ARTICLE
Journal of Cellular Biochemistry 119:424–430 (2018)

Journal of Cellular Biochemistry

Effect of rMnSOD on Sodium Reabsorption in Renal Proximal Tubule in Ochratoxin A–Treated Rats

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ABSTRACT

Ochratoxin A (OTA) is a mycotoxin produced by *Aspergillus* and *Penicillium* that represent toxic real threat for human beings and animal health. In this study we evaluated the effect of a new recombinant mitochondrial manganese containing superoxide dismutase (rMnSOD) on oxidative stress and on the alterations of fluid reabsorption in renal proximal tubule (PT) as possible causes of OTA nephrotoxicity. Finally, we have measured the concentration of O_2^- in the kidney through dihydroethidium assay (DHE) and nitric oxide (NO) concentration through nitrites and nitrates assay. Male Sprague Dawley rats weighing 120–150 g were treated for 14 days by gavage, as follows: Control group, 12 rats received a corresponding amount of saline solution (including 10% DMSO); rMnSOD group, 12 rats treated with rMnSOD (10 μg/kg bw); OTA group, 12 rats treated with OTA (0.5 mg/kg bw) dissolved in 10% DMSO and then scaled to required volume with corn oil; rMnSOD + OTA, 12 rats treated with rMnSOD (10 μg/kg bw) plus OTA (0.5 mg/kg bw). Our results have shown that rMnSOD restores the alteration of reabsorption in PT in rats treated with OTA plus rMnSOD, probably through the response to pressure natriuresis, where nitric oxide plays a key role. Moreover, rMnSOD prevents the nephrotoxicity induced by OTA probably restoring the balance between superoxide and NO that is most probably the cause of hypertension and renal functional alterations through the inhibition of NO synthase. In conclusion these data provide important information for understanding of mechanism of toxic action of OTA. J. Cell. Biochem. 119: 424–430, 2018.

KEY WORDS: OCHRATOXIN A; rMnSOD; NITRIC OXIDE; MICROPUNTURE; ANTIOXIDANTS

Ochratoxin A (OTA) is a natural mycotoxin produced by filamentous mold species belonging to the genera *Aspergillus* and *Penicillium*. It is distributed in food and feedstuffs due to fungi contamination in susceptible agricultural commodities [Wu et al., 2011]. Several studies have shown that OTA is nephrotoxic, hepatotoxic, teratogenic, neurotoxic, immunotoxic, genotoxic, and carcinogenic in several animal species, with the longest half-life in human blood [Ringot et al., 2006; El Khoury and Atoui, 2010; Malir et al., 2013]. In addition, chronic exposure to low doses could be more dangerous that acute exposure to high doses [Pfohl-Leszkowicz and Manderville, 2007]. OTA is absorbed in small intestinal absorption, enters in the bloodstream where it binds serum proteins and it is subsequently distributed to different tissues and organs [Hagelberg et al., 1989]. Humans are exposed to OTA through various routes, especially through dietary intake, as well as through skin contact or inhalation exposure, although they are less important than the food [Iavicoli et al., 2002; Degen et al., 2007].

The kidney is the primary target of OTA. It has been shown to induce Balkan Endemic Nephropathy, kidney tumors and chronic interstitial nephropathy [Maaroufi et al., 1995; Fuchs and Peraica, 2005]. Kidney lesions have been observed in the proximal tubule (PT), regarded the main intrarenal target of the OTA, such as damage of epithelial cells, reduction of the size and the density of brush border. Moreover, the hystological data shows an enlargement of tubular membrane [Schaaf et al., 2002].

The mechanism of OTA toxicity has not yet been clearly elucidated. Several studies have demonstrated that OTA induced nephrotoxicity and hepatotoxicity through oxidative DNA damage

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Manuscript Received: 5 June 2017; Manuscript Accepted: 6 June 2017
Accepted manuscript online in Wiley Online Library (wileyonlinelibrary.com): 7 June 2017
DOI 10.1002/jcb.26197 • © 2017 Wiley Periodicals, Inc.

in vitro [Kamp et al., 2005] and in vivo [Meki and Hussein, 2001; Di Giacomo et al., 2007]. Physical, chemical, and biological methods have been developed to reduce the toxic action of OTA [Zinedine et al., 2007; Battacone et al., 2010]. In several studies, the attention has been focused on natural antioxidant constituents, as such as polyphenols, vitamin C, vitamin E, and retinol (vitamin A) [Bose and Sinha, 1994; Kumari and Sinha, 1994; Hoehler and Marquardt, 1996; Grosse et al., 1997]. Polyphenols are secondary metabolism products of plants, and constitute one of the most numerous and widely distributed group of natural antioxidants that actually appear reduce the toxic effects caused by OTA. In contrast, vitamin E seems to not counteract the toxicity of OTA in HepG2 cells [Gayathri et al., 2015].

In an our previously paper we have evaluated the mechanism of chronic nephrotoxic diseases induced by OTA. In particular, we have demonstrated, in a rat animal model treated for 2 weeks with OTA that the increase in Reactive Oxigen Species (ROS) productions was linked to a strong reduction of glomerular filtration rate (GFR) and with a significant increase in blood pressure (BP). Moreover, in rat treated with OTA plus the new recombinant mitochondrial manganese superoxide dismutases (rMnSOD), the kidney functions and the lipidic peroxidation levels were preserved [Ciarcia et al., 2016].

rMnSOD ia a new isoform of human MnSOD that was isolated and obtained in a synthetic recombinant form. It is able to enter into cells and it appears to have antioxidant and antitumor activities [Mancini et al., 1999, 2006]. Moreover, rMnSOD is radioprotective for healthy cells and radiosensitive for cancer cells [Borrelli et al., 2009], it displays a specific and selective cytotoxic activity against tumor cells expressing the oestrogen receptor [Mancini et al., 2008]. We have also demonstrated, that rMnSOD provides protection to rat kidneys treated with cyclosporine A, allowing for the recovery of 80% of their GFR [Damiano et al., 2013].

In the present paper, the effects of rMnSOD on renal kidney injury induced by OTA were evaluated. In particular, we have studied the involvement of PT through in vivo micropuncture studies. In addition, we have analyzed the alteration of the most important sodium transport in the proximal tubule, the sodium-hydrogen exchangers (NHE3) to determine whether the change in absorption of liquids in PT was linked to sodium reabsorption change. Finally, we have measured the concentration of O_2^- in the kidney through dihydroethidium assay (DHE) and nitric oxide (NO) concentration through nitrites and nitrates assay.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

The rats were obtained from Harlan Laboratories Srl (San Pietro al Natisone, Udine, Italy); Ochratoxin A (OTA), FITC-inulin and all the reagents were provided by Sigma–Aldrich (Milan, Italy), rMnSOD was obtained by Laedhexa Biotechnologies Inc., San Francisco, CA and the, Department of Experimental Oncology, Istituto Nazionale Tumori "Fondazione G. Pascale," IRCCS (Naples, Italy) [Mancini et al., 2006]. Anti-NHE3 antibody were purchased by Tema Ricerca srl (Bologna, Italy).

ETHICS STATEMENT

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by Italian Ministry of Health (Permit Number: 2015-261-PR).

EXPERIMENTAL PROTOCOL

Experiments were performed on a total of 48 adult male Sprague Dawley rats weighing 120–150 g. They were housed under constant environmental conditions (temperature 22°C and a 12 h light–dark cycle). Animals were fed a standard diet; food and water were given ad libitum. The rats, randomly divided into four groups, were treated for 14 days by gavage, as follows: Group 1 (Control group), 12 rats received a corresponding amount of saline solution (including 10% DMSO); Group 2 (rMnSOD group), 12 rats treated with rMnSOD (10 μg/kg bw); Group 3 (OTA group): 12 rats treated with OTA (0.5 mg/kg bw) dissolved in 10% DMSO and then scaled to required volume with corn oil; Group 4 (rMnSOD + OTA), 12 rats treated with rMnSOD (10 μg/kg bw) plus OTA (0.5 mg/Kg bw). The dose and length of rMnSOD and OTA administration was chosen according to previous experiments [Hoehler and Marquardt, 1996]. Blood Pressure (BP) was recorded in anaesthetized rats, during the in vivo micropuncture experiments, through the femoral artery using a blood pressure recorder (Pressure Monitor BP-1, Word Precision Instruments).

PREPARATION OF ANIMALS FOR IN VIVO MICROPUNCTURE EXPERIMENT

The rats were anaesthetized with 2% isoflurane (Isotec 4, Ohmeda), placed on a thermo-regulated table (37°C) and prepared for renal micropuncture evaluation. The right femoral artery was then cannulated with polyethylene tubing (PE 50) connected to a blood pressure transducer (Statham) for continuous monitoring. A second polyethylene tube was placed in the left jugular vein and connected to an infusion pump (Braun, Germany) that delivered a modified Ringer's solution (NaCl 125 mmol/L^{-1}; $NaHCO_3$ 25 mmol . L^{-1}) at a rate of 1.8 ml . h^{-1}. The left kidney was then exposed through a flank incision, cleared of perirenal fat and immobilized in a plastic kidney cup with agar (3% in saline), and then bathed with warmed mineral oil. Thirty to sixty minutes was allowed after surgery before beginning tubule micropuncture.

IN VIVO MICROPUNCTURE STUDY

The proximal tubule (PT) site was identified by injections from a "finding" pipette containing dye-stained artificial tubular fluid. The flow was blocked by injection of T grease (T grade, Apiezon Products, Manchester, UK) via a micropipette (10–12 μm OD) proximal to the perfusion site. The tubule was perfused with a micropipette (tip diameter 8–10 μm OD) connected to a micro-perfusion pump (model A1400, World Precision Instruments Inc, Sarasota, FL) at 20 ± 3 nl/min. The perfusion solution contained FITC-inulin (Sigma) as volume marker and 0.1% FD&C green dye for identification of the perfused loops. Tubules were perfused for 2 to 8 min before fluid collections, which were made at a downstream site with a micropipette (7–10 μm OD) after placement of a column of oil to block downstream flow.

Samples were collected for 3–5 min and transferred into a constant-bore capillary tube for which the length was measured with a micrometer to calculate the tubular fluid volume. Thereafter, FITC-inulin fluorescence was determined in samples and standards using a 96-well plate reader with excitation and emission wavelengths of 490 and 520 nm, respectively. Collected samples with 95% and 105% of microperfused inulin were discarded. To determine the lengths of the perfused segments, tubules were filled with high-viscosity microfil (Flow Tech, Inc. Carver, MA). At the end of the experiments, the kidney was partially digested in 20% NaOH, and the casts were measured under a dissecting microscope. The Absoluted fluid reabsorption (Jv) was calculated by the difference in the perfusion rate and the collection rate factored by the length of the nephron: $Jv = V_{perf}$ (nanoliters for minute) $- V_{coll}$ (nanoliters per minute)/PT length (millimeters) where V_{perf} indicates perfusion rate and V_{coll} indicates collection rate and expressed as nanoliters per minute per millimeter. The composition of the perfusion fluid was as follows in mM: 125 NaCl, 20 $NaHCO_3$, 5 KCl, 1 $MgSO_4$, 2 CaCl, 1 $NaHPO_4$, 5 glucose, and 4 urea. At the end of each experiment, tubular fluid samples were transferred individually to 0.5 ml constant-bore Microcaps to determinate the volume. Then FITC-labeled inulin fluorescence was determined in samples and standards using a Spectra Max2 plate reader with excitation and emission wavelengths of 490 and 520 nm, respectively, according to Lorenz and Gruenstein (1999).

ANTI-NHE3 WESTERN BLOT EXPERIMENT

Western blot analysis was performed to determine the relative amounts of the protein present in different samples. Samples, prepared from cortical kidney tissues, were homogenized in a lysis buffer (sucrose 0.3 M, imidazole 0.5 M, EDTA 0.5 M,) with a protease inhibitor Mix (cat. n. 39102.01, SERVA) which protects proteins of interest from degradation. The samples were loaded on NuPage Bis-Tris gel 4–12% (Novex by Lifetech) with sample reducing buffer 10× (Invitrogen) and fourX LDS NuPAGE Sample Buffer (Invitrogen) after boiling for 15 min at 70°C. The 20× Mops SDS Buffer (Novex by Lifetechnologies) and NuPAGE Antioxidant (Invitrogen) were used for proteins electrophoretic separation for 60 min at 200 volts in a Novex Mini-cell electrophoresis chamber (Invitrogen). Later proteins were transfered to Invitrolon PVDF filter paper sandwich (0.4 μm pore size, Invitrogen), activated with methanol for 5 min, using 20× Transfer Buffer NuPAGE (Novex by Lifetech) for 70 min at 30 volts. The PVDF membrane was incubated with casein I-block (Tropix) for 1 h; then it was incubated with the primary rabbit polyclonal antibody anti NHE-3 (cat. n.TA 309445, OriGene), diluited 1:1000 in I-block over night at 4°C and with the primary rabbit polyclonal antibody anti actin (cat. n. TA 349206, OriGene), diluited 1:1000 in I-block over night at 4°C. The following day the blot, after washing with I-block three times for 5 min per time, was exposed to secondary antibody-enzyme conjugate to alkaline phosphatase (Applied Biosystems) for 1 h. The membrane was washed again as above and incubated with Western Light Plus Kit, CSPD, and Nitroblock (Tropix), for 5 min to develop the chemiluminescent signal. Proteins were visualized in the darkroom using photographic plate (X-RAY FILM, FUJIFILM) for some minutes. The molecular weight standards, SeeBlue Plus® Prestained Standard 11× (Invitrogen), was used to identify proteins of interest on PVDF filter paper. Densitometric analysis of the bands, normalized to B-actin, was performed with ImageJ.

SUPEROXIDE ASSAY IN THE DISSECTED AORTA

Superoxide concentrations within the cells of the abdominal aorta were evaluated by the oxidation of dihydroethidium (DHE; Molecular Probes). DHE can enter the cell and be oxidized by superoxide to yield ethidium (Eth), which binds DNA producing bright red fluorescence. The increase in Eth-DNA fluorescence indicates peroxide production within cells (30). The Aorta was dissected and frozen at −80°C. Sections (10 μm thick) were cut at the cryostat (Leica CM 1850) and then incubated with a 20 μM DHE solution for 30 min at room temperature. Cover slips were mounted with Dako Fluorescence Mounting Medium. Pictures were acquired at the Leica DMI6000 B inverted microscope. Fluorescence intensity (IF) was quantified by Imagej software. Briefly, average pixel intensity was quantified from three equal random areas from each sample. The average pixel intensity was compared among groups. The data were expressed as IF/g of tissue x micrograms of proteins.

NITRITE AND NITRATE ASSAY

The production of nitrite (NO_2) and nitrate (NO_3), stable metabolites of NO production, was determined in the supernatant of kidney cytosols by Griess reagent. Nitrate was reduced to nitrite by addition of nitrate reductase (0.4 U/ml) in the presence of 10 mM NADPH and 2.5 mM flavin adenin dinucleotide and then assayed as nitrite. The plates were incubated with the Griess reagent at 25°C under reduced light for 20 min. Absorbance was read at 550 nm using a Perkin Elmer UV Spectrophotometer. The concentration of NO_2 was calculated on a calibration curve (range: 0.125–16 μg/ml), prepared using dilutions of sodium nitrate in the plating medium. The data were expressed as picomoles of nitrite for milligrams of proteins.

STATISTICAL ANALYSIS

All data are mean ± SD. Statistical analysis was performed by one-way ANOVA followed by the unpaired *t*-test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

EFFECT OF OTA ON BLOOD PRESSURE

As shown in the Figure 1, OTA significantly increased blood pressure (BP), expressed as mmHg, at 14 days of the treatment compared to control. In fact BP values shifted by 98 ± 4 control to 130 ± 2 OTA ($^{*}P < 0.05$ respect to control). However, the administration of rMnSOD plus OTA restored this function from 130 ± 2 OTA to 96 ± 2 rMnSOD + OTA ($^{\#}P < 0.05$). rMnSOD, utilized alone, had no effect on BP (104 ± 4).

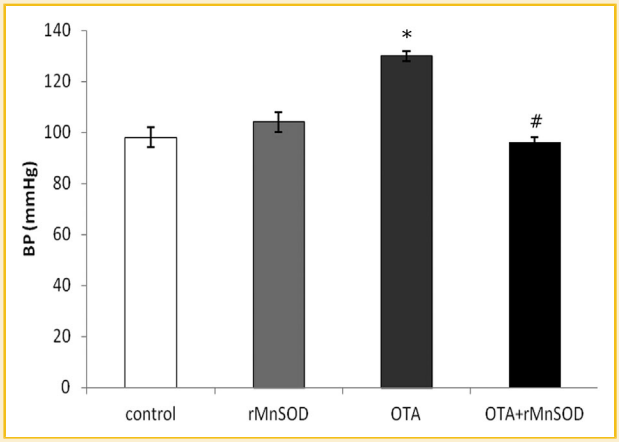

Fig. 1. Effects of OTA and rMnSOD used alone or in association on blood pressure (BP) in rats treated for 2 weeks. OTA treatment significantly increased BP, while co-administration with rMnSOD restored this effect (values mean ± SD; $^{*}P < 0.05$ vs. control group; $^{\#}P < 0.05$ vs. OTA group).

IN VIVO MICROPUNCTURE STUDY EVALUATION

As shown in Figure 2, the absoluted fluid reabsorption (Jv) measured in the PT averaged 2.5 ± 0.3 nl/min/mm in control rats. However, Jv was lower in PT of rat treated with OTA (1.6 ± 0.2 nl/min/mm, ($^{*}P < 0.05$ respect to control). Treatment with rMnSOD plus OTA restored the Jv to normal levels (2.3 ± 0.3 nl/min/mm respect to 2.5 ± 0.3 nl/min/mm) ($^{\#}P < 0.05$ respect OTA alone). RMnSOD alone had no effect on Jv (2.4 ± 0.4 nl/min/mm).

WESTERN BLOT ANALYSIS OF NHE3

NHE3 protein abundance was determined by western blot analysis, as shown in Figure 3. The antibody recognized a protein with a molecular mass between 66 and 116 kDa. Densitometric analysis have demonstrated that NHE3 abundance, normalized for β-actin, is decreased in OTA group (control 17589 ± 818 respect to OTA 6607 ± 1961, $^{*}P < 0.05$). Moreover, animals treated with OTA plus rMnSOD were similar to the control group (17516 ± 1391). rMnSOD alone had no effect on NHE3 protein abundance respect to the control (22907 ± 1403).

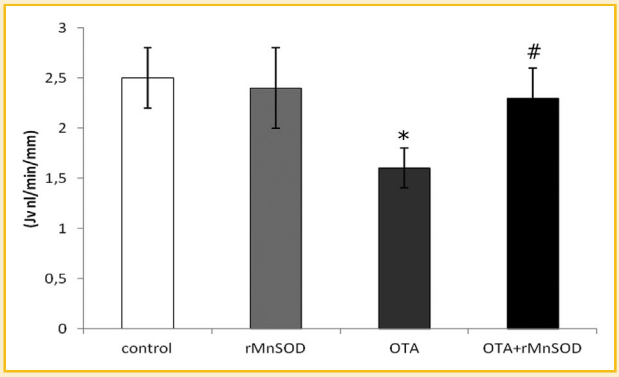

Fig. 2. Effects of OTA and rMnSOD used alone or in association on absoluted fluid reabsorption (Jv) in proximal tubule after 2 weeks of treatment. OTA treatment significantly decreased Jv in PT, while co-administration with rMnSOD partially restored this effect (values mean ± SD; $^{*}P < 0.05$ vs. control group; $^{\#}P < 0.05$ respect OTA group).

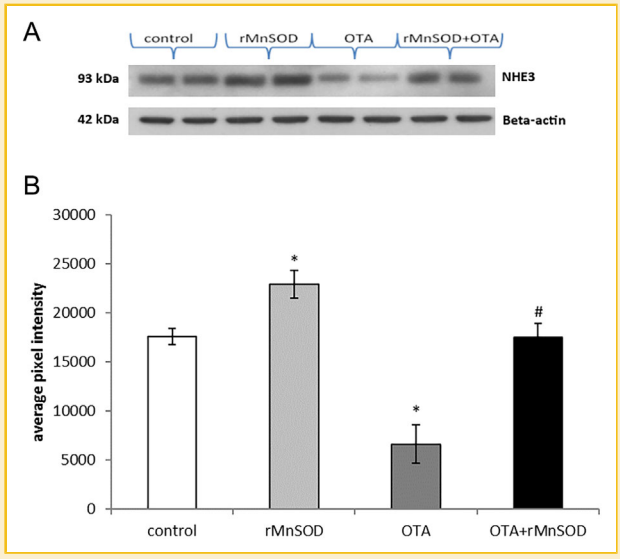

Fig. 3. Effects of OTA and rMnSOD used alone or in association on sodium-hydrogen exchanger isoform 3 (NHE3) expression in the cortical rat kidney after 2 weeks of treatment. OTA treatment significantly decreased the NHE3 expression, while co-administration with rMnSOD partially prevent this effect, on the contrary rMnSOD used alone significantly increased the NHE3 expression (values mean ± SD; $^{*}P < 0.05$ vs. control group; $^{\#}P < 0.05$ respect OTA group). (A) shows the band intensity NHE3/beta-actin.Western blot analysis of sodium transporter (NHE3) in the cortical kidney tissue. (B) Summary graphs represent the average relative density of the bands (normalized to β-actin) in the groups. $^{*}P < 0.05$ versus control rats.

EFFECTS OF rMnSOD ON OTA-INDUCED OXIDATIVE STRESS

The red fluorescence generated by the binding of the Eth-DNA complex was showed that the abdominal aorta of OTA treated animals presented a red fluorescent signal significantly brighter than control (52532 ± 4614 vs. 22522 ± 5715, $^{*}P < 0.05$) (Fig. 4A, C). When the rats were treated with OTA plus rMnSOD, the fluorescence intensity was significantly lower than the OTA-treated group (33101 ± 3251) (Fig. 4D), indicating that rMnSOD was able to completely prevent the production of superoxide induced by OTA. rMnSOD treatment had no effect on the fluorescence intensity (31251 ± 4210) (Fig. 4B).

NITRITE AND NITRATE ASSAY

As shown in the Figure 5, OTA decreased the NO production (express as pmoles/mg of proteins) in rat kidney cytosol respect to control (12.51 ± 0.8 OTA respect to 25.08 ± 1.0 control, $^{*}P < 0.05$). Treatment with rMnSOD plus OTA restored the levels of NO concentrations respect to OTA from 12.51 ± 0.8 OTA to 23.02 ± 2 rMnSOD + OTA group. rMnSOD used alone, did not show any significant difference respect to control (25.08 ± 1.0 and 24.09 ± 1.1, respectively).

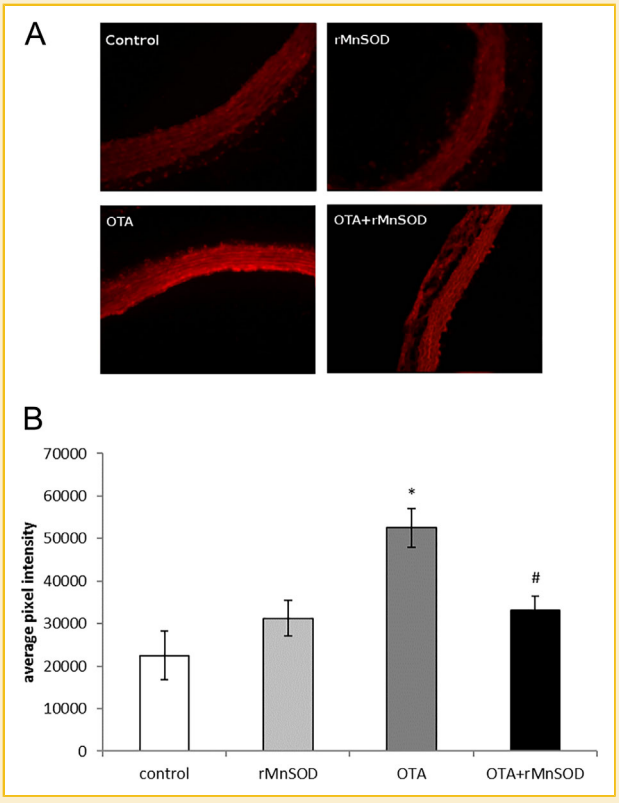

Fig. 4. Effects of OTA and rMnSOD used alone or in association on O_2^- production in rat kidney after 2 weeks of treatment by dihydroethidium (DHE) assay. (A) Representative pictures of DHE staining on the rat aorta. OTA treatment induces an increase of DHE fluorescence signal as the expression of superoxide-mediated intracellular injury. rMnSOD prevents OTA-induced injury on the aorta. (B) Fluorescence intensity quantification. Fluorescence intensity was quantified by Imagej software. Briefly, average pixel intensity was quantified from three equal random areas from each sample (values mean ± SD; $^*P < 0.05$ vs. control group; $^\#P < 0.05$ respect OTA alone).

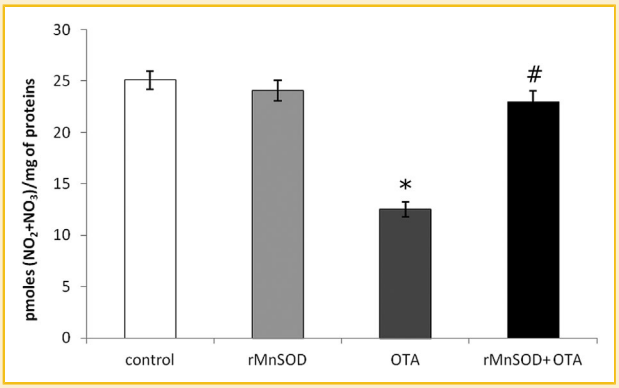

Fig. 5. Effects of OTA and rMnSOD used alone or in association on nitric oxide (NO) production in rat kidney after 2 weeks of treatment measured by Griess reagent. NO production is reduced OTA treatment showed a significant reduction in NO respect to control. (values are mean ± SD, $^*P < 0.05$ vs. control group; $^\#P < 0.05$ respect OTA alone).

DISCUSSION

Although the correct mechanism of OTA nephrotoxicity is yet unclear, several studies have proposed the involvement of Reactive Oxigen Species (ROS) [Bhat et al., 2016; Ciarcia et al., 2016]. It has been shown a significant increase of ROS levels and a severe reduction of antioxidant enzymes in OTA treated cells showing lesions in the cell membrane, proteins, and DNA [Baudrimont et al., 1997; Liu et al., 2012]. In accordance with these authors, we have shown, in an our previous paper, that three important parameters of oxidative stress, such as malondialdehyde, SOD and catalase were altered in the kidney of OTA treated rats [Ciarcia et al., 2016]. In the present study we have measured the O_2^- and NO production. DHE can enter the cell and could be oxidized by superoxide to yield ethidium which binds DNA producing red fluorescence that indicates peroxide production within the cell [Carter et al., 1994]. Our results have shown that rMnSOD reduces the O_2^- production induced by OTA, confirming the ability of rMnSOD to scavenge many species of free radicals, probably through the presence of the leader peptide that allows the rMnSOD to enter the cell preventing its degradation by circulating proteases. We have also investigated the link between the O_2^- and NO production, finding that the increase of O_2^- was linked to the reduction of the NO concentration. The link O_2^- /NO is most probably the cause of hypertension and renal functional alterations through the inhibition of NO synthase (NOS). In our animal model of rat treated with OTA for 14 days, we have found a significant increase in blood pressure, such finding agree with the result of Wang et al. (2016) who observed a decrease of the vasodilator NO during some pathophysiological processes, such as hypertension.The most important finding of this work is that the fluid reabsorption in the PT of OTA treated group was impaired compared to control group. Treatment with rMnSOD restored the lower value of Jv in PT, suggesting that the hypertensive effect of OTA could be related to O_2^-. Moreover, our data have shown a decrease of NHE3 expression in hypertensive rats treated with OTA. NHE3 is the major transporter of sodium in the PT and it is involved in the pathogenesis of hypertension [Dagher and Sauterey, 1992]. Thus, our data demostrated that the decrease in PT by NHE3 plays a crucial role in the hypertension induced by OTA. In fact, it has been demostrated that sodium transport is inibited in the PT when the blood pressure increases and NO has been identified as pivotal candidate to mediate this process, though the pressure–natriuresis response [Salom et al., 1992]. Some experiments have demonstrated that the block of the basal synthesis of NOS by Ng-monomethyl-L-arginine induced a significant decrease of Jv and HCO3 in the PT in the rat kidney [Wang, 1997; Wang et al., 2000] and a similar result has been observed by Wang [2002] in NOS knockout mice. The physiological importance of the regulation in PT by NOS is unclear. A previous study has demonstrated a stimulatory pathway at the low NO concentration and an inhibitory pathway at high NO concentrations [Dagher and Sauterey, 1992], indicating that NO regulates the transport of Na^+ and HCO_3^- in the PT.

Our data, together, suggest that O_2^- inhibits the activity of NHE3 probably through the link O_2^-/NO. In fact, some authors have demonstrated that the NHE3 activity is inactivated in Spontaneously Hypertensive Rat, probably through the involvement of the regulatory factor for NHE3. In accordance with this hypertensive model, in this paper we have shown a severe decrease of NHE3 activity in PT in OTA hypertensive rats. Therefore, it is possible that high renal levels of O_2^- lead to translocation of NHE3, and that the antioxidant rMnSOD could reactivate this pathway. In conclusion, in this paper have demostrated that O_2^- could reduces NHE3 activity and also inhibits fluid uptake. These data suggest that increased reactive oxygen species alters proximal tubular reabsorption during hypertension induced by OTA and impacts renal regulation of fluid and solute balance.

ACKNOWLEDGMENTS

We thank Leonida Manco for technical assistance during the experiments.

REFERENCES

Battacone G, Nudda A, Pulina G. 2010. Effects of ochratoxin a on livestock production. Toxins 2(7):1796–1824.

Baudrimont I, Ahouandjivo R, Creppy EE. 1997. Prevention of lipid peroxidation induced by ochratoxin A in Vero cells in culture by several agents. Chem Biol Interact 104(1):29–40.

Bhat PV, Pandareesh MD, Khanum F, Tamatam A. 2016. Cytotoxic effects of ochratoxin a in neuro-2a cells: Role of oxidative stress evidenced by N-acetylcysteine. Front Microbiol 7:1142.

Borrelli A, Schiattarella A, Mancini R, Morrica B, Cerciello V, Mormile M, d'Alesio V, Bottalico L, Morelli F, D'Armiento M, D'Armiento FP, Mancini A. 2009. A recombinant MnSOD is radioprotective for normal cells and radiosensitizing for tumor cells. Free Radic Biol Med 46(1):110–116.

Bose S, Sinha SP. 1994. Modulation of ochratoxin-produced genotoxicity in mice by vitamin C. Food Chem Toxicol 32(6):53–37.

Carter WO, Narayanan PK, Robinson JP. 1994. Intracellular hydrogen peroxide and superoxide anion detection in endothelial cells. J Leukoc Biol 55:253–258.

Ciarcia R, Damiano S, Squillacioti C, Mirabella N, Pagnini U, Florio A, Severino L, Capasso G, Borrelli A, Mancini A, Boffo S, Romano G, Giordano A, Florio S. 2016. Recombinant mitochondrial manganese containing superoxide dismutase protects against ochratoxin A-Induced nephrotoxicity. J Cell Biochem 117(6):1352–1358.

Dagher G, Sauterey C. 1992. H_ pump and Na-H exchange in isolated single proximal tubules of spontaneously hypertensive rats. J Hypertens 10:969–978.

Damiano S, Trepiccione F, Ciarcia R, Scanni R, Spagnuolo M, Manco L, Borrelli A, Capasso C, Mancini R, Schiattarella A, Iervolino A, Zacchia E, Bata-Csere A, Florio S, Anastasio P, Pollastro R, Mancini A, Capasso G. 2013. A new recombinant MnSOD prevents the cyclosporine A-induced renal impairment. Nephrol Dial Transplant 28(8):2066–2072.

Degen G, Mayer S, Blaszkewicz M. 2007. Biomonitoring of Ocratoxin A in grain workers. Micotoxin Res 23:88–93.

Di Giacomo C, Acquaviva R, Piva A, Sorrenti V, Vanella L, Piva G, Casadei G, La Fauci L, Ritieni A, Bognanno M, Di Renzo L, Barcellona ML, Morlacchini M, Galvano F. 2007. Protective effect of cyanidin 3-O-beta-D-glucoside on ochratoxin A-mediated damage in the rat. Br J Nutr 98(5):937–943.

El Khoury A, Atoui A. 2010. Ochratoxin A: General overview and actual molecular status. Toxins 2:461–493.

Fuchs R, Peraica M. 2005. Ochratoxin A in human kidney diseases. Food Addit Contam 22(Suppl1):53–57.

Gayathri L, Dhivya R, Dhanasekaran D, Periasamy VS, Alshatwi AA, Akbarsha MA. 2015. Hepatotoxic effect of ochratoxin A and citrinin, alone and in combination, and protective effect of vitamin E: In vitro study in HepG2 cell. Food Chem Toxicol 83:151–163.

Grosse Y, Chekir-Ghedira L, Huc A, Obrecht-Pflumio S, Dirheimer G, Bacha H, Pfohl-Leszkowicz A. 1997. Retinol, ascorbic acid and alpha-tocopherol prevent DNA adduct formation in mice treated with the mycotoxins ochratoxin A and zearalenone. Cancer Lett 114(1-2):225–229.

Hagelberg S, Hult K, Fuchs R. 1989. Toxicokinetics of ochratoxin A in several species and its plasma-binding properties. J Appl Toxicol 9(2):91–96.

Hoehler D, Marquardt RR. 1996. Influence of vitamins E and C on the toxic effects of ochratoxin A and T-2 toxin in chicks. Poult Sci 75(12):1508–1515.

Iavicoli L, Brera C, Carelli G, Caputi R, Marinaccio A, Miraglia M. 2002. External and internal dose in subjects occupationally exposed to Ochratoxin A. Int Arch Occup Environ Health 75:381–386.

Kamp HG, Eisenbrand G, Janzowski C, Kiossev J, Latendresse JR, Schlatter J, Turesky RJ. 2005. Ochratoxin A induces oxidative DNA damage in liver and kidney after oral dosing to rats. Mol Nutr Food Res 49(12):1160–1167.

Kumari D, Sinha SP. 1994. Effect of retinol on ochratoxin-produced genotoxicity in mice. Food Chem Toxicol 32(5):471–475.

Liu J, Wang Y, Cui J, Xing L, Shen H, Wu S, Lian H, Wang J, Yan X, Zhang X. 2012. Ochratoxin A induces oxidative DNA damage and G1 phase arrest in human peripheral blood mononuclear cells in vitro. Toxicol Lett 211(2): 164–171.

Lorenz JN, Gruenstein E. 1999. A simple, nonradioactive method for evaluating single-nephron filtration rate using FITC-inulin. Am J Physiol 276(1Pt 2):F172–F177.

Maaroufi K, Achour A, Hammami M, el May M, Betbeder AM, Ellouz F, Creppy EE and Bacha H. 1995. Ochratoxin A in human blood in relation to nephropathy in Tunisia. Hum Exp Toxicol 14(7):609–614.

Malir F, Ostry V, Pfohl-Leszkowicz A, Novotna E. 2013. Ochratoxin A: Developmental and reproductive toxicity-an overview. Birth Defects Res 98:493–502.

Mancini A, Borrelli A, Formisano S, Masucci MT, Maffeo A, Perla G, De Martino L, Bevilacqua N, Botti G, Maggino T. 1999. Establishment and growth regulation of a novel ovarian cancer cell line from a poorly-differentiated adenocarcinoma: Proposal for a new treatment. Eur J Gynaecol Oncol 20(1):45–52.

Mancini A, Borrelli A, Schiattarella A, Aloj L, Aurilio M, Morelli F, Pica A, Occhiello A, Lorizio R, Mancini R, Sica A, Mazzarella L, Sica F, Grieco P, Novellino E, Pagnozzi D, Pucci P, Rommelaere J. 2008. Biophysical and biochemical characterization of a liposarcoma-derived recombinant MnSOD protein acting as an anticancer agent. Int J Cancer 123(11): 2684–2695.

Mancini A, Borrelli A, Schiattarella A, Fasano S, Occhiello A, Pica A, Sehr P, Tommasino M, Nüesch JP, Rommelaere J. 2006. Tumor suppressive activity of a variant isoform of manganese superoxide dismutase released by a human liposarcoma cell line. Int J Cancer 119(4):932–943.

Meki AR, Hussein AA. 2001. Melatonin reduces oxidative stress induced by ochratoxin A in rat liver and kidney. Comp Biochem Physiol C Toxicol Pharmacol 130(3):305–313.

Pfohl-Leszkowicz A, Manderville R. 2007. Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans. Mol Nutr Food Res 51:61–99.

Ringot D, Chango A, Schneider YJ, Larondelle Y. 2006. Toxicokinetics and toxicodynamics of Ochratoxin A, an update. Chem Biol Interact 159:18–46.

Salom MG, Lahera V, Miranda-Guardiola F, Romero JC. 1992. Blockade of pressure natriuresis induced by inhibition of renal synthesis of nitric oxide in dogs. Am J Physiol 262:718–722.

Schaaf GJ, Nijmeijer SM, Maas RF, Roestenberg P, de Groene EM, Fink-Gremmels J. 2002. The role of oxidative stress in the ochratoxin A-mediated toxicity in proximal tubular cells. Biochim Biophys Acta 1588(2): 149–158.

Wang T, Inglis FM, Kalb RG. 2000. Defective absorption of fluid and bicarbonate in the proximal tubule of mice lacking neuronal nitric oxide synthase (nNOS). Am J Physiol Renal Physiol 279:518–524.

Wang T. 2002. Role of iNOS and eNOS in modulating proximal tubule transport and acid-base balance. Am J Physiol Renal Physiol 283(4): F658–F662.

Wang T. 1997. Nitric oxide regulates HCO3 and Na transport by a cGMP mediated mechanism in the kidney proximal tubule. Am J Physiol Renal Physiol 272:242–248.

Wang X, Chandrashekar K, Wang L, Lai EY, Wei J, Zhang G, Wang S, Zhang J, Juncos LA, Liu R. 2016. Inhibition of nitric oxide synthase 1 induces salt-sensitive hypertension in nitric oxideSynthase 1α knockout and wild-type mice. Hypertension 67(4):792–799.

Wu Q, Dohnal V, Huang L, Kuča K, Wang X, Chen G, Yuan Z. 2011. Metabolic pathways of ochratoxin A. Curr Drug Metab 12(1):1–10.

Zinedine A, Soriano JM, Juan C, Mojemmi B, Moltó JC, Bouklouze A, Cherrah Y, Idrissi L, El Aouad R, Mañes J. 2007. Incidence of ochratoxin A in rice and dried fruits from Rabat and Salé area, Morocco. Food Addit Contam 24(3):285–291.