

Enteral ventilation technology to combat severe respiratory failure

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Introduction

In 2020, coronavirus disease 2019 (COVID-19) brought about an unprecedented crisis in humanity. As of July 2021, of the 196 million infected people worldwide, approximately 14% and 5% were estimated to be severely ill and fatal, respectively. Sadly, more than 4 million patients died, with an increased fatality rate of 26.9% in the elderly population. In critically ill patients with respiratory failure, mechanical ventilation and extracorporeal membrane oxygenation are used as standard treatments to provide vital oxygenation. However, in general, their use requires advanced medical resources and highly invasive procedures, potentially making the elderly contraindicated. Thus, developing another supportive oxygenation approach is urgently needed to help countless patients under respiratory distressed conditions.

To this end, our group recently established an “enteral ventilation via anus (EVA)” technique with transanal oxygen delivery by leveraging the highly vascularized distal bowel region [1]. This emerging medical technology can assist respiratory function with minimal invasiveness independent of airway or extracorporeal ventilation. Herein, we discuss the state-of-the-art development and clinical potential of EVA techniques.

Diverse Breathing Modes in Living Organisms

Four billion years ago, there was no oxygen on the ancient earth, and living organisms evolved in an anaerobic environment. About 2.5 to 2 billion years ago, the oxygen concentration began to increase gradually. The reason behind this is thought to be that cyanobacteria and a variety of plants that emerged later began to produce organic matter and vast amounts of oxygen gas from carbon dioxide and water through photosynthesis. The increase in the concentration of oxygen in the atmosphere provides an aerobic environment that allows organisms to acquire new energy

metabolism, resulting in the flourishing of multicellular and terrestrial organisms with the emergence of various respiration methods [2–11] (Table 1).

The respiratory system of mammals, including humans, is primarily via the lungs; however, by comparing the breathing systems of diverse organisms, various modes of respiration independent of the lungs were acquired during the process of adaptive evolution. For example, in birds and dinosaurs, air flows through the lungs in one direction and is absorbed into the bloodstream. Some frogs and salamanders in Southeast Asia do not have lungs and sustain themselves through cutaneous respiration [2, 4, 5]. Furthermore, among mammals, particular species, such as naked mole rats, obtain energy by activating specialized fructose metabolic pathways under hypoxia; and the genetic approach revealed that sulfur respiration, as well as oxygen, can be utilized for energy metabolism in laboratory mice [6, 7].

Some aquatic organisms acquire accessory modes of respiration that do not involve the gills or skins. Loaches, such as *Misgurnus anguillicaudatus*, and catfish, such as *Corydoras* species, use their gut to maintain systemic oxygenation under hypoxic conditions [8–10]. In particular, the loach develops a lung-ventilation-independent intestinal breathing system that works in hindgut area under extremely hypoxic conditions. The histopathological requirements for intestinal respiration include: 1. abundant capillaries; 2. thinning of the intestinal epithelium; and 3. anatomical proximity to areas of air uptake.

The intestine of the loach is anatomically divided into three regions, cranial to caudal, namely, the foregut, midgut, and hindgut, each of which is approximately 2 cm in length. Some reports have evaluated the hindgut area responsible for intestinal breathing activity at the molecular and cellular levels. In the 1960s in Japan, for example, histopathological studies on the distance between intestinal epithelial cells and capillaries in the hindgut and comparison with the foregut and midgut showed that the hindgut has a characteristic structure with a more capillary-rich and thinner intestinal epithelium [3, 12]. Recently, extended

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Table 1. Organisms breathe in a wide variety of ways

Generic name	Scientific name	Methods of respiration and metabolism	Method of accessory respiration	Reference
Loach	<i>Misgurnus anguillicandatus</i> etc.	Intestinal respiration	Oxygen is taken into the (posterior) intestine under hypoxia.	Arch Histol Jpn, 1963 [3] etc.
Corydoras	<i>Corydoras paleatus</i>	Intestinal respiration	Oxygen is taken into the (posterior) intestine under hypoxia.	Rev Biol Trop, 2016 [8]
Sea cucumber	<i>Apostichopus japonicus</i>	Intestinal respiration	Takes in oxygen from the respiratory tree in the intestines.	PeerJ, 2018 [9]
Sea spider	<i>Colossendeis megalonyx</i> etc.	Intestinal respiration	Takes in oxygen from the intestines in the legs.	Curr Biol, 2017 [10]
Naked mole rat	<i>Heterocephalus glaber</i>	Fructose metabolism	Energy is produced by fructose metabolism under hypoxic and anoxic conditions.	Science, 2017 [6]
Mouse	<i>Mus musculus</i>	Sulfur metabolism	Use sulfur metabolites for energy production.	Nat Commun, 2017 [7]
Frog	<i>Borbourula kalimantanensis</i>	Cutaneous respiration	Has no lungs and sustains life through skin respiration.	Curr Biol, 2008 [2]
Salamander	<i>Onychodactylus japonicus</i> etc.	Cutaneous respiration	Has no lungs and maintains life by breathing through the mouth and skin.	J Anat, 2017 [4]; Proc Biol Sci, 2018 [5]

Adapted from reference [11]. Okabe, R., Yoneyama, Y., Takebe, T. Enteral ventilation via anus for severe respiratory failure. *Cardioangiolog* 89, 212–218, 2021 (in Japanese).

molecular analyses have been reported, including analysis of vascular endothelial growth factor (VEGF) expression and signaling molecules related to intestinal breathing in the hindgut, comparative transcriptome analysis of the gut at developmentally different stages before and after the acquisition of intestinal breathing function, and analysis of ammonia secretion capacity along the digestive tract [13–16]. Notably, in adult loaches, the *Vegfa* and *Vegfb* genes are highly expressed in the hindgut, and their expression is suppressed when sufficient oxygen is supplied. In addition, the expression of transporters related to digestion, including H⁺: peptide cotransporter (PEPT1a; *Slc15a1*), is suppressed specifically in the hindgut compared to the foregut and midgut [17], indicating that loach regulates digestive and respiratory functions in the hindgut to survive under hypoxia.

While some exciting progress has been made in the biology of aquatic organisms, few attempts have been made to translate such mechanisms of intestinal respiration in mammals. Thus, we explored intestinal respiration in mammals and examined the feasibility of supplying oxygen to the blood via the gut.

Mechanism of Enteral Ventilation via the Anus (EVA)

The loach takes oxygen gas from the atmosphere or oxygen contained in the mud through the hindgut and supplies oxygen to the bloodstream through the thin hindgut mucosa. One of the histopathological findings of the loach hindgut is that the mucosal epithelium is extremely thin, with abundant capillaries in physical proximity to the blood vessels. When we analyzed murine distal rectum corresponding to the hindgut, there are histological similarities in the distal end of the rectum, a region of transition to the anal canal. In this region, the thickness of the epithelium thins abruptly with abundant blood circulation, called the

superior rectal venous plexus. We also revealed that in mice, mechanical abrasion to thin the mucosal epithelial layer of the rectal area transiently induced the expression of *Vegfa* and *Anxa1*, which are reportedly involved in angiogenesis and epithelial remodeling, respectively, in a similar way as the loach hindgut.

Therefore, we first designed an intestinal gas ventilation (hereafter abbreviated as gas-EVA or g-EVA) system in which pure oxygen is directly administered into the large intestine of mice. The rectum of the mice was mechanically abraded within 4 cm of the anus side, and pure oxygen gas was then administered to the same area. Using a type I respiratory failure mouse model induced by ventilatory control, we found that the administration of pure oxygen gas to the intestinal lumen improved respiratory symptoms, such as a marked improvement in arterial blood oxygen concentration, the release of hypoxic symptoms in peripheral organs, and a significant improvement of behavioral findings and survival rate (Fig. 1A–C). Even in a lethal hypoxic state, the g-EVA method allowed survival for more than 50 min and marked improvement in arterial oxygen saturation. These attempts to mimic the histopathological features of the loach hindgut indicated that systemic oxygenation was possible in mice by the administration of pure oxygen to a physically thinned intestine. We also consider the possibility that changes in digestive function, such as a decrease in transporter activity due to abrasion, contribute to successful oxygenation, as observed in loach. Further studies at the physiological and molecular levels are the key to maximizing efficient oxygen in the gut.

We then devised liquid ventilation (hereafter abbreviated as liquid-EVA or l-EVA) in mice and micro mini pigs using perfluorocarbon (PFC) enema towards clinical applications. PFC is a fluorinated compound with excellent oxygen and carbon dioxide transport and dissolution capacities (50 ml O₂/dl and 160–210 ml CO₂/dl, respectively). In mice, without requiring intestinal abrasion, the ratio of measured

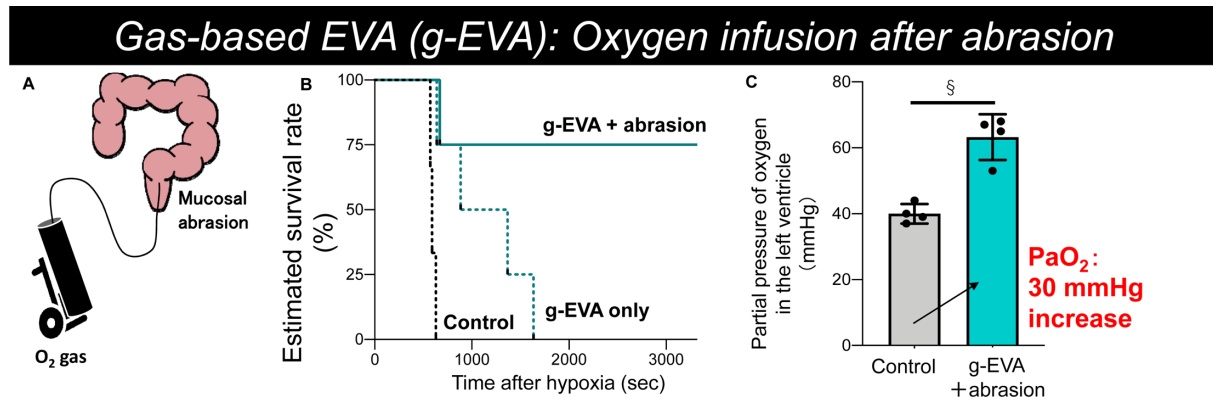


Fig. 1. Resolving fatal hypoxemia by gas-based EVA (g-EVA). Results are cited from [1]. **A.** Overview of g-EVA with mucosal abrasion. **B.** Survival rate of control, g-EVA, and g-EVA with mucosal abrasion groups in 8% lethally hypoxic ventilated mice. **C.** Partial pressure of oxygen in the left ventricle of the heart by g-EVA with mucosal abrasion.

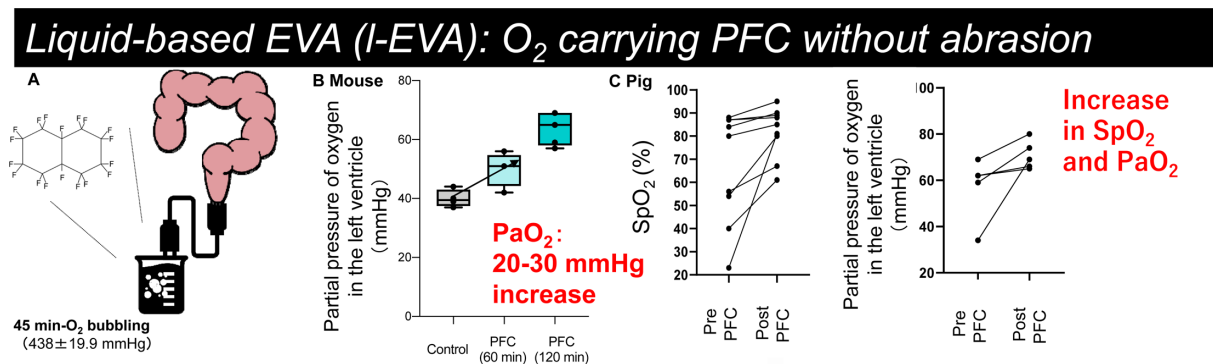


Fig. 2. Resolving fatal hypoxemia using liquid-based EVA (l-EVA). Results are cited from [1]. **A.** Overview of l-EVA using perfluorocarbon (PFC). **B.** Improvement of the partial pressure of oxygen in the left ventricle at 60 and 120 min after PFC administration in lethally hypoxic ventilated mice. **C.** Percutaneous oxygen saturation and partial pressure of oxygen before and after PFC administration in lethally hypoxic-ventilated pigs.

partial pressure of oxygen in the left heart ventricle was approximately 1.6 times higher in the l-EVA treatment group than in the control group (40.0 ± 2.94 mmHg and 63.8 ± 5.59 mmHg, respectively), and the lethal type I respiratory failure was dramatically improved (Fig. 2A, 2B). In micro mini pigs, a single intrarectal administration of PFC improved severe respiratory failure. Furthermore, repeated intrarectal administration of PFC was found to improve respiratory failure for approximately 60 min. The ratio of partial pressure of oxygen measured in arterial blood was approximately 1.2-fold higher in the l-EVA groups (57.2 ± 13.5 mmHg and 70.8 ± 6.22 mmHg, respectively), and hypoxemia was improved (Fig. 2C). Thus, the proof of concept was obtained at the large animal level, where the circulating plasma volume is similar to that of humans, suggesting the practicality of this application to patients. The improvement of arterial blood oxygenation demonstrated in our study using mice and micro-mini pigs would be effective in the treatment of patients with severe respiratory failure when applied to humans.

History of Clinical Application and Future Development

Historically, attempts to improve systemic oxygenation by oxygen gas insufflation into the digestive tract have been made for a long time. For example, in Europe in the 1700s, there is a record that cigarette smoke, aka Tobacco Enema, was put into the rectum of patients who were drowned or suffocated. According to a featured article published at the Lancet magazine, the above-mentioned tobacco enema therapy saved 990 patients [19], until the standard of care, such as ventilators, was established. Later, in the 1950s, the literature indicated that gastric and rectal oxygenation was effective for asphyxia in newborns [18–24]. However, intestine-mediated therapy at that time was poor on a medical basis, and there were risks associated with gas insufflation. To date, no follow-up studies have been conducted [25].

In contrast, after Clark *et al.* first reported liquid respiration via the airways using PFC with excellent oxygen solubility in 1960, studies with various types of PFC have been reported, including basic experiments on local or systemic oxygenation via the intestine [26–29]. The use of PFC

to treat hypoxic environments is also being studied in the field of diving medicine, which is used for the treatment of diving diseases as well as for the treatment and prevention of barometric pressure disorders [30]. In addition, PFC has been tested as artificial blood in multiple phases (I, II, and III) clinical trials. Some types of PFC have been administered to more than 35,000 people, and their safety has been confirmed [31–33]. Clinical trials are currently underway for intracranial diseases such as cerebral infarction. As more extensive research is being conducted on PFC as an oxygen carrier, it is expected to deliver a new treatment for ischemic tissues such as ischemic heart disease and cerebral ischemia (ClinicalTrials.gov Identifier: NCT03463551).

A wide variety of clinical applications will be envisioned once intestinal breathing is established. For example, in the field of emergency and intensive care, potential indications, including pneumonia, heart failure, airway burns, bronchial asthma, severe attacks, and cases where neither intubate nor ventilate are expected. Furthermore, although lung transplantation has been established as a curative procedure for patients with chronic respiratory failure, there are multiple exclusion criteria due to limited organ availability. The donor shortage is becoming increasingly serious, and patients on the waiting list need adjunctive respiratory treatment. Thus, there is an unmet need for respiratory support for acute/chronic respiratory failure, such as the need to expand the indications of conventional methods and the search for strategies to protect autologous lungs.

Although there are currently some unsolved issues, including safety and tolerability of PFC enema in humans [34], EVA technology has the potential to act as a bridge to invasive ventilation procedures and, in the long-term, potentially extended towards chronic respiratory problems.

Conflict of Interest

Y.Y., R.O. and T.T. are inventors for EVA technology and equity holders for EVA Therapeutics, Inc.

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