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ORIGINAL ARTICLE

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Quasi-total-body exposure to an oxygen-ozone mixture in a sauna cabin

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Abstract We have investigated the effects of quasi-total-body exposure of healthy volunteers to either an oxygen-ozone mixture (O_2-O_3) or to oxygen (O_2) alone during a short period in a sauna cabin. The subjects underwent both an experimental and a control examination, separated by a 3.5-month interval. Body mass, blood pressure, body temperature changes, electrocardiograms, venous blood gas and haemocytometric analyses, total antioxidant status and plasma levels of protein thiol groups, thiobarbituric acid reactive substances (TBARS), plasma cytokine, hepatic enzymes and creatine were determined before, immediately after the 20-min period in the cabin and then 0.5, 1.0 and 24 h afterwards. We observed statistically significant variations of body temperature, venous partial pressure of O_2 values, TBARS and plasma levels of interleukin 8, particularly after O_2-O_3 exposure. The increase in TBARS plasma levels concomitant with protein oxidation has been tentatively interpreted as being attributable to the transcutaneous passage of some reactive O_2 species, which should be considered if this approach is to be used as a biological response modifier. However, in the present study no adverse effects were noted after one session.

Key words Ozone · Hyperthermia · Oxygenation · Oxydative stress · Lipid peroxidation

Introduction

During the last 3 decades a gas mixture composed of medical oxygen and ozone (O_2-O_3: ≈97%:3%) has been used widely in Germany and Italy for the treatment of torpid ulcers in patients with ischaemic limbs (Viebahn 1994; Werkmeister 1995). The treatment is performed by enclosing the limb for about 20 min in a polythene bag, where a water-vapour-saturated environment appears to enhance healing, owing to the powerful disinfectant and stimulating action of O_2-O_3. This limited exposure of the skin to O_3 does not appear to cause any local damage, even after very prolonged therapy. In reviewing the routes of administration of O_3 (Bocci 1996b), exposure of the whole body, excluding the neck and head to avoid the associated respiratory toxicity (Lippman 1989; Devlin et al. 1991; Aris et al. 1993; Kelly et al. 1995), to a humidified O_2-O_3 mixture in a thermostatically controlled cabinet has been considered as one possible option for patients who, for lack of venous access, cannot undergo conventional autohaemotherapy. However, it was pointed out that this method should be approached carefully by evaluating the possible increase in lipid peroxidation products in the plasma before, during, and after O_3 exposure.

Recently, we became aware that quasi-total-body exposure to O_2-O_3 is already being carried out in Canada and the United Kingdom with the aid of a commercially available sauna cabin. To the best of our knowledge, there is a total lack of scientific data regarding the effects of heat combined with exposure to O_2-O_3 in humans, and we felt it worth while to carry out a limited (one exposure to O_2-O_3) investigation in healthy volunteers to evaluate the metabolic changes and potential adverse effects of this non-conventional procedure.

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Methods

Blood samples

The study was authorised in May 1998 by the Ethical Committee of the University of Siena. Seven healthy male volunteers gave their informed consent to participate in the study, but they allowed only the withdrawl of venous blood. A recent complete check up showed

no abnormality in any of the subjects. They agreed to undergo a period of exposure O_2-O_3 and, after 3.5 months, to O_2 alone, so that they could act as their own controls. The mean (SD) body mass of the subjects was 68 (5.4) kg, their height was 1.77 (0.79) m, and their body surface area was 1.88 (0.15) m^2. They were aged between 32 and 48 years [mean 41 (5)]. One of the subjects had to be excluded from the study due to technical problems that arose during blood sampling. Depending upon the test, either heparin or ethylene diaminetetraacetic acid was used as an anticoagulant. For each sample, the volume (15 ml) of blood was always withdrawn from the basilic vein. None of the subjects were smokers.

Experimental procedure

The cabin (Sonnet II, kindly donated by Plasmafire International, Langley, BC, Canada) is made of laminated plastic and, after subtracting the body's volume, has an internal residual volume of about 440 l. The flow of gas through the cabin (either a gas mixture composed of about 97% O_2 and 3% O_3, or pure O_2) is 1 litre/min, and is delivered by a Ozonosan-Hansler PM 100 K (Iffezheim, Germany). The concentration of O_3 was assessed in real time using a portable Ozonosan-Hansler (Iffezheim, Germany) photometer. Any internal increase in barometric pressure in the cabin is prevented by an external silicon tubing that is connected to an O_3 destroyer (Ozonosan-Hansler catalyser). The maximum O_3 concentration is reached at the end of the session and has been estimated to be no higher than 0.90 μg/ml, a value that is about 12-fold lower than the minimal O_3 concentration used during local treatment of torpid ulcers in the leg for the same period (Werkmeister 1995). Steam is generated in the cabin by a thermostatically controlled heater which, during preliminary experiments, was set at 90°C. The heater was turned on 10 min before the subject entered the cabin, and then two towels and one polythene sheet were wrapped around the subject's neck. Although the doors were tightly closed using O_3-resistant gaskets, they were further insulated with the polythene sheet and towels to avoid any leakage of O_3 into the room. The session lasted 20 min, during which the maximal temperature inside the cabin reached 46–50°C. Just before opening the doors, the gas flow was interrupted and the internal gas was rapidly aspirated via the outlet to prevent any breathing of O_3 by the subject and the assistant. Determination of several variables was performed before and at the end of the session, and then 0.5, 1.0 and 24 h later. Body (oral) temperature was also measured in the middle of the session. Standard 12-lead electrocardiograms were recorded before and after the session. Body mass was assessed with an electronic balance with an error of ± 50g. Blood gas analysis was performed with an IL-1620 blood gas analyser (Instrument Laboratory, Lexington, Mass., USA). Systolic and diastolic arterial blood pressures were measured with a standard cuff sphygmomanometer. Haemocytometric determinations were performed by an automated haematology analyser.

Biochemical determinations

1. Total antioxidant status (TAS) in plasma samples was measured according to the method of Rice-Evans and Miller (1994). Values are reported as mM/ml of plasma. The reproducibility of the assay against a standard was lower than 8%.
2. Protein thiol groups (PTG) in the plasma were measured according to Hu (1994), using procedure 1 with 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) dissolved in absolute methanol. Values are reported as μM/ml of plasma. Measurement series repeated against a standard varied within ± 3%.
3. The thiobarbithuric acid (TBA) assay was carried out using plasma, as described by Buege and Aust (1994). Values (μM) are reported as TBA-reactive substances (TBARS). The reproducibility of the assay was lower than 6%.
4. The haemoglobin determination was carried out using 20 μl of original blood and an equal volume of the plasma that was collected after the ozonation. Samples were mixed with 5 ml of the cyanide-methahaemoglobin reagent (Sclavo Hemoglobin test kit). Optical density, read spectrophotometrically at 540 nm, was converted to units of haemoglobin according to a standard curve, and is referred to as a percentage of total haemoglobin. The reproducibility of the assay was lower than 3%.
5. Plasma levels of hepatic enzymes and creatine were determined using standard clinical laboratory tests. An internal standard was run every day.

Determination of cytokines

Plasma samples kept at −70°C were thawed, mixed by vortex and then centrifuged. Clear plasma samples were diluted 1:1 with the appropriate diluent. Levels of interleukin 8 (IL-8) were determined using an enzyme-linked immunosorbent assay kit (ELISA) produced by Medgenix Fleurus, Belgium; myeloperoxidase (MPO) and transforming growth factor (TGFβ1) were quantitated by an ELISA kit produced by R&D, Minneapolis Minn., USA. Latent TGFβ1 was determined after acid activation. A three-cycle automatic washing was routinely performed. Negative plasma samples, in the absence or presence of haemoglobin, were spiked with the cytokine's standards to assess the reliability and precision of the various assays. Yields ranged between 93% and 105%.

Statistical analyses

Data were collected and the results are expressed as the mean (SD). A software package was used for data collection and statistical analysis (Statview SE, Abacus Concepts, Berkeley, Calif., USA). The significance of the differences between the means at different times in each group was analysed by one-way analysis of variance and, when appropriate, the Sheffè test was used to compare pairs of means. The significance of the differences between means for the two groups at different times was analysed by Student's *t*-test. The level of statistical significance was set at $P < 0.05$.

Results

Each volunteer was subjected to one 20-min exposure in the cabin, either in the presence of O_2-O_3 or in O_2 alone. Modifications of body mass, oral temperature, and systolic and diastolic pressures are shown in Fig. 1. As expected, there was a significant increase in body temperature, which reached a peak at the end of the treatment and declined rapidly thereafter. The temperature increase ranged between 37.5°C and 39.3°C. There was a concomitant reduction in body mass within the range of 200–600 g. Similarly, blood pressure decreased slightly, but recovered within the next 30–60 min. Other data are summarised in Table 1. Results relative to the venous partial pressure of O_2 (P_vO_2) and CO_2 (P_vCO_2) are shown diagrammatically in Fig. 2. There was a significant increase and decrease of P_vO_2 and P_vCO_2 values, respectively, at the end and for 1 h post-exposure to both O_2-O_3 and O_2 alone, and the increase in P_vO_2 values observed after exposure to O_2 alone was not significantly higher than that obtained after exposure to O_2-O_3. Values for both erythrocytes and haematocrit increased immediately after the 20-min exposure. They decreased thereafter, probably due to rehydration, and were almost normal after 24 h (Table 1).

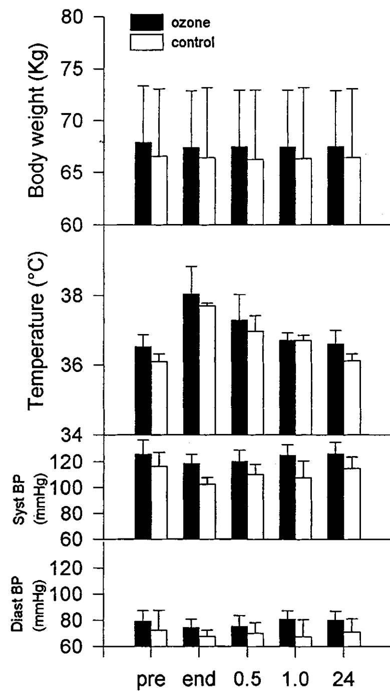

Fig. 1 Modification of body mass, oral temperature, diastolic and systolic blood pressure (*diast BP* and *Syst BP* respectively) of six subjects before (*pre*), at the end (*end*), and 0.5, 1.0 and 24 h after a period in the sauna cabin in the presence of either O_2-O_3 (*black bars*) or O_2 only (control, *white bars*). Values represent the mean ± SD. No significant intragroup or intergroup differences were found

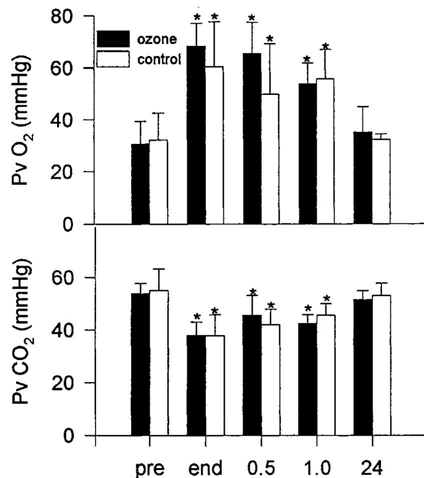

Fig. 2 Modification of partial pressure of O_2 in the venous blood (P_vO_2) and partial pressure of CO_2 in the venous blood (P_vCO_2) of six subjects before (*pre*), at the end (*end*), and 0.5, 1.0 and 24 h after a period in the sauna cabin in the presence of either O_2-O_3 (*black bars*) or O_2 only (control, *white bars*). Values represent the mean ± SD. *Asterisks* indicate statistical differences ($P < 0.05$) for the intragroup comparison

We noted an initial significant increase in leucocytes, followed by a decrease after 60 min of O_2-O_3 exposure. (Fig. 3). Figure 4 shows the interesting results observed after O_2-O_3 exposure. Both TAS and PTG decreased

Table 1 A summary of parameters measured before (PRE), at the end (END), and 0.5 h, 1.0 h and 24 h after a period in the sauna cabin in the presence of either O_2-O_3 (experimental) or O_2 only (control). Values are given as the mean (SD). Intragroup and intergroup comparisons revealed no significant differences. (*n.d.* Not determined, *RBC* red blood cells, *Ht* haematocrit, *PLT* platelets, [*HCO*$_3$] bicarbonate concentration, S_vO_2 venous oxygen saturation, *TGF*β transforming growth factor β1, *AST* aspartate amino transferase, *ALT* alanine amino transferase)

Parameters	PRE		END		0.5 h		1.0 h		24 h	
	O_2-O_3	O_2	O_2-O_3	O_2	O_2-O_3	O_2	O_2-O_3	O_2	O_2-O_3	O_2
RBC (×10^6 mm^3)	4.94 (0.22)	4.66 (0.16)	5.06 (0.22)	4.73 (0.1)	4.71 (0.14)	4.54 (0.1)	4.62 (0.21)	4.39 (0.1)	4.87 (0.26)	4.56 (0.2)
HT (%)	45.37 (1.91)	42.54 (2.02)	46.5 (1.92)	43.3 (1.8)	43.27 (1.19)	41.68 (1.8)	42.5 (1.82)	40.5 (1.82)	44.13 (2.91)	42.36 (1.58)
PLT (×10^3 mm^3)	183.5 (17)	187.3 (16)	207 (20)	199.5 (20)	182.5 (19)	162.8 (3.9)	173.2 (23)	169 (11.7)	191.5 (26)	178.3 (12.3)
pH	7.33 (0.03)	7.3 (0.02)	7.43 (0.04)	7.4 (0.02)	7.38 (0.04)	7.38 (0.01)	7.39 (0.03)	7.37 (0.01)	7.38 (0.03)	7.36 (0.04)
[HCO_3] mmol/l	28.8 (0.98)	27.68 (1.40)	25.3 (1.24)	24.03 (1.20)	26.9 (2.32)	25.30 (1.31)	26.07 (1.34)	26.70 (1.18)	27.32 (1.02)	27.20 (1.60)
S_vO_2 sat (%)	54.2 (21.8)	50.1 (19.3)	88.3 (8.7)	93.2 (2.8)	76.3 (20.3)	91.1 (4.3)	86.5 (6.4)	81.7 (12.6)	62.4 (32.2)	60.1 (18.25)
TGFβ (pg/ml)	54.5 (10.9)	n.d.	32.8 (9.7)	n.d.	32.7 (9.2)	n.d.	30.5 (7.3)	n.d.	45.7 (8.7)	n.d.
AST (U/l)	23.2 (3)	21 (4)	24 (2.5)	22 (2)	23.7 (6.3)	22 (4)	23.2 (4.7)	23 (6)	24 (2.9)	24 (4.8)
ALT (U/l)	19.8 (3.5)	20 (2)	21.3 (4.6)	22 (3)	21.7 (3.9)	21 (3)	20.3 (3.8)	20 (4)	22.6 (4)	20.8 (4.5)
Creatine (mg/dl)	1.03 (0.2)	1.01 (0.1)	1.17 (0.2)	1.08 (0.3)	0.97 (0.1)	1 (0.2)	0.92 (0.1)	0.98 (0.1)	1.02 (0.45)	0.99 (0.38)

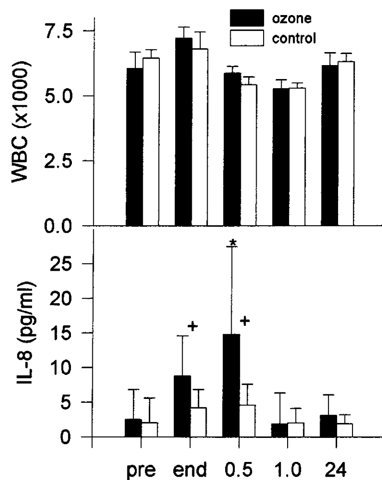

Fig. 3 Modification of leukocyte (*WBC*) and interleukin 8 (*IL-8*) plasma levels of six subjects before (*pre*), at the end (*end*), and 0.5, 1.0 and 24 h after a period in the sauna cabin in the presence of either O_2-O_3 (*black bars*) or O_2 only (control, *white bars*). Values represent the mean ± SD. *Asterisks* indicate statistical differences ($P < 0.05$) for the intragroup comparison. *Crosses* indicate statistical differences ($P < 0.05$) for the intergroup comparison

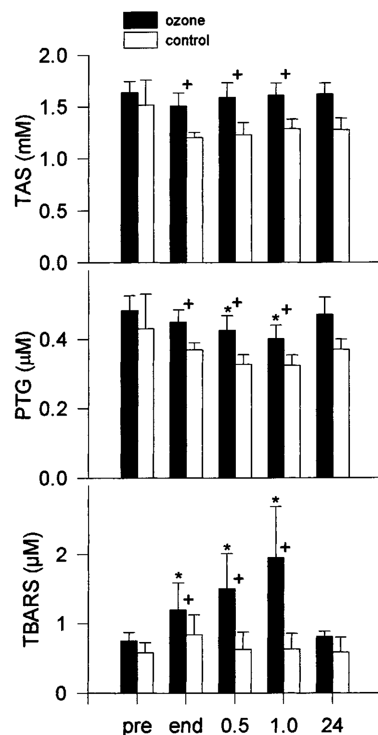

Fig. 4 Modification of total antioxidant status (*TAS*), protein thiol groups (*PTG*) and thiobarbituric acid reactive substances (*TBARS*) in the plasma of six subjects before (*pre*), at the end (*end*), and 0.5, 1.0 and 24 h after a period in the sauna cabin in the presence of either O_2-O_3 (*black bars*) or O_2 only (control, *white bars*). Values represent the mean ± SD. *Asterisks* indicate statistical differences ($P < 0.05$) for the intragroup comparison. *Crosses* indicate statistical differences ($P < 0.05$) for the intergroup comparison

after both O_2-O_3 and O_2 exposure, but PTG values only became significantly lower after O_2-O_3 exposure. Surprisingly, baseline values of both TAS and PTG have been found to be lower in mid-September (O_2 alone) than 3.5 months before (O_2-O_3), in spite of the fact that the same subjects were under study. There was a concomitant, progressive and significant increase of TBARS in the plasma after O_2-O_3 exposure, which receded completely 24 h after the end of the exposure. In spite of this increase, it is important to emphasise that no haemolysis was noted at any time.

We have investigated whether the plasma levels of three representative markers changed after the O_2-O_3 exposure: levels of IL-8 increased significantly 30 min after exposure (Fig. 3). Conversely, levels of MPO and TGFβ1 either did not change or tended to decrease (Table 1).

Plasma levels of hepatic enzymes and creatine remained within the normal range. No abnormalities (except an increase systolic pressure after exposure) were noticeable from the electrocardiographic tracings that were carried out before and after the session. All subjects tolerated the exposure to both O_2-O_3 and O_3 well, without reporting either immediate or subsequent adverse effects. Four subjects enjoyed the sauna, but two subjects reported that they would find it difficult to tolerate a period longer than 20 min in the cabin.

Discussion

There is no doubt that inhaled O_3 is particularly toxic for the respiratory tract, because of the enormous expanse of the mucosa and the very limited antioxidant capacity of the lining fluid (Kelly et al. 1995). By comparison, whole blood is more resistant to O_3 because of the great reservoir of antioxidant compounds (Halliwell 1994; Yu 1994) and the polyvalent and effective antioxidant system present in erythrocytes (Galleano and Puntarulo 1995; Mendiratta et al. 1998). On the other hand, washed erythrocytes or ghosts resuspended in buffered saline, without any natural antioxidants, are extremely sensitive to O_3 (Goldstein and Balchum 1967).

There are a few interesting reports regarding the oxidative damage caused by O_3 to murine and human skin (Thiele et al. 1997a, b; Podda et al. 1998): on one hand, it has been shown that exposure to O_3 causes depletion of vitamins C and E, while on the other hand, the increased epidermal malondialdehyde (MDA) production that occurs suggests that chronic exposure to O_3, as it is found in urban smog, has implications for skin health.

For these reasons we cautiously undertook an investigation, using human volunteers, to assess some biochemical, physiological, immunological and toxicological modifications that may occur during one session of quasi-total-body exposure to O_2-O_3. In this first exploratory study, we exposed the volunteers first to a low O_3 concentration for a short time (while avoiding any

inhalation of the gas), and second, to O_2 alone. Technical pitfalls can be excluded once the cabinet is tightly closed; the flow of the gas into the cabin did not exceed 1 l/min, the room was suitably ventilated, and the gaseous content of the cabin was quickly aspirated before opening it. During the 20-min period of the exposures, the body temperature reached its peak when the temperature in the cabin approached 46–50°C, but then decreased rapidly once the subject lay down for the next hour (Fig. 1). The loss of body water and slight decline in blood pressure that were observed have been attributed to intense sweating and cutaneous vasodilation.

Owing to the brief period of exposure we did not anticipate such a marked increase of oxygenation, which was slightly lower during O_2-O_3 exposure (Fig. 2). Other surprising results were the modifications observed in venous plasma regarding the lowering of the TAS in conjunction with the striking increase of lipid peroxidation products (Fig. 4). In comparison to control values, there is no doubt that these effects are due to exposure to O_3, but how they come about is a matter of conjecture.

It has been established that O_3, when dissolved in biological fluids, decomposes immediately by acting mostly on unsaturated fatty acids, and is unable to pass through the bimolecular layer of the cell membrane (Viebahn 1994; Kelly et al. 1995). It is therefore not possible for the O_3 to pass through either the superficial sebaceous film (Nicolaides 1974) and the multiple layers of the skin, or via the cutaneous glands. Indeed Thiele et al. (1997b) have observed that following exposure of mouse skin to O_3, MDA levels increase mostly in the external layers of the epidermis. At the present time we can envisage that O_3 acts on the superficial layer of human skin, but an array of lipid oxidation products and reactive oxygen species, such as unionised hydrogen peroxide (Bocci et al. 1998a), can pass through the transcutaneous barrier and, enhanced by the vasodilation that has occurred enter the circulation. If this assumption is correct, part of the O_3 activity will be transferred into the blood pool that can, at least in part, counteract it with its large reservoir of antioxidant compounds. The increase in plasma levels of TBARS observed in the present study appears to be transient, since 1 day after the session they had returned to baseline levels. The hypothesis that the induced oxidative stress has a short lifetime appears to be supported by the lack of acute or late side effects. Moreover, neither haemolysis, nor modifications of the plasma levels of MPO, a sensitive marker of the activity of neutrophils (Weissman et al. 1980; Boxer and Smolen 1988), were noted throughout the experimental period.

An initial leucocytosis followed by a modest leucopenia was observed after exposure to O_2-O_3 in the present study, and was probably due to a transient release of IL-8. This result agrees well with our previous data (Bocci et al. 1998b) showing that IL-8 is a chemokine that is released rapidly by leucocytes in blood that has been briefly exposed to O_2-O_3.

A final remark must be made regarding the practical usefulness of this approach in the field of O_3 therapy. Advantages of this new procedure are that it does not involve the handling of potentially infectious blood, and it is simple, inexpensive, and non-invasive compared with ozonated authohaemotherapy. It does, however, increase levels of peroxidation, and this must be taken into account when treatments are repeated. Although we now know that it is possible to induce a state of adaptation to the oxidative stress or "ozone tolerance", (Bocci 1996a, b; Leon et al. 1998), this point needs to be carefully evaluated in future studies in which repeated treatments are performed. As it is, the treatment may represent a tool for modifying the biological response to some pathologic states, but whether it will become really useful remains to be established.

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