Neuroanesthesia and Intensive Care

Olprinone, a phosphodiesterase III inhibitor, does not affect hypoxia-induced pial arteriolar dilatation in rabbits

[L'olprinone, un inhibiteur de la phosphodiesterase III, n'a pas d'effet sur la dilatation artériolaire pie-mérienne chez les lapins]

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Purpose: Olprinone, a phosphodiesterase III inhibitor, is used for the treatment of heart failure or asthma. Such patients may suffer from hypoxia. However, the effect of olprinone on the cerebral vasodilator response to hypoxia remains unclear.

Methods: Rabbits were anesthetized and ventilated mechanically. The pial arteriolar diameter was determined using a cranial window and intravital microscopy. Hypoxia was induced twice in the same animal by reducing FiO_2 to 0.1. The first episode was induced during an infusion of saline, and the second during an infusion of saline (saline group; $n = 8$) or olprinone ($1 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, OLPI group; $n = 8$ or $10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, OLPI0 group; $n = 8$). The pial arteriolar responses to hypoxia were recorded and compared between the two episodes of hypoxia in each group.

Results: Blood gas data in the first hypoxic challenge were identical to those in the second challenge in each group. Pial arteriolar diameter increased significantly during hypoxia. In arterioles between 50–100 μm diameter, first and second hypoxia-induced pial arteriolar dilatation in OLPI were $13 \pm 6\%$ and $10 \pm 7\%$ respectively ($P = 0.574$) and those in OLPI0 were $16 \pm 6\%$ and $15 \pm 7\%$ respectively ($P = 0.606$). In arterioles between 25–50 μm , the results were the same as in arterioles between 50–100 μm .

Conclusion: Olprinone does not affect the hypoxia-induced dilatation of pial arterioles in pentobarbital anesthetized rabbits.

Objectif : L'olprinone, un inhibiteur de la phosphodiesterase III, est utilisé comme traitement de l'insuffisance cardiaque ou l'asthme. L'hypoxie est aussi possible dans ces cas. Cependant, l'effet de l'olprinone sur la réponse vasodilatatrice cérébrale à l'hypoxie n'est pas encore très clair.

Méthode : Des lapins ont été anesthésiés et ventilés mécaniquement. Le diamètre artériolaire pie-mérien a été déterminé grâce à une fenêtre crânienne et à la microscopie intravital. L'hypoxie a été induite deux fois chez le même animal en réduisant la FiO_2 à 0,1. Le premier épisode a été induit pendant la perfusion d'une solution saline et le second, pendant la perfusion d'une solution saline (groupe saline ; $n = 8$) ou d'olprinone ($1 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, groupe OLPI ; $n = 8$ ou $10 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, groupe OLPI0 ; $n = 8$). Les réponses artérielaires pie-mériennes à l'hypoxie ont été enregistrées et comparées pour les deux épisodes d'hypoxie dans chacun des groupes.

Résultats : Les données sur la gazométrie du premier épisode d'hypoxie ont été identiques à celles du second épisode dans les deux groupes. Le diamètre artériolaire pie-mérien a augmenté de façon significative pendant l'hypoxie. Dans les artérioles de 50–100 μm de diamètre, la première et la seconde dilatation artériolaire pie-mérienne induite par l'hypoxie ont été, dans le groupe OLPI, de $13 \pm 6\%$ et de $10 \pm 7\%$ respectivement ($P = 0,574$) et dans le groupe OLPI0, de $16 \pm 6\%$ et de $15 \pm 7\%$ respectivement ($P = 0,606$). Dans les artérioles de 25–50 μm , les résultats ont été les mêmes que pour les artérioles de 50–100 μm .

Conclusion : L'olprinone ne modifie pas la dilatation des artérioles pie-mériennes induite par l'hypoxie chez des lapins anesthésiés au pentobarbital.

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SEVERE asthma may be life-threatening and is treated with a variety of drugs. Theophylline, a widely used bronchodilator attenuates hypoxia-induced cerebral vasodilatation,¹⁻⁴ which supports the oxygen supply to the brain during a hypoxic state by increasing cerebral blood flow.^{5,6} Therefore, we aim to use bronchodilators that do not affect the hypoxia-induced cerebral vasodilatation. Recently, it was reported that olprinone, a phosphodiesterase III (PDE III) inhibitor, has a bronchodilator effect in human patients suffering an asthma attack,⁷ and in dogs with bronchoconstriction induced by 5HT.⁸ These reports suggest that olprinone could be a safe drug to use to treat severe asthma. However, the effect of olprinone on the vasodilator response to hypoxia, which is sometimes associated with severe asthma, remains unclear. The aim of this study was to examine the effect of olprinone on pial vessel diameter during hypoxia in a rabbit model of hypoxia.

Materials and methods

Animal preparation

This study was approved by the Animal Use and Care Committee of the University of Tsukuba. Twenty-four adult Japanese white male rabbits weighing between 2.5 and 3.0 kg were used in this experiment. The rabbits were purchased from the Tokyo Laboratory Animals Science CO., Ltd. (Tokyo, Japan), and were inbred.

The rabbits were anesthetized with pentobarbital (50 mg *iv*). A tracheostomy was then performed, and the lungs were ventilated mechanically with an inspired oxygen concentration of 30–35%. End-tidal CO₂ was monitored continuously (Datex Normocap CO₂ monitor, Datex Instrumentarium Co., Helsinki, Finland), and tidal volume and respiratory rate were adjusted to maintain the end-expiratory CO₂ level between 30–40 mmHg. Additional doses of pentobarbital (25 mg each) were administered when it was necessary during the tracheostomy, and anesthesia was then maintained with a continuous *iv* infusion of pentobarbital sodium (10 mg·hr⁻¹) and pancuronium (0.4 mg·hr⁻¹).

A catheter was placed in a femoral artery to measure mean arterial blood pressure (MAP) and obtain arterial blood samples. A femoral vein was cannulated for infusion of drugs. Arterial blood pH, PCO₂, and PO₂ were measured with a blood gas analyzer (288 Blood Gas System, Ciba Corning Diagnostics Co., MA, USA) and maintained at normal levels, except during the experimental induction of hypoxia.

Each animal was immobilized in a stereotactic frame, and a closed cranial window was placed over

the right parietal cortex for visualization of the pial microcirculation as reported elsewhere.⁹ The scalp was incised and retracted, and the temporal muscle was removed. A hole approximately 8 mm in diameter was made in the parietal bone. The dura was reflected and removed and a plastic ring with a glass coverslip was placed over the hole and secured with dental acrylic. Two ports served as an inlet and an outlet for artificial cerebrospinal fluid (CSF). The composition of artificial CSF was Na⁺ 151, K⁺ 4, Ca²⁺ 3, Cl⁻ 110 mEq·dl⁻¹, and glucose 100 mg·dl⁻¹.

We studied two arterioles (diameters between 25–50 µm and 50–100 µm) in each animal. Diameters of pial arterioles were observed with intravital microscopy (Olympus BX50WI, Olympus Optical Co., Ltd., Tokyo, Japan). The image was projected by a charge-coupled device video camera (model CS 900; Sony Co., Tokyo, Japan) onto a colour high-resolution video monitor (model CPDE220; Sony Co., Tokyo, Japan) by use of a recording lens (PE 2.5x; Olympus Optical Co., Ltd., Tokyo, Japan) and an objective lens (UplanFl 4x; Olympus Optical Co., Ltd, Tokyo, Japan). The images were stored in a personal computer system for subsequent analysis. The diameters of pial arterioles were measured using Canvas™ (Deneba Systems Inc., Miami, Florida, USA).⁸

Experimental protocol

After surgery, we waited a minimum of 30 min before initiating the experimental protocol. At each measurement time, the arteriolar diameters, MAP, heart rate (HR), and blood gas data (pH, PaCO₂, PaO₂) were obtained.

Hypoxia was induced by administering 10% O₂ in nitrogen to the animals during five minutes. In each experiment hypoxia was induced twice. The first hypoxic episode served as control and was induced during saline infusion (5 mL·hr⁻¹). The second was induced during olprinone infusion. We waited a minimum of 30 min between hypoxic episodes. Two different concentrations of olprinone in saline were infused at a rate of 1 µg·kg⁻¹·min⁻¹ (OLP1 group; *n* = 8) or 10 µg·kg⁻¹·min⁻¹ (OLP10 group; *n* = 8), and hypoxia was induced 20 min after infusion of olprinone. In the saline time control group (saline group; *n* = 8), hypoxia was induced twice during saline infusion (5 mL·hr⁻¹).

We also examined the effects of olprinone on pial arterioles by comparing diameters before and ten minutes after infusion of olprinone.

Statistical analysis

Data are presented as a mean ± SD. Hemodynamic and blood gas data under control conditions and dur-

FIGURE The effect of olprinone on hypoxia-induced cerebral vasodilation. Pial arterial diameter increased significantly during hypoxia in all groups. The second response was not different from the first response in all groups. First: first hypoxic episode; second: second hypoxic episode; saline: both hypoxic episodes during saline infusion; OLP1; second hypoxic episode during infusion of olprinone 1 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; OLP10; second hypoxic episode during infusion of olprinone 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. * $P < 0.05$ vs normoxia.

ing interventions were compared using analysis of variance (ANOVA), and post-hoc testing by the Scheffe's F test. The change in diameter from baseline after olprinone infusion was compared using a paired t test. The percent changes in diameter during first and second hypoxia were compared using Wilcoxon test. A value of $P < 0.05$ was considered significant.

Results

Effects of olprinone on hypoxia-induced vasodilation

Hypoxia dilated cerebral arterioles significantly (Figure). In arterioles between 50–100 μm diameter, first and second hypoxia-induced pial arteriolar dilatation in saline group were $12 \pm 6\%$ and $14 \pm 6\%$ ($P = 0.292$), those in OLP1 were $13 \pm 6\%$ and $10 \pm 7\%$ ($P = 0.574$), and those in OLP10 were $16 \pm 6\%$ and $15 \pm 7\%$ respectively ($P = 0.606$). In arterioles between 25–50 μm , the results were similar (Figure). Olprinone did not affect dilatation of pial arterioles in response to hypoxia (Figure).

Exposure of rabbits to 10% inspired oxygen reduced PaO_2 significantly without affecting pH or PaCO_2 in all groups (Table I). Hypoxia increased MAP and decreased HR significantly (Table I).

Responses of pial arterioles to olprinone

Intravenous infusion of olprinone 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ but not 1 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ decreased MAP and increased HR slightly (Table I). Olprinone did not change PaO_2 ,

PaCO_2 , and pH (Table I). Both infusion rates of olprinone produced dilatation of pial arterioles of 25–50 μm diameter (Table II).

Discussion

The major novel finding of the present study is that, in pentobarbital anesthetized rabbits, olprinone does not affect hypoxia-induced cerebral vasodilatation. In addition, we found that olprinone produces dilatation of cerebral arterioles 25–50 μm in diameter.

Olprinone, a PDE III inhibitor, has positive inotropic and vasodilator effects and has been used to treat patients with heart failure.^{10–13} Recently, it also has been reported that olprinone has a bronchodilator effect, and that bronchial asthma may be treated by *iv* olprinone in humans⁷ and animals,⁸ and by inhalation of olprinone in humans.¹⁴ Although the role of olprinone in the treatment of asthma remains to be established, it has more potent spasmolytic effect on the pulmonary vasculature and is less arrhythmogenic compared to aminophylline.⁸ As we have shown, olprinone has few effects on cerebral arterioles during hypoxia, a possible additional benefit.

Although theophylline has been used widely to treat bronchial asthma, it has been shown to attenuate cerebral vasodilatation in response to hypoxia.^{1–4} Since the vasodilator response during hypoxia helps maintain blood supply to the brain,^{5,6} theophylline may not be recommended for hypoxic patients.⁴ From this point of view, olprinone may be a better choice than theophylline to treat severe asthma in hypoxic patients.

Theophylline is a phosphodiesterase inhibitor and also an adenosine receptor antagonist.^{15,16} Theophylline affects the cerebral vasodilatory response to hypoxia by blocking the adenosine receptor.^{1–3} The effect of olprinone or other PDE III inhibitors on the adenosine receptor of the cerebral vessels remains unclear. Based on our results, we speculate that olprinone does not antagonize the adenosine receptor in the cerebral arteriole.

In this study the animals were each exposed to hypoxia twice and results compared between the first and second hypoxic episode. Since the reduction of FiO_2 to 0.1 does not necessarily result in the same level of PaO_2 in each animal, variations in cerebral vasodilatation may have been important. However, by exposing each animal to hypoxia twice, we were able to compare vasodilatation in the same vessel before and after the infusion of olprinone.

In this study we tested 1 and 10 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ doses of olprinone. It remains possible that larger doses of olprinone may affect cerebral vasodilatation during hypoxia. However the doses of olprinone

TABLE I Hemodynamic and arterial blood gas analysis data

		Saline (n = 8)			OLP1 (n = 8)			OLP10 (n = 8)		
		Baseline	Infusion	Hypoxia	Baseline	Infusion	Hypoxia	Baseline	Infusion	Hypoxia
MAP	First	92 ± 10	92 ± 8	116 ± 14*#	90 ± 12	90 ± 10	107 ± 23*#	92 ± 7	92 ± 7	108 ± 6*#
(mmHg)	Second	88 ± 10	88 ± 7	104 ± 19*#	87 ± 12	86 ± 12	106 ± 22*#	90 ± 10	78 ± 10*§	97 ± 13#
HR	First	274 ± 54	268 ± 54	132 ± 35*#	279 ± 60	287 ± 53	129 ± 34*#	298 ± 33	308 ± 36	123 ± 44*#
(beats·min ⁻¹)	Second	293 ± 48	300 ± 44	142 ± 39*#	306 ± 30	307 ± 49	138 ± 33*#	297 ± 50	331 ± 51	183 ± 66*#§
pH	First	7.39 ± 0.04	7.39 ± 0.04	7.42 ± 0.03	7.40 ± 0.03	7.40 ± 0.03	7.41 ± 0.04	7.39 ± 0.03	7.40 ± 0.03	7.42 ± 0.05
	Second	7.40 ± 0.03	7.40 ± 0.03	7.42 ± 0.04	7.40 ± 0.03	7.40 ± 0.03	7.42 ± 0.04	7.41 ± 0.03	7.40 ± 0.04	7.40 ± 0.05
PaCO ₂	First	36 ± 2	36 ± 2	34 ± 4	36 ± 3	35 ± 3	35 ± 3	35 ± 2	35 ± 2	33 ± 3
(mmHg)	Second	35 ± 2	34 ± 2	34 ± 3	36 ± 2	36 ± 2	35 ± 3	35 ± 2	35 ± 2	34 ± 4
PaO ₂	First	118 ± 9	118 ± 9	36 ± 10*#	117 ± 10	117 ± 10	33 ± 6*#	121 ± 9	119 ± 11	38 ± 8*#
(mmHg)	Second	117 ± 8	116 ± 10	35 ± 9*#	123 ± 9	122 ± 10	33 ± 6*#	123 ± 6	120 ± 5	38 ± 9*#

Baseline = before infusion of saline or olprinone; Infusion = at ten minutes infusion of saline or olprinone; Hypoxia = data during hypoxia; First = first hypoxia episode during saline infusion (5 mL·hr⁻¹); Second = second hypoxia episode during infusion of saline 5 mL·hr⁻¹ (saline group), olprinone 1 µg·kg⁻¹·min⁻¹ (OLP1 group), or olprinone 10 µg·kg⁻¹·min⁻¹ (OLP10 group); MAP = mean arterial pressure; HR = heart rate; OLP = olprinone. Values are mean ± SD. *P < 0.05 *vs* baseline, #P < 0.05 *vs* infusion, §P < 0.05 *vs* First.

TABLE II Diameters of pial arterioles after olprinone infusion

		Arteriole (50–100 µm)		Arteriole (25–50 µm)	
		Baseline	Infusion	Baseline	Infusion
Saline	(n = 8)				
	saline	66 ± 16	67 ± 17	36 ± 8	37 ± 9
	saline	69 ± 19	68 ± 18	38 ± 10	37 ± 9
OLP1	(n = 8)				
	saline	80 ± 15	79 ± 14	40 ± 10	40 ± 9
	olprinone	77 ± 14	79 ± 15	38 ± 7	42 ± 8*
OLP10	(n = 8)				
	saline	77 ± 16	77 ± 17	39 ± 11	39 ± 10
	olprinone	79 ± 13	81 ± 13	37 ± 10	39 ± 11*

Baseline = diameter before infusion of saline or olprinone; infusion = diameter during infusion of saline or olprinone; saline = data during infusion of saline 5 mL·hr⁻¹; olprinone = data during infusion of olprinone 1 µg·kg⁻¹·min⁻¹ (OLP1 group), or olprinone 10 µg·kg⁻¹·min⁻¹ (OLP10 group). OLP = olprinone. Values are mean ± SD. *P < 0.05 *vs* baseline.

which produce bronchodilatation in humans⁷ and in animals⁸ were similar or smaller than the doses administered in this study.

The dilatory effect of olprinone was significant in arterioles of 25–50 µm diameter. The effect of olprinone on pial vessels of different diameters remains unclear and might be related to differences in the distribution of PDE III in the cerebral arterioles.

In summary, olprinone does not affect hypoxia-induced dilatation of pial arterioles in pentobarbital anesthetized rabbits. This characteristic of the drug, if documented in humans, may prove to be beneficial when treating heart failure or asthma in hypoxic patients.

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