SUPPORTING INFORMATION

Evidence for the Involvement of NEIL1 in Nucleotide Excision Repair of (5'*R*)- and (5'*S*)-8,5'-Cyclo-2'-deoxyadenosines

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EXPERIMENTAL METHODS

Materials. Nuclease P1 (from *Penicillium citrinum*) was purchased from United States Biological (Swampscott, MA). Snake venom phosphodiesterase was obtained from Sigma Chemical Co. (St. Louis, MO). Alkaline phosphatase was purchased from Roche Applied Science (Indianapolis, IN). Acetonitrile (HPLC grade) was from Burdick and Jackson (Muskegon, MI). Biomax5 ultrafiltration membranes (5 kDa molecular mass cutoff) from Millipore (Bedford, MA) were used to filter hydrolyzed DNA samples. Water (HPLC-grade) for analysis by LC/MS/MS was from J. T. Baker (Phillipsburg, NJ). Water purified through a Milli-Q system (Millipore, Bedford, MA) was used for all other applications. N,O-bis(trimethylsilyl)trifluororacetamide containing 1% trimethylcholorosilane was obtained from Pierce Chemicals (Rockford, IL). *S*-cdA was purchased from Berry & Associates (Ann Arbor, MI). The concentration of *S*-cdA in aqueous solution was determined by measuring its absorption spectrum between 210 nm and 350 nm, and using the absorption coefficient of 15000 $M^{-1}cm^{-1}$ at 265 nm (*1*). Stable isotope-labeled analogues of FapyAde and FapyGua, i.e., FapyAde-$^{13}C,^{15}N_2$ and FapyGua-$^{13}C,^{15}N_2$, were purchased from Cambridge Isotope Laboratories (Cambridge, MA). The stable isotope-labeled analog of 8-OH-Gua, i.e., 8-OH-Gua-$^{13}C,^{15}N_3$ was obtained as described (*2*). *E. coli* Fpg was prepared according to a published procedure (*3*).

Knockout mice. The four *neil1*$^{+/+}$ and eight *neil1*$^{-/-}$, along with twelve *ogg1*$^{-/-}$ mice used in this study were housed in ALAC approved facilities with 12 hr/12 hr light/dark cycles and given *ad libitum* access to food (Purina 5053 chow) and water. Nine of the *neil1* mice were derived from *neil* $^{+/-}$ breeders that had been backcrossed 7 generations into C57BL/6. Control C57Bl/6 mice were purchased from Charles River Laboratories. The remaining three are *neil1*$^{-/-}$, two of which are offspring of 7th and 9th backcrossed generation *neil1*$^{-/-}$ breeding pairs, and the third mouse was born from 12th backcrossed generation *neil1*$^{+/-}$ parents. The average age of the *neil1*$^{+/+}$ and *neil1*$^{-/-}$ mice was 18 months, while the *ogg1*$^{-/-}$ mice were from a F1 generation that were 8 months of age at the time of euthanasia. All animals were euthanized by CO_2 and livers harvested immediately and chilled to 4 °C, but not frozen. The liver samples were blinded before shipment.

Construction and characterization of the *neil1*$^{-/-}$ mice were previously reported (*4*). The *ogg1*$^{-/-}$ were originally constructed by Klungland et al. (*5*), and we obtained our colony from Dr. Fernando Cardozo-Pelaez, University of Montana, with permission from Dr. Tomas Lindahl.

Isolation of nuclear DNA from liver of mice. Livers were not frozen and sent on ice by overnight express mail. They were processed immediately upon arrival. Livers were cut and homogenized 10 times with Wheaton Dounce Tissue Grinders in a buffer consisting of 0.25 M sucrose, 1 mM EDTA and 10 mM Tris-HCl (pH 7.4) at 4 ºC. The mixture was then centrifuged at 600g for 15 min. The supernatant fraction was removed. A 2 mL aliquot of the lysis buffer (10 mM Tris at pH 8.2, 2 mM EDTA, 0.4 NaCl, 1% SDS) and proteinase K in a final concentration of 2 mg/mL were added to the pellet. The mixture was incubated for 18 h at 37 ºC, and saturated NaCl solution (1/4 of total volume) was added. The mixture was vortexed well, and then

centrifuged at 12000g for 15 min at 4 ºC. Cold ethanol (96%) was added to the supernatant fraction in a ratio of 2.5 to 1. The mixture was kept –20 ºC for 18 h. The pellet was separated and re-suspended in 1 mL TE buffer. RNase A at a final concentration of 0.2 mg/mL was added and the mixture was incubated at 37 ºC for 1 h. Lysis buffer (1 mL) and proteinase K (final 2 mg/mL) were added. After incubation for 1 h at 55 ºC, an aliquot of saturated NaCl solution (1/4 of total volume) was added. The mixture was vortexed well and then centrifuged at 12000g for 15 min at 4 ºC. DNA was precipitated with cold ethanol (96%) at –20 ºC for 18 h. The supernatant fraction and DNA pellet were separated by centrifugation. The DNA pellet was washed with 80% ethanol several times, and then dried under vacuum. Subsequently, DNA was dissolved in water at 4 °C for 18 h. The DNA concentration was measured by recording the UV spectrum of each sample using an absorption spectrophotometer between the wavelengths of 200 nm and 350 nm both to ascertain the quality of DNA and to accurately determine the DNA concentration. The absorbance at 260 nm is used to measure the DNA concentration (absorbance of 1 = 50 µg of DNA/mL). The absorbance at 340 nm, which was generally negligible, was subtracted from that at 260 nm. Subsequently, 50 µg aliquots of DNA samples are dried in a SpeedVac under vacuum for 2 h.

Preparation of stable isotope-labeled internal standards. Stable isotope-labeled analogues of *R*-cdA and *S*-cdA, i.e., *R*-cdA-$^{15}N_5$ and *S*-cdA-$^{15}N_5$, were prepared according to the published procedures using dATP-$^{15}N_5$ as the starting material (*6*). The concentrations of the solutions of *R*-cdA-$^{15}N_5$ and *S*-cdA-$^{15}N_5$ were determined using LC/MS/MS and an aliquot of the 0.1 mM solution of *S*-cdA as the internal standard.

Analysis of DNA samples. The measurement of *R*-cdA and *S*-cdA was achieved using LC/MS/MS with isotope-dilution. Aliquots of *S*-cdA-$^{15}N_5$ and *S*-cdA-$^{15}N_5$ were added as internal standards to 50 µg aliquots of DNA samples. The samples were hydrolyzed with nuclease P1, snake venom phosphodiesterase and alkaline phosphatase at 37 ºC for 24 h, and then filtered and analyzed by LC/MS/MS as described (*7*). FapyGua and 8-OH-Gua were identified and quantified using GC/MS with isotope-dilution, following hydrolysis of DNA samples with *E. coli* Fpg to release these lesions (*8*).

Statistical analysis of the data. Statistical analysis was performed by using GraphPad Prism (Version 3.02, San Diego, CA, USA). The statistical analysis of the significance between groups of DNA base lesions was carried out using the nonparametric Kruskal-Wallis one-way Analysis of Variance (ANOVA) by ranks and Mann-Whitney *U* tests. A p-value < 0.05 was considered statistically significant.

REFERENCES for EXPERIMENTAL METHODS

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