A Phase I, Dose-escalation Trial Evaluating the Safety and Efficacy of Emulsified Isoflurane in Healthy Human Volunteers

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ABSTRACT

Background: This first-in-human volunteer phase I clinical trial aimed to evaluate the safety, tolerability, and anesthesia efficacy of emulsified isoflurane (EI), an intravenously injectable formulation of isoflurane.

Methods: Seventy-eight healthy volunteers were recruited in this open-label, single-bolus, dose-escalation, phase I trial and were allocated into 16 cohorts. Each volunteer received a single bolus injection of EI. The dose started with 0.3 mg/kg (for isoflurane) and was planned to end with 64.6 mg/kg. Postdose vital signs, physical examination, laboratory tests, chest radiograph, 12-lead electrocardiogram, and development of any adverse event were closely monitored as safety measurements. Effectiveness in producing sedation/anesthesia was assessed by Modified Observer's Assessment of Alertness/Sedation and Bispectral Index.

Results: The dose escalation ended as planned. The most common adverse events associated with EI were injection pain (77 of 78, 98.7%) and transient tachycardia (22 of 78, 25.6%). Only at high doses (≥38.3 mg/kg) did EI cause transient hypotension (5 of 78, 6.4%) or apnea (11 of 78, 14.1%), but all the affected volunteers recovered uneventfully. Fast onset of unconsciousness (typically 40 s after injection) was developed in all volunteers receiving doses of 22.6 mg/kg or greater. Waking-up time and depression in Modified Observer's Assessment of Alertness/Sedation correlated well with EI dose. None of the postdose tests revealed any abnormal result.

Conclusions: EI is safe for intravenous injection in human volunteers in the dose range of 0.3 to 64.6 mg/kg. At doses of 22.6 mg/kg or higher, EI produced rapid onset of unconsciousness in all volunteers followed by fast, predictable, and complete recovery. **(Anesthesiology 2014; 120:614-25)**

IT has been more than 160 yr since Morton's first successful demonstration of general anesthesia with ether inhalation. With the introduction of modern potent volatile anesthetics,[1,2] inhaled anesthesia continues to be used extensively for surgical anesthesia. However, drug delivery remains dependent on inhalation, which leads to delayed onset of drug effects and larger drug consumption because extra time and drug are needed to fill the lung capacity and breathing circuit. If volatile anesthetic could be given by intravenous injection, these shortcomings would be avoided. In addition, anesthesia could be rapidly deepened with intravenous injection or infusion and readily lightened by adjusting ventilation because contemporary volatile anesthetics, such as isoflurane, undergo little biotransformation *in vivo*, and are removed *via* respiration.[3]

Direct intravenous injection of halothane in humans[4] and dogs[5] caused severe pulmonary edema and death. The animal study[5] showed that halothane induced direct pulmonary damage because the solubility of halothane is low in blood, and mixing would not have sufficiently occurred until the liquid passed through the lung capillaries. However, predissolved (be emulsified) in fat/Intralipid® (Huarui Pharmacy, Ltd., Wuxi, Jiangsu, China), volatile anesthetics could be safely injected into animals to produce anesthesia.[6,7] Our preclinical studies further showed that intravenous injection of emulsified isoflurane (EI) had a greatly shortened onset time compared with inhaled isoflurane[8] and a similar short onset time but a much shorter

What We Already Know about This Topic

- Emulsified isoflurane is a new intravenous preparation of an anesthetic that is conventionally inhaled
- The authors tested efficacy and safety of emulsified isoflurane in 78 volunteers, using a dose-escalation approach

What This Article Tells Us That Is New

- Virtually all volunteers experienced pain at the injection site, and a quarter experienced tachycardia
- Subjects given more than 23 mg/kg lost consciousness within 40 s
- Recovery was rapid and correlated with dose

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recovery time compared with propofol,[9] which indicated a potential clinical application. The successful application of EI in animals for organ protection,[10] for intravenous regional anesthesia,[11] and for elucidating action mechanisms of anesthetics[12,13] further justified the need for systematic assessment of EI in human.

Therefore, the primary aim of this open-label, single-bolus, dose-escalation, phase I clinical trial was to assess the safety and tolerability of EI in healthy human volunteers. Sedative/anesthesia effect of EI was also observed without comprising subjects' safety.

Materials and Methods

All volunteers gave written informed consent before enrollment. This study was approved by the Chinese Food and Drug Administration of China (No. 2009L01628) after review of our preclinical studies[8,9] and West China Ethic Committee (West China Hospital, Chengdu, China) approval. This study was also registered with clinical trial (NCT01302353). EI injection was provided by Yichang Humanwell Pharmaceutical Co., Ltd. (Hubei, P. R. China); it is prepared by dissolving liquid isoflurane into 30% Intralipid® at the volume ratio of 1:11.5 with an isoflurane concentration of 8% (vol/vol).

Inclusion and Exclusion Criteria

Healthy volunteers were recruited by local advertisement and were screened before enrollment. All the volunteers aged between 18 and 45 yr and having a normal medical history and a body mass index between 19 and 24 were included. The screening included: medical history, physical examination, routine lab tests including hematology test, clinical chemistry test, urine test, and stool test, 12-lead electrocardiograph, and chest radiograph test. Particularly, volunteers with suspected difficult airway, hyperlipidemia or malignant hyperthermia in family medical history were excluded.

Study Design and Dose Escalation

This was an open-label, single-bolus, dose-escalation, phase I clinical trial. Up to 78 healthy volunteers were planned for enrollment. Each volunteer received a single bolus injection of 8% (vol/vol) EI. The dose escalation started with 0.3 mg/kg (for isoflurane) and was planned to end with 64.6 mg/kg. Within the preset dose range, 16 dose cohorts were arranged with decreasing increments of dose (table 1).

Clinic Protocol

One day before the study, volunteers were required to follow a diet without alcohol or caffeine, and with limited amount of fat, till 24 h after EI administration. All the volunteers were asked to fast for 8 h before the study started.

After entering the study room, all volunteers received 10 l/min O_2 *via* a face mask connecting to an anesthesia machine in order to minimize rebreathing during study. Standard anesthetic monitoring was applied to the volunteers, including 12-lead electrocardiogram, noninvasive blood pressure, pulse oximetry, respiratory rate, body surface temperature, Bispectral Index (BIS XP® version 2.1; Aspect Medical Systems, Newton, MA), and end-tidal carbon dioxide and isoflurane monitoring (T5; Mindray Medical International Ltd., Shenzhen, China). Vital signs was recorded continuously by the automatic monitor and manually at predefined time points in Case Reports Forms: baseline before insertion of an intravenous catheter; every minutes for the first 15 min postdose; every 2 min from 15 to 30 min postdose; every 5 min from 30 to 60 min postdose; every 1 h from 1 to 4 h postdose; at the eighth hour and 24th hour postdose, respectively.

An intravenous catheter was inserted in forearm for drug and fluid administration. Before EI injection, 10 ml/kg Ringer's solution was given to restore volume deficit caused by overnight fasting. Ringer's solution infusion was continued at a rate of 10 $ml \cdot kg^{-1} \cdot h^{-1}$ for the first hour after EI injection.

Emulsified isoflurane was injected at the rate of 4 ml/10 s right after a stopwatch was started. The volunteers were asked to keep their eyes open so that consciousness could be assessed. Upon EI injection, volunteers were asked to report any discomfort sensed on injection site in a yes-or-no pattern (nodding or shaking their heads to the question "do you feel any discomfort"). One hour after injection, volunteers were asked to recall the nature of the discomfort. The intensity of the discomfort was further evaluated by Visual Analog Scale from 0 to 10, in which 0 stands for normal condition with no discomfort and 10 for the worst possible situation.

After EI injection, apnea was defined as loss of end-tidal carbon dioxide waveform for 15 s. Once apnea was confirmed, manual ventilation *via* face mask would be initiated at the rate of 12 cycles per minute. The effectiveness of mask ventilation was ensured by good chest movement, regular end-tidal carbon dioxide waves, and well-maintained oxygen saturation. For every 45 s, manual ventilation would be suspended for 15 s to assess the recovery of spontaneous respiration, which was implied by reappearance of regular carbon dioxide waveform.

Volunteers rested in bed for 1 h after EI injection and resided in the clinic ward for 24 h. Physical examination, routine lab test including hematology test, clinical biochemistry test, urine test, and stool test, chest radiograph and 12-lead electrocardiogram were retested before discharge. The preset 24-h observation period would be extended if abnormality was found in any of these postdose tests.

Safety Measurements

Safety was monitored by repeated measurement of vital signs, postdose physical examination, postdose routine laboratory tests including hematology test, clinical biochemistry test,

Table 1. Dose Escalation and Demographics

Cohort	Isoflurane (mg/kg)	Lipid Volume (ml/kg)	Number of Volunteers (Male/Female)	Age (yr)	Weight (kg)	Height (cm)	BMI (kg/m^2)
1	0.3	0.0024	4 (2/2)	24.5 (21.0–25.0)	54.8 (6.4)	164.0 (8.8)	20.30 (0.51)
2	0.7	0.0060	4 (2/2)	23.0 (20.0–24.0)	59.5 (4.9)	168.3 (6.7)	21.03 (1.31)
3	1.4	0.0120	4 (2/2)	20.5 (19.0–26.0)	54.5 (6.2)	164.0 (5.5)	20.21 (1.06)
4	2.4	0.0200	4 (2/2)	24.5 (23.0–26.0)	60.0 (10.8)	166.0 (11.2)	21.63 (1.30)
5	3.6	0.0301	4 (2/2)	23.0 (22.0–24.0)	57.6 (8.1)	169.3 (7.4)	20.04 (1.44)
6	4.7	0.0391	4 (2/2)	21.5 (21.0–25.0)	58.9 (9.3)	166.8 (10.6)	21.05 (1.14)
7	6.1	0.0508	4 (2/2)	23.5 (21.0–24.0)	58.8 (5.9)	167.5 (8.8)	20.90 (0.74)
8	7.9	0.0660	4 (2/2)	23.0 (20.0–28.0)	57.0 (12.3)	164.5 (9.5)	20.83 (2.10)
9	10.3	0.0859	4 (2/2)	23.0 (21.0–23.0)	62.3 (11.9)	167.8 (10.0)	21.93 (1.82)
10	13.4	0.1116	6 (3/3)	22.0 (20.0–27.0)	60.5 (8.8)	166.8 (6.9)	21.62 (1.47)
11	17.4	0.1451	6 (3/3)	21.0 (20.0–24.0)	53.8 (7.2)	162.7 (9.3)	20.25 (0.72)
12	22.6	0.1886	6 (3/3)	23.5 (20.0–28.0)	59.6 (8.2)	166.5 (10.4)	21.45 (1.63)
13	29.4	0.2452	6 (3/3)	23.0 (19.0–24.0)	60.0 (7.0)	167.2 (7.6)	21.43 (1.36)
14	38.3	0.3188	6 (3/3)	21.0 (20.0–25.0)	53.3 (5.9)	163.8 (6.1)	19.80 (0.97)
15	49.7	0.4144	6 (3/3)	22.0 (20.0–27.0)	54.8 (6.4)	163.2 (9.0)	17.24 (7.75)
16	64.6	0.5387	6 (3/3)	22.5 (19.0–29.0)	56.8 (9.2)	163.2 (9.1)	21.20 (1.50)
P value				0.6464	0.8920	0.9956	0.3961

There was no difference observed in demographics between volunteers in the 16 dose levels. Age is presented as median (range); weight, height, and BMI are presented as mean (SD).

BMI = body mass index.

urine test, and stool test, postdose chest radiograph, and postdose 12-lead electrocardiogram. Discomfort reported by volunteers was also documented in Case Reports Forms and details were included in safety assessment, such as nausea or vomiting. Right after each volunteer completed the study protocol, the data-monitoring committee reviewed his or her Case Reports Form. The severities of abnormal vital signs were graded as mild, moderate, severe, or life-threatening, according to the criteria in table 2. The severities of other adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events v4.0.[14]

The dose escalation would be terminated if any of the following criteria was met:

- Severe adverse events developed in more than half of the volunteers at any dose level;
- Life-threatening adverse event or death developed in any volunteer at any dose level;
- The planned maximal dose of 64.6 mg/kg was reached.

Efficacy Evaluation

After EI injection, sedative level was evaluated at 30-s intervals by using Modified Observer's Assessment of Alertness/Sedation[15] (MOAA/S), till 30 min postdose. Loss of consciousness (LOC) was defined as failure to follow loud verbal command to open his or her eyes. Once LOC was confirmed, a noxious electrical stimulus was applied to one hand (50 mA, 0.2 ms). Prevention of purposeful movement in unstimulated extremities to this electrical stimulus was regarded as immobility. Recovery of consciousness was defined as following loud verbal command to reopen his/her eyes. Waking-up time ($T_{waking\text{-}up}$) was defined as the period between LOC and recovery of consciousness. Time from waking-up to full recovery ($T_{waking\text{-}up\ to\ full\ recovery}$) was defined as the time elapsed from recovery of consciousness to the first of five consecutive MOAA/S = 5. The reduced conscious level during the 30-min observation phase was expressed quantitatively by the area above the MOAA/S *versus* time curve. Bispectral index was monitored continuously for 30 min after EI injection.

Statistical Analysis

Data analysis was performed by using Graphpad Prsim Software (version 5.01; GraphPad Software, Inc., La Jolla, CA). Quantitative data between dose cohorts were analyzed by one-way ANOVA test, followed by Student–Neumann–Keuls test when indicated, except for age. Age was analyzed by Kruskal–Wallis test, followed by Nemenyi test when necessary. Probit analysis was carried out to estimate ED_{50}. Hemodynamic parameters were compared by ANOVA for repeated measurements, followed by *post hoc* Bonferroni test when needed. All the reported *P* values were two-detailed and *P* value less than 0.05 was considered a statistical significance.

Results

This study was conducted in the Good Clinical Practice center of West China Hospital, Sichuan University, Chengdu, P. R. China. The volunteer enrollment started on May 17, 2010 and the last volunteer finished his study protocol on September 22, 2010. Dose escalation ended up with the planned maximal dose of 64.64 mg/kg and all the 78 enrolled volunteers completed study protocol. Therefore, data of all the

Table 2. Assessment Criteria of Abnormal Vital Signs

	Intensity	Assessment	Treatment
Hypotension (SBP <90 mmHg)	Mild	SBP 80–89 mmHg >3 min	Close monitoring
	Moderate	SBP 70–79 mmHg >2 min	Ephedrine 6 mg × 2
	Severe	SBP 70–79 mmHg >2 min and unresponsive to ephedrine *or* SBP 60–69 mmHg	Metaraminol 10 μg × 2
	Life-threatening	SBP 60–69 mmHg and unresponsive to metaraminol *or* SBP <60 mmHg	Intensive intervention and terminate the trial
Hypertension (SBP >140 mmHg) or (DBP >90 mmHg)	Mild	SBP 141–160 mmHg/DBP 91–100 mmHg >3 min	Close monitoring
	Moderate	SBP 161–170 mmHg/DBP 101–105 mmHg >2 min	Urapidil 12.5 mg × 2
	Severe	SBP 161–170 mmHg/DBP 101–105 mmHg >2 min and unresponsive to urapidil *or* SBP 171–180 mmHg/DBP 106–110 mmHg	Nitroglycerin 0.1–1.0 μg kg^{-1} min^{-1}
	Life-threatening	SBP 171–180 mmHg/DBP 106–110 mmHg and unresponsive to nitroglycerin *or* SBP >180 mmHg/DBP 110 mmHg	Intensive intervention and terminate the trial
Bradycardia (HR <56 beats/min)	Mild	HR 51–55 beats/min	Close monitoring
	Moderate	HR 46–50 beats/min >5 min	Atropine 0.5 mg × 2
	Severe	HR 46–50 beats/min >5 min and unresponsive to atropine *or* HR 41–45 beats/min >3 min	Isoproterenol 0.05–0.1 μg kg^{-1} min^{-1}
	Life-threatening	HR 41–45 beats/min >3 min and unresponsive to atropine *or* HR <41 beats/min	Intensive intervention and terminate the trial
Tachycardia (HR >100 beats/min)	Mild	HR 101–119 beats/min	Close monitoring
	Moderate	HR 120–139 beats/min >5 min	Esmolol 25 mg × 2
	Severe	HR 120–139 beats/min >5 min and unresponsive to esmolol 25 mg *or* HR 140–159 beats/min >3 min	Esmolol 50 mg × 2
	Life-threatening	HR 140–159 beats/min >3 min and unresponsive to esmolol 50 mg *or* HR >159 beats/min	Intensive intervention and terminate the trial
Hypoxia (Spo_2 <95%)	Mild	Spo_2 92–95%	Close monitoring
	Moderate	Spo_2 90–91%	Mask ventilation
	Severe	Spo_2 90–91% and unresponsive to mask ventilation *or* Spo_2 85–89%	Eliminating possible causes of hypoxia*
	Life-threatening	Spo_2 85–89% and unresponsive to treatment *or* Spo_2 <85%	Intensive intervention and terminate the trial

Abnormal vital signs are classified according to the severity.

* The treatment would be determined according to the volunteer's symptoms: *e.g.*, chin lift or oropharyngeal airway would be applied in volunteers with obstructed upper airway; aminophylline would be administrated in case of bronchospasm. However, the volunteer would not be intubated unless his or her hypoxia was determined as life-threatening.

DBP = diastolic blood pressure; HR = heart rate; SBP = systolic blood pressure; Spo_2 = oxygen saturation measure by pulse oximetry; ×2 = treatment could be given twice at an interval of 5–10 min.

78 volunteers were included for statistical analysis. Demographic data were similar among the 16 cohorts (table 1).

Effect of EI on Safety

Overall, vital signs were kept stable after injection of EI. In 22 volunteers the abnormal vital signs were detected 25 times as tachycardia and hypotension. All were graded as mild in severity except one hypotension case, which was graded as moderate (table 3). Heart rate reached the peak in 2 min after injection ($P < 0.0001$ *vs.* baseline) and spontaneously returned to baseline within 4 min (fig. 1A and table 4). The maximum increase in heart rate correlated well with EI dose (fig. 1A; $R^2 = 0.618$; $P < 0.001$). Tachycardia (heart rate >100 beats/min) was observed in 20 of the 78 volunteers (25.6%). Among these 20 cases, 12 were found in the 18 volunteers (66.7%) receiving EI of 38.3 mg/kg or higher. Synchronous with increase in heart rate, there was a transient increase in blood pressure lasting for maximal 2 min. This increase was very slight and no hypertension (systolic blood pressure >140 mmHg) was observed. No hypotension was observed in volunteers receiving doses less than 38.3 mg/kg either. Approximately 6 min after EI injection, 5 of 18 volunteers (27.8%) receiving EI of 38.3 mg/kg or higher developed hypotension (systolic blood pressure <90 mmHg) without accompanying bradycardia, as showed in figure 1B and table 4. Blood pressure returned to the baseline spontaneously in four volunteers, and one volunteer received EI of 38.3 mg/kg required 6 mg ephedrine according to our predefined protocol (systolic blood pressure lower than 80 mmHg in two consecutive measurements at 1-min intervals) to restore her blood pressure. Other vital signs, such as oxygen saturation and body temperature, were within the normal range after EI injection and were unchanged compared with the baseline.

Eleven of 18 volunteers (61.1%) receiving EI of 38.3 mg/kg or higher developed apnea. Oxygen saturation was well maintained at 99% or higher with mask ventilation in these 11

Table 3. Abnormal Vital Signs following EI Injection

Cohort	Isoflurane (mg/kg)	Abnormal Vital Signs			
		Mild	Moderate	Severe	Life-threatening
1	0.3	0	0	0	0
2	0.7	1 (*1 Tachy*)	0	0	0
3	1.4	0	0	0	0
4	2.4	0	0	0	0
5	3.6	0	0	0	0
6	4.7	0	0	0	0
7	6.1	0	0	0	0
8	7.9	0	0	0	0
9	10.3	0	0	0	0
10	13.4	0	0	0	0
11	17.4	3 (*3 Tachy*)	0	0	0
12	22.6	1 (*1 Tachy*)	0	0	0
13	29.4	3 (*3 Tachy*)	0	0	0
14	38.3	2 (*1 Tachy* and *1 HoTN*)	1 (*1 HoTN*)	0	0
15	49.7	7 (*6 Tachy* and *1 HoTN*)	0	0	0
16	64.6	7 (*5 Tachy* and *2 HoTN*)	0	0	0
Total	—	24 (*20 Tachy* and *4 HoTN*)	1 (*1 HoTN*)	0	0

The abnormal vital signs were counted by times be observed, and it would be recorded as two if both hypotension and tachycardia were observed in one volunteer.

EI = emulsified isoflurane; HoTN = hypotension (systolic blood pressure <90 mmHg); Tachy = tachycardia (heart rate >100 beats/min).

volunteers. A dose-dependent increase in duration of apnea was observed (table 5) and respiration was recovered spontaneously.

The following airway responses to the pungency of isoflurane were observed: (1) all the 78 volunteers reported sensing pungent smell after receiving EI injection; (2) paradoxical abdominal movement during inspiration even after lifting the chin manually was observed in 11 of 18 volunteers (61.1%) receiving EI of 38.3 mg/kg or higher. Later, apnea occurred and mask ventilation was initiated in 10 of these 11 volunteers. The paradoxical movement resolved spontaneously in the other volunteer whose spontaneous respiration was well maintained after EI injection; and (3) cough was observed in six volunteers (7.7%, 6 of 78). All the affected volunteers recovered uneventfully without any medication.

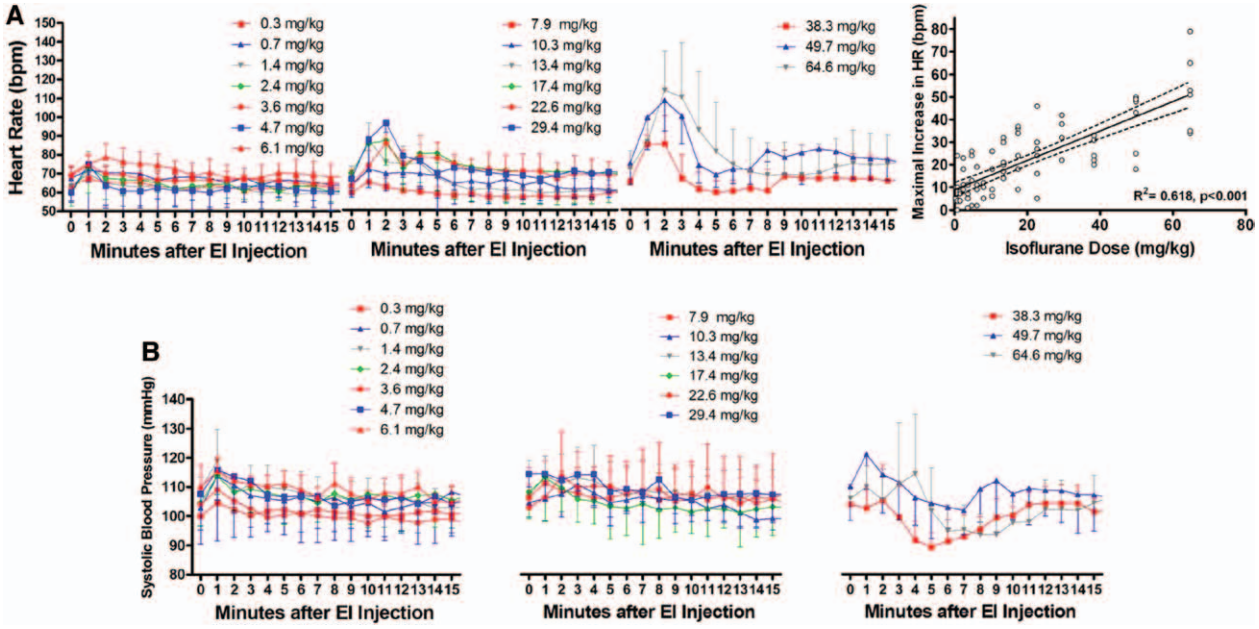

Fig. 1. Hemodynamic change after injection of emulsified isoflurane (EI). There was a transient hyperdynamic response after injection of EI, manifested as a synchronous increase in heart rate (HR) (*A*) and blood pressure (*B*) immediately after injection. The maximal increase in HR correlated well with dose of EI, where the *solid line* indicates the best-fit line and the *dash lines* indicate the 95% confidence band of the best-fit line. bpm = beats/min.

Table 4. Hemodynamic Changes after EI Injection

Cohort	Isoflurane, mg/kg (Folds of ED_{50})	HR, beats/min			SBP (mmHg)			
		Baseline	HR_{Max} (Maximal Change)	Tachycardia, n	Baseline	SBP_{MAX}	SBP_{MIN}	Hypotension, n
1	0.3 (0.03)	64±6	69±8 (6±5%)	0/4 (0.0%)	100±7	107±7	98±4	0/4 (0.0%)
2	0.7 (0.07)	68±13	76±24 (4±17%)	1/4 (25.0%)	103±12	117±21	100±9	0/4 (0.0%)
3	1.4 (0.14)	63±10	70±12 (10±3%)	0/4 (0.0%)	109±4	120±10	103±6	0/4 (0.0%)
4	2.4 (0.24)	60±7	75±12 (22±13%	0/4 (0.0%)	105±8	115±6	103±7	0/4 (0.0%)
5	3.6 (0.35)	69±5	75±5 (9±7%)	0/4 (0.0%)	104±4	110±7	97±4	0/4 (0.0%)
6	4.7 (0.46)	60±5	76±10 (26±21%)	0/4 (0.0%)	108±12	117±12	103±10	0/4 (0.0%)
7	6.1 (0.60)	70±4	80±7 (8±8%)	0/4 (0.0%)	110±8	117±5	104±3	0/4 (0.0%)
8	7.9 (0.78)	60±3	66±4 (10±13%)	0/4 (0.0%)	107±10	114±10	104±8	0/4 (0.0%)
9	10.3 (1.01)	63±6	78±14 (14±12%)	0/4 (0.0%)	105±5	113±5	99±3	0/4 (0.0%)
10	13.4 (1.31)	66±7	89±7 (33±15%)	0/6 (0.0%)	108±12	117±9	105±9	0/6 (0.0%)
11	17.4 (7.71)	70±10	96±10 (27±26%)	3/6 (50.0%)	109±9	118±15	97±6	0/6 (0.0%)
12	22.6 (2.22)	63±8	88±11 (38±26%)	1/6 (16.7%)	104±8	124±13	101±9	0/6 (0.0%)
13	29.4 (2.89)	67±9	100±8 (47±22%)	3/6 (50.0%)	116±5	123±5	101±6	0/6 (0.0%)
14	38.3 (3.75)	65±7	92±9 (32±14%)	1/6 (16.7%)	105±6	114±5	89±5	2/6 (33.3%)
15	49.7 (4.88)	75±12	114±15 (46±20%)	6/6 (100%)	111±12	124±15	99±11	1/6 (16.7%)
16	64.6 (6.34)	72±9	125±21 (60±32%)	5/6 (83.3%)	107±5	124±16	89±6	2/6 (33.3%)
Total				20/78 (25.6%)				5/78 (6.4%)

Data are presented as mean ± SD. Changes are given in % compared with baseline. ED_{50} (median effective dose) for producing unconsciousness was 10.2 mg/kg.

EI = emulsified isoflurane; HR_{Max} = maximal heart rate after EI injection; Hypotension, n = cases of SBP <90 mmHg; SBP_{Max} = highest systolic blood pressure after EI injection; SBP_{MIN} = lowest systolic blood pressure after EI injection; Tachycardia, n = cases of HR >100 beats/min.

Injection pain was observed in 77 of the 78 volunteers (98.7%), and pain severity was not associated with EI doses (table 6). No edema or redness was found at the injection site.

Mild nausea without vomiting was reported by four volunteers (5.1%, 4 of 78) after recovery from anesthesia. No significant change was found in postdose hematology test (table 7) or postdose biochemistry test (table 8). Single bolus injection of EI had no effect on blood lipid level, as shown in table 9. Postdose chest radiograph or 12-lead electrocardiogram found no abnormal results either.

Anesthetic Effect of EI

A dose-dependent reduction in MOAA/S scores was observed (fig. 2A), and the degree of MOAA/S reduction correlated

Table 5. Respiratory Adverse Events Associated with EI Injection

Cohort	Isoflurane, mg/kg (Folds of ED_{50})	Apnea		Airway Responses to Pungency		
		Incidence	Duration (s)	Pungent Smell	Para Abdominal Movement	Cough
1	0.3 (0.03)	0/4 (0.0%)	NA	4/4 (100%)	NA	NA
2	0.7 (0.07)	0/4 (0.0%)	NA	4/4 (100%)	NA	NA
3	1.4 (0.14)	0/4 (0.0%)	NA	4/4 (100%)	NA	NA
4	2.4 (0.24)	0/4 (0.0%)	NA	4/4 (100%)	NA	NA
5	3.6 (0.35)	0/4 (0.0%)	NA	4/4 (100%)	NA	NA
6	4.7 (0.46)	0/4 (0.0%)	NA	4/4 (100%)	NA	NA
7	6.1 (0.60)	0/4 (0.0%)	NA	4/4 (100%)	NA	NA
8	7.9 (0.78)	0/4 (0.0%)	NA	4/4 (100%)	NA	NA
9	10.3 (1.01)	0/4 (0.0%)	NA	4/4 (100%)	NA	NA
10	13.4 (1.31)	0/6 (0.0%)	NA	6/6 (100%)	NA	NA
11	17.4 (7.71)	0/6 (0.0%)	NA	6/6 (100%)	NA	2/6 (33.3%)
12	22.6 (2.22)	0/6 (0.0%)	NA	6/6 (100%)	NA	1/6 (16.7%)
13	29.4 (2.89)	0/6 (0.0%)	NA	6/6 (100%)	NA	NA
14	38.3 (3.75)	3/6 (50.0%)	31±10	6/6 (100%)	4/6 (66.7%)	1/6 (16.7%)
15	49.7 (4.88)	4/6 (66.7%)	174±151	6/6 (100%)	4/6 (66.7%)	2/6 (33.3%)
16	64.6 (6.34)	4/6 (66.7%)	508±216	6/6 (100%)	3/6 (50.0%)	NA
Total		11/78 (14.1%)	—	78/78 (100%)	11/78 (14.1%)	6/78 (7.7%)

Time data are presented as mean ± SD. ED_{50} (median effective dose) for producing unconsciousness was 10.2 mg/kg.

EI = emulsified isoflurane; NA = not applicable; Para abdominal movement = paradoxical abdominal movement during inspiration.

Table 6. Injection Pain Associated with EI Injection

Cohort	Isoflurane (mg/kg)	Incidence	Onset (s)	VAS		Radiation	
				Median	Range	Median	Range
1	0.3	4/4 (100%)	5 ± 5	6	5–7	2	2–2
2	0.7	4/4 (100%)	7 ± 4	7	6–8	2	2–3
3	1.4	4/4 (100%)	4 ± 5	7	5–8	3	2–3
4	2.4	4/4 (100%)	11 ± 5	5	4–8	2	1–2
5	3.6	4/4 (100%)	9 ± 4	6	4–7	3	1–3
6	4.7	4/4 (100%)	8 ± 1	4	2–7	3	2–3
7	6.1	4/4 (100%)	7 ± 3	6	4–6	3	3–3
8	7.9	4/4 (100%)	7 ± 1	7	4–8	2	2–3
9	10.3	4/4 (100%)	7 ± 3	6	5–8	3	2–3
10	13.4	6/6 (100%)	11 ± 3	7	4–7	2	1–3
11	17.4	6/6 (100%)	7 ± 4	4	1–6	2	2–3
12	22.6	6/6 (100%)	5 ± 1	7	4–10	2	1–3
13	29.4	6/6 (100%)	10 ± 4	3	2–7	1	1–2
14	38.3	5/6 (83.3%)	6 ± 2	5	0–6	2	1–2
15	49.7	6/6 (100%)	5 ± 3	5	5–8	3	1–3
16	64.6	6/6 (100%)	6 ± 2	6	3–8	1	1–2
Total		77/78 (97.7%)					
P value			0.5874	0.4514		0.5162	

Time data are presented as mean ± SD. VAS score is presented as median and range.

EI = emulsified isoflurane; Radiation = areas to which the pain radiated, 1 = pain localized at the venous cannulation site and below ipsilateral wrist joint; 2 = pain reached the ipsilateral wrist joint but below the ipsilateral elbow joint; 3 = pain reached or spread beyond ipsilateral shoulder joint; VAS = Visual Analog Scale.

linearly with EI dose (R^2 = 0.9224; P < 0.0001; fig. 2B). The first case of LOC was observed in the dose group of 6.1 mg/kg, and LOC was seen in all the volunteers receiving at doses of 22.6 mg/kg or higher (table 10). The onset time was similar among all the subjects with LOC, typically 40 s after initiation of EI injection. The waking-up time increased with escalation of EI dose, from 30 s (6.1 mg/kg) to approximate 716 s (64.6 mg/kg), following an exponential growth (R^2 = 0.9125), as showed in figure 2C. It is worth noting that a more than 10-fold increase in EI dose (6.1–64.6 mg/kg) caused only a very slight increase in time from waking-up to fully recovery, from 60 s to approximate 110 s. The estimated ED_{50} for LOC was 10.2 mg/kg, with 95% CI from 9.2 to 11.6 mg/kg (fig. 3A). In the 43 subjects with LOC,

Table 7. Hematology Test before and after Bolus Injection of EI

Group	Erythrocytes (10^{12}/l)		Hemoglobin (g/l)		Leukocytes (10^9/l)		PLT (10^9/l)	
	Before	After	Before	After	Before	After	Before	After
1	4.94 ± 0.78	4.67 ± 0.47	138 ± 29	132 ± 23	5.37 ± 1.23	5.36 ± 0.64	188 ± 93	183 ± 83
2	4.79 ± 0.42	4.72 ± 0.24	145 ± 17	143 ± 10	5.63 ± 1.39	6.35 ± 0.63	252 ± 51	246 ± 39
3	4.70 ± 0.30	4.71 ± 0.35	144 ± 8	145 ± 10	5.75 ± 0.74	5.56 ± 0.81	197 ± 17	194 ± 15
4	4.61 ± 0.47	4.74 ± 0.32	136 ± 21	140 ± 17	6.44 ± 1.57	5.79 ± 0.59	233 ± 67	205 ± 47
5	4.80 ± 0.52	4.92 ± 0.33	142 ± 17	146 ± 11	6.42 ± 1.66	5.66 ± 0.81	218 ± 45	207 ± 36
6	4.82 ± 0.48	4.88 ± 0.49	143 ± 16	147 ± 16	5.48 ± 0.85	5.74 ± 1.32	236 ± 50	231 ± 66
7	4.71 ± 0.47	4.76 ± 0.51	140 ± 10	143 ± 10	5.95 ± 1.31	6.38 ± 1.27	231 ± 32	231 ± 32
8	4.78 ± 0.47	4.89 ± 0.48	143 ± 19	146 ± 18	5.75 ± 1.12	6.59 ± 1.55	220 ± 55	207 ± 61
9	4.89 ± 0.50	4.89 ± 0.60	147 ± 14	149 ± 17	5.77 ± 0.72	5.45 ± 0.84	211 ± 27	224 ± 27
10	4.83 ± 0.42	4.75 ± 0.32	145 ± 14	143 ± 10	4.99 ± 0.72	6.16 ± 1.18	247 ± 40	226 ± 36
11	4.78 ± 0.39	4.50 ± 0.56	144 ± 12	136 ± 17	5.85 ± 1.48	6.18 ± 1.79	221 ± 32	232 ± 21
12	4.75 ± 0.42	4.82 ± 0.29	137 ± 16	139 ± 19	5.78 ± 1.47	5.41 ± 0.90	175 ± 35	183 ± 34
13	4.83 ± 0.35	4.59 ± 0.64	147 ± 11	140 ± 18	5.82 ± 1.51	6.29 ± 1.44	223 ± 70	215 ± 74
14	4.83 ± 0.41	4.80 ± 0.39	140 ± 16	138 ± 14	6.39 ± 1.76	5.44 ± 0.91	205 ± 27	214 ± 23
15	4.89 ± 0.61	4.68 ± 0.61	147 ± 16	140 ± 17	6.16 ± 1.37	6.10 ± 0.67	200 ± 58	195 ± 49
16	4.85 ± 0.65	4.66 ± 0.51	144 ± 19	139 ± 17	4.44 ± 0.51	5.62 ± 1.93	191 ± 37	171 ± 36

Data are presented as mean ± SD.

EI = emulsified isoflurane; PLT = platelet.

Table 8. Biochemistry Test before and after Bolus Injection of EI

	ALT (U/l)		AST (U/l)		BUN (mM)		Cr (μM)	
Group	Before	After	Before	After	Before	After	Before	After
1	15±4	14±6	17±4	17±4	3.48±1.26	3.95±1.09	76.3±13.5	64.5±6.5
2	9±4	11±4	18±4	18±5	4.19±1.24	3.40±0.98	86.8±21.4	83.4±29.4
3	10±3	11±3	18±3	15±2	4.94±0.19	3.96±0.77	77.6±13.0	63.7±35.2
4	14±4	14±2	18±3	18±3	4.01±0.19	3.61±0.92	81.4±19.6	67.2±21.4
5	13±2	12±3	15±1	17±4	4.78±0.83	4.03±0.89	76.3±23.9	67.2±15.6
6	13±5	13±5	15±3	15±4	3.32±1.05	3.82±0.74	70.8±15.7	66.0±11.9
7	12±3	12±1	16±4	14±3	2.49±0.89	3.22±0.62	68.8±16.0	67.9±15.5
8	9±3	9±4	14±4	11±1	3.80±1.09	3.38±1.02	64.6±16.5	65.8±19.2
9	12±6	15±7	19±5	15±2	4.88±1.43	3.67±0.66	72.6±19.4	72.8±15.5
10	14±3	15±5	18±2	17±4	3.76±1.06	3.69±1.02	78.1±11.5	71.6±7.5
11	14±4	12±2	17±2	16±3	4.49±1.24	3.82±0.81	82.0±17.8	77.8±12.1
12	14±4	14±6	20±7	19±6	4.52±1.97	3.81±1.13	72.3±15.7	64.0±14.2
13	16±8	15±9	18±3	19±4	4.70±0.95	3.98±0.59	77.1±17.0	71.6±15.8
14	13±2	13±2	16±2	16±3	4.10±0.70	3.65±0.89	70.9±14.0	69.3±9.1
15	13±3	14±3	17±1	16±2	4.22±0.56	4.19±0.49	74.2±10.6	70.0±11.1
16	15±5	14±5	19±6	20±5	4.48±0.94	3.93±0.45	72.3±12.8	62.2±13.2

Data are presented as mean ± SD.

ALT = alanine transaminase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; Cr = creatine; EI = emulsified isoflurane.

purposeful movement to standard electrical stimulus was prevented in 30 subjects and the estimated ED_{50} for immobility was 18.1 mg/kg with 95% CI from 14.7 to 22.3 mg/kg (fig. 3B).

Discussion

In this open-label, single-bolus dose, dose-escalation phase I clinical trial, we showed that (1) single bolus injection of EI from 0.3 to 64.6 mg/kg was well tolerated by healthy human volunteers with a satisfactory safety profile, and (2) EI at doses of 22.6 mg/kg or higher was effective in producing rapid onset of general anesthesia, followed by a predictable, fast, and complete recovery in all the healthy human volunteers.

For all tested volunteers, all the adverse events and most abnormal vital signs (24 of 25 in total, 96%) were graded as mild in severity, indicating that the LD_{50} of EI in human should be larger than the maximum dose tested (64.6 mg/kg). With the estimated ED_{50} for LOC of 10.2 mg/kg, the therapeutic index (therapeutic index = LD_{50}/ED_{50}) of EI should be larger than 6 (64.6/10.2), which attested the safety of EI. In animal studies,[9,16] the efficacy endpoint for calculating

Table 9. Blood Lipid Level before and after Bolus Injection of EI

	Blood Glucose (mM)		TG (mM)		Tchol (mM)		HDL (mM)		LDL (mM)	
Group	Before	After	Before	After	Before	After	Before	After	Before	After
1	4.17±0.45	3.66±0.81	0.94±0.27	1.11±0.49	3.83±0.49	3.60±0.18	1.37±0.1	1.41±0.27	2.02±0.38	1.92±0.2
2	4.31±0.63	4.21±0.39	0.82±0.43	0.76±0.26	3.97±1.00	3.84±0.88	1.52±0.62	1.43±0.43	2.11±0.67	2.05±0.63
3	4.30±0.49	3.83±0.33	0.71±0.18	0.71±0.26	4.24±0.68	4.23±0.89	1.67±0.24	1.59±0.14	2.52±0.61	2.38±0.94
4	4.48±0.43	4.52±0.49	0.58±0.10	0.65±0.17	3.87±0.47	4.00±0.99	1.33±0.17	1.59±0.28	2.22±0.31	2.23±0.83
5	4.40±0.58	4.36±0.38	0.86±0.34	0.93±0.27	3.48±0.46	3.47±0.24	1.24±0.14	1.21±0.14	1.88±0.44	2.06±0.3
6	4.61±0.25	4.13±0.72	1.18±0.60	2.18±2.48	3.78±0.72	4.15±0.73	1.23±0.08	1.36±0.27	2.13±0.5	2.19±0.44
7	4.55±0.29	3.86±0.39	1.01±0.50	0.92±0.31	4.14±0.47	4.18±0.42	1.41±0.11	1.49±0.18	2.52±0.46	2.53±0.4
8	4.45±0.24	4.05±0.51	0.70±0.29	0.77±0.31	3.36±0.42	3.07±0.55	1.33±0.37	1.23±0.19	1.92±0.49	1.73±0.63
9	4.51±0.49	4.15±0.36	0.71±0.2	0.88±0.45	3.72±0.20	3.92±0.83	1.48±0.3	1.34±0.2	2.17±0.37	2.44±0.87
10	4.57±0.48	4.38±0.44	0.89±0.34	0.84±0.24	3.88±0.37	3.86±0.11	1.34±0.21	1.39±0.19	2.32±0.4	2.42±0.19
11	4.48±0.24	3.92±0.46	0.94±0.35	0.72±0.20	3.63±0.76	3.65±0.89	1.22±0.29	1.26±0.29	2.28±0.64	2.18±0.66
12	4.23±0.28	3.83±0.52	0.76±0.39	0.61±0.23	3.73±0.46	3.94±0.7	1.65±0.47	1.42±0.23	2.14±0.47	2.06±0.45
13	4.18±0.59	3.81±0.43	1.13±0.40	1.06±0.53	4.20±1.03	3.87±1.03	1.33±0.17	1.21±0.16	2.33±0.84	2.16±0.88
14	4.20±0.60	3.79±0.52	0.76±0.19	0.74±0.3	3.65±0.37	3.66±0.71	1.54±0.28	1.58±0.31	1.88±0.35	2.01±0.64
15	3.83±0.54	4.12±0.68	0.77±0.16	0.67±0.14	3.84±0.32	3.39±0.29	1.66±0.34	1.55±0.27	1.92±0.47	1.69±0.35
16	3.93±0.65	3.40±0.54	0.73±0.35	0.85±0.26	4.02±0.41	3.47±0.50	1.46±0.18	1.28±0.08	2.38±0.42	2.04±0.46

Data are presented as mean ± SD.

EI = emulsified isoflurane; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Tchol = total cholesterol; TG = total glyceride.

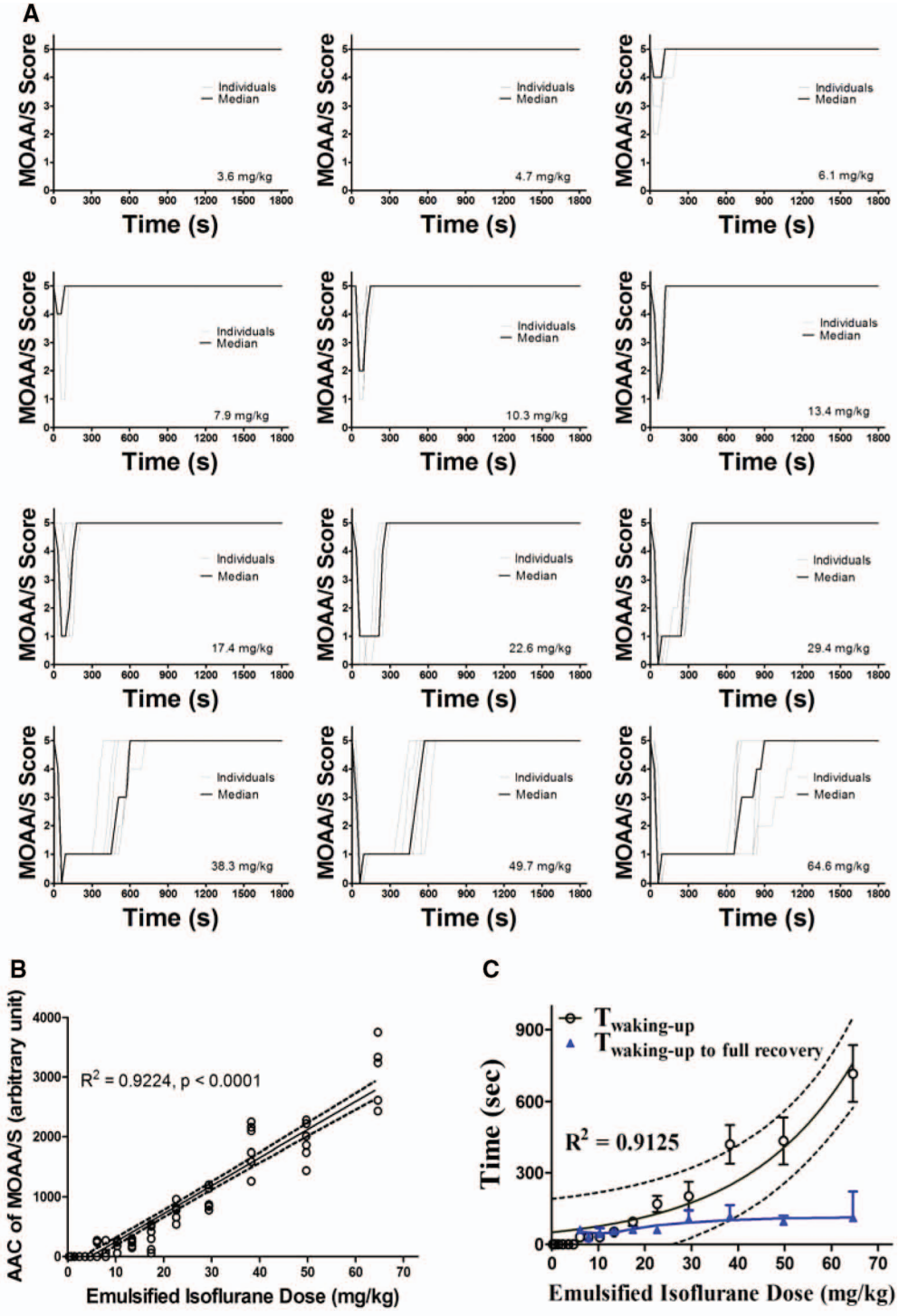

Fig. 2. Dose-dependent sedative/anesthetic effect of emulsified isoflurane. (*A*) Individual Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scores were summarized by dose levels, in which *gray lines* stand for individual volunteers whereas *black lines* stand for the mean values. At dose level of 4.7 mg/kg or smaller, no change in MOAA/S was observed (data not shown for doses lower than 3.6 mg/kg). (*B*) A linear correlation was found between the reduced consciousness, reflected by the area above MOAA/S curve and the dose of emulsified isoflurane ($R^2 = 0.9224$; $P < 0.0001$). The *solid line* indicates the best-fit line and the *dash lines* indicate the 95% confidence band of the best-fit line. (*C*) Larger dose of emulsified isoflurane caused statistically longer waking-up time ($T_{Waking\text{-}up}$) in anesthetized volunteers ($P < 0.0001$ when waking-up times were compared between dose levels). The increase in waking-up time was well simulated by an exponential growth function (*black line*, $R^2 = 0.9125$), where the *solid line* indicates the best-fit line and the *dash lines* indicate the 95% confidence band of the best-fit line. The time from waking-up to full recovery ($T_{waking\text{-}up\ to\ full\ recovery}$) was only slightly increased and was similar between dose levels (blue line, $P = 0.5348$). AAC = area above the curve; $T_{Waking\text{-}up}$ = waking-up time; $T_{waking\text{-}up\ to\ full\ recovery}$ = time from waking-up to full recovery.

Table 10. Clinical Pharmacology of EI

Isoflurane (mg/kg)	LOC	LOC			ET_{ISO} Peak (%)	$T_{ET\ Iso,\ Peak}$ (s)	BIS_{Lowest}	$T_{BIS,\ Lowest}$ (s)	ROC		$T_{Waking-up}$ (s)	$T_{waking-up\ to\ full\ recovery}$ (s)	Immobility
	LOC	Time (s)	BIS	ET_{ISO} (%)					BIS	ET_{ISO} (%)			
6.1	1/4 (25.0%)	30	95	0.95	0.95	60	79	120	84	0	30	60	0/1 (0%)
7.9	1/4 (25.0%)	60	97	0.95	0.95	120	87	90	97	0.1	30	30	0/1 (0%)
10.3	2/4 (50.0%)	30 ± 8	97 ± 1	1.12 ± 0.34	1.29 ± 0.11	90 ± 42	64 ± 21	87 ± 17	64 ± 21	0.15 ± 0.11	40 ± 21	45 ± 21	1/4 (25.0%)
13.4	5/6 (83.3%)	49 ± 9	97 ± 1	1.04 ± 0.17	1.38 ± 0.10	47 ± 8	55 ± 29	90 ± 19	49 ± 38	0.12 ± 0.10	51 ± 12	55 ± 12	2/6 (33.3%)
17.4	4/6 (66.7%)	36 ± 4	93 ± 7	1.65 ± 0.52	1.71 ± 0.49	43 ± 7	44 ± 24	106 ± 30	63 ± 20	0.05 ± 0.11	93 ± 18	60 ± 0	3/6 (50.0%)
22.6	6/6 (100%)	42 ± 6	95 ± 2	1.72 ± 0.39	2.21 ± 0.40	55 ± 4	26 ± 15	130 ± 16	65 ± 20	0.06 ± 0.1	168 ± 34	60 ± 0	3/6 (50.0%)
29.4	6/6 (100%)	44 ± 10	94 ± 2	1.97 ± 0.36	2.15 ± 0.41	56 ± 9	22 ± 9	123 ± 17	73 ± 14	0.08 ± 0.09	201 ± 60	105 ± 37	5/6 (83.3%)
38.3	6/6 (100%)	41 ± 6	87 ± 16	1.47 ± 0.85	2.31 ± 0.44	62 ± 20	17 ± 3	140 ± 37	76 ± 4	0.15 ± 0.08	419 ± 81	115 ± 48	5/6 (83.3%)
49.7	6/6 (100%)	40 ± 11	93 ± 5	2.1 ± 0.59	2.37 ± 0.35	58 ± 35	22 ± 12	176 ± 39	78 ± 6	0.15 ± 0.12	433 ± 99	95 ± 23	5/6 (83.3%)
64.6	6/6 (100%)	47 ± 12	91 ± 5	1.85 ± 0.35	3.13 ± 0.86	114 ± 21	20 ± 11	166 ± 80	78 ± 2	0.16 ± 0.09	716 ± 118	110 ± 110	6/6 (100%)
P value		0.4035	0.4710	0.0959	0.0004	<0.0001	0.0031	0.01112	0.1067	0.5100	<0.0001	0.5348	NA
General		42 ± 9	92 ± 8	1.74 ± 0.56					71 ± 17	0.11 ± 0.099		84 ± 68	–

Data are presented as mean ± SD, starting from the dose level at which first case of LOC was observed.

BIS_{Lowest} = lowest BIS value after EI administration; EI = emulsified isoflurane; ET_{Iso} = end-tidal isoflurane concentration; $ET_{ISO,\ Peak}$ = peak end-tidal isoflurane concentration after EI administration; LOC = loss of consciousness; immobility = prevention of purposeful movement to single standard electrical stimulus of 50 mA for 0.2 ms; ROC = recovery of consciousness; $T_{BIS,\ Lowest}$ = time needed for lowest BIS value; $T_{ET\ Iso,\ Peak}$ = time needed for development of peak end-tidal isoflurane concentration; $T_{Waking-up}$ = waking-up time; $T_{waking-up\ to\ full\ recovery}$ = time from waking-up to full recovery.

ED_{50} was loss of righting reflex and the therapeutic index was estimated to be 3. Here, loss of righting reflex was considered comparable/equivalent[17] to LOC in our study. Lack of effective anesthetic care in face of EI-induced respiratory/circulatory depression may account for smaller therapeutic index in animals. In this phase I clinical trial, some volunteers did require intervention (mask ventilation for 11 suffering apnea and vassopressor for one suffering moderate hypotension). But the dose required to trigger these interventions (≥38.3 mg/kg) was higher than the minimal 100% effective dose of 22.6 mg/kg. More importantly, with routine anesthetic care all the affected volunteers were easily maintained as stable and recovered uneventfully. Therefore, in our tested dose range from 0.29 to 64.64 mg/kg, EI could be safely administrated by trained anesthesiologists.

Paradoxical abdominal movement was observed in 11 of 18 volunteers (61.1%) receiving dose of 38.3 mg/kg or higher, among which apnea developed and mask ventilation was required in 10 volunteers. The cause of this paradoxical abdominal movement was not clear but it was unlike to be laryngospasm or bronchospasm, because no stridor was heard and no abnormal high resistance was sensed with mask ventilation. It would not be something more ominous because oxygen saturation in these affected volunteers was well maintained with mask ventilation and all the affected volunteers recovered uneventfully without any other intervention.

The normal results found in all the postdose tests further confirmed the safety of EI in humans. Special attention was given to blood lipid level, because the solvent of EI is 30% Intralipid®. With the maximal Intralipid® load of 0.6 ml/kg applied in this study, no change in triglyceride, cholesterol, low-density lipoprotein, or blood glucose level was detected in the 24-h postdose test.

As mentioned in a recent editorial,[18] the solubility, carrying capacity, and long-term stability should be considered for volatile anesthetic emulsion safety. EI of 8% used in this study has following advantages: (1) the solvent itself, 30% Intralipid®, has been safely used in clinical practice for decades; (2) as described in our previous article,[9] concentration of 8% EI is lower than the saturated concentration of 8.24% of isoflurane in 30% Intralipid® at 20°C; therefore, the possibility for liquid isoflurane being separated from the emulsion was minimal; (3) for intravenous anesthesia induction, a 20-ml syringe usually is the biggest. In this study, we found that the minimal 100% effective volume of 8% EI for producing unconsciousness is 0.19 ml/kg, and 20 ml of 8% EI should carry enough isoflurane for producing unconsciousness in patients weighing 100 kg or less. (4) With 2-yr storage at room temperature for 8% EI tested in this study, no particle growth and no phase separation was detected (unpublished data provided by Nan Luo, Bachelor of Science, Yichang Humanwell Pharmaceutical Co., Ltd., Yichang, Hubei, P. R. China. These data were collected from September 2003 to September 2005 to investigate the long-term stability of 8% EI. These data have been reported

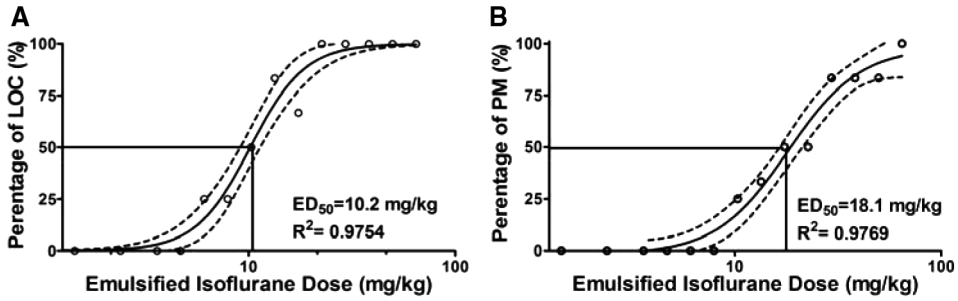

Fig. 3. Estimation of ED_{50} of emulsified isoflurane for producing loss of consciousness (LOC) and immobility. (*A*) *Circles* indicate the percentage of volunteers in whom LOC was observed. LOC was defined as failure to follow loud verbal command to open his or her eyes. The *solid line* shows the result of probit analysis and the *dashed lines* indicate the 95% CIs of the best-fit line. (*B*) *Circles* indicate the percentage of volunteers in whom immobility to standard electrical stimulation (50 mA, 0.2 ms) was observed. Prevention of purposeful movement in unstimulated extremities to this electrical stimulus was regarded as immobility. The *solid line* shows the result of probit analysis and the *dashed lines* indicate the 95% CIs of the best-fit line. ED_{50} = median effective dose; PM = Prevention of purposeful Movement in unstimulated extremities.

to Chinese Food and Drug Administration in China for approval of this phase I clinical trial). In addition, no animal or human volunteers developed any complication related to instability of 8% EI, such as chest or back pain, cyanosis, or dyspnea.

In this trial, EI produced dose-dependent sedation/anesthesia in human subjects. No change in MOAA/S was observed in subjects receiving doses of 4.7 mg/kg or lower. Doses in range of 6.1 to 17.4 mg/kg produced anesthesia (MOAA/S = 0) in some volunteers but minimal sedation in others (MOAA/S = 4), with a large interindividual variability, which suggests that bolus injection of EI at subanesthetic doses may not be suitable for sedation. Doses of 22.6 mg/kg or higher produced 100% LOC with a stable onset time of 40 s. The duration of LOC correlated well with the dose of EI from 168 ± 34 s at 22.6 mg/kg to 716 ± 118 s at 64.6 mg/kg (table 10 and fig. 2). This good correlation between duration of LOC and single dose of EI suggests that general anesthesia could be well controlled for short anesthesia cases, for example, endoscopic examination. Theoretically, apnea induced by EI could delay the recovery from LOC due to pulmonary elimination of isoflurane. But in this trial, the longer duration of LOC was unlikely to be associated with apnea because effective mask ventilation was initiated once apnea was noted. Therefore, the duration of LOC is mainly determined by the dose of EI.

In conclusion, this first-in-human trial showed that EI could be safely injected by anesthesiologists in the dose range of 0.3 to 64.6 mg/kg. At 22.6 mg/kg or higher, EI was effective in producing rapid onset and predictable general anesthesia in healthy human volunteers.

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Competing Interests

The authors declare no competing interests.

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