WISMERLL News Letter No. 7

Combined July/August Issue Featuring the 4th Free Radical Seminar --Thank you for the large attendance--

The 4th Free Radical Seminar was held on July 9, 2006 in the Tower Hall on the 49th floor of Roppongi Hills with about 250 participants including those from Singapore and Korea with trilingual simultaneous interpretation among Japanese, Italian, and English. This issue of the Wismerll News presents digests of the 3 keynote addresses and 11 lectures. It is our pleasure if this issue conveys the value of the research results presented by the lecturers and the lively atmosphere of the seminar.

Keynote Lecture 3

Overview of 15 years of research on measuring oxidative stress

 \sim The history of d-ROMs test and FRAS since its development, application, and the future \sim *Dr. Mauro Carratelli*, Developer of FRAS/FREE System President of Diacron srl Italy



I was born in Toscana, Italy where I spent a happy childhood. Since I was very small, I had been tempted to challenge "mysteries of the world", which are metaphysical problems such as "What is a man?", "Why was he born?", and "Where does he go?" In my youth, I frequently changed my research themes, which included chemistry, physics, and biology, but I soon settled into analyzing data on medical tests, and continued it for 15 years. However, I got tired of this work and began to devote myself to research on free radicals. In those days, I recalled existential problems that I had cherished since childhood such as "Why does a man age?" and "Why does he die?" and kept pondering them. "Oxidation" generally requires oxygen and hydrogen. Rusting of machines made of iron is basically the same as rusting of man. Man ages by rusting and eventually dies. Attention to this process was the origin of FRAS and F.R.E.E.

I started the development of the d-ROMs test in 1988 and obtained a patent in 1994. During those 6 years, I was so absorbed in research that I occasionally neglected my family. The d-ROMs test is like my child. I named the unit of the values obtained by the d-ROMs test U. CARR after my name, Carratelli, to indicate that I take full responsibility for the test. Now, I am confident that this device will contribute to the future of medicine and that my family will pardon me for this.

Keynote Lecture l

What's news

 \sim The latest information about the oxidative stress researches with d-ROM, BAP, and OXY adsorbent tests \sim

Prof. Eugenio Iorio President of International Observatory of Oxidative Stress, Free Radicals and Antioxidant Systems:(Parma Italy)



(1) Neurological diseases: Physiologically, defense of the brain against oxidation is very weak, and its anti-oxidation system is reported to be depressed. The brain is vulnerable to oxidative stress, because it consumes 20% of the body's oxygen while it accounts for only 2% of the body weight.

(2) Amyotrophic lateral sclerosis (ALS): The d-ROMs test has shown high oxidative stress levels. Mutation of SOD has been revealed.

(3) Parkinson's disease: Oxidation of dopamine is reported to damage neurons. Polyphenol, which is a potent antioxidant, is occasionally used for the treatment.

(4) Alzheimer's disease: A decline of the anti-oxidation capacity, which is detected by the OXY adsorption test, has been suggested to induce this disease. Its progression is known to be delayed by the use of antioxidants.

(5) Multiple sclerosis: Oxidative stress is known to play a major role, and changes in the oxidation balance have been shown to markedly affect the progression of the disease.

(6) Down syndrome: Increased expression of free radicals is being disclosed.

(7) Cardiovascular disorders: The values yielded by the d-ROMs test have been suggested to be useful for the prediction of the disorders.

(8) Veterinary medicine: Oxidative stress has been measured in pigs, cows, and fish, and the meat quality has been shown to decline at a high oxidative stress level.

Keynote Lecture 2

FREE Radical Theory of Evolution and Life

 \sim Examining life/aging/disease/death from biological evolutionary viewpoint \sim

Prof. Masayasu Inoue M.D., Ph.D., Biochemistry and cellular pathology, Medical Faculty, Osaka

Municipal University



Active oxygen and NOs, which are the basis of various diseases and aging, continue to be produced in the body under a strict control system, and play important roles in defense against infections and circulatory regulation. We can eat food contaminated to some degree by bacteria without problems thanks to active oxygen and NOs, and man cannot live a day without them. If we survive today by eating, the desire to hand over genes for tomorrow grows in us. Activities to transmit genes are controlled by the brain in a sophisticated manner. Not only the reproductive center in the brain but also erection and ejaculation reflexes, and ovulation reflex under central nervous control are regulated by crosstalk among NOs and superoxides.

By these processes, life is reset as genes of a male and female are recombined, and the risk management against pathogens is strengthened by the acquired new diversity. Thus, active oxygen and NOs constitute a supersystem that integratingly control defense against infections, circulatory energy metabolism, reproduction, differentiation and growth, brain functions, and even instinct. Mitochondria, necessary for survival, induce localized apoptosis via active oxygen, write a scenario of development, growth, and reproduction in an early stage, and improvise individual death via age-associated lesions in a late stage. This supersystem underlies the expansion of niches by life from the ocean to rivers, to the land, and to the air through its long history of evolution.

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Lecture l

Current utilization and future development of FRAS 4 in NICU in Saitama Medical University General Medical Center

 \sim Based on the oxygen administration and free radical values in cardiac resuscitation of premature babies, and chronological changes of antioxygen potential of breast milk \sim

Dr. Katsuichi Ezaki M.D., Ph.D., New Born Division, General Prenatal Medicine Center, Saitama Medical University, General Medical Center



For resuscitation of asphyxic neonates in an ischemia-reperfusion state, 100% oxygen used to be used. However, this method, which results in oxygen overdosing, has been shown to increase free radicals in term neonates, but its effects in preterm infants remain to be clarified. We divided preterm infants who were born at a gestational age of less than 35 weeks and developed asphyxia into those resuscitated with 100% oxygen and those in whom oxygen was reduced to maintain SpO₂ at 95% or less (oxygen reduction group) and compared the results of d-ROMs test. d-ROMs were reduced in the oxygen reduction group, suggesting that development disorders may be prevented by the oxygen-reduction treatment. When the anti-oxidation capacity was compared between mother's milk and artificial milk by the BAP test, it was higher in mother's milk. However, the anti-oxidation capacity of mother's milk decreased about 100 days after delivery. It has been shown that preterm infants show better growth and that the DNA damage marker levels are lower, when they are fed with mother's milk for at least 3-4 months, and the results of our study suggest an involvement of the anti-oxidation capacity.

Lecture 2

Trials in redox researches in women's infertility area, and application of FRAS 4

Dr. Toshihiro Fujiwara M.D., Ph.D., Women's Medicine and Obstetrics Division, Tokyo University Hospital



In the treatment of infertility, age of the female patient is known to be an important factor of the therapeutic outcome, because the fertilizing capacity and embryogenic capacity (after fertilization) of ova are reduced as well as because the number of ova itself is reduced with aging. In consideration of the fact that oxidative stress is related to age-associated changes in many other organs, it is also considered to be involved in female infertility. Part of the causes of poor therapeutic results in high-age infertile females may, therefore, be oxidative stress at the ovary level. Informative results may be obtained in such patients by performing fertilization treatments after the administration of antioxidants (vitamin C, vitamin E, CoQ10, catechin, etc.), measuring d-ROMs and BAP in serum or folliculi using FRAS4 before and after administration, and evaluating their relationships with the clinical results.

Lecture 3

Reactive oxygen and antioxidative potential in high pressure oxygen and scuba diving

Dr. Harumi Nakayama M.D., Ph.D., Director of Harumi Clinic, Health Education Division, Healthcare

Hygieiology, Tokyo Medical and Dental University



Hyperbaric oxygenation (HBO) is a treatment aimed to induce recovery of hypoxic or damaged tissues by increasing oxygen absorption in the body. Oxygen poisoning is one of the side effects of HBO, and the involvement of reactive oxygen species (ROS) has been suggested. In this study, we measured serum d-ROMs, serum BAP, and urinary 8-OHdG before and after HBO, and evaluated whether they are useful for the assessment of the effects of ROS. As a result, serum d-ROMs increased immediately after exposure to ROS but decreased after 1 day. No effect of HBO was noted in the serum BAP or urinary 8-OHdG level. The serum d-ROMs level is also considered to be a test item useful for the assessment of the effect of ROS due to HBO. When d-ROMs were measured before and after scuba diving, they were found to be reduced during a period after a stress-free dive.

Lecture 4 Proposal of biomarker in evaluation of forestry therapy effect

Prof. Nobuo Suzuki M.D., Ph.D., Mr. Shigeru Sugaya, Environmental Biochemistry Div., Medical Faculty,



The stress relieving effect of a therapeutic walk in the forest is well known, but there have been few physiologic or biochemical studies of this effect. We attempted to clarify this effect using oxidation-damaged materials as indices. We measured the serum d-ROMs, urinary 8-OHdG, and salivary IgA (an immunological capacity marker) in subjects including rheumatic patients and blood MMP-3 (a rheumatic marker) as well in patients with mental rheumatism before and after a walk in the forest. As a result, serum d-ROMs decreased, urinary 8-OHdG increased, salivary IgA increased, and blood MMP-3 decreased in about half the subjects. From these results, we concluded: (1) The amount of oxidization-damaged materials in the body decreases after a walk in the forest, (2) excretion of oxidation-damaged materials increases after a walk in the forest, and (3) these changes are observed more clearly in both healthy people and rheumatic patients living in wooded regions than in those living in urban regions.

Lecture 5 Measuring oxidative stress of sports athletes – Association between FRAS 4 and Urine 8 OHdG

Prof. Terushige Kouno M.D., Ph.D., Sports Medicine, St. Marianne University



Exercise increases the oxygen uptake and oxygen supply to active muscle tissues. This is reported to cause an associated increase in the active oxygen production and possibly damage body tissues. To evaluate the oxidative stress level before and after high-intensity intermittent exercise, we measured d-ROMs and BAP using FRAS4 and urinary 8-OHdG and examined their relationships. In college American football players, d-ROMs and BAP increased immediately after high-intensity intermittent exercise on the Powermax, and urinary 8-OHdG increased after 1 hour, compared with the levels before the exercise. Also, the resting d-ROMs value and urinary 8-OHdG level were significantly correlated. Since both d-ROMs and BAP increased immediately after high-intensity intermittent exercise and decreased after 1 hour, oxidative stress and anti-oxidation capacity (oxidative stress eliminating ability) may be balanced in athletes.

Lecture 6 The significance of oxidative stress measurement in Type 2 Diabetes

Dr. Naoki Sakane M.D., Ph.D., Preventive Medicine Division, Clinical Research Center, National Kyoto Medical Center



In this study, we examined the body composition, d-ROMs and BAP levels using FRAS4, lifestyle, and diet using a digital camera in patients with type II diabetes mellitus, and evaluated their relationships with oxidative stress. As a result, the mean d-ROMs value was higher, and the mean BAP value was lower, than the respective normal levels. According to various parameters, d-ROMs were increased in those not exercising regularly (at least for 30 minutes, 2 times a week). Also, d-ROMs were increased in those without regular bowel movements. Concerning the diet, d-ROMs tended to be high in those who often ate "pastry", "fish", and "eggs" and low in those who often ate "other vegetables". BAP tended to be low in those who did not consider nutritional balance of their diet. These results suggest that d-ROMs and BAP may be biomarkers useful for the assessment of dietary and exercise therapies and hygienic guidance in patients with type II diabetes.

Lecture 7 The significance of oxidative stress in chronic heart failure

Dr. Kazuo Komamura M.D., Ph.D., Cardiac Dynamism Research Division, Circulation Dynamism Function, National Cardiovascular Center



To evaluate the validity of measurement of the d-ROMs value in cardiovascular disorders, we compared it with the plasma 8-isoprostane (ISO) concentration, which is widely adopted as a typical oxidative stress marker in cardiovascular diseases. In 43 patients with chronic heart failure with stable hemodynamics, we measured d-ROMs (using F.R.E.E.) and ISO. The severity of heart failure was evaluated according to the NYHA Functional Class and plasma BNP. Five healthy subjects were evaluated as controls. Correlations between d-ROMs and ISO and correlations of d-ROMs and ISO with NYHA class and BNP were analyzed. A significant correlation was noted between d-ROMs and ISO. The d-ROMs level differed significantly between NYHA classes I-II and classes III-IV, and ISO showed a similar tendency. Also, both d-ROMs and ISO were correlated with BNP. These results suggest that d-ROMs are useful as a bedside cardiovascular oxidative stress marker.

Lecture 8

Significance and application of oxidative stress monitoring in the field of neurological emergency/intensive medicine

Dr. Kenjii Doi M.D., Ph.D., Emergency Medicine, Showa University Medical School



The clinical effectiveness of brain hypothermia in emergency neurology treatment and intensive care has recently been recognized, and the therapy is becoming a standard brain protection procedure. In this study, we evaluated hydroperoxide concentration in internal jugular venous blood and anti-oxidation capacity in patients with head trauma treated by brain hypothermia. Patients with severe head injuries were divided into those treated by mild hypothermia at a brain temperature of 33-34°C and those who received aggressive normothermic management at 35-37°C, blood was sampled at 3 points, i.e., "before induction of brain hypothermia", "maintenance period", and "after temperature recovery", and the anti-oxidation capacity was measured using FRAS4. In the "maintenance period", the blood hydroperoxide concentration and anti-oxidation capacity were reduced in the hypothermia group compared with the normothermia group. Also, the hydroperoxide concentration increased markedly in the temperature recovery period in the hypothermia group. FRAS4 is considered to be useful for simple monitoring of the free radical level and anti-oxidation capacity during the temperature recovery period, the management of which is reported to be difficult in brain hypothermia therapy.

Lecture 9 The role of FRAS in anti-aging treatment

Dr. Tadashi Mitsuo M.D., Ph.D., President of the association for Chelation Therapy in Japan, Director, Mitsuo Clinic



At our hospital, FRAS has been used for 3 years. We reported at the last year's Free Radical Seminar that the d-ROMs level tended to be higher in females than in males, but we were not sure about its cause. Since whole blood is assayed by this analytical method, we considered that the hematocrit may affect the results and, in this study, evaluated the data of d-ROMs by correcting them to a standard mean hematocrit (Htc) of 43% (corrected d-ROMs = d-ROMs ? Htc/43). As a result, a significant gender difference in the d-ROMs value disappeared after correction. In females, however, d-ROMs tended to increase with age. The BAP value tended to be higher in females than in males. The results of analysis using FRAS4 are expected to be of great clinical use if the relationship between the d-ROMs value and BAP value is clarified, and a golden standard is established.

Lecture 10 Diagnosis and treatment evaluation dry mouth utilizing oxidative stress

Prof. Ichiro Saitoh D.D.S, Oral Pathology Division, Dental Faculty, Tsurumi University



Dry mouth is a state in which the mouth is dried due to a decrease in saliva secretion. According to epidemiological surveys in Western countries, this condition is observed in 25% of the population. Diseases caused by dry mouth vary widely and include serious conditions such as aspiration pneumonia. Dry mouth may be caused by destruction of salivary glands, but, assuming that it may often be caused by environmental factors related to lifestyle-related diseases, we started studying its relationship with oxidative stress. Using F.R.E.E. for this purpose, we found that d-ROMs were increased in the dry mouth group compared with the normal group and that the d-ROMs level was correlated with the values of conventional oxidative stress markers. A clinical study of CoQ10 intake yielded encouraging results that d-ROMs decreased, and BAP increased, with an increase in salivary secretion 2 weeks after the beginning of CoQ10 intake.

Lecture 11 Cancer and oxidative stress

Dr. Katsutaro Nagata, M.D., Ph.D., Psychosomatic medicine, Hamamatsu Medical University Hospital



We evaluated oxidative stress and anti-oxidation capacity in cancer patients. First, d-ROMs were significantly increased, but BAP showed no significant difference, in cancer patients compared with healthy controls. Also, the d-ROMs value was poor in those with metastasis, those undergoing chemotherapy, and those undergoing radiotherapy and poorest during the period of palliative care, but BAP showed no significant difference. When the d-ROMs and BAP values were compared between before and after the use of CoQ10 (1 month), no significant difference was noted in the d-ROMs value, but BAP increased markedly. In cancer patients, the d-ROMs value was generally high, but the BAP value showed no marked change. The d-ROMs value appeared to closely reflect the state of progression of cancer, so that the d-ROMs test is considered to be useful for the prognosis. In this study, we particularly noted that the ratio between the anti-oxidation capacity and oxidative stress level (BAP/d-ROMs) was correlated with the QOL index in cancer patients.