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Negative air ions created by water shearing improve erythrocyte deformability and aerobic metabolism

Abstract To elucidate a potential mechanism by which negative air ions improve aerobic metabolism, changes in venous blood lactate levels, pH, erythrocyte deformability, and plasma superoxide dismutase activity and ceruloplasmin levels were examined during a 1-h exposure to negative air ions created by water shearing or corona discharge in nine adult healthy volunteers. The blood lactate level decreased from 1.3 ± 0.3 to 1.0 ± 0.2 mmol/l, pH increased from 7.388 ± 0.025 to 7.417 ± 0.036, and erythrocyte deformability improved from 37.0 ± 2.2 to 35.1 ± 3.0 s, expressed as the mean ± s.d., when exposed to negative air ions created by water shearing, but did not change when exposed to negative air ions created by corona discharge. Other variables did not change in either exposure. The results obtained suggest that negative air ions created by water shearing improve aerobic metabolism by improving erythrocyte deformability.

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Key words: Negative air ions; Water shearing; Corona discharge; Erythrocyte deformability; Aerobic metabolism; Lactate; Superoxide dismutase.

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Practical Implications
The paper shows that negative air ions created by water shearing method improve aerobic metabolism only during a 1-h exposure, which may be caused by improvement of erythrocyte deformability, but negative air ions created by corona discharge have no effects. A potential mechanism is that negative air ions enter the circulating blood via the lungs and electrons of these ions are delivered to the plasma protein. Why negative air ions created by corona discharge have no effects is considered that water binding does not exist so that the lifetime of these ions is markedly short, by which the ions cannot reach the alveoli of the lungs sufficiently.

Introduction

Negative air ions are natural components of atmospheric air and are generated by certain natural phenomena, such as the minute shearing of water droplets in waterfalls or rain and, rarely, cosmic radiation, ultraviolet rays, and corona discharge or lightning (Krueger and Reed, 1976; Yates et al., 1986). As it is thought that negative air ions have beneficial biologic actions, commercially available systems for generating these air ions have been used in homes, hospitals, perishable food factories, and various sterile rooms (Yates et al., 1986). There are two types of generating methods: one is by corona discharge, and the other is by water shearing similar to the 'Lenard's effect'. The former method charges every substance in the air negatively, generating ozone as a by-product. The latter method only generates superoxide ions (O_2^-) attached to microclusters of water showing a structure of $O_2^-(H_2O)_n$ (Kosenko et al., 1997), and is essentially considered a natural source of negative air ions. Negative air ions in the form of $O_2^-(H_2O)_n$ have a half-life of about 60 s, and are markedly different from those created by corona discharge, which disappear within several seconds, probably because of the absence of water binding (Iwama et al., 2002). High levels of negative air ions created by water shearing are observed near waterfalls, ranging from 2000 to 10,000 ions/cm^3 in my experience.

Superoxide, O_2^-, causes oxidative damage to various tissues and is suppressed by superoxide dismutase (SOD) (Huang et al., 1999; Li et al., 1995). However, recently an interesting study was reported in which a low intensity of superoxide increased SOD activity, whereas a high intensity decreased SOD activity. Thus, there was a biphasic action, where negative air ions exerted the former effect (Kosenko et al., 1997). Intrinsic superoxide is generated mainly during oxida-

tive phosphorylation in the mitochondria, and SOD is among the most prominent of the intramitochondrial free radical scavengers (Huang et al., 1999; Li et al., 1995). If SOD activity is increased by exposure to negative air ions, it is possible that oxidative phosphorylation would be accelerated, thus improving aerobic metabolism. In fact, an attenuating effect of negative air ions created by water shearing on blood lactate levels, indicative of an improved effect on aerobic metabolism, has recently been demonstrated. This finding may be caused by the effect on SOD activity. Alternatively, another action such as an effect on erythrocyte function may be considered (Iwama et al., 2002). To elucidate a potential mechanism of improvement of aerobic metabolism, the effect of exposure to negative air ions created by water shearing or corona discharge on SOD activity and erythrocyte deformability were examined.

Materials and Methods

After approval of our Institutional Committee, seven male and two female adult healthy volunteers with no medical history and not in any medication were studied. The age, height, and weight were 27 ± 5 years (23–38), 170 ± 6 cm (160–178), and 62 ± 7 kg (55–77), as the mean ± s.d. (range) respectively. The volunteer entered a study room of about 30 m^3 space, that was maintained at around 26°C by an automated air-conditioner, and was placed in a recumbent position. After closing the door, a sensor and sampling tube for measuring percutaneous oxygen saturation (SpO_2) and end-tidal carbon dioxide tension ($ETCO_2$) (Handheld Capnograph Pulseoximeter NPB-75; Nellcor Puritan Bennett, Pleasanton, CA, USA) were attached to the right digital finger and nasal cavity, followed 30 min later by aspirating 9.5 ml venous blood from the left cubital vein using a plastic syringe containing 0.5 ml heparin (500 U). At this time, SpO_2 and $ETCO_2$ values were recorded. Subsequently, two commercially available electric air purified humidifiers for household use (Aqua Air Rich; Matsushita Seiko, Osaka, Japan, Figure 1) were placed about 2 m from the volunteer and operated at 1800 ml/min airflow. These appliances provide high humidity and negative air ions by means of water shearing. One hour after producing negative air ions, venous blood was collected in the same way, and SpO_2 and $ETCO_2$ values were recorded. On a different day, the same volunteer received a similar treatment, but where negative air ions were generated by means of corona discharge, using an electric appliance (Anion Program 2000 SI-01; I.P.S., Tokyo, Japan) placed about 2 m away from the volunteer and with an electric pole at an angle of 30°, was operated instead of the water shearing appliance. In the study room, an ion detector (Ion Tester KST-900; Kobe Denpa, Kobe, Japan) placed around the

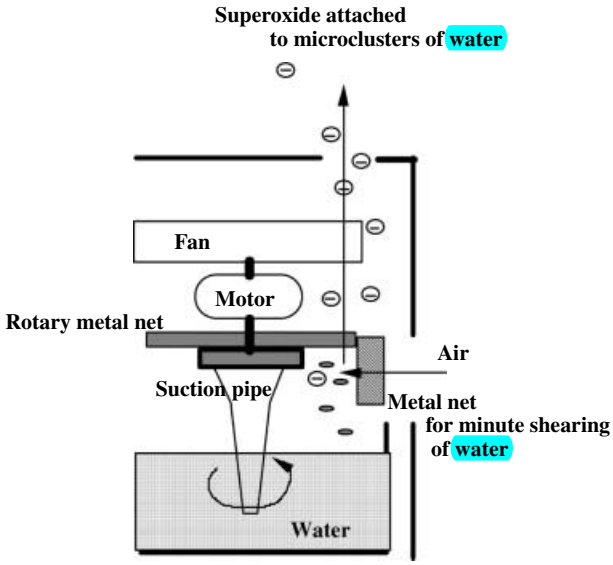

Fig. 1 Diagram of water shearing appliance creating negative air ions. Water sucked via the rotary pipe jets out to the metal net and is then crushed minutely, when superoxide attached to microclusters of water is created

volunteer showed approximately 0 levels of both negative and positive air ions before operating these appliances. The water shearing appliance produced 3000 ions/cm^3 of negative air ions and 100 ions/cm^3 of positive air ions, and the corona discharge appliance generated 10,000 ions/cm^3 of negative air ions and no positive ions. Almost no air ions were detected in the usual use of this room. The temperature and humidity of the room environment were also measured using a digital high-performance thermohygrometer (SK-110 TRH; Sato Keiryoki, Tokyo, Japan) at the times of collecting venous blood.

Immediately after collecting the venous blood, the blood lactate level was measured using a portable testing device (Lactate Pro LT-1710; Kyoto Daiichi Kagaku, Kyoto, Japan), and the blood pH was measured by a blood gas analyzer (288 Blood Gas System; Ciba Corning, Medfield, MA, USA). Subsequently, erythrocyte deformability was determined using a microchannel array flow analysis (Microchannel Array Flow Analyzer, Simple Type, Hitachi-Haramachi Electronics, Hitachi, Ibaragi, Japan, Figure 2) as an artificial model of human capillary vessels. This is composed of a 15 × 15 mm crystal silicon plate on which 8736 V-shaped grooves measuring 7 μm width, 30 μm length and 4.5 μm depth (Bloody 6–7 Tip, Hitachi-Haramachi Electronics) are etched and covered tightly with an optically flat glass plate. Fluids can be passed from the penetrating hole at the center of the plate to outside the plate via the grooves. A glass cylinder of about 200 μl volume is connected at right angles to the outer end of the penetrating hole. After filling the artificial passages with saline, a 100-μl

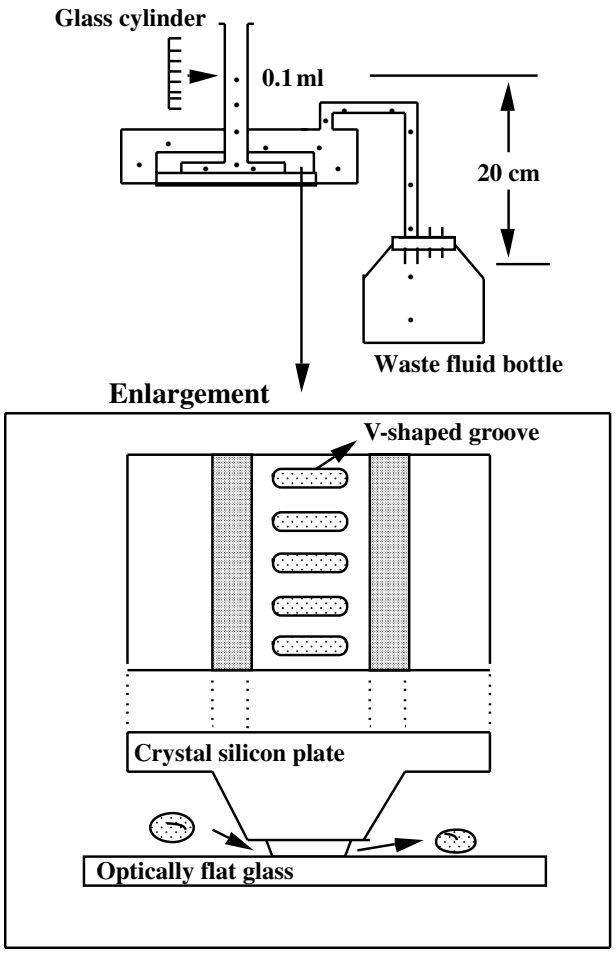

Fig. 2 Diagram of microchannel array flow analysis

sample introduced into this cylinder passes through the grooves by the pressure produced from the 20 cm height difference between the cylinder and the bottle for waste fluids. The standard measurement for blood, particularly for estimation of erythrocyte deformability, is described by the manufacturers as follows: after setting up this system, the passage time of 100 μl saline is first measured by a stopwatch, followed by measurement of the passage time of 100 μl blood treated with 5% heparin (1000 U/ml). The value obtained by the formula [sample time (s) × 12 (s)/saline time (s)] is taken as the passage time of the blood, and is an index of erythrocyte deformability, because the average passage time of saline is around 12 s when using this microchannel system. The normal passage time of 100 μl whole blood ranges from 30 to 60 s (Kikuchi et al., 1992, 1994a,b). The remaining blood was centrifuged, and total plasma SOD activity and ceruloplasmin levels were assayed using a modified nitrite method (Davison and Woolf, 1978; Misra and Fridovich, 1972) and nephelometry method (Emancipator et al., 1992; Montagne et al., 1992) respectively.

Statistical analysis was performed by paired *t*-test for each exposure to negative air ions, and $P < 0.05$ was considered significant.

Results

The results obtained are shown in Table 1. The SpO_2 and $ETco_2$ were not changed throughout the 1-h exposures to negative air ions created by either water shearing or corona discharge. Room temperature and humidity increased slightly when negative air ions were created by water shearing, but did not change when negative air ions were created by corona discharge. The blood lactate levels, venous blood pH and erythrocyte deformability decreased, increased and improved respectively, during 1-h exposure to negative air ions created by water shearing, but did not change during 1-h exposure to negative air ions created by corona discharge. Total plasma SOD activity and plasma ceruloplasmin levels were not changed in either type of exposure.

Table 1 Changes in measured variables during 1-h exposure to negative air ions created by water shearing or corona discharge

	Water shearing		Corona discharge	
Generation of negative air ions	Pre	Post (1 h)	Pre	Post (1 h)
SpO_2 (%)	98 ± 1	98 ± 1	98 ± 1	98 ± 1
$ETco_2$ (mmHg)	41 ± 4	41 ± 4	41 ± 4	41 ± 3
Room temperature (°C)	26.1 ± 0.2	26.6 ± 0.2*	26.7 ± 0.9	26.1 ± 0.3
Room humidity (%)	51.4 ± 4.2	69.7 ± 6.8*	47.7 ± 6.0	48.4 ± 7.5
Blood lactate (mmol/l)	1.3 ± 0.3	1.0 ± 0.2*	1.3 ± 0.3	1.2 ± 0.3
Venous blood pH	7.388 ± 0.025	7.417 ± 0.036*	7.384 ± 0.023	7.389 ± 0.026
Erythrocyte deformability (s)	37.0 ± 2.2	35.1 ± 3.0*	36.2 ± 4.1	35.9 ± 3.3
Total plasma SOD activity (u/ml)	4.3 ± 1.7	3.7 ± 0.6	3.8 ± 0.7	3.8 ± 0.9
Plasma ceruloplasmin (mg/dl)	22 ± 4	21 ± 3	21 ± 3	20 ± 3

SpO_2, percutaneous oxygen saturation; $ETco_2$, end-tidal carbon dioxide tension; SOD, superoxide dismutase.
Values are mean ± SD. $n = 9$.
* $P < 0.05$ vs. pre-value.

Discussion

The diameter of human capillary vessels is around 6 μm in average, whereas the erythrocyte diameter is around 8 μm. Erythrocytes flatten to pass through these capillary vessels, an activity termed 'erythrocyte deformability', so as to deliver oxygen to the peripheral tissues. Therefore, erythrocyte deformability is an important factor for aerobic metabolism (Chien, 1987; Mokken et al., 1992), but it has not been thoroughly examined because of the difficulty of measurement. However, recently it has become possible to form precise micron-sized grooves on a crystal silicon plate by application of minute manufacturing technology for semiconductors, providing several artificial microchannels similar to human capillary vessels. By passing whole blood through this artificial vessel and measuring the passage time, it is possible to evaluate erythrocyte deformability simply and precisely (Kikuchi et al., 1992, 1994a,b). This measurement system was used to determine erythrocyte deformability in my study.

The difference between the production of negative air ions by water shearing and corona discharge is whether water binding exists or not. It has been demonstrated that the former air ions reach the alveoli of the lungs (Duan et al., 1994). Water binding may cause a marked difference in the half time of these ions, which is about 60 s for the ions produced by water shearing and several seconds for those produced by corona discharge (Iwama et al., 2002). The present results show that only the negative air ions created by water shearing have a biologic action, decreasing blood lactate levels, increasing blood pH and improving erythrocyte deformability. Thus it seems that water binding is necessary to exert a biologic action. The markedly long lifetime of this type of negative air ions allows them to enter the living body after reaching the alveoli of the lung. However, because it is possible that superoxides created by corona discharge could combine with atmospheric water to produce similar air ions to the water shearing method, it cannot be said that negative air ions created by corona discharge have no biologic effects. If a high intensity of these air ions are provided for a long time, similar biologic effects may be expected. Further study to elucidate whether negative air ions created by corona discharge have a biologic action is necessary to establish this unequivocally.

Active oxygen species are adsorbed and changed into water and oxygen by plasma proteins, especially ceruloplasmin, and SOD. The structure of negative air ions suggests that there are active oxygen species, but their action may exert deoxidation rather than oxidation by means of providing electrons to the living body. In fact, exposure to negative air ions is reported to induce augmentation of SOD activity (Kosenko et al., 1997). Although the present results showed no changes in SOD activity or ceruloplasmin levels during 1-h exposures, it is possible that negative air ions, especially those created by water shearing, entered the circulating blood via the alveoli of the lungs and provided some electrons to plasma proteins. As a plasma protein is an amphoteric electrolyte, an electric charge may advance negatively. A combination of erythrocyte membrane and plasma proteins provides protection for the erythrocyte, whereas its deformability tends to be restrained (Kikuchi and Koyama, 1984). As the erythrocyte membrane is also charged negatively, the negative advancement of plasma proteins may weaken this combination, resulting in improvement of erythrocyte deformability and thus aerobic metabolism. This mechanism may result in a decrease in blood lactate levels, followed by an increase in blood pH. Consequently, the present results suggest that negative air ions improve aerobic metabolism because of their effect on erythrocyte function, improving erythrocyte deformability, rather than on SOD activity.

The present study was designed to compare the biologic effects of negative air ions created by water shearing with those created by corona discharge. The environment of the study room was controlled by an automated air-conditioner, so that various air parameters such as oxygen and carbon dioxide content, temperature and humidity would not be changed. However, the temperature and humidity was increased slightly when negative air ions were produced by water shearing, whereas there were no changes when they were created by corona discharge. Regarding respiratory variables, there were no changes either of the exposures, suggesting that there was no change in oxygen and carbon dioxide content in the study room. Although the aforementioned changes in temperature and humidity might affect the present results, to my knowledge there are no studies showing that these parameters affect aerobic metabolism. Furthermore, the results obtained from exposure to corona discharge can be regarded as a control group, because measured variables of aerobic metabolism in this study did not change during a 1-h resting position. Taking these discussions into consideration, the study design and results obtained are appropriate.

Although some speculation is involved, it is concluded that negative air ions created by water shearing improve aerobic metabolism by means of improvement of erythrocyte deformability during a 1-h exposure. A potential mechanism for this is that negative air ions enter the circulating blood via the lungs and electrons of these ions are delivered to the plasma protein, where its electric charge advances negatively and weakens the combination with the erythrocyte membrane, increasing deformability. However, it cannot be unequivocally stated that negative air ions created by corona discharge have no biologic effects. If these air ions are provided at a high intensity for a long time, similar effects may be expected.

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