Medical treatment using biomedical plasma techniques

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We performed to clarify the mechanism by which the irradiation / inhalation using atmospheric-pressure plasma source promotes disease treatments such as burn healing, lungs and heart disease treatment, bone regeneration, and recovery of hypoxic ischemic encephalopathy (HIE).

1. Introduction

Many studies have been conducted on novel plasmas in the fields of chemistry, solid-state physics, and nanomaterials. Such plasmas have a boundary reaction field in a liquid or a gas-liquid phase. Examples include liquid plasmas, which are plasmas generated in media (e.g., water and ionic solutions) that are denser than gases, and atmospheric pressure or in a gas flow. Atmospheric-pressure plasmas are indispensable for sterilizing, disinfecting, decomposing hazardous materials, and modifying material surfaces. They are also used in new fields of biomedical science. Clarifying the mechanisms of plasma technologies that are used in practical applications is of critical importance. To achieve this, it is important to understand plasma technology. Plasma technologies include plasma medicine such as medical sterilization and treatment using floating-electrode dielectric barrier discharge (FE-DBD), and the introduction of genetic material (DNA), proteins, and low-molecular-weight pharmaceutical compounds into cells using plasma-based molecular introduction devices. These technologies have been attracting considerable attention in recent years as practical applications of plasma.

2. Disease treatments with irradiation and inhalation by atmospheric pressure plasma source

2.1. Atmospheric pressure plasma source

A schematic diagram of the experimental setup as shown in Fig.1, the plasma source has a coaxial structure consisting of a 1-mm-diameter tungsten wire in a glass capillary (diameter of plasma generation area: 8 mm; tip diameter: 1 mm) with a grounded tubular electrode wrapped around the outside of the capillary. A high-voltage power supply provides a high voltage for plasma generation. The following conditions were used to generate the plasma: applied pulse voltage: 8 kV; frequency: 3 kHz; helium (He) gas flow rate: 1 L/min; plasma irradiation time: 1–300 s

2.2. Healing burns by plasma irradiation

From the background described in the introduction, we are trying to clarify the mechanism by which the plasma irradiation activates regeneration of living tissues, i.e., plasma irradiation of burns [1-3].

A separate group of six new male Wistar rats (SPF,

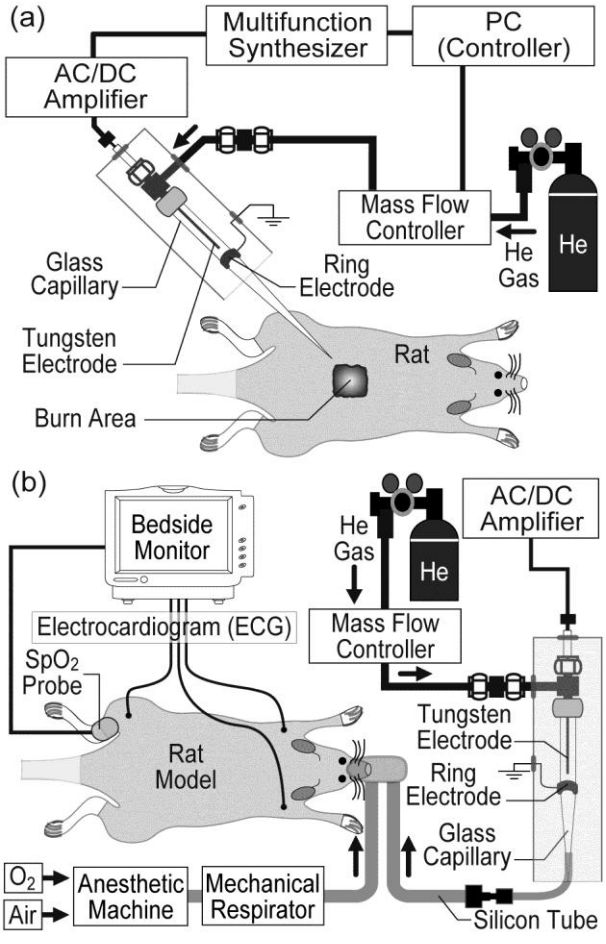

Fig.1. Schema of experimental setup. (a) plasma irradiation, (b) plasma inhalation.

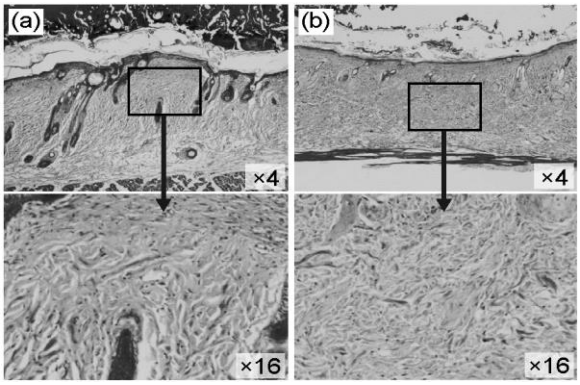

Fig.2. Histological findings from rat skin.

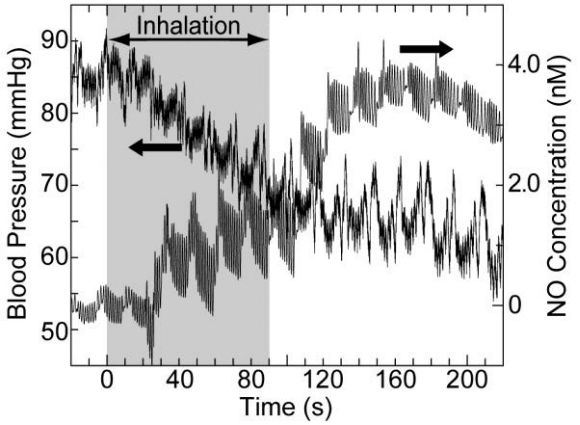

Fig. 3 Blood pressure and NO concentration in abdominal aorta as a function of the duration of plasma inhalation.

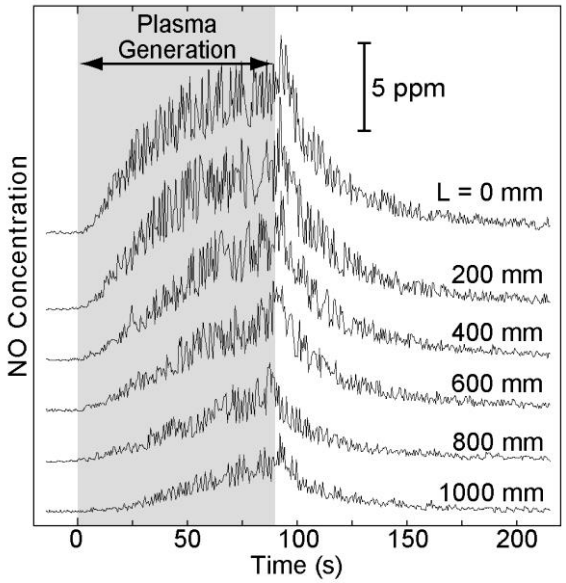

Fig. 4 NO concentrations in silicon tubes of various lengths as a function of the duration of inhalation of plasma

8 weeks old, weight: 200–220 g) was used to observe histological findings from rat skins. A full thickness burn (5 mm^2) was made a rat's back, using an electric scalpel. One was then irradiated with the plasma once a day; the other one was not irradiated. Seven days after injury, fluorescein isothiocyanate (FITC)-labeled tomato-lectin was injected into the rats for visualization of neo-vascular vessels in the burn tissues. The burn tissues were subsequently extracted, and the tomato-lectin labeled neo-vascular vessels in the tissue specimens were observed using a confocal laser microscope. The histopathological findings from the burn area are shown in Figure 2. In the control example shown in Figure 2(a), there is marked proliferation of blue-stained connective tissue between hair follicles (tissue surrounding the coat is below the pores). The arrangement of the hair follicles has become irregular. On the other hand, in the plasma irradiation example shown in Fig.2(b), the arrangement of the hair follicles is regular. These results suggest that plasma irradiation promotes the healing sequence of hair follicles, as denoted by their regular arrangement in the irradiated example. Plasma irradiation may facilitate re-formation of normal tissue building in the process of post-burn skin regeneration.

2.3. Treatment of cardiac disease by plasma inhalation

Plasmas contain many neutral molecules, ions, and radicals and oxidative nitrogen compounds such as Nitric oxide (NO) are generated under atmospheric conditions. NO in mammals including humans is an important cellular signaling molecule for many physiological and pathological processes. Especially, NO inhalation is used to treat persistent pulmonary hypertension of the newborn, heart load reduction during open-heart surgery and primary pulmonary hypertension. We aimed to distinguish endogenous and exogenous NO in a porcine model pulmonary of hypertension to clarify the relationship between NO concentration in the bloodstream and hypotension. Especially, we examined saturation pulse oxygen (SpO_2) in the blood under plasma inhalation at atmospheric pressure using a rat heart failure model of myocardial infarction (MI) [4].

A schematic diagram of the experimental setup for the plasma inhalation at atmospheric pressure using a rat heart failure model of myocardial infarction (MI) is shown in Figure 1(b). The MI model used here involved ligating the left coronary artery to induce ischemia in the coronary artery of left ventricle of the heart.

The simultaneous measurements of blood pressure and NO concentration in the blood were performed to clarify the relationship between SpO_2 and NO concentration. Figure 3 shows the relationship between blood pressure and NO concentration in the abdominal aorta as a function of the duration of plasma inhalation in a normal rat. Here, the reason for

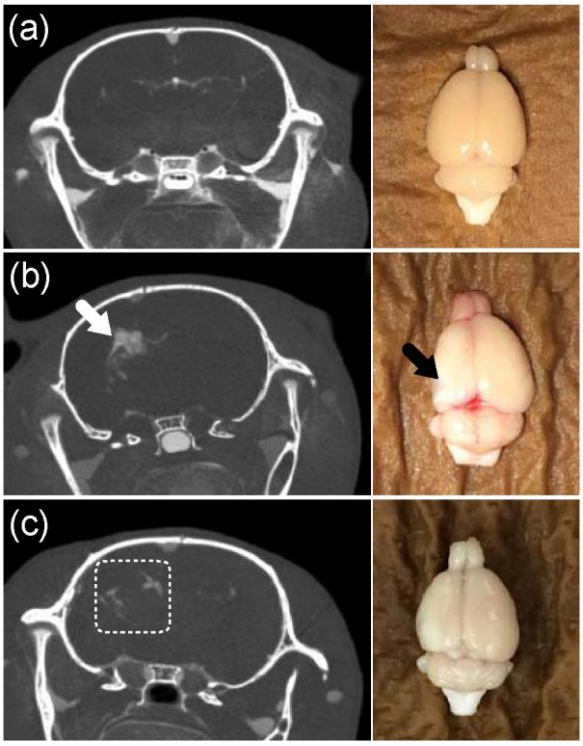

Fig. 5. CT images and photographs of the rat's brain. (a) Sham operation, (b) Inhalation-1, and (c) Inhalation-2.

Table. l. The volume of left hemisphere

Inhalation conditions	Volume (cm^3)
Sham operation (n:4)	0.0626 ± 0.00217
Inhalation-1 (n:3)	0.0540 ± 0.00227
Inhalation-2 (n:2)	0.0494 ± 0.00153

using a normal rat is that the rat MI model cannot endure SpO2 measurements that require surgical operation. The NO concentration started to increase 20 s after the start of plasma inhalation, reaching a maximal value at ≈ 160 s. Blood pressure did not decrease during He gas inhalation, but decreased from 89/81 to 73/60 mmHg after plasma inhalation. Blood pressure started to fall about 20 s after starting plasma inhalation and reached a nadir after 160 s.

To verify a direct effect, NO could be measured with a NO monitor due to NO being generated along with plasma. The effect of silicon tube length on NO concentration without inhalation in rats is shown in Fig. 4. The peak values observed ranged from 3.6 to 6.1 ppm, with NO concentrations being inversely proportional to the length of the silicon tube (L = 1000 mm), compared with the glass capillary tip (L = 0). Therefore, it is thought that an immediate effect of elevated NO concentrations is responsible for the observed decreases in blood pressure following plasma inhalation.

Plasma inhalation was conducted immediately preceding endocardial ischemia, and could potentially ameliorate decreases in blood flow due to the onset of cardiac dysfunction.

2.4. Functional recovery of hypoxic ischemic encephalopathy by plasma inhalation

The hypoxic ischemic encephalopathy (HIE) is a condition in which the brain does not receive enough oxygen. HIE can be fatal. Brain cells can begin dying within as little as five minutes without oxygen. There are a variety of causes of HIE. Although any injury and many health conditions can cause a lack of oxygen to the brain, there is no cure for HIE[1]. We accomplished the experiment concerning the functional recovery of HIE by atmospheric pressure plasma inhalation [5].

A HIE model rat (Levine rat model) undergoing plasma inhalation is shown in Fig.1(b)[3]. The rat HIE model used here involved ligating the left common carotid artery with 3-0 silk to induce ischemia in the brain. The 7-day-old rats were allowed to recover for an hour and placed for 2 h in hypoxia chamber (oxygen (O_2): 8%, nitrogen (N_2): 92 %, temperature: 37°C). HIE model rats (n: 5, initial body weight range: 10.3–14.9 g) were anesthetized with 1.5% sevoflurane, nitrous oxide (N_2O): 6 L/min, and O_2: 2 L/min using an anesthesia device with a mechanical respirator. The 3-week-old HIE model rats were anesthe-tized by sevoflurane inhalation, they were done the plasma inhalation for two weeks. The experimental conditions of the plasma inhalation are follows; Inhalation-1: plasma including O_2 gas and Inhalation-2: plasma including O_2 + N_2O gases.

The six-week-old rats were anesthetized with pentobarbital sodium (0.8 ml/kg) and perfused transcardially with saline followed by ioamidol and formalin. Before doing the paraffin treatment, the rat head and the brain were diagnosed by using X-ray computed tomography (CT). After perfusion, we made CT imaging for rat's head. Here, the CT scanner for experimental animals (Latheta LCT-200, Hitachi Aloka Medical, Ltd., Tokyo, Japan) was used for the rat's head imaging. The scanning conditions are following; 1 pixel: 80×80 μm, slice thickness: 80 μm, X-ray tube voltage: 50 kV, respectively. We measured left hemisphere volume of 5-6 mm from interaura.

Figure 5 shows removed brain and CT images obtained the position at 6 mm from interaura. The rat's left brain in case of the Inhalation-1 is larger than that of Inhalation-2. The brain for Inhalation-1 is bigger than Inhalation-2. The rat's head had an aneurysm and a brain edema in left side (white and black arrows) as shown in Fig.5(b), had

intricately-shaped blood vessels (dotted line square area) in left brain [Fig.5(c)]. The volume of left hemisphere is shown in Table.1. The rat's brain is bigger than Inhalation-2. The atmospheric pressure plasma including mixture of O_2 and N_2O gases has the possibility of influencing the cerebral blood vessel. Therefore, it is thought that nitrogen oxides (NOx) such as NO, nitrogen dioxide (NO_2), nitrate (NO_3), and N_2O etc. can be expected of the inhibition or improvement of the hypoxic ischemic encephalopathy.

3. Acknowledgments

The authors thank Mr. T. Komachi, T. Kishimoto, S. Murata, M. Osawa, M. Oga, C. Tokita, G. Takahashi, M. Lee, H. Saitou, Y. Funaki, R. Kagawa, K. Kobayashi (Plasma Regenerative Medicine Collaboration Team, Department of Medical Engineering, Tokyo City University, Japan) for technical assistance and Y. Kiriyama and N. Nakano (Riken Keiki Co., Ltd.) and D. Ichizawa (Society of Semiconductor Industry Specialists) for advice regarding NO and NOx measurements in plasma flow. This study was supported by a Grant-in-Aid for Scientific Research on Innovative Areas (No. 24108010) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan.

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