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Full Length Research Paper

Identification of the window period for oxidative stress intervention

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A window period was proposed for oxidative stress attenuating intervention, which explained controversial results from using an anti-oxidation treatment in chronic disease prevention. In this study, a method is reported for identifying the WPOS using a NIDDM diabetes animal model when using any single oxidative stress biomarker. Abnormal oxidative stress was initiated using streptozotocin, and the concentration of TBARS in plasma was used as the biomarker. The results confirmed the existence of WPOS and revealed a very easy way to identify it.

Key words: Oxidative stress, WPOS, diabetes.

INTRODUCTION

Oxidative stress indicates the balance status of living things at the molecular level. It has been proven as the major factor in the development of many chronic human diseases (Halliwell and Gutteridge, 2007), such as cardiovascular diseases, cancers, aging-related diseases, and diabetes (Clarke and Armitage, 2002; Finkel and Holbrook, 2000). Although all the tests (including animal tests) have proved the effectiveness of oxidative stress intervention in preventing chronic diseases *in vitro* and *in vivo*, most clinical trials have shown either ineffective or adverse results. These clinical trials were geared towards normal population although only safe antioxidants such as vitamins and minerals were used. So, research on oxidative stress has encountered a bottleneck. Window Period for Oxidative Stress Intervention (WPOS) (Zhu and Zhongguo, 2006; Zhu, 2009) was proposed almost a decade ago, which explains the controversial results of human clinical trials. WPOS suggests that there exists a microbalance in all living things under healthy conditions,

$$[EQ]_{RN} = M\, C_R\, C_N \quad (1)$$

or

$$\frac{\triangle [EQ]_{RN}}{M} = C_R\, d\, C_N + C_N\, d\, C_R \quad (2)$$

EQ=microbalance of the living thing, M=mass number of the living thing, R=reactive species, N=counterpart of reactive species or neutralizing species, C=concentration. Inside a micro-environment such as that of a single cell, or a specific organ such as the liver, kidney or pancreas, each reactive species has its own neutralizing species. Both reactive species and their own neutralizing species are important for living beings, converse to our original belief that reactive species are “bad” for us.

For individuals with a constant body mass under healthy conditions, $\Delta EQ=0$

$$C_R dC_N = -C_N dC_R \quad (3)$$

Equation (3) indicates that although many biomarkers of oxidative stress exist inside living beings under healthy conditions, the concentration in fluctuation of any reactive species equal the changes of their counterpart neutralizing species insides WPOS. This simplifies the identification of WPOS, because the fluctuation of any single oxidative stress biomarker should show the window. By using time to monitor the course of concentration change, we should be able to observe the plot, as seen in Figure 1.

The WPOS hypothesis gives reasonable explanations to the controversial results of human clinical trials. We always consider only the damages caused by excess

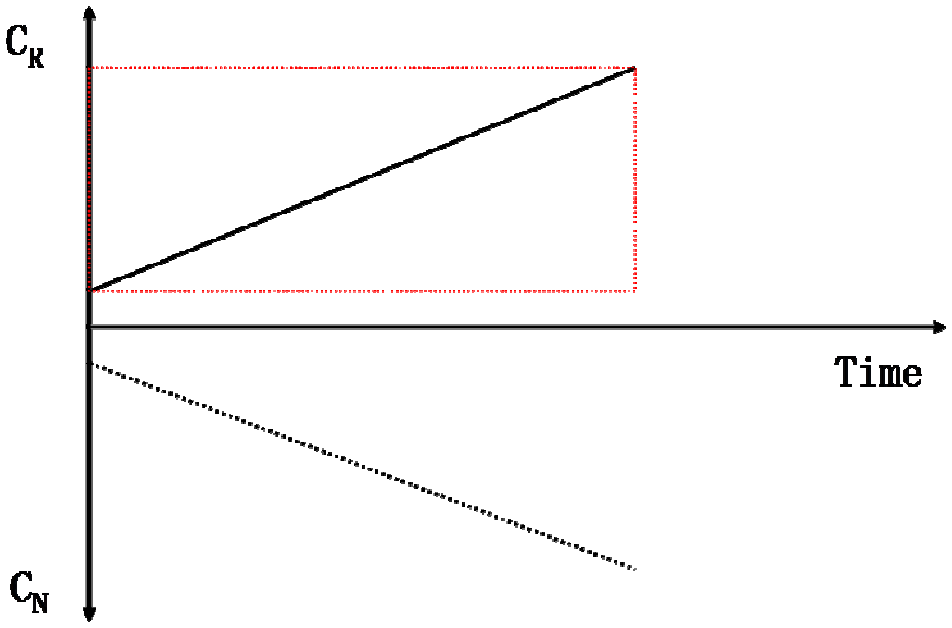

Figure 1. Illustrative demonstration diagram of WPOS. Inside WPOS, the microbalance is maintained; increased concentration of reactive species will be neutralized by counterparts.

oxidants (such as free radicals); in fact, too much neutralizing species (such as antioxidants) is also the cause of microbalance collapse, according to Equation 3 of WPOS. Because reactive species play the key roles in metabolism, immune functions, etc., antioxidant supplements for healthy persons will be problematic.

METHODS

In this study, the author demonstrated how to identify the WPOS using an animal model. For the last two decades, significant progress has been made in the development of biomarkers for the purpose of detecting oxidative stress level (Dalle-Donne et al., 2006). As animal models of diabetes, including insulin-dependent (Kolb, 1987) and non-insulin-dependent (Masiello et al., 1998) are well-established and reliable, the author chose the NIDDM diabetes animal model for the purpose. The well-known lipid-oxidation product, thiobarbituric acid-reacting substance (TBARS) in plasma (Bar-Or et al., 2001), is chosen as oxidative stress biomarkers for the experiments.

Seven week old male Wistar rats were housed in individual cages in an air-conditioned room (25°C±2) with a 12 h light/12 h dark cycle. Care of experimental animals is in accordance with the Animal Protection Law (November 4, 1998, China). They were acclimatized for 7 days with a standard chow diet and free access to water before experimental application. On day eight, oxidative stress was initiated by using a 0.1 M solution of streptozotocin (0.9%) in saline at one dose of 65 mg/kg body weight. Rat tail vein blood was taken every four hours and the concentration of TBARS in plasma was determined.

Subsequently, a figure was plotted using the changes of TBARS in plasma *vs* the time, using the data from streptozotocin-induced diabetes rats.

Confirmation of WPOS was carried out using antioxidant intervention. A combination of lipoic acid, nicotinamide, and amino-acid-chelated selenium in a ratio of 200/100/1(weight) was used to attenuate oxidative stress caused by streptozotocin. One dose of the combination (200 mg lipoic acid/kg body weight) was administered to rats at different time points. Each group has ten rats and the results were tabulated in Table 1.

RESULTS

As shown in Figure 2, the changes of TBARS in plasma were minimal throughout the experimental period for the control groups (no streptozotocin was administrated). Three levels of TBARS were observed for the experimental group. The levels were defined arbitrarily as normal oxidative stress (NOS) with TBARS levels around 2.5 nM/ml for all rats before streptozotocin administration, semi-health level (SHL) with TBARS levels between 2.5-7.6 nM/ml for all rats in the time period of 0-24 h after streptozotocin administration, and disease-caused oxidative stress (DOS) with TBARS levels higher than 7.6 nM/ml for all rats 24 h after streptozotocin administration. Blood glucose levels were also monitored and no diabetes symptoms were observed until 40 h (the incubation period of disease) after streptozotocin administration.

If time is used as one variable and the concentration level of TBARS as another, a two-dimensional window can be seen within the time period of 0 to 24 h as one edge, and TBARS levels between 2.5-7.6 nM/ml as

Table 1. The time effect of intervention by the combination.*

Time point (Hour) of STZ -streptozotocin	Number of rats with induced diabetes	Number of rats with no diabetes
10 h before STZ	9	1
6 h before STZ	3	7
10 min after STZ	4	6
4 h after STZ	3	7
8 h after STZ	4	6
16 h after STZ	4	6
20 h after STZ	3	7
24 h after STZ	9	1
28 h after STZ	10	0
36 h after STZ	9	1

* Lipoic acid, nicotinamide, and amino acid chelated selenium.

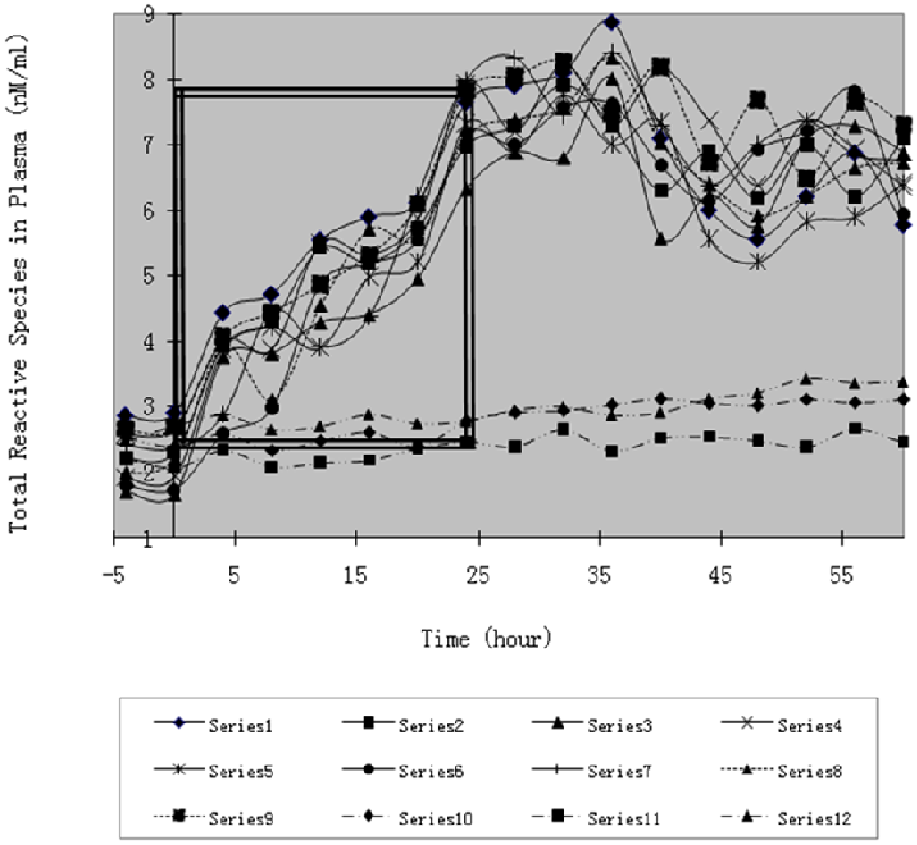

Figure 2. Time course of plasma TBARS level of rats. Series 10-12 are the rats without streptozotocin administration, as control; Series 1-9 are the rats with administered streptozotocin in one dose of 65 mg/kg body weight. Time 0 is when streptozotocin was administered. Tail vein blood was taken every four hours. Double-lined rectangle is the Window Period for Oxidative Stress attenuating intervention of diabetes of Wistar rat.

another edge (double-lined rectangle in Figure 2). This experimental result demonstrates the existence of WPOS.

The experimental results that confirm WPOS (Table 1) demonstrate that in a time period of 28 h, from 6 h before

to 22 h after streptozotocin administration, the numbers of rats with induced diabetes were significantly reduced as a result of attenuating oxidative stress by the combination of lipoic acid, nicotinamide, and amino acid chelated selenium. Detailed analysis revealed that not all rats' oxidative stress were attenuated to normal oxidative stress level. For the rats whose oxidative stress was regulated to normal oxidative stress level, no diabetes was observed. But if the combination was used outside this 28-h period, the onset of diabetes was inevitable even at much higher dose (up to 3 times dosage). This period of 28 h, from 6 h before oxidative stress outbreak normal oxidative stress (NOS) level to the time point when oxidative stress reach disease-caused oxidative stress (DOS) level, is defined as the window period for oxidative stress attenuating intervention (WPOS). One more critical observation is that although the oxidative stress reached disease-caused oxidative stress (DOS) level 24 h after streptozotocin administration, no diabetes was observed until 40 h. This result suggests that the window period for oxidative stress attenuating intervention is much shorter than the incubation period of the disease. The time between the initiations of the damage to the onset of the disease is normally considered as the incubation period of the disease.

DISCUSSION

WPOS explains why all the tests (including lab experiments and animal tests) proved the effectiveness of oxidative stress intervention in preventing chronic diseases *in vitro* and *in vivo* while most clinical trials showed either ineffective results or adverse ones. During careful analysis of all published results, it is revealed that oxidative stress levels were elevated for lab experiments and animal tests before anti-oxidant intervention (the concentration of reactive species C_R was higher than normal). The attenuating interventions were carried out inside WPOS. However, most clinical trials were geared towards a normal population and the majority of the normal population has a normal level concentration of reactive species. Long periods of antioxidants dosing meant the concentration elevation of the neutralizing species C_N. Therefore, clinical trials showed either ineffective or adverse results. When clinical trials of antioxidant intervention are designed, WPOS should be considered. First, participants should have elevated oxidative stress level; second, antioxidants used should be the proper neutralizing species.

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