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Cu,Zn-superoxide dismutase deficiency in mice leads to organ-specific increase in oxidatively damaged DNA and NF-κB1 protein activity*

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Earlier experimental studies have demonstrated that: i) Cu,Zn-superoxide dismutase deficiency leads to oxidative stress and carcinogenesis; ii) dysregulation of NF-κB pathway can mediate a wide variety of diseases, including cancer. Therefore, we decided, for the first time, to examine the level of oxidative DNA damage and the DNA binding activity of NF-κB proteins in *SOD1* knockout, heterozygous and wild-type mice. Two kinds of biomarkers of oxidatively damaged DNA: urinary excretion of 8-oxodG and 8-oxoGua, and the level of oxidatively damaged DNA were analysed using HPLC-GC-MS and HPLC-EC. The DNA binding activity of p50 and p65 proteins in a nuclear extracts was assessed using NF-κB p50/p65 EZ-TFA transcription factor assay. These parameters were determined in the brain, liver, kidney and urine of *SOD1* knockout, heterozygous and wild-type mice. The level of 8-oxodG in DNA was higher in the liver and kidney of knockout mice than in wild type. No differences were found in urinary excretion of 8-oxoGua and 8-oxodG between wild type and the SOD1-deficient animals. The activity of the p50 protein was higher in the kidneys, but surprisingly not in the livers of SOD1-deficient mice, whereas p65 activity did not show any variability. Our results indicate that in Cu,Zn-SOD-deficient animals the level of oxidative DNA damage and NF-κB1 activity are elevated in certain organs only, which may provide some explanation for organ-specific ROS-induced carcinogenesis.

Keywords: Cu,Zn-SOD deficiency, oxidative stress, NF-κB pathway

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INTRODUCTION

Oxidative stress is produced in cells by reactive oxygen species (ROS) resulting from cellular metabolism and from interaction with exogenous sources such as cigarette smoke, redox-cycling drugs and ionizing radiation. DNA damage caused by ROS is the most frequent type encountered by aerobic cells. An increase in the amount of oxidatively generated DNA lesions has been demonstrated in DNA exposed to ROS-generating systems *in vitro* (Douki *et al.,* 1997); oxidatively modified DNA bases have also been found in the DNA of animal and human tissues under conditions usually referred to as oxidative stress (Kasprzak *et al.*, 1997). Data accumulated over many years clearly show that oxidative DNA damage plays an important role in a number of disease processes. Thus, oxidatively damaged DNA is implicated in aging and in neurodegenerative diseases such as Alzheimer's disease. There is also strong evidence for a role of this type of DNA damage in carcinogenesis (Djuric *et al.*, 2001; Olinski *et al.*, 2003).

The level of oxidative damage to DNA can be assessed directly, by measuring 8-oxodG abundance in cellular DNA, and this parameter is the most studied and is widely acknowledged as a robust biomarker of oxidative stress (Kasai, 1997). An alternative approach to assessing oxidative stress/DNA damage at the level of the whole organism is determination of urinary excretion of oxidatively modified bases/nucleosides (for review see Shigenaga *et al.*, 1989; Olinski *et al.*, 2006).

The analysis of 8-oxoGua in urine presents particular difficulties (e.g., poor solubility can cause a loss of the analyte) (Helbock *et al.,* 1998) and until recently no reliable assay was available for its detection. New techniques based on mass spectrometric detection (MS) have been developed which allow simultaneous determination of 8-oxodG and 8-oxoGua in the same urine sample (Ravanat *et al.,* 1999; Gackowski *et al.,* 2001; Weimann *et al.,* 2004). One such method involves HPLC prepurification followed by gas chromatography with isotope dilution MS detection (Gackowski *et al.*, 2001). In addition to unequivocal identification of the analysed compounds and high sensitivity, the use of isotopically labeled internal standards compensates for potential losses of the analyte during sample work-up.

To limit the adverse effects of ROS, cells possess a variety of defense mechanisms including antioxi-

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★Preliminary results of this work were presented at a poster session during the 7th Parnas Conference in Jalta, Ukraine.

Abbreviations: ALS, amyotrophic lateral sclerosis; Cu,Zn-SOD, Cu,Zn-superoxide dismutase; dG, 2'-deoxyguanosine; GC-MS gas chromatography with isotope dilution mass spectrometric detection; Gua, guanine; HPLC, high-performance liquid chromatography; LPS lipopolysaccharide; 8-oxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine; 8-oxoGua, 8-oxo-7,8-dihydroguanine; OGG1, 8-oxo-7,8-dihydroguanine glycosylase; ROS, reactive oxygen species; TNF, tumor necrosis factor.

dant enzymes. A major component of this defense are superoxide dismutases (SODs) (Busuttil *et al.,* 2005). SODs are among the first line of defense in the detoxication of products resulting from oxidative stress (Johnson & Giulivi, 2005). They are a family of enzymes that very efficiently catalyze the dismutation of the superoxide radical anion to hydrogen peroxide and molecular oxygen. Cu,Zn-SOD (SOD1), located in the cytosol, is responsible for the majority of total SOD activity. Cu,Zn-SOD (*SOD1, Sod1*) transgenic and mutant mice have been widely used to study the role of ROS in different experimental systems (Huang *et al.,* 2002; Kruidenier *et al.,* 2003). According to ample data, a deficiency in various forms of SOD promotes oxidative damage in a wide range of organisms (Fridovich, 1997). In addition, mice deficient in Cu,Zn-SOD have a decreased life span and elevated incidence of liver cancer (Elchuri *et al.,* 2005) as well as defective energy homeostasis and may develop an ALS-like pathology (Gonzalez de Aguilar *et al.,* 2005). Mice lacking this enzyme exhibited a pronounced susceptibility to paraquat toxicity, and female homozygous knock-out mice showed a markedly reduced fertility in comparison with wild type and heterozygous once (Ho *et al.,* 1998). Increased levels of Cu,Zn-SOD usually confer resistance to oxidative impact, whereas decreased expression renders mutant mice more susceptible (Elchuri *et al.,* 2005). Interestingly, the effects of oxidative stress imposed by Cu,Zn-SOD deficiency are restricted to certain organs (Elchuri *et al.,* 2005), while few reports concerning oxidative stress at the level of the whole organism in such mutants have been published (Sentman *et al.,* 2006).

NF-κB is a transcription factor discovered by Sen and Baltimore in 1986 in B cells (Sen & Baltimore, 1986). This dimeric transcription factor is composed of different members of the Rel family, such as p65 (Rel A), p50, p52, c-Rel and Rel-B, which can activate a great number of genes involved in stress responses, inflammation, carcinogenesis, apoptosis, etc. (Wang *et al.,* 2002). The NF-κB pathway may be activated *via* at least two distinct routes termed the canonical and the noncanonical pathway, respectively (Oliver *et al.,* 2009). Unlike most transcription factors, proteins of this family reside in the cytoplasm in a resting state through interaction with IκB inhibitory proteins, and must be activated and translocated into the nucleus in order to function (Schoonbroodt & Piette, 2000). In response to proinflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-1 (IL-1), or to bacterial lipopolysaccharide (LPS) the IκBs are rapidly phosphorylated and undergo ubiqutination and proteolysis. Because NF-κB can be activated in many cells by a diverse set of stimulating agents with redox regulation properties, reactive oxygen species have been proposed to be involved in the activation of the NF-κB pathway (Schreck *et al.,* 1991). This model was found not to be universal, since a relation between NF-κB activation and generation of intracellular reactive oxygen species was only detected in certain cell lines (Schoonbroodt & Piette, 2000). However, reactive oxygen species are considered to be a second messenger that can be implicated in NF-κB pathway modulation. Until now, there has been no study concerning the NF-κB binding activity and the level of oxidative stress in *SOD1* knockout mice. Likewise, no study of oxidative damage to DNA at the level of individual organs and the whole organism in the mutant mice has been published.

8-oxodG level is a good biomarker of oxidative stress and, as it was mentioned above, there are some inconsistencies concerning the influence of the stress on NF-κB binding activity. In the present study we examined a possible relationship between the aforementioned parameters in Cu,Zn-SOD knock-out, heterozygous and wild-type mice. To provide a better insight into the issue concerning organ-specific carcinogenesis, the level of 8-oxodG was analysed in DNA isolated from: (i) liver and kidney (since in the deficient animals increased mutation accumulation was observed in those organs (Busuttil *et al.,* 2005)), and (ii) brain (since the high rate of oxygen consumption per unit mass of tissue renders this organ especially vulnerably to the deleterious effects of oxidative stress.)

Oxidative stress/DNA damage at the level of the whole organism was determined as urinary excretion of 8-oxodG/8-oxoGua.

MATERIALS AND METHODS

Animals. A breeder pair of mice (strain B6; 129S7-Sod1^{tm1Leb}) heterozygous for the *SOD1*^{tm1Leb}-targeted mutation (Matzuk *et al.*, 1998) and their progeny were provided by the Jackson Laboratory (Bar Harbor, ME, USA). Males and females heterozygous for the non-functional *SOD1* allele (*SOD1*$^{-/+}$) were intercrossed, and their progeny were kept at 24–25 °C, in 80 % humidity with a light-dark cycle of 12 h. Mice received a standard laboratory diet (Labofeed, Kcynia, Poland) and water *ad libitum*. Genotyping using DNA isolated from mouse tails was performed by PCR analysis according to the protocol provided by The Jackson Laboratory. Mice homozygous for the non-functional *SOD1* allele (*SOD1*$^{-/-}$), heterozygous and control mice homozygous for the wild-type *SOD1* allele (*SOD1*$^{+/+}$) were used in the study at the age of 12 months. We used 28 mice of each genotype for every experiment. All the experimental procedures involving animals were approved by the Local Ethical Commission.

Preparation of nuclear extracts and NF-κB DNA binding assay. Liver, kidney and brain were excised immediately after death from mice killed by cervical dislocation, flash frozen in liquid nitrogen and stored at –75 °C. Nuclear extracts were obtained using Nuclear Extraction Kit (Marligen Biosciences) according to the manufacturer's instructions. Protein concentration in nuclear extracts was determined by the Bradford method (Bradford, 1976). DNA binding activity of p50 and p65 proteins in nuclear extracts was assessed using NF-κB p50/p65 EZ-TFA transcription factor assay (Upstate). Briefly, 12.5 μg of nuclear extract was mixed with a double stranded biotinylated oligonucleotide containing the consensus sequence for NF-κB binding (5'-GGGACTTTCC-3'). The obtained mixture was transferred to a streptavidin-coated 96-well plate and incubated for 1 h at room temp. After washing, plates were incubated with rabbit primary antibodies specific for mouse p65 or p50 for 1 h at room temp. Development was done with HRP-conjugated anti-rabbit secondary antibody and tetramethylbenzidine (TMB) as substrate. Absorbance was measured at 450 nm in a microplate spectrophotometer. Results were normalized to absorbance/mg protein. Whole-cell extract from TNFα-stimulated HeLa was used as a positive control.

DNA analysis. DNA from tissues was isolated using the method described earlier (Siomek *et al.,* 2007) with some modifications. 8-OxodG and dG in hydrolysates were determined using HPLC with two detectors working in series: UV-VIS (UVD 340S) and Coulochem II 5200A electrochemical detector (ESA, Inc., Chelmsford, MA, USA). Detection of dG was performed at 254 nm. 8-OxodG was determined by the electrochemical detector: guard cell +750 mV, detector 1: +130 mV (as a screening electrode), detector 2: +450 mV (as a measuring electrode set to sensitivity of 20 nA/V).

Urine sample analysis. Internal standards, 0.5 nmol of $^{15}N_5$, ^{13}C 8-oxoGua and 0.05 nmol of $^{15}N_5$ 8-oxodG, and 10 μl of acetic acid (Sigma, HPLC grade), were added to 2 ml of murine urine. After centrifugation (2000×*g*, 10 min) supernatant was filtered through a Millipore GV13 0.22 μm syringe filter and 500 μl of this solution was injected into the HPLC system. Purification of 8-oxoGua and 8-oxodG was performed according to the method described by Ravanat *et al.* (1999) with some modifications. The effluent was monitored with a UV detector at 220–360 nm. The collected fractions were dried by evaporation under reduced pressure in a Speed–Vac system. 8-oxodG was treated with 400 ml of 60% formic acid (Sigma) for 30 min at 130°C. Subsequently, samples were prepared for GC-MS analysis performed according to the method described by Dizdaroglu (1994), adapted for additional $^{15}N_5$ 8-oxoGua analyses (*m/z* 445 and 460 ions were monitored).

Statistical analysis. NF-κB activity, the level of 8-oxodG in DNA as well as urinary level of 8-oxoGua and 8-oxodG were analyzed with one-way analysis of variance (ANOVA) followed by post-hoc Tukey's test. To estimate correlation between NF-κB activity and 8-oxodG level, Pearson's correlation coefficient (r) was computed. A $p \leq 0.05$ was considered statistically significant. All statistical analyses were performed using Statistica 7 software (StatSoft).

RESULTS

NF-κB activity

In order to verify the hypothesis that basal DNA-binding activity of NF-κB is affected by Cu,Zn-SOD deficiency we assessed the p50 (NF-κB1) and p65 (RelA) DNA binding activity in nuclear extracts from brain, kidney and liver of wild type ($^{+/+}$), Cu,Zn-SOD deficient ($^{-/-}$) as well as heterozygous ($^{+/-}$) mice.

We observed a significant increase in p50 but not p65 DNA binding activity in the kidneys of Cu,Zn-SOD deficient mice compared with the wild-type and heterozygous animals (Fig. 1A, B). No genotype-related differences were observed in p50 or p65 DNA-binding activity in livers and brains.

Level of oxidative stress markers

To check the assumption that Cu,Zn-SOD deficiency can lead to oxidative stress/oxidative damage to DNA the level of the biomarkers 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in DNA isolated from brain, liver and kidney and the urinary excretion of 8-oxo-7,8-dihydroguanine (8-oxoGua) and 8-oxodG were determined in wild type ($^{+/+}$), Cu,Zn-SOD-deficient ($^{-/-}$) and heterozygous ($^{+/-}$) mice.

A significantly higher level of 8-oxodG was observed in cellular DNA of Cu,Zn-SOD-deficient ($^{-/-}$) mice compared with wild-type animals in both liver and kidney (Fig. 2A, B).

We found no significant influence of the genotype on the level of 8-oxoGua and 8-oxodG in urine (Fig. 3A, B) or on the level of 8-oxodG in the brain.

Correlation between NF-κB activity and 8-oxodG level

In order to assess the direction and strength of a possible correlation between the NF-κB DNA-binding activity and the level of 8-oxodG in DNA, Pearson's correlation coefficients (r) were computed. A strongest negative correlation was found in the liver and the kidney of heterozygous mice with statistically significant r values reaching about (–0.5) in the liver and over (–0.6) in the kidney for both p50 and p65 (Table 1). In wild-type animals this correlation was also negative but the r values were lower and, in the case of liver, not statistically significant. Interestingly, correlation coefficient values in the liver and kidney of Cu,Zn-SOD-deficient mice were much lower than those in the wild type and heterozygous mice and not statistically significant. This result suggests that the weak/moderate negative correlation between p50/p65 activity and 8-oxodG level observed in wild type and het-

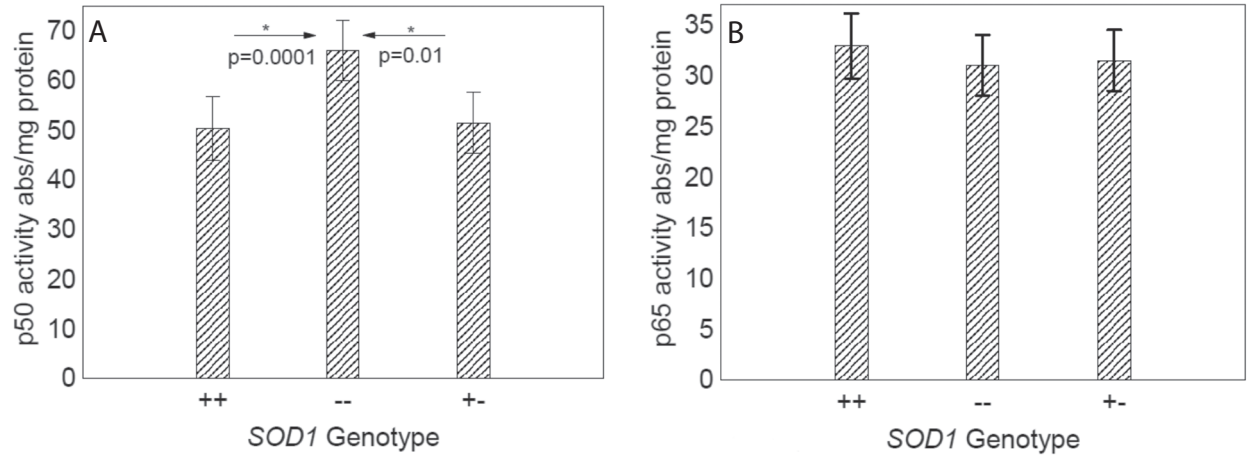

Figure 1. DNA-binding activity of p50 (A) and p65 proteins (B) in kidney
The activity is expressed as absorbance/mg protein.

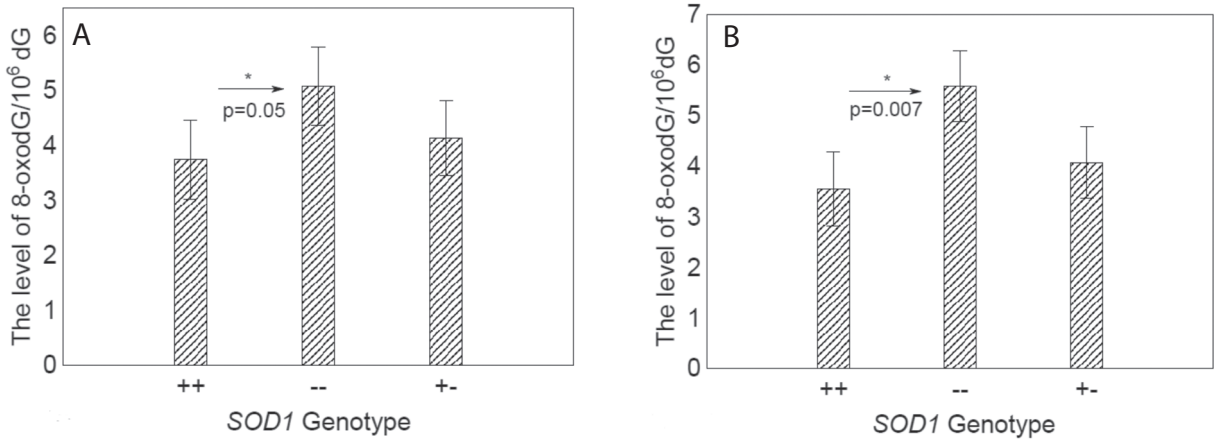

Figure 2. Level of 8-oxodG/10⁶dG in kidney (A) and liver (B)

erozygous mice was absent in Cu,Zn-SOD-deficient animals.

In the case of brain the tendency was less clear, nevertheless, we still observed a weak, significant negative correlation between 8-oxodG level and p50 (heterozygous animals) or p65 activity (wild type animals) whereas no statistically significant r values were observed in Cu,Zn-SOD-deficient animals.

DISCUSSION

Oxidative stress is a result of an imbalance between oxidant production and the cellular antioxidant capacity. Thus, oxidative stress may occur as a result of increased production of ROS or by deterioration of antioxidant mechanisms, or both.

Whatever the reason, oxidative stress leads in many cases to an age-dependent increase in the cellular level of oxidatively modified macromolecules, including DNA (Sohal *et al.,* 2002). Damage to DNA is particularly harmful since it may be fixed into mutation, if not repaired in a proper time, and passed onto daughter cells. Moreover, unlike proteins and lipids, entire new molecules of DNA cannot be synthesized to replace those containing damage. Although background levels of oxidatively damaged DNA always exist, oxidative stress can lead to an increase in the damage, and this increase has been linked to various pathophysiological conditions, such as aging, carcinogenesis and neurodegenerative and cardiovascular disease (reviewed: Cooke *et al.,* 2006).

Cu,Zn-SOD is believed to play a major role in the first line of antioxidant defense and it was previously shown that mice deficient in SOD1 exhibited a reduced life span and a high incidence of liver tumors. Simultaneously a significant increase of 8-oxodG was observed in the liver of *SOD1*$^{-/-}$ mice (Elchuri *et al.,* 2005).

In accordance with the aforementioned finding we found a statistically significant about 60%-increase of 8-oxodG level in DNA isolated from livers of mice deficient in SOD1 in comparison with age-matched controls (Fig. 2A). However, Elchuri *et al.* (2005) did not report on the level of oxidatively damaged DNA in other tissues. Our results demonstrate that this level was also significantly elevated in kidneys of the *SOD1*$^{-/-}$ mice, albeit the change was not as distinct as that in the livers. No increase was found in DNA extracted from the brain. We also observed no difference in the level of 8-oxodG between the wild type and heterozygous animals in any of the tissues studied. Interestingly, when mutation frequencies were determined in different tissues of *SOD1* $^{-/-}$ mice, statistically significant increase was observed in the liver and kidney but not in brain (Busuttil *et al.,* 2005).

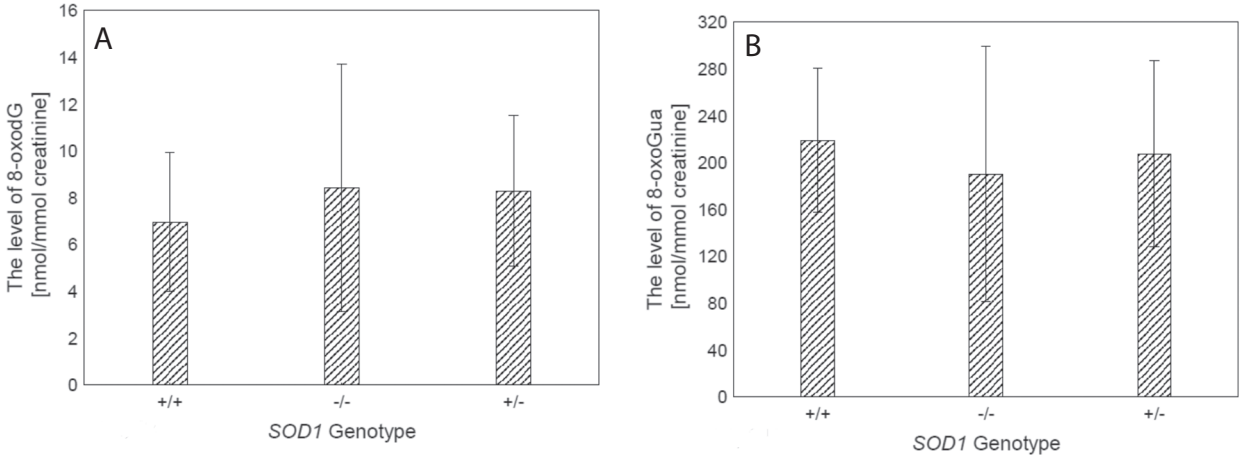

Figure 3. Urinary level of 8-oxodG (A) and 8-oxoGua (B)

Table 1. Correlation between NF-κB DNA-binding activity and 8-oxodG in DNA
Underlined values r are statistically significant at $p<0.05$.

A	The level of 8–oxodG/10^6dG in liver cells		
	SOD1$^{+/+}$	*SOD1*$^{+/-}$	*SOD1*$^{-/-}$
p50 protein activity	–0.34	–0.52; p=0.005	0.15
p65 protein activity	–0.27	–0.45; p=0.015	0.16
B	The level of 8–oxodG/10^6dG in kidney cells		
	SOD1$^{+/+}$	*SOD1*$^{+/-}$	*SOD1*$^{-/-}$
p50 protein activity	–0.41; p=0.032	–0.63; p<0,001	–0.15
p65 protein activity	–0.49; p=0.009	–0.47; p<0,001	–0.24
C	The level of 8–oxodG/10^6dG in brain cells		
	SOD1$^{+/+}$	*SOD1*$^{+/-}$	*SOD1*$^{-/-}$
p50 protein activity	–0.39	–0.42; p=0.043	–0.22
p65 protein activity	–0.44; p=0.03	–0.11	–0.28

Studies with *OGG1*-knockout animals also revealed organ-specific accumulation of 8-oxodG and increased frequencies of mutation in the liver (Epe, 2002) and kidney (Arai *et al.,* 2006). Therefore, it is likely that these organs are especially sensitive to oxidative damage.

Despite a plethora of studies which have dealt with Cu,Zn-SOD-deficient animals an important question concerning oxidative stress at the level of the whole animal has not been answered yet, and only conflicting results have been published concerning urinary/plasma excretion of oxidative stress biomarkers, 8-isoprostaglandin $F_{2\alpha}$ and thiobarbituric acid reactive substances (TBARS), which are markers of lipid peroxidation (Sentman *et al.,* 2006).

In our work specific markers of oxidative stress were determined. In addition to measuring damage in specific cells, a whole-body burden of oxidative stress was assessed by the measurement of urinary excretion of 8-oxoGua and its deoxynucleoside equivalent 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) (Cooke *et al.,* 2006). It is understood that, following excision from DNA, the oxidatively modified bases/nucleosides are released into the bloodstream and consequently appear in the urine. Therefore, there is no doubt that the urinary assay which measures 8-oxoGua and 8-oxodG reflects oxidative stress at the level of the whole organism.

No statistically significant differences in urinary excretion of 8-oxoGua and 8-oxodG were found between wild type and SOD1-deficient animals (Fig. 3A, B). Therefore, our results indicate that in the SOD1-deficient animals oxidative stress is restricted to certain organs (liver and kidney) and is not characteristic for the whole organism.

NF-κB, a transcription factor known to regulate expression of antiapoptotic genes and to activate different proinflammatory cytokines and chemokines, is also likely to be a crucial link between inflammation and initiation and progression of carcinogenesis (Naugler & Karin, 2008). The major, canonical pathway responsible for NF-κB activation and proinflammatory responses, depends mainly on phosphorylation and degradation of the sequestering proteins that releases a transcriptionally active NF-κB p65/p50 heterodimer which translocates to the nucleus and activates gene expression. It has been shown that p50 protein may also act as a homodimer in an alternative pathway, where it represses or activates transcription depending on the binding of additional proteins such as hostone deacetylase-1 (HDAC1) or Bcl3 (Yu *et al.,* 2009).

Our study demonstrated a statistically significant increase in the DNA-binding activity of NF-κB1 (p50) protein in Cu,Zn-SOD-deficient mice without any changes in the activity of the RelA/p65 subunit. Moreover, the aforementioned increase was restricted to kidney, while no statistically significant changes were found in livers or brains.

Constitutively active NF-κB has been found in many types of cancer, and there is experimental evidence with mouse models for a causative role of NF-κB in malignant conversion and progression (Luedde *et al.,* 2007; Sakurai *et al.,* 2008). Cu,Zn-SOD-deficient animals developed organ-specific, widespread oxidative stress/oxidative damage. Since NF-κB is recognized to be redox-regulated it is possible that in some organs of *SOD1*$^{-/-}$ animals (the liver) this pathway is constitutively activated, which may be an additional factor responsible for organ-specific cancer development. The results presented in this work suggest that the increased DNA-binding activity of NF-κB p50 protein in kidney may be linked to the inhibition of proinflammatory activity of canonical NF-κB pathway, which in turn may protect this organ from cancer development in Cu,ZnSOD-deficient animals. In the original paper Huang and co-workers (Elchuri *et al.,* 2005) reported that spontaneous tumors in the SOD1-deficient animals were only observed in livers. Those authors hypothesized that that selectivity could be linked with the "special environment and the characteristics of the hepatic tissue". However, our results demonstrate a significantly higher level of the promutagenic 8-oxodG not only in the liver but also in the kidney. Moreover, as mentioned above, increased mutation accumulation was observed in the kidney as well as in the liver of the deficient animals (Busuttil *et al.,* 2005). Our results concerning NF-κB suggest yet another explanation of why the kidney is protected from cancer development despite an increased DNA damage/mutation frequency; a specific increase in NF-κB p50 protein activity may inhibit potentially pro-inflamatory/pro-carcinogenic activity of the "canonical" NF-κB pathway in the organ. The recently published results of Panzer *et al.* (2009) support this notion. In that work the authors demonstrated that p50 was responsible for the switch from the inflammatory process toward resolution in renal inflammatory disorder. Moreover, p50 protein plays an important role in the resolution of LPS-induced renal inflammation since NF-κB p50 knockout mice demonstrate a significantly higher chemokine expression and prolonged renal inflammatory leading to consecutive tissue injury, and reduced survival.

We found a significant negative correlation between 8-oxodG level in cellular DNA and NF-κB activity, albeit only in the wild type and heterozygous animals. This result suggests that in physiological conditions NF-κB activity may be regulated by oxidative stress. This result is well understood since the NF-κB transcription factor regulates expression of genes coding for antioxidant proteins such as ferritin heavy polypeptide, glutathione S-transferase, heme oxygenase 1, Mn-SOD and Cu,Zn-SOD (Pahl, 1999; Rojo *et*

al., 2004). p50 protein may be involved in activation of their expression *via* the classical pathway (as a p50/p65 heterodimer) or the alternative pathway as a p50/p50 homodimer which can be transcriptionally active upon binding coactivators such as Bcl3. Alternatively, our results suggest that under physiological conditions NF-κB activity may be regulated by oxidative stress.

The Cu,Zn-SOD-deficient animals lacked a correlation between 8-oxodG level and NF-κB DNA-binding activity. Obviously, an increased NF-κB activity cannot result in increased Cu,Zn-SOD level in these animals to counteract DNA damage caused by ROS. Another possibility is that p50/p50 homodimers act here as repressors of transcription and prevent NF-κB-dependent expression of antioxidative genes which finally leads to increased level of oxidative DNA damage. It is also possible that an aberrant oxidation process in organs of the *SOD1*$^{-/-}$ animals may obscure the subtle relationship observed in the wild-type strain.

Summing up, our results demonstrated enhanced organ-specific oxidative damage to DNA restricted to liver and kidney of *SOD1*$^{-/-}$ mice and a lack of an effect of SOD1 deficiency on urinary excretion of 8-oxodG and 8-oxoGua, markers of oxidative DNA damage at the level of the whole organism. Moreover, we also demonstrated an increase in NF-κB1 (p50) DNA-binding activity in kidneys of Cu,Zn-SOD-deficient mice and a negative correlation between this activity of NF-κB and 8-oxodG level in the wild-type and heterozygous but not *SOD1*$^{-/-}$ animals.

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