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COMPARISON OF PLASMA LEVELS OF LIPID HYDROPEROXIDES AND ANTIOXIDANTS IN HYPERLIPIDEMIC NAGASE ANALBUMINEMIC RATS, SPRAGUE-DAWLEY RATS, AND HUMANS

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Summary: The levels of lipid hydroperoxides and antioxidants in plasma samples from Nagase analbuminemic rats (NAR) and control Sprague-Dawley rats (SDR) were measured in comparison with those from normal human subjects. Cholesteryl ester hydroperoxide (CE-OOH) was detected, but phosphatidylcholine hydroperoxide was not. The levels of CE-OOH and the ratios of CE-OOH/CE were found to increase significantly in the order of human < SDR < NAR, suggesting that oxidative stress increases in the same order. NAR have a significantly lower level of ascorbate and lower ratio of ubiquinol/ubiquinone concentrations than SDR. This also suggests that NAR are subject to more oxidative stress than SDR, since ascorbate and ubiquinol are the most effective plasma antioxidants against oxygen radicals. © 1992 Academic Press, Inc.

Oxygen radicals have been suggested to be important in aging and degenerative diseases, including cancer [1,2]. It is, therefore, of interest to compare the degrees of oxidative stress of species with different life spans and sensitivities toward carcinogens. We selected three groups of animals for this purpose: Nagase analbuminemic rats (NAR), Sprague-Dawley rats (SDR), and healthy humans. Humans live the longest and NAR are more susceptible than SDR to the inductions of cancers of the urinary bladder [3], kidney [4], and stomach [5] by carcinogens such as *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, *N*-dimethylnitrosamine, and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, respectively.

NAR were discovered among hypercholesterolemic SDR and established through breedings by Nagase *et al.* [6]. NAR have extremely low levels of serum albumin, but their total amount of serum proteins is almost the same as that of SDR [7]. NAR show

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only mild abnormalities with the exception of hypercholesterolemia [8], elevated plasma GOT, GPT, and LDH activities [9], and occasional edema [10].

We measured the plasma levels of lipid hydroperoxides as markers of oxidative stress, since lipids are highly susceptible to oxidation and lipid hydroperoxides are the primary oxidation products. We also measured antioxidant concentrations because antioxidants prevent oxidation by sacrificing themselves.

MATERIALS AND METHODS

Male NAR were kindly supplied by Dr. S. Nagase, Sasaki Institute, Tokyo, Japan (n = 13, 8-72 weeks of age). Male SDR were obtained from Charles River Japan Inc. (Atsugi, Japan) (n = 16, 8-32 weeks of age). Rats were fed on basal diet (CE-2, CLEA Japan, Tokyo). Samples of human plasma were obtained from healthy male volunteers (n = 23, 24-63 years of age). Blood was collected in a heparinized syringe and promptly centrifuged at 1500 *g* for 10 min. Plasma samples were stored at -80°C.

Plasma antioxidants, lipid hydroperoxides, and selected lipids were analyzed using an HPLC column equipped with UV and chemiluminescence (CL) detectors as previously described [11,12]. Two HPLC pumps (type 880-PU), a UV detector (type 875-UV), and a CL detector (type 825-CL) were purchased from Japan Spectroscopics (Tokyo). Plasma was extracted by shaking it vigorously with 4 volumes of methanol and 20 volumes of *n*-hexane, and the two phases were separated by centrifugation at 1500 *g* for 10 min. A sample of the aqueous methanol phase was injected into an aminopropylsilyl column monitored at 265 nm for the measurement of ascorbate and urate. The aqueous methanol phase was also injected into a silica gel column to analyze phosphatidylcholine hydroperoxide (PC-OOH) by CL detection. The eluent for the above two systems was methanol/40 mM monobasic sodium phosphate (9/1, v/v). The hexane phase was evaporated under reduced pressure and the residue was redissolved in methanol/*tert*-butyl alcohol (1/1 by volume). A sample was injected into an octylsilyl column to measure α-tocopherol and ubiquinones from the absorption at 276 nm and ubiquinols and CE-OOH by CL detection. The eluent was methanol/*tert*-butyl alcohol (19/1 by volume). The above solution was also injected into an octadecylsilyl column monitored at 210 nm to measure free cholesterol (FC) and cholesteryl esters (CE). The eluent was methanol/*tert*-butyl alcohol (1/1 by volume). All HPLC columns (5 μm, 4.6 x 250 mm) were obtained from Supelco Japan (Tokyo). Methyl linoleate hydroperoxide was used as a standard for the measurement of lipid hydroperoxides.

RESULTS AND DISCUSSION

Figure 1 shows chemiluminescence chromatograms of hexane extracts from plasma samples of NAR and SDR. CE-OOH, ubiquinol-9, and ubiquinol-10 gave positive peaks [12] whereas α-tocopherol gave a negative peak [11]. The positive peak at about 3.8 min has not been characterized. NAR showed a higher level of CE-OOH than SDR, but gave a similar level of ubiquinol to the latter. Figure 2 shows the

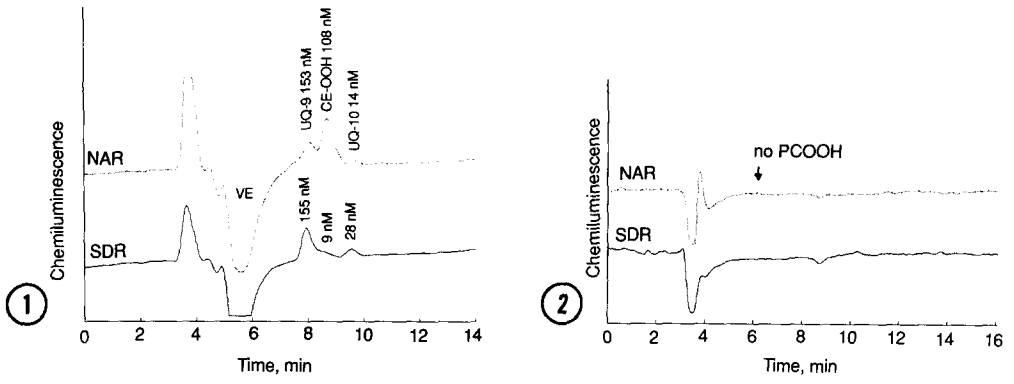

Fig. 1. Chemiluminescence chromatograms of hexane extracts from plasmas of NAR and SDR analyzed with an octylsilyl column using methanol/*tert*-butyl alcohol (19/1, v/v) as the mobile phase. VE, α-tocopherol; UQ-9, ubiquinol-9; UQ-10, ubiquinol-10.

Fig. 2. Chemiluminescence chromatograms of methanol extracts from plasmas of NAR and SDR analyzed with an aminopropylsilyl column using methanol/40 mM monobasic sodium phosphate (9/1, v/v) as the mobile phase.

chemiluminescence chromatograms of methanol extracts from plasma samples of NAR and SDR. No peak of PC-OOH was detected in either. This finding is not surprising, since PC-OOH readily decomposes in human plasma [13,14], and this was also demonstrated in rat plasma (data not shown). Judging from our analyses, the PC-OOH concentrations in plasma were less than 10 nM, given that the detection limit for PC-OOH is 0.1 pmol and the volume of sample injected was 10 μl. CE-OOH, however, has been shown to be stable in human plasma [13]. Table 1 summarizes results on the plasma levels of FC, CE, total cholesterol (TC) as the sum of FC and CE, CE-OOH, CE-OOH/CE, ubiquinol, ubiquinol/TC, ubiquinone, ubiquinol/ubiquinone, α-tocopherol, α-tocopherol/TC, ascorbate, and urate in human, SDR, and NAR plasmas. Data for human plasma were consistent with those reported before [12] except for the urate level, for which a miscalculation has been corrected.

Comparison of NAR and SDR plasmas

NAR plasma has higher levels of FC and CE than SDR plasma, as observed before [3,4]. The mean levels of CE-OOH in SDR and NAR were significantly different, being 9.5 and 39.8 nM, respectively. The CE-OOH/CE ratios in SDR and NAR were 7.5×10^{-6} and 15.5×10^{-6}, respectively, the difference being significant ($p = 0.01$ by Student' t test). These results suggest that oxidative stress is greater in NAR than in SDR. This possibility was supported by a comparison of the plasma levels antioxidants, especially ascorbate and ubiquinol. These two antioxidants have been

Table 1. Levels of selected lipids, antioxidants, and lipid hydroperoxide in human, SDR, and NAR plasmas

	humans (n=23)	SDR (n=16)[b]	NAR (n=13)[c]
FC, mM	1.47 ± 0.22	0.52 ± 0.12	0.94 ± 0.20
CE, mM	2.96 ± 0.47	1.45 ± 0.64	2.59 ± 1.03
TC, mM	4.42 ± 0.61	1.97 ± 0.73	3.53 ± 1.11
CE-OOH, nM	3.4 ± 1.9	9.5 ± 7.1	39.8 ± 31.8
10^6 CE-OOH/CE	1.0 ± 0.6	7.5 ± 5.9	15.5[d] ± 9.7
Ubiquinol[a], μM	0.62 ± 0.39	0.16 ± 0.07	0.18 ± 0.06
10^3 Ubiquinol[a]/TC	0.14 ± 0.09	0.09 ± 0.05	0.06 ± 0.03
Ubiquinone[a], μM	0.47 ± 0.12	0.16 ± 0.06	0.40 ± 0.15
Ubiquinol/ubiquinone[a]	1.4 ± 0.9	1.2 ± 0.4	0.4 ± 0.2
α-Tocopherol, μM	25.7 ± 8.1	21.0 ± 8.6	31.1 ± 9.7
10^3 α-Tocopherol/TC	5.8 ± 1.6	12.6 ± 7.3	9.5 ± 4.2
Ascorbate, μM	37.4 ± 14.5	34.7 ± 13.0	9.4 ± 7.3
Urate, μM	498 ± 104	156 ± 110	276 ± 169

[a] Ubiquinol (ubiquinone)-10 in humans and ubiquinol (ubiquinone)-9 in rats.

[b] An underline shows a significant difference from humans by Student's t ($p < 0.01$).

[c] A double underline shows a significant difference from SDR by Student's t ($p < 0.01$).

[d] Significantly different from SDR by Student's t ($p = 0.011$).

Numbers indicate means ± S.D.

shown to decrease initially during oxidation of plasma initiated by aqueous peroxyl radicals [13,15], activated neutrophils [13], and cupric ion [14]. As shown in Table 1, NAR have a significantly lower level of ascorbate and lower ratio of reduced to oxidized ubiquinone than SDR. On the contrary, there were no significant differences in the ratios of α-tocopherol to TC or the urate levels in the two strains. This is consistent with the observation that α-tocopherol and urate decreased only after the consumption of ascorbate and ubiquinol during plasma oxidation [13-15].

Comparison of human and SDR plasmas

The plasma levels of ascorbate were the same in humans and SDR. Furthermore, the ratios of ubiquinol to TC and ubiquinol to ubiquinone were also the same. The α-tocopherol levels in the two groups were similar, but, the ratio of α-tocopherol to TC was higher in SDR plasma than in human plasma. The CE-OOH level and CE-

OOH/CE ratio were significantly greater in SDR than in humans. SDR have about 3 times more CE-OOH and about a 7.5-times higher CE-OOH/CE ratio than humans. Furthermore, the difference in the total amounts of CE-OOH formed would be much more if the half lives of lipoproteins in circulating plasma in the two groups were taken into account. For example, the half lives of low density lipoprotein in rat and human plasma are 10 min and 3 days, respectively [16]. These results clearly indicate greater oxidative stress in SDR than in humans. A similar conclusion can be deduced from results with other markers of oxidative stress. Rats excrete about 15 times more oxidized DNA products, thymine glycol and thymidine glycol, than humans [17]. Mice excrete about 3 times more of another type of oxidized DNA product, 8-hydroxy-2'-deoxyguanosine than humans [18]. Rats expire about 10 times more pentane, which is believed to be an end product of lipid hydroperoxide, than do humans [19].

In summary, measurements of plasma lipid hydroperoxides and antioxidants, especially ascorbate and ubiquinol, suggest that oxidative stress increases in the order of human < SDR < NAR. This order is consistent with the differences in life spans of humans and rats, and with the finding that NAR are more sensitive than SDR to carcinogens. These results are also compatible with the deleterious effects of unquenched reactive oxygen species in the process of aging and in carcinogenesis.

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