Anti-Cancer Drugs 2000, **11,** pp. 727–730

Preclinical report

Expression of myeloperoxidase in the inner ear of cisplatin-treated guinea pigs

Ken-ichi Watanabe[1] and Toshiaki Yagi[1]
[1]Department of Oto-Rhino-Laryngology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan.

Cisplatin is known to cause inner ear damage (ototoxicity). The role of myeloperoxidase (MPO) in the inner ear of the guinea pigs after injections of cisplatin i.p. was examined immunohistochemically. Three days after the injection, the animals were sacrificed with intracardiac perfusion of fixative, and temporal bones were removed and processed for immunohistochemistry with the anti-MPO antibody. MPO could be detected after 3 days in the lateral wall, the organ of Corti, supporting cells of the sensory epithelium and dark cells. These results suggest that MPO and reactive oxygen species are involved in the inner ear dysfunction after the application of cisplatin. [© 2000 Lippincott Williams & Wilkins.]

Key words: Cisplatin, immunohistochemistry, inner ear dysfunction, myeloperoxidase.

Introduction

The anticancer drug cisplatin shows severe side effects, e.g. renal toxicity, myelosuppression and ototoxicity, which limit its administration. However, details of ototoxicity are not yet clarified. Recent reports indicate that reactive oxygen species (ROS) are detected in the cisplatin-treated inner ear.[1] We have reported that the administration of cisplatin activated the inducible nitric oxide synthase in the inner ear.[2,3] There are many reactions of importance in relation to oxidants. Myeloperoxidase (MPO) produces hypohalous acid using hydrogen peroxide and halides.[4,5]

MPO is present at high concentrations in cytoplasmic granules of neutrophils. When some foreign bodies, e.g. bacteria or virus, invade into the tissue, a variety of chemical substances are released from neutrophils. MPO reacts with hydrogen peroxide (H_2O_2).[6] The toxicity of H_2O_2 is accelerated by reaction with MPO and chloride.[7] The complex of MPO and H_2O_2 oxidizes many substances and produces hypochlorous acid (HOCl) and OCl^-. HOCl and OCl^- are strong oxidants, and have a defensive property against invading foreign bodies. It is known that the patients with genetic deficiency of MPO suffer recurrent infections.[8] On the other hand, ROS are reported to be responsible for the cytotoxic effect under pathological conditions.[9–11] It is supposed that MPO generated ROS have ototoxic effects and could injure normal tissues.

The purpose of this study is to evaluate the influence of cisplatin on the inner ear by means of immunohistochemistry in an animal model.

Materials and methods

Materials

Twelve guinea pigs weighing 250–350 g with a positive Preyer's reflex were used in this study. All animals were confirmed to be free from otitis media. All procedures were performed under anesthesia with 5% ketaminhydrochloride (50 mg/kg body weight) and 2% xylazinhydrochloride (10 mg/kg body weight).

The animals were divided into two groups, cisplatin and control (NaCl 0.9% (w/v)). In the cisplatin group (n=6) 10 mg/kg body weight of cisplatin (0.5 mg/ml, Bristol-Myers Squibb, Tokyo, Japan) dissolved in physiological saline [NaCl 0.9% (w/v)] was injected i.p. In the control group (n=6), only physiological saline [10 ml/kg, NaCl 0.9% (w/v)] was injected. The care and use of the animals reported on in this study were approved by the ethical committee of animal experimentation of Nippon Medical School.

Correspondence to K-i Watanabe, Department of Oto-Rhino-Laryngology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan.
Tel: (+81) 3 3822 2131; Fax: (+81) 3 5685 0830;
E-mail: bxp02646@nifty.ne.jp

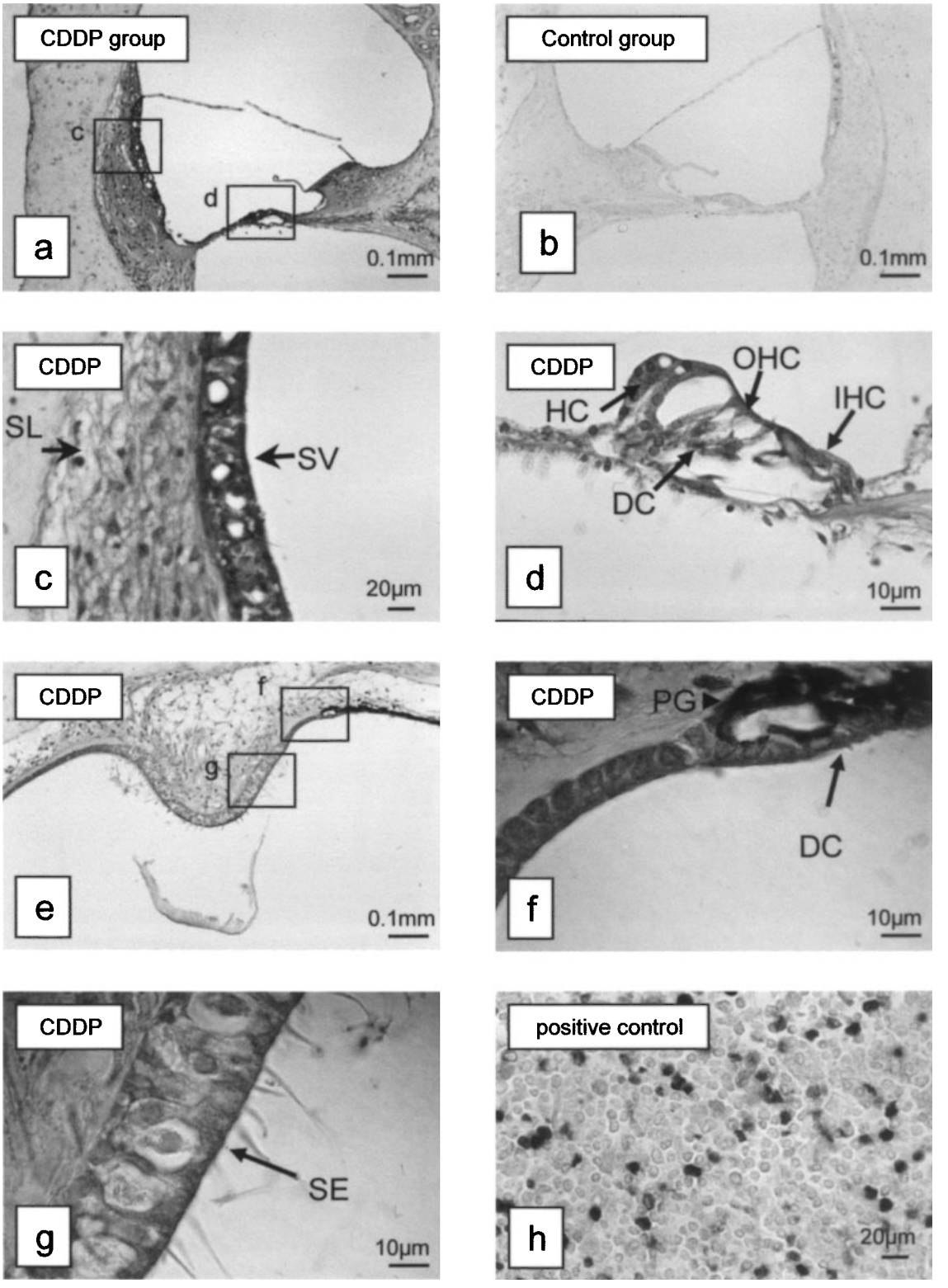

Figure 1. Paraffin sections of the inner ear 3 days after the administration of cisplatin, 8 μm. Immunohistochemistry, anti-MPO antibody. (a) Cisplatin group, anti-MPO antibody: MPO is mainly expressed in the organ of Corti (CO) and stria vascularis (SV), $\times$15. (b) Control group, anti-MPO antibody: no MPO immunoreactivity was seen in the tissues which received the injection of physiological saline (control group), $\times$15. (c) Cisplatin group, anti-MPO antibody: in the lateral wall, the SV show positive staining. The spiral ligament (SL) exhibited weak immunoreactivity for MPO, $\times$150. (d) Cisplatin group, anti-MPO antibody:

Immunohistochemical examination

All animals were sacrificed after 3 days. The tissues were fixed via cardiac perfusion with 4% paraformaldehyde (pH 7.4) after flushing out red blood cells with phosphate buffer solution (PBS). Temporal bones were removed. The inner ears were immersed in the same fixative overnight. The specimens were embedded in paraffin after decalcification with 10% EDTA solution in Tris at pH 7.0 for 7 days. Each specimen was sectioned into slices of 8 μm thickness. After removing paraffin, the sections were immersed in 5% H_2O_2 for 30 min and then in 0.25% Triton 100 for 10 min. Subsequently, they were incubated with the primary antibody to MPO at 1:4000 dilution (rabbit polyclonal antibody; Dako, Glostrup, Denmark) overnight. After rinsing with 0.1% Tris buffer solution and normal goat serum, the tissues were incubated with the second antibody at 1:400 dilution (anti-rabbit, Dako). Processing was ultimately performed with a horseradish peroxidase complex at 1 : 100 dilution for 1 h and a nickel-enhanced DAB (Wako, Osaka, Japan).

Results

Expression of MPO

Immunoreactivity for MPO could not be observed in all tissues of the control group (Figure 1b). The spleen exhibited a positive staining for MPO (Figure 1h). In the cisplatin group, immunoreactivity was detected after the injection of cisplatin (Figure 1a and e). MPO immunoreactivity was evident in the structures of the stria vascularis (Figure 1c), in the supporting cells of the organ of Corti and Hensen's cell (Figure 1d). This immunoreactivity for MPO was observed in all turns of the cochlea.

In the vestibule, MPO immunoreactivity became evident in the structures of the supporting cells of the sensory epithelium (Figure 1g) and dark cells (Figure 1f). Immunoreactivity for MPO was detected in the cytoplasm of the supporting cells. Deformity of sensory hairs was also detected (Figure 1g).

Discussion

Cisplatin is widely used in the treatment of patients with cancer. Cisplatin reacts with DNA and exerts cytotoxicity by inducing a rapid intra-strand cross-linking.[12] In the tumor cells, cisplatin inhibits the replication of cells. After the clinical application of cisplatin, many authors reported the ototoxic effects of cisplatin, such as heating disturbance, vertigo or tinnitus.[13–15] The normal cells which organize the inner ear do not have much chance to proliferate. Recently ROS have become known to be involved in many tissues after the injection of cisplatin.

ROS are known to have cytotoxic properties. It is well known that neutrophils release MPO when they respond to some stimuli. MPO catalyzed ROS are capable of not only killing bacteria or tumor cells but also injuring the surrounding tissues. MPO reacts with H_2O_2. The MPO–H_2O_2 complex generates HCOCl, OCl^- and Cl_2. The toxicity of H_2O_2 is accelerated by reaction with MPO. Johnson *et al.*[16,17] stated that cell injury by ROS might involve lipid peroxidation, oxidation of sulhydryl, iron sulfur group, chloramines and aldehyde. In the kidney, cisplatin induces the generation of free radical species and subsequent lipid peroxidation.[18] Clerici *et al.*[1] reported that ROS were detected in cochlear explants after exposure to ototoxic substances. From these reports, it is supposed that part of the cytotoxicity of cisplatin is due to the ROS.

Our study revealed that the cochlea and vestibule exhibited immunoreactivity for MPO. In the cochlea, the stria vascularis, supporting cells of the organ of Corti and in the vestibule, the dark cells and the sensory epithelium of the crista ampularris showed immunoreactivity to MPO. Injected cisplatin exerts its toxicity via blood vessels. The stria vascularis supply energy to the inner ear. MPO catalyzed ROS might influence the function of the stria vascularis. The degeneration of supporting cells precedes the damage of sensory cells, both in the cochlea and vestibule.[19] Then, the homeostasis of the inner ear could be lost.

The immediate timing of the expression of MPO after the application of cisplatin is still obscure. Oyanagi[20] reported cisplatin-stimulated neutrophils to produce superoxide radicals. On the other hand, Hara *et al.*

CO is shown. MPO is positive in the supporting cells, Hensen's cells (HC) and Deiter's cell (DC). There is a weak immunoreactivity in the sensory cells (outer and inner hair cells, OHC/IHC), $\times$150. (e) Cisplatin group, anti-MPO antibody: the crista ampullaris is shown, $\times$15. (f) Cisplatin group, anti-MPO antibody: dark cells of vestibule exhibited a positive reactivity for MPO. Normal pigmentation was observed in the dark cells (arrow head), $\times$150. (g) Cisplatin group, anti-MPO antibody: in the sensory epithelium, MPO immunoreacitivity was detected in the supporting cells and sensory cells, $\times$150. (h) Positive control, anti-MPO antibody: the tissue of normal spleen exhibits an immuno-reactivity for MPO, $\times$30.

reported that cisplatin inhibited the production of superoxide by neutrophils. ROS are generated via a variety of pathways associated with many chemical substances, enzymes and signal messengers. Hara *et al.*[21] speculated that the cell membrane of neutrophils might be influenced by the action of cisplatin. Ueta *et al.*[22] also pointed out that the combined administration of 5-FU and cisplatin suppressed the generation of ROS and MPO activity. Cisplatin has cytotoxic effects. Many chemical mediators could be released from damaged cells as a result of the cytotoxic effects of cisplatin. Cytotoxicity leads a release of cytokines from damaged cells. Macrophages treated with cisplatin and cytokines produced larger amounts of ROS than those treated with only cisplatin or cytokine.[23] Thus, cytokines accelerate the release of MPO by neutrophils.

ROS plays an important role under pathological conditions. Inducible nitric oxide synthase catalyzes high amounts of nitric oxide (NO) and NO affects inner ear dysfunction.[9–11]. NO also regulates the catalytic activity of MPO.[24] A variety of reactions cascades and acts mutually. Our study showed that MPO was expressed in the inner ear after the injection of cisplatin. It is supposed that the MPO–ROS pathway could be one of pathways of inner ear disturbance during chemotherapy.

Conclusion

MPO was expressed in the organ of Corti, the stria vascularis, and the supporting cells and dark cells of the crista ampullariss of the cisplatin group. Our results suggest, in accordance with immunohistological observation, that MPO expression and ROS could contribute to the ototoxic effect of cisplatin.

References

1. Clerici WJ, Hensley K, DiMartino DL, Butterfield DA. Direct detection of ototoxicant-induced reactive oxygen species generation in cochlear explants. *Hear Res* 1996; **98**: 116–24.
2. Watanabe K, Hess A, Bloch W, Michel O. Expression of inducible of guinea pigs after the application of cisplatin. *Anti-Cancer Drugs* 2000; **11**: 29–32.
3. Watanabe K, Hess A, Bloch W, Michel O. Nitric oxide synthase inhibitor suppresses the ototoxic side effect of cisplatin in guinea pigs. *Anti-Cancer Drugs* 2000; **11**: 401–6.
4. Murray RK. Red and white blood cells. In: Murray RK, Granner DK, Mayes PA, Rodwell VW, eds. *Harper's biochemistry*. Stanford, CA: Appleton & Lange 1996: 766–75.
5. Hampton MB, Kettle AJ, Winterbourn. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* 1998; **92**: 3007–17.
6. Klebanoff SJ. Myeloperoxidase. *Proc Ass Am Physic* 1999; **111**: 383–9.
7. Hazen SL, Hsu FF, Mueller DM, Crowley JR, Heinecke JW. Human neutrophils employ chlorine gas as an oxidant during phgocytosis. *J Clin Invest* 1996; **98**: 1283–9.
8. Kitahara M, Eyre HJ, Simonian Y, Atkin CL, Hasstedt SJ. Hereditary myeloperoxidase deficiency. *Blood* 1981; **57**: 888–893.
9. Beckmann J, Koppenol W. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and the ugly. *Am J Physiol* 1996; **271**: 1424–37.
10. Takumida M, Zhang D M, Anniko M. Localization of nitric oxide synthase isoforms (I, II and III) in the endolymphatic sac of the guinea pig. *Otorhinolaryngol* 1997; **59**: 311–3.
11. Watanabe K, Hess O, Bloch W, Michel O. Inhibition of inducible nitric oxide synthase lowers the cochlear damage by lipopolysaccharide in guinea pigs. *Free Radic Res* 2000; **32**: 363–70.
12. Tognella S. Pharmacological interventions to reduce platinum-induced toxicity. *Cancer Treat Rev* 1990; **17**: 139–42.
13. Moroso MJ, Blair RL. A review of *cis*-platinum ototoxicity. *J Otolaryngol* 1983; **12**: 365–9.
14. Komune S, Asakuma S, Snow JB. Pathophysiology of the ototoxicity of *cis*-diamminedichloroplatinum. *Otolaryngol Head Neck Surg* 1981; **89**: 275–82.
15. Laurell G, Bagger-Sjöbäck D. Dose-dependent inner ear changes after i.v. administration of cisplatin. *J Otolarngol* 1991; **20**: 158–67.
16. Johnson RJ, Couser WG, Chi EY, Adler S, Klebanoff SJ. A new mechanism of glomerular injury: the myeloperoxidase-hydrogen peroxide halide system. *J Clin Invest* 1987; **79**: 1379–87.
17. Johnson RJ, Klebanoff SJ, Ochi RF, *et al*. Participation of the myeloperoxidase–H_2O_2–halide system in immune complex nephritis. *Kidney Int* 1987; **32**: 342–9.
18. Srivastava RC, Farookh A, Ahmad N, Misra M, Hasan SK, Husain MM. Evidence for the involvement of nitric oxide in cisplatin-treated toxicity in rats. *BioMetals* 1996; **9**: 139–42.
19. Estrem SA, Babin RW, Ryu JH, Moore KC. *Cis*-diamminedichloroplatinum (II) ototoxicity in the guinea pig. *Otolaryngol Head Neck Surg* 1981; **89**: 638–45.
20. Oyanagi Y. Stimulatory effect of platinum (IV) ion on the production of superoxide radical. *Biochem Pharmacol* 1977; **26**: 473–6.
21. Hara N, Ichinose Y, Motohiro A, Kuda T, Aso H, Ohta M. Influence of chemotherapeutic agents on superoxide anion production by human polymorphonuclear leukocytes. *Cancer* 1990; **66**: 684–8.
22. Ueta E, Osaki T. Suppression by anticancer agents of reactive oxygen generation from polymorphonuclear leukocytes. *Free Radic Res* 1996; **24**: 39–53.
23. Ranjan P, Sodhi A, Singh SM. Murine peritoneal macrophages treated with cisplatin and interferon-γ undergo NO-mediated apoptosis via activation of an endonuclease. *Anti-Cancer Drugs* 1998; **9**: 333–41.
24. Abu-Soud HM, Hazen SL. Nitric oxide modulates the catalytic activity of myeloperoxidase. *J Biol Chem* 2000; **275**: 5425–30.

(Received 4 July 2000; accepted 18 July 2000)