International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2018.796.801

Research Article
Loading of Gentamicin and Alpha Lipoic Acid on a Biodegradable Polymer for More Effective and Less Nephrotoxic Formula

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Abstract

Background and Objective: Gentamicin sulphate (GN) is a broad-spectrum antibiotic used for treatment of several types of infection. However, it can cause vasoconstriction, leading to serious adverse effects, such as kidney damage and inner ear problems. The aim of this study was to suppress GN nephrotoxicity and to sustain its release. **Methodology:** Polycaprolactone (PCL) nanoparticles (NPs) were loaded with GN and alpha lipoic acid (ALA) using the solvent evaporation technique. The prepared NPs were assessed for particle size, zeta potential, morphology, entrapment efficiency percentage and GN release. Finally, an *in vivo* nephrotoxicity study was carried out to assess the protective effect of ALA on the kidneys of rabbits. **Results:** Data revealed that the prepared GN-ALA-PCL NPs were in the nano size range. GN was released more slowly than pure GN, thus sustaining its effect. Creatinine increased 1.4-fold in the pure GN group in comparison with the control group and other electrolytes (sodium, calcium and potassium) showed abnormal results for the pure GN group. There was no significant difference in creatinine and the other electrolytes between the GN-ALA-PCL NPs group and the control group. Data confirm the protective effect of ALA against GN nephrotoxicity. **Conclusion:** Loading of GN with ALA on PCL NPs could be a successful strategy to inhibit GN nephrotoxicity and extend GN release, which enhances its safety and dose frequency profiles.

Key words: Aminoglycosides, gentamicin, alpha lipoic acid, polycaprolactone, nanoparticles, neuropathy, nephrotoxicity

Received: November 27, 2017 Accepted: March 26, 2018 Published: July 15, 2018

Citation: Bader Mubarak Aljaeid and Mohamed Ahmed El-Moselhy, 2018. Loading of gentamicin and alpha lipoic acid on a biodegradable polymer for more effective and less nephrotoxic formula. Int. J. Pharmacol., 14: 796-801.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Gentamicin sulphate (GN) is an antibiotic related to the aminoglycoside group. It inhibits bacterial protein synthesis by binding irreversibly with the 30_S subunit, causing bacterial death. It is a broad-spectrum antibiotic used especially for Gram-negative bacteria and for the treatment of urinary tract, soft tissue and upper respiratory tract infections[1]. Many adverse effects are associated with GN use, such as nerve damage, kidney damage and neuromuscular problems[1,2]. GN potentially affects the proximal tubular cells. It is freely filtered by the glomeruli and then moved to the proximal tubular epithelial cells, after initial exposure to phospholipids on the brush border membranes. GN alters the phospholipid metabolism of the tubular cell. Moreover, GN causes renal vasoconstriction[3,4].

Alpha lipoic acid (ALA) is an antioxidant made by the body. Several studies have suggested that ALA has many medical uses, including lowering blood glucose levels and antimicrobial activity. It is able to interact with free radicals, which decreases the risk of diabetic complications, such as tingling, pain, burning, itching and numbness in the arms and legs as a result of nerve damage ending in peripheral neuropathy[4,5]. Other preliminary studies have reported that ALA helps in the treatment of glaucoma[6,7]. ALA also plays a role in the treatment of other disorders, such as erectile dysfunction and cancer. It has been reported that 5% ALA cream tightens fine lines from sun damage in aging skin.

Previous studies have reported that ALA has a protective effect against nephrotoxicity induced by GN. Sandhya and Varalakshmi[8] reported that ALA protects against basolateral and brush border membrane damage induced by GN. However, no previous reports have investigated full kidney function for the protective effect of ALA against GN-induced nephrotoxicity. In addition, no reports have explored the combined formulation of ALA and GN. This study investigates the formulation of ALA and GN, loaded in a biodegradable nanoparticulate formulation that has the specific characteristic of a small size that facilitates cellular entrapment, binding and stabilization of proteins and lysosomal escape after endocytosis, thus leading to improved efficacy and bioavailability of the formulated drug[9-11]. Furthermore, a delay in GN release will decrease dosing frequency and improve convenience for patients. The aim of this study was to protect the kidney from GN-induced nephrotoxicity by loading GN with ALA on a biodegradable polymer.

MATERIALS AND METHODS

The study was carried out at the Nanotechnology Lab of the Faculty of Pharmacy, King Abdulaziz University, Jeddah in 2016. GN and ALA powder was purchased from Xian Sunny Biochemical Technology (Shaanxi, China). Polyvinyl alcohol (PVA) and polycaprolactone (PCL) (Mn = 10,000 Da) were purchased from Sigma-Aldrich (Dusseldorf, Germany).

Formulation of GN-ALA-PCL nanoparticles: Amounts of GN, ALA and PCL were determined using different ratios of all components to achieve the smallest particle size and highest entrapment efficiency percentage (EE %). ALA was loaded with GN on PCL nanoparticles (NPs) using the solvent evaporation technique[12]. About 150 mg of GN was dissolved in 15 mL of 1% polyvinyl alcohol (PVA) solution. ALA (300 mg) and PCL (450 mg) were dissolved together in 20 mL of chloroform and then added dropwise to the GN solution under stirring. Stirring was continued overnight to evaporate the organic solvent. The prepared NPs were ultrasonicated by ultrasonicator (Sonics, USA), then washed with water twice to remove the PVA and unentrapped drugs by centrifugation at 15000 rpm and collecting the precipitate, after that the filtrate was discarded and the distilled water was added to resuspend the NPs, finally NPs were frozen at -80°C for 24 h followed by lyophilization for 48 h.

Particle size and zeta potential measurements: The prepared formulations were assessed for particle size and zeta potential using a Microtrac® particle size analyzer (Microtrac Inc., Montgomeryville, PA, USA). GN-ALA-PCL NPs were diluted with a certain volume of water. Particle size was expressed using three replicate samples.

GN-ALA-PCL NPs morphology: The prepared NPs were imaged using Transmission Electron Microscopy (TEM). A drop of the prepared GN-ALA-PCL NPs was mounted on a carbon-coated grid and left for 10 min to allow better adsorption on the carbon; excess liquid was removed. A drop of phosphotungstic acid (1%) then was added and the prepared sample was imaged by TEM (Model JEM-1230, JOEL, Tokyo, Japan).

Entrapment efficiency (EE %): A specified weight of the lyophilized GN-ALA-PCL NPs was dissolved in chloroform. Then water was added and water and solution were mixed by vortex for 10 min. ALA was dissolved in a chloroform layer

and quantitative using the High-Performance Liquid Chromatography (HPLC) method[13]; GN was derivatized and determined by HPLC according to Ahmed's method[9].

Stability study of the formulation: The physical stability of the prepared formula was evaluated for its size, zeta potential and EE % after three cycles of freezing at -20°C and thawing.

Drug release study: To evaluate the release rate of GN from GN-ALA-PCL NPs, a Franz cell diffusion apparatus (MicroettePlus™; Hanson Research, Chatsworth, USA) was used. GN-ALA-PCL NPs containing 1 mg of GN were placed in a donor chamber and allowed to release GN into a receptor chamber through a silicon membrane with a diameter of 0.2 µm. A 0.01 M potassium dihydrogen phosphate buffer (pH 5.5) was used as a medium and samples were withdrawn automatically after 1, 2, 4, 6, 8 and 12 h and then determined by HPLC.

Antimicrobial evaluation: The antimicrobial activity of GN-ALA-PCL NPs and pure GN was tested against standard strains of four bacteria, which were obtained from the Microbiology Laboratory of King Abdulaziz University Hospital, Jeddah, KSA[14]. These strains included Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Bacillus subtilis* ATCC 6633) and Gram-negative bacteria (*Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853).

The agar diffusion method[15] was used to evaluate the antimicrobial activity of GN-ALA-PCL NPs compared with pure GN. Petri dishes (150 mm) containing 25 mL Mueller-Hinton agar containing 1 mL bacterial culture (1×10^6 CFU mL^{-1}) were used. Each strain was immunized independently. Gaps measuring 10 mm were made in seeded agar plates. The openings were then loaded with 200 µL of pure GN and GN-ALA-PCL NPs with a GN concentration of 0.5 mg mL^{-1} for each sample. Dishes were then incubated for 24 h at 37°C. The inhibitory zone was characterized as the non-attendance of bacterial development in the territory encompassing the gaps and was measured utilizing a caliper. All specimens were triplicates and data were expressed as Mean±SD.

Nephrotoxicity study: Groups of six male rabbits (weight: 2.5±0.8 kg) were injected intramuscularly with (a) Saline only, as a control; (b) Pure GN (20 mg kg^{-1}) and (c) GN-ALA-PCL NPs (equivalent to 20 mg kg^{-1}). Animals were acquired from the animal house, Faculty of Science, BeniSuef University, BeniSuef, Egypt. All exploratory conventions were affirmed by the Animal Ethics Committee of the Faculty of Science of BeniSuef University and complied with the Declaration of Helsinki, the Guiding Principle in Care and Use of Animals (DHEW production NIH 80-23) and the Standards of Laboratory Animal Care (NIH distribution #85-23, reconsidered in 1985).

The rabbits were accustomed for at least 14 days in naturally controlled enclosures (20±1°C and 12/12 h dark/light cycle) with free access to standard feed and water. Blood samples were collected on days 1, 5 and 10. Blood samples were collected from the medial canthus of the eye by means of capillary tubes. Creatinine, urea, sodium, potassium and calcium were measured as indicators for nephrotoxicity. The collected serum was kept at -80°C for further investigation and the method has been previously described[16].

Statistical analysis: Data were expressed as Mean±SE and were statistically evaluated using one-way analysis of variance to express the differences between the parameters, followed by Tukey's pairwise *post hoc* test. The confidence level was set at $p<0.05$ using GraphPad Prism 6 (GraphPad Software, San Diego, CA).

RESULTS

GN causes kidney damage as a result of oxidative stress. Nephrotoxicity increases blood urine nitrogen and creatinine and causes acute tubular necrosis; all of these effects were noted on administration of a nephrotoxic dose of GN in this study. The size of GN-ALA-PCL NPs was in the nano range (540.4±40.43 nm) with a zeta potential of 21.2±2.1 mV, indicating high stability with no aggregation. The EE % of GN and ALA were 42.32±5.43 and 91.21±6.43%, respectively.

The GN-ALA-PCL NPs formulation produced spherical particles with regular edges, as shown in Fig. 1. Surface morphology and particle size are essential factors to control cellular uptake and tissue penetration. Figure 2 shows the percentage of cumulative GN released from the prepared GN-ALA-PCL NPs. Pure GN was dissolved quickly in the medium with no resistance and reached 98.2±1.1% after 1 h, whereas the GN-ALA-PCL NP formulation displayed a biphasic drug release pattern, with a burst release within 1 h (34.2%) attributed to drug adsorption on the NP surface, followed by a sustained release pattern (88.43%). After three cycles of freeze and thaw, there was no significant change in particle size, zeta potential or EE %.

Antimicrobial activity was carried out to evaluate the effect of ALA on GN activity. There was no significant difference between the antimicrobial activity of pure GN and GN-ALA-PCL NPs against the four types of bacteria.

Table 1: Kidney function parameters measured in rabbit plasma (Mean±SD) for pure GN and GN-ALA-PCL NPs

Parameters	Creatinine	Urea	Sodium	Potassium	Calcium
	----------	----------	($mmol\ L^{-1}$)	----------	----------
Control group					
Day 1st	0.100±0.002	5.20±2.20	138.60±1.40	4.20±0.12	5.43±0.70
Day 5th	0.100±2.3	5.10±2.50	146.10±1.20	4.10±1.60	5.30±1.50
Day 10th	0.110±1.6	5.20±2.50	139.10±3.10	4.10±1.20	5.20±1.10
Pure GN group					
Day 1st	0.091±0.03	4.95±1.80	136.00±9.20	4.65±1.30	4.95±1.20
Day 5th	0.125±0.03*	6.06±2.30*	143.00±6.40	5.35±1.30	1.75±0.50*
Day 10th	0.155±0.0*	6.93±2.20*	156.00±5.12*	6.73±5.80*	1.22±0.72*
GN-ALA-PCL NPs group					
Day 1st	0.086±0.03	5.78±1.58	135.00±9.97	4.13±1.26	3.08±0.35
Day 5th	0.110±0.03	5.05±2.08	143.83±6.30	4.30±1.30	4.75±0.60
Day 10th	0.100±0.05	4.78±2.23	142.00±1.20	4.90±1.20	4.63±0.50

p-value<0.05, significant difference between any parameter in comparing with control group

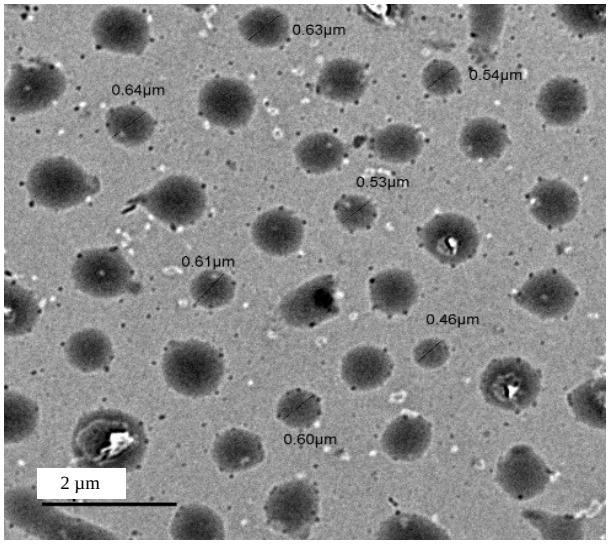

Fig. 1: GN-ALA-PCL NPs morphology showing a spherical particle in nano-size range

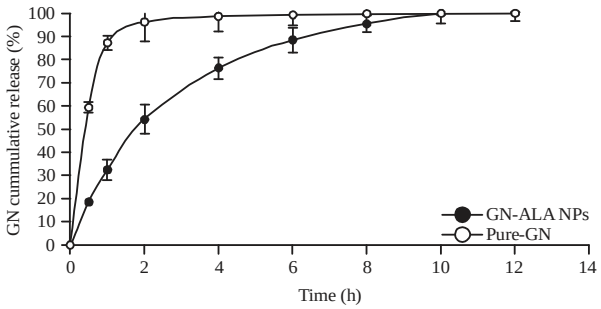

Fig. 2: GN cumulative release (%) from pure GN and GN-ALA-PCL NPs

An *in vivo* nephrotoxicity study revealed no significant change in creatinine, urea, sodium, potassium and calcium levels between GN-ALA- PCL NPs and the control group (gp), as shown in (Table 1). The pure GN group showed a significant increase in the levels of creatinine, urea, sodium and potassium ($p<0.05$) in comparison with the pure control group. However, calcium showed a significant decrease ($p<0.05$) of about 30% in the pure GN group in comparison with the control group, thus confirming the nephrotoxicity of GN.

DISCUSSION

The study aimed to load GN and ALA on a PCL-based nanoparticulate formulation to increase the circulation time and yield a sustained GN release that allows more GN to penetrate at the site of action, which enhances therapeutic efficacy. Furthermore, incorporation of ALA, which has potent antioxidant effects, into the NP formula reduces the nephrotoxicity of GN. The relatively high particle size of GN-ALA-PCL NPs is a result of the high viscosity of the organic phase; incorporating PCL and ALA to form an emulsion after evaporation of the organic phase results in the production of a larger globule that is hard to break by ultrasonication. These data agree with Fahmy *et al.*[17], who used the same polymer with the same concentration. The low EE % of GN (42.32±5.43) is rationalized by its high water solubility. After formation of the NPs, it is necessary to remove the PVA by washing with distilled water. Therefore, GN is predicted to escape during washing. However, the EE % of ALA was higher compared to GN due to viscous forces that oppose the diffusion of ALA molecules into the aqueous phase from the organic phase. ALA is insoluble in water but soluble in organic solvents, which facilitates its escape toward insoluble PCL and the formation of aggregate NPs[18]. The slow GN release from GN-ALA-PCL NPs could be attributed to deep entrapment of GN in the core of NPs and the low porosity and solubility of the PCL NP matrix in the buffered medium[19].

With regard to the nephrotoxicity study, these data completely agree with previous study. Dworacka *et al.*[20] measured levels of glutathione (GSH) and the activity of three antioxidant systems-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) after administration of GN alone and GN with ALA in two groups of rats. They observed a significant increase in the GSH level and in the activity of SOD, CAT and GPx in the GN-ALA group.

ALA has unique properties; it has hydrophilic and lipophilic characteristics that enhance its ability to penetrate tissue of vital organs, in addition to its ability to fight ROS. In a previous study[18], ALA played a nephroprotective role in cisplatin-induced renal toxicity in mice via a triple mechanism that depended on antiapoptotic antioxidant properties and initiation of antioxidant gene expression. Some studies have indicated that superoxide radicals can inhibit GPx and singlet oxygen and peroxyl radicals can inhibit SOD and CAT activity. Cisplatin-induced toxicity in mice exhibited a significant elevation in kidney function biomarkers, accompanying a significant depletion of CAT, SOD and GPx. ALA may play a renoprotective role in cisplatin-induced nephrotoxicity through antioxidant and antiapoptotic mechanisms combined with the onset of mRNA expression of antioxidant genes[8].

In the present study, urea and creatinine were significantly decreased in the GN-ALA-PCL NPs group compared with the GN group. In this study, only the pure GN group had an increased level of creatinine and urea (renal damage indicators). Elevations were significantly reduced in the GN-ALA-PCL NPs group compared to the control group. A previous study demonstrated that ALA supplementation attenuates renal injury in rats with obstructive nephropathy via its beneficial antioxidant properties. ALA both improves renal dysfunction and decreases abnormal levels of MDA and GSH during rat renal ischemia/reperfusion[19,20]. This study recommends administration of GN and ALA loaded on PCL polymer to avoid GN nephrotoxicity and to sustain GN release, leading to decreased dosing frequency and enhanced patient convenience.

CONCLUSION

The study confirms the protective effect of ALA on GN nephrotoxicity. Moreover, the prepared GN-ALA-PCL NPs achieved relatively moderate and high EE %, with a relatively extended release profile and good, stable formula. All kidney parameters were within the normal range after administration of GN-ALA-PCL NPs, in contrast to pure GN. Data confirmed ALA protection of renal tubular cells. It can be concluded that loading GN and ALA on PCL NPs may provide a novel combination for protection of the kidneys during treatment with GN.

SIGNIFICANCE STATEMENT

This study suggests a novel combination of GN and ALA that can be a beneficial to protect from GN-induced nephrotoxicity. Furthermore, it will help researchers to uncover an important topic on utilizing and exploring combinations of other aminoglycosides and potent anti-oxidants.

ACKNOWLEDGMENT

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant No. (G-267-166-38). The authors, therefore, acknowledge with thanks DSR for technical and financial support.

REFERENCES

1. Elsayed, M.G.A., A.A.A. Elkomy, M.S. Gaballah and M. Elbadawy, 2014. Nephrotoxicity of cefepime: A new cephalosporin antibiotic in rats. J. Pharmacol. Pharmacother., 5: 33-38.
2. Shamsuyarova, A., 2015. Overexpression of catalase prevents gentamicin-induced apoptosis of renal proximal tubular cells in transgenic mice. Master's Thesis, Department of Biomedical Sciences, Faculty of Medicine, Université de Montréal, Canada.
3. Nasri, H., M. Nematbakhsh and M. Rafieian-Kopaei, 2013. Ethanolic extract of garlic for attenuation of gentamicin-induced nephrotoxicity in Wistar rats. Iran. J. Kidney Dis., 7: 376-382.
4. Tavafi, M., 2013. Protection of renal tubules against gentamicin induced nephrotoxicity. J. Renal Injury Prevent., 2: 5-6.
5. Notarnicola, A., G. Maccagnano, S. Tafuri, A. Fiore, V. Pesce and B. Moretti, 2015. Comparison of shock wave therapy and nutraceutical composed of *Echinacea angustifolia*, alpha lipoic acid, conjugated linoleic acid and quercetin (Perinerv) in patients with carpal tunnel syndrome. Int. J. Immunopathol. Pharmacol., 28: 256-262.
6. Hermann, T., 2007. Aminoglycoside antibiotics: Old drugs and new therapeutic approaches. Cell. Mol. Life Sci., 64: 1841-1852.
7. Ismail, A.F.H., F. Mohamed, L.M.M. Rosli, M.A.M. Shafri, M.S. Haris and A.B. Adina, 2016. Spectrophotometric determination of gentamicin loaded PLGA microparticles and method validation via ninhydrin-gentamicin complex as a rapid quantification approach. J. Applied Pharm. Sci., 6: 7-14.

8. Sandhya, P. and P. Varalakshmi, 1997. Effect of lipoic acid administration on gentamicin induced lipid peroxidation in rats. J. Applied Toxicol., 17: 405-408.
9. Karagoz, B., L. Esser, H.T. Duong, J.S. Basuki, C. Boyer and T.P. Davis, 2014. Polymerization-Induced Self-Assembly (PISA)-control over the morphology of nanoparticles for drug delivery applications. Polym. Chem., 5: 350-355.
10. Li, J.H., G.X. Ju, J.L. Jiang, N.S. Li, J. Peng and X.J. Luo, 2016. Lipoic acid protects gastric mucosa from ethanol-induced injury in rat through a mechanism involving aldehyde dehydrogenase 2 activation. Alcohol, 56: 21-28.
11. Monastra, G., S. De Grazia, S.C. Micili, A. Goker and V. Unfer, 2016. Immunomodulatory activities of alpha lipoic acid with a special focus on its efficacy in preventing miscarriage. Expert Opin. Drug Deliv., 13: 1695-1708.
12. Sreelola, V. and A.K. Sailaja, 2014. Preparation and characterisation of ibuprofen loaded polymeric nanoparticles by solvent evaporation technique. Int. J. Pharm. Pharm. Sci., 6: 416-421.
13. Rajkumar, B., T. Bhavya and A.A. Kumar, 2014. Reverse phase HPLC method development and validation for the simultaneous quantitative estimation of alpha lipoic acid and allopurinol in tablets. Int. J. Pharm. Pharm. Sci., 6: 307-312.
14. Balouiri, M., M. Sadiki and S.K. Ibnsouda, 2016. Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharma. Anal., 6: 71-79.
15. Ahmed, O.A., U.A. Fahmy, A.S. Al-Ghamdi, B.M. Aljaeid and H. Aldawsari *et al*., 2017. Finasteride-loaded biodegradable nanoparticles: Near-infrared quantification of plasma and prostate levels. J. Bioact. Compatible Polym., (In Press). 10.1177/0883911517694397.
16. Kurakula, M., O.A.A. Ahmed, U.A. Fahmy and T.A. Ahmed, 2016. Solid lipid nanoparticles for transdermal delivery of avanafil: Optimization, formulation, *in-vitro* and *ex-vivo* studies. J. Liposome Res., 26: 288-296.
17. Fahmy, U.A., O.A.A. Ahmed and K.M. Hosny, 2015. Development and evaluation of avanafil self-nanoemulsifying drug delivery system with rapid onset of action and enhanced bioavailability. AAPS PharmSciTech, 16: 53-58.
18. Melli, G., M. Taiana, F. Camozzi, D. Triolo and P. Podini *et al*., 2008. Alpha-lipoic acid prevents mitochondrial damage and neurotoxicity in experimental chemotherapy neuropathy. Exp. Neurol., 214: 276-284.
19. Poongothai, S., R. Ilavarasan and C.M. Karrunakaran, 2010. Simultaneous and accurate determination of vitamins B1, B6, B12 and α-lipoic acid in multivitamin capsule by reverse-phase high performance liquid chromatographic method. Int. J. Pharm. Pharm. Sci., 2: 133-139.
20. Dworacka, M., S. Iskakova, E. Krzyzagorska, A. Wesolowska, Y. Kurmambayev and G. Dworacki, 2015. Alpha-lipoic acid modifies circulating angiogenic factors in patients with type 2 diabetes mellitus. Diabetes Res. Clin. Pract., 107: 273-279.