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INFLUENCE OF BLOOD PLASMA LIPOPROTEINS BEFORE AND AFTER PUVA-EXPOSITION ON PRODUCTION OF REACTIVE OXYGEN SPECIES BY HUMAN NEUTROPHILS *IN VITRO*

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The influence of LDL and HDL blood plasma lipoproteins before and after 3 hrs PUVA-exposition, on production of reactive oxygen species (ROS) by human neutrophils stimulated by opsonized zymosan, was studied. A measure of ROS production by neutrophils was the intensity of luminol-enhanced chemiluminescence which method was applied for the determination of ROS level. This research revealed that non-exposed LDL and HDL lipoproteins at concentration 2.0 mg × L^{-1} decrease production of neutrophils in vitro with reference of neutrophils non-treated by lipoproteins. In the presence of PUVA-exposed LDL a significant decrease of ROS production vs. non-exposed LDL effect was observed, while HDL after 3 hrs PUVA-exposition showed no effect on this production. This result was explained by lipoprotein lipids activity, both their antioxidative properties and capability of ROS producing stimulation, which modulate by lipid photodegradation during PUVA-exposition.

INTRODUCTION

PUVA-therapy is an accepted photochemotherapy method applied in the treatment of many autoimmune diseases, such as psoriasis, vitiligo or scleroderma (Morison, Honig & Karp, 1996). As beneficial effect of PUVA-therapy is strongly connected with photointeractions between furocoumarins and cellular lipids (Beijersbergen van Henegouven, Wijn & Schoenderwoerd, 1989), thus efficacy of PUVA-method may be related to furocoumarins interactions with blood plasma lipoproteins, which level and chemical composition strongly change in these diseases (Offidani, Ferretti, Taus, Simonetti, Dousset, Valdiguie, Curatola & Bossi, 1994; Seckin, Tokgozoglu & Akkaya, 1994; Seishima, Seishima, Mori & Noma, 1994), suggesting a risk of atherogenesis. According to recent studies (Schwartz, Valente, Sprague, Kelley & Nerem, 1991; Wick, Scheck, Amberger, Kleindienst & Xu, 1995) atherosclerosis also appears to be an autoimmune disease which makes a reason to the application of PUVA-method in the treatment of atherosclerosis.

It has been also well documented that oxidative stress and excessive production of reactive oxygen species (ROS), produced by human leukocytes play an important role in atherogenesis (Catchard, Morel & Chilsholm, 1985; Korpela, 1990; Nakahara, Sato, Ishisaka, Kanno, Yoshioka, Yasuda, Inoue & Utsumi, 1998).

In this work, an influence of human LDL and HDL lipoproteins before and after PUVA-exposition on production of ROS by human neutrophils *in vitro*, was studied. In course of PUVA-exposition in the presence of oxygen, LDL and HDL are oxidized (Fossell, Fletcher, McDonagh & Hui, 1991; Bugaj, Masiakowski, Hulka-Soroka & Bartosz, 1998). Whether that changed under photooxidation lipoproteins would modify ROS production by neutrophils, has been not yet clearly elucidated.

MATERIALS AND METODS

Lipoproteins (VLDL, LDL and HDL fractions) were prepared by sequential ultracentrifugation of human blood collected from healthy donors (Havel, Eder & Bragdon, 1955) In several fractions total protein concentration was determined using a colorimetry with copper ions, and then all these fractions were diluted by PBS to final concentration 10.0 mg·L^{-1} (*vs.* total protein).

The PUVA-exposition was performed for 1.93 mL of each diluted fractions, mixed with 70.0 µL of 8-methoxypsoralen (Sigma-Aldrich) stock solution (6.0 mg·L^{-1}) to final concentration 210 µg·L^{-1}. The obtained reaction mixtures were

Table 1. Statistical analysis of the differences between the data for response of neutrophils non-treated with lipoproteins (AUC_s) *vs.* neutrophils treated with lipoproteins (AUC)

Sample	AUC (mV·min)	AUC_s/ AUC	p-value (AUC_s *vs.* AUC)
non-treated cells	337.93[1] ± 143.87*	-	-
cells+non-exposed LDL	283.50 ± 150.10	1.19	0.09
cells+ PUVA-exposed LDL	258.27 ± 148.34	1.31	0.02
cells+non-exposed HDL	245.53 ± 156.74	1.38	0.06
cells+PUVA-exposed HDL	261.49 ± 155.96	1.29	0.25

*± SD; [1]AUC_s

Table 2. Statistical analysis of the differences between the data for response of neutrophils treated by non-exposed blood plasma lipoproteins (AUC_n) vs. the neutrophils treated by lipoproteins PUVA-exposed (AUC_e)

lipoproteinfraction	AUC_n (mV·min)	AUC_e (mV·min)	AUC_n / AUC_e	p-value AUC_n / AUC_e
LDL	280 ±150*	260 ± 150	1.10	0.03
HDL	246 ± 160	260 ± 160	0.94	0.08

* ± SD

incubated for 15 min and subsequently irradiated using UVA-lamp ($\lambda = 365$ nm, energy dosis 10 J × cm^{-2}) for up to 3 hrs (Fossell *et al.*, 1991).

Neutrophils were isolated from heparinized blood of 10 healthy donors by one-step Gradisol G (Ficoll) gradient centrifugation. Obtained cells after hemolization of resting erythrocytes by 0.84% ammonium chloride solution (pH = 7.4) were washed twice and suspended in PBS.

For the determination of reactive oxygen species (ROS), produced by granulocytes *in vitro*, a luminol-enhanced chemiluminescence method was applied. A 1250 Luminometer (BioOrbit, Finland) was used for the measurement of chemiluminescence intensity. Examined samples contained 5×10^5 cells per mL, 1.0 mg × L^{-1} of opsonized zymosan. 26.55 mg·L^{-1} of luminol and 2.0 mg L^{-1} (*vs.* total protein concentration) of blood plasma lipoproteins before and after PUVA-exposition. As a standard probe, a sample without lipoproteins was applied. All investigated samples were added with PBS to final volume 1.0 ml. The investigations were carried out at a temperature 37°C. Each measurement was expressed in mV·min as the area under the curve (AUC) of chemiluminescence intensity *vs.* time. This was a measure of ROS production by neutrophils (Baj, Kantorski, Kowalski, Kośmider, Tchórzewski, Pawlicki & Ciećwierz, 1994).

Values are expressed as mean ± SD. The alpha-error probability (p-value) of Student's t-test was applied for analysis of the differences between the data for response of neutrophils non-treated with lipoproteins (AUC_s) *vs.* neutrophils treated with PUVA-exposed or non-exposed lipoproteins (AUC), as well as for response of neutrophils treated with non-exposed lipoproteins (AUCn) *vs.* neutrophils treated with PUVA-exposed lipoproteins (AUC_e).

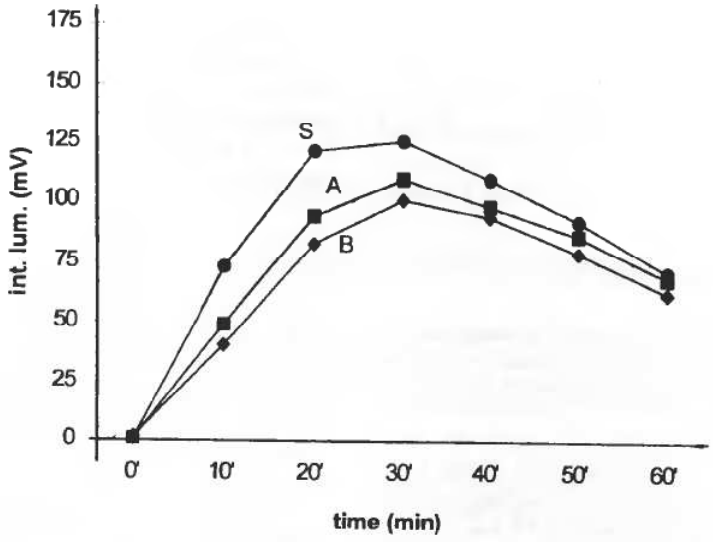

Fig. 1. Changes of the chemiluminescence intensity vs. time for neurophiles treated by LDL lipoproteins. S – non-treated neutrophils (standard probe); A – neutrophils treated by non-exposed LDL; B – neutrophils treated by PUVA-exposed LDL.

RESULTS AND DISCUSSION

Despite of the wide distribution range of obtained AUC-data (high values of SD), some statistically significant differences between response of non-treated and treated neutrophils were found. As was shown in Table 1, non-exposed LDL and HDL fractions added at 2.0 mg × L^{-1} concentration to the suspension of stimulated neutrophils, decreased a level of produced ROS, with reference to neutrophils non-treated by lipoproteins. In the case of HDL this decrease was greater than in the case of LDL (27.34 and 16.11%, respectively). On the other hand, after addition of PUVA-exposed HDL fractions, no statistically significant changes of ROS level *vs.* a control probe were observed (Table 2). These effects may be explained by antioxidative properties of some unsaturated lipoprotein lipids, such as fatty acids (Harris, 1992; Baroni, Amelio, Sangiorgi, Gaddi & Battino, 1999), glycerides (Araujo, Barbosa, Hsin, Maranhão & Abdalla, 1995) and phospholipids (Galella, Maragnoni, Risé, Colombo, Galli & Galli, 1993). These compounds act as scavengers of ROS but during 3 hrs PUVA-exposition their antioxidative activity can decrease due to photodegradation. In fact, earlier research revealed that lipoprotein lipids during PUVA-exposition undergo photodegradation and HDL lipids (probably unsaturated phospholipids) are *ca.* twice photolabile than LDL lipids (Bugaj *et al.*, 1998).

As lipid components of LDL fractions are considerably more photostabile, they should decrease a level of produced ROS, due to partially maintenance of their antioxidative properties. Whereas, LDL lipoproteins after 3 hrs decrease a level of ROS more strongly than the same lipoproteins non-exposed, as was shown in Fig. 1. This difference was statistically significant (Table 2). According to recent studies (Araujo *et al.*, 1995), there is a positive correlation between the concentration of triglycerides in LDL fraction and the level of ROS, which production by human leukocytes is strongly stimulated by these lipids, probably due to the activation of some oxidative enzymes, such as NADPH oxidase (Morel, Doussiere & Vignais, 1991; Henderson, Moule & Chappel, 1993). Therefore, an observed decrease of ROS level in presence of PUVA-treated LDL may be caused by photodegradation of LDL triglycerides.

However, it is necessary to account also an influence of proteinic components of blood plasma lipoproteins on production of such ROS as oxygen radical anion O_2^- or its disproportionation product H_2O_2. These species very effective oxidize amino acids of proteins, while their capability of penetration through non-polar lipid structures is very small (Cogny, Paul & Atger, 1994). An influence of other lipoprotein concentration as well as of other stimulators action (fMLP, PMA) should be also examined questions will be a subject of the later studies, to better explain of observed phenomena.

This work showed that LDL and HDL lipoproteins before PUVA-exposition decrease at concentration 2.0 mg × L^{-1} production of reactive oxygen species (ROS) by neutrophils stimulated by opsonized zymosan. After 3 hrs PUVA-exposition, LDL significantly decreases producing of ROS by stimulated neutrophils, while HDL showed no effect on this production.

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