Analysis of the salivary levels of immune biomarkers after inhalation of a steam mixed gas containing hydrogen gas

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Abstract

In this preliminary study, we examined the effects of inhalation of a steam mixed gas containing hydrogen gas on the salivary levels of immune biomarkers, especially interleukin-1β and soluble-form CD44, in healthy adult volunteers. This gas was generated by the decomposition of superheated steam using a machine (Suisonia) manufactured by Earth Engineering Co., and was designated as "XEN" in our laboratory. The salivary levels of interleukin-1β and soluble-form CD44 were measured by enzyme immunoassay 15 min after the subjects had inhaled "XEN" via a nasal cannula. The results revealed a significant increase in salivary interleukin-1β levels ($P < 0.01$), but no significant change ($P = 0.093$) in salivary soluble-form CD44 levels at 15 min after "XEN" inhalation, compared to the values measured before inhalation. These findings indicate that "XEN" rapidly induces salivary interleukin-1β secretion and that elevated salivary interleukin-1β levels after "XEN" inhalation may reflect the activation of both natural and acquired immune responses in the living body.

Key words : IL-1β, salivary immune biomarker, sCD44, steam mixed gas containing hydrogen gas, XEN

Introduction

It has been reported that reactive oxygen species (free radicals) are responsible for the development of many diseases and also the process of aging, and that removal of reactive oxygen species by molecular hydrogen (H_2) may be the best approach to prevent many diseases[1, 2]. A number of studies have demonstrated that H_2 ameliorates the clinical and pathological manifestations in numerous human diseases[3], as also in experimental animal models of disease[4]. In fact, it has been reported, based on these findings, that H_2 could be used for antioxidant therapy, for example, through oral administration of H_2 water, intravenous drip infusion of H_2-rich saline, or administration by inhalation of 2-4% H_2 gas[5]. In particular, H_2 gas has been vigorously demonstrated as an antioxidant in various fields. Since oxidative stress develops during the acute and chronic inflammatory phases of many diseases, H_2 gas has been demonstrated as an effective therapy for symptom relief in these diseases[6, 7]. However, the precise mechanism(s) of action of H_2 gas, in particular, its immunological effects (such as induction of cytokine and soluble-protein production), in the living body remain(s) very poorly understood.

Recently, a machine, termed Suisonia, which generates steam mixed gas containing H_2 gas by decomposition of superheated steam was developed by Earth Engineering Co. This gas was designated as "XEN" in our laboratory. Furthermore, this gas is known to be stable and safe, and is widely used in some national medical institutions.

The aim of the present study was to analyze the immunological effects of "XEN" by investigating the

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changes in the levels of some immune biomarkers, interleukin-1β (IL-1β) and soluble-form CD44 (sCD44) in the saliva, which have been shown to be closely associated with various immunological reactions in healthy adult volunteers administered "XEN" by inhalation via a nasal cannula. In addition, we shall also discuss the beneficial effects of "XEN" on the biological and immunological reactions in the living body.

Materials and Methods

Reagents

Enzyme immunoassay (EIA) kits for interleukin-1β (IL-1β) and soluble-form CD44 (sCD44) were purchased from Diaclone laboratories Co. (USA).

Steam mixed gas containing H_2 gas generator machine

A machine (Suisonia, FRJ-003) developed by Earth Engineering Co. (Kitakyushu, Japan) that decomposes superheated steam to produce a steam mixed gas containing H_2 gas was used in this study (Fig. 1). When superheated steam produced by heating water is further superheated, it decomposes into hydrogen (H_2) and oxygen (O_2), at a decomposition ratio of 67% H_2 and 33% O_2 (water: 100%). Since air is also present inside the machine, the concentration of H_2 gas produced within the machine is approximately 2.4%. Because the H_2 produced is delivered by introducing air, the H_2 concentration is approximately 0.1% to 0.3% when inhaled. O_2 is also produced by the decomposition, but the H_2 is isolated by adsorbing the O_2 with a cartridge. If no additional O_2 were present, the decomposed H_2 and O_2 would return to water by a cooling process, but air is introduced, the O_2 in the air (approximately 21%) intermingles with them. This steam mixed gas containing H_2 gas is designated as "XEN" in our laboratory, and was administered by inhalation to healthy volunteers via a nasal cannula. This steam mixed gas containing H_2 gas is known to be stable and safe, and is frequently used in some national medical institutions.

Ethics statement

The study protocol was approved by the institutional review board (IRB) of Kyushu University of Health and Welfare (IRB number 15-058). Informed consent was obtained from each of the volunteers prior to their participation in this study.

Volunteers and saliva collection

Ten healthy volunteers without any abnormalities of the oral cavity (6 males and 4 females; age 37.6 ± 10.8 yr) were selected for this study. Prior to the administration of "XEN" by inhalation, the subjects were asked to gargle and rinse their mouth (oral cavity) 10 times with water. Approximately 30 min later, with the subjects seated on a chair, "XEN" was administered by the inhaled route via a nasal cannula for 15 min. Thereafter, saliva samples were obtained from the 10 volunteers. Briefly, the saliva specimens were collected in 15-mL sterile tubes for two minutes. All the samples were then centrifuged at 2,500 rpm for 30 min, and the supernatants were collected and stored at -30°C until use.

Measurement of IL-1β and sCD44 in the saliva

The IL-1β and sCD44 levels in the salivary samples were measured using the IL-1β detection EIA kit and sCD44 detection kit, respectively. Each of the measurements was repeated three times.

Statistical analysis

Data were analyzed by Wilcoxon's *t*-test. Differences at $P < 0.05$ were considered to be statistically significant.

Results and Discussion

We first examined the effect of "XEN" inhalation on IL-1β production/secretion in the living body. The 10 normal healthy volunteers inhaled "XEN" for 15 min, following which the salivary IL-1β level was measured by EIA. As shown in Figure 2, the salivary IL-1β level was significantly increased ($P < 0.01$) after "XEN" inhalation as compared to the level recorded before the "XEN" inhalation. On the other hand, no significant change in the salivary IL-1β level was observed without "XEN" inhalation (data not shown).

IL-1β (molecular mass about 17 kDa) is one of the cytokines that belongs to both the natural and acquired immune systems, and is mainly produced by monocytes and macrophages. In regard to its functions, IL-1β is

known to exert a variety of biological actions, including activation of T and B lymphocytes, granulocytes, natural killer cells and endothelial cells. As a result, productions of IL-8, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), colony-stimulating factor (CSF) and nitric oxide (NO), as also the expressions of various adhesion molecules are strongly induced[8]. Thus, IL-1β in specimens such as the blood and salivary fluid is widely used as an important biomarker of various biological and immunological reactions[9]. In this study, the enhancement of salivary IL-1β levels after "XEN" inhalation strongly indicates activation of the nasal mucosal immune system.

We then examined the salivary sCD44 level after "XEN" inhalation by EIA in the 10 normal healthy volunteers. As shown in Figure 3, no significant change in the salivary sCD44 level was observed following "XEN" inhalation under the same conditions.

CD44 (molecular mass about 80 kDa) is expressed on cells such as lymphocytes, macrophages, granulocytes, fibroblasts and endothelial cells, and there are three types of CD44 molecules (CD44H, CD44E and sCD44) that differ in their biological functions. In particular, the sCD44 level in the serum/plasma has been reported to be increased in some patients with inflammation[10]. Thus, the sCD44 level in the serum/plasma and saliva has come to be recognized as an excellent biomarker of inflammatory reactions in the living body[11]. As there was no significant change in the salivary sCD44 level between before and after "XEN" inhalation, it was concluded that the "XEN" inhalation via the nasal cannula did not trigger an inflammatory reaction in the nasal mucosa.

The precise mechanism(s) underlying the increase in the salivary IL-1β level after "XEN" inhalation remains unclear at the present time. However, we speculate on some possible mechanism(s), as follows. The steam mixed gas containing H_2 gas, so-called "XEN", was administered by inhalation via a nasal cannula to the subjects in this study; thus, it would appear that the nasal and oral mucosa-associated lymphoid tissues (MALTs)[12], immune-related organs, were stimulated and activated by "XEN", resulting in the increased production/secretion of IL-1β from the lymphocytes, monocytes and macrophages in the nasal and oral MALTs, with a consequent increase of the salivary IL-1β concentration.

H_2 has been recognized as a medical gas with beneficial effects against pathophysiological disorders caused by oxidative stress[13], inflammation with tissue damage[14], apoptosis induced by various substances[15] and disorders of fat metabolism[16], and as a regulator of intracellular signaling pathway(s)[17]. In addition, this preliminary study clearly indicated that the steam mixed gas containing H_2 gas, so-called "XEN", had the ability to increase the salivary IL-1β levels derived from the nasal mucosal immune system, suggesting that the salivary level of IL-1β reflects activation of the immune system in the living body, for example, for immune regulation and protection against infections and tumor development. These findings indicate that the steam mixed gas containing H_2 gas, or "XEN", may come to be recognized widely as being useful for various clinical applications in the future.

In conclusion, we are the first to report that inhalation of a steam mixed gas containing H_2 gas, so-called "XEN", rapidly increased the salivary concentration of IL-1β. Further analyses, at both the basic and clinical levels, are needed to clarify in detail, the mechanism(s) underlying the increase of the salivary IL-1β levels following "XEN" inhalation.

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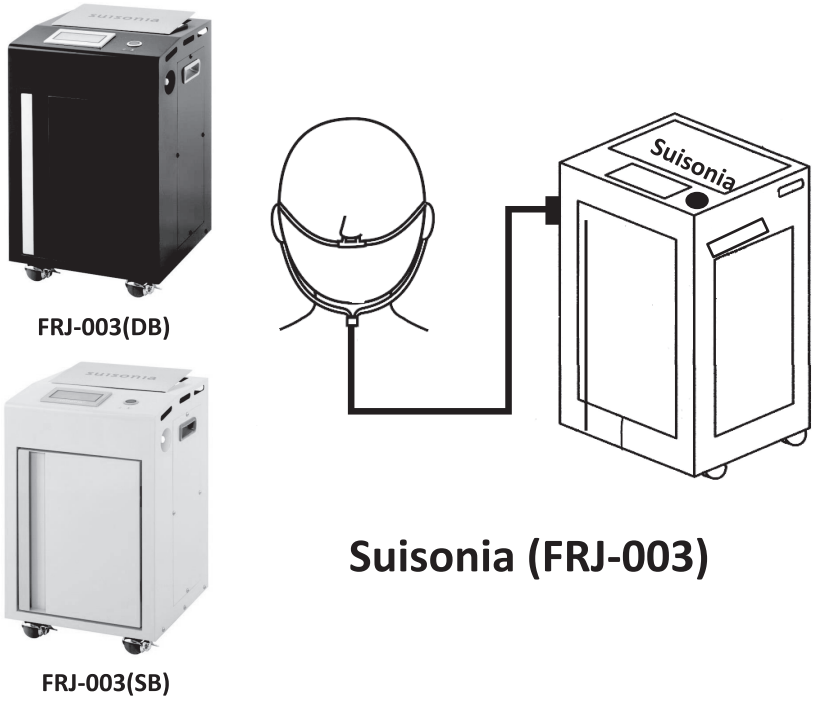

Figure 1. Summary of the steam mixed gas containing H_2 gas obtained using the machine generator.

A steam mixed gas containing H_2 gas was generated using a machine (Suisonia, FRJ-003) that decomposed superheated steam. This gas was designated as "XEN" in our laboratory, and administered to the study subjects by the inhaled route via a nasal cannula. In addition, this gas is stable and safe, and is used frequently in some national medical institutions. Earth Engineering Co. owns the copyrights to the photographs of Suisonia, and we obtained the company's approval for publishing it here.

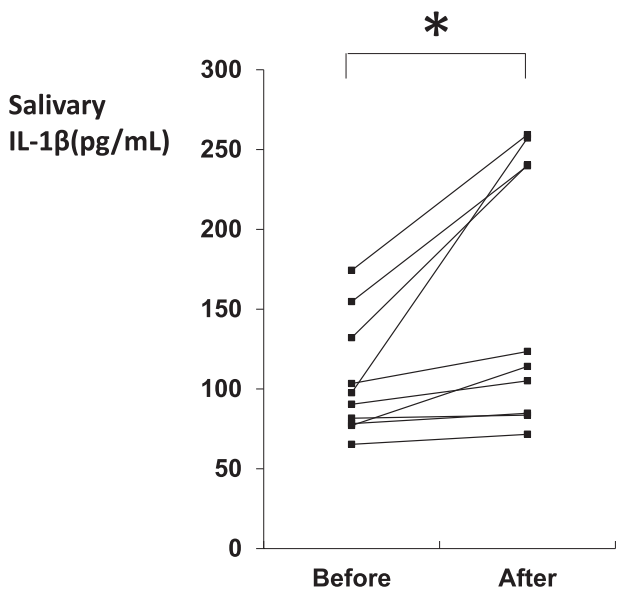

Figure 2. Measurement of IL-1β in the saliva.

The measurement of IL-1β in the saliva samples (n = 10) was performed using an IL-1β detection EIA kit. The measurements were repeated three times. *P < 0.01 (before inhalation vs. after inhalation).

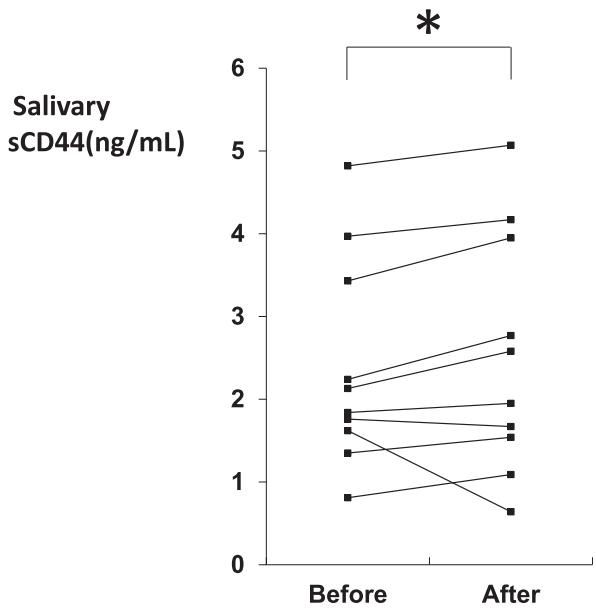

Figure 3. Measurement of sCD44 in the saliva.

The measurement of sCD44 in the saliva samples (n = 10) was performed using a sCD44 detection EIA kit. The measurements were repeated three times. *P = 0.093 (before inhalation vs. after inhalation).

水素ガスを含む蒸気混合ガス吸入後の唾液バイオマーカーの解析

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要　旨

スイソニアから発生する水素ガス（濃度0.1～0.3%）を含む蒸気混合ガス“XEN”を鼻カニューラで吸入した。吸入後、唾液中の免疫バイオマーカーの動態を酵素抗体法（enzyme immunoassay：EIA）で解析した。その結果、“XEN”吸入15分後、唾液中のインターロイキン－１β（IL－１β）の濃度が吸入前と比較して有意に増加した（P＜0.01）。一方、唾液中の可溶性CD44分子（sCD44）は吸入前後で有意な差は認められなかった。以上の結果は、“XEN”が鼻粘膜免疫系を活性化し、生体の免疫力を増強させる作用があることを示唆するものである。

キーワード：インターロイキン１β，唾液バイオマーカー，可溶性CD44，水素ガス含有蒸気混合ガス，XEN