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Influence of the Environment in Space on the Biochemical Characteristics of Human Low Density Lipoproteins

NICOLE DOUSSET[1]*, JEAN PIERRE MOATTI[2], NICOLLE MOATTI[3], MICHEL DEGRÉ[4], BRIGITTE ECHE[5], GILBERT GASSET[5] and RENÉ TIXADOR[6]

[1]*INSERM U 305, Technologie Biomédicale, Hôtel Dieu, Toulouse;* [2]*Laboratoire de Biochimie III, CHU La Grave, Toulouse;* [3]*Laboratoire de Bactériologie, CHU Rangueil, Toulouse;* [4]*BIOSERAE, Montolieu, 11170 Alzonne;* [5]*Groupement Scientifique de Biologie et Médecine Spatiales, Université Paul Sabatier, Toulouse;* [6]*Groupe de Recherches Cliniques de Radiobiologie et Biologie Gravitationnelle, Université Paul Sabatier, Toulouse*

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The purpose of this experiment was to study the efficiency of protective substances on the effects of cosmic radiation in space on low density lipoproteins. This environment induced modifications in LDL consisting of an increase of lipid peroxidation markers (hydroperoxides, thiobarbituric acid reactive substances). In contrast, apo B was not affected by cosmic radiation as shown by the stability of the trinitrobenzenesulfonic acid reactivity and the tryptophan content. Furthermore, oxidation of LDL was partially inhibited by the addition of cysteamine or/and probucol before the spaceflight experiment. The hydroperoxide formation was almost completely inhibited by cysteamine. It was concluded that antioxidants can exert a protective effect against peroxidative stress induced by the space environment.

Key words: space, radiation, oxidation, low density lipoproteins, antioxidant

INTRODUCTION

Experiments have shown that the space environment (characterized by microgravity and cosmic radiation) acts on biological materials.[1,2] Moreover, radiation is known to produce oxygen radicals that damage proteins, lipids, and nucleic acids. Antioxidants and radioprotectors are thought to give protection against such radiation damage.[3–7] Because the lipoproteins are acellular biological systems which have no repair processes, it is interesting to evaluate the radiation effects and the protective efficiency of antioxidant or/and radioprotector substances on low density lipoproteins (LDL), which are sensitive to free radicals and involved in atherogenesis.[8] Lipid radicals and/or highly reactive aldehyde products derived from peroxidized lipids in lipoproteins

*To whom correspondence should be addressed: Dr Nicole Dousset, Biochemistry, CHU Rangueil, 1 Avenue J. Poulhès, 31054, Toulouse, France. Fax: 33 61 32 29 53

could be among the culprits responsible for cell membrane alterations. In fact, Esterbauer *et al.*[8] have shown that peroxides and especially lipid-derived aldehydes play a key role in the deleterious effects of lipid peroxidation in lipoproteins. LDL oxidation is followed by changes such as increased lipid hydroperoxides and thiobarbituric acid reactive substances (TBARS) contents and a decrease in endogenous antioxidants and polyunsaturated fatty acids. Apolipoprotein B becomes progressively altered during oxidation with the loss of reactive amino groups.

Our study points to the potential effects of a space-flight environment on lipoprotein oxidation. This experiment called: 'Vitamin' (mission BIOPAN) was conceived as an attempt to examine the effect of cosmic radiation on an acellular biological system (indirect effect on biological objects in space experiments). We additionally investigated the protective effect of antioxidant and radioprotector supplementation. In fact, probucol has been suggested to have antioxidant properties, because its presence in LDL prevents oxidative modification.[9–11] It is known that radioprotectors and antioxidants play a role in the protection against radiation.[12] Thiol groups (glutathione) play an important role in the maintenance of protein sulfhydryl groups and protect enzymes against radical injury[13] and erythrocytes against oxidative hemolysis.[14] Oxidative modification of LDL was monitored by assaying intermediates and end products of lipid peroxidation, lipid hydroperoxides and TBARS.

The results clearly indicate that a space environment for 15 days induces LDL lipid peroxidation. It is shown that exposure of LDL to a space environment (cosmic radiation) resulted in the formation of lipid hydroperoxides and TBARS.

MATERIALS AND METHODS

Materials

Probucol was a gift from Marion Merrell Dow (Cincinnati, OH). Cysteamine was from Sigma (St. Louis, MO). Low density lipoproteins (LDL) from human plasma were purchased from Sigma. These lipoproteins were sterile-filtered.

Methods

Preparation of lipoproteins

Just before their use, LDL were diluted in a 0.15 M NaCl, 0.01% EDTA, pH 7.4 buffer to a protein concentration of 1 mg/ml. Three LDL solutions were studied: one without protective substance, one with cysteamine (5 mM final concentration) and one with probucol (5 μM final concentration). Incorporation of probucol into LDL was performed as described by Barnhart *et al.*[15]

Preparation of the different batches

The same volume (0.4 ml) of each solution was delivered in 24 polyethylene ampullae, the volume of which was about 1 milliter. After sealing, each ampulla contained 0.4 ml of LDL solution and about 0.6 ml of air. The LDL were dissolved in aqueous medium (water radiolysis) and the solutions were kept in the presence of air (oxygen effect). The first batch comprised 12 ampullae as the 'Ground' control and the second consisted of 12 ampullae as the 'Flight' batch. In each batch, there were 4 ampullae containing LDL without protective substance (LO), 4 ampullae containing LDL with probucol (LA), and 4 containing LDL with cysteamine (LC). The sealing of the ampullae was checked by vacuum test.

Flight conditions

The experiment was prepared in the laboratory 4 days before the flight and the batches 'flight' and 'ground' transported in the same conditions (4°C) to the launch site. The ampullae were housed in aluminium boxes of 8 × 4 × 2 cm (aluminium thickness = 0.5 mm) in the ESA's Biopan.[16] Biopan is a circular, pan shaped container, with a hinged lid which opens through 180°, exposing the biological samples accommodated on the fixed

bottom plate and in the lid to open space. Each box contained 12 ampullae: 6 on a top layer and 6 on a bottom layer (the bottom layer was the nearest to the Foton satellite surface). Three thermoluminescent detectors ($CaSO_4$) were also placed in each box (two on the top, one on the bottom). The experiment was thermostated throughout the flight at about 20°C. The 'flight' boxes were housed in Biopan's 'experiment tray', which provided a combination of minimal shielding conditions plus normal pressure throughout the flight. The Foton retrievable satellite was carried into space on a Soyouz 7 launcher from Plesetsk launch site and retrieved in Kazakhstan. The flight duration was 17.5 days. The experiments were directly exposed (lid open) to the harsh space environment for 15 days. During the flight the 'ground-control' aluminium boxes were placed at the same temperature as the 'flight' boxes.

Determination of peroxidation products

Peroxidation products were assayed by the measurement of the thiobarbituric acid-reactive substances (TBARS) following the method of Yagi[17] and lipid peroxides were assayed by ferrous ion oxidation, as described by Jiang *et al.*[18]

Determination of lipid substrate modifications

For the analysis of polyunsaturated fatty acids, lipids from LDL were extracted and transmethylated, following the technique of Lillington *et al.*,[19] in the presence of heptadecanoic acid as internal standard. The methyl esters were analyzed by gas chromatography (Intersmat, model DFL, carbowax column). Vitamin E was evaluated by HPLC.[20]

Apolipoprotein modifications

The reactive amino groups of LDL (except the cysteamine batch) were measured by the 2,4,6-trinitrobenzene sulfonic acid (TNBS) method as described by Steinbrecher.[21] Intrinsic fluorescence of tryptophan in LDL was studied at 20°C using 290 nm as excitation wavelength and 330 nm as emission wavelength.[22]

RESULTS

The space-dependent oxidation of LDL is illustrated in Figures 1 and 2. There was a significant increase in lipid peroxidation products such as lipid peroxides and TBARS for the lipoproteins exposed to the harsh space environment.

The hydroperoxide functions in the 'flight' LDL without protective substance (LO) were significantly higher than in the 'ground' ones (Figure 1). If we look at the hypothetical efficiency of antioxidant and radioprotector addition on the LDL, we observe that both substances protected against the effects of space, since the hydroperoxide level was similar in the LDL

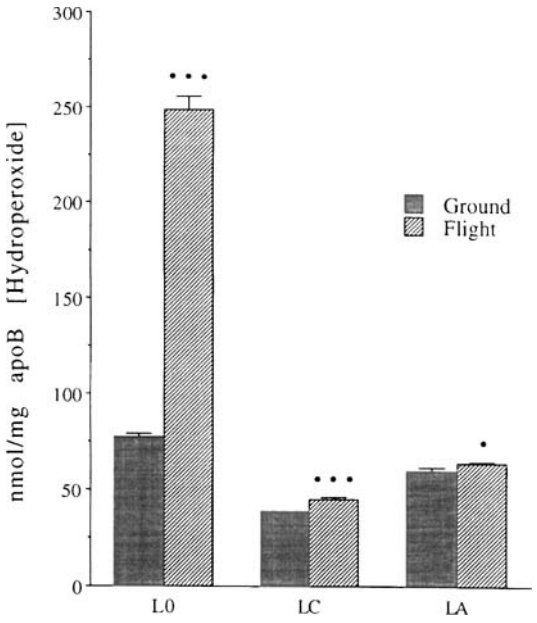

FIGURE 1 Effects of 'flight' on the formation of hydroperoxides in LDL without protective substance (LO), in the presence of protector: cysteamine (LC) and probucol (LA). Results are expressed as nmol hydroperoxide per mg apolipoprotein B and are the means ± S.E.M. of four results obtained for each batch. Values significantly different ($p < 0.05$*), ($p < 0.001$***) from their corresponding 'ground control' are indicated.

remaining at the ground and those subjected to flight (Figure 1). There was a significant difference between 'ground' and 'flight' ampullae in the presence of cysteamine or probucol (Figure 1).

The TBARS concentrations of the 'flight' ampullae were always much higher than the 'ground' TBARS, without (LO) or with protector (LC and LA) (Figure 2). It is noteworthy that LDL which remained on the ground demonstrated only a moderate increase in TBARS content. The results concerning 'flight' LDL indicate a significant increase of TBARS which is not prevented by probucol or cysteamine addition (Figure 2).

The two predominant polyunsaturated fatty acids of human LDL are linoleic acid and arachidonic acid. Exposure of LDL to cosmic radiation did not result in a depletion of arachidonic acid or linoleic acid contents (Table 1).

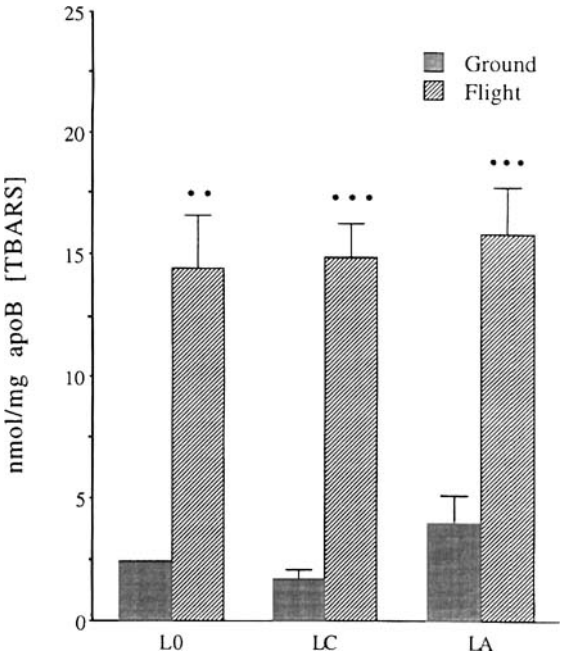

FIGURE 2 Effects of 'flight' on the TBARS formation in LDL without protective substance (LO), in the presence of protector: cysteamine (LC) and probucol (LA). Results are expressed as nmol TBARS per mg apolipoprotein B and are the means ± S.E.M. of four results obtained for each batch. Values significantly different ($p < 0.01$**), ($p < 0.001$***) from their corresponding 'ground control' are indicated.

The evaluation of tocopherol in LDL (Table 1) demonstrates that the 'ground control' LDL containing cysteamine had a higher level of vitamin E than lipoproteins without protector or with probucol.

Concerning the modifications of the protein moiety of LDL, due to the fact that, in spatial experiments, it is not possible to multiply the samples, we chose to evaluate the free amino groups and tryptophan of apo B located at the boundary of the lipid core.[23] The TNBS reactivity measured in the samples in the presence or in the absence of probucol did not demonstrate any modification (results not shown), indicating that the lysine residues were not modified. Also, the tryptophan residues appeared not to be sensitive to cosmic radiations, since the tryptophan content remained the same in the different batches of LDL.

DISCUSSION

The present results are consistent with the concept that radiation alters lipoproteins. In fact, the absorbed dose was 0.135 mGy for the ground control and 19.57 mGy at the top and 12.66 mGy at the bottom of the hardware for the in flight experiment.

Cosmic radiation cannot be reproduced on the ground, due to the complex variety of particles and electromagnetic radiation and the energy spectrum involved. This is why experimental calibration before flight was performed using ^{60}Co radiation (12 mGy for 15 days of experiment) and the results showed no hydroperoxide or TBARS increases in LDL.

This experiment suggests that addition of antioxidants such as probucol or radioprotectors such as cysteamine will make the lipoproteins less susceptible to oxidative damage. Thus, the addition of such compounds may have beneficial effects for lipoproteins against oxidative injury induced by space radiation.

However, our results show that probucol does not completely prevent peroxidation of LDL

TABLE 1 Fatty acid and vitamin E composition (expressed as μg lipid/mg apo B) of LDL without protective substance (LO) and in the presence of protector: cysteamine (LC) and probucol (LA). G: ground; F: flight. Mean of 2 results obtained for each batch.

	LO_G	LO_F	LC_G	LC_F	LA_G	LA_F
Linoleic acid	82	94	93	77	90	70
Arachidonic acid	17	19	19	16	20	19
α-tocopherol	5.2	5.7	6.6	5.5	5.8	6.0
γ-tocopherol	0.25	0.20	0.33	0.19	0.20	0.28

lipids. Probucol has antioxidant properties and its presence in LDL prevents oxidative modifications by copper ions.[9,10] It acts as an antioxidant by scavenging oxygen radicals as described by Daugherty *et al.*[24] and Hiramatsu *et al.*[7] In the present report we show that probucol, at the concentration studied, is much less potent than cysteamine in inhibiting LDL oxidation in the ground control and in the flight experiments. To explain these data, two possibilities may be envisaged, first the concentration and second the lipophilic nature of probucol: probably only part of the probucol is incorporated into the core of the particle as discussed by Esterbauer *et al.*[8]

In this study, the modifications of the properties of the LDL submitted to cosmic radiation were extensive, in terms of hydroperoxide and TBARS contents. These modifications were not accompanied by any modification of the protein moiety, essentially reactive amino groups and tryptophan residues. The discrepancy which seems to exist between the high level of hydroperoxide formation and the moderate TBARS formation in the 'flight' samples can explain the absence of modifications of TNBS reactivity; in fact only aldehydes are able to react with apolipoprotein B such that the electrophoretic and immunologic behaviours of LDL are modified.[21] Our results are consistent with those observed when LDL are exposed to ultraviolet radiation.[25,26] In fact UV induces the oxidation of the lipids present in the LDL core, whereas external monolayer phospholipids are relatively less oxidized and apo B undergo minor structural modifications.[25–27] The present experiment, i.e. exposure of LDL to cosmic radiation, resulted in lipid peroxidation affecting only the core of the lipoproteins.

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References

1. L. Lapchine, N. Moatti, G. Gasset, G. Richoilley, J. Templier and R. Tixador (1986) Antibiotic activity in space. *Drugs Experimental Clinical Research*, **12**, 933–938.
2. R. Tixador, G. Gasset, B. Eche, N. Moatti, L. Lapchine, C. Woldringh, P. Toorop, J.P. Moatti, F. Delmotte and G. Tap (1994) Behavior of bacteria and antibiotics under space conditions. *Aviation, Space, and Environmental Medicine*, **65**, 551–556.
3. N. Gotoh, K. Shimizu, E. Komuro, J. Tsuchiya, N. Noguchi and E. Niki (1992) Antioxidant activities of probucol against lipid peroxidations. *Biochimica et Biophysica Acta*, **1128**, 147–154.
4. L.R. Mc Lean and K.A. Hagaman (1990) Antioxidant activity of probucol and its effects on phase transitions in phosphatidylcholine liposomes. *Biochimica et Biophysica Acta*, **1029**, 161–166.
5. A.W.T. Konings, J. Damen and W.B. Trieling (1979) Protection of liposomal lipid against lipid radiation induced oxidative damage. *International Journal of Radiation Biology*, **35**, 343–350.
6. A.A. Shaheen and S.M. Hassan (1991) Radioprotection of whole-body γ-irradiation-induced alteration in some haematological parameters by cysteine, vitamin E and their combination in rats. *Strahlenterapy Onkology*, **167**, 498–501.
7. M. Hiramatsu, J. Liu, R. Edamatsu, S. Ohba, D. Kadowaki and A. Mori (1994) Probucol scavenged 1,1-diphenyl-2-picrylhydrazyl radicals and inhibited formation of thiobarbituric acid reactive substances. *Free Radical Biology & Medicine*, **16**, 201–206.
8. H. Esterbauer, J. Gebicki, H. Puhl and G. Jûrgens (1992) The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology & Medicine*, **13**, 341–390.
9. S. Parthasarathy, S.G. Young, J.L. Witzum, R.C. Pittman

and D. Steinberg (1986) Probucol inhibits oxidative modification of low density lipoprotein. *The Journal of Clinical Investigation*, **77,** 641–644.
10. L.R. Mc Lean and K.A. Hagaman (1989) Effect of probucol on the physical properties of low-density lipoproteins oxidized by copper. *Biochemistry*, **28,** 321–327.
11. J. Regnstrom, G. Walldius, L.A. Carlson and J. Nilsson (1990) Effect of probucol treatment on the susceptibility of low density lipoprotein isolated from hypercholesterolemic patients to become oxidatively modified *in vitro*. *Atherosclerosis*, **82,** 43–51.
12. A.S. Khouw, S. Parthasarathy and J.L. Witzum (1993) Radioiodination of low density lipoprotein initiates lipid peroxidation: protection by use of antioxidants. *Journal of Lipid Research*, **34,** 1483–1496.
13. X. Shan, T.Y. Aw and D.P. Jones (1990) Glutathione-dependent protection against oxidative injury. *Pharmacology and Therapeutics*, **47,** 61–71.
14. E. Niki, E. Komuro, M. Takahashi, S. Urano, E. Ito and K. Terao (1988) Oxidative hemolysis of erythrocytes and its inhibition by free radical scavengers. *The Journal of Biological Chemistry*, **263,** 19809–19814.
15. R.L. Barnhart, S.J. Busch and R.L. Jackson (1989) Concentration-dependent antioxidant activity of probucol in low density lipoproteins in vitro: probucol degradation precedes lipoprotein oxidation. *Journal of Lipid Research*, **30,** 1703–1710.
16. R. Demets (1994) Successful first operational flight of ESA's Biopan. *Microgravity News*, **7,** 21–23.
17. K. Yagi (1987) Lipid peroxides and human diseases. *Chemistry and Physics of Lipids*, **45,** 337–351.
18. Z.Y. Jiang, J.V. Hunt and S.P. Wolff (1992) Ferrous ion oxidation in the presence of xylenol orange for detection of lipid hydroperoxide in low density lipoprotein. *Analytical Biochemistry*, **202,** 384–389.
19. J.M. Lillington, D.J.H. Trafford and H.L.J. Makin (1981) A rapid and simple method for the esterification of fatty acids and steroid carboxylic acids prior to gas-liquid chromatography. *Clinica Chimica Acta*, **11,** 91–98.
20. A.P. Leenheer, V.O. De Bevere, A.A. Cruyl and A.E. Claeys (1978) Determination of serum α-tocopherol (vitamin E) by high-performance liquid chromatography. *Clinical Chemistry*, **24,** 585–590.
21. U.P. Steinbrecher (1987) Oxidation of human low density lipoprotein results in derivatization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. *The Journal of Biological Chemistry*, **262,** 3603–3608.
22. N. Dousset, G. Ferretti, M. Taus, P. Valdiguié and G. Curatola (1994) Fluorescence analysis of lipoprotein peroxidation. In *Methods in Enzymology*, (ed. L. Packer) Academic Press, New York 233, pp. 459–469.
23. G.E. Dobretsov, M.M. Spirin, O.V. Chekrygin, I.M. Karmansky, V.M. Dmitriev and Y.A. Vladimirov (1982) A fluorescence study of apolipoprotein localization in relation to lipids in serum low density lipoproteins. *Biochimica et Biophysica Acta*, **710,** 172–180.
24. A. Daugherty, B.S. Zweifel and D.G. Schnfeld (1991) The effects of probucol on the progression of atherosclerosis in mature Watanabe heritable hyperlipidaemic rabbits. *British Journal of Pharmacology*, **103,** 1013–1018.
25. R. Salvayre, A. Nègre, M. Lopez, N. Dousset, T. Levade, M.T. Pierraggi, and L. Douste-Blazy (1990) A new model system for studying the cytotoxicity of oxidized lipoproteins in cultured cells, in *Free Radicals, Lipoproteins and Membrane lipids*, (eds A. Crastes de Paulet, L. Douste-Blazy and R. Paoletti) Plenum Press, London, NATO-ASI Series A 189, pp. 249–256.
26. N. Dousset, A. Nègre-Salvayre, M. Lopez, R. Salvayre and L. Douste-Blazy (1990) UV-treated lipoproteins as a model system for the study of the biological effects of lipid peroxides on cultured cells. 1. Chemical modification of UV-treated LDL. *Biochimica et Biophysica Acta*, **1045,** 219–223.
27. A. Nègre-Salvayre, N. Paillous, N. Dousset, J. Bascoul and R. Salvayre (1992) Wavelength dependence of photo-induced peroxidation and cytotoxicity of human LDL. *Photochemistry and Photobiology*, **55,** 197–204.