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The oxidative DNA lesions 8,5′-cyclopurines accumulate with aging in a tissue-specific manner

Jin Wang[1], **Cheryl L. Clauson**[2], **Paul D. Robbins**[2], **Laura J. Niedernhofer**[2], and **Yinsheng Wang**[1]

Jin Wang: jinwang@ucr.edu; Cheryl L. Clauson: clausoncl@upmc.edu; Paul D. Robbins: probb@pitt.edu; Laura J. Niedernhofer: niedlx@upmc.edu; Yinsheng Wang: yinsheng.wang@ucr.edu

[1]Department of Chemistry, University of California, Riverside, CA 92521-0403, USA

[2]Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15219, USA

Summary

Accumulation of DNA damage is implicated in aging. This is supported by the fact that inherited defects in DNA repair can cause accelerated aging of tissues. However, clear-cut evidence for DNA damage accumulation in old age is lacking. Numerous studies report measurement of DNA damage in nuclear and mitochondrial DNA from tissues of young and old organisms, with variable outcomes. Variability results from genetic differences between specimens or the instability of some DNA lesions. To control these variables and test the hypothesis that elderly organisms have more oxidative DNA damage than young organisms, we measured 8,5′-cyclopurine-2′-deoxynucleosides (cPu), which are relatively stable, in tissues of young and old wild-type and congenic progeroid mice. We found that cPu accumulate spontaneously in the nuclear DNA of wild-type mice with age and to a greater extent in DNA repair-deficient progeroid mice, with a similar tissue-specific pattern (liver>kidney>brain). These data, generated under conditions where genetic and environmental variables are controlled, provide strong evidence that DNA repair mechanisms are inadequate to clear endogenous lesions over the lifespan of mammals. The similar, although exaggerated, results obtained from progeroid, DNA repair-deficient mice and old normal mice support the conclusion that DNA damage accumulates with, and likely contributes to aging.

Keywords

ageing; progeria; DNA damage; nucleotide excision repair; oxidative DNA lesion

DNA damage is implicated in contributing to the aging process. This is largely supported by the fact that inherited defects in DNA repair lead to accelerated aging of tissues (Hasty *et al.*

Correspondence: Yinsheng Wang, Department of Chemistry, University of California, Riverside, CA 92521-0403, USA. Tel.: 951 827 2700; fax: 951 827 4713; yinsheng.wang@ucr.edu. Correspondence should be sent to Yinsheng Wang prior to publication.

Author Contributions

J. W., C. L. C., P. D. R., L. J. N. and Y. W. designed research; J. W., C. L. C., L. J. N. and Y. W. wrote the paper; and J. W. conducted experiments.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Structures of cdA and cdG.

Fig. S2 Representative LC-MS/MS/MS data.

Table S1 Levels of 8,5'-cyclo-2'-deoxyguanosine (cdG) and 8,5'-cyclo-2'-deoxyadenosine (cdA) in nuclear DNA of tissues of *Ercc1*$^{-/\Delta}$ mice and age-matched littermates (n = 3).

2003). For example, XFE progeroid syndrome is caused by mutations in *XPF*, one subunit of the XPF-ERCC1 endonuclease, required for several DNA repair pathways including nucleotide excision repair (NER) (Sijbers *et al.* 1996). XFE patients display premature aging of several organ systems, which is recapitulated in mice with reduced expression of XPF-ERCC1 (*Ercc1*$^{-/\Delta}$ mice) (Niedernhofer *et al.* 2006; Gregg *et al.* 2011). However, it is not known which spontaneous endogenous DNA lesions drive accelerated aging when repair is attenuated and whether the same lesions accumulate in normal organisms with age.

Numerous studies have investigated the accumulation of DNA damage with age in nuclear and mitochondrial genomes from mammalian tissues with varying results (Bohr 2002; Loft *et al.* 2012). This variability can be attributed to the inherent difficulties in measuring endogenous oxidative DNA lesions (Collins *et al.* 2004). 8,5′-cyclopurine-2′-deoxynucleosides (cPu, Fig. S1) can be induced by endogenous reactive oxygen species (Jaruga & Dizdaroglu 2008), and they are relatively stable. Generation of cPu during sample preparation is greatly inhibited by O_2 (Jaruga & Dizdaroglu 2008), rendering these lesions reliable biomarkers of oxidative stress and DNA damage.

cPu are substrates for NER (Brooks *et al.* 2000; Kuraoka *et al.* 2000); therefore, we predict that cPu lesions should accumulate in tissues of NER-deficient organisms. In addition, cPu strongly block DNA replication and transcription, causing replication and transcriptional mutagenesis (Kuraoka *et al.* 2000; Marietta & Brooks 2007; Yuan *et al.* 2011). Thus, upon accumulation, cPu lesions are anticipated to induce deleterious consequences to living cells thereby potentially contributing to aging.

We employed LC-MS/MS/MS (Wang *et al.* 2011) and measured both 5′*R* and 5′*S* diastereomers of 8,5′-cyclo-2′-deoxyadenosine (cdA) and 8,5′-cyclo-2′-deoxyguanosine (cdG) (Fig. 1, Table S1, and Fig. S2) in nuclear DNA from liver, kidney and brain from congenic wild-type and *Ercc1*$^{-/\Delta}$ mice. Adduct levels were measured in young adult mice (10 weeks of age), at 21 weeks of age, by which time *Ercc1*$^{-/\Delta}$ mice have profound progeroid symptoms (Gregg *et al.* 2011), and in old wild-type mice (3 years old). In the liver, there were significantly more cPu in *Ercc1*$^{-/\Delta}$ mice than normal littermates at both 10 and 21 weeks of age, providing further evidence that NER is critical for clearing cPu *in vivo*. In both normal and progeroid animals, there were significant increases in the levels of all four lesions with aging (at 21 weeks of age compared to 10 weeks), indicating that, even with a normal complement of DNA repair, NER is unable to completely clear cPu from the nuclear genome and the adduct levels increase over time. Surprisingly, there were significantly lower levels of the *R* diastereomers of cdG and cdA in the liver of old wild-type mice (3 years) compared to 21 week-old mice, possibly reflecting the fact that the liver can undergo regeneration under stress (Michalopoulos 2007).

In kidney, like the liver, cPu levels increased significantly with age in both normal and *Ercc1*$^{-/\Delta}$ mice (from 10 to 21 weeks of age). However, not until 21 weeks of age was there a significant difference in cPu levels between normal and ERCC1-deficient mice, suggesting that accumulation of cPu lesions begins later in the kidney than in the liver. Between the ages of 10 to 21 weeks, the levels of *S*-cdG increased 2–3-fold in the liver and kidney of normal mice as well as the liver from *Ercc1*$^{-/\Delta}$ mice, but >5-fold in *Ercc1*$^{-/\Delta}$ kidney. Similarly, the increase in *S*-cdA in *Ercc1*$^{-/\Delta}$ kidney from 10 to 21 weeks dramatically exceeded that of the liver and kidney of normal mice, perhaps suggesting an acute aging-related degenerative process in the kidney of *Ercc1*$^{-/\Delta}$ mice that causes oxidative stress. Overall, the levels of all of the cPu adducts were at least 2-fold greater in the liver than the kidney in *Ercc1*$^{-/\Delta}$ mice, at any age.

In the brains of normal mice, a significant increase in cPu levels was detected in the 3 year-old mice, illustrating a time-dependent accumulation of cPu with age. In contrast, *Ercc1*$^{-/\Delta}$ mice had significantly more *S*-cdG than normal mice at 10 weeks of age, but the levels of cPu decreased as the animals aged and were significantly lower at 21 weeks than 10 weeks. This could be because neurons harboring DNA damage are particularly vulnerable to cell death. Indeed, there is evidence of neuron attrition with aging in rodents (O'Callaghan & Miller 1991), which is accelerated in progeroid *Ercc1*$^{-/\Delta}$ mice (Gregg *et al.* 2011). Interestingly, the 5'*S* diastereomers of cPu were present at higher levels than the corresponding 5′*R* diastereomers in kidney and brain of both normal and *Ercc1*$^{-/\Delta}$ mice, likely reflecting the more efficient repair of the 5′*R* isomers (Kuraoka *et al.* 2000).

These data, generated from samples in which genetic and environmental variables were optimally controlled, establish that cPu lesions spontaneously accumulate in nuclear DNA *in vivo* with aging. DNA repair-deficient mice with accelerated aging generally have more lesions than normal mice. Remarkably, the levels of cPu lesions increase significantly as wild-type mice age, indicating that DNA repair mechanisms are inadequate to cope with endogenous DNA damage over a lifetime. Moreover, the pattern of cPu accumulation was different among the three tissues studied, with liver accumulating the most damage. The levels of cPu lesions are ~2 orders of magnitude lower than 8-oxoG under aerobic conditions (Chatgilialoglu *et al.* 2011). However, since cPu block transcription and replication, and are mutagenic, there is a strong likelihood that cPu could contribute to the degenerative aging-related changes seen in progeroid *Ercc1*$^{-/\Delta}$ mice (Gregg *et al.* 2011) and with normal aging.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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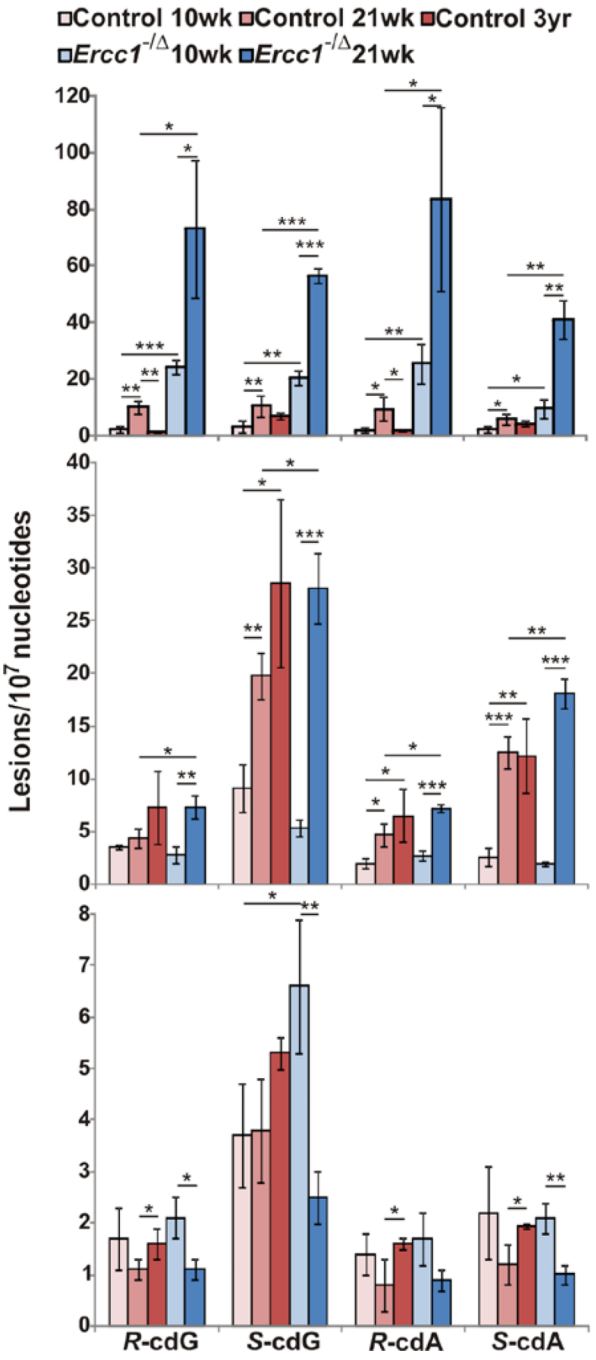

Fig. 1. Levels of 8,5′-cyclopurine-2′-deoxynucleosides in nuclear DNA of mouse tissues

Liver (top), kidney (middle), and brain (bottom) of *Ercc1*$^{-/\Delta}$ mice and age-matched littermates were examined. '*', $p < 0.05$; '**', $p < 0.01$; '***', $p < 0.001$. The p values were calculated using unpaired two-tailed t-test. The values represent the mean and standard error of results obtained from tissues of three different animals per group.