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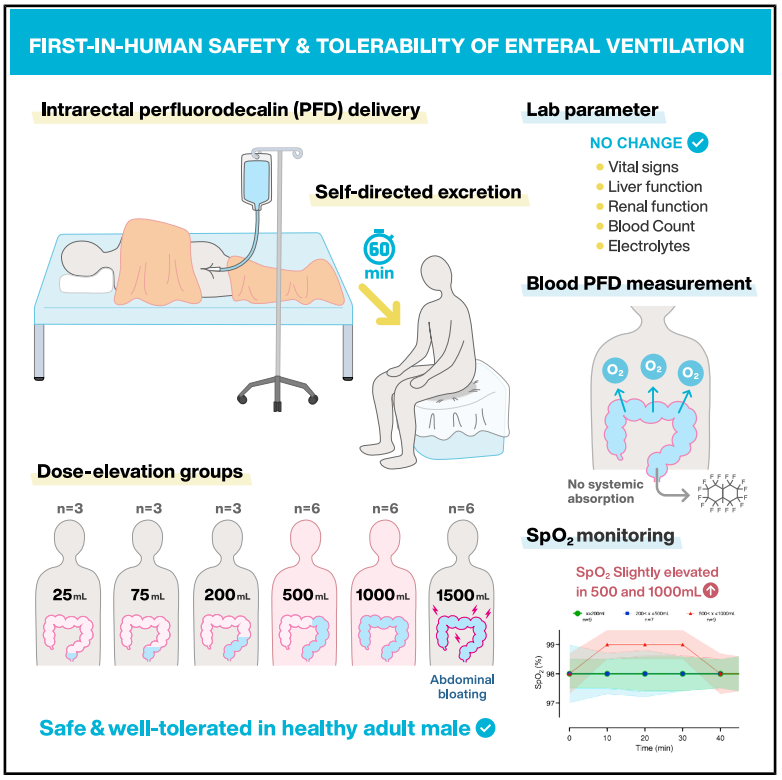
Safety and tolerability of intrarectal perfluorodecalin for enteral ventilation in a first-in-human trial

Graphical abstract



Highlights

- First-in-human, dose-escalation trial for intrarectal perfluorodecalin (PFD)
- Favorable tolerability profile up to 1,000 mL PFD, with all adverse events being mild
- No detectable systemic absorption of PFD (<1.0 μg/mL)
- Dose-dependent oxygen transfer predicted by pig pharmacokinetic model

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In brief

This first-in-human, dose-escalation trial demonstrates that intrarectal administration of perfluorodecalin is safe and well tolerated in healthy volunteers, with no systemic absorption. These findings provide the foundational safety data to advance enteral ventilation as a novel therapeutic strategy for patients with respiratory failure.

Translation to Humans

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Safety and tolerability of intrarectal perfluorodecalin for enteral ventilation in a first-in-human trial

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CONTEXT AND SIGNIFICANCE Patients with severe respiratory failure often need mechanical ventilation to survive, but these therapies can cause further lung injury. Scientists are exploring a new method called "enteral ventilation" to deliver oxygen through the intestine, which could give the lungs a chance to rest and heal. This study evaluated the safety of this method in humans for the first time, using a special liquid called perfluorodecalin with exceptional oxygen-carrying ability. In a trial with 27 healthy male volunteers, the authors found that administering this liquid rectally was safe and well tolerated. This important safety milestone paves the way for future studies to see if this technique can help patients with respiratory failure.

SUMMARY

Background: Enteral ventilation is an emerging approach that provides partial systemic oxygenation independent of pulmonary gas exchange, enabling lung rest. Perfluorodecalin, a clinically approved liquid with high oxygen solubility, is a promising vehicle for enteral oxygen delivery. The primary endpoint of this first-in-human trial was to assess the safety and tolerability of intrarectal perfluorodecalin administration.

Methods: This was a phase 1, single-site, open-label, non-controlled, dose-escalation trial in 27 healthy adult males aged 20–45 years. Participants received a single intrarectal dose of non-oxygenated perfluorodecalin (escalating from 25 to 1,500 mL) retained for 60 min. Safety and tolerability were assessed through monitoring of adverse events, vital signs, clinical laboratory tests, and systemic perfluorodecalin exposure. A pharmacokinetic model using large-animal data was employed to predict potential oxygen transfer.

Findings: No serious adverse events or dose-limiting toxicities occurred. Mild gastrointestinal symptoms, such as abdominal bloating and pain, were transient, dose dependent, and resolved without intervention. All clinical laboratory parameters, including liver and renal function markers, remained within normal limits. Perfluorodecalin concentrations were undetectable in blood (<1.0 μg/mL). The pharmacokinetic model predicted a dose-dependent oxygenation effect, consistent with a modest increase in peripheral oxygen saturation observed in the higher-dose group.

Conclusions: This first-in-human study demonstrates that intrarectal administration of non-oxygenated perfluorodecalin is safe, feasible, and well tolerated. These findings establish a critical safety foundation and

support the continued development of enteral ventilation with fully oxygenated perfluorodecalin as an adjunctive strategy to support respiratory failure patients.

Funding: This work was funded by EVA Therapeutics, Inc.

INTRODUCTION

Each year, just in the United States, over 1.5 million critically ill adults undergo tracheal intubation.[1,2] Hypoxemia occurs in 10%–20% of these cases in emergency departments and intensive care units, often leading to cardiac arrest or death.[3–5] Despite advancements in invasive and non-invasive ventilation,[6] oxygenation strategies remain limited and may exacerbate lung injury through barotrauma and volutrauma. A strategy that enables partial oxygenation independent of pulmonary gas exchange could offer a paradigm shift, providing "lung rest" to mitigate ventilator-induced lung injury.

Inspired by hydroponic organisms, such as loaches,[7,8] recent preclinical studies indicate enteral ventilation offers a means to transfer oxygen into and remove carbon dioxide from blood in the distal intestine in hypoxic environments.[9] While intrarectal delivery of oxygenated perfluorocarbons (PFCs) demonstrated efficacy in animal models of systemic[10,11] and local[12,13] tissue hypoxia, its safety, tolerability, and feasibility in humans remain untested.

PFCs are synthetic compounds with exceptional capacity to dissolve and transport gases, functioning as liquid oxygen carriers via diffusion driven by partial pressure gradients.[9,11] While the solubility of oxygen in water is approximately 10 mL O_2/L and in blood is around 200 mL O_2/L, PFCs can dissolve as much as 400–500 mL O_2/L at 1 atmosphere.[14,15] Among PFCs, perfluorodecalin (PFD) stands out for its chemical and biological inertness, biocompatibility, and established safety profile. Accordingly, it is biocompatible, chemically inert, and clinically used in ophthalmic and surgical settings.[14–17] Delivering PFD enterally may offer a practical, targeted means of systemic oxygenation without relying on pulmonary function.

EVA101 is a first-in-class therapeutic candidate that repurposes PFD as a gas-exchange vehicle for enteral ventilation. To translate this concept toward clinical application, a foundational understanding of optimal dosing, intestinal retention capacity, and safety in humans is essential. This study reports the first-in-human evaluation of EVA101, assessing its intrarectal administration across escalating doses, with systematic monitoring of tolerability, adverse events (AEs), PFD biodistribution, and pharmacokinetic (PK) parameters. The findings establish the feasibility of this novel route, setting the stage for future efficacy trials in hypoxic patient populations.

RESULTS

Demographics and baseline characteristics

91 participants attended a single center (Medical Corporation Heishinkai OPHAC Hospital, Japan) for screening. 28 participants were excluded for not meeting the eligibility criteria, and 63 participants were enrolled in the trial. Finally, 27 participants received non-oxygenated PFD (Figure 1). The decision to increase the dose was based on dose-limiting AEs as a safety assessment at each stage. The 27 participants had a mean ± standard deviation (SD) age of 26.4 ± 6.0 years and a mean ± SD BMI of 20.9 ± 1.3 kg/m^2. Baseline demographics and clinical characteristics showed no significant differences between safety populations (Table 1).

Tolerability

Non-oxygenated PFD administration was achieved up to 1,500 mL, with no obvious leakage of PFD from the anus in any group. One participant (of six) in the planned 1,000 mL group discontinued PFD administration due to defecation urgency, and four patients (of six) in the planned 1,500 mL group discontinued PFD administration due to abdominal pain. Of the remaining patients, 21 (78%) completed more than 95% of the planned PFD dose (Tables 2 and S1). Three of the four participants in the planned 1,500 mL PFD group who discontinued PFD administration had no defecation within 24 h prior to PFD administration (Figure 1; Table S1). In addition, one participant (of three) each in the planned 75 and 200 mL groups and one (of six) and four (of six) participants in the 1,000 and 1,500 mL groups described above discontinued PFD retention into the intestine due to defecation urgency or abdominal pain (Table 2). The remaining 20 patients (74%) completed PFD intestinal retention for 60 min.

Safety outcome

All participants completed 3 days of follow-up after non-oxygenated PFD administration. AEs were determined according to the Common Terminology Criteria for Adverse Events v.5.0. In the context of this clinical trial, no severe or life-threatening AEs (grade $\geq$3) or gastrointestinal symptoms (grade $\geq$2) were observed up to 1,500 mL. During the study, mild AEs (grade = 1), including defecation urgency, abdominal pain, and bloating, were reported by 16 participants (59%). The incidence of AEs was more frequent in the high-dose group, especially abdominal pain and bloating (Table 2). All events resolved following excretion of PFD without intervention. AEs in individual participants are shown in Table S1. Post-trial assessment indicates that abdominal pain is associated with defecation status on the prior day. Of the six cases who reported abdominal pain due to PFD administration in the 1,000 and 1,500 mL groups, four participants discontinued administration of the planned dose due to abdominal pain (all 1,500 mL), and three of the four had no defecation within 24 h before the PFD administration. Additionally, no clinically significant vital sign change was considered to be related to administration, and the doses of non-oxygenated PFD were described (Table S2). Thus, no dose-limiting AEs related to PFD administration were noted up to 1,000 mL.

Clinical laboratory assessment

Clinically significant alterations did not occur throughout the study. Hematological and biochemical parameters remained

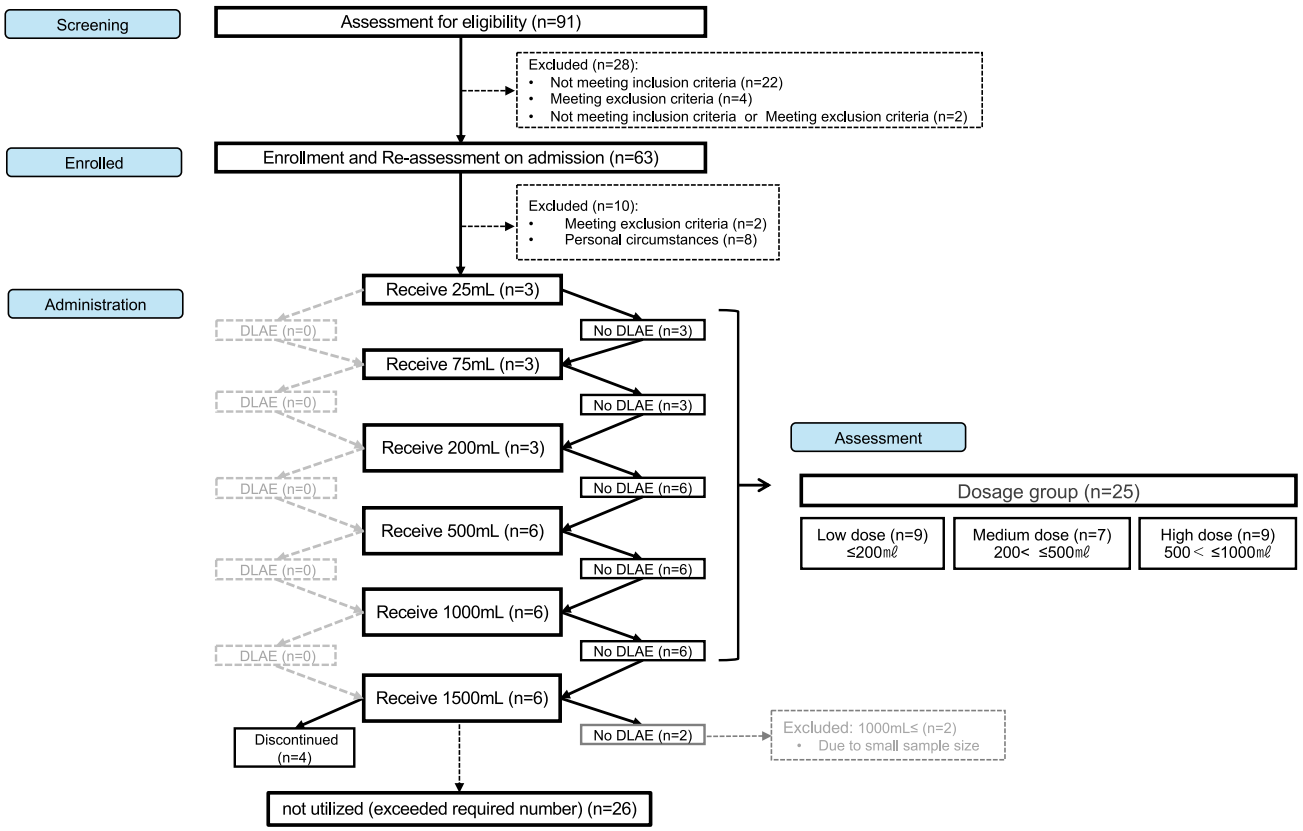

Figure 1. Clinical trial design

This dose escalation study has a 3+3 design as a rule-based design for the first-in-human clinical trial. 91 healthy males are screened, 63 of whom enrolled. Cohort sizes of the low-dose group (≤200 mL) and the medium-high-dose groups (≥500 mL) are 3 and 6 participants, respectively. There were no dose-limiting adverse events up to the 1,500 mL cohort. Finally, 27 participants received a single administration of non-oxygenated perfluorodecalin in several dosage groups up to 1,500 mL, and the tolerability and safety were evaluated as the safety population. They were further regrouped according to the actual perfluorodecalin dose (low, medium, or high), and the oxygenation effect was evaluated between dosage groups. DLAE, dose-limiting adverse events.

within normal ranges throughout the study (Table 3). In particular, the liver enzymes AST and ALT did not show elevations in 27 cases from the day before to 1 day after PFD administration, with mean ± SD values ranging from 20.1 ± 7.3 to 18.1 ± 5.9 ($p = 0.002$) and from 17.8 ± 7.2 to 17.2 ± 7.6 ($p = 0.294$), respectively, and both remained within the reference ranges.

PFD absorption

PFD concentrations in whole blood determined by gas chromatography-mass spectrometry were below the lower limit of quantification (LLOQ; 1.0 μg/mL) at all measurement points 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, and 12 h after PFD administration up to 1,500 mL.

Ventilation

Transcutaneous carbon dioxide pressure ($tcpCO_2$) was also stable across all dose groups ($p = 0.491$). In the high-dose group, 500–1,000 mL PFD administration resulted in a slight increase of approximately 2 mmHg from the baseline (Figure S1). However, no significant changes were observed in respiratory rate in each PFD administration group by repeated-measures ANOVA ($p = 0.091$).

Oxygenation

PK modeling based on large-animal data suggested that even ambient (non-oxygenated) PFD administration could produce small, dose-dependent increases in oxygen saturation, with changes of about 1% at higher volumes (Figures S2A and S2B). We use this PK modeling approach to test whether there is additive oxygenation effect in healthy human participants. There were a few cases ($n = 2$) in which more than 1,000 mL of PFD could be administered, and therefore, these patients were excluded from oxygenation and ventilation evaluation. Baseline characteristics showed no significant differences between the actual dosage groups: low- (≤200 mL), medium- (200 ≤ 500 mL), or high- (500 ≤ 1,000 mL) dose group (Table S2). Changes in time series of peripheral oxygen saturation (SpO_2) depending on the non-oxygenated PFD dose for each group were not significantly different by repeated-measures ANOVA ($p = 0.203$). However, the time course of the mode of SpO_2 was described as a graph, which showed that the administration of PFD of less than 500 mL resulted in no change from baseline, whereas administration of 500–1,000 mL PFD resulted in a 1% increase (Figure 2). 200, 500, or 1,000 mL administration resulted in areas under the curve (AUC) of 2.4, 5.9, and 11.7 and SpO_2 upshifts of 0.08%, 0.2%, and 0.4%, respectively. The

Table 1. Baseline demographics and clinical characteristics

	All (*n* = 27)	25 mL (*n* = 3)	75 mL (*n* = 3)	200 mL (*n* = 3)	500 mL (*n* = 6)	1,000 mL (*n* = 6)	1,500 mL (*n* = 6)	*p* value
Age, years	26.4 ± 6.0	24.0 ± 4.4	25.7 ± 6.4	26.7 ± 6.1	27.5 ± 9.4	26.7 ± 6.0	26.3 ± 4.1	0.985
Height, cm	170.7 ± 6.4	172.6 ± 6.9	170.5 ± 10.2	172.3 ± 5.1	166.6 ± 2.4	174.7 ± 7.1	169.0 ± 6.4	0.360
Weight, kg	61.1 ± 6.5	65.5 ± 9.2	58.9 ± 7.1	65.9 ± 2.8	57.0 ± 3.6	62.1 ± 6.8	60.7 ± 7.5	0.332
BMI, kg/m^2	20.9 ± 1.3	21.9 ± 1.6	20.2 ± 0.3	22.2 ± 1.1	20.5 ± 1.2	20.3 ± 1.1	21.2 ± 1.6	0.190
Non-drinker	11 (41%)	0 (0%)	2 (67%)	1 (33%)	4 (67%)	2 (33%)	2 (33%)	0.469
Never smoker	19 (70%)	1 (33%)	2 (67%)	2 (67%)	3 (50%)	6 (100%)	5 (83%)	0.247
12ch ECG abnormal	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1.000
sBP, mmHg	97.6 ± 7.2	96.7 ± 4.7	94.3 ± 5.9	98.3 ± 9.7	100.7 ± 9.2	99.8 ± 6.3	93.8 ± 6.5	0.581
dBP, mmHg	54.7 ± 6.7	56.3 ± 4.2	49.7 ± 6.4	55.3 ± 7.2	58.8 ± 6.8	56.8 ± 7.4	50.0 ± 4.3	0.160
HR, beats/min	60.9 ± 9.1	56.7 ± 10.6	57.3 ± 5.1	56.3 ± 4.2	60.3 ± 12.8	64.7 ± 8.5	63.7 ± 8.7	0.670
RR, breaths/min	15.3 ± 2.0	16.3 ± 0.6	16.0 ± 2.0	16.3 ± 0.6	16.0 ± 1.8	14.7 ± 3.0	14.0 ± 1.3	0.324
BT, °C	36.3 ± 0.3	36.1 ± 0.1	36.5 ± 0.0	36.4 ± 0.5	36.1 ± 0.2	36.3 ± 0.3	36.4 ± 0.4	0.416
SpO_2, %	98.4 ± 0.7	99.0 ± 0.0	98.0 ± 0.0	98.3 ± 0.6	98.3 ± 1.0	98.5 ± 0.8	98.5 ± 0.5	0.674
$tcpCO_2$, mmHg	43.1 ± 1.9	42.7 ± 1.2	42.3 ± 2.9	42.7 ± 2.5	43.2 ± 1.5	43.8 ± 0.8	43.2 ± 2.9	0.908

Values are presented as mean ± SD or number (proportion). BMI, body mass index; ECG, electrocardiogram; BP, blood pressure; HR, heart rate; RR, respiratory rate; BT, body temperature; SpO_2, percutaneous oxygen saturation; $tcpCO_2$, transcutaneous carbon dioxide pressure.

higher elevation of SpO_2 led to a higher frequency of 1% in SpO_2 monitoring, which supported the findings of the graph in Figure 2. Though the prime goal of this trial is to determine the safety and tolerability of non-oxygenated PFD material, this may indicate possible oxygen transfer when the high-dose protocol is applied because the intrinsic oxygen pressure in ambient PFD (approximately 200 mmHg) is higher than the one found in venous drainage (approximately 40 mmHg).

DISCUSSION

This study assessed the safety and tolerability of EVA101 at dosages up to 1,500 mL of PFD. Mild AEs were consistent with the route of administration and increased in frequency with the dosage of PFD. At the highest dose (1,500 mL), more than half of the participants discontinued PFD administration due to mild abdominal pain. The abdominal pain was CATAE grade 1; however, we decided to discontinue PFD administration to prevent potential severe AEs. By contrast, doses up to 1,000 mL were generally well tolerated. All AEs were transient in nature and disappeared following the excretion of PFD without intervention. In addition, defecation within 24 h prior to PFD administration may affect the PFD dose. Based on the results of this clinical trial and the consensus on the safe initiation of transanal irrigation,[18] PFD can be administered at doses up to 1,000 mL without prior bowel preparation.

Table 2. Tolerability and adverse events

	All (*n* = 27)	25 mL (*n* = 3)	75 mL (*n* = 3)	200 mL (*n* = 3)	500 mL (*n* = 6)	1,000 mL (*n* = 6)	1,500 mL (*n* = 6)
Completed administration of ≥95% of planned PFD dose	21 (78%)	3 (100%)	3 (100%)	2 (67%)	6 (100%)	5 (83%)	2 (33%)
Retained the PFD for 60 min	20 (74%)	3 (100%)	2 (67%)	2 (67%)	6 (100%)	5 (83%)	2 (33%)
Severe AEs[a] (grade ≥3)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Moderate AEs[a] (grade 2)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Mild AEs[a] (grade 1)	16 (59%)	0 (0%)	1 (33%)	1 (33%)	3 (50%)	5 (83%)	6 (100%)
Anal discomfort	1	0	1	0	0	0	0
Anal pain	1	0	1	0	0	0	0
Desire to defecate	5	0	1	1	1	2	0
Abdominal bloating	12	0	0	1	2	5	4
Abdominal pain	6	0	0	0	0	2	4

Values are presented as number (proportion). AEs were defined using the Common Terminology Criteria for Adverse Events v.5.0. AEs, adverse events; PFD, perfluorodecalin.
[a]Multiple choices allowed.

Table 3. Hematological and biochemical parameters

	All (n = 27)	p value	25 mL (n = 3)	75 mL (n = 3)	200 mL (n = 3)	500 mL (n = 6)	1,000 mL (n = 6)	1,500 mL (n = 6)
TP, g/dL								
Pre	7.2 ± 0.4	<0.001**	7.2 ± 0.3	7.4 ± 0.3	7.3 ± 0.3	7.2 ± 0.5	7.2 ± 0.2	6.9 ± 0.2
Post	6.7 ± 0.3	<0.001**	6.6 ± 0.4	6.5 ± 0.1	6.8 ± 0.4	6.6 ± 0.3	7.0 ± 0.3	6.8 ± 0.2
Alb, g/dL								
Pre	4.7 ± 0.3	<0.001**	4.6 ± 0.1	4.8 ± 0.3	4.7 ± 0.2	4.8 ± 0.4	4.7 ± 0.2	4.7 ± 0.2
Post	4.4 ± 0.3	<0.001**	4.2 ± 0.2	4.2 ± 0.3	4.3 ± 0.2	4.4 ± 0.3	4.4 ± 0.1	4.5 ± 0.2
T-Bil, mg/dL								
Pre	0.9 ± 0.4	0.687	1.0 ± 0.6	1.0 ± 0.4	1.2 ± 0.3	0.7 ± 0.2	1.0 ± 0.2	0.7 ± 0.2
Post	0.8 ± 0.3	0.687	0.9 ± 0.3	1.0 ± 0.3	1.1 ± 0.2	0.8 ± 0.2	0.9 ± 0.3	0.7 ± 0.2
ASL, IU/L								
Pre	20.1 ± 7.3	0.002*	22.3 ± 7.5	16.3 ± 1.9	20.7 ± 3.9	23.3 ± 12.6	18.5 ± 2.6	18.8 ± 1.3
Post	18.1 ± 5.9	0.002*	19.0 ± 5.7	13.3 ± 1.9	16.7 ± 1.2	19.3 ± 9.9	17.8 ± 3.5	19.7 ± 2.6
ALT, IU/L								
Pre	17.8 ± 7.2	0.294	24.7 ± 13.5	12.7 ± 2.1	18.7 ± 3.1	15.7 ± 7.1	15.3 ± 2.7	21.0 ± 3.8
Post	17.2 ± 7.6	0.294	23.0 ± 12.6	10.0 ± 2.2	16.3 ± 4.0	15.3 ± 8.3	15.7 ± 3.9	21.8 ± 2.5
BUN, mg/dL								
Pre	10.3 ± 2.9	0.439	7.8 ± 0.7	12.1 ± 1.7	13.3 ± 2.8	10.6 ± 3.3	10.2 ± 2.5	8.9 ± 1.3
Post	10.6 ± 2.0	0.439	9.6 ± 1.6	10.9 ± 0.4	12.3 ± 0.1.2	10.2 ± 2.0	10.9 ± 2.2	10.2 ± 2.1
Cre, mg/dL								
Pre	0.82 ± 0.10	0.018*	0.95 ± 0.08	0.72 ± 0.07	0.96 ± 0.06	0.79 ± 0.06	0.81 ± 0.07	0.80 ± 0.04
Post	0.80 ± 0.10	0.018*	0.88 ± 0.13	0.71 ± 0.06	0.93 ± 0.02	0.76 ± 0.07	0.77 ± 0.04	0.80 ± 0.09
Na^+, mmol/L								
Pre	141.4 ± 1.7	0.847	140.0 ± 1.6	141.0 ± 1.4	141.0 ± 0.8	142.5 ± 1.5	141.7 ± 1.2	141.2 ± 1.9
Post	141.3 ± 1.2	0.847	141.3 ± 1.2	141.0 ± 0.8	140.3 ± 0.9	142.0 ± 0.6	141.2 ± 1.3	141.5 ± 1.3
K^+, mmol/L								
Pre	4.0 ± 0.2	0.052	3.9 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.2	3.9 ± 0.2
Post	4.1 ± 0.2	0.052	4.0 ± 0.0	4.1 ± 0.2	4.1 ± 0.1	4.1 ± 0.2	4.2 ± 0.2	4.0 ± 0.2
CRP, mg/dL								
Pre	0.07 ± 0.09	0.141	0.08 ± 0.07	0.03 ± 0.00	0.07 ± 0.05	0.04 ± 0.03	0.07 ± 0.05	0.11 ± 0.16
Post	0.05 ± 0.06	0.141	0.04 ± 0.03	0.03 ± 0.02	0.09 ± 0.05	0.03 ± 0.01	0.07 ± 0.08	0.06 ± 0.08
ESR, mm/h								
Pre	1.9 ± 1.2	0.449	2.3 ± 0.9	2.3 ± 0.5	1.7 ± 0.5	1.7 ± 1.1	2.0 ± 1.4	1.7 ± 1.5
Post	2.0 ± 1.4	0.449	2.3 ± 0.5	2.0 ± 0.8	2.3 ± 0.5	1.3 ± 0.7	2.3 ± 1.7	2.0 ± 1.8
WBC, $\times 10^2/\mu L$								
Pre	61.3 ± 12.9	0.110	57.0 ± 10.6	53.7 ± 9.5	74.7 ± 7.7	62.8 ± 13.3	62.3 ± 12.7	58.0 ± 10.4
Post	57.2 ± 15.5	0.110	55.0 ± 3.7	65.7 ± 25.8	58.3 ± 6.6	58.7 ± 11.1	53.3 ± 15.5	55.8 ± 16.1
Hb, g/dL								
Pre	14.8 ± 0.9	0.948	15.4 ± 0.5	14.4 ± 0.7	15.3 ± 0.6	15.1 ± 1.0	14.7 ± 0.8	14.6 ± 1.1
Post	14.8 ± 1.0	0.948	14.7 ± 0.9	14.1 ± 0.9	15.5 ± 0.5	14.9 ± 1.1	14.8 ± 1.0	14.9 ± 0.8
Plt, $\times 10^4/\mu L$								
Pre	24.7 ± 3.8	0.023*	23.9 ± 0.8	29.9 ± 3.9	21.6 ± 2.5	24.1 ± 3.3	25.1 ± 1.8	24.3 ± 4.2
Post	23.9 ± 3.8	0.023*	22.6 ± 1.2	29.2 ± 5.5	20.6 ± 4.1	22.7 ± 3.1	24.6 ± 1.8	23.8 ± 2.1

Values are presented as mean ± SD. Significant difference: *$p < 0.05$ or **$p < 0.001$. "Pre" indicates blood test results 1 day before PFD administration, and "post" indicates blood test results 1 day after PFD administration. TP, total protein; Alb, albumin; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; Cre, creatinine; Na^+, natrium ion; K^+, potassium ion; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; WBC, white blood cell; Hb, hemoglobin; Plt, platelet.

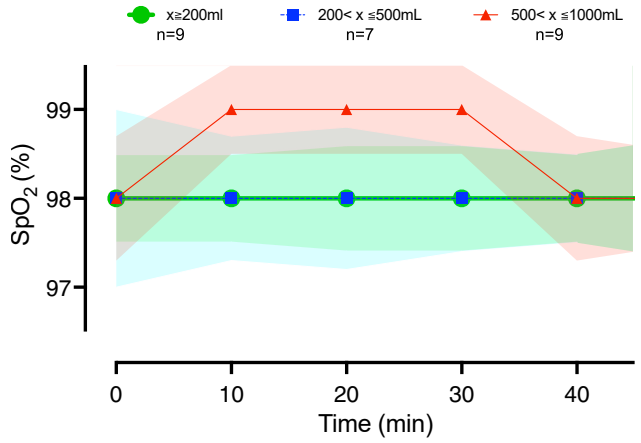

Figure 2. SpO_2 profiles for the low-, medium-, or high-dosage group
Changes in time series of the SpO_2 values depending on the non-oxygenated perfluorodecalin dose for each group average. The green, blue, and red lines represent the mode of SpO_2. Each shaded area represents the standard deviation.

In this study, intestinal administration of PFD did not result in any clinically significant changes in organ damage markers, including liver and renal function, as assessed by comprehensive blood tests. This contrasts with prior studies in hypoventilated rat models, where continuous 4 h perfusion of oxygenated PFCs through the small intestine was associated with elevations in liver enzymes such as AST and ALT.[11] Our findings demonstrate that single-dose, bolus administration of PFD via the rectum is hematologically and biochemically safe in humans.

Furthermore, all blood samples showed PFD concentrations below the LLOQ (1.0 μg/mL). This threshold is several orders of magnitude lower than doses known to cause toxicity. For example, the no-observed-adverse-effect level (NOAEL) of intravenous Fluosol-DA 20% in a 14-day study in rats was a fractionated dose of 50 mL/kg (containing 7 g PFD and 3 g perfluorotripropylamine/kg).[19] Three hours after intravenous administration of an emulsion containing 7 g PFD and 3 g perfluorotripropylamine/kg, blood concentrations reached 77,360 μg/mL (a total of PFD and perfluorotripropylamine concentrations), which is far above the LLOQ.[20] These comparisons emphasize the wide safety margin relative to the non-detectable systemic levels observed in our study. PFD also has a well-established clinical safety record, having been used as an oxygen carrier[21] and as an intraoperative aid in ophthalmic surgery.[22,23] Moreover, animal studies relevant to enteral administration have demonstrated no AEs even when small amounts were absorbed.[9,10] Taken together, these findings support the conclusion that even if trace quantities entered the circulation in our study (<1.0 μg/mL), they would not pose a safety risk, further reinforcing the favorable safety profile of PFD.

To guide and validate appropriate O_2-PFD dosing in future clinical studies, we developed a structural PK model using ΔSaO_2% and ΔSvO_2% data from pigs administered rectal O_2-PFD. Incorporating the pig dataset was primarily intended to provide a quantitative context for interpreting patient data through the PK model of oxygen transfer. Given the novelty of this approach and the absence of established clinically relevant physiological models for this oxygenation outcome, porcine data were considered a pragmatic reference. The simulations demonstrated dose-dependent increases in SaO_2 and SvO_2, predicting a maximal ~1% increase at a 1,000 mL dose of non-oxygenated PFD, consistent with observed clinical findings. This concordance provides support that porcine data may offer useful insights. Taken together, these preliminary findings support the clinical feasibility of enteral PFD delivery and lay the groundwork for its application in designing O_2-PFD dosing strategies in future clinical studies.

Limitations of the study

This study has several limitations inherent to its nature as a first-in-human, phase 1 trial. First, the study population was a small cohort of 27 young, healthy Japanese males, which limits the generalizability of our findings to women, older adults, other ethnic populations, and, most importantly, the target population of critically ill patients with respiratory failure, whose underlying physiology and comorbidities may alter tolerability and systemic response. Second, the small sample size, dictated by the 3+3 dose-escalation design, is appropriate for identifying common dose-limiting toxicities but lacks the statistical power to detect rare AEs. Third, our assessment of oxygenation relied on SpO_2 as a non-invasive surrogate. The observed 1% increase in the high-dose group is a promising signal but falls within the precision limits of pulse oximetry and requires confirmation with direct arterial blood gas measurements in future patient-based studies. Fourth, this trial utilized non-oxygenated PFD to primarily establish the safety and tolerability of the vehicle and administration procedure; therefore, the therapeutic potential of fully oxygenated PFD remains to be evaluated. Finally, while the PK model developed from porcine data provided a useful predictive framework, the direct quantitative extrapolation of parameters from animal models to human physiology should be interpreted with caution.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, Takanori Takebe (takanori.takebe@cchmc.org).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

- The clinical trial data generated in this study are not publicly available due to privacy and ethical restrictions related to participant confidentiality. De-identified data may be shared by the lead contact upon reasonable request.
- This paper does not report original code; no custom code was used.
- Any additional information required for reanalyzing the data presented in this paper can be provided by the lead contact upon request.

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AUTHOR CONTRIBUTIONS

H.N., C.Y., and H.M. designed and oversaw the clinical trial. T.F., Y.K., and T.T. had unrestricted access to all data. T.F., Y.T., and T.T. performed and verified the statistical analyses. T.F. and T.T. prepared the first draft of the manuscript. All authors contributed to the review and editing of the manuscript, approved the final draft for submission, and take full responsibility for its content, including the accuracy of the data.

DECLARATION OF INTERESTS

T.T. is an inventor of intellectual properties for enteral ventilation technology and founder of EVA Therapeutics, Inc. T.F., H.N., C.Y., and H.M. are scientific and clinical advisors/consultants for EVA Therapeutics, Inc.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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REFERENCES

1. Cairns, C., and Kang, K. (2022). National hospital ambulatory medical care survey: 2019 Emergency Department Summary Tables (CDC). https://stacks.cdc.gov/view/cdc/115748.
2. Pfuntner, A., Wier, L.M., and Stocks, C. (2013). Most frequent procedures performed in US hospitals, 2011. In Healthcare Cost and Utilization Project (HCUP) Statistical Briefs [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); Statistical Brief #165.
3. Driver, B.E., Semler, M.W., Self, W.H., Ginde, A.A., Trent, S.A., Gandotra, S., Smith, L.M., Page, D.B., Vonderhaar, D.J., West, J.R., et al. (2021). Effect of use of a bougie vs endotracheal tube with stylet on successful intubation on the first attempt among critically ill patients undergoing tracheal intubation: a randomized clinical trial. JAMA *326*, 2488–2497.
4. Prekker, M.E., Driver, B.E., Trent, S.A., Resnick-Ault, D., Seitz, K.P., Russell, D.W., Gaillard, J.P., Latimer, A.J., Ghamande, S.A., Gibbs, K.W., et al. (2023). Video versus direct laryngoscopy for tracheal intubation of critically ill adults. N. Engl. J. Med. *389*, 418–429.
5. Russotto, V., Myatra, S.N., Laffey, J.G., Tassistro, E., Antolini, L., Bauer, P., Lascarrou, J.B., Szułdrzyński, K., Camporota, L., Pelosi, P., et al. (2021). Intubation practices and adverse peri-intubation events in critically ill patients from 29 countries. JAMA *325*, 1164–1172.
6. Gibbs, K.W., Semler, M.W., Driver, B.E., Seitz, K.P., Stempek, S.B., Taylor, C., Resnick-Ault, D., White, H.D., Gandotra, S., Doerschug, K.C., et al. (2024). Noninvasive Ventilation for Preoxygenation during Emergency Intubation. N. Engl. J. Med. *390*, 2165–2177. https://doi.org/10.1056/NEJMoa2313680.
7. Jasinski, A. (1973). Air-blood barrier in the respiratory intestine of the pond-loach, Misgurmus fossilis L. An ultrastructural study. Acta Anat. *86*, 376–393.
8. Park, J.Y., Kim, I.S., and Kim, S.Y. (2003). Structure and mucous histochemistry of the intestinal respiratory tract of the mud loach, (Cantor). J. Appl. Ichthyol. *19*, 215–219. https://doi.org/10.1046/j.1439-0426.2003.00452.x.
9. Okabe, R., Chen-Yoshikawa, T.F., Yoneyama, Y., Yokoyama, Y., Tanaka, S., Yoshizawa, A., Thompson, W.L., Kannan, G., Kobayashi, E., Date, H., and Takebe, T. (2021). Mammalian enteral ventilation ameliorates respiratory failure. Méd. Sur *2*, 773–783.e5. https://doi.org/10.1016/j.medj.2021.04.004.
10. Fujii, T., Yoneyama, Y., Kinebuchi, A., Ozeki, N., Maeda, S., Saiki, N., Chen-Yoshikawa, T.F., Date, H., Nishiwaki, K., and Takebe, T. (2023). Enteral liquid ventilation oxygenates a hypoxic pig model. iScience *26*, 106142. https://doi.org/10.1016/j.isci.2023.106142.
11. Miyaguchi, N., Nagahiro, I., Kotani, K., Nakanishi, H., Mori, H., Osaragi, T., and Shimizu, N. (2006). Transintestinal systemic oxygenation using perfluorocarbon. Surg. Today *36*, 262–266. https://doi.org/10.1007/s00595-005-3135-z.
12. Papadimitriou, D.K., Pitoulias, G.A., Papaziogas, B.T., Tachtsi, M.D., and Kalaitzis, E.D. (2007). Intraluminal intestinal administration of oxygenated perfluorocarbon improves acid-base and cardiopulmonary parameters after acute mesenteric ischemia. An experimental study in rabbits. Surg. Today *37*, 298–304. https://doi.org/10.1007/s00595-006-3390-7.
13. Joh, D., Morreau, M., Lee, A., Pendharkar, S., Stokes, B., Warren, R., Hickey, A., Phillips, A.J., and Windsor, J.A. (2023). Intraluminal oxygen mitigates acute mesenteric ischaemia: a systematic review of methods and outcomes in animal studies. ANZ J. Surg. *93*, 859–868. https://doi.org/10.1111/ans.18211.
14. Jagers, J., Wrobeln, A., and Ferenz, K.B. (2021). Perfluorocarbon-based oxygen carriers: from physics to physiology. Pflügers Archiv *473*, 139–150. https://doi.org/10.1007/s00424-020-02482-2.
15. Krafft, M.P., and Riess, J.G. (2021). Therapeutic oxygen delivery by perfluorocarbon-based colloids. Adv. Colloid Interface Sci. *294*, 102407. https://doi.org/10.1016/j.cis.2021.102407.
16. Feng, H., Geronemus, R.G., and Brauer, J.A. (2019). Safety of a Perfluorodecalin-Infused Silicone Patch in Picosecond Laser-Assisted Tattoo Removal: A Retrospective Review. Dermatol. Surg. *45*, 618–621. https://doi.org/10.1097/DSS.0000000000001522.
17. Ferrara, M., Steel, D.H., and Romano, M.R.; European Intraocular Tamponades Study Group (2024). A minimum specification dataset for liquid ocular endotamponades: recommendations by a European expert panel. Graefes Arch. Clin. Exp. Ophthalmol. *262*, 1141–1149. https://doi.org/10.1007/s00417-023-06289-6.
18. Emmanuel, A., Krogh, K., Perrouin-Verbe, B., Krassiukov, A., Koch, S., Mosiello, G., Bazzocchi, G., Christensen, P., Rodriguez, G., and Brugaletta, C. (2025). Consensus on Safe Initiation and Monitoring of Transanal Irrigation to Optimize Adherence With Therapy. Neuro Gastroenterol. Motil. *37*, e15011. https://doi.org/10.1111/nmo.15011.
19. West, L., McIntosh, N., Gendler, S., Seymour, C., and Wisdom, C. (1986). Effects of intravenously infused Fluosol-DA 20% in rats. Int. J. Radiat.

Oncol. Biol. Phys. *12*, 1319–1323. https://doi.org/10.1016/0360-3016(86)90163-x.

20. Lutz, J., and Stark, M. (1987). Half life and changes in the composition of a perfluorochemical emulsion within the vascular system of rats. Pflügers Archiv *410*, 181–184. https://doi.org/10.1007/BF00581913.
21. Castro, C.I., and Briceno, J.C. (2010). Perfluorocarbon-based oxygen carriers: review of products and trials. Artif. Organs *34*, 622–634. https://doi.org/10.1111/j.1525-1594.2009.00944.x.
22. Joussen, A.M., and Wong, D. (2015). Egress of large quantities of heavy liquids from exposed choroid: a route for possible tumor dissemination via vortex veins in endoresection of choroidal melanoma. Graefes Arch. Clin. Exp. Ophthalmol. *253*, 177–178. https://doi.org/10.1007/s00417-014-2911-0.
23. Chang, S., Ozmert, E., and Zimmerman, N.J. (1988). Intraoperative perfluorocarbon liquids in the management of proliferative vitreoretinopathy. Am. J. Ophthalmol. *106*, 668–674. https://doi.org/10.1016/0002-9394(88)90698-8.
24. Hansen, A.R., Graham, D.M., Pond, G.R., and Siu, L.L. (2014). Phase 1 trial design: is 3 + 3 the best? Cancer Control *21*, 200–208. https://doi.org/10.1177/107327481402100304.
25. Yamaoka, K., and Nakagawa, T. (1983). A nonlinear least squares program based on differential equations, MULTI (RUNGE), for microcomputers. J. Pharmacobio-Dyn *6*, 595–606. https://doi.org/10.1248/bpb1978.6.595.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Perfluorodecalin, PFD	Shandong Zhongshan Photoelectric Materials Co., Ltd. Kanto Denka Kogyo Co., Ltd.	Cas: 306-94-5
PFD filling container (200 mL, 500 mL)	Asuka Company (AS16 spout & screw cap), Yamato Material (Ibis200-SP), Mikasa Sangyo (16MS spout), Kaupac Co. (DP16-TN0500)	Custom device, EO sterilized by Steritech
Silicone tube	Tigers Polymer Corporation	ID 4 mm, OD 7 mm, length 1 m; EO sterilized
Rectal balloon catheter	Create Medic Co., Ltd.	Medical Device Approval No. 21600BZZ00638000
Oxygen bubbling catheter kit	Nipro Corporation	Safelet Catheter Kit PU, Approval No. 15700BZZ01125000
Experimental models: Organisms/strains		
Human participants	volunteer	N/A
Software and algorithms		
GraphPad Prism 9	Graphpad Software	https://www.graphpad.com/
SAS®9.4	SAS Institute Inc.	https://www.sas.com/en_us/home.html
R version 4.1.2	R Project	https://www.r-project.org
Other		
Pulse oximeter (SpO_2 monitor)	Hospital clinical equipment (standard)	N/A
Hospital clinical equipment (standard)	Hospital clinical equipment (standard)	N/A
Vital signs monitor	Hospital clinical equipment (standard)	N/A

EXPERIMENTAL MODELS AND STUDY PARTICIPANT DETAILS

Human participants

This study was a single-site, non-controlled, non-blinded Phase 1 clinical trial conducted at Medical Corporation Heishinkai OPHAC Hospital in Japan. The study was registered with the Japan Registry of Clinical Trials (jRCT2052240047). The protocol was approved by the relevant institutional review board, and written informed consent was obtained from all participants prior to any study-related procedures. The trial enrolled healthy adult males aged 20–45 years who met all eligibility criteria. Participant information on sex (ascribed at birth), age, and race was self-reported during the screening process. All participants were identified as Japanese males. Information on gender (self-identification) and socioeconomic status was not collected as part of this study's protocol. Because the effects of perfluorodecalin on pregnancy and the reproductive system are unknown, enrollment was limited to men to avoid any potential risk of accidental fetal exposure, a conservative safety strategy often adopted in early-phase clinical trials.

Study design

This was a single-site, non-controlled, non-blinded Phase 1 clinical trial. This dose escalation study was 3 + 3 design as rule-based design for the first-in-human clinical trial.[24] The sample size was determined based on practical considerations and the 3 + 3 design, rather than on a powered efficacy endpoint. The study was registered with jRCT2052240047. Written informed consent was obtained from all study participants.

Inclusion criteria.

(1) Healthy Japanese males aged 20 to 45 years at the time of providing informed consent, with a body mass index between 18.5 and 25.0.
(2) No clinically significant abnormalities in laboratory tests (including hematology, blood biochemistry, erythrocyte sedimentation rate, and urinalysis), electrocardiogram, or vital signs at screening.

(3) Able to abstain from smoking and alcohol consumption throughout the hospitalization period.
(4) Capable of understanding and voluntarily sign the informed consent form, and willing to comply with all study procedures and restrictions.

Exclusion criteria.

(1) Presence of clinically significant medical conditions (e.g., metabolic, endocrine, hepatic, renal, hematologic, cardiovascular, gastrointestinal, urologic, immunologic, neurologic, or psychiatric disorders) that the investigator deemed inappropriate for study participation.
(2) History of lower gastrointestinal diseases (e.g., hemorrhoids, irritable bowel syndrome, inflammatory bowel disease, colon polyps, or diverticulosis), abdominal surgery, or gastrointestinal conditions such as chronic constipation, chronic diarrhea, or acute diarrhea.
(3) Known or suspected hypersensitivity or allergic reaction to any materials used in the investigational device.
(4) Use of medications or dietary supplements within 2 weeks prior to device use, or anticipated need for such during the study period.
(5) Positive test results for alcohol, drugs of abuse, hepatitis B, hepatitis C, HIV, or syphilis; or history of alcohol or drug dependence.
(6) History of whole blood drawing of 400 mL within 12 weeks, 200 mL within 4 weeks, or component blood drawing (plasma and platelet) within 2 weeks prior to the use of the test device
(7) Participation in another clinical trial involving an investigational product within 3 months prior to consent, or planned participation in another clinical trial during the study period.
(8) Any relationship with the sponsor that could present a conflict of interest or lead to coercion.
(9) Persons who are judged by the investigator or subinvestigator to be inappropriate to participate in this clinical trial

The trial enrolled healthy adult males aged 20–45 years who met eligibility criteria, including no significant medical history or laboratory abnormalities. Because the effects of perfluorodecalin on pregnancy and the reproductive system are unknown, enrollment was limited to men to avoid any risk of accidental fetal exposure. This male-only enrollment represents a conservative safety strategy commonly adopted in early-phase trials when potential fetal risks are uncertain.

The study assessed six dosage levels of non-oxygenated perfluorodecalin (PFD): 25 mL, 75 mL, 200 mL, 500 mL, 1000 mL, and 1500 mL. Dose escalation proceeded following safety evaluation by an independent committee.

The trial included five phases.

(1) Screening Phase: Conducted 28 to 2 days prior to admission.
(2) Pre-observation Day: Day 1 of admission.
(3) Intervention Day: Day 2 of admission.
(4) Post-observation Day: Day 3 of admission.
(5) Follow-up Day: Three days post-discharge.

In protocol #3 (intervention), the assigned PFD dose was instilled rectally and retained for 60 min. Participants were instructed not to void or defecate during this period. The retention time was predefined to maximize mucosal contact for potential gas exchange while maintaining tolerability. After 60 min, the rectal catheter was removed, and participants were allowed to evacuate the PFD.

The primary endpoint was the safety and tolerability of intrarectal PFD administration, assessed by the incidence of serious adverse events (SAEs).

Secondary endpoints included.

(1) Incidence of adverse events (AEs), rectal tolerability, and PFD recovery rate
(2) Laboratory evaluations: hematology, biochemistry, and urinalysis
(3) Vital signs: blood pressure, heart rate, respiration rate, and temperature
(4) Oxygenation parameters: peripheral oxygen saturation and transcutaneous carbon dioxide pressure
(5) Rectal tolerability and PFD recovery rate
(6) Pharmacokinetics of PFD concentration in plasma.

AEs were defined using Common Terminology Criteria for Adverse Events v5.0. Tolerability was defined as the completion of $\geq$95% of the planned PFD dose and the ability of $\geq$50% of participants to retain the PFD for 60 min without requiring early termination due to discomfort or SAEs.

Exploratory endpoints.

(1) Device performance issues.
(2) Impact on blood lactate and pyruvate levels.

METHOD DETAILS

Pharmacokinetic modeling based on large animal studies

The primary aim of this trial was to assess the safety and tolerability of non-oxygenated PFD; however, the intrinsic oxygen pressure in ambient PFD is higher than that in venous blood. To gain quantitative, dose-dependent insights, we developed a structural pharmacokinetic (PK) model based on oxygen mass balance and the time-course changes in arterial (SaO_2) and venous oxygen saturation (SvO_2) following O_2-PFD administration.Time-course data of ΔSaO_2% and ΔSvO_2% from mini-pigs receiving rectal O_2-PFD were used for model development. Human values were applied where pig-specific data were unavailable, including total blood volume per body weight (7.7%), arterial and venous fractions (20% and 75%), hemoglobin concentration (14 g/dL), and oxygen-binding capacity (1.34 mL O_2/g Hb). Based on these values, estimated arterial and venous blood volumes in a 24.2 kg pig were 0.372 L and 1.40 L, respectively, corresponding to oxygen amounts of 0.70 mL and 2.62 mL per 1% change in SaO_2 and SvO_2.The structural model was developed using the average ΔSaO_2 and ΔSvO_2 values obtained from 21 pigs (Figure S3). The theoretical curves generated using optimized parameters generally aligned with observed data in a separate pig dataset. While the applicability of porcine findings to humans remains uncertain, pig data were used in this study to provide supportive context for model development, reflecting their frequent use in critical care research and the possibility that oxygen transfer mechanisms may share broad similarities.

Figure S2A illustrates the conceptual PK model with the corresponding differential equations for oxygen dynamics are defined as follows:

$$\frac{dAO_2}{dt} = Kca \times AcapO_2 - Kac \times AO_2 - CLO_2 \times AO_2 \quad \text{(Equation 1)}$$

$$\frac{dVO_2}{dt} = Kcv \times AcapO_2 - Kvc \times VO_2 \quad \text{(Equation 2)}$$

For the period up to 30 min post-administration

$$\frac{dAcapO_2}{dt} = SL + Ka \times O_2PDF - Kca \times AcapO_2 + Kac \times AO_2 - Kvc \times AcapO_2 + Kvc \times VO_2 \quad \text{(Equation 3)}$$

for the period beyond 30 min post-administration

$$\frac{dAcapO_2}{dt} = - Kca \times AcapO_2 + Kac \times AO_2 - Kcv \times AcapO_2 + Kvc \times VO_2 \quad \text{(Equation 4)}$$

$$\frac{dO_2PFD}{dt} = - KA \times PFDO_2 \quad \text{(Equation 5)}$$

AO_2, VO_2, $AcapO_2$, and O_2PFD denote the amount of oxygen in arterial blood, venous blood, rectal capillaries, and the administered PFD, respectively. The intercompartmental transfer rates were defined as follows: Kca and Kac for capillary-to-arterial and arterial-to-capillary transfer; Kcv and Kvc for capillary-to-venous and venous-to-capillary transfer. For oxygen absorption from rectal PFD into capillaries, both an immediate absorption rate constant (Ka) and a slow-release component (SR) were introduced.

The initial AO_2 and VO_2 were set to 0 mL. The initial O_2PFD value was calculated based on the theoretical oxygen content estimated from PFD vapor pressure, multiplied by a bioavailability parameter (BA). Parameter estimation was performed using a nonlinear least-squares fitting program, MULTI Runge,[25] incorporating the Runge-Kutta-Gill numerical integration method.

Simulation of clinical data using pig PK model and human parameters

Using the develped pig PK model, optimized parameters, and human blood volume values, we simulated the time-dependent changes in ΔSaO_2% and ΔSvO_2% following rectal administration of non-oxygenated PFD at volumes of 20, 75, 200, 500, 1000, and 1500 mL. Human values of 7.7% for total blood volume per body weight, and 20% and 75% for arterial and venous blood fractions were used. The oxygen content per 1% ΔSaO_2 and ΔSvO_2 was calculated using Hb values of 14 g/100 mL and oxygen-binding capacity of 1.34 mL/g. In a 60 kg human, arterial and venous blood volumes were calculated as 0.924 L and 3.460 L, respectively, resulting in 1.73 mL and 6.49 mL of oxygen per 1% SaO_2 and SvO_2.

The oxygen content in non-oxygenated PFD was estimated from PFD vapor pressure, multiplied by the BA parameter obtained from the pig model. The slow-release (SR) rate was similarly defined based on a proportional relationship with vapor pressure. Simulations were performed using Equations 1, 2, 3, 4, and 5 and the Runge-Kutta-Gill method to compute ΔSaO_2 and ΔSvO_2, which were then converted to % changes based on the calculated 1% oxygen capacities.

Simulated increases in SaO_2 and SvO_2 were dose dependent, and in clinical trials involving 1000 mL rectal PFD administration, observed changes in both SaO_2 and SvO_2 were approximately 1% (Figures S2B and S2C). These results support the hypothesis that, although modest without oxygen bubbling, intestinal oxygen uptake can occur at higher doses.

Validation of oxygen transfer and required PFD volume

The fractional part of the mean of SpO_2 did not have meaning enough to be representative of the group because SpO_2 was obtained as an integer and the change, if any, was small (due to its originally high value in healthy participants). The median was also not optimal due to the small number of participants. So, the variation of the mode of SpO_2 was described as a graph and validated by calculating the elevation of SpO_2 using area under curve (AUC).

When the blood got oxygenated, the oxygen content of arterial blood (ΔCaO_2 [mL/dL]) and the oxygen delivery (ΔDO_2 [mL/min]) elevated:

$$\Delta CaO2 = 1.34 \times Hb \times \Delta SpO2 + 0.003 \times \Delta PaO2 \quad \text{(Equation 6)}$$

$$\Delta DO2 = \Delta CaO2 \times CO \quad \text{(Equation 7)}$$

Where Hb is hemoglobin [g/dL] and CO is cardiac output [L/min], into which 14.0 g/dL and 5 L/min are substituted respectively. The term "$0.003 \times \Delta PaO_2$" in the EQ6 was sufficiently small enough to be omitted.

How much oxygen PFD can deliver (X [mL/mL]) was given as follows:

$$X = c \times d \times (P_{before} - P_{after}) / P_{std} \quad \text{(Equation 8)}$$

Where c is oxygen solubility [mL/g] into PFD at 25°C, 760 mmHg, d is density [g/mL], P_{before} and P_{after} are partial oxygen pressure [mmHg] in PFD before and after administration, and P_{std} is the atmospheric pressure (760mmHg). When the partial oxygen pressure of nonoxygenated PFD changed from 220 mmHg to 40 mmHg, the volume of the PFD required for AUC = 1 (ΔSpO_2 = 1%, time = 1 min) was calculated to be 85 mL.

To simplify, assuming the graph of the SpO_2 changes was in the shape of the isosceles trapezoid with 40 min of lower bottom and 20 min of upper bottom, the height, or the SpO_2 upshift, was calculated as AUC/30.

QUANTIFICATION AND STATISTICAL ANALYSIS

The statistical analysis will be conducted using SAS 9.4, Microsoft Excel 2019, and Microsoft Word 2019. Continuous variables were summarized using the mean, standard deviation, median, minimum, and maximum, while categorical variables will be presented as frequencies and proportions, with statistical summaries conducted by planned and actual administration volumes. The Safety Analysis Population consisted of all subjects exposed to the device, excluding those with major Good Clinical Practice (GCP) violations. An adjudication committee reviewed all deviations and missing data, and analyses included evaluations conducted at withdrawal points where applicable. Key statistical considerations includes the application of subgroup analysis for primary endpoints, with no adjustments for covariates, interim analysis, multiplicity adjustment, or sensitivity analysis planned.

The baseline demographics and clinical characteristics of safety population or each dosage group were compared using one-way analysis of variance (ANOVA) or Fisher's exact test. Differences in hematological and biochemical parameters before and after PFD administration were compared using paired t-test. Vital sign parameters, SpO_2, and $tcpCO_2$ during PFD administration were compared using repeated measures ANONA. Categorical variables were expressed as numeric values (proportion), and continuous variables were presented as mean ± standard deviation (SD). Delta (Δ) represents the difference in blood gas analysis values before non-oxygenated PFD administration. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using Graph Pad Prism10 software and R software, version 4.4.0 (The R Foundation for Statistical Computing, Vienna, Austria).