7

UDC 547.785.5
STRUCTURES AND BIOLOGICAL ACTIVITY OF CUPROPHYLLINS

Martynov A.V., Bomko T.V., Nosalskaya T.N., Kabluchko-Baruah T.V.

Mechnikov Institute of Microbiology and Immunology

Chlorophylls (a, b) are the porphyrin compounds and most common chemicals in the plant's world. In fact, these compounds are an obligatory intermediate product both in energy metabolism and in plant catabolism. At the same time, currently there are few pharmaceutical preparations on the pharmaceutical market based on chlorophylls. Dyes based on hydrolyzed chlorophyll are successfully used in the food industry. Commercial chlorophylline is a copper complex of hydrolyzed chlorophylls. As shown earlier in TLC, the chlorophyllin mixture contains a large number of different compounds [1].

It is like water-soluble saponified derivatives in the form of sodium-magnesium complexes, and similar structures in the form of a complex with copper. The latter are more brightly colored, soluble in water and widely used as coloring agents in cooking. In this case, if the initial chlorophyll was not found to have a pronounced biological activity, the substituted derivatives in the form of copper complexes possessed a number of new unique biological properties. Non-hydrolyzed hydrophobic cuprophylline obtained from eucalyptus leaves possessed high antimicrobial activity to most strains of staphylococci, inclusion resistant to antimicrobials and multiresistant strains[2].

This drug is called Chlorophyllipt, it is allowed to be used as a medicinal product and is one of the oldest antibacterial drugs from plants on the market.

Chlorophyllipt had a bacteriostatic effect on antibiotic-resistant staphylococci in a dose 6.2 ± 0.072 μg / ml and bactericidal - in a dose 12.6 ± 0.153 μg / ml. During an experiment in laboratory on animals, the beneficial effect of chlorophyllipt on hematopoiesis, reparative tissue regeneration, phagocytic activity of blood leukocytes and connective tissue cells, as well as an increase in vitamin PP level, lysozyme and catalase activity in animal tissues were found [3]. Specifically for Chlorophyllipt several methods for determining antimicrobial activity have been developed [4].

It is marketed as ethanoic and oily solutions for topical use, and as an alcohol solution for intravenous injections. Its main purpose is the fight against staphylococcal infections [5].

Recently, found that the oral administration of chlorophyllipt activates cellular immunity and indirectly exhibits antiviral activity. Another compound of cuprophyllin is water-soluble chlorophyllin [6].

Some authors show the variability of the structure and biological activity of cuprophyllins.

The variability of the cuprophyllins structure

There are variants of cuprophylline for a composition with one copper atom per porphyrin ring to one atom per 6 rings. This variability in the structure led to the manifestation of directly opposite biological effects of cuprophyllins. In one experiment, cuprophylline inhibited dimethylhydrazine colorectal cancer, and in another it was a promoter of carcinogenesis [7].

The results obtained earlier were contradictory. In one case, it was shown that copper-porphyrin e6 (CuChle6) is a tricarboxylic derivative, and it dominates in the mixture [8, 9, 10].

In another study, it was shown that the derivative e4 (CuChle4) is a dicarboxylic derivative and dominates in most samples according to the analysis results. In another experiment it was shown that not all porphyrin cycles in the structure of cuprophylline are in copper form as a complex, and sometimes are represented by "empty" porphyrins [11].

Purified derivatives of chlorophyllin had completely different types of biological activity. For example, CuChle 6 stimulates lipase activity, while chlorin e6 which is its metal-free counterpart, inhibits this enzymatic reaction [12]. Chemical structures of chlorophyll -a and -b are show at Fig. 1 and 2. Chemical structures of well-known marketed derivatives of chlorophyll with Cu as ligand are show at Fig. 3 and 4.
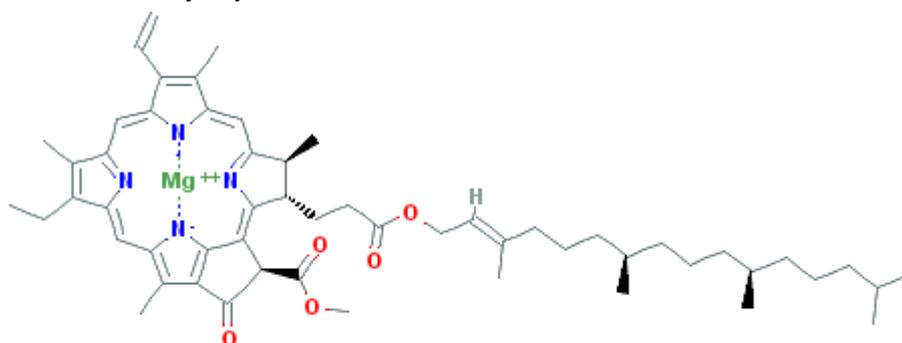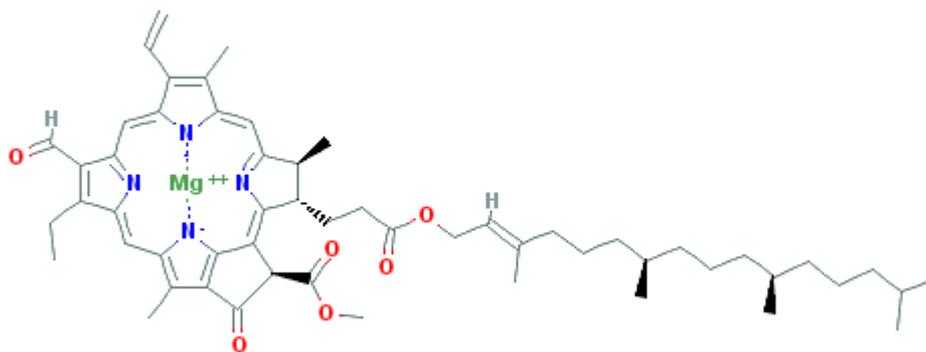

Fig.1. Chlorophyll a
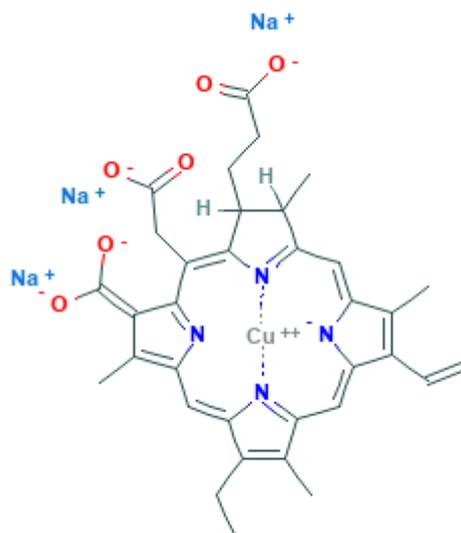
Fig.2. Chlorophyll b
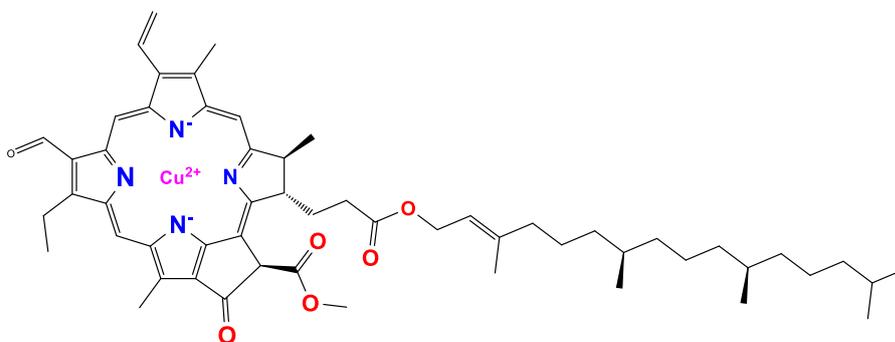
Fig.3. Chlorophyllin, cuprophyllin 3 sodium

Fig.4. Chlorophyllipt

In addition, it shown that the antioxidant activity of e4 was 20% higher than that of the e6 derivative [13]. The photodynamic properties of the chlorophyll's derivatives, containing and not containing copper atom as a ligand also differed significantly [14].

Toxic properties of cuprophyllins
Chlorophyllin (CHL) has proven that there is antimutagenic and anticarcinogenic activity in several organisms without causing lethal effects. However, there is no information about its effects when it is administered in gestation. In addition, it was shown the assessed possible effects of CHL when it administered to CD-1 mice on the 8th day of gestation using the same doses and administration route used in previous antimutagenic and antigenotoxic studies. Females were exposed to a single dose of CHL by i.p. injection (20, 40, 50, or 100mg/kg b.w.). On day 18 all wombs were subjected to cesarean section and the fetuses were examined with common teratological methods. Results show that CHL-treatment induced dose-dependent total litter loss and is dose-dependent, probably due to either the interaction between CHL and some general control mechanisms of embryo development or by an impairment of maternal-fetal interactions. The analysis of uterine horns of the CHL-treated females with total litter loss revealed the presence of green rings in the uterus. Results show the inverse relationship between the number of live implants and the frequency of green rings, indicating implantation sites

where embryo death and early resorptions occurred. Although CHL was given in a single dose on day 8 in this study, the results indicate that CHL is associated with significant embryo lethality [15].Chlorophyll-Copper-complex, prepared by extraction from green plants has very high lethal doses. Its oral LD50 for rats > 10000 mg/kg [16].

Influence of cuprophyllins on immunity
Previous findings demonstrated that chlorophyllin inhibits inducible nitric oxide gene expression in macrophages. It was shown that CHL inhibited IL-1beta production and its mRNA expression in a lipopolysaccharide (LPS)-stimulated murine macrophage cell-line, RAW 264.7. The inhibitory effect of CHL on IL-1 beta gene expression was further supported by an in vitro transfection assay using a pIL-1(870 bp)-CAT construct, where CHL inhibited the activation of the IL-1beta promoter. Furthermore, CHL attenuated the activation of NF-kappa B, NF-IL6 and AP-1, which are known to be responsible for IL-1 beta gene expression, as determined by an electrophoretic mobility shift assay and an in vitro transfection assay using p(NF-kappaB)3-CAT, p(NF-IL6)3-CAT, and p(AP-1)3-CAT, respectively. However, it was evident that the inhibitory activity of CHL on IL-1 beta expression in the LPS-stimulated macrophages was independent of CRE/ATF. The immunoblot experiment demonstrated that CHL also caused a substantial decrease in the phosphorylation of p38 MAP kinase in LPS-stimulated RAW 264.7. These results suggest that CHL inhibits IL-1 beta production in macrophages stimulated with LPS at transcriptional level by blocking the phosphorylation of p38 and by suppressing the activation of transcription factors, NF-kappaB, NF-IL6, and AP-1 [17].

Effect of CHL on experimental allergic reaction was also investigated. IgE antibody mediated reactions, homologous passive cutaneous anaphylaxis (PCA) in rats and the release of anaphylactic mediators (histamine and/or slow reacting substance of anaphylaxis (SRS-A) from sensitized guinea pig lung tissues or rat peritoneal mast cells classified as a Type I reaction were clearly inhibited by CHL at a similar potency as N-(3',4'-dimethoxy cinnamoyl) anthranilic acid (N-5'). The increase of vascular permeability in rat skin caused by autacoids or enzymes that participate in the Type I reaction was also inhibited by CHL. Type II or III, complement dependent, reactions including reversed cutaneous anaphylaxis in rats and Forssman cutaneous vasculitis in guinea pigs were inhibited by CHL. Prednisolone inhibited RCA in rats, but did not inhibit FCV in guinea pigs. Two experimental types of glomerulonephritis, nephrotoxic serum nephritis in rats and immune complex nephritis in F1 mice, in which Type II and III reactions might participate in the onset and the development of the disease, were slightly inhibited by CHL in terms of the biochemical changes of blood and urine parameters and histopathological scores. A moderate remission of the onset and development of these two experimental types of nephritis was recognized by the administration of prednisolone. Delayed hypersensitivity

reaction as a Type IV reaction caused by sheep red blood cells in sensitized mouse footpad was not affected by CHL. Prednisolone clearly inhibited the SRBC induced footpad reaction in mice. IgM antibody production in mice and IgE antibody production in rats were not influenced by daily injection of CHL [18].

In another study, it was shown that CHL was earlier shown to reduce the level of intracellular ROS and apoptosis induced by ionizing radiation and 2,2'-azobis(2-propionimidinedihydrochloride). In the present studies, the effect of CHL on radiation-induced immunosuppression and modulation of immune responses in mice was examined. Chlorophyllin inhibited the in vitro lymphocyte proliferation induced by concanavalin A (Con A) in a dose dependent manner at doses ≥50µM. At lower doses (10µM) CHL significantly inhibited activation inducing cell death in Con A stimulated spleen cells. Spleen cells obtained from CHL treated mice showed an inhibition of response to Con A depending on dose of CHL and the time after its administration. Spleen cells obtained from CHL treated mice (24 h) showed lower inhibition of response to Con A following in vitro (5 Gy) as well as whole body irradiation (2 Gy). The expression of antiapoptotic genes bcl-2 and bcl-xL was up-regulated in these cells. Chlorophyllin treatment of mice led to splenomegaly and increase in the number of peritoneal exudate cells (PEC). The numbers of T cells, B cells and macrophages in the spleen were also increased. Increased phagocytic activity was seen in PEC obtained from CHL treated mice. Most importantly, CHL administration to mice immunized with sheep red blood cells augmented both humoral and cell-mediated immune responses [19].

Also was investigate the effects of CHL on the proliferation, differentiation and immunomodulatory function of mesenchymal stem cells (MSCs) from mice with aplastic anemia. Peripheral blood cells were counted and colony-forming fi broblasts (CFU-F) in the bone marrow were assayed. The ability of MSCs to form calcium nodes after culture in osteoinductive medium was also observed. The immunosuppressive effect of MSCs on T lymphocytes was analyzed by enzyme-linked immunosorbent assay and flow cytometry, to evaluate the efficacy of CHL in mice with aplastic anemia. In this study were shown, that peripheral blood white cell and platelet counts were increased by medium and high CHL doses, compared with the untreated control. CFU-Fs were also increased compared with the untreated control, and the numbers of calcium nodes in MSCs in osteoinductive medium were elevated in response to CHL treatment. The percentage of Forkhead box protein 3 T cells was increased in T cell-MSC cocultures, and the cytokine transforming growth factor β1 was up-regulated in SCC-treated groups. The results of this study suggest that CHL not only promotes the proliferation and differentiation of MSCs, but also improves their immunoregulatory capacity in mice with aplastic anemia [20].

In 2005 year, some researchers investigated the effect of CHL on the activation of murine splenocytes stimulated with lipopolysaccharide (LPS). RT-PCR analysis showed that LPS-activated IFN-gamma

expression gradually declined by CHL treatment in a dose dependent manner while mRNA production of TNF-alpha, IL-2, and FasL was not changed. CHL also suppressed IL-12 production (p70, a heterodimer of p40 and p35) and the mRNA expression of IL-12 p40 and IL-12 receptors (both IL-12Rbeta1 and IL-12Rbeta2), which are involved in the induction of IFN-gamma expression. Furthermore, an electrophoretic mobility shift assay showed that CHL inhibited DNA binding activity of NF-kappaB, STAT-3, and STAT-4 to their cognate DNA recognition motifs, all of which contribute to the IL-12-induced IFN-gamma transcription. Exogenous addition of recombinant IL-12 abrogated the inhibitory effect of CHL on IFN-gamma and its mRNA expression in LPS-activated splenocytes. Collectively, these results show that CHL inhibits IFN-gamma production by LPS-stimulated splenic mononuclear cells due to down-regulation of IL-12 production [21].

Radioprotective antimutagenic properties of cuprophyllins

By delaying the time of gamma irradiation of 72 h larvae, pretreated at 48 h with 5% CHL, it was established that the overall inhibiting effect of CHL in somatic cells of Drosophila, as measured in the wing spot test, persists for about 4 days or until the time of cessation of the proliferation of wing anlagen. In the same population of cells, some spot classes gave evidence of an inhibitory effect whereas others did not arguing against the suggestion that the radioprotective effect of CHL is a consequence of an induced delay in development, shrinking of the potential radiation target and lowering the probability of induced events [22].

Another study presented evidence that treating the Drosophila female with chlorophyllin (CHL) has a marked effect on the yield of dominant lethals induced by the irradiation of sperm. The yield is significantly greater in the embryonic period (between the egg and the first instar) and is significantly reduced in postembryonic stages compared with a sucrose control [23].

In other study it was shown the significantly antimutagenic properties of CHL: in the study to examine the interaction of calf-thymus DNA with CHL and CHL in aqueous solution at physiological pH, with pigment/DNA (phosphate) molar ratios (r) of 1/80 to 1/2. Fourier transform infrared difference spectroscopic method was used to determine the pigment binding mode, binding constant, sequence selectivity, DNA secondary structure and structural variations of the pigment-DNA complexes in aqueous solution [24].

The antimutagenic effect of CHL in somatic cells monitored by the wing spot test persisted for 3 days after completion of the pretreatment and appeared to terminate at a time corresponding to the cessation of mitotic divisions of wing anlagen cells. Within the same population of cells, CHL demonstrated both an inhibitory effect as measured in mwh single spot classes, and contrarily, a promoting effect in the class of mwh/flr twin spots and to an extent in the class of large flr spots. The reason for the contrasting effects of CHL remains to be determined [25].

Antiviral properties of cuprophyllins

In earlier studies shown that the compound CHL obtained from conifers has a marked veridical action on certain viruses and gives marked therapeutic effect in herpetic keratitis, herpes zoster, and herpes simplex [26].

Dengue, a mosquito-borne viral infection, is one of the major public health concerns in the tropical and subtropical regions of the world. Approximately, 2.5 billion people across the world are at risk from dengue and 50 to 100 million new infections of dengue occur annually. There is yet no vaccine or medicine available against dengue, and treatment remains only supportive. Targeting its vector by a combination of biological and chemical approaches and management of breeding sites are currently the only existing approaches to control or eliminate dengue. Chlorophyll derivatives like chlorophyllin and pheophorbide have been reported as effective natural photosensitizers against larvae of several insects including flies. Chlorophyll derivatives were also reported effective against larval stages of freshwater snails as well as against certain parasites of fish. CHL can prove to be a good contributor in an integrated approach against dengue [27].

Also was shown the antiviral effect of CHL on the poliovirus replication in cell culture. The drug was tested for the virucide, prophylactic and therapeutic activities on the replication of the poliovirus in HEp-2 cells cultures, at concentrations of 0.5 and 2.5 µg/mL. Virus titration and an indirect immunofluorescence test were used for the evaluation. The CHL inhibited poliovirus replication in all treatment protocols; however, it was more effective on virucide treatment, with a 95.7% reduction in viral multiplication at concentration of 2.5 µg/mL. CHL reduced the number of specifically fluorescent infected cells in both virucide and therapeutic treatments, 8h and 10h post-infection, at both concentrations (0.5 and 2.5 µg/ mL). It is suggested that CHL either has a direct action on the virus particles or acts on the initial stage of the poliovirus replication [28].

CHL exerted antiviral activities against influenza virus and HIV as the major ingredient of bamboo leaf extract solution by blocking adsorption. This mechanism of action is different completely from the one of povidone-iodine [29].

Chlorin e6, a metal-free chlorophylline-like molecule, showed the strongest antiviral activity against HBV as well as profound antiviral effects on other enveloped viruses, such as hepatitis C virus (HCV), human immunodeficiency virus (HIV), dengue virus (DENV), Marburg virus (MARV), Tacaribe virus (TCRV), and Junin viruses (JUNV). Remarkably, chlorin e6 inactivated DENV at subnanomolar-level concentrations. However, the compound had no antiviral effect against encephalomyocarditis virus and adenovirus, suggesting that chlorin e6 may be less active or inactive against nonenveloped viruses. Although other porphyrin derivatives have been previously reported to possess antiviral activity, this is the first analysis of the biochemical impact of CHL and chlorin e6 against HBV and of the dramatic anti-infectivity impact upon DENV.

The possible application of this family of compounds microbicides and systemic virus neutralizing agents, is discussed [30].

CHL was assayed for its capacity to prevent nuclear fragmentation in HEp-2 cells infected with poliovirus. CHL was assayed at concentrations of 0·5 and 2·5 μg ml−1, and NF was monitored using the comet assay and acridine orange staining. In this study demonstrated that CHL reduced the percentage of NF in poliovirus-infected HEp-2 cells, when cells were treated with drug before infection or exposed continuously to drug. However, the highest degree of protection was achieved when the virus was exposed to CHL before infection followed by protocol where infected cultures were continuously exposed to the drug after infection. It is suggested that CHL primarily binds to the virus which inhibits cell penetration, thereby maintaining nuclear integrity. Considering that CHL has several beneficial properties and no significant toxic effects in humans and animals, it would be an ideal candidate drug to test for antiviral activity [31].

Antimicrobial properties of cuprophyllins

Chlorophyllin antibacterial properties observed in vitro on staphylococci, streptococci, lactobacilli, anaerobic spore-bearers, pneumococci, Bacillus pumilus, patogenic clostridia - Cl. welchii, Cl. sporogenes. It was found to inhibit the growth of Gram-positive bacteria and no effect on Gram-negative organisms [32].

Chlorophyllin from the leaf extract of Mimosa pudica recorded potential antimicrobial activity with the range of 9 mm-18 mm at 25-100 μg ml-1 against human pathogenic bacteria and fungi, viz., two Gram-negative bacteria: Pseudomonas aeruginosa and Escherichia coli, and two Gram-positive bacteria: Staphylococcus aureus and Klebsiella pneomoniae, and one fungal pathogen, Candida albicans [33].

Chlorophyll-solution presents effective antimicrobial activity on C. albicans but did not present any activity on E. faecalis [34].

Cotton fabrics were dyed with the natural CHL from Japanese bamboo leaves. It improves the textile coloration and antimicrobial activity tested against two common pathogens: Staphylococcus aureus and Klebsiella pneumoniae [35].

CHL had the ability to inhibit the clotting of human plasma by the coagulase of the *S.aureus*. The critical concentration of the water-soluble derivatives of chlorophyllin for the test system which was used was between a 0.040 per cent solution and a 0.045 per cent solution [36]. Chlorin-e and chlorophyll in in concentrations of .025 and .050% in glycerine broth and Sauton's media inhibited the growth of H-37 and avian tubercle bacilli. Copper chlorin-e, deuteroporphyrin, copper deuteroporphyrin, and pyrroporphyrin sulfonic acid-sodium salt showed no growth retarding effects in concentrations of 0.025 and 0.050%. Evidence is presented to suggest that the H-37 tuberculoprotein of the living bacillus is capable of binding copper chlorin-e. The absorption spectrum of the copper chlorin-e-bacillary protein complex when compared with that of CHL in

aqueous solution lends support to the assumption that chemical union between protein and pigment exists [37].

1.6% of clinically healthy women active in certain professions have been found to be carriers of pathogenic species of *Listeria*. The study of the sensitivity of 73 strains of *Listeria spp.* to the preparations of Chlorophyllipt has revealed that in usual therapeutic concentrations Chlorophyllipt produces a pronounced antibacterial effect on these organisms. The use of Chlorophyllipt for the sanitation of carriers of pathogenic Listeria is proposed [38].

Influence of chlorophyllin on atherogenesis

The effect of CHL, was assessed in rats with experimental atherosclerosis. The study was focused on changes in serum cholesterol, lipids, and triglycerides concentration as well as on serum and abdominal aorta Cu and Zn values. It has been ascertained that after 90 d in animals fed a rich lipid diet there was a statistically significant increase in serum cholesterol, triglycerides, and lipid concentration ($p < 0.01$). A significant augmentation of serum Cu values ($p < 0.01$) accompanied by a marked lowering of the same element in abdominal aorta ($p < 0.01$) was also found, as compared to the results registered in the control group. However, CHL, administered for 90 d in the group of animals with experimental atherosclerosis, significantly decreased the serum cholesterol, triglycerides, and serum lipid values ($p < 0.01$), increased copper content in aortic tissue ($p < 0.01$) and lowered serum copper concentration ($p < 0.01$) as compared to the untreated group. Moreover, in the aorta of administered animals the lipid infiltration has been demonstrated to be significantly diminished vs. the untreated group [39].

The oxidative modification of low density lipoproteine (LDL) may play an important role in the early events of atherogenesis. Thus the identification of antioxidative compounds may be of therapeutic and prophylactic importance regarding cardiovascular disease. CHL, has been reported to inhibit lipid oxidation in biological membranes and liposomes. Hemin (Fe^{3+}-protoporphyrin IX) has been shown to bind to LDL thereby inducing lipid peroxidation. As Cu-CHL has a similar structure as hemin, one may assume that Cu-CHL may compete with the hemin action on LDL. Therefore, in the present study Cu-CHL and the related compound magnesium-chlorophyllin (Mg-CHL) were examined in their ability to inhibit LDL oxidation initiated by hemin and other LDL oxidizing systems. LDL oxidation by hemin in presence of H_2O_2 was strongly inhibited by both CHLs. Both CHL were also capable of effectively inhibiting LDL oxidation initiated by transition metal ions (Cu^{2+}), human umbilical vein endothelial cells (HUVEC) and tyrosyl radicals generated by myeloperoxidase (MPO) in presence of H_2O_2 and tyrosine. Cu- and Mg-CHL showed radical scavenging ability as demonstrated by the diphenylpicrylhydracyl radical (DPPH)-radical assay and estimation of phenoxyl radical generated diphenyl (dityrosine) formation. As assessed by ultracentrifugation

the chlorophyllins were found to bind to LDL (and HDL) in serum. Copper chlorophyllin (Cu-CHL) and its magnesium analog could act as potent antagonists of atherogenic LDL modification induced by various oxidative stimuli. As inhibitory effects of the CHLs were found at concentrations as low as 1 μmol/l, which can be achieved in humans, the results may be physiologically/therapeutically relevant [40].

Despite uses of titanium dioxide (TiO₂) particles in various consumer and medical products therapy increasing human daily exposure, little studies were conducted on its cardiotoxicity. Thus, this study was designed to investigate the possible modulation of nanoTiO₂ induced genotoxicity, mutagenicity and apoptosis by CHL coadministration in mice cardiac cells. Mice were injected into the abdominal cavity with TiO₂ (500, 1000 and 2000 mg/kg b.w) suspended either in deionized dist. water or in CHL (40 mg/kg b.w) solution for five consecutive days and scarified 24 hour after the last injections. CHL co-administration resulting in significant reductions in tail length, %DNA in tail and tail moment that was highly elevated in nano-TiO₂ treated groups in a dose dependent manner, also the appearance of both apoptotic fragmentized Laddered and smeared DNA on agarose gel was returned to the normal unfragmentized appearance. The observed dose dependent high incidence of mutations inductions in p53 exons (5-8) and myocardial cells infiltrations by inflammatory cells, hemorrhage and congested blood vessels by nano-titanium was attenuated by CHL co-administration. Finally, CHL improved the antioxidant defense system indicated by significant reduction in malondialdiahyde level and increases in reduced glutathione level and glutathione peroxidase activity that were disrupted in nano-titanium treated groups. In conclusion: nano TiO2 particles induced genotoxicity, mutagenicity and apoptosis were attenuated by CHL co-administration in mice cardiac cells. It is recommended for further studies to detect the CHL dose that completely normalize nano-titanium induced cardiotoxicity [41].

Conclusion
1. Different derivatives of chlorophyll have different biological activity.
2. Hydrophilic cuprophyllin - chlorophyllin very good studied and have good profile of toxicity and using in cooking as colorant.
3. Chlorophyllin has such biological effects: antiviral, antimicrobial, immunomodulating, antioxidant, antiatherosclerotic, antimutagenic and anticancerogenic. Most of the studies were carried out as in vitro and in vivo with statistically trustworth results.
4. Derivatives of chlorophyll are very perspective candidates for fight with multiresistant strains of mnicroorganisms and viral infections.
5. Derivatives of chlorophyll must be more studied for using as active pharmaceutical ingredients for development a new drugs in the form of injection, tablets, ointments.

STRUCTURES AND BIOLOGICAL ACTIVITY OF CUPROPHYLLINS

Martynov A.V., Bomko T.V., Nosalskaya T.N., Kabluchko-Baruah T.V.

Chlorophylls (a, b) are the porphyrin compounds and most common chemical in the plant's world. In fact, these compounds are an obligatory intermediate product both in energy metabolism and in plant catabolism. At the same time, currently there are few pharmaceutical preparations on the pharmaceutical market based on chlorophylls. Dyes based on hydrolyzed chlorophyll are successfully used in the food industry. Commercial chlorophylline is a copper complex of hydrolyzed chlorophylls. As shown earlier in TLC, the chlorophyllin mixture contains a large number of different compounds. It is like water-soluble saponified derivatives in the form of sodium-magnesium complexes, and similar structures in the form of a complex with copper. The latter are more brightly colored, soluble in water and widely used as coloring agents in cooking. In this case, if the initial chlorophyll was not found to have a pronounced biological activity, the substituted derivatives in the form of copper complexes possessed a number of new unique biological properties. Non-hydrolyzed hydrophobic cuprophylline obtained from eucalyptus leaves possessed high antimicrobial activity to most strains of staphylococci, inclusion resistant to antimicrobials and multiresistant strains. This drug is called Chlorophyllipt, it is allowed to be used as a medicinal product and is one of the oldest antibacterial drugs from plants on the market. It is marketed as ethanoic and oily solutions for topical use, and as an alcohol solution for intravenous injections. Its main purpose is the fight against staphylococcal infections. Recently, found that the oral administration of chlorophyllipt activates cellular immunity and indirectly exhibits antiviral activity. Another compound of cuprophyllin is water-soluble chlorophyllin. Some authors show the variability of the structure and biological activity of cuprophyllins. Different derivatives of chlorophyll have different biological activity. Hydrophilic cuprophyllin chlorophyllin very good studied and have good profile of toxicity and uses in cooking as colorant. Chlorophyllin have such biological effects: antiatherosclerotic, antiviral, antimicrobial, immunomodulating, antimutagenic and anticancerogenic, antioxidant. Most of the studies were carried out as in vitro and in vivo with statistically trustworth results. Derivatives of chlorophyll are very perspective candidates for fight with multiresistant strains of mnicroorganisms and viral infections. Derivatives of chlorophyll must be more studied for using as active pharmaceutical ingredients for development a new drugs in the form of injection, tablets, ointments.

References

1 Sato M., Fujimoto I., Sakai T.et al. Effect of sodium copper chiorophyllin on lipid peroxidation. IX. On the antioxidative components in commercial preparations of sodium copper chlorophyllin// Chem. Pharm. Bull. 1986. Vol. 34. P. 2428-2434.

2 Nadtokova VL The domestic antibacterial drug chlorophyllipt // Antibiotics. 1970. N. 10. P. 888-892.

3 Nadtoka V.L. Chlorophyllipt. Experimental-clinical research // Avtoref. Diss. For the competition Degree of Doct. M. Sciences. - Kharkov, 1970. - 23 p.

4 Olefir Yu. V., Luttseva AI, Gunar O V. et al. Experimental evaluation of methods for determining the antimicrobial activity of chlorophylliptine preparations// Statements of the scientific center of expertise of means of medical application. 2015. Vol. 4. P. 47-50.

5 Elizarova V.E.. Chlorophyllipt as an effective antiseptic in the treatment of periodontitis// Stomatol. Children. Age and prevention. 2003. Vol. 1-2. P. 17-18.

6 Young R. W., Beregi J. S. Use of chlorophyllin in the care of geriatric patients// Journal of the American Geriatrics Society.1980. Vol. 28. N. 1.P. 46-47.

7 Nelson R.L. Chlorophyllin, an antimutagen, acts as a tumor promoter in the rat-dimethylhydrazine colon carcinogenesis model// Anticancer Res. 1992. Vol. 12. P. 737-740.

8 Wall M.E. Preparation of chlorophyll derivatives for industrial and pharmaceutical use// US Agricultural and Chemistry Bureau.1951. Vol. 299. P. 1- 6

9 Eddy W.H. Chlorophyll, Strong Cobb and Co., Inc., Lake Worth, Florida. 1953. P. 1-60

10 Kephart J.C. Chlorophyll derivatives: their chemistry, commercial preparation and uses// Econ Bot. 1955. Vol. 0. P.3-38.

11 Chernomorsky S.A. Chiorophyllin copper complex: quality control// J. Soc. 1993. Vol. 44. P. 235-238.

12 Kurnygina V.T., Nikitina T.V. Effect of metal-chlorophyll complexes on pancreatic lipase activity. 1981. Biol Nauki. Vol. 6. P. 31-34.

13 Sato M., Fujimoto I., Sakai T., et al. Effect of sodium copper chiorophyllin on lipid peroxidation, IX, On the antioxidative components in commercial preparations of sodium copper chlorophyllin// Chem. Pharm. Bull. 1986. Vol. 34. P. 2428-2434.

14 Spikes J.D., Bommer J.C. Chlorophyll and related pigments as photosensitizers in biology and medicine, in: H. Scheer (Ed.), Chlorophylls, CRC Press, London. 1990. P. 1185-1189.

15 Garcia-Rodriguez M. C., Morales-Ramirez P., Altamirano-Lozano M. Effects of chlorophyllin on mouse embryonic and fetal development in vivo// Teratog Carcinog Mutagen. 2002. Vol. 22. N. 6. P. 461–471. https://doi.org/10.1002/tcm.10042

16 Chlorophyll-Copper-complex. Material Safety Data Sheet according to Regulation (EC) No. 1907/2006 (REACH) Revision Date 16.11.2015

17 Yun C. H., Jeon Y. J., Yang Y., Ju H. R., Han S. H. Chlorophyllin suppresses interleukin-1 beta expression in lipopolysaccharide-activated RAW 264.7 cells// Int Immunopharmacol. 2006. Vol.6. N. 2. P. 252–259. https://doi.org/10.1016/j.intimp.2005.08.012

18 Nagai H., Nishiyori T., Daikoku M., Koda A.. Immunopharmacological studies of sodium copper chlorphyllin (SCC)// Jpn J Pharmacol. 1983. Vol.33. N. 4. P. 819–828. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6195383

19 Sharma D., Kumar S. S., Sainis K. B.. Antiapoptotic and immunomodulatory effects of chlorophyllin// Mol Immunol. 2007.Vol. 44. N.4). P. 347–359. https://doi.org/10.1016/j.molimm.2006.02.031

20 Yin L. M., Jiang H. F., Wang X. et al.. Effects of sodium copper chlorophyllin on mesenchymal stem cell function in aplastic anemia mice// Chin J Integr Med. 2013. Vol. 19. N. 5. P. 360–366. https://doi.org/10.1007/s11655-012-1210-z

21 Yun C. H., Son C. G., Chung D. K., Han S. H.. Chlorophyllin attenuates IFN-gamma expression in lipopolysaccharide-stimulated murine splenic mononuclear cells via suppressing IL-12 production// Int Immunopharmacol. 2005. Vol. 5. N. 13–14. P. 1926–1935. https://doi.org/10.1016/j.intimp.2005.06.014

22 Pimentel E., Cruces M. P., Zimmering S.. On the persistence of the radioprotective effect of chlorophyllin (CHL) in somatic cells of Drosophila// Mutat Res. 1999. Vol. 446. N. 2. Vol. 189–192. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10635341

23 Olvera O., Arceo C., Zimmering S.. The effect of treating Drosophila females with chlorophyllin on the yield of dominant lethals recovered from irradiated sperm// Mutat Res. 2003). Vol.534. N. 1–2. P. 201–202. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12504769

24 Tajmir-Riahi H. A., Neault J. F., Diamantoglou S.. DNA adducts with chlorophyll and chlorophyllin as antimutagenic agents: synthesis, stability, and structural features// Methods Mol Biol.2004. Vol 274. P. 159–171. https://doi.org/10.1385/1-59259-799-8:159

25 Pimentel E., Cruces M. P., Zimmering S.. Evidence that chlorophyllin (CHL) may behave as an inhibitor or a promoter of radiation-induced genetic damage in somatic cells of drosophila// Mutat Res. 2000. Vol. 472, N. 1–2. P. 71–74. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11113699

26 Krikun B. L., Chernomorskii S. A., Gurinovich L. K.. Study of the antiviral properties of chlorophyllin sodium preparations. Bulletin of Experimental Biology and Medicine. 1969. Vol. 68. N. 5. P. 1271–1273.

27 Azizullah A., Rehman Z. U., Ali I., et al. Chlorophyll derivatives can be an efficient weapon in the fight against dengue//Parasitol Res. 2014. Vol.113. N. 12. P. 4321–4326. https://doi.org/10.1007/s00436-014-4175-3

28 https://www.mysciencework.com/publication/show/d9bd0ae1a11b10e498ff05752e689953

29 Ito A., Tsuneki A., Yoshida Y., et al. In Vitro Inhibition of Cytopathic Effect of Influenza Virus and Human Immunodeficiency Virus by Bamboo Leaf Extract Solution and Sodium Copper Chlorophyllin// Yonago acta medica. 2016. Vol. 59. N. 1. P. 61.

30 Guo H., Pan X., Mao R., et al. Alkylated porphyrins have broad antiviral activity against hepadnaviruses,

flaviviruses, filoviruses, and arenaviruses// Antimicrobial agents and chemotherapy. 2011.Vol. 55. N.2.P. 478-486.
31 Botelho M. V. J., Orlandi J. M., Melo F. L., et al. Chlorophyllin protects HEp-2 cells from nuclear fragmentation induced by poliovirus// Letters in applied microbiology. 2004. Vol.39. N.2.P.174-177.
32 Mowbray S. The antibacterial activity of chlorophyll// Br Med J. 1957. Vol.1. N. 5013P. 268–270.
33 Rajalakshmi K., Banu N. Antimicrobial activity of natural chlorophyllin from endangered medicinal plant Mimosa pudica l// Int J Farm & Farm Sci. 2016. Vol.8, N.4.
34 Maekawa L.E., Lamping R., Marcacci D. et al. Chlorophyll-solution presents effective antimicrobial activity on C. *albicans* but did not present any activity on *E. faecalis*// RSBO. 2007. Vol.4. P.37-40.
35 Park S. J., Park Y. M. Eco-dyeing and antimicrobial properties of chlorophyllin copper complex extracted from Sasa veitchii //Fibers and Polymers. 2010. Т. 11. №. 3. С. 357-362
36 Miller J. M., Jackson D. A., Collier C. S. The inhibition of the coagulase of the Staphylococcus aureus by the water-soluble derivatives of sodium-copper chlorophyllin// Am J Surg. 1959. Vol.97. N. 3. P. 321–323. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/13627357
37 Daly S., Heller G., Schneider E. Effect of Chlorophyll Derivatives and Related Compounds on the Growth of M. tuberculosis// Experimental Biology and Medicine. 1939. Vol. 42. N. 1. P. 74-78
38 Krasovskiy V. V., Nadtoka V. L., Pokhil S. I. Listeria bacterial carriage: a method for sanative treatment// Zhurnal mikrobiologii, epidemiologii, i immunobiologii. 1999. Vol .3. P. 18-20.
39 Vlad M., Bordas E., Caseanu E., et al. Effect of cuprofilin on experimental atherosclerosis// Biological trace element research. 1995. Vol. 48. N. 1. P. 99.
40 Kapiotis S., Hermann M., Exner M., et al. Copper-and magnesium protoporphyrin complexes inhibit oxidative modification of LDL induced by hemin, transition metal ions and tyrosyl radicals. Free radical research. 2005. Vol. 39. N. 11. P. 1193-1202.
41 Mohamed H. R. H. Attenuation of nano-TiO2 induced genotoxicity, mutagenicity and apoptosis by chlorophyllin in mice cardiac cells// Int J Sci Res. 2014. Vol.3. N.6. P. 2625-2636.