PROCEEDING

Biomarkers for oxidative stress: measurement, validation, and application

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Abstract : Biomarkers are essential for assessment of oxidative stress and evaluation of antioxidant capacity *in vivo*. Total hydroxyoctadecadienoic acid (HODE) and7-hydroxycholesterol measured after reduction and saponification of biological fluids may be used as reliable biomarker.
J. Med. Invest. 52 Suppl. : 228-230, November, 2005

Keywords **:** *oxidative stress, antioxidant, biomarker, HODE, hydroxycholesterol*

INTRODUCTION

"Oxidative stress" has been defined as an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage (1) . Oxidative stress may be induced by not only oxidants but also non-oxidants. For example, gastric stress ulcer induced by water immersion may also be relevant to oxidative stress. Furthermore, it may be noteworthy that, even if the level of stress is smaller than that of antioxidant capacity, the stress acts as a signal, to which the body responds irrespective of its level. When the stress level exceeds defense capacity, it may induce oxidative damage, whereas the low level stress may stimulate defense network and induce adaptive response. Under such case, stress acts as a good stress , "*eustress*."

It is now widely accepted that the oxidative stress is involved in various disorders and diseases. The beneficial effects of antioxidants against oxidative damage have also received much attention. Biomarkers are important to assess the extent of oxidative stress *in vivo*. They are also necessary for evaluation of antioxidant capacity *in vivo*. Various methods have been proposed and applied to evaluate the antioxidant capacity *in vitro*, but it has been still difficult to evaluate antioxidant capacity *in vivo* quantitatively. Several biomarkers have been used : they include TBARS (thiobarbituric acid reactive substances) , ethane and pentane in exhaled gas, protein carbonyl, aldehyde-modified proteins, and 8-oxodeoxyguanosine. However, they are not satisfactory from the viewpoints of sensitivity, specificity, reproducibility, simplicity and speed.

LIPID PEROXIDATION PRODUCTS AS BIOMARKER

Lipids, in particular polyunsaturated fatty acids and their esters (PUFA) , are quite susceptible to oxidation and their oxidation products may serve as appropriate biomarkers. Fatty acids and their esters and cholesterol are important lipid substrates. They are oxidized by three distinct mechanisms: enzymatic oxidation, non-enzymatic, free radical-mediated oxidation, and non-enzymatic and non-free radical oxidation. Each type of oxidation gives specific products. In Table 1 are shown the characteristics of the oxidation by three mechanisms, taking linoleates as the representative PUFA substrate.

F2-isoprostanes (isoP) and neuroprostanes which consist of series of chemically stable prostaglandin F 2-like compounds formed from arachidonates by a mechanism independent of the cyclooxygenase pathway have been widely accepted as one of the

Received for publication September 9, 2005 ; accepted September 16, 2005.

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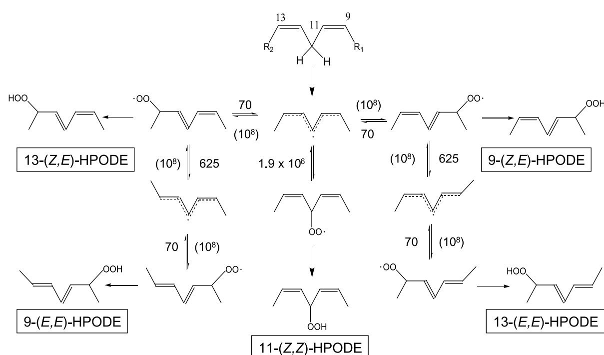
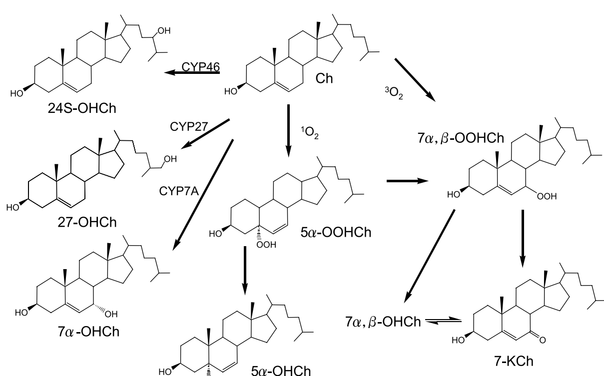
Table 1. Lipid peroxidation (products from linoleate)

Type	Characteristics	Isomers of HPODE		
		Regio	Stereo	Enantio
1. Enzymatic (15-LOX)	Specific Catalytic	13	cis, trans	*S*
2. Non-enzymatic, free redical chain oxidation ($LO_2\cdot$)	Random Chain reaction	9.13	cis, trans trans, trans 9-ct=13-ct 9-tt=13-tt	*R*=*S* (racemic)
3. Non-enzymatic, non-radical oxidation ($^1O_2$)	Random Stoichiometric	9,10,12,13	cis, trans	

most reliable biomarkers of oxidative stress *in vivo* (2) . Linoleates, although less reactive toward free radicals than arachidonates, are in general more abundant than arachidonates and oxidized by a straight forward mechanism to give four conjugated diene hydroperoxides as primary product almost quantitatively (Scheme 1). Cholesterol is also oxidized by the three mechanisms described above (Scheme2). We have recently proposed a method for the measurement of lipid peroxidation *in vivo*, where total hydroxyoctadecadienoic acids (tHODE) and 7-hydroxycholesterol (t 7-OHCh) were determined after reduction with sodium borohydride followed by saponification with potassium hydroxide of biological samples such as plasma, erythrocytes, urine, and tissues (3) . In this method, both free and ester forms of hydroperoxides and ketones as well as hydroxides of linoleic acid and cholesterol are measured as tHODE and t 7-OHCh respectively. This method enables us to measure the ratio of stereo-isomers of HODE, (Z, E)-HODE/(E, E)-HODE, from which the activity of antioxidants can be assessed.



Scheme1. Reaction pathways of the peroxidation of linoleate



Scheme 2. Oxidation pathway of cholesteroll

We have measured the levels of tHODE and t 7-OHCh together with isoP for plasma, erythrocytes and urine from healthy volunteers and also patients of several kinds of diseases. This method has been also applied to the experimental animals under various oxidative stress with and without added antioxidant.

The administration of carbon tetrachloride to mice induced the increase in tHODE in liver and plasma, which was followed by an increase in plasma glutamic oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) . Total isoP was also increased similarly, but its level was about 2 to 3 orders of magnitude smaller than that of tHODE. Similarly, a remarkable increase in tHODE and iso-P was observed when mice were fed choline-deficient diet (CDD) for 1 month. tHODE level was about 2 to 3 orders larger than iso-P. Stereo-isomer ratio of HODE, (Z, E)/(E, E), was decreased by CDD. An administration of water-soluble radical initiator to rats and mice increased the levels of tHODE and iso-P in plasma and liver. The increase in tHODE and isoP and the decrease in (Z, E)/(E, E) ratio of tHODE described above were suppressed by the administration of natural and synthetic antioxidants. tHODE was found to be a good biomarker for the assessment of oxidative stress and also antioxidant capacity *in vivo*.

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