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# Prevention of free-radical mediated tissue damage and carcinogenesis induced by low-molecular-weight iron

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**Abstract**

The unique model of iron-induced oxidative nephrotoxicity and renal cancer can be used to screen possible bioprotective substances *in vivo*. Ferric iron complexed with nitrilotriacetic acid (Fe-NTA) in this model is thought to be a tumor initiator as well as a promoter through the production of active oxygen species/free radicals. In the present paper, I have summarized the animal studies using this model of chemoprevention, and present some of new studies.

## Introduction

In the previous workshop I showed that iron complexes of nitrilotriacetic acid (Fe-NTA) and of ethylenediaminediacetic acid (Fe-EDDA) are potent mediators of free radical production, and upon injection intraperitoneally in rats and mice, they induce acute tubular necrosis (due to secondary apoptosis), and renal cancer. This unique model of iron-induced oxidative nephrotoxicity and renal cancer can be used to screen possible scavengers *in vivo*. Iron in this model is thought to be an initiator as well as a tumor promoter. In the present paper, I have summarized the animal studies using this model of chemoprevention, and present some new studies. Here I present data that some of the food supplements actually prevent oxidative damage and cancer.

## Animal studies using Fe-NTA model

References were collected through PubMed using ferric nitrilotriacetate (Fe-NTA) and nephrotoxicity or renal cell carcinoma as keywords. Table 1 shows the summary of the chemoprevention studies of nephrotoxicity induced by Fe-NTA. Most of the effective additives are lipid soluble substances. As far as we investigated, water-soluble extract of Ginseng is a potent radical scavenger *in vitro*, however it could not prevent acute tubular necrosis induced by Fe-NTA in rats. Inhibitory effect of carcinogenesis that was exerted by food additives is summarized in Table 2. Since we found vitamin E ($\alpha$-tocopherol) is a potent inhibitor of nephrotoxicity and carcinogenesis induced by Fe-NTA, we can assess the effectiveness of antioxidants relative to vitamin E. Therefore, our model is a convenient tool, with some limitations, to screen chemopreventive agent *in vivo*.

## Typical relevant studies

Food supplemented with vitamin E, a known radical scavenger, could inhibit tissue lipid peroxidation, apoptosis, 8-hydroxydeoxyguanosin (8-OH-dG) formation, a known DNA oxidative modifications, in Wistar rats. On repeated injections of Fe-NTA, renal cancer developed in 11 of 25 rats (44%) for vitamin E-sufficient, and only 1 of 20 rats (5%) for vitamin E-supplemented rats. Vitamin E also prevented the Fe-NTA-induced iron deposition in the developing bones in Wistar rats.

The protection of renal damage and carcinogenesis by Brazilian propolis and its extract Artepillin C

*Table 1.* Inhibitory agents of tissue damage induced by ferric nitrilotriacetate *in vivo*.

| Agents used | Organ | Markers for the inhibitory effects | Authors | Reference |
|---|---|---|---|---|
| Brazilian propolis and Artpillin C | Kidney | Thiobarituric acid-reactive substances, 4-hydroxy-2-nonenal -modified proteins and 8-hydroxy-2′-deoxyguanosine | Kimoto T, Koya S, Hino K *et al.* | Pathol Int 50, 679–89, 2000 |
| T-0970, synthesized ureidophenol derivative | Liver | Thiobarbituric acid-reactive substances, GOT, GPT | Suzumura K, Hashimura Y, Kubota H, Ohmizu H, Suzuki T, | Free Radic Res 32, 255–64, 2000 |
| A single post whole-body low-dose irradiation (50 cGy of gamma-ray) | Mice liver | GOT, GPT | Yamaoka K, Kojima S, Nomura T | Free Radic Res 32, 213–21, 2000 |
| Nordihydroguairetic acid (NDGA), 2 mg NDGA/day/animal | Liver Kidney | Ornithine decarboxylase activity [$^3$H]thymidine incorporation | Ansar S, Iqbal H, Athar M | Carcinogenesis 20, 599–606, 1999 |
| Melatonin | Kidney | Malondialdehyde, 4-hydroxyalkenals, 8-hydroxydeoxyguanosine | Qi W, Reiter RJ, Tan DX *et al.* | Toxicology 139, 81–91, 1999 |
| Alpha-Tocopherol | Kidney | Renal antioxidants and antioxidant enzymes, ornithine decarboxylase activity [3H]thymidine incorporation | Iqbal M, Rezazadeh H, Ansar S, Athar M | Hum Exp Toxicol 17, 163–171, 1998 |
| Garlic oil | Kidney | Microsomal lipid peroxidation, hydrogen peroxide, renal antioxidant enzymes | Iqbal M, Athar M | Food Chem Toxicol 3, 485–95, 1998 |
| Chinese ant extract (CAE) | Kidney | Schiff's reagent, TBARS | Ma Y, Wang X, Zhao Y, Kawabata T, Okada S | Res Commun Mol Pathol Pharmacol 96, 169–178, 1997 |
| Methylene dioxybenzenes, particularly isosafrole | Kidney Liver | Histology | Zhao ZS, Khan S, O'Brien PJ | Chem Biol Interact 108, 107–118, 1997 |
| alpha G-Rutin | Mice kidney | Renal lipid peroxidation | Shimoi K, Shen B, Toyokuni S *et al.* | Jpn J Cancer Res 88, 453–460, 1997 |
| Vitamin E | Kidney | Lipid peroxidation, apoptosis, 8-hydroxydeoxyguanosine, cold Schiff staining, staining by terminal deoxynucleotidyl transferase-mediated nick end labeling | Zhang D, Okada S, Yu Y *et al.* | Cancer Res, 57, 2410–2414, 1997 |
| 2-mercaptoethanesulfonate, N-acetylcysteine | Kidney | 8-OHdG, lipid peroxides as thiobarbituric acid-reactive substances | Umemura T, Hasegawa R, Sai-Kato K *et al.* | Jpn J Cancer Res, 87, 882–886, 1996 |
| Probucol | kidney | Schiff's staining | Qin X, Zhang S, Zarkovic M 1995 *et al.* | Carcinogenesis. 16, 2549–2552, |
| Pyridinecarboxamide (picolinamide (2-pyridine carboxamide)) | Kidney | Histology | Kawabata T, Ogino T, Mori M, Awai M | Acta Pathol Jpn 42, 469–475, 1992 |
| GSH and Cysteine | Kidney | 8-hydroxydeoxyguanosine | Umemura T, Sai K, Takagi A, Hasegawa R, Kurokawa Y | Cancer Lett 58, 49–56, 1991 |
| Vitamin E | Kidney Liver | Thiobarbituric acid reactive substance | Hamazaki S, Okada S, Ebina Y, Li JL, Midorikawa O | Toxicol Appl Pharmacol 92, 500–506, 1988 |

*Table 2.* Inhibitors of Renal Carcinogenesis Induced by Ferric Nitrilotriacetate.

| Inhibitory agents | Authors | Reference and year |
|---|---|---|
| Brazilian propolis and artepillin C | Kimoto T, Koya S, Hino K *et al.* | Pathol Int 50, 679–689, 2000 |
| Nordihydroguairetic acid (NDGA), 2 mg NDGA/day/animal | Ansar S, Iqbal M, Athar M | Carcinogenesis, 20, 599–606, 1999 |
| Vitamin E | Zhang D, Okada S, Yu Y *et al.* | Cancer Res 57, 2410–2414, 1997 |
| Estrogen | Deguchi J, Miyamoto M, Okada S | Jpn J Cancer Res 86, 1068–1071, 1995 |

was studied by late Prof. Kimoto's group in ddY mice. They reported propolis and Artepillin C prevent oxidative renal damage, as measured by thiobarituric acid reactive substances (TBARS), histochemical findings of 4-hydroxynonenal-modified proteins and 8-OH-deoxyguanosine, known products of lipid peroxidation. As to the incidence of tumors treated with Fe-NTA alone, pre-cancerous cyst was found in 3, and cancer was found in 4 out of 8 effective mice. However, in mice receiving Fe-NTA with either propolis or Artepillin C pretreatment, pre-cancerous cyst and cancer was zero in both groups ($P < 0.05$ vs. Fe-NTA alone group by Fisher's exact probability test).

Iqbal *et al.* found that Fe-NTA is a potent inducer of renal ornithine decarboxylase (ODC) activity. ODC is a rate limiting enzyme of polyamine biosynthesis pathway and a marker enzyme to assess the tumor promoting potential of an agent. The treatment of Fe-NTA to uninitiated animals led to the development of renal cell tumors in 17% of animals studied. However, treatment of Fe-NTA to N-diethyl nitrosamine (DEN)-initiated rats led to development of renal cell tumors in 71% of animals. They also reported a potent lignin-derived herbal antioxidant, nordihydroguairetic acid (NDGA), against Fe-NTA-mediated tissue toxicity. The administration of NDGA, afforded $> 80\%$ protection against DEN- and Fe-NTA-mediated renal tissue injury *in vivo*. Their data showed that NDGA can abrogate the toxic and tumor-promoting effects of Fe-NTA.

The promotion action of iron was also abrogated by the simple procedure of repeated phlebotomy after prolonged initiation with Fe-NTA. We performed phlebotomy in Wistar rats having received Fe-NTA in the initiation stage, and compared them with the non-phlebotomized animals. As a result, incidence of RCC was not significantly different between phlebotomy and non-phlebotomy animals (9 out of 17 effective rats vs. 9 out of 16 rats, respectively), the total weight of RCC was significantly heavier in animals of non-phlebotomy group than those of phlebotomy group ($23.6 \pm 18.5$ g vs. $54.4 \pm 42.4$ g, $P < 0.05$).