Reciprocal protection of LDL and HDL oxidised by $^{\bullet}OH$ free radicals in the presence of oxygen

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Abstract The aim of this work was to compare the behaviour of HDL oxidised by $^{\bullet}OH$ or $^{\bullet}OH/O_2^{\bullet-}$ free radicals produced by gamma radiolysis in the absence or in the presence of LDL at the same concentration of 3 g·l^{-1}, in order to specify the possibility of reciprocal protection of HDL and LDL towards lipid peroxidation. This oxidation was quantitatively evaluated by the decrease of endogenous α-tocopherol and the formation of oxidation products (thiobarbituric acid-reactive substances and conjugated dienes) and by the determination of initial radiation yields. Our results demonstrated that HDL could be protected by LDL against in vitro radical oxidation only in the presence of oxygen (action of $^{\bullet}OH/O_2^{\bullet-}$ free radicals). This observation addresses new questions about the interaction between HDL and LDL, especially the possibility of a reciprocal protection.

Key words: Lipoprotein, high density; Lipoprotein, low density; Peroxidation; Radiation; Free radical

1. Introduction

Human high density lipoproteins (HDL) are well-known for their protective effect in the atherosclerotic process, by their ability to remove the cellular cholesterol from peripheral tissues [1]. Besides this classical role of HDL, protection by HDL against LDL peroxidation has been reported by several authors [2–4]. However, both HDL and LDL are susceptible in vivo to oxidative modifications in the subendothelial space [5], so that the question of a reciprocal protection of these lipoproteins can be addressed. In a recent study [6], Tribble et al. observed an antioxidant effect of LDL on HDL oxidation when these lipoproteins were co-incubated in the presence of Cu^{2+}. A convenient method to study lipoprotein peroxidation initiated by free radicals is gamma radiolysis of water, which allows selective production of oxygenated free radicals with well-known radiolytic yields (number of free radicals produced per unit of energy absorbed, that is per Joule). We have previously used this method to initiate the peroxidation of human LDL [7,8] and HDL [9,10] and we proposed kinetic schemes of degradation of these lipoproteins under free radical attack. The aim of the present study was to compare the behaviour of HDL oxidised by $^{\bullet}OH$ or $^{\bullet}OH/O_2^{\bullet-}$ free radicals in the absence or in the presence of LDL at the same concentration of 3 g·l^{-1}, in order to specify the possibility of reciprocal protection of HDL and LDL towards lipid peroxidation. The oxidative modifications induced by $^{\bullet}OH$ or $^{\bullet}OH/O_2^{\bullet-}$ free radicals have been quantitatively analysed by monitoring the decrease in α-tocopherol (major lipoprotein endogenous antioxidant), and the formation of thiobarbituric acid-reactive substances (TBARS) and of conjugated dienes, as a function of increasing radiation doses, and by determining initial radiolytic yields (decrease in α-tocopherol, formation of TBARS and conjugated dienes) at pH 7.

2. Materials and methods

2.1. Isolation of lipoproteins

LDL ($1.019 < d < 1.063$) and HDL ($1.063 < d < 1.21$) were isolated from the serum of normolipidaemic donors by sequential ultracentrifugation [11] in the presence of EDTA (1.08 mmol·l^{-1}) and further dialysed for 18 h against a 10^{-2} mol·l^{-1} sodium phosphate buffer (pH 7), just prior to use. For the radiolysis experiments, the dialysed solutions of HDL and LDL were adjusted to a concentration of 3 g·l^{-1} (expressed as total lipoprotein), by dilution in the same buffer. For the experiments where HDL and LDL were both subjected to irradiation, HDL and LDL were separated after irradiation by ultracentrifugation at $d = 1.063$ and HDL concentration was adjusted again to 3 g·l^{-1}. The analysis of the markers of lipid peroxidation (see below) was thus done on the HDL and LDL fractions. Apolipoprotein A-I and B concentrations were obtained by laser immunonephelometry [12]. Lipoprotein cholesterol, phospholipids and triacylglycerols were assayed by enzymatic methods [13–15].

2.2. Gamma radiolysis

Gamma irradiations were performed with a ^{60}Co irradiator (activity approx. 50 Ci). After washing, the vessels used for irradiation were heated at 400°C for 4 h. Dosimetry was determined by Fricke's method [16] (radiooxidation of 10^{-3} mol·l^{-1} ferrous sulfate (Mohr's salt) solutions in 0.4 mol·l^{-1} H_2SO_4 under air atmosphere) taking λ_{max} (Fe^{3+}) = 304 nm, $\varepsilon_{304} = 2204$ mol^{-1}·l·cm^{-1} at 25°C and $G = 16.2 \times 10^{-7}$ mol·J^{-1}. Irradiations were performed on 5 ml of lipoprotein solutions in 10^{-2} mol·l^{-1} sodium phosphate buffer, at pH 7. Before irradiation, the lipoprotein solutions were saturated with either N_2O or O_2 for 40 min at 25°C. Under these conditions, in N_2O-saturated solutions, hydroxyl free radicals were generated with a radiolytic yield (number of $^{\bullet}OH$ free radicals produced by unit of energy absorbed) of 5.6×10^{-7} mol·J^{-1} [16]. In O_2-saturated solutions, hydroxyl and superoxide radicals were simultaneously produced with respective yields of 2.8×10^{-7} mol·J^{-1} and 3.4×10^{-7} mol·J^{-1} [17]. The dose rate was 2.4×10^{-2} Gy·s^{-1} and the radiation doses were 0–800 Gy. For each experimental set, 5 ml of N_2O- or O_2-saturated non-irradiated lipoprotein solution was taken as control.

2.3. Biochemical markers of lipid peroxidation

The decrease in endogenous vitamin E and the formation of TBARS were systematically analysed in lipoproteins one day after irradiation. Results were identical to those obtained immediately after

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Abbreviations: EDTA, ethylene diamine tetraacetic acid; HDL, high density lipoproteins; HPLC, high performance liquid chromatography; LDL, low density lipoproteins; TBARS, thiobarbituric acid-reactive substances

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irradiation. To monitor the formation of conjugated dienes, differential absorbance spectra were always recorded immediately after irradiation.

Endogenous α-tocopherol was assayed before and after irradiation, by reverse phase HPLC, with spectrophotometric detection at 292 nm [18]. TBARS were determined as end-products of lipid peroxidation by a spectrofluorimetric method (λ_{exc} = 515 nm, λ_{em} = 548 nm) using 1,1,3,3-tetraethoxypropane as the standard [19]. The concentration of TBARS produced was calculated as the difference between the TBARS concentration in the irradiated samples and the non-irradiated controls. Differential absorbance spectra of oxidised lipoproteins were recorded between 200 and 700 nm, taking control HDL as reference, with a 1-mm optical pathway. An increase in the differential absorbance at 236 nm constituted evidence for the formation of conjugated dienes [20].

For the decrease in α-tocopherol and TBARS formation, initial radiation yields (G) were respectively determined as the initial slopes of the curves and as the slopes of the linear part of the curves, and expressed in mol of disappeared α-tocopherol and formed TBARS, per unit of energy (Joule) absorbed, respectively. To some extent, these yields reflected the rates of α-tocopherol disappearance and of TBARS formation as a result of the action of the oxygenated free radicals selectively produced at steady-state concentrations and hence at a constant rate by gamma radiolysis of aqueous HDL solutions.

3. Results

3.1. Action of $^{\bullet}OH$ free radicals

Decrease in endogenous vitamin E. Fig. 1 shows the decrease in endogenous vitamin E in HDL (N_2O-saturated solutions) as a function of the radiation dose, depending of the presence of 3 g·l^{-1} LDL. When 3 g·l^{-1} HDL were irradiated in the presence of 3 g·l^{-1} LDL, then isolated from LDL, vitamin E was consumed as a function of the radiation dose, with an initial yield of $(1.38 \pm 0.10) \times 10^{-7}$ mol·J^{-1}, which was a little lower than if the same HDL were irradiated in the absence of LDL ($(1.40 \pm 0.10) \times 10^{-7}$ mol·J^{-1}). In addition, when the HDL/LDL mixture was irradiated, vitamin E remained in HDL, from 100 Gy to 700 Gy, at a level about 2×10^{-6} mol·l^{-1}, whereas vitamin E totally disappeared at about 500 Gy when HDL were irradiated alone.

Formation of TBARS. As can be seen in Fig. 2, TBARS appeared in irradiated HDL (alone or with LDL) with the same yield, i.e. $(0.25 \pm 0.05) \times 10^{-7}$ mol·J^{-1}. However, when HDL was irradiated with LDL, TBARS formation exhibited a maximum for a radiation dose of 200 Gy, then decreased, so that TBARS concentration in HDL at high radiation doses (> 300 Gy) was about 2–3-fold lower than that obtained when HDL were irradiated alone.

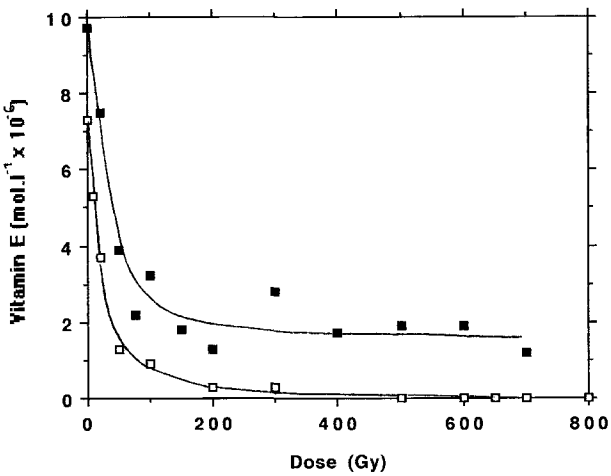

Fig. 1. Disappearance of vitamin E in 3 g·l^{-1} HDL as a function of the radiation dose, depending on the presence of 3 g·l^{-1} LDL. Action of $^{\bullet}OH$ free radicals. 10^{-2} mol·l^{-1} sodium phosphate buffer, pH 7. N_2O-saturated solutions. Dose rate = 2.4×10^{-2} Gy·s^{-1}. ■, 3 g·l^{-1} HDL in the presence of 3 g·l^{-1} LDL; □, 3 g·l^{-1} HDL in the absence of LDL. Results are the means of two separate experiments performed on HDL isolated from a pool of sera of 10 normolipidaemic donors.

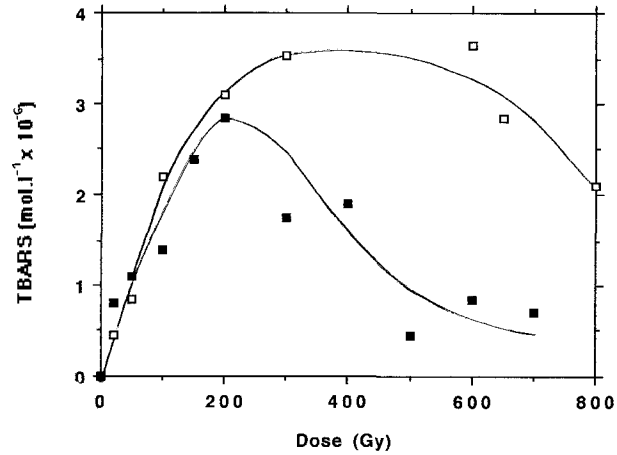

Fig. 2. TBARS formation in 3 g·l^{-1} HDL as a function of the radiation dose, depending on the presence of 3 g·l^{-1} LDL. Action of $^{\bullet}OH$ free radicals. 10^{-2} mol·l^{-1} sodium phosphate buffer, pH 7. N_2O-saturated solutions. Dose rate = 2.4×10^{-2} Gy·s^{-1}. ■, 3 g·l^{-1} HDL in the presence of 3 g·l^{-1} LDL; □, 3 g·l^{-1} HDL in the absence of LDL. Results are the means of two separate experiments performed on HDL isolated from a pool of sera of 10 normolipidaemic donors.

Formation of conjugated dienes. The yield of conjugated diene formation in HDL irradiated alone was $(14.0 \pm 1.00) \times 10^{-7}$ mol·J^{-1}, whereas it was $(11.1 \pm 1.00) \times 10^{-7}$ mol·J^{-1} when HDL were irradiated in the presence of LDL (Fig. 3). It is noteworthy that, in both cases (in the absence or in the presence of HDL), this yield was much higher than $G(^{\bullet}OH)$. In the case of the HDL/LDL mixture, we observed at high radiation doses (> 200 Gy) a higher dispersion of the experimental values than when HDL were alone in the irradiated medium.

3.2. Action of $^{\bullet}OH/O_2^{\bullet-}$ free radicals

Decrease in endogenous vitamin E. Fig. 4 shows the decrease in endogenous vitamin E in 3 g·l^{-1} HDL (O_2-saturated solutions), as a function of the radiation dose, depending on the presence of 3 g·l^{-1} LDL. When 3 g·l^{-1} HDL were irradiated in the presence of 3 g·l^{-1} LDL, vitamin E in HDL was consumed with an initial yield of $(0.65 \pm 0.10) \times 10^{-7}$ mol·J^{-1}, which was about 2-fold lower than that obtained when HDL were irradiated alone ($(1.50 \pm 0.10) \times 10^{-7}$ mol·J^{-1}).

Formation of TBARS. Fig. 5 shows TBARS formation in 3 g·l^{-1} HDL when they were irradiated either in the presence or in the absence of 3 g·l^{-1} LDL (O_2-saturated solutions). The yield of TBARS formation was 3-fold lower when HDL and LDL were simultaneously present in solution than that observed when HDL were alone in solution: $(0.21 \pm 0.05) \times 10^{-7}$ vs. $(0.70 \pm 0.10) \times 10^{-7}$ mol·J^{-1}.

Formation of conjugated dienes. When 3 g·l^{-1} HDL were subjected to the action of $^{\bullet}OH/O_2^{\bullet-}$ free radicals in the presence of 3 g·l^{-1} LDL, the yield of conjugated diene formation (Fig. 6) was $(2.90 \pm 0.50) \times 10^{-7}$ mol·J^{-1}, i.e. more than 2-fold

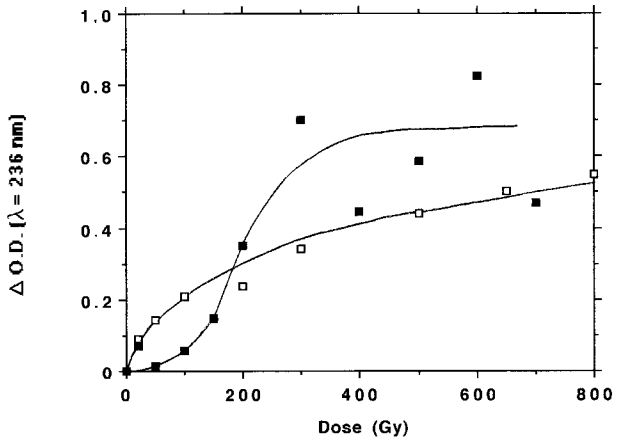

Fig. 3. Differential absorbance at 236 nm in 3 $g \cdot l^{-1}$ HDL as a function of the radiation dose, depending on the presence of 3 $g \cdot l^{-1}$ LDL. Action of $^{\bullet}OH$ free radicals. 10^{-2} $mol \cdot l^{-1}$ sodium phosphate buffer, pH 7. N_2O-saturated solutions. Dose rate = 2.4×10^{-2} $Gy \cdot s^{-1}$. ■, 3 $g \cdot l^{-1}$ HDL in the presence of 3 $g \cdot l^{-1}$ LDL; □, 3 $g \cdot l^{-1}$ HDL in the absence of LDL. Results are the means of two separate experiments performed on HDL isolated from a pool of sera of 10 normolipidaemic donors.

lower than that obtained when HDL were irradiated alone ($(6.30 \pm 0.50) \times 10^{-7}$ $mol \cdot J^{-1}$).

Table 1 summarises the comparative results obtained with 3 $g \cdot l^{-1}$ HDL subjected to $^{\bullet}OH$ or $^{\bullet}OH/O_2^{\bullet-}$ free radicals, depending on the simultaneous presence of 3 $g \cdot l^{-1}$ LDL. It clearly shows the major consequences of the presence of LDL when HDL were irradiated:

– when $^{\bullet}OH$ free radicals were generated alone, LDL did not modify the yields of vitamin E decrease, of TBARS formation or of conjugated diene production. Nevertheless, the presence of a remaining vitamin E steady-state at high radiation doses (up to 800 Gy) was observed in the presence of LDL and not in their absence;

– by contrast, when $^{\bullet}OH$ and $O_2^{\bullet-}$ free radicals were simultaneously generated in solution, all determined radiation yields were clearly lowered by the presence of LDL during the HDL irradiation.

Table 1 also makes it possible to compare the behaviour of the 3 $g \cdot l^{-1}$ LDL reisolated from the irradiated HDL/LDL mixtures, with that of 3 $g \cdot l^{-1}$ LDL irradiated alone. Similarly to what was observed for HDL:

– $^{\bullet}OH$ free radicals led to a yield value of vitamin E decrease in LDL irradiated in the presence of HDL which was close to that obtained in LDL irradiated alone ($(0.80 \pm 0.10) \times 10^{-7}$ vs. $(0.70 \pm 0.10) \times 10^{-7}$ $mol \cdot J^{-1}$). A steady-state vitamin E level (1.5×10^{-6} $mol \cdot l^{-1}$) was observed in LDL only under the former conditions. The yield of TBARS formation was not significantly different in the presence or in the absence of HDL ($(0.15 \pm 0.05) \times 10^{-7}$ vs. $(0.10 \pm 0.05) \times 10^{-7}$ $mol \cdot J^{-1}$), and the TBARS concentrations at high radiation doses were very similar (1.5×10^{-6} vs. 2.0×10^{-6} $mol \cdot l^{-1}$);

– by contrast, in the presence of oxygen, the yield of vitamin E decrease in LDL was lowered by the presence of HDL ($(0.70 \pm 0.10) \times 10^{-7}$ vs. $(1.00 \pm 0.10) \times 10^{-7}$ $mol \cdot J^{-1}$), and vitamin E disappeared at a higher radiation dose (350 Gy vs. 150 Gy). With regard to TBARS, the presence of HDL dramatically decreased both G(TBARS) ($(0.15 \pm 0.01) \times 10^{-7}$ vs. $(1.10 \pm 0.20) \times 10^{-7}$ $mol \cdot J^{-1}$) and the plateau of TBARS obtained at high radiation doses (6.5×10^{-6} vs. 17.0×10^{-6} $mol \cdot l^{-1}$).

4. Discussion

Kinetic mechanisms of HDL oxidation have been developed elsewhere [10]. Briefly, it has been previously shown [10] that free radicals could compete towards the different molecular targets of HDL (lipids, apolipoproteins, antioxidants), but each kind of free radical reacted in different ways. $^{\bullet}OH$ free radicals were able to attack non-specifically all protein and lipid sites, which is not the case for $O_2^{\bullet-}$ free

Table 1
Comparative results obtained with 3 $g \cdot l^{-1}$ HDL subjected to $^{\bullet}OH$ or $^{\bullet}OH/O_2^{\bullet-}$ free radicals, depending on the simultaneous presence of 3 $g \cdot l^{-1}$ LDL

		3 $g \cdot l^{-1}$ HDL in the absence of LDL	3 $g \cdot l^{-1}$ HDL in the presence of 3 $g \cdot l^{-1}$ LDL	3 $g \cdot l^{-1}$ LDL in the presence of 3 $g \cdot l^{-1}$ HDL	3 $g \cdot l^{-1}$ LDL in the absence of HDL [8]
$^{\bullet}OH$ pH 7	G(−vit.E) ($\times 10^{-7}$ $mol \cdot J^{-1}$)	1.40 ± 0.10	1.38 ± 0.10	0.80 ± 0.10	0.70 ± 0.10
	Dose for [vitamin E] $\leq 2 \times 10^{-7}$ $mol \cdot l^{-1}$	500 Gy	none (vitamin E steady-state: 2×10^{-6} $mol \cdot l^{-1}$)	none (vitamin E steady-state: 1.5×10^{-6} $mol \cdot l^{-1}$)	600 Gy
	G(TBARS) ($\times 10^{-7}$ $mol \cdot J^{-1}$)	0.25 ± 0.05	0.25 ± 0.05	0.15 ± 0.05	0.10 ± 0.05
	Plateau of TBARS ($\times 10^{-6}$ $mol \cdot l^{-1}$)	3.5	2.8	1.5	2.0
	G(conjugated dienes) ($\times 10^{-7}$ $mol \cdot J^{-1}$)	14.0 ± 1.00	11.1 ± 1.00	Not determined	Not determined
$^{\bullet}OH/O_2^{\bullet-}$ pH 7	G(−vit.E) ($\times 10^{-7}$ $mol \cdot J^{-1}$)	1.50 ± 0.10	0.65 ± 0.10	0.70 ± 0.10	1.00 ± 0.10
	Dose for [vitamin E] $\leq 2 \times 10^{-7}$ $mol \cdot l^{-1}$	100 Gy	200 Gy	350 Gy	150 Gy
	G(TBARS) ($\times 10^{-7}$ $mol \cdot J^{-1}$)	0.70 ± 0.10	0.21 ± 0.05	0.15 ± 0.01	1.10 ± 0.20
	Plateau of TBARS ($\times 10^{-6}$ $mol \cdot l^{-1}$)	10	5.0	6.5	17.0
	G(conjugated dienes) ($\times 10^{-7}$ $mol \cdot J^{-1}$)	6.30 ± 0.50	2.90 ± 0.50	Not determined	Not determined

Data relating to the 3 $g \cdot l^{-1}$ LDL reisolated from the HDL/LDL mixtures after irradiation are compared to those of the 3 $g \cdot l^{-1}$ LDL irradiated alone.

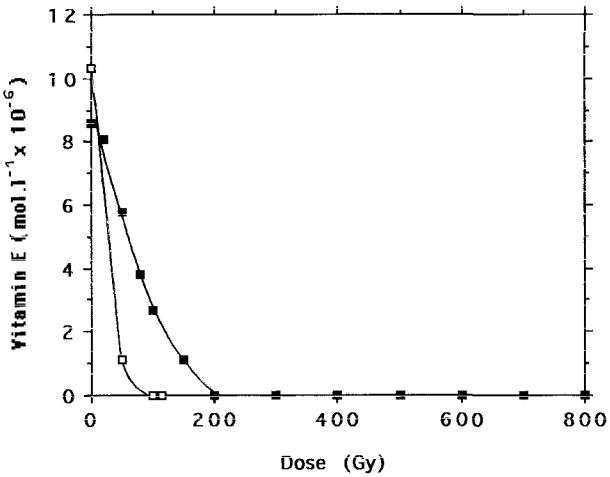

Fig. 4. Disappearance of vitamin E in 3 g·l^{-1} HDL as a function of the radiation dose, depending on the presence of 3 g·l^{-1} LDL. Action of $^{\bullet}OH/O_2^{\bullet-}$ free radicals. 10^{-2} mol·l^{-1} sodium phosphate buffer, pH 7. O_2-saturated solutions. Dose rate = 2.4×10^{-2} Gy·s^{-1}. ■, 3 g·l^{-1} HDL in the presence of 3 g·l^{-1} LDL; □, 3 g·l^{-1} HDL in the absence of LDL. Results are the means of two separate experiments performed on HDL isolated from a pool of sera of 10 normolipidaemic donors.

radicals, which are poor initiators of lipid peroxidation. Moreover, the action of $^{\bullet}OH$ free radicals was enhanced by the presence of oxygen, leading for example to radiation yields of TBARS and to plateaus of TBARS at high radiation doses that were higher in the presence of oxygen than in its absence.

Our results show that, when $^{\bullet}OH$ free radicals were generated alone, the presence of 3 g·l^{-1} LDL in the 3 g·l^{-1} HDL solutions did not disturb the HDL oxidation (for yield values, see below), except for the residual vitamin E level at high radiation doses, which constituted the major difference with the irradiation of 3 g·l^{-1} HDL without LDL (Table 1). The presence of LDL thus seems responsible for the remaining of a steady-state vitamin E level in HDL, but the mechanism involved is unknown.

Radiation yields of all the markers studied were not modified by the presence of LDL when 3 g·l^{-1} HDL were oxidised by $^{\bullet}OH$ free radicals. Indeed, these radicals led to a yield of vitamin E decrease which was very similar to that obtained in the absence of LDL. This implies that there was no protection of HDL by LDL towards the attack of endogenous vitamin E by $^{\bullet}OH$ free radicals. Similarly, with regard to TBARS (Table 1), experimental values of G(TBARS) did not exhibit any difference depending on the presence of LDL, but these values were very low, so that their determination could lack precision. However, at high radiation doses, TBARS formed in 3 g·l^{-1} HDL irradiated in the presence of 3 g·l^{-1} LDL tended to a maximum concentration which was slightly lower than that obtained with 3 g·l^{-1} HDL irradiated alone. Such a phenomenon could result from a protection of HDL towards the formation of TBARS, by the steady-state vitamin E level obtained from 200 Gy. With regard to conjugated dienes (Fig. 3), at low radiation doses, only the curve relating to the mixture HDL+LDL exhibited a weak inflexion point which could reflect a weak protection of HDL by the presence of LDL, but the yield remained very close to that obtained without LDL. The observation that radiation yields were not modified by the presence of LDL could be explained by the fact that there was no effect of the lipoprotein concentration (constant yields regardless of the HDL concentration). As an example, for the yield of vitamin E decrease, only the vitamin E/HDL components ratio has to be taken into account, and this ratio is the same whatever the HDL concentration. When LDL are added, this ratio is not modified, so that the yield remains unchanged.

By contrast, in the presence of oxygen, that is under conditions close to the physiological ones, the most important differences appeared between HDL irradiated alone or simultaneously with LDL. As shown in Fig. 4, the action of $^{\bullet}OH/O_2^{\bullet-}$ free radicals on 3 g·l^{-1} HDL irradiated in the presence of 3 g·l^{-1} LDL led to a complete disappearance of vitamin E at higher radiation doses than in the absence of LDL. This involves a protection of vitamin E, in relation with the presence of LDL. Besides, it is noteworthy that the complete disappearance of vitamin E at high radiation doses contrasts with the steady-state vitamin E level observed with $^{\bullet}OH$ free radicals. The possible regeneration of oxidised vitamin E seems inhibited by the presence of oxygen.

TBARS formation in HDL was markedly reduced when HDL were irradiated in the presence of LDL. This can be clearly noted (Table 1) by the decrease in the yield value and in the plateau values obtained at high radiation doses. There would be a protection of HDL by the LDL simultaneously present during the irradiation process, not only with regard to the yields, but also to the total oxidation products (TBARS) formed at high radiation doses. A similar protection can be observed for the yields of conjugated diene formation, thus leading to the hypothesis that the lipid moiety of HDL is partly protected from the radical attack.

Nevertheless, significant protection of HDL by LDL was only observed in the presence of oxygen (action of $^{\bullet}OH/O_2^{\bullet-}$ free radicals, which could be assimilated to the action of $^{\bullet}OH$ free radicals in the presence of oxygen, as in the case of LDL [8]). This led us to suppose that a simple competition of $^{\bullet}OH$ free radicals between the different components of HDL and

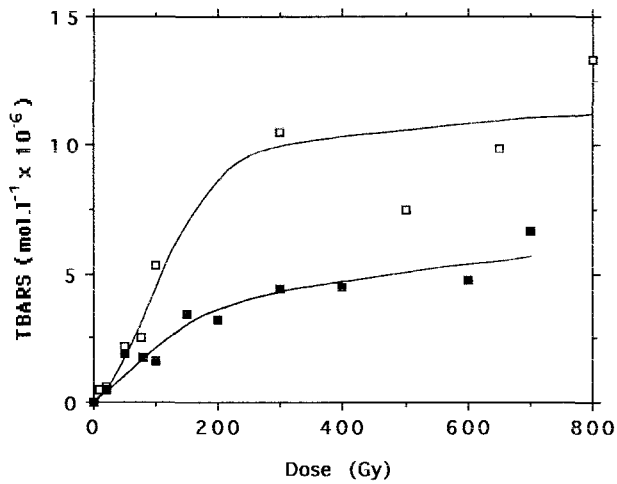

Fig. 5. TBARS formation in 3 g·l^{-1} HDL as a function of the radiation dose, depending on the presence of 3 g·l^{-1} LDL. Action of $^{\bullet}OH/O_2^{\bullet-}$ free radicals. 10^{-2} mol·l^{-1} sodium phosphate buffer, pH 7. O_2-saturated solutions. Dose rate = 2.4×10^{-2} Gy·s^{-1}. ■, 3 g·l^{-1} HDL in the presence of 3 g·l^{-1} LDL; □, 3 g·l^{-1} HDL in the absence of 3 g·l^{-1} LDL. Results are the means of two separate experiments performed on HDL isolated from a pool of sera of 10 normolipidaemic donors.

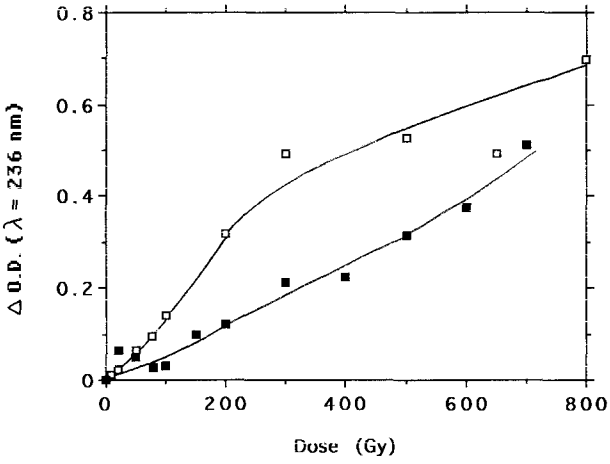

Fig. 6. Differential absorbance at 236 nm in 3 $g \cdot l^{-1}$ HDL as a function of the radiation dose, depending on the presence of 3 $g \cdot l^{-1}$ LDL. Action of $^{\bullet}OH/O_2^{\bullet-}$ free radicals. 10^{-2} $mol \cdot l^{-1}$ sodium phosphate buffer, pH 7. O_2-saturated solutions. Dose rate = 2.4×10^{-2} $Gy \cdot s^{-1}$. ■, 3 $g \cdot l^{-1}$ HDL in the presence of 3 $g \cdot l^{-1}$ LDL; □, 3 $g \cdot l^{-1}$ HDL in the absence of LDL. Results are the means of two separate experiments performed on HDL isolated from a pool of sera of 10 normolipidaemic donors.

LDL cannot be evoked. Indeed, there is no HDL concentration effect from 1 to 5 $g \cdot l^{-1}$ (data not shown). Nevertheless, the mechanism does not seem as simple as in the case of the action of $^{\bullet}OH$ free radicals alone. Indeed, the formation of new oxidation products could be initiated and amplified, via chain reactions, by oxygen. It could be hypothesised that only the oxidation products formed in the presence of oxygen could be exchanged from HDL to LDL, so affording protection of HDL by LDL simultaneously present. The assessment of LDL behaviour in the LDL/HDL mixtures after irradiation and further isolation of LDL (Table 1) led us to the conclusion that, similarly to what was observed for HDL, LDL irradiated in the presence of HDL seemed to be partly protected against oxidation, only in O_2-saturated solutions.

A role of HDL in the protection of LDL against lipid peroxidation has been generally reported [2–4]. In this way, Parthasarathy et al. [4] explored the potential of HDL-LDL interactions in culture that might affect the generation of oxidised LDL and their subsequent metabolism by macrophages. Indeed, co-incubation of HDL and LDL with endothelial cells had a profound inhibitory effect on the subsequent degradation of the incubated macrophages, while having no effect on the generation of TBARS or the formation of conjugated dienes. These authors suggested an explanation based on a rapid exchange of oxidised phospholipids from LDL with unoxidised HDL phospholipids for oxidation. Our hypothesis is compatible with that proposed by Parthasarathy et al. [4].

As mentioned above, such a reciprocal protection of HDL by LDL has been previously evoked by Tribble et al. [6]. According to these authors, the unexpected antioxidant effects exhibited by LDL in the presence of Cu^{2+} may reflect specific properties of these particles. Our original method, i.e. water gamma radiolysis, allowed us to show a reciprocal protection of HDL and LDL against in vitro $^{\bullet}OH$ induced peroxidation in the presence of oxygen. This observation leads to a new approach of the interaction between HDL and LDL towards oxygenated free radicals.

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