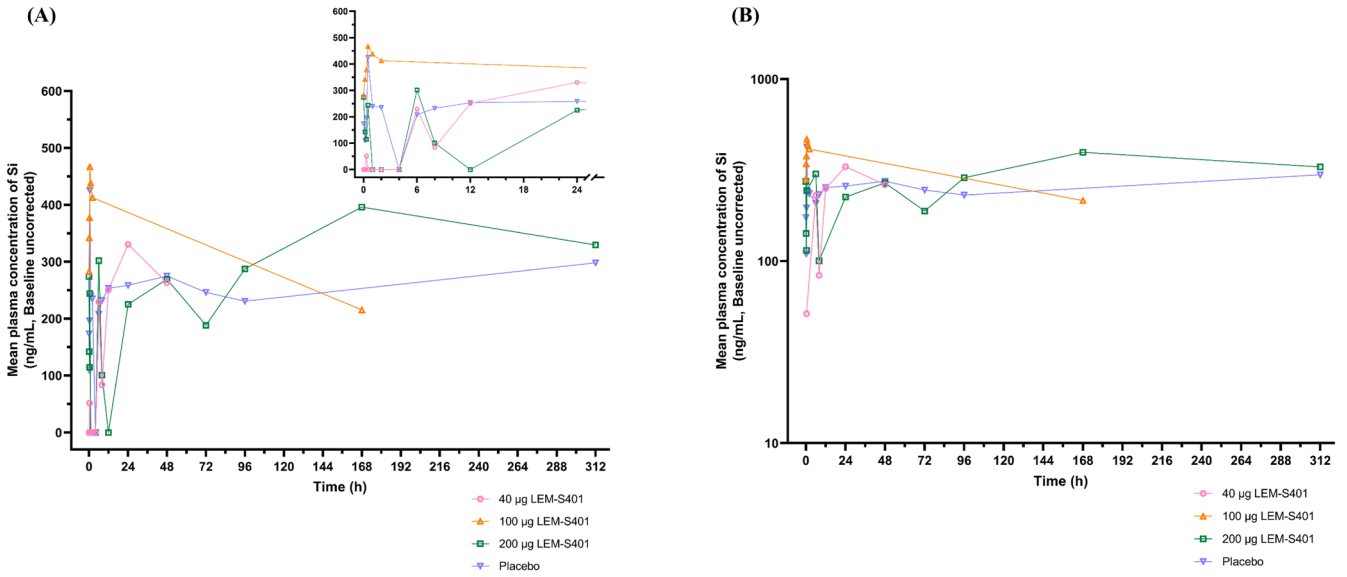

FIGURE 3 | Mean plasma concentration-time curves (baseline-uncorrected) after a single subcutaneous injection of 40 μg LEM-S401 (-○-), 100 μg LEM-S401 (-△-), 200 μg LEM-S401 (-□-), and placebo (-▽-) in healthy participants. The inset shows the mean plasma concentration of DegradaBALL from 0 h to 24 h after administration. (A): Linear scale; (B): Semi-log scale.

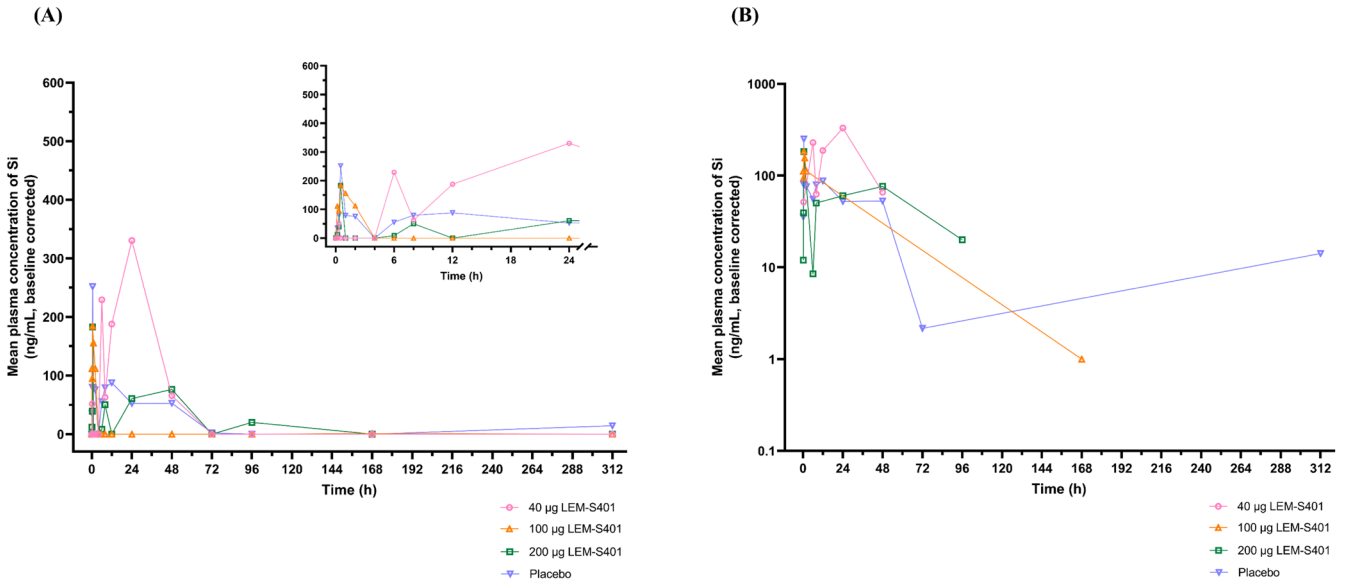

FIGURE 4 | Mean plasma concentration-time curves (baseline-corrected) after a single subcutaneous injection of 40 μg LEM-S401 (-○-), 100 μg LEM-S401 (-△-), 200 μg LEM-S401 (-□-), and placebo (-▽-) in healthy participants. The inset shows the mean plasma concentration of DegradaBALL from 0 h to 24 h after administration. (A): Linear scale; (B): Semi-log scale.

may act as an external immunogenic agent. However, all cases were rated as mild and resolved within an average of 2 days for injection site pain and 2 h for injection site erythema without additional medication. Furthermore, despite the dose escalation in Cohort 2 and Cohort 3, adverse events and blood concentrations about LEM-17234 and Si did not significantly increase in participants. Furthermore, by referring to the nonclinical and clinical studies of an already approved siRNA-based therapeutic, patisiran (ONPATTRO), this clinical trial also selected and analyzed cytokines as indicators for assessing the safety and tolerability of LEM-17234 (siRNA), and no significant differences were observed between treatment groups [16]. This trend is consistent with the results observed in other phase 1 trials of subcutaneous siRNA in healthy participants [15]. These data suggest that the DegradaBALL drug delivery technology minimizes the systemic distribution of the active pharmaceutical ingredients (APIs), enabling safe administration to humans.

Keloids and hypertrophic scars are caused by the abnormal proliferation of collagen fibers in the reticular dermis, a layer of the skin [12]. Dermal ointments are sometimes used to treat keloids and hypertrophic scars, but since penetration into the skin is a prerequisite for effective treatment, internal local injections are generally used [1, 13]. However, in patients with hypertrophic scars and keloids, abnormal enlargement and destruction of the dermal layer are prominent, and the dermis is often thicker

than normal [6]. In addition, the formation of keloids is often accompanied by angiogenesis due to uncontrolled angiogenesis, and reports indicate that the margins of keloids are highly pro-angiogenic [14]. Therefore, in this study, considering the pathophysiology of these keloids, the investigational product was administered subcutaneously to ensure safety and tolerability against additional unexpected systemic exposure.

siRNA is a promising therapeutic option that can resolve unexpected side effects and off-target issues resulting from extensive signal pathway regulation raised by small molecule treatment. However, naked and unmodified siRNA are easily degraded by RNase, which is abundant in biological fluid, and have obvious limitations such as unsatisfactory stability and poor pharmacokinetic behavior [17]. As a result, developmental strategies for existing siRNA gene therapeutics have focused on developing high-concentration doses or chemically modified siRNA, but even these have a major limitation in that most siRNA are distributed to the liver when administered by IV or SC. Even if not degraded, IV-administered siRNA undergoes glomerular filtration and can be rapidly excreted. Therefore, an efficient local administration strategy for siRNA can improve its stability and efficacy by preventing degradation from RNase distributed in biological fluids. Also, a local administration strategy for siRNA using a nano-drug delivery system can be applied to various indications by enabling extra-hepatic delivery.

The primary clearance pathway of siRNAs is nuclease-mediated metabolism in tissues, and plasma concentrations are known to be transient [18]. In this study, as well as in the preclinical study, the plasma concentrations of LEM-17234 were not detected. Most of LEM-17234 is slowly released from the drug delivery vehicle DegradaBALL after reaching the target organ and cells. Consequently, the amount of LEM-17234 that is systemically circulated or absorbed is expected to be minimal. During systemic circulation and/or after absorption, LEM-17234 is presumed to be rapidly biodegraded by RNases present in the body and thus, systemic exposure to LEM-17234 is likely to be minimal. Even when administered intravenously, siRNA is characterized by a rapid decrease in plasma concentrations below the detection limit 30 min after the end of infusion. Therefore, the absence of detectable concentrations of LEM-17234 in this study is considered an expected outcome given the characteristics of the siRNA formulation and route of administration [19].

The baseline concentration of Si, the main component of DegradaBALL, was detected in all participants, including those in the placebo group, prior to administration. Silicon is an essential nutrient in human biology and is primarily obtained from various food sources, particularly grains. It is naturally present in the human body, and its concentration can be influenced by dietary intake, including food and water consumption, as well as environmental and occupational exposure. Previous studies have also reported significant inter-individual variability in silicon levels [20]. The reference range for serum silicon in males aged 18–59 years has been reported to be 272.43–285.63 ng/mL [21], and the median Si concentration detected in the 0 h blood samples in this study was 281 ng/mL. Therefore, the Si concentrations detected in blood samples collected at 0 h are presumed to result from dietary intake rather than exogenous exposure to the investigational product. In this study, analyzing systemic exposure and blood concentration-time profiles of Si revealed no clear dose-proportionality or trend with dose escalation, and no significant differences were identified when comparing the coefficients of variability (%) for the mean C_{max} of each dose group with those of the placebo group. Furthermore, DegradaBALL may not entirely follow a systemic circulation elimination pathway, and previous nonclinical studies have suggested that removal occurs partly via phagocytes and lymphatics [22]. Additionally, an analysis of individual pharmacokinetic profiles revealed that in Cohort 2 and Cohort 3, the majority of participants exhibited a distinct increase within 0.5 h post-dosing. Meanwhile, in the placebo group, participant 0305 had a C_{max} of 1289 ng/mL, which was significantly higher than that of other participants. This value is considered an outlier and may contribute to statistical distortion (Figure S1). These findings indicate that a single subcutaneous administration of LEM-S401 is expected to have a negligible impact on systemic exposure, further supporting its potential as a safe and effective drug delivery system.

In a nonclinical study, when mice were administered a high dose (20 mg/kg) of DegradaBALL, Si was detected up to 11 days (264 h) post-dose and disappeared by Day 15. Therefore, in this study, PK blood sampling was performed until the PSV (approximately 312 h), a time point beyond 264 h, when the persistence of concentrations was observed in the nonclinical study. Although most foods contain Si, no dietary restrictions were imposed in this study, nor did it include blood draws to correct for baseline levels. In the groups receiving 40 μg LEM-S401 and 100 μg LEM-S401, concentrations were not detected during the PSV but were detected in the groups receiving 200 μg LEM-S401 and placebo. Si is known to have a fasting blood concentration of 100 to 300 ng/mL [23]; therefore, baseline correction values were calculated based on predose concentrations, and 200 μg LEM-S401 was also undetectable at the PSV. Considering that MSNs between 20 and 110 nm in diameter were mostly eliminated in the urine and feces within 2–7 days in nonclinical studies, Si concentrations were detected in the placebo group during the PSV, and the effect of food and DegradaBALL on Si concentrations during the PSV is unlikely [24, 25].

5 | Conclusions

LEM-S401 was well tolerated and safe when administered as a single subcutaneous dose in healthy adults, and no clinically significant systemic exposure was observed. These results support the clinical utility of LEM-S401 in this patient population. Furthermore, this study demonstrates a promising formulation for extra-hepatic application via subcutaneous injection, suggesting its potential applicability across multiple indications.

Author Contributions

Ha-Yeon Kim and Jaeso Cho wrote the manuscript. Jaeso Cho, Jun Gi Hwang, Dal-Hee Min, and Cheolhee Won designed the research.

Ha-Yeon Kim, Min Kyu Park, and Jun Gi Hwang performed the research. Ha-Yeon Kim and Jun Gi Hwang analyzed the data.

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Conflicts of Interest

Lemonex owns patents related to LEM-S401. All other authors declared no competing interests for this work.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.