European Journal of Biology and Medical Science Research
Vol.3, No.6, pp.57-64, December 2015
Published by European Centre for Research Training and Development UK (www.eajournals.org)

PROTECTIVE EFFECTS OF HYDROGEN MOLECULE AND LABILE IRON REMOVAL THERAPY AGAINST NEPHROTOXICITY BY CIS-PLATIN

Yuzo Nishida

Medical Research Institute, Kanazawa Medical University,
Uchinada, 920-0293 Japan.

ABSTRACT: *Many facts are demonstrating that the protective effect of the hydrogen molecule against the nephrotoxicity induced by cis-platin should be attributed to that H2 molecule decomposes the peroxide adduct of the iron(III) species, which has been demonstrated to be highly electrophilic, and proposed to be an intrinsic active species to induce nephrotoxicity induced by the several iron(III) chelates, and that labile iron removal therapy by the use of our super-polyphenols should be very useful to depress the side-effects induced by cis-platin. .*

KEYWORDS: Cis-Platin, Nephrotoxicity, Hydrogen Medicine, Labile Iron Removal Therapy.

INTRODUCTION

Cis-platin is one of the most effective chemotherapeutic agents and plays a major role in the treatment of a variety of human solid tumors (N.-Kamimura et al, 2009). But, it is well known that cis-platin induces cytotoxicity in LLC-PK1 cells (renal proximal tubular epithelial cells), an *in vitro* model, and also acute renal failure in rats, an *in-vivo* model (Baliga et al, 1998). This adverse effect limiting the efficacy of the cis-platin is nephrotoxicity, and the mechanism underlying this nephrotoxicity is not well understood.

Baliga et al. have demonstrated that catalytic iron, as measured by bleomycin assay, was significantly increased in LLC-PK1 cells exposed to cisplatin and in the kidney of rats treated with cisplatin. As the iron chelator including DFO and 1,10-phenanthroline completely prevented cis-platin induced cytotoxicity in LLC-PK1 cells and DFO also provided marked protection against cis-platin-induced acute renal failure in rats, they suggested that the catalytic iron plays an important role in the pathogenesis of this model of renal injury (Baliga et al, 1998). As the protective effects of iron chelators and hydroxyl radical scavengers in several models of renal injury have been generally taken as evidence for the participation of hydroxyl radical in the tissue damage, they concluded that their data have lend strong support to a critical role for iron in mediating tissue injury via hydroxyl radical formation in cis-platin-induced nephrotoxicity. Very recently, Ohta et al have reported that hydrogen efficiently mitigates the renal injury of cis-platin (N.-Kamimura et al, 2009), and concluded that this should be due to the unique property of hydrogen molecule to neutralize OH-radical (Ohsawa et al, 2007).

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

We have investigated the mechanism of nephrotoxicity induced by the artificial iron(III) chelates (Nishida et al, 2007; Nishida, 2012a, 2012b) and concluded that the active species to induce the renal nephrotoxicity is a peroxide-iron(III) species which exhibits strong electrophilicity towards several organic compounds, and that responsibility of the hydroxyl radical in the nephrotoxicity induced by the iron(III) chelates is negligible; our results are supported by the recent works by Eami et al. (Eanmi et al, 2014). In this report, we will give new insight into the relationship between the protective effects by hydrogen molecule and the iron chelator on the nephrotoxicity due to cis-platin.

SIMILARITY IN RENAL INJURY BY ARTIFICIAL IRON(III) CHELATES AND CIS-PLATIN

Ferric nitrolotriacetate (Fe(III)-nta) is a well-known renal carcinogen, and Fe(III)-nta-injected animals have been used as a model of carcinogenesis (Mizuno et al, 2006). When Fe(III)-nta is intraperitoneally injected into animals, lipid peroxidation and oxidative modification of proteins and DNA occur in renal proximal tubules, and tubular epithelial cells are damaged. Thiobarbituric acid reactive substance (TBARS; these include malondialdehyde and other aldehyde derivatives) has also been shown to increase in kidneys, and cold Schiff staining showed lipid peroxidation in renal proximal tubules in Fe(III)-nta-treated animals (Mizuno et al, 2006; Nishida et al, 2007; Nishida, 2009). Increases in 4-hydroxy-2-nonenal (4-HNE)-modified proteins and 8-hydroxy-deoxyguanosine (8-OH-dG) were also demonstrated using biochemical methods. In Fe(III)-nta-injected mice, amount of reduced glutathione decreased, and oxidized form increases when metabolic rate of glutathione was accelerated. Repeated injections of with Fe(III)-nta result in appearance of atypical epithelial cells in renal tubules, and finally in induction of renal carcinoma (Mizuno et al, 2006).

It should be noted here that similar renal proximal tubular epithelial cells damages are induced by cis-platin, as described in **INTRODUCTION**. As stated before, the proximal tubules necrosis and renal carcinoma induced by iron(III)-(nta) and other related compounds are observed mainly in the renal proximal tubules, but any injury was not observed in the distal position, although much iron(III) ions are present in that position. It should be noted here that the glutathione cycle is highly operating in the renal proximal position, and this may demonstrate that glutathione cycle should be closely related with the iron(III)-induced injuries (Nishida et al, 2007; Nishida, 2012a). Based on the facts that the proximal tubules necrosis induced by artificial iron(III)-chelates in rat kidneys are highly dependent on the chelate structure, and pH dependency is observed for the solution of iron(III)-(ida) chelate (see Table 1), we have concluded that 1) iron(III) ions of the iron(III) chelates are not reduced to an iron(II) state by glutathione reductase (the direct interaction between the glutathione cycle and iron(III) ions is negligible), and 2) the intrinsic active species to induce the renal injury should be a peroxide adduct of binuclear iron(III) complex (right side of the Figure 1), which exhibits strong electrophilicity towards organic compounds (Nishida, 2012a, 2012b). The formation of the peroxide adduct of binuclear iron(III) complex (right side of the Figure 1) is greatly accelerated through the *indirect interaction* with the glutathione cycle.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

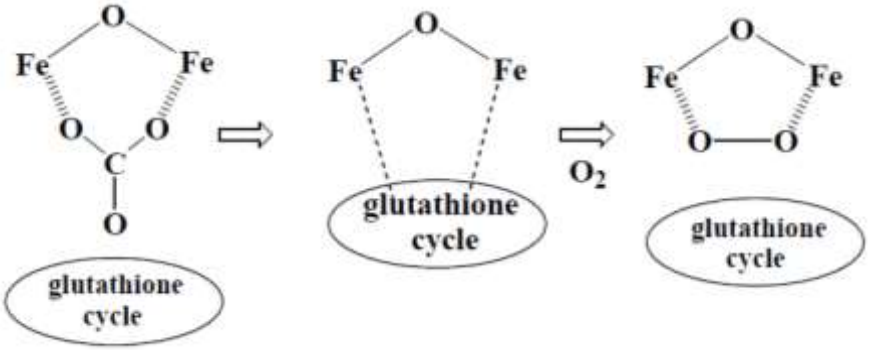


Figure 1. Indirect interaction between the binuclear iron(III)-(nta) complex and glutathione cycle in the presence of oxygen gives an iron(III)-peroxide adduct as illustrated in the right side (Nishida et al, 2007; Nishida, 2012a).

Table 1. Effects of Iron Chelates on renal tubular injuries (Nishida, 2012a)

Iron chelates	pH 6.2	pH 7.2	pH 8.2
Fe-(nta)	+	+	+
Fe-edda	+	+	+
Fe-ida	+	+	—
Fe-edta	—	—	—
Fe-pac	—	—	—
Fe-hida	—	—	—

(+, active; —, inactive)

The above conclusion, *i.e.*, the direct interaction between the glutathione cycle and iron(III) ions is negligible, may also valid for the case of the cis-platin compounds, *i.e.*, it seems reasonable to assume that platinum (II) ions are not directly interacting with the –SH group of the glutathione protein, leading to th at nephrotoxicity by cis-platin should be induced by the iron(III) ions as detected by Baliga et al (Baliga et al, 1998).

HYDROGEN MOLECULE REACTS WITH THE PEROXIDE ADDUCT OF METAL CHELATES AND OXO-METAL SPECIES

Nagano et al. have measured the fluorescence spectra of the *O*-dearylated HPF molecule derived from HPF in the solution containing Fe(II) ion, H2O2, HPF and phosphate buffer, and concluded that HPF should be a specific compound for detecting the OH radical formation (Setsukinai et al, 2003), because it has been believed that the system containing the ferrous ion and hydrogen peroxide gives a hydroxyl radical (Burkitt & Mason, 1991). Ohta et al. used the HPF molecule in the culture cell, and concluded that H2 can neutralize the OH-radical according to the results by Nagano (Ohsawa et al, 2007).

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

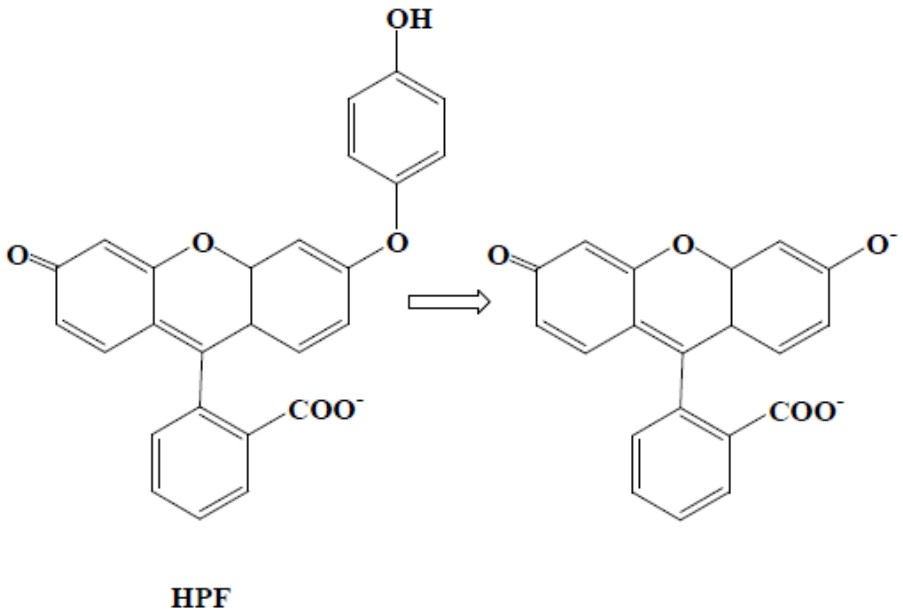


Figure 2. Conversion of HPF molecule(left side) to the O-dearylated HPF molecule(right side) (Setsukinai et al, 2003)

In 2014, Enami et al. have reported that several oxo-iron(IV) species are formed, but hydroxyl radical formation is negligible in the Fenton reaction solution (Enami et al, 2014). Since the formation of the oxo-species detected by Enami et al. can be elucidated by the use of Nishida's concerted mechanism (Nishida, 2012b) (see the Figure 3), and that reactive intermediates of peroxidase and cytochrome P-450 react with HPF to give an *O*-dearylated HPF (Setsukinai et al, 2003), where for both cases an oxo-iron(IV) species is believed to be a reactive species, it seems reasonable to assume that an oxo-iron(IV) species should be an intrinsic species to give an *O*-dearylated HPF in the reaction with HPF in both the reactions investigated by Nagano et al and Ohta et al.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

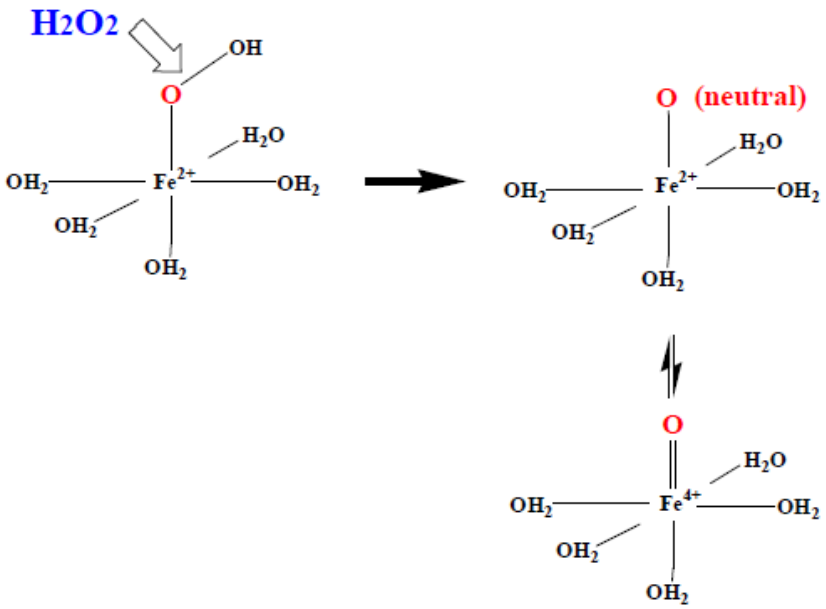
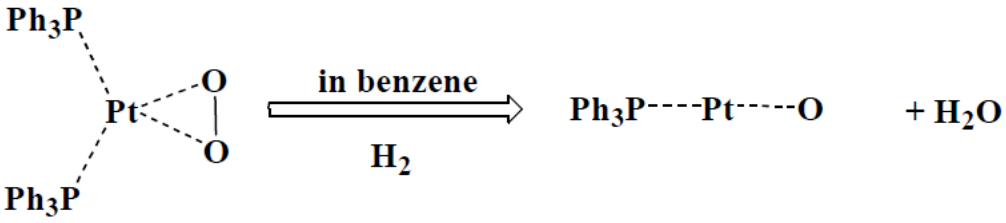


Figure 3. Concerted heterolytic cleavage of the peroxide ion coordinated to the iron atom gives an O(-2) and O(neutral state), and the formed Fe(II)-O(neutral state) is identical to Fe(IV)=O(oxo ion), detected by Enami et al. (Nishida, 2012b)

In 1982, Kushi et al. have reported that H2 molecule reacts readily with peroxide adduct of Pt(II) compound (see below), to decompose the original peroxide adduct (Kushi et al, 1982).



This can be elucidated as follows: The [(PPh3)2Pt(II)-peroxide complex] (Lanci et al, 2005) has an unoccupied orbital consisting of dx2-y2 orbital and □*-orbital of the peroxide ion, as depicted in Figure 4. As this unoccupied orbital exhibits high electrophilicity, approaching of H2 molecule (see Figure 5) leads to the heterolytic cleavage of the peroxide ion into O (double negative) and O (neutral state). Since the formed Pt(II)-O(neutral state) is identical to Pt(IV)=O(oxo ion), and this species exhibits also high electrophilicity (this complex also has an unoccupied orbital), it reacts readily with H2 molecule to give water, which is consistent with the observed fact by Kushi et al.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

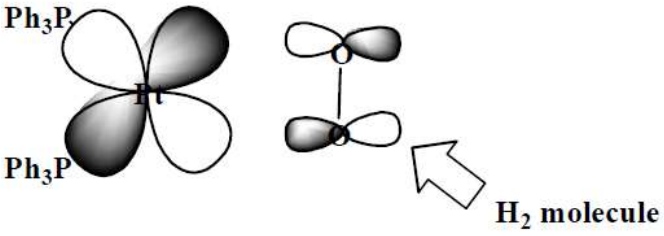


Figure 5. Schematic illustration of the reactin between Pt(II)-Peroxide adduct and hydrogen molecule

Since the electronic property of the Fe(IV)=O(oxo ion) species observed in the reactive intermediate of peroxidase and cytochrome P-450 (Himo and Siegbahn, 2003) is similar to that of Pt(IV)=O(oxo ion) species, it seems reasonable to assume that the Fe(IV)=O(oxo ion) species should react with H2 in the reactions performed by Ohta et al. (Ohsawa et al, 2007); this gives reasonable explanation for the observed facts.

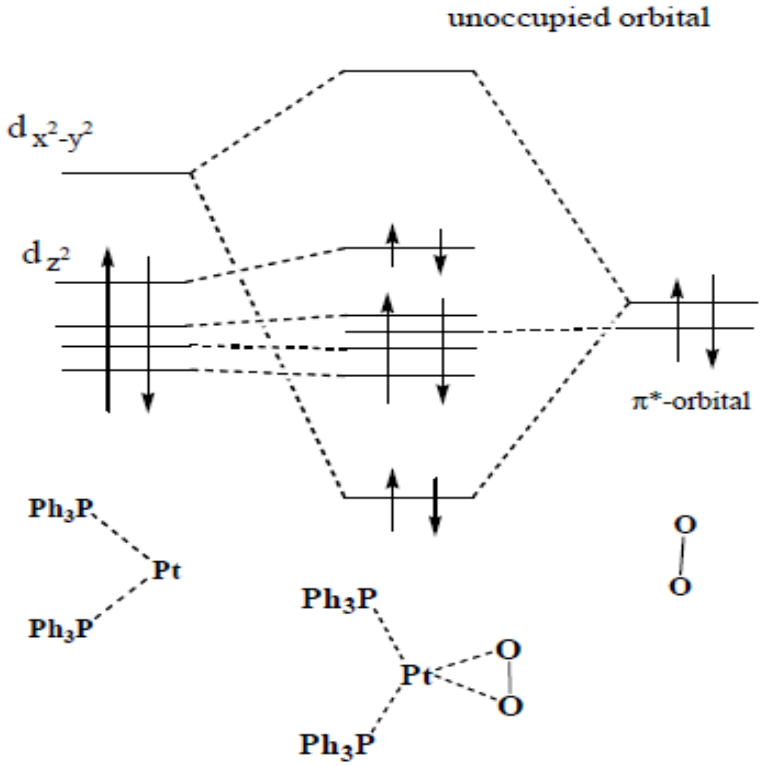


Figure 4. MO scheme of [(PPh3)2Pt(II)-peroxide]. The presence of the unoccupied orbital demonstrates that this species should react with H2 molecule according to the scheme shown in Figure 5.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

The high electrophiliity of the peroxide adduct of the binuclear iron(III) complexes has been already confirmed by our works (Nishida & Takeuchi, 1987; Nishida, 2009; Nishida 2012a, 2012b), and thus this suggests that the protective effect of the hydrogen molecule against the nephrotoxicity induced by cis-platin should be attributed to that H2 molecule decomposes the peroxide adduct of the iron(III) species, which has been shown to be highly electrophilic, and proposed to be an intrinsic active species to induce nephrotoxicity by Nishida, and that labile iron removal therapy by the use of our super-polyphenols (Nishida, 2012a; Nishida, 2015) should be very useful to depress the side-effects induced by cis-platin. Based on the discussion developed in this paper we would like to point out that Nagano's conclusion on the HPF molecule is doubtful, *i.e.*, we cannot mention the OH-radical formation by the use of HPF molecule.

REFERENCES

Baliga, R. Zhang Z., Baliga, M., Ueda, N., and Shah, S. V. (1998) *Kidney International*. 53, 394-401.

Burkitt, M. J. and Mason, R. P. (1991) Direct evidence for in vivo hydroxyl-radical generation in experimental iron-overloar: An ESR spin-trapping investigation. *Proc. Natl. Acad. Sci. USA*. 88, 8440-8444.

Enami, S., Sakamoto, Y. and Collusi, A.J. (2014): Fenton chemistry at aqueous interfaces. *Proc. Natl. Acad. Sci. USA*. 111, 623-629. doi:10.1073/pnas.1314885111.

Himo, F. and Siegbahn, P. E. M. (2003) Quantum chemical studies of radical-containing enzymes. *Chem. Rev*. 103, 2421-2456. doi:10.1021/cr020436s.

Kushi, K., Matsunuma, Y., Kanai, H., Tarama, K. & Yoshida, S. (1982) Hydrogenation of olefins catalyzed by the platinum-triphenylphosphine complex containing oxygen atom. *Nippon Kagaku Zasshi*, 347-351.

Lanci, M. P., Brinkley, P. W., Stone, K. L., Smirnov, V. V., Roth, J. P. (2005) Structure of transition state in the metal-mediated O2-activation Reactions. *Angew. Chem. Int. Ed*. 44, 7273-7276. doi:10.1002/anie.20052009.

Mizuno, R., Kawabata, T., Sutoh, Y., Nishida, Y., and Okada, S. (2006) Oxidative renal tubular injuries induced by aminocarboxylato-type iron(III) coordination compounds as candidate renal carcinogens", *BioMetals*, 19, 675-683. doi:10.1007/s10534-006-9004-4.

Nishida, Y. & Takeuhci, M. (1987) Unique reactivity of peroxide ion trapped by binuclear iron(III) complex. *Z. Naturforsch*., 42b, 52-54.

Nishida, Y., Itoh, Y. and Satoh, T. (2007): Origin of renal proximal tubular injuries by Fe(III)-nta chelate. *Z. Naturforsch*. 62c, 608-612. http://www.znaturforsch.com/ac/v62c/s62c0608.pdf

Nishida, Y. (2009). Structural characteristics of iron(III) chelates to induce tissue damage and renal carcinoma: Chemical origin of the iron toxicity. *TCIMail, No. 141*, 2-15. Retrieved from http://www.tciamerica.com/tcimail/backnumber/article/141drE.pdf

Nishida, Y. (2012a): The chemical mechanism of oxidative stress due to non-transferrin-bound iron (NTBI). *Adv. Biosci. Biotech*. 3, 1076-1086. doi: 10.4236/abb.2012.327131.

Nishida, Y. (2012b): "Oxygen Activation, Oxidative Stress and Human Health", LAP Lambert Academic Publishing, Saarbrucken, Germany (2012), Chapter 4, pp34-41.

Nishida, Y. (2015): Abnormal iron accumulation in brain and new iron chelator for labile iron removal therapy. *Int. J. Chem*. 7(1), 104-110. doi:10.5539/ijc.v7n1p104.

N.-Kamimura, N, Mori, T, Ohsawa, I, Asoh, S. & Ohta, S. (2009) Molecular hydrogen

ISSN 2053-406X(Print) ISSN 2053-4078(Online)

alleviates nephrotoxicity induced by an anti-cancer drug cisplatin without compromising activity in mice. *Cancer Chemother Pharmacol*. 64(4), 753-761. doi:10.1007/s00280-008-0924-2

Ohsawa, H., Ishikawa, M., Takahashi, K., Watanabe, M., Nishimaki, K., Yamagata, K., Katsuura, K., Katayama, Y., Asoh, S. and Ohta, S. (2007) Hydrogen acts as a therapeutic antioxidant by selecting reducing cytotoxic oxygen radical. *Nature Medicine*, 13, 688-694. doi:10.1038/nm1577.

Setsukinai, K., Urano, Y., Kakinuma, K., Majima, J., Nagano, T. (2003) Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species. *J. Biol. Chem.*, 278, 3170-3175. doi:10.1074/jbc.M209264200.

ISSN 2053-406X(Print) ISSN 2053-4078(Online)