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Salivary and Serum 8-Hydroxydeoxyguanosine Level in Simulated Microgravity

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Abstract

Background. Microgravity is associated with an increased of peroxidative. The effect is more pronounced after long duration space flight and can even last for several weeks after landing.

Aim. To determine the influence of a simulated microgravity on antioxidant status of the human body.

Material and Methods. 10 healthy volunteers were studied in condition before, during, and just after the simulated microgravity of -6 head –down –tilt (HDT) bed rest for 10 days. We measured the salivary and serum 8-hydroxydeoxyguanosine before, during and recovery of HDT.

Results. The 8-hydroxydeoxyguanosine showed significant increase in simulating microgravity.

Conclusion. The data provides evidence that oxidative stress is among critical nutritional concerns for long duration space travellers.

Introduction

On long duration space flights such as mars mission, astronauts undergo many physiological changes such as loss of bone mass, muscle strength, and cardiovascular fitness, as a result of reduced metabolic activities and lower cellular and tissue oxygen demand [1]. There is a balance within the body between oxidant production and antioxidant defences, with the balance shifted slightly in favour of oxidants. Mainly products of this "leakage" are the two ROS: superoxide radical (O_{2}–) and H_2O_2. Other ROS include free radicals such as nitric oxide and compounds such as ozone and HOCl. ROS can attack and damage cellular constituents such as DNA, proteins, and membrane lipids. Oxidative damage from free radicals to DNA and lipids has been implicated in the etiology of a wide variety of chronic diseases and acute pathologic states. The chronic diseases range from oral disease such as periodontitis and oral cancer to cardiovascular disease and neurodegenerative disease including Alzheimer and Parkinson diseases [2-12]. It has been observed that increased lipid peroxidation in human erythrocyte membranes and reductions in some blood antioxi-

dants after long-duration space flight [13-15]. It has been observed that the urinary excretion of 8-iso-prostaglandin F2_ and 8-oxo-7,8 dihydro-2 deoxyguanosine (8-OH dG) in six subjects during and after long-duration space flight (90 to 180 d). Isoprostane 8-isoprostaglandin F2_ and 8-OH dG are markers for oxidative damage to lipids and DNA, respectively [16-17]. Both non-radical and radical species have been demonstrated to be capable of degrading *in vivo* proteoglycans, which are the main components of the basic substance of connective tissue. Hyaluronic acid depolymerization caused by ROS may be characterized by glycoside bond fragmentation between monomers (between glucuronic acid and acetylglucosamine) [18-19]. Many other extracellular matrix components (collagen,fibronectin, laminin) have been found to be degraded to low-molecular-weight peptides. For homeostasis, extra- and intra-cellular preventive mechanisms must exist. This function is played by antioxidants, which may be regarded as those substances which, when present at low concentrations compared with those of an oxidizable substrate, will significantly delay or inhibit oxidation of that substrate [19-20].

There is no study on the correlation of blood and saliva 8-OHdG in simulating microgravity environments. Hence, this study was designed to examine oxidative marker 8 dihydro-2 deoxyguanosine (8-OH dG) concentration in the blood and saliva of Normal healthy subject in simulated microgravity condition of -6° head-down-tilt (HDT) bed rest.

Materials and Methods

The subjects of this investigation were 10 male volunteers aged (18-22 years, mean weight of 72.5 ± 3.2 kg and mean height of 174.9 ± 3.4 cm) participated in 6°HDT bed-rest exposure and who had not participated in systemic endurance training for 10 day prior to study and. Each Subject was given a detailed explanation of the experimental protocol and provided written and verbal consent. Each subject completed a medical and dental history questionnaire to determine the status of systemic diseases, smoking, alcoholic and drugs history as well as clinical examination for systemic disease, chronic diseases and oral & dental diseases. Persons were excluded from study who has systemic diseases, chronic diseases, oral& dental disease, smoking, alcoholic and drugs history. The average energy and calcium expended by the subjects during the simulation was 2300 kcal/ day (range 2080-3010 kcal/day) and 1200 mg/day, respectively.

Blood and saliva samples were taken just before HDT, throughout the time course of the HDT, during and recovery. Subjects were asked to awaken at 6 A.M. on the day of the study and to remain seated or standing until arrival at research centre. Baseline control measurements were obtained during the hour before HDT. At -9 A.M. the subjects were transferred supine to a gurney and tilted to 6' HDT, where they remained for the next 8 h. At -5 P.M. till 10 day, after 10 day the subjects were returned to a chair and remained in a seated position for the 4-h recovery period. Blood and saliva samples were prepared at the same time.

Whole unstimulated saliva was collected over a five-min period from subjects with instructions to allow saliva to pool in the bottom of the mouth and drain into a collection tube, when necessary. Unstimulated whole saliva produced in a 5-min period (about 3 mL) was collected, allowed to drain into a plastic container, and centrifuged at 3,000 × *g*, in 4°C for 5 min to remove bacterial and cellular debris. Saliva samples were stored at –80°C until analysis. Blood samples were collected into Vacutainer tubes. The blood was centrifuged at 1,700 *g* for 10 min and the plasma was separated. Plasma was stored at –80°C until analysis. Quantitative measurement of the oxidative DNA adduct 8-OHdG was performed according to the method described by Toyokuni et al. [21]. Briefly, the saliva samples were centrifuged at 10,000g for 10 minutes and the supernatant was used to determine 8-OHdG levels with a competitive ELISA kit (Japan Institute for the Control of Aging, Shizuoka, Japan). The determination range was 0.5–200 ng/mL. Serum 8-OHdG levels were measured in duplicate by a competitive ELISA kit (OXIS, Portland, OR, USA) according to the manufacturer's instructions. The sensitivity of the method was 1 ng/ml.All data were statistically analyzed using SPSS statistical package (SPSS, version13, Chicago,IL,USA). Data are expressed as mean ± standard deviation. Differences between pre,during and after microgravity simulation were analyzed for significance using one-way ANOVA test. Correlation assessment was performed using the Spearman correlation analysis. Statistical significance was defined as $p < 0.05$.

Results

The 8-OH dG level was statistically significant increased in simulating microgravity condition as compared to before and recovery stage , also relatively higher in recovery stage as compared to before simulation of microgravity (Table-1, $P<0.05$). Serum and salivary cor-

relation ananlysis revealed strong and highly significant correlation for 8 –OHdG (r=0.89) in before, during and in recovery stimulated microgravity.

Table 1: Salivary and Serum 8 dihydro-2 deoxyuanosine (8-OH dG) concentration in 10 Normal healthy subject in before HDT (A), thought the time course of the HDT experiment (B), and during recovery (C).

Sr. no	8-OH dG	A	B	C
1	Salivary (ng/ml)	0.45±0.02 **(0.34-0.65)**	0.67±0.06 **(0.56-0.87)**	0.48±0.08 **(0.41-0.68)**
2	Serum	2.21±1.08 **(1.98-2.67)**	3.45±1.12 **(2.98-3.89)**	2.78±1.15 **(2.01-2.89)**

P<0.05.

Discussions

In present study, serum and salivary 8-OHdG levels were increased in simulated microgravity environments as compared to before & recovery simulated microgravity as in previous studies [21-23]. The simplest explanation for the increased oxidative damage simulated microgravity in humans is that the increase is due to a combination of 1) the consequences of the loss of protein secondary to simulated reductive remodeling of skeletal muscle from the decreased work load on the antigravity muscles, 2) the simulated microgravity protein depletion from inadequate dietary intake, and 3) the increased anabolism associated with protein repletion. With increased generation of adenosine triphosphate, leakage of ROS from the mitochondrial electron transport chain will be increased [2]. There is another factor that can lead to decreased production of antioxidant defenses postflight, which is the suboptimal synthesis of host (defense) proteins. There is some evidence that the synthesis of host antioxidant protein defenses could be suboptimal due to competition for amino acids occurring between repleting muscle and other tissues [24]. The change we identified after the exposure to microgravity are transient and reversible. Four hours the end of the exposure to microgravity 8-OHdG, we examined had returned to approximately same as that of before. If enough time is given to marsonaunts or astronauts to become its initial conditions that would result in an undetectable condition of risk. The combination of muscle decompensation and nutritional depletion would place astronauts in this category. The principal difference between the ground-based exercise studies and the space-flight situation is that the ground-based studies lack the undernutrition component. Another difference between the space-flight situation and the groundbased exercise studies is that the concerns of the latter are directed toward performance, whereas for the astronauts the concern is for long-term damage. So, in Simulated microgravity may show less oxidatative stress as compared to real microgravity condition. Providing additional dietary antioxidants during and recovery process may decrease the oxidative damage.

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