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IRON CHELATORS FOR LABILE IRON REMOVAL THERAPY AND ENHANCEMENT OF LONGEVITY

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ABSTRACT : *Abnormally high levels of non-transferrin-bound iron ions (NTBI), or labile iron ion, have been demonstrated in a number of neurodegenerative disorders including dementia, Parkinson's disease (PD) and Alzheimer's disease (AD), and oxidative stress due to NTBI is believed to be associated with neuronal death in these diseases. We have prepared the new iron chelators, so-called super-polyphenols, in order to delete these dangerous NTBI. Our super-polyphenols are characterized by the following four points, i.e., 1) the super-polyphenols are water-insoluble, but can catch iron(III) ion in the aqueous solution with ease, 2) their iron (III) chelates are also water-insoluble, 3) these are not metabolized in the human body due to its insolubility in water and its polymeric structure (MW~90,000), and 4) they do not interact with the iron ions in the transferrin. Since our super-polyphenols can excrete only NTBI effectively from the plasma and give no damage to human body, our super-polyphenols may be one of the most important substances for labile iron removal therapy, including the prevention of dementia, Alzheimer's and Parkinson's diseases, and also for the enhancement of longevity.*

KEYWORDS: NTBI, Super-polyphenols, Hydrogen medicine, Longevity

INTRODUCTION

It is well known that the iron ions not associated with transferrin generally termed as non-transferrin-bound iron (NTBI), or labile iron ion, is detected in the plasma of patients with thalassemia, hemochromatosis and other iron-overloading disorders, and is present at concentration up to 10 μM (Hershko et al, 2005; Evans et al, 2008). Recently the origin of the iron toxicity due to NTBI has been elucidated by us on the chemical point of view (Nishida, 2004, 2011, 2012a, 2021b); in our mechanism the contribution of hydroxyl radical is completely denied, which has been supported by the recent work by Enami et al. (Enami et al, 2014); the formation of the Fe(IV)=O species observed by Enami et al. can be reasonably explained in terms of concerted mechanism proposed by Nishida (Nishida, 2012b). In addition to this, we have succeeded in elucidating the mechanism of accumulation of iron ion and formation of iron deposition in the brain of patients with neurodegenerative disorders (Nishida, 2012c; Abe et al, 2015a).

For the prevention and treatment of many diseases related with NTBI, especially dementia, Alzheimer's and Parkinson' s diseases (Stankievicz et al, 2007; Gaeta and Hider, 2005), we have prepared new iron chelators which remove *only* NTBI from the plasma *effectively and without toxicity*; these chelates are called as super-polyphenols (Nishida, 2015; Nishida et al, 2012). In this report we have discussed the high possibility of the super-polyphenols as a drug for enhancement of longevity.

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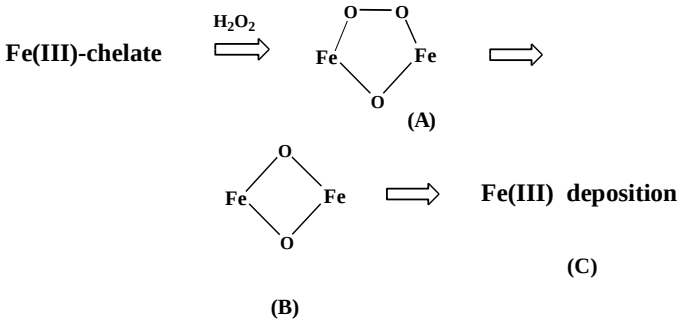
ROLE OF HYDROGEN PEROXIDE ON OXIDATIVE STRESS DUE TO NTBI

We already reported that hydrogen peroxide promotes the formation of di-μ-oxo-diiron(III) species (species (B) in Scheme I) from several iron(III) chelates with amino acid derivative in the presence of reducing agents (see Scheme I) (Sutoh et al, 2006; Nishida, 2012c); this should be due to the strong electrophilicity of the (μ-peroxo)(μ-oxo)-diiron(III) species (species (A) in Scheme I) formed in the solution (Nishida, 2012a, 2012b, 2012c), which turns to the di-μ-oxo-diiron(III) species by oxidizing the peripheral organic compounds in the solution. The further aggregation of the di-μ-oxo-diiron(III) species may proceed to give the iron deposition (see (C) in Scheme I), and these processes are completely exemplified by the recent our work (Abe et al, 2015a

Scheme I



As these polymeric iron(III) ions are not transferred to apo-transferrin (Nishida, 2012c; Nishida et al, 2007), Scheme I explains the marked iron accumulation in the brain as well as visceral tissue despite low serum iron levels observed for patient of aceruroplasminemia, where in the patient of aceruroplasminemia, Fe^{2+} ions are oxidized by apo-transferrin to Fe^{3+} ions with the formation of hydrogen peroxide (Nishida, 2012c). Similar ferroxidase-like function was also observed for APP, amyloid precursor protein (Duce et al, 2012), and thus promoted production of APP should be closely related with the increase of AD patients as observed (Roberts et al, 2012). The fact that increasing of pathogenesis of AD is induced by ferritin abnormality (Roberts et al, 2012) can be elucidated by the increased H_2O_2 formation from the abnormal ferritin as described above, which gives serious oxidative stress in the presence of NTBI as shown in Scheme I. There are many reports to show that high level of H_2O_2 are present in the brain of both the patients of PD and AD (Gaeta and Hider, 2005), and these H_2O_2 may lead to the formation of polymeric iron(III) compounds which are not transferred to transferrin, and also act as an active oxidant towards proteins or DNA through the formation of (μ-peroxo)-diiron(III) species (A) shown in Scheme I (Nishida, 2015).

NEW CHELATING AGENTS TO REMOVE NTBI FROM THE PLASMA

As shown in our previous papers the formation of H_2O_2 is greatly promoted by NTBI (Nishida, 2004; Abe et al, 2015b). Thus, if we can delete NTBI from the plasma, the concentration of H_2O_2 should decrease. For the removal of NTBI the iron chelation method has been used, but almost all the chelates used until now are less effective or have certain adverse effects

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(Agarwal, 2006). A new synthetic oral iron chelator, Exjade, is high effective (Cappellini, 2007), but serious side effects are also reported for Exjade (Nishida, 2015).
We have prepared the so-called *super-polyphenols* which contain more than 100 molecules of catechol derivative in one polymeric compound, chitosan (Nishida et al, 2012; Nishida, 2015) (see Figure 1). We found that water-insoluble super-polyphenols can eliminate NTBI effectively *in vitro study*. Our super-polyphenols are characterized in the following four points, 1) the super-polyphenols are water-insoluble, 2) these are not metabolized in the human body due to its insolubility in water and its polymeric structure (MW~90,000), 3) their iron (III) chelates are also water-insoluble, and 4) they do not interact with the iron ions in the transferrin. The property 4) clearly indicates that our super-polyphenols can discriminate the necessary iron ion and unnecessary iron ion (NTBI) in the human body, and can remove only the NTBI from the body without giving damage to the human body.

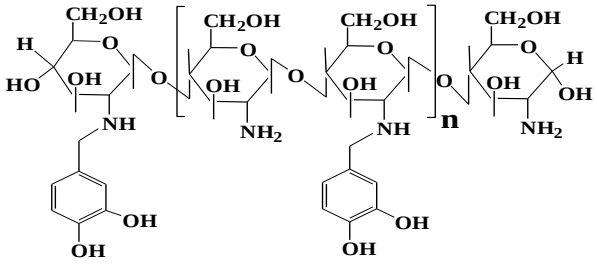

Figure 1. An example of super-polyphenols, FC-Cate2.

Recently research with curcumin has increased significantly (Belkacemi, et al, 2011). In vitro and in vivo studies have demonstrated that curcumin could target pathways involved in the pathophysiology of AD, suggesting that curcumin might be a promising compound for the development of AD therapy. We have found that the water-insoluble super-polyphenol containing vanillin derivative shows high ability to catch NTBI in solution through chelation (see Figure 2) (Nishida, 2015), implying that the antioxidant, anti-inflammatory, antiproliferative properties of curcumin and ferulic acid (Calabrese, et al, 2006) should be due to its ability to excrete NTBI from the plasma (Nishida, 2015), and thus it seems quite likely that our super-polyphenols can be one of the most hopeful substances for labile iron removal therapy, including the prevention of dementia, Alzheimer's and Parkinson's diseases, and also for the enhancement of longevity (see the discussion below).

NEW IDEA ON THE DRUGS FOR ENHANCEMENT OF LONGEVITY

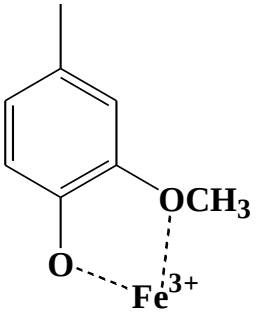

Figure 2. Iron(III) chelate with vanillin derivative (Nishida, 2015)

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Levels of cellular and organismal dysfunction increase dramatically with old age, and aging is the greatest risk factor for numerous pathologies, including most forms of cancer, stroke, neurodegenerative disorders, heart disease, and diabetes (Niccoli and Partridge, 2011). Hence delaying aging therapeutically promises immense benefits to human health (Kennedy et al, 2014). It is well known that iron accumulates as the brain ages and has been linked to motor and cognitive dysfunction in the elderly, and a growing body of data suggests that brain iron accumulation *in vivo* contribute to tissue damage in a variety of chronic neurologic disorders; these are consistent with the histologic and MRI data (Stankievicz et al, 2007). These facts lead to the idea that removal of the accumulated NTBI with age from the human body and prevention of the accumulation of NTBI should be one of the best ways to enhance the longevity.

Very recently, He *et al*. reported that the common non-steroidal anti-inflammatory drug ibuprofen (see Figure 3) increased the life span of *Saccharomyes cerevisiae*, *Caenothatbditis elegans and Drasophilia melanogaster*, and pointed out that enhanced longevity by ibuprofen occurs in Yeast through inhibition of tryptophan import (He et al, 2014). Here, I would like to point out that the enhanced longevity by ibuprofen observed by Polymenis *et al*. may be partially related with the decreased NTBI and H_2O_2 in plasma; the facts to support the above idea will be developed below. We observed that the addition of H_2O_2 to the solution of iron(III) complex with benzylamine-N,N-diacetic acid induces the facile hydroxylation of benzene ring of the benzylamine-N,N-diacetic acid, as illustrated in Figure 4 (Nishida and Nishino, 2011).

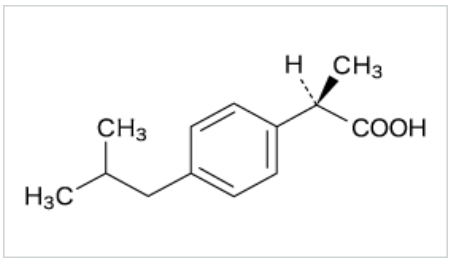

Figure 3. Ibuprofen

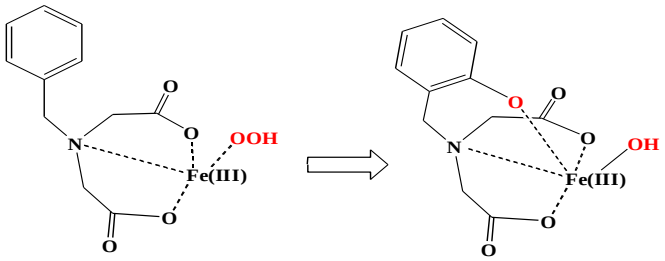

Figure 4. Reaction of iron(III)-OOH species and phenol ring:
Heterolytic cleavage of OOH group into O (neutral) and OH^- is promoted through the interaction with phenol ring (Nishida, 2012b)

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Based on the results of Mass spectral data using $H_2{}^{18}O_2$, the oxygen atom of the hydroxylated group is derived from the H_2O_2 added (Nishida and Nishino, 2011), indicating that the hydroxylation of the benzene ring proceeds through heterolytic cleavage of H_2O_2, i.e., **$H_2O_2 \longrightarrow O$(neutral) and + OH_2** (Nishida, 2012b); in this case, the electrophilic hydrogen peroxide coordinated to the iron(III) ion is very important, which reacts with the HOMO of the benzene ring (Nishida and Nishino, 2001). It should be noted here that in the above process both the dangerous iron(III) ions and hydrogen peroxide are deleted from the solution through the formation of iron(III) complex with 2-hydroxybenzylamine-N,N-diacetic acid.

DFT calculations (Nishida, 2012d) on the ibuprofen derivative (see Figures 5 and 6) imply that similar reaction observed for the iron(III) complex with benzylamine-N,N-diacetic acid may proceed as illustrated in Figure 6. The decrease of both the NTBI and H_2O_2 by ibuprofen as described in Figure 6 should be related with the fact observed by He et al. (He et al, 2014), but the formed iron(III) species here may be dangerous (Nishida and Nishino, 2001; Nishida, 2012a) which are quite consistent with the many serious side effects by ibuprofen reported until now.

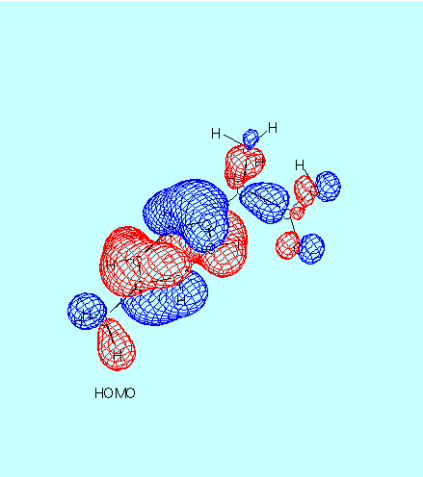

Figure 5. HOMO of ibuprofen derivative shown in Figure 4

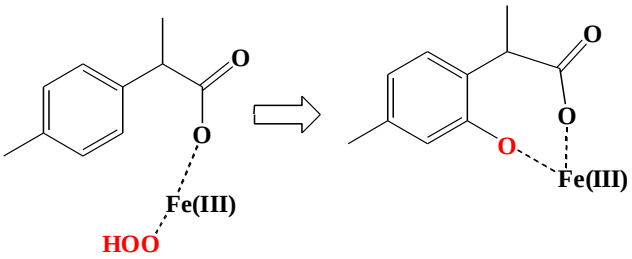

Figure 6. Assumed reaction of Fe(III)-OOH species and ibuprofen derivative.

Donepezil, so-called as Aricept, is used for the treatment of mild, moderate, or severe dementia associated with Alzheimer's disease (Sabbagh et al, 2013). It belongs to a class of drugs called cholinesterase inhibitors. Scientists believe that Alzheimer's disease may result from a

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deficiency in neurotransmitters used by nerves in the brain to communicate with one another. Donepezil inhibits acetylcholinesterase, an enzyme responsible for the destruction of acetylcholine, leading to increased concentrations of acetylcholine in the brain, which is believed to be responsible for the improvement seen during treatment with donepezil. It improves the symptoms, but does not slow the progression of Alzheimer's disease. DFT calculations on the donepezil derivative (see Figure 7) imply that similar reaction observed for the iron(III) complex with benzylamine-N,N-diacetic acid may proceed as illustrated in Figure 8. Many side effects are reported for donepezil as follow; headache, generalized pain, fatigue dizziness, nausea, vomiting, and these should be due to the toxicity by the iron(III) species formed in the step (see Figure 8), similar to the cases of ibuprofen and also Exjade.

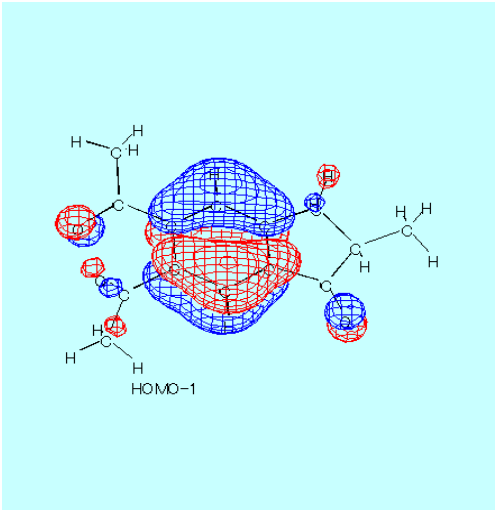

Figure 7. The next HOMO of donepezil derivative molecule (see Figure 8)

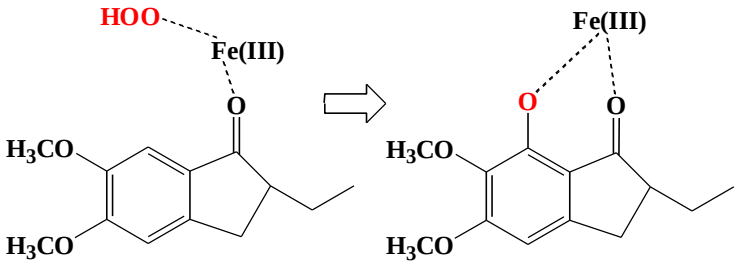

Figure 8. Assumed reaction of Fe(III)-OOH species and donepezil derivative

Satoh *et al*. reported that NMN, nicotinamidemononucleotide, (see Figure 9) extends life span and delays aging in mice through the regulation of Nk2 homebox1 in the DMH and LH (Satoh et al, 2013), implying that decrease of NMN should be closely related with the aging. As to the NMN, we should notice that NMN contains the phosphoric group, as indicated in Figure 9. As pointed out by Nishida, the phosphoric group may react with NTBI, to give a dangerous

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binuclear iron(III) species illustrated in Figure 10, which is consistent with the fact that excess ADP strongly promotes the peroxidation of fatty acids (Nishida and Akamatsu, 1992). Thus, it seems quite likely that presence of much NTBI interferes the usual function of NMN, and also excess NMN may be very harmful under the presence of much NTBI because several dangerous binuclear iron (III) species would form (Nishida, 2012b).

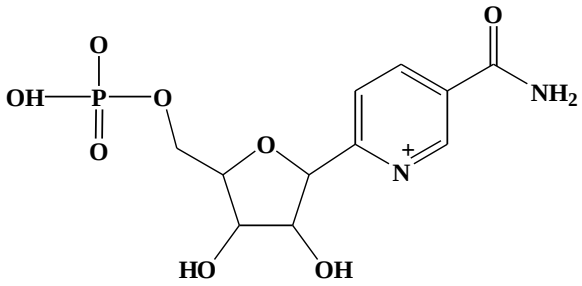

Figure 9. Structure of NMN

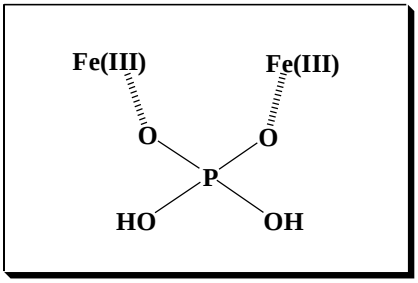

Figure 10. Formation of a binuclear Fe(III) species by phosphoric acid

HYDROGEN MEDICINE AND NTBI

Recently Ohta et al. have proposed that molecular hydrogen (H_2) has potential as a "novel" antioxidant in preventive therapeutic applications (Ohsawa et al, 2007). According to their papers, H_2 has a number of advantages as an antioxidant: H_2 rapidly diffuses into tissues and cells, and it is mild enough neither to disturb metabolic redox reactions nor to affect reactive oxygen species that function in cell signaling. They reported that H_2 selectively reduce the hydroxyl radical, the most cytotoxic of reactive oxygen species. But, we have demonstrated that the hydroxyl radical does not form in the human body, and pointed out that the most oxidative stress are induced by the electrophilic oxygen or hydrogen peroxide adduct of the iron species (see Figure 4) (Nishida, 2012b). Thus, it may be probable that H_2 may react with the electrophilic oxygen atom in Fe-O (neutral) (see Figure 11), to give a water molecule.

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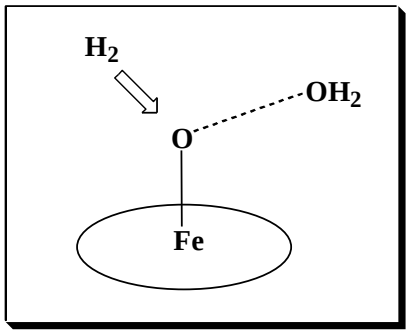

Figure 11. Reaction scheme between Fe(III)-OOH_2 and H_2. Heterolytic cleavage of peroxide molecule into O (neutral) and OH_2 is promoted through interaction with H_2 molecule, to give H_2O.

CONCLUSION

Based on the discussions described above, it seems quite clear that for the enhancement of longevity, the most necessary is to decrease the NTBI! Since our super-polyphenols can excrete only NTBI *effectively and without toxicity*, I would like to point out that our super-polyphenols are the most important substances for this purpose. Of course, we must pay attention to the iron-deficiency, and thus the use of our super-polyphenols should be limited to the patients with iron-overloading disorders. *Our project is now under progress in Japan.*

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