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Fate of Lipid Hydroperoxides in Blood Plasma

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Cholesteryl ester hydroperoxide (CE-OOH) and phosphatidylcholine hydroperoxide (PC-OOH) are the major primary oxidation products of lipoproteins. CE-OOH is present in human and rat plasmas while PC-OOH is undetectable. This is likely due to the enzymatic (plasma glutathione peroxidase) and the nonenzymatic (apolipoproteins A and B-100) reducing activities of PC-OOH in plasma, and to the enzymatic conversion of PC-OOH to CE-OOH by lecithin:cholesterol acyltransferase in high density lipoproteins. The regioisomeric distribution of CE-O(O)H in human plasma indicates that free radical-mediated chain oxidation is an ongoing process, even in healthy young individuals.

DETECTION OF LIPID HYDROPEROXIDES IN HUMAN PLASMA

Lipid hydroperoxides are the primary stable products of lipid peroxidation. For example, cholesteryl ester hydroperoxide (CE-OOH) and phosphatidylcholine hydroperoxide (PC-OOH) are formed as the major oxidation products when low density lipoproteins (LDL) are exposed to oxygen radicals.[1,2] Typical chemical structures for some of these hydroperoxides are shown in Fig. 1. Because of the importance of lipid peroxidation in pathophysiological conditions, we have developed an ultrasensitive method for the detection of lipid hydroperoxides using isoluminol chemiluminescence in connection with a post HPLC column detection.[3] The method has been used widely, since the detection limit is picomole or less, and biological antioxidants do not interfere with the quantitation of lipid hydroperoxides. The characterization of lipid hydroperoxides can be achieved by HPLC migration profiles.

We were able to detect about 3 nM CE-OOH in blood plasma obtained from healthy individuals.[4] It is also noteworthy that the plasma levels of CE-OOH and the ratios of CE-OOH to cholesteryl esters (CE) increase significantly in the order of humans < Sprague-Dawley rats < Nagase analbuminemic rats as shown in Fig. 2,[5] and that this order is generally correlated to the comparative life spans of humans and rats, and is consistent with the observation that Nagase analbuminemic rats are more sensitive than Sprague-Dawley rats to carcinogens.

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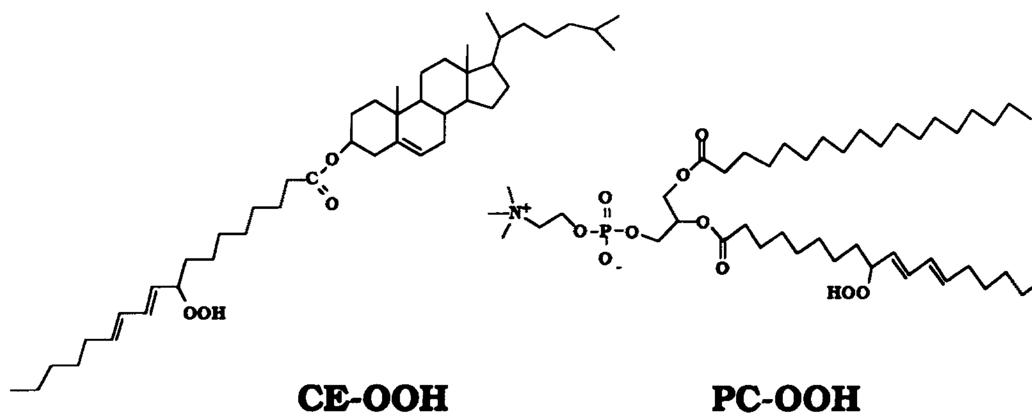

FIGURE 1 Typical chemical structures for cholesteryl ester hydroperoxide (CE-OOH) and phosphatidylcholine hydroperoxide (PC-OOH)

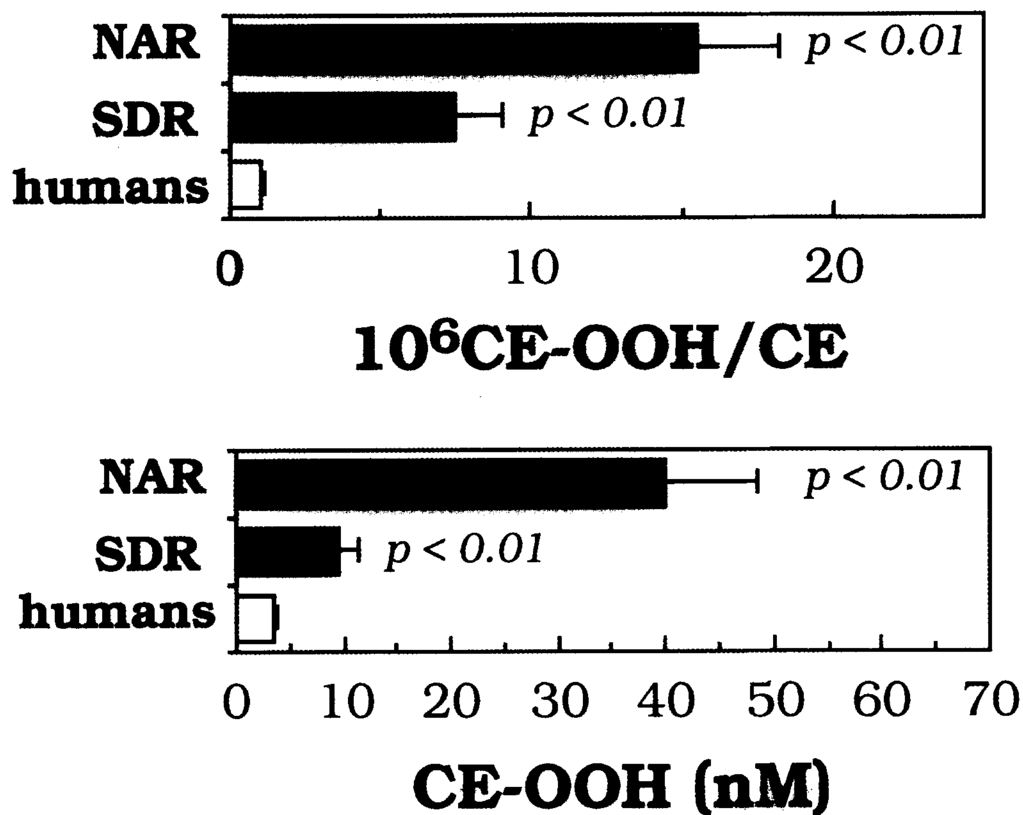

FIGURE 2 Plasma levels of cholesteryl ester hydroperoxide (CE-OOH) and the ratios of CE-OOH to cholesteryl esters (CE) in humans, Sprague-Dawley rats (SDR), and Nagase analbuminemic rats (NAR)

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ENZYMATIC AND NONENZYMATIC REDUCTION OF LIPID HYDROPEROXIDES

In addition, we have failed to detect PC-OOH in human and rat plasmas.[5,6] It is is likely that this is due to the presence of plasma glutathione peroxidase which can reduce PC-OOH but not CE-OOH.[7–9] Glutathione peroxidase requires two molecules of glutathione for the reduction of one molecule of hydroperoxide. This implies that *in vitro* human plasma is capable of reducing only about 2.5 μM hydroperoxides because the levels of glutathione usually available in human plasma is 5 μM.[10] However, we have noticed that human plasma has a capacity to reduce 20 μM PC-OOH or more.[11,12] We isolated two hydroperoxide-reducing protein fractions from human plasma by means of a sequential purification scheme, which involves ammonium sulfate precipitation followed by sequential chromatography on anion exchange, hydrophobic interaction, and heparin columns. One of the proteins was identified as apolipoprotein A-I by N-terminal amino acid sequence analysis.[12] The other protein was identified as apolipoprotein B-100 by amino acid sequence analysis of its tryptic peptides.[13] Stocker *et al.* also found apolipoprotein A-I and A-II reduced CE-OOH.[14,15] The reduction by these apolipoproteins is methionine dependent[13–15] and 3, 3, and 78 methionines are present in apolipoproteins A-I, A-II, and B-100, respectively. On the other hand, albumin cannot reduce PC-OOH despite the presence of 6 methionine residues in albumin. Free methionine is also inactive. Therefore, the accessibility and binding of lipid hydroperoxides to the protein methionine residues are crucial for the reduction of lipid hydroperoxides.

CONVERSION OF PC-O(O)H TO CE-O(O)H

Lecithin:cholesterol acyltransferase (LCAT) is present in high density lipoprotein (HDL) and converts PC to CE and lysoPC in the presence of free cholesterol (FC). We have demonstrated LCAT is able to convert PC-O(O)H to CE-O(O)H, as well.[11] If we consider that CE-OOH in HDL is preferentially taken up by the liver,[16] LCAT may play a beneficial role in the removal of PC-O(O)H from human plasma. In fact, LCAT deficiency is associated with atherosclerosis, renal failure, corneal opacities, and other degenerative diseases.[17] Figure 3 summarizes the fate of lipid hydroperoxides in blood plasma, based on the above discussion.

REGIOISOMERS OF CHOLESTERYL LINOLEATE HYDRO(PERO)XIDES

The presence of CE-OOH, largely cholesteryl linoleate hydroperoxides (Ch18:2-OOH), in human plasma[4] is thought to constitute direct evidence for the free radical-mediated chain oxidation of lipids *in vivo,* since CE is not considered to be a substrate for enzymatic oxidation. However, it has been shown that 15-lipoxygenase is capable of oxidizing cholesteryl linoleate (Ch18:2) to give a regiospecific 13ZE-Ch18:2-OOH even when Ch18:2 is located in low density lipoprotein.[18] On the other hand, free radical chain oxidation of Ch18:2 gives four regioisomers (13ZE-, 13EZ-, 9EZ-, and 9EE-Ch18:2-OOH) as shown in Fig. 4.[19]We therefore measured these regioisomers in human plasma from healthy subjects to distinguish between nonenzymatic and enzymatic oxidation. Little oxidation during the analytical procedure was confirmed by using cholesteryl 11Z, 14Z-eicosadienoate (Ch20:2) as an external probe.[20]We found that all regioisomers were present and that the enzymatic oxidation product (13ZE-form) was not the major product as shown in Fig. 5, indicating that free radical chain oxidation of linoleate occurs, even in healthy young individuals.[20] This conclusion is supported by the presence of isoprostanes, nonenzymatic oxidation products of arachidonic acid, in

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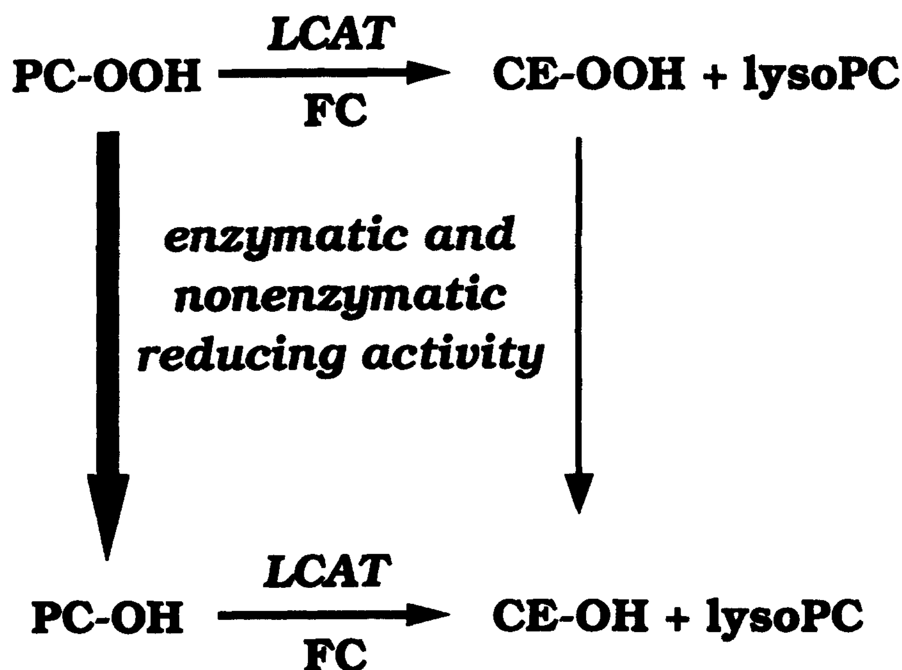

FIGURE 3 Fate of lipid hydroperoxides in human plasma

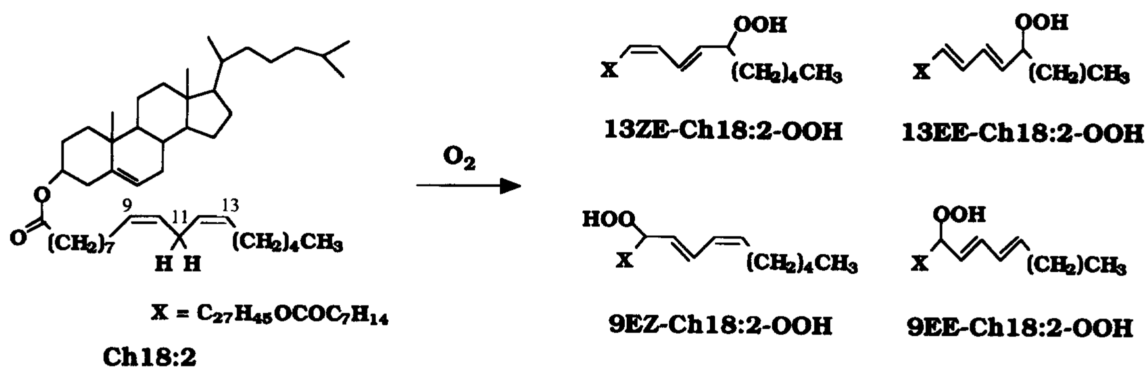

FIGURE 4 Autoxidation products derived from cholesteryl linoleate (Ch18:2)

plasma and urine from healthy humans. Levels of isoprostanes in plasma from healthy humans are reported to be 0.1–0.3 nM[21–23] which are significantly lower than the plasma Ch18:2-O(O)H levels found here; 13.6 nM. This is not surprising since CE-OOH are the major products while isoprostanes are minor products during the copper-induced oxidation of low density lipoprotein.

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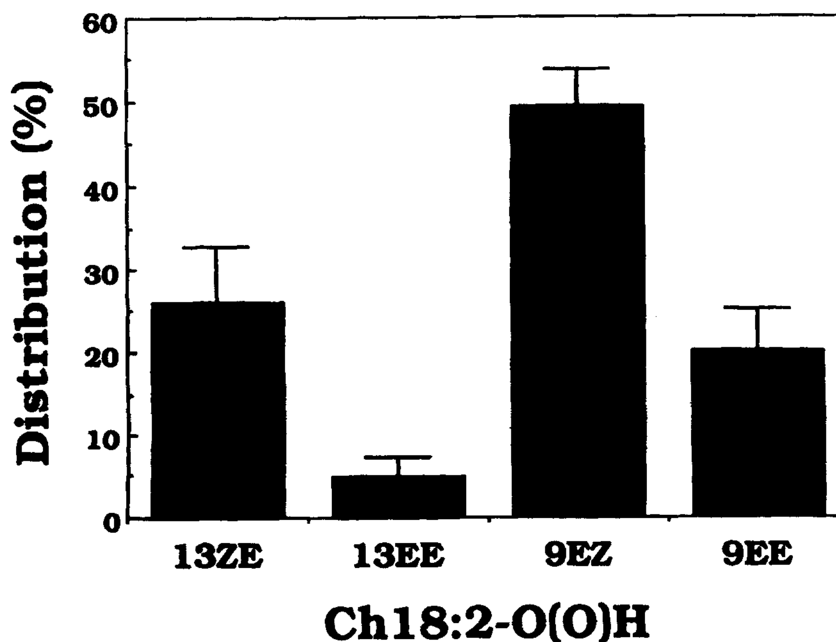

FIGURE 5 Distribution of cholesterol linoleate hydro(pero)xide regioisomers in plasma from nine non-smoking Japanese males (21–29 years old)

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