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Nanoparticles of lipoic acid esters: preparation and antioxidant effect

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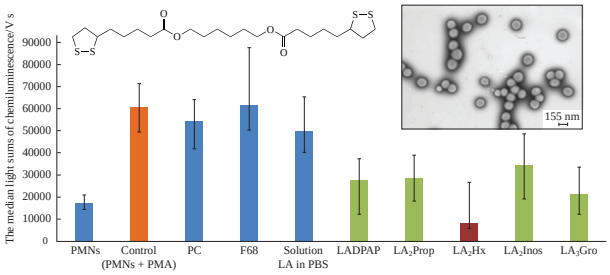
Five lipoic acid esters were synthesized, and their nanoforms based on copolymer Pluronic F68 and phosphatidylcholine were obtained. The most pronounced antioxidant effect on neutrophils activated by phorbol-12-myristate-13-acetate was shown by the nanodispersion containing the derivative of lipoic acid with hexane-1,6-diol, reducing the concentration of reactive oxygen species by 7.5 times.



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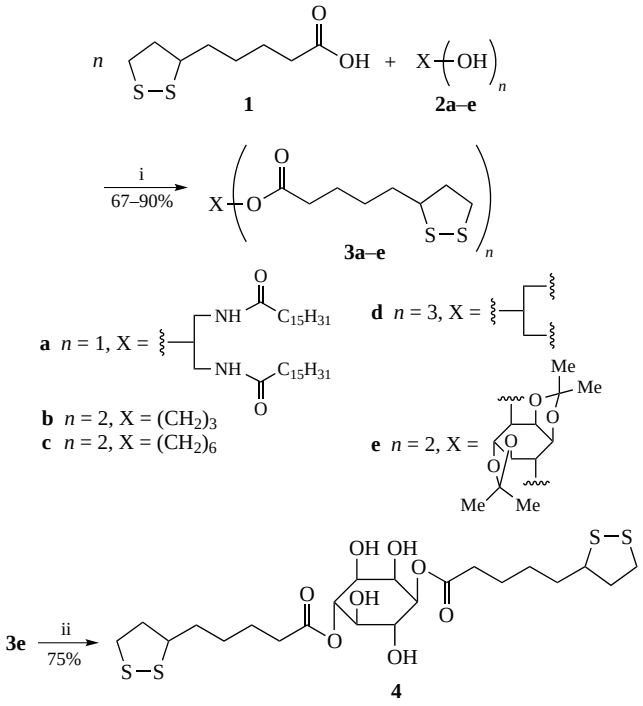
The change in the functional activity of neutrophils (polymorphonuclear cells, PMNs) is one of the factors determining the development and progression of pathological processes, including cerebral ischemia. Excessive accumulation of reactive oxygen species (ROS) causes a dramatic increase in the metabolic activity of PMNs and subsequently their death.[1–3] Therefore, it is necessary to suppress these processes using antioxidants and to study the mechanisms of their action.[4–6] One of the most universal and promising antioxidants is lipoic acid (LA). As was found using various experimental model systems, LA efficiently suppressed the formation of lipid peroxidation products and ROS *via* the regulation of the activity of enzymes (peroxidases, oxidases), nuclear transcription factors (Nrf2 and NF-κB) and *via* metal chelation.[7–9]

Lipoic acid is poorly soluble in water. Therefore, it is used in medications in the forms of meglumine or ethylenediamine salts, which are administered in high doses. Because of the fast biodegradation of LA (oxidation of its side chain and binding to plasma proteins[10]), its concentration in the blood plasma rapidly decreases (20–25 μg ml^{-1} over 20–30 min), thus leading to a lower antioxidant and therapeutic effect. The excretion half-life of LA is 25 min. Therefore, to achieve a slow release of LA, to maintain its high concentration in the circulating blood after the administration, and to protect it from biodegradation, preparation of nanoparticles (NPs) based on LA or its derivatives is an actual problem. In addition, as is known from literature, various nanodispersions (liposomes or nanoemulsions) can improve the penetration of drugs to cells *via* their fusion with the plasma membrane, in the process of endocytosis or using other mechanisms, and promote the transport of LA through the cell membrane.[11–14]

In this work, to modify LA **1**, we used alcohols **2a–e** [1,3-di(palmitoylamino)propan-2-ol, propane-1,3-diol, hexane-1,6-diol, glycerol, and *myo*-inositol] forming esters with different numbers of LA residues (Scheme 1). They are nontoxic and biocompatible; once in the body, they can participate in the lipid metabolism, enzyme activity regulation, and other biological processes. Esters **3a–e** were synthesized by the Steglich esterification of the corresponding alcohols **2a–e** with LA **1** (1.2 equiv. per hydroxy group) using the activation with 4-dimethylaminopyridine (DMAP) and 1,3-dicyclohexylcarbodiimide (DCC) in dichloromethane. These reactions were performed in the dark at room temperature for 24 h (see Scheme 1). After further processing of the reaction mixtures, the target compounds **3a–e** were isolated using column chromatography on silica gel; the product yields were 90, 85, 85, 67 and 80%, respectively. The



Scheme 1 *Reagents and conditions*: i, DCC (1.2 equiv.), DMAP (1.5 equiv.), CH_2Cl_2, dark, room temperature, 24 h; ii, Dowex H^+, MeOH.

Table 1 Characteristics of LA ester nanoparticles.

NPs	C_{F68}/ mg ml^{-1}	C_{PC}/ mg ml^{-1}	$C_{LA\ ester}$/ mg ml^{-1}	Size/nm	PDI	ζ/mV
PC	–	3	–	135 ± 45	0.246 ± 0.020	–1.3 ± 0.5
F68	18	–	0.5–1	7 ± 2 (80%) 60 ± 10 (20%)	0.497 ± 0.050	–14 ± 5
3a	18	–	0.5–1	375 ± 80	0.158 ± 0.030	–17 ± 5
3b	18	–	0.5–1	360 ± 65	0.147 ± 0.060	–21 ± 5
3c	18	–	0.5–1	340 ± 50	0.153 ± 0.070	–20 ± 5
3d	18	–	0.5–1	354 ± 60	0.069 ± 0.004	–22 ± 5
4	–	3	0.25	45 ± 25 (25%) 167 ± 45 (75%)	0.376 ± 0.037	–22 ± 5

isopropylidene protecting groups of derivative **3e** were removed by acid hydrolysis using a Dowex H^+ ion-exchange resin to afford LA ester **4** with a 75% yield. The structures of compounds obtained were confirmed using ^{1}H NMR spectroscopy and mass spectrometry.

The nanoparticles of the LA esters were prepared using the method of phase inversion under intense stirring of the suspension formed by the injection of polyoxyalkylene copolymer Pluronic F68 or distilled water into a methanol solution of derivatives **3a**–**d** or a mixture of phosphatidylcholine (PC) with compound **4**. The organic solvent and the excess water were then removed under reduced pressure (Table 1).

In this way we obtained homogeneous (PDI < 0.3) Pluronic F68-based nanodispersions of LA esters **3a**–**d** (0.5–1 mg ml^{-1}) consisting of mostly spherical particles with a size ranging from 290 to 455 nm [Figure 1(*a*)]. We also prepared the phosphatidylcholine-based heterogeneous nanosuspension with the derivative of LA and *myo*-inositol **4** (0.25 mg ml^{-1}), consisting of two fractions of differently shaped particles [Figure 1(*b*)]: 20–70 nm (25%) and 122–212 nm (75%). The resultant NPs with LA esters were stable during long-term storage (over 18 months) at room temperature (see Online Supplementary Materials, Figure S2).

One of the most popular models for studying the biological properties of antioxidants is represented by neutrophils (PMNs), which are capable of generating ROS in the presence of various activators. In this study, we evaluated the effect of the resultant nanoparticles on the oxidative burst in these cells compared to nanodispersions without antioxidants and to LA in the phosphate buffer solution (PBS). As the activator of free radical processes in PMNs, we used phorbol-12-myristate-13-acetate (PMA), which, in turn, leads to an increase in the formation of ROS by regulating the activity of various cellular enzymes (NADPH oxidase, superoxide dismutase, peroxidase) and signaling pathways of the Nrf2 and NF-κB factors.[15,16] The studies were performed *in vitro* using PMNs isolated from the blood of nominally healthy donors aged from 20 to 30.

The NPs of the LA esters **3a**–**d** and **4** were shown to decrease the concentration of ROS in PMA-activated neutrophils by a factor of 1.5 to 7.5 compared to the control (PMNs + PMA) – among other things, because of the suppression of NADPH oxidase activity (twofold, $p < 0.05$, see Online Supplementary Materials, Figures S3 and S4). The most significant antioxidant effect was seen when we used the nanodispersion of the derivative LA with hexane-1,6-diol **3c**. It should also be noted that there are no significant differences when comparing the luminol luminescence intensity values obtained by exposing of nanoparticles without antioxidants and nanosuspension with compound **4** to neutrophils (see Figure S3, $p > 0.05$). At the same time, it was also found that neither the nanoparticles without antioxidants nor the solution of LA in the PBS (pH 7.4) had any significant effect on the functional activity of these cells. As is described in literature, LA is reduced to dihydrolipoic acid in cells by various enzymes (glutathione reductase, thioredoxin reductase) in the presence of NADPH, thus increasing the antioxidant status of the cells and reducing the concentration of free radicals.[17] Therefore, one can assume that LA esters, after penetrating into the PMNs, can undergo reduction *via* their interaction with NADPH, thus reducing the activity of NADPH oxidase and the formation of ROS.

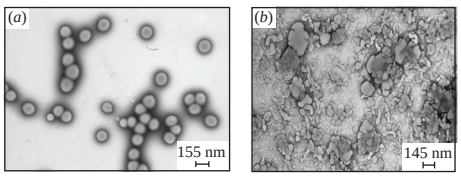


Figure 1 Electron micrographs of the LA ester nanoparticles: (*a*) compound **3c**, (*b*) compound **4**.

Thus, the resultant nanoparticles with the LA derivatives can, after further studies *in vivo*, form the bases for the creation of drugs with a higher therapeutic efficiency. To conclude, we have prepared nanoparticles of the synthesized LA esters **3a**–**d** and **4**. The resultant nanoforms were stable for over 18 months at room temperature. The NPs of the LA esters were shown to suppress the activity of NADPH oxidase in PMA-activated neutrophils, thus decreasing the concentration of the ROS.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.07.023.

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