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Review article

Chelation therapy for Alzheimer's disease: Nanoparticles as new components of this therapy

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Abstract

***Objective*:** Modern treatment of Alzheimer's disease (AD) with acetylcholinesterase (AChE) inhibitors and N-methyl-D-aspartate (NMDA) receptor antagonists, due to only a slightly indicated moderate alleviation of symptoms, as well as the impossibility of the stagnation and a more significant remission of the disease, or complete recovery, is in constant search for new medications that would be more effective. Considering that we are becoming increasingly aware of the pathophysiology of this disease, with emphasis on metal-induced oxidative stress, the accumulation of transitional metals (especially iron), oxidative compounds, free radicals, as well as AGE compounds, the research studies are increasingly directed to the role of the so-called chelators, *i.e.* the catchers and cleaners of metal ions. The validity of the opinion about the crucial role of transitional metals in the induction of oxidative stress and the consequential neurodegenerative damages has been confirmed by a large concentration of iron, zinc, copper and aluminium in the pathologically affected areas of the brain (parietal and frontal cortex, hippocampus, amygdala) and by the close relation between the concentration levels and clinical features of the disease progression.

***Methods*:** With a comprehensive study of the available literature related to AD therapy, relevant data linked to so-called chelation therapy has been thoroughly analysed, particularly the interesting bonding between chelators and nanoparticles that enables a better penetration of chelators into the brain and the elimination of the formed complexes by exiting from the brain through otherwise hardly permeable blood brain barrier (BBB). Thus, this study has in this way not only contributed to the insight into modern AD therapy, but has also greatly contributed to the knowledge of the complex pathology and pathophysiology of this disease.

***Results*:** Results of the analysis of available studies undoubtedly indicate the crucial role of metal-induced oxidative stress in AD pathophysiology, with a significant role of iron ions. Drawn by electrostatic forces and bound to the β1 chain of the apical amyloid beta (Aβ) monomer that forms the protofilament, the non-toxic ferric iron (Fe^{3+}) is reduced to the toxic form of ferrous iron (Fe^{2+}), which is oxidized in the Fenton reaction through H_2O_2, forming the extremely toxic hydroxyl radical (*OH) with consequent severe oxidative damage in the surrounding molecular structures. The results of the analysis suggest the beneficial therapeutic effect of metal chelators that are specially bound to the nanoparticles system.

***Discussion*:** The discussion elaborates the detailed analysis of the comparison of the obtained results with the data from a number of studies that focus on the same problem area. It is noticed that the author's considerations closely coincide with the opinions of renowned experts. This particularly refers to the crucial role of metal ions, especially iron, in AD pathophysiology, as well as the importance of iron chelator therapy combined with nanoparticles.

***Conclusion*:** AD is a severe, chronic, neurodegenerative disease with a fluctuating progressive course, and with a lethal end. The current therapy with AChE inhibitors and NMDA receptor inhibitors does not show satisfying results. The increasingly frequent therapy with metal chelators, especially iron chelators, combined with nanoparticles that are particles built from natural or artificial polymers capable of easily passing through the BBB, has given promising results and requires additional and more intensive research.

***KEY WORDS*:** Alzheimer's disease, oxidative stress, chelators, nanoparticles, chelation therapy

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Introduction

Following the enormous rise of the worldwide elderly population (*e.g.* senectual explosion – relative and absolute rise of the elderly portio, 65 years and more, in the general population), there is a concomitant rise in the incidence and prevalence of chronic degenerative diseases, among them especially neurodegenerative, with a marked position of Alzheimer's disease (AD). AD is a chronic, progressive and lethal disease, still mostly of unknown polygenetic etiology. It is closely connected with the aging process and old age. Due to the typical progressive loss of memory, thought and speech ability, it presents a very serious disease which enormously burdens the patient's family, as well as the complete society. The costs for its prevention, and nowadays not a very successful treatment, have an exponential trend [1-4].

The yet not adequately explained etiology, and a number of recent investigations lead to the evident fact that oxidative stress is the crucial link in AD pathophysiology. This complex event, according to a number of investigations, is connected with metal metabolism disregulation, especially with the rise of intracerebral concentration of transition metals, among them especially iron (Fe) and copper (Cu). Their ions, particularly the nontoxic, redox-inactive ferric ion (Fe^{3+}), whose concentration in the pathology affected brain regions is several times higher than in the nonaffected regions, have a great affinity toward the beta amyloid (Aβ), an important AD marker. Fe^{3+} is by Aβ reduced into the toxic redox-active Fe^{2+}, which enters in the Fenton reaction, and through the oxidation by H_2O_2 (hydrogen peroxide) generates the extremely toxic and destructive hydroxyl radical (*OH). This radical induces the wasting oxidative deteriorations of neuronal lipid membranes (lipid peroxidation), of protein structures and of DNA molecules. The Fe^{3+} brain interstitial concentration (ISF, interstitial fluid) and its disorders are now under intensive investigations. Actually, there are a number of unsolved questions as to this problem. According to some opinions, it seems that an inverted transferin (Tf) route is possible, which during this event attracts Fe^{3+} from the subarachnoid space (SAS) in the superior sagital sinus venous blood flow (passage across the arachnoid granulations). This inverted route is in AD probably damaged by Aβ sedimentation. Additionally, in AD there is also a decline in Tf (oligodendrocytes) and TfR1 (Transferin receptor protein 1 - vascular endothelium of brain capillaries) production. On the other hand, it seems that Tf generated in the brain cannot pass through the arachnoid layer. It is also possible that in AD there exists an actual defect of Fe^{3+} efflux by perivascular lymphatic routes. All these events certainly require further investigations [1, 3, 5-12].

AD, as a serious disease, is the target of a number of therapeutic attempts. However, with regard to the poor results of all former procedures, among them the actual therapy by acetylcholinesterase (AChE) inhibitors (AChEI), Aricept (donezepil hydrochloride), Exelon (rivastigmine), and Reminyl (galantamine hydrochloride), as well as N-methyl-D-aspartate (NMDA) receptor antagonists (Ebixa, memantine), there is an increased importance of chelation therapy, based on the blockade of the transition metals effect on oxidative stress. Along with the isolated chelators treatment, the combination of chelators and the nanoparticle system is becoming more actualized.

Nanoparticles are small particles, composed of natural or artificial polymers which, conjugated with chelators, can easily pass across the blood brain barrier (BBB), and after the capture of metals they can, without any problem, bring the formed complex out from the brain. Chelation therapy, combined with the nanoparticle system, today is increasingly under intensive investigations and is adequately applied in AD therapy, as well as in other neurodegenerative and even several malignant diseases. The results are very good, and indicate a promising perspective of this therapy [1, 3, 7, 13, 14].

By the method which includes the analysis of accessible literature involved in AD therapy, this study in detail elaborates a variety of relevant data about the mentioned therapy, and especially the interesting connection between chelators and nanoparticles. It is evident that this connection enables a much better influx of chelators into the brain, and also a better elimination of generated complexes (metal-chelator-nanoparticle) out from the brain, across the otherwise hardly permeable BBB. In this way the study contributes, not only to the insight into actual AD therapy, but also gives a great contribution to the knowledge and understanding of the complex pathology and pathophysiology of this disease (***Fig. 1***).

The results of the available reviewed studies undoubtedly indicate the crucial role of oxidative stress, induced by metals, in AD pathophysiology. The role of iron ions is especially emphasized. By electrostatic forces attracted and bound to the β1 chain of the appical Aβ monomer of the forming protofilament (Aβ monomers fibrillization), nontoxic ferric ion (Fe^{3+}) is reduced into toxic ferrous ion (Fe^{2+}), which in the Fenton reaction ($Fe^{2+} + H_2O_2 = Fe^{3+} + {}^{-}OH + {}^{*}OH$) is oxidized by hydrogen peroxide (H_2O_2), with the formation of the extremely destructive and toxic hydroxyl radical (*OH). The final results are the wasting oxidative deteriorations of the surrounding molecular structures. The results of the analysis indicate the favourable therapeutic effect of metal chelators, especially conjugated with nanoparticles.

The following two tables (***Tables 1, 2***) show important desirable and undesirable effects of actually utilized chelators, especially iron chelators. ***Table 3*** shows the effects of metal chelation therapy through the use of nanoparticles.

Fig. 1 shows the schematic presentation of the nanoparticle-chelator conjugate passage, enabled by means of endocytosis, across the BBB.

It is clearly visible that ***Fig. 1*** is divided into three parts, A, B, and C. In the upper part (A) on the left side visible is the isolated nanoparticle (green circle) with designated functional groups necessary for the conjugation with the chelator (black points). Nanoparticle surfaces can be coated with Polysorbate 80. It is a nonionic surfactant and emulsifier often used in foods and cosmetics, and is a water soluble synthetic compound. Nanoparticles coated with this compound (Tween 80, T-Ω80) can be very easily delivered to the brain. The surfactant (nanoparticle stabilizer) adsorbs on the surface of the nanoparticles and provides steric stabilization to them. During the brain targeting, the interaction between T-80 coating and brain micro-vessel endothelial cells is essential. APOE is a fat binding protein, very important for the formation of lipoproteins. It has a crucial role in the onset and course of EOAD, it accelerates the delivery of the full complex chelator-nanoparticle across the BBB. Transport across the BBB is enabled by the low-density lipoprotein (LDL) receptor and the complete complex of endocytosis. On the left lower part (B), the full complex partially enclosed in the endosome is visible. Additionally, nanoparticles have a very thin surrounding interfacial layer. This layer is essential for thermal conductivity. Recently, nuclear magnetic resonance (NMR) experiments indicate that the thickness

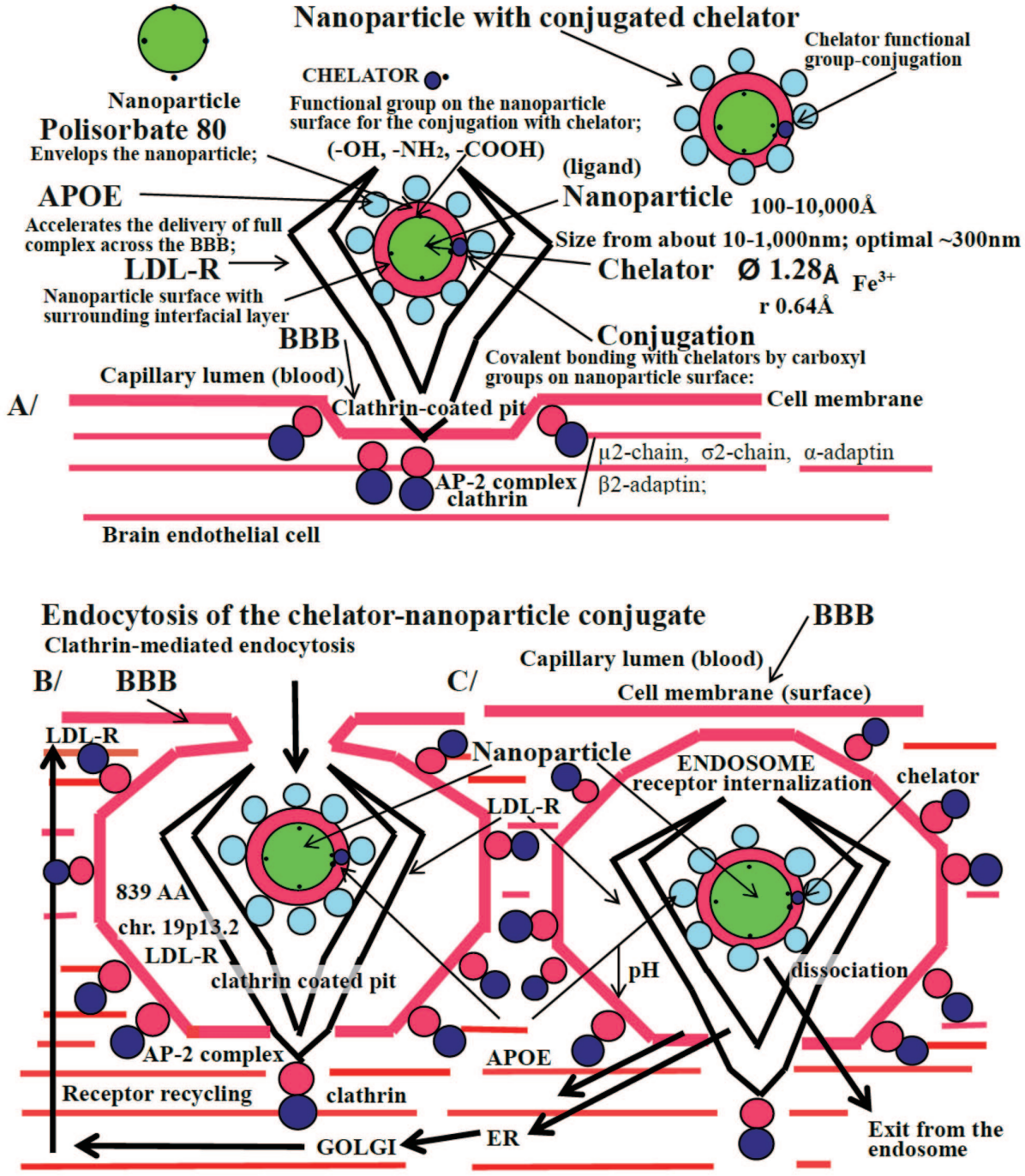

Fig. 1. **Schematic presentation of nanoparticle-chelator conjugate entry across the BBB: transcellular pathway.**

AD, Alzheimer's disease; BBB, blood brain barrier; APOE gene, protein coding gene, important for making the protein apolipoprotein E; ApoE, Apolipoprotein E, a class of proteins important in cardiovascular and AD; clathrin; AP-2 complex; endocytosis; LDL-R, low-density lipoprotein-receptor.

Table 1. Desirable effects of iron chelators.

High affinity for iron; **Some benefits in AD therapy;** **(L1, Deferiprone), high oral activity, marked lipophilicity;** **High BBB penetration ability;**
Curcumin: ability for binding toxic metals and formation of tight and inactive complexes; anti-inflammatory property; strong radical scavenging efficacy; strong neuroprotective role; strong protection against oxidative stress; it leads to various improvements in the AD pathology; it can clear amyloid plaques; it easily passes across the BBB; relatively safe; biologically active polyphenolic compound;
clioquinol (iodochlorhydroxyquin) copper and zinc specific chelator; BBB penetrable; dissolution of β-amyloid plaques; delay of cognitive decline in treated AD patients; it significantly decreases iron ion - induced aggregation of Aβ42;
(DFO), desferrioxamine; (EDTA), ethylenediaminetetraacetic acid; (clioquinol), iodochlorhydroxyquin; clinical improvement in AD patients;
(DFO) prevents formation of β-pleated sheets of Aβ42 - in the presence of Al (III) and Fe(III); EDTA prevents this formation only in the presence of Zn(II) and Cu(II);
metal chelation can prevent Aβ aggregation; it can also safely dissolve precipitated Aβ peptides;
inhibition of amyloid fibrillization and disintegration of matured amyloid fibrils; decreasing of amyloidogenic cytoxicity; relatively easy passage across the BBB (metal-chelator-nanoparticle);

Presentation of the desirable effects of iron chelators. The crucial points are high affinity for iron and high BBB penetration ability. The table also points out the decrease of amyloid aggregation and inhibition of amyloid fibrillization and disintegration of matured amyloid fibrils. There are some signs of improved status in AD patients.

Table 2. Undesirable effects of iron chelators.

Accompanying undesirable chelation of metals in various tissues leading to serious side effects;
They have a host of adverse effects and substantial obstacles; (DFO), neurotoxicity, poor absorption in the gastrointestinal tract; (DFO), quick degradation after administration, hydrophilic nature; (DFO) induces often weak penetration across the BBB;
(L1, Deferiprone), strong hydrophilicity of the L1-iron complex induces difficulty in removing the complex across the BBB;
clioquinol (iodochlorhydroxyquin), copper and zinc specific chelator; association with subacute myelo-optic neuropathy; the second generation of this chelator is under clinical investigations;

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Table 3. Metal chelation therapy through the use of nanoparticles.

Nanoparticles are composed of natural or artificial polymers, they range in size from 10 – 1,000 nm, optimally around or less than 300 nm; particles coated with surfactants *e.g.* polysorbate 80 have the optimal ability to cross the BBB;
A number of studies indicate the endocytosis of polysorbate coated nanoparticles as an optimal way for transfer across the BBB; In this case very important is the absorption of apolipoprotein E (ApoE) on the surface of nanoparticles coated with polysorbate 80; LDL-receptor mediated endocytosis;
Chelator (chelating agent) is a compound which can form several bonds to a single metal ion (*e.g.* DFO, Deferiprone or L1, Deferasirox, Curcumin etc.);
Chelators conjugated to nanoparticles have the ability to cross the BBB;
This complex by the same route can relatively easily exit from the brain;
A chelate is a chemical compound composed of a metal ion and a chelator (chelating agent);
Nanoparticles have the potential to transfer chelators in and out of the brain;
Chelation therapy is a safer and more effective method of reducing the metal load in the brain;
Evident attenuation of harmful effects of oxidative damage (oxidative stress);
Reduced drug toxicity;
Improved biodistribution;
Improved therapeutic efficacy;
Evident good effects in AD therapy;
Nanoparticles also have a thin surrounding interfacial layer;

Clearly evident desirable effects are achieved by the combination of chelators and nanoparticles. It is important to emphasize some points: obvious attenuation of harmful effects of oxidative damage, reduced drug toxicity, improved biodistribution, improved therapeutic efficacy, and evident good effects in the course of therapy.

of the ordered layer is around 1.4 nm (14Å). It is important to emphasize that the clathrin-mediated endocytosis is the essential internalization pathway for the nanoparticle-chelator conjugate. Clathrin is a protein and has a fundamental role in the formation of coated vesicles. AP2-adaptor complex is crucial in the process of clathrin mediated endocytosis. It is composed of four components, α-adaptin, β2-adaptin, μ2-chain, and σ2-chain. The right lower part ofof ***Fig.1*** presents the fully developed endosome, which completely encircles the nanoparticle-LDL complex.

Liu G *et al.* [1)] in their study present the mechanism by which nanoparticles deliver chelators across the BBB. They emphasize the great importance of clathrin-mediated endocytosis and apolipoproteins E and B (ApoE, ApoB), and especially of the nanoparticle mimicry with LDL. ***Fig.1*** exactly explains the first phase of events. The second phase, the removal of the formed complex, the nanoparticle-chelator-metal, out from the brain, can be explained by the analogous carrier-mediated transport system, but with the absorbed Apo-A1. LDL receptors have been found on both luminal and abluminal sides of the BBB endothelial cells.

By NMR technology measurement of silica (SiO_2, silicon diokside) nanoparticle (203 nm Ø) surface functional groups, Yuan L *et al.*[15)] have determined that on a surface area of 1 nm2 there are 4 hydroxyl (-OH), 3,7 amine (-NH_2), and 2,3 carboxyl (-COOH) groups. They emphasise the crucial role of these groups for binding with chelator adequate structures.

Fig.2 shows the chemical structure of some chelators tested in AD.

Discussion

The etiology of AD

In a number of studies about the etiology of AD, it has been evidently established that the unusual aggregation of Aβ peptide monomers into highly ordered-β-sheet structures, and consequent amyloid fibrils, are the crucial cause in these events [16, 17)]. Without entering the detailed polygenetic etiology of this disease, it is necessary to emphasize the existence of its two essential forms: the early form (EOAD, early onset AD, 5% of all cases) which develops before the age of 65, and the late form (LOAD, late onset AD, 95% of all cases) which develops after the age of 65. They both provide practically the same clinical picture [4, 18)].

The mutations of the gene complexes: *APP* (β-amyloid precursor protein gene, 21q21.3); *PSEN1* (presenilin-1 protein coding gene, 14q24.2); *PSEN2* (presenilin-2 protein coding gene, 1q42.13), and *BACE1* (βsecretase-1 protein coding gene, 11q23.3) lead to EOAD, and mutations of *APOE4* (apolipoprotein E coding gene, 19q13.32) and *ADAM10* (ADAM metallopeptidase Domain 10 protein coding gene-regulator of α-secretase activity-gene, 15q21.3) lead to LOAD [4, 19)].

In general, quite inefficient results of the recent standard AD therapy (AChE inhibitors: Aricept, donepezil hydrochloride; Exelon, rivastigmine; Reminyl, galantamine hydrochloride; and NMDA receptor antagonists, Ebixa, memantine) have implemented the necessity of searching for new medicaments, and then relatively successful effects of metal chelators were shown. Unfortunately, a number of their undesirable and side effects interlope the search for more successful solutions, and these were obtained by the use of the combination of metal chelators with nanoparticles. The composite of a chelator with a nanoparticle induces a relatively easy passage of this complex across the BBB, the binding of the metal ion, and a very easy efflux of the full complex, chelator-nanoparticle-metal, out of the brain [1, 3, 7, 13, 14, 19, 20)].

What are metal chelators and what is the essence of their activity? Iron chelator, the chemical compound, by natural or artificial origin, has a strong affinity for iron ions, and also shows a certain affinity for other metals, which can lead to undesirable effects. By binding with free iron ions, primarily with Fe^{3+}, and by their drawing out from the formed amyloid fibrils, the chelator reduces their concentration in the neuropil, with concomitant reduction decline of the Fe^{3+} in Fe^{2+} and the generation of harmful hydroxyl radical *OH. Bandaged by the covalent bond with the chelator, metal ions can be, through the BBB, thrown out of the brain. Which characteristics must a good chelator have? According to Budimir A [2)], a good chelator must have small molecular weight, small electrical charge, selective affinity for specific ions, good bonding ability with metals, good ability for metal bonding in protein aggregates with their dissolution, and finally small toxicity and minimal side effects. Again, it is important to emphasize, which is often confusing, that the chelate is a conjugate of a metal and a chelator (***Tables 1, 2***).

Metal-chelator-nanoparticles

What are nanoparticles? In the introduction it is mentioned that they are small particles formed from natural or artificial polymers (a large molecule made up of many repeated subunits), which bound with chelators can easily pass through the BBB and adequately bind (chelation) metal ions with consequent efflux of the formed complex (metal-chelator-nanoparticle) also through the BBB out from the brain. These nanoparticles are produced by various companies. Dimensions of these particles fluctuate between 10-1,000 nm (100-10,000Å - the optimal value is around 300 nm [3,000 Å]). If a thin coat, as with polysorbate 80 (nonionic surfactant and emulsifier often used in foods and consmetics-synthetic compound) is added, this enables nanoparticles to covalently bind with a chelator, and later also with a metal, and an easy penetration through the BBB [1)]. Nanoparticles have a surrounding interfacial layer with functional groups responsible for binding with the chelator (carboxylic groups, amine groups). Generally, the thickness of the ordered layer is approximately around 1-1.4 nm (10-14 Å). It is an integral part of the nanoparticle, fundamentally affecting its properties. It consists of ions and inorganic and organic molecules. Organic molecules are nanoparticle stabilizers and protectors. Nanoparticles are the bridge between bulk materials and atomic or molecular structures. In relation to the bulk material, they have different and usually unexpected properties. On the other hand, chelators also have functional groups for binding with nanoparticles. The interfacial layer has nanoscale pores. Through this layer there is a sustained ionic flux, so it is also important for thermal conductivity (***Fig. 1***).

Today, there are many methods for nanoparticle production, but their description surpasses the theme of the presented study. The reader interested in this problem can find adequate materials in other literature or in the producer's information.

Natural chelators

Without entering the detailed description of a number of chelators, this study is limited only to the effects of their three representatives, green tea polyphenol (epigallocatechin-3-gallate, EGCG), curcumin, and desferrioxamine B (DFOB) (***Fig. 2-6***).

Debnath K *et al.* [14] elaborate in their study how the molecular EGCG binds with β amyloid and during this process induces the inversion of fibrillation kinetics, the remodelling of formerly formed fibrils, and the decrease of amyloid induced toxicity. Due to the weak chemical stability and substandard bioavailability, the practical utilization of EGCG is very difficult. On the other hand, EGCG conjugated with nanoparticles (nano EGCG), is even up to 100 times more effective in amyloid inhibition, disintegration of formed plaques, conjugate stability, and in decreased amyloid toxicity. The authors also emphasize the much easier crossing through the BBB. Disintegration of formed fibrils on the molecular level happens through EGCG auto-oxidation, which then subsequently reacts with free primary amyloid amine groups accompanied with the formation of Schiff bases. The nano-EGCG complex is composed of 25-160 nm (250-1,600 Å) polyaspartic acid-based micelles, connected with EGCG by covalent bonds and noncovalent interactions.

The analysis of ***Fig. 2*** clearly shows the explanation of strong anti-oxidative EGCG effects. Abundantly present hydroxyl groups (-OH) on the three phenol rings (A, B, D), especially on B and D rings, make free radical targets, and give a hydrogen atom to the radicals, neutralize their unpaired electron, and generate neutral water (H_2O). The loss of hydrogen atom induces the generation of the new free radical on the EGCG backbone. This new radical has a tendency for binding with a new incoming hydrogen atom and for the hydroxyl group reparation. After these events, it is possible that the new oxido/reductive cycle repeats in limitless manners, with permanent free radicals destruction, especially the extremely dangerous hydroxyl radicals. Clearly visible are the double effects of EGCG: the anti-oxidative effect and binding with Aβ. The later effect was mentioned in the upper paragraph [14].

It is also possible that after the EGCG auto-oxidation, which is induced by molecular oxygen (O_2), the produced reactive quinone complex (quinones = oxidized derivatives of aromatic compounds), through the Schiff base addition, involving alanine side chain, generates a covalent complex R2C=RN'. In more detail, as a result of the EGCG auto-oxidation, one hydron (H^+), one EGCG radical (EGCG*), and one superoxide radical anion (O_2^{*-}) originate. O_2 accepts one electron (e^-) and transforms into O_2^{*-}. Hydron (it has a very short life), which is very reactive, also accepts one electron and transforms into the H atom. It is also possible that two hydrons form a H_2 molecule. The double bond (=) between the two nearest C atoms changes its position and binds to the active oxygen. The carbonyl group is formed. Consequently, the Schiff base formation and cross link with free amine group on the adjacent Aβ monomer alanine side chain is possible.

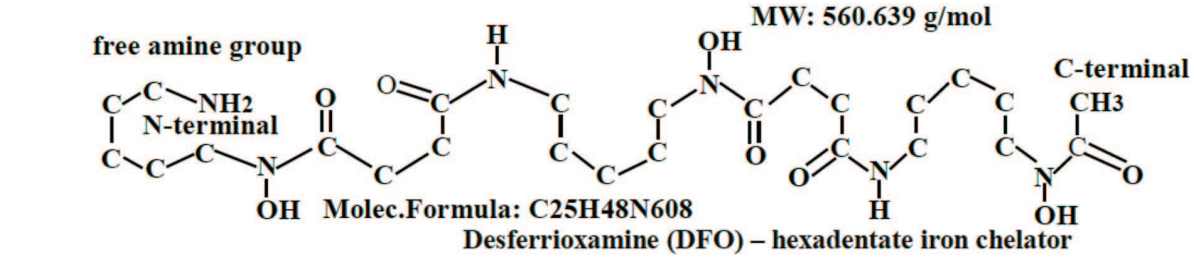
EGCG binds directly with an unfolded natural Aβ peptide and inhibits the process of peptide aggregation and fibrillization. The result is the formation of nontoxic disordered Aβ oligomers. The EGCG binding to the Aβ peptide can occur in three ways: through hydrophobic interactions, through H-bonds, and through the formation of Schiff bases. The Schiff base formation with the consequent cross-linking of fibrils, prevents the fibril dissociation and the discharge of the build-up of amyloid monomers. In this way, the occurence of the new aggregation of free monomers, their fibrillization, and the generation of additional amounts of harmful hydroxyl radicals is prevented. It is important to emphasize once again that free Aβ-monomers have a strong intrinsic tendency to aggregate.

Additionally, it is evident that EGCG remodels mature amyloid fibrils and converts them to amorphous protein aggregates, less toxic to surrounding structures. It seems that during this remodelling, the accompanying Fe^{3+} chelation by EGCG has a very important role in this process. Also, investigations demonstrate that the hydrophobic binding sites in the amyloid fibril are important for EGCG binding and the mentioned remodelling. EGCG amyloid fibril remodelling is dependent on its auto-oxidation. Oxidized EGCG molecules react with amyloid fibril free amine groups by the formation of Schiff bases and by the cross linking of these fibrils [21-23].

The EGCG binding place with nanoparticles can be attributed to the hydrogen bond interactions between N atoms on the nanoparticle surface and H atoms in hydroxyl groups of EGCG (C-N.....HO-C) (***Fig. 2***).

EGCG is a polyphenol component obtained from the leaves of the plant *Camellia sinensis*. Other components are some polyphenols (epicatechin gallate and epicatechin) and flavanols, three kinds of flavonoids (kaempherol, quercetin, and the highest quantity of Myricetin). Extracts from the Camellia sinensis leaves were for a long time used in traditional Indian and Chinese medicine in the treatment of AD (***Fig. 2***).

In their study, Hedge ML *et al.* [7] present a detailed analysis of the curcumin natural compound, which, as it is observed, can signifficantly diminish the intracerebral iron concentration and slow down the course of AD. It is found that curcumin is the dominant component in the old indian spice tumeric, together with the two curcuminoids, demethoxycurcumin and bisdemethoxycurcumin. Recent investigations of this compound indicate its ability to strongly bind metal ions, at the same time forming compact inactive complexes. The strong anti-inflammatory efficacy of curcumin is also observed. The analysis of the curcumin molecular structure indicates that the enol and phenol hydroxyls have the ability to form metal complexes. The formation ofthese complexes with Cu and Fe, accompanied with the marked cleaning of free radicals shows curcumin's strong neuroprotective ability. Its significant decrease of metal toxicity is evident, as well as a high anti-oxidative efficacy. All facts indicate that curcumin can be effective in AD therapy; it is also valued for the mentioned EGCG. The authors of the study emphasize the yet unknown etiology of AD, and the evident role of metal ions (Cu^{2+}, Fe^{3+}, Zn, and Al) and oxidative stress, as crucial factors in the AD pathophysiology. They stress again the importance of the brain accumulation of these ions in a number of neurodegenerative diseases, especially in AD.



N-[5-[[4-[5-[acetyl(hydroxy)amino]pentylamino]-4-oxobutanoyl]-hydroxyamino]pentyl]-N'-(5-aminopentyl)-N'-hydroxybutanediamide; great molecular weight; strong hydrophyllicity;

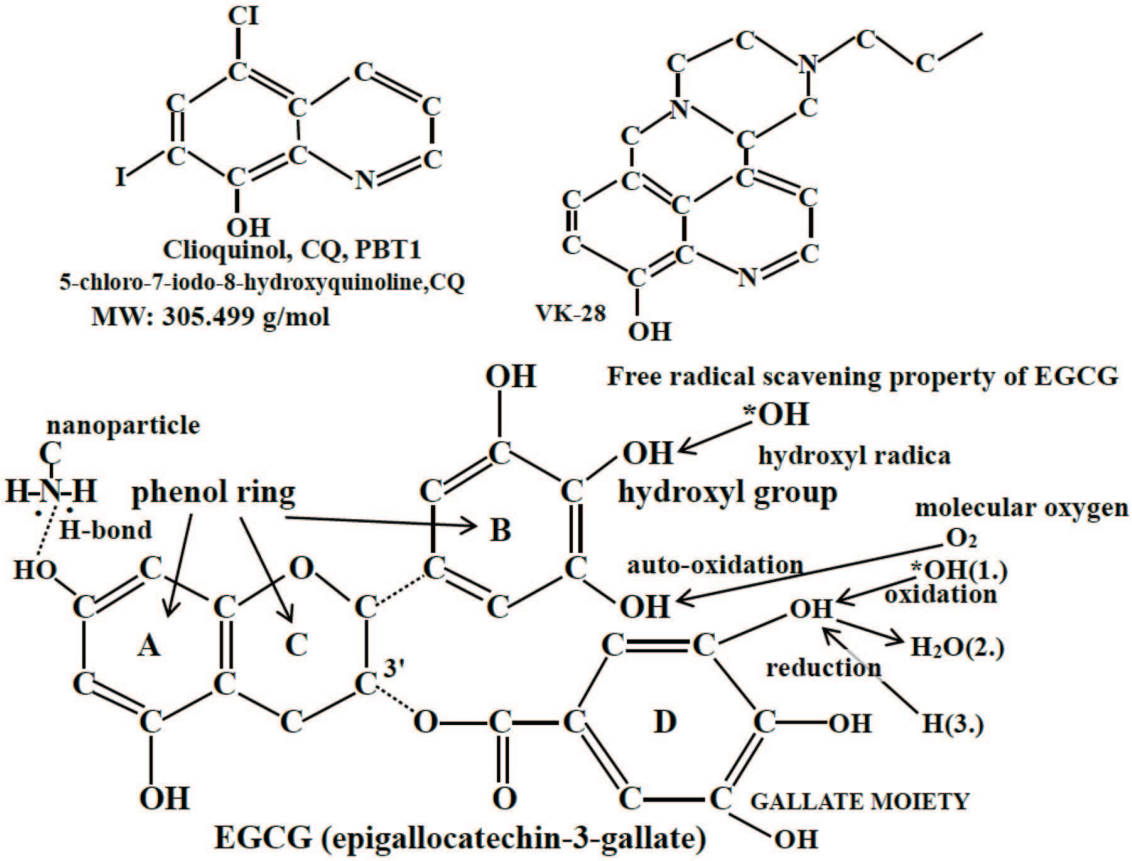


Fig. 2. **Chemical structure of some chelators tested in AD.**

Presentation of the chemical structure of some chelators tested in AD. Clearly visible is the structure of desferrioxamine (DFO), clioquinol (CQ, PBT1), VK-28, EGCG; some facts about DFO and clioquinol are presented in **Table 1-3**. AD, Alzheimer's disease; MW, molecular weight.

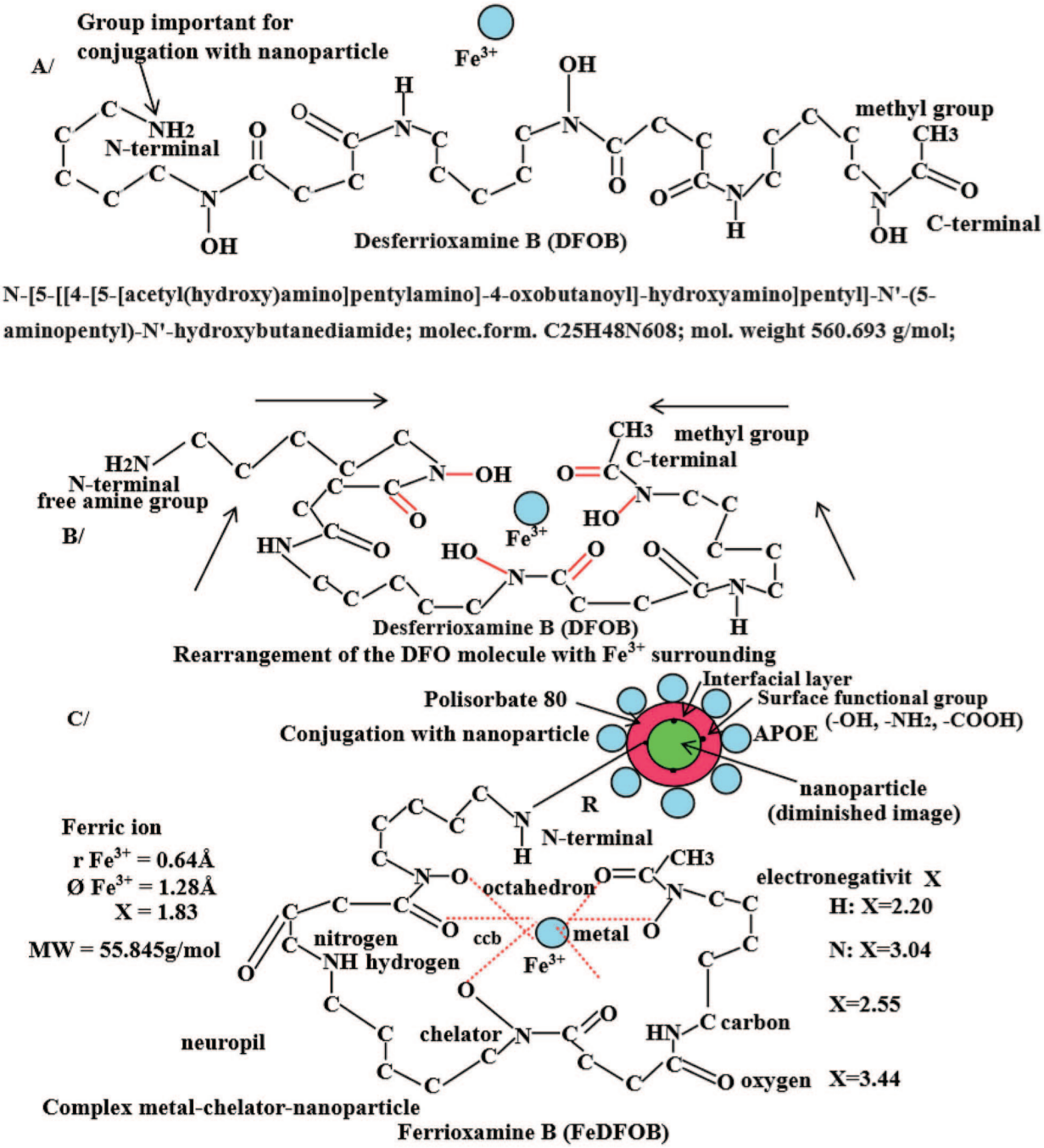


Fig. 3. **Chemical structures of (A, B) desferrioxamine B (DFOB) and (C) ferrioxamine B: Formation of hexadentate octahedral complex.**

r, radius; Ø, diameter; metal; chelator, nanoparticles; ccb, coordinate covalent bond.

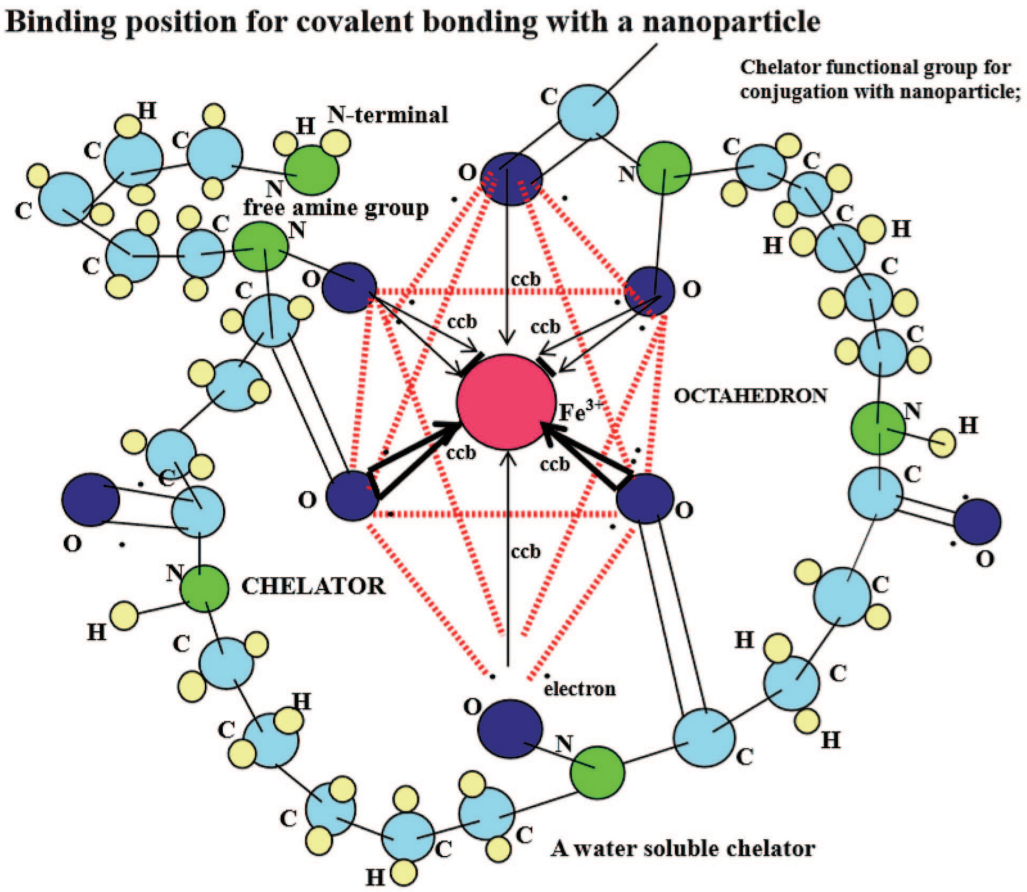


Fig. 4. **Schematic presentation of ferrioxamine molecular structure.**

A metal ion is visible in the center of the octahedron; six oxygen atoms comprise a square. O, oxygen; N, nitrogen, H, hydrogen; C, carbon; small dots, electrons; ferrioxamine is desferrioxamine with conjugated Fe^{3+} ion; ccb, coordinate covalent bond.

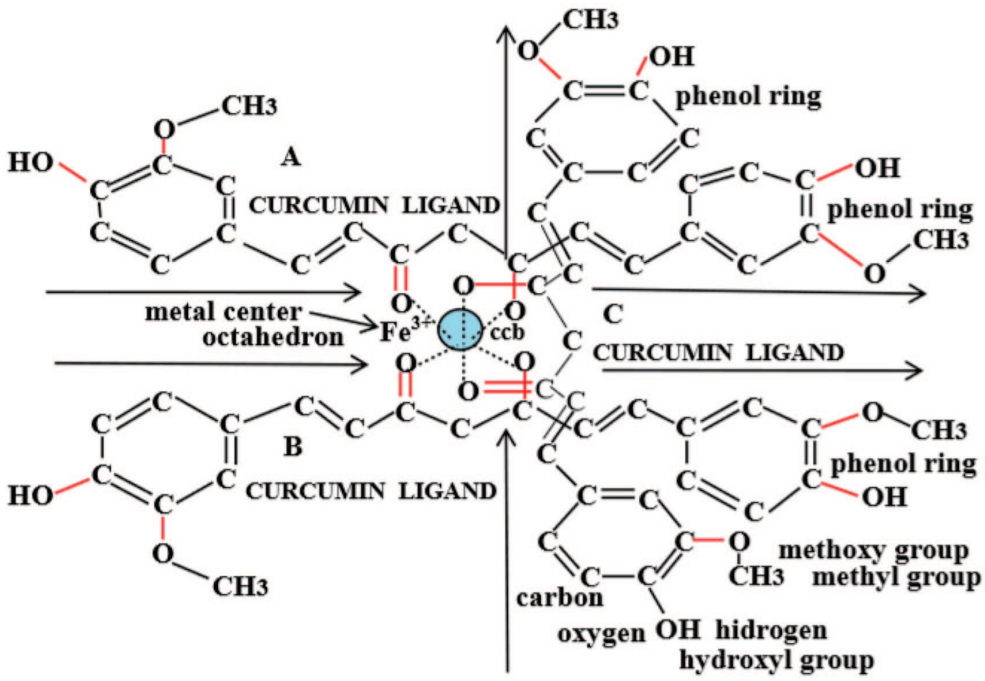


Fig. 5. **A, B, C coordination of the three curcumin ligands to the Ferric ion (Fe^{3+}).**

Curcumin (polyphenol compound (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5 -dione); It can cross the blood-brain-barrier (BBB), strong anti-inflammatory and anti-oxidative effects, can dissolve Amyloid plaques in AD, beneficial effects on the course of AD, chelation effects binds effectively with redox-active metals like Fe and Cu, formation of curcumin-metal complexes, strong neuroprotector. Curcumin is found in turmeric, the spice that comes from the root Curcuma longa, a member of the Ginger family, Zingiberaceae. The curcumin bidentate fashion and octahedral geometry is visible; ccb, coordinated covalent bond; AD, Arzheimer's disease.

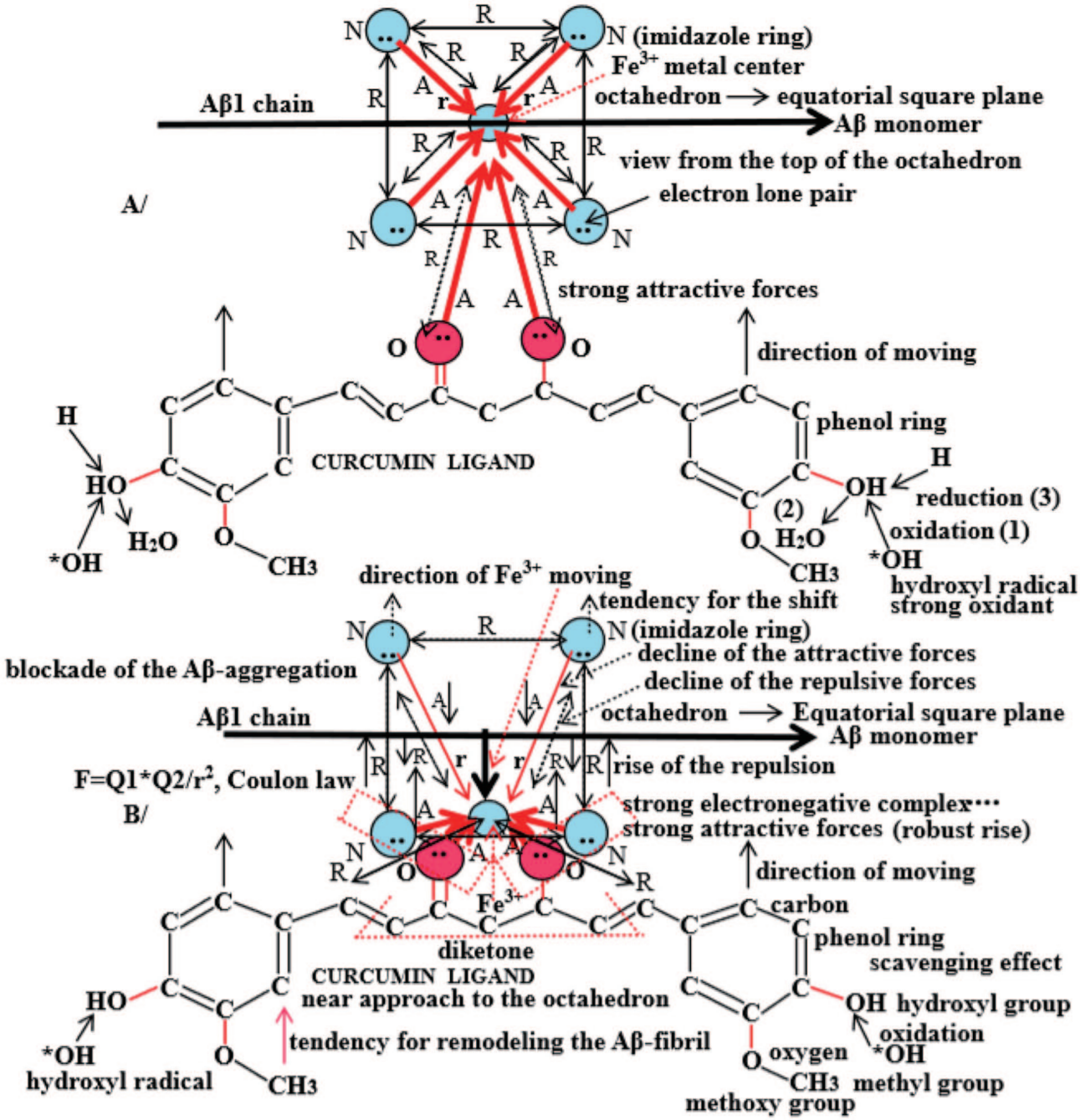


Fig. 6. **Hypothetical schematic presentation of curcumin approach to the amyloid beta (Aβ) protofilament.**

The perpendicular view is visible from the protofilament longitudinal axis; Z-axis is not presented; electrons; curcumin is a polyphenol compound; R, repulsion; A, attraction; N, nitrogen; O, oxygen; C, carbon; H, hydrogen; Fe^{3+}, ferric ion; F, electrostatic force; Q, charge; r, distance; (1), hydroxyl group oxidation; (2), Hydrogen attraction-water generation; (3), new H approach;

β-Amyloid accumulation

They also accentuate the connection of AD with β-amyloid accumulation.The role of Al (aluminium) is still quite unknown. The mentioned researchers are especially included in the problem of the two natural metal chelators, curcumin and epigallocatechin, which, as it is confirmed, decrease the level of Fe in the brain and suppress the course of AD.

Bonda DJ *et al.* [3] also consider that the current AD therapy with AchE inhibitors is not satisfactory. This therapy can only weakly mitigate AD symptoms, with the delay of the inevitable decline of important cognitive functions. They also emphasize the significant rise of costs connected with its prevention and therapy. According to them, all this indicates a strong necessity for a new approach to AD therapy, which accentuates chelation therapy. Oxidative stress, induced by a number of undiserable events, is a crucial factor in the AD etiology. Oxidative damage of mitochondrial DNA, important proteins and phospholipids, are significantly greater than in healthy people. Due to the significance of transition metals in the induction and strength of oxidative stress, the importance of metal chelators and chelation therapy is obvious. Among these compounds, the authors especially emphasize desferrioxamine (DFO), the hexadentate iron chelator, which strongly binds Fe^{3+} and moderately Al, Zn, and Cu. Clinical trials indicate that DFO slows down the rate of AD progression. However, the great molecular weight and hydrophylicity, which is typical for hexadentate chelators, disrupts the passage across the BBB. The authors consider that smaller metal chelators can pass the BBB much more easily, but present signs of toxicity. The solution to this problem is found in metal chelators and in their conjugations with nanoparticles. The earlier mentioned nanoparticles are in fact polymer particles, of magnitude 10-1,000 nm (100 -10,000Å), optimally around 300 nm (3,000Å). They are made from natural or artificial polymers. With the ability to strongly bind with chelators, they as conjugates can easily pass across the BBB into the brain, and then out of it. This crossing through the BBB is enabled by endocytosis (***Fig. 1***). In this way, hydrophylic chelators can also pass across the BBB. Conjugates are significantly less toxic and side effects are rare and weak. They also successfully prevent Aβ-aggregation, which, as is more and more evident, is the primary cause for AD toxicity. According to these authors, chelation therapy has a promising future.

Liu G *et al.* [6] demonstrate that DFO has shown some good effects applied to AD therapy, but as a lot of other chelators, has shown many undesirable side effects and important obstacles for an undisturbed chelation therapy. Consequently, they combined DFO with nanoparticles, which evidently facilitates the treatment and improves the effects. Among the undesirable effects, it is important to mention the pain in the place of injection, postinjection febrility, hearing loss and problems with sight, diarrhoea and vomiting, pressure drop and allergic reactions. DFO has been on the market in USA since 1968.

Fig. 2 shows the chemical structure of some chelators tested. Electrostatic forces around the central ferric ion (Fe^{3+}) form strong octahedral complexes, which, conjugated with nanoparticles, relatively easily pass across the BBB. For the complex formation, DFO needs one of its own molecule (hexadentate chelator), deferasirox (DFX) needs two of its own molecules (tridentate chelator), and deferiprone (DFP) needs three of its own molecules (bidentate chelator) (***Fig. 3-5***). The formation of the octahedral metal complex is explained in detail by Barić and Cetina [24].

The exact understanding of the chelation process requires a detailed insight into the metal ion complex structure and into the electrostatic forces involved in these processes. It is evident that at the begining there is a dominance of attractive electrostatic forces developed in the electrostatic field between the positive central metal ion and corresponding negative chelator points. Repulsive forces between metal d-electrons and negative ligand charges (chelator components) are in this phase evidently small. Due to the increasing approach of the future octahedron elements, the ligand field and complex with Fe3+ in the centre acquire the form of an octahedron, energetically the most favourable structure (***Fig. 3-5***). In fact, the octahedral complex is the result of composed electrostatic interactions between the positive metal centre (here Fe^{3+}) and negative charges of ligand ion pairs. If the ligands are neutral polar molecules, the metal centre reacts with the ligands negative ends (ion-dipole interaction – attractive force). Furthermore, ligands interact with one another in an electrorepulsive manner [24].

Analysis of ***Fig. 3*** and ***Fig. 4*** clearly shows the position for binding with the nanoparticle, the NH2 group on one end of DFOB molecule. As an example, the conjugation is possible with gold nanoparticles (colloid). Data from the nanoparticle producer,"BBI Solutions", serve for the practical use of gold nanoparticles which range from 2nm to 250 nm. Nanoparticles with the diameter of 2 nm have a mass of one particle 8.08 x 10^{-20} g , and with a diameter of 200 nm, a mass of 8.08 x 10^{-14} g.

ROS involvement

The analysis of the formation of the chelate composed of the metal ion Fe^{3+} and curcumin (chelator) indicates that in fact there exists a synchronised approach and binding of three bidentate chelator units (***Fig.5***). Two units approach the central Fe^{3+} mutually parallel in the altitude of the equatorial square plane ofof the complex, and the third unit, located in the square equally distant from the first two, approaches perpendicularly towards the two units.The distance from the complex Z-axis is determined by the electrostatic equilibrium in the complex. According to recent literature, two ways of binding to Fe^{3+} are possible.

Khalil MI *et al.* [25] in their paper present the example of the bidentate coordination of curcumin ligand to the Fe^{3+} ion. According to their opinion, the three curcumin ligands are coordinated to the Fe^{3+} ion in a bidentate fashion forming the octahedral geometry. In fact, here the proton discharge from the curcumin enol form occurs (***Fig. 5***). The speculation about the possibility of the metal complex formation through the phenolic hydroxyls may be also correct [7]. FTIR (Fourier Transform Infrared Spectroscopy) studies of metal complexes by Banerjee R [26], indicate that although the metal chelation can occur through the o-methoxy phenol and the β-diketo group, in most cases the complexation of curcumin with metal ions involves the diketo group. Although the mechanism of curcumin action in the therapy of AD is yet insufficiently known, the author emphasizes its anti-oxidative, anti-inflammatory, anti-cancer, and neuroprotective characteristics. She also emphasizes the crucial role of oxidative stress and reactive oxidative species (ROS), which further induce ROS generation and detrimental neurodegenerative damage. The author also emphasizes the possibility of good effects of curcumin metal complexes on

the course of aggregation and amyloid peptide fibrillization.

The slowing down of the course of aggregation and β-amyloid fibrillization demand a separate analysis. In this sense, the author of this study (Barić N) presents some of his own reflections. Attracted by the attractive electrostatic forces (Coulomb's law) of the central metal ion (Fe^{3+}), curcumin approaches the octahedral complex ion which is formed between two neighbouring harmonized monomers. The approach occurs from the side of the Aβ β1 strand in the level of the octahedron equatorial square plane. There exists the attractive electrostatic force between the central metal ion (Fe^{3+}) and two lone pairs on the chelator (curcumin) oxygens (electrostatic field). When chelator (curcumin) oxygens have arrived (movement from position A to B) near the octahedral complex ligands on the equatorial plane-square (N atoms on the two imidazole rings), a rise in the repulsion between electrons of O and N occurs, and also a rise in the value of the negative charge (q+q) due to the two closely approached negative charges (O and N). Now we have a rise in the attractive value between the four (4) attractive forces and the central metal ion (the anterior part of the complex ion). At the same time, there is no rise in the attractive forces in the posterior part of the complex. The small movement of the attracted Fe3+ in the anterior direction induces the decline in the strength of the two posterior and Z-axial attractive forces. Now there is the possibility of the Fe^{3+} movement in the anterior direction toward the curcumin, and its binding with it. Generally, the repulsive forces are weak. This movement induces protofilament destabilisation and destruction, and Fe^{3+} bound with the chelator and nanoparticle, are additionally removed from the brain (***Fig. 6***).

Fig. 6, among other things, shows the hydroxyl radical (*OH) attack on the curcumin phenol ring hydroxyl group (1), its oxidation (hydrogen loss), water formation (2), and finally the new hydrogen atom approach - reduction (3). The oxidation/reduction process continues.

β-Amyloid aggregation

In their study, Kochi A *et al.* [27] declare that AD progression is connected with the accumulation of protein aggregates, metal ion dyshomeostasis, and oxidative stress. All these events are accompanied with metals bound with amyloid β (Aβ), which clearly indicates the connection of AD pathology with these complexes. Curcumin and its derivatives show evident anti-amyloidogenic activity. On the other hand, their weak solubility and stability in physiological conditions lead to their difficult application. The results of this group of authors indicate that specific curcumin derivates, especially Gd (gadolinium, at. Number 64) – Cur, have prevailed over these deffects with the evident decrease of the Cu (II)-triggered Aβ aggregation intensity.

Yang F *et al.* [28] also emphasize the close connection between the Fe^{3+} ions chelation process and Aβ aggregation as central events, both in the pathophysiology, and in AD therapy. By aggregation from the isolated Aβ monomer, dimers, olygomers, protofibrils and fibrils can be generated. Curcumin can also, without any contact with Aβ and with connected Fe^{3+}, bind the free Fe^{3+} and stop its binding with Aβ. The authors present the following curcumin characteristics: strong binding with Aβ, strong aggregation inhibition, anti-inflammatory and anti-oxidative effects. They also emphasize the blockade of Aβ accummulation in the brain accompanied with the disaggregation of fibrilar Aβ. The delay of cognitive defficit is evident.

Aβ aggregation as the central link in the AD pathophysiology is also quoted by a number of investigators [14, 16, 20, 24, 26]. Finder VH *et al.* [29], Etienne MA *et al.* [30], Ahmed M *et al.* [31] are also interested in this problem.

In their study, House E *et al.* [32] present an excellent example of the importance of Aβ aggregation on the course of AD, as well as the effects of metal chelators DFO and EDTA on these events. Following the course of Aβ42 fibrillization connected with the addition of Al (III), Fe(III), Zn(II), and Cu(II), during 32 weeks, the authors have observed the disolution of formed peptide aggregates in the presence of desferrioxamine (DFO) and ethylenediaminetetraacetic acid (EDTA). Either alone, or in the presence of Al(III) or Fe(III), Aβ has formed aggregates which have been dissolved with one or the other chelator. This indicates that chelation of Al(III) and Fe(III) makes a protective mechanism against the occurence of AD and its progression.

During the aggregation process, the oblique, nonparallel approach of two Aβ monomers most probably occurs, with close contact of a MBD (metal binding domain – primarily His13-His14) on one, and MetS35 (methionine sulphur 35) on the other monomer. During these events, an electron "hop" from MetS35 on the incoming monomer to the Fe^{3+} bound on the MBD of the fixed monomer is possible. The result is the reduction of Fe^{3+} into Fe^{2+}, the Fenton reaction, and generation of extremely aggressive and toxic *OH. After the establishment of the parallel congruent relation of the approaching monomers, the distance between the two redox centers becomes greater and the oxido/reduction events come to an end. Fe^{3+} remains fixed in the interspace between two congruent monomers forming the center of the octahedron. The situation is now mature for the curcumin attack on this last balanced Fe^{3+} position, with its extraction, chelation, and destruction of the formed protofilament, respectively the fibrile [24].

In their tutorial review, Wanninger S *et al.* [33] present an overview of metal complexes containing curcumin and its derivatives. In detail they elaborate on the synthesis, crucial characteristics, and application of curcumin metal complexes and curcumin derivatives as ligands. The metal complex is built from the central metal ion to which are bound one or more ligands. The authors are especially interested in the medical use of these complexes. They emphasize three spheres, which are included in the ivestigation of malignant diseases, AD, and anti-oxidative/neuroprotective activity. The study presents a detailed figurative review of the curcumin molecule and its derivatives as the components of metal complexes. The authors consider that curcumin, alone or bound with specific metals, in the future will have a growing importance in the dignostics and therapy of a number of diseases, especially AD.

Ferrari E *et al.* [34] consider that the most recent actual curcuminoids make a new perspective chapter in AD treatment, and especially emphasize their complexing ability. Their biological properties can be compared with the properties of pure curcumin, only their stability is higher. K^2T derivatives are obtained by the inclusion of the $-CH_2COOC(CH_3)_3$ group onto the central atom of the diketone group. Depending on the solvent, their diketo-ketoenol tautomerism is retained. The formation of metal complexes is analysed by NMR and ultra violet (UV)-vis spectroscopy.

Ferrari E *et al.* [35] in another study show how curcuminoids coordinate metal ions through the keto-enol

function. They also emphasize that curcumin anti-oxidative ability is mostly dependent on the phenol group presence, while the coordination of the keto-enol part does not have a major influence.

Priya RS *et al.* [36] in their study explain how the two hydroxyl groups attached to the phenol ring and β-diketo part of the molecule, by the conjugation system connected with rings, are the active centres of curcumin. Two phenol centres with their -OH groups have anti-oxidative properties, and the β-diketo part makes the metal chelating center. This part has a chelation ability for a lot of metal ions with the possibility of metal complex formation. In this way, the prevention of extremely deteriorative lipid peroxidation is possible. As a strong anti-oxidant, curcumin effectively declines the formation of amyloid plaques, which is very important in the development and course of AD. Curcumin biological activities, which are anti-inflammatory, anti-cholesteroletic, and anti-Alzheimeric, are the consequence of its anti-oxidative properties. At the same time curcumin can act as a metal chelator and as an anti-oxidant. Curcumin metal complexes also have an anti-oxidative activity and they do not decrease its biological ability.

Lee WH *et al.* [37] consider that the brain, due to its great oxygen consumption, the great concentration of polyunsaturated fatty acids, and of redox active transition metal ions, makes a system very sensitive to oxidative stress. In the situation of the evident rise of pro-oxidative ROS compounds, which are present in the case of AD, an imbalance of the sensitive equilibrium of ROS/defensive anti-oxidative compounds occurs, with the phenomenon of dangerous brain structure deterioration. These damages are accompanied with strong Aβ aggregation, more expressed by the consequent formation of ROS, and with the decline of the ACh level. Curcumin is presented as a strong anti-oxidative compound, able to cleanse brain tissue of free radicals generated by oxygen, and to stop Aβ plaque formation and activation of fibril disintegration. Curcumin also has favourable effects on Parkinson's disease (PD) by decreasing the concentration of toxic α-synuclein aggregates and ROS compounds. It is also effective in the treatment of malignant brain tumors. The authors in detail analyse the chemical structure of curcumin. By tables they present the fascinating facts of curcumin therapy in traditional medicine and in recent applications.

Elmegeed GA *et al.* [38] based on their investigations, consider that hybrid drugs which simultaneously act on more targets are much better than monofunctional drugs in the therapy of the complex AD pathophysiology. During these investigations this group has made complex compounds composed of the steroid part and the curcumin molecule. By adequate synthesis of specific compounds with the heterocyclic nucleus and the necessary curcumin component, they tried to elucidate the effects of these new AD therapeutics. Their experiments were performed on albino rat females, and they showed the clearly favourable effect of specific compounds on the animals diseased by AD. These experiments revealed elevated values of brain ACh, GSH (glutathione reduced), paraoxenase, and BCL2 (2B-cell lymphoma). At the same time, decreased activity of cerebral AChE and the level of P53 (tumor protein) was found. A decline of 8-OHG (8-hydroxy guanosine) values was found in the urine, and a decline of Caspase-3 was found in the blood serum. The hystological analysis of the brain tissue indicates a strong rise of ChAT (cholineacetyl transferase) positive cells. All of these compounds have shown a marked anti-cholinesterase potential with a strong anti-oxidative and anti-apoptotic activity. Future investigations are necessary, as well as considering the possibility of the use of these compounds in the therapy of diseased people.

In the end, after this detailed presentation of chelators and chelation therapy, it is necessary to emphasize the great importance of the daily intake of accessible natural or synthetic chelators, anti-oxidants and neuroprotectors, especially for the middle aged and aged population. The possibility of their purchase on the pharmaceutical and natural markets is evidently getting better. However, the author of this study considers that professional medical consultation is necessary when deciding about the use of these compounds. This preventive approach is effective not only in the treatment of AD, but also in the treatment of the accelerated pathological aging process.

Conclusion

AD is a wasting chronic neurodegenerative disease, with a fluctuating progressive course and a lethal end. The current therapy with cholinesterase blockers and NMDA receptor blockers is not satisfactory. The increasingly frequent therapy with metal chelators, especially iron chelators which can also be combined with nanoparticles, formed from natural and artificial polymers and with the ability to easily pass across the BBB, gives promising results and requires further investigations.

Conflict of interest

The authors declare no conflict of interest in this study.

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