Endogenous Deoxyribonucleic Acid (DNA) Damage in Human Tissues: A Comparison of Ethenobases with Aldehydic DNA Lesions

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Abstract

Two types of endogenous DNA lesions, ethenobases [1,N6-ethenoadenine (εA), 3,ε4-ethenocytosine (εC)] and aldehydic DNA lesions (ADLs), were measured in several tissues (liver, lung, kidney, colon, colon mucosa, cerebellum, and gray and white matter of the cerebrum) obtained postmortem during autopsy examinations of 12 individuals (6 males, 6 females; ages, 58–87 years). Issues relating to changes in levels of DNA damage with disease and after death were addressed. The extent of DNA damage in autopsy samples was not associated with the length of the postmortem interval and was similar to levels observed in surgery samples, suggesting that endogenous, steady-state levels of etheno adducts and of ADLs are relatively stable during the hours immediately after death. In this limited series of samples, and with a few possible exceptions, the disease status before death was not associated with increased endogenous DNA damage in the affected tissue. DNA ethenobases were lowest in the cerebellum (median molar ratios: εA/A = 1.0 × 10−8; εC/C = 1.9 × 10−6) and highest in the gray matter (εA/A = 2.9 × 10−8; εC/C = 4.8 × 10−6) and white matter (εA/A = 2.4 × 10−8; εC/C = 5.2 × 10−8) of the cerebrum. In other tissues, median values were 1.2–1.9 × 10−5 for εA/A and 2.0–3.3 × 10−5 for εC/C. There was a good correlation between the levels of εA and εC (r = 0.80, P < 0.0001). Levels of ADLs were similar in the liver, lung, kidney, and white matter of the cerebrum (median values: 5.7–7.9 ADLs/106 nucleotides), higher in the colon (11.3 × 10−6) and gray matter of the cerebrum (9.0 × 10−6) and lower in the cerebellum (3.7 × 10−6). There was no correlation between levels of ethenobases and amounts of ADLs (εA versus ADLs: r = 0.12, P = 0.33; εC versus ADLs: r = 0.024, P = 0.85). Although there was an interindividual variability in the extent of endogenous DNA damage (4-fold for εA and εC, 2-fold for ADLs), which may be determined, in part, by the capacity to repair DNA and may be related to the pathology or treatment of the patients, these results suggest that the cerebrum contains higher endogenous DNA damage than the other tissues. These data are in line with previous studies showing that brain tissues are more susceptible to oxidative stress and lipid peroxidation than other tissues.

Introduction

The possible role of endogenous DNA damage in human diseases (degenerative diseases, cancer) is receiving increasing attention (1, 2). Several lesions that can lead to mutations have been characterized, the most common being oxidized bases (3) and abasic sites (4). More recently, aldehydic DNA adducts derived from LPO products were detected in DNA from humans and rodents (5, 6). Enals such as HNE derived from PUFA yield proanabases with DNA; if further oxidized into epoxy-aldehydes, such as 4-hydroxy-2,3-epoxynonanal, they yield ethenobases (7). Another major DNA lesion formed by LPO products is M1G, a pyrimidopurinone adduct of malondialdehyde with guanine (8). These exocyclic DNA adducts exhibit promutagenic properties (8–10) and, thus, could be involved in genomic instability and carcinogenesis. Several known cancer risk factors increase the level of these DNA lesions in target organs. Elevated levels of εA and εC have been found in hepatic DNA from patients with metal storage diseases (Wilson’s disease, primary hemochromatosis; Ref. 11) and in colonic polyps of familial adenomatous polyposis patients (12). A diet rich in ω-6 PUFA’s has been shown to increase etheno DNA adducts in WBCs of female subjects (13). In a mouse model, overproduction of nitric oxide induced high levels of etheno adducts in spleen DNA (14). Fulminant hepatitis in Long Evans Cinnamon rats, a strain with abnormal copper metabolism, coincided with a peak in the number of etheno adducts in liver DNA (15). Administration of ethanol to rats enhanced the formation of endogenous etheno adducts in hepatic DNA (16). Altogether, these data suggest that etheno DNA adducts could be useful biomarkers of DNA damage associated with oxidative stress and LPO (17).

AP or abasic sites are the most common DNA lesions. It has been estimated that ∼10,000 AP sites arise every day in the DNA from a mammalian cell (18, 19). These sites can result from spontaneous depurination of oxidized bases (20), activity

4 The abbreviations used are: LPO, lipid peroxidation; HNE, trans-4-hydroxy-2-nonenal; PUFA, polyunsaturated fatty acid; εA, 1,N6-ethenoadenine; εC, 3,ε4-ethenocytosine; M1G, pyrimido[1,2-a]purin-10(3H)-one; AP, apurinic/apyrimidinic; ARP, aldehyde reactive probe; ADL, aldehydic DNA lesion.
of DNA polymerases on oxidized or alkylated bases (20), or from attack of deoxyribose moieties by hydroxyl radicals (21). If not repaired, AP sites can be lethal or mutagenic (4). However, because of a lack of a reliable and sensitive assay, only a few studies have been performed on AP sites in vivo. Recently, a sensitive slot-blot assay was developed using ARP that can react with the aldehydic forms of AP sites caused by DNA glycosylases or spontaneous depurination/depyrimidination, resulting in the formation of stable biotin-tagged complexes (19). The adduction of aldehydic AP sites using ARP enabled us to quantitate the total number of regular AP sites by the ARP-slot blot assay. However, ARP is not a specific probe for AP sites, because it can also react with aldehydic bases and probably aldehydic oxidized deoxyribose lesions (22). In this study, the lesions detected by the ARP-slot blot assay in human tissue DNA will be referred to as ADLs, instead of AP sites, unless there is clear evidence that indicates that they are real AP sites. Furthermore, the DNA isolation step was improved to avoid artifactual formation or degradation of ADLs (23).

There have been few systematic studies of endogenous levels of DNA damage in different tissues from humans. To improve our knowledge on the extent of background DNA damage in humans, tissue samples were collected at autopsy, a few hours after death. These samples were analyzed for two ethenobases, εA and εC, and for ADLs.

Materials and Methods

Tissue Samples. Human tissue samples were obtained from the University Hospital of Zurich, Switzerland. The tissues (liver, lung, kidney, colon, colon mucosa, gray and white matter of the cerebrum, and cerebellum) were collected through a medical examiner’s office, snap frozen in liquid nitrogen, and kept at −80°C. Gender, age, and cause of death were determined by the medical examiner. This study was approved by the IARC Ethical Review Committee.

Clinical data of the patients are shown in Table 1. There were six women and six men, their ages ranged from 58 to 87 years. The postmortem interval (time at which the tissue samples were collected and frozen) ranged from 3 to 17.5 h (median ~ 8 h).

Isolation of DNA. For analysis of etheno adducts, DNA was isolated using the phenol/chloroform extraction method (24). For analysis of ADLs, a modified phenol/chloroform extraction method was used as described elsewhere (23). Briefly, to prevent Fenton reactions and oxidation of DNA, 2,2,6,6-tetramethylpiperidine-N-oxyl was added to the buffers; digestion with proteinase K was carried out at a low temperature (4°C) to prevent degradation of ADLs.

Analysis of εA and εC. Fifty micrograms of DNA were hydrolyzed enzymatically into nucleoside 3′-monophosphates. 3′-εdAMP and 3′-εdCMP were purified on specific immunogels and postlabeled on the 5′-position with [γ-32P]ATP and T4 polynucleotide kinase (6). The labeled nucleoside 5′-monophosphates were separated by two-dimensional thin-layer chromatography on polyethyleneimine cellulose and quantitated with a PhosphorImager (Molecular Dynamics). Normal nucleotides in DNA hydrolysates were quantitated by UV detection, after reverse-phase HPLC separation. Levels of etheno adducts were expressed as molar ratios of ethenonucleotide to parent nucleotide in DNA.

Analysis of ADLs. The ARP-slot-blot assay was performed as described by Nakamura et al. (23). Eight micrograms of DNA in 30 μl of PBS were incubated with 1 mM aldehyde reactive probe at 37°C for 10 min. After precipitation with cold ethanol, DNA was resuspended in TE buffer (10 mM Tris-HCl (pH 7.4), containing 1 mM EDTA). The DNA concentration was measured by a UV spectrophotometer, and the DNA solution was then prepared at 0.275 μg/220 μl of TE buffer. Heat-denatured DNA was immobilized on a nitrocellulose membrane (Protran BA85, Schleicher & Schuell). The nitrocellulose membrane was soaked with 5X SSC and then baked in a vacuum oven for 30 min. The membrane was preincubated with 10 ml of hybrid mix for 15 min and then incubated in the same solution containing streptavidin-conjugated horseradish perox-
Results

Etheno Adducts. Levels of εA and εC were analyzed in DNA from liver, lung, kidney, cerebellum, and cerebrum from 10 individuals (a–j, Table 1), and in colon tissue and colon mucosa from 8 individuals (c–j, Table 1). Data were grouped by tissue (Figs. 1A and 2A) or by individual (Figs. 1B and 2B).

Levels of etheno adducts differed significantly between the eight tissues analyzed: \( P = 0.0015 \) for εA, \( P = 0.0203 \) for εC (Kruskal-Wallis test). The cerebellum exhibited lower levels of εA than the gray matter and white matter of the cerebrum \( (P < 0.01, \text{ Dunn's test}) \) and lower levels of εC than the gray matter of the cerebrum \( (P < 0.05, \text{ Dunn's test}) \). In most cases, ranges and median values of both etheno adducts differed only marginally among the liver, lung, kidney, colon, and colon mucosa. εA/A levels ranged from 0.83 to 4.08 \( \times 10^{-8} \) in these tissues, with median values between 1.20 and 1.86 \( \times 10^{-8} \) (Fig. 1A). The molar ratio of εC/C varied from 0.97 to 8.88 \( \times 10^{-8} \), with median values of 2.05 to 3.34 \( \times 10^{-8} \) (Fig. 2A).

Two exceptions were one liver sample (patient a) and one lung sample (patient a), which contained high levels of both εA \( (\varepsilon A/A = 8.27 \text{ and } 12.2 \times 10^{-8}, \text{ respectively}) \) and εC \( (\varepsilon C/C = 15.3 \text{ and } 16.7 \times 10^{-8}, \text{ respectively}) \). The cerebellum exhibited the lowest levels of εA \( (\varepsilon A/A, \text{ from } 0.40 \text{ to } 1.65 \times 10^{-8}, \text{ median, } 1.00 \times 10^{-8} \) and εC \( (\varepsilon C/C, \text{ from } 0.91 \text{ to } 6.81 \times 10^{-8}, \text{ median, } 1.92 \times 10^{-8} \). Levels of etheno adducts were found to be higher in gray and white matter of the cerebrum: εA/A ranged from 1.0 to 11 \( \times 10^{-8} \) (median, 2.9 \( \times 10^{-8} \)) in the gray matter, and from 1.1 to 8.6 \( \times 10^{-8} \) (median, 2.4 \( \times 10^{-8} \)) in the white matter; εC/C ranged from 2.3 to 14 \( \times 10^{-8} \) (median, 4.8 \( \times 10^{-8} \)) in the gray matter, and from 1.4 to 15 \( \times 10^{-8} \) (median, 5.2 \( \times 10^{-8} \)) in the white matter.

When these data were grouped by individual \( (i.e., \) grouping the levels of etheno adducts measured in the six or eight different tissues for each individual), interindividual differences in the range and average levels of etheno adducts were obvious (Figs. 1B and 2B): \( P = 0.0204 \) for comparison of εA levels and \( P = 0.0012 \) for comparison of εC levels (Kruskal-Wallis test). In addition, Dunn’s multiple comparisons test showed a significant difference between patient a, on the one hand, and patients j and g on the other hand, with regard to the levels of εC \( (P < 0.05) \). Patients a and b and, to a lesser extent, patients c and d, exhibited a larger range and higher median values of εA/A (Fig. 1B) and εC/C (Fig. 2B) in their DNA than did patients e to j.

Comparison of the levels of εA and εC measured in each DNA sample showed a significant linear correlation: \( r = 0.80, \text{ } P < 0.0001 \) \( (n = 76; \text{ Fig. 3}) \). The slope of the correlation

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**Fig. 1.** Levels of εA in human DNA (autopsy tissue samples from 10 individuals identified by letters a–j). Each value is the average of two or three analyses. Horizontal bars represent medians.

**Fig. 2.** Levels of εC in human DNA (autopsy tissue samples from 10 individuals identified by letters a–j). Each value is the average of two or three analyses. Horizontal bars represent medians.
indicated that the average ratio of εA/A to εC/C in human DNA was 0.49 ± 0.04.

ADLs. ADLs were measured in liver, lung, kidney, cerebellum, gray and white matter of the cerebrum from 12 individuals (a–l; Table 1; except for white matter of the cerebrum from patient b and liver from patient k, which were not analyzed for ADLs) and in colon tissue from 8 individuals (c–j). This included the same tissue samples that were analyzed for etheno adducts, as well as tissues from two other individuals (k and l). Data are shown in Fig. 4, A and B. Statistical analysis indicated that the levels of ADLs differed significantly among the six tissues examined: \( P = 0.0019 \) (Kruskal-Wallis test; Fig. 4A). The number of ADLs in the cerebellum was significantly different from those in the gray matter of the cerebrum and colon (\( P < 0.05 \) and \( P < 0.01 \), respectively; Dunn’s test). The colon exhibited the highest number of ADLs (from 6.0 to 42 ADLs per 10^6 nucleotides; median value, 11.35), followed by the gray matter of the cerebrum (4.0 to 19 ADLs per 10^6 nucleotides; median value, 9.05). The number of ADLs in the liver, lung, kidney, and white matter of the cerebrum were similar, ranging from 3.1 to 17 ADLs per 10^6 nucleotides (median values from 5.75 to 7.90 ADLs per 10^6 nucleotides). The cerebellum had the lowest number: 2.80 to 11.3 ADLs per 10^6 nucleotides (median value, 3.70).

When grouped by individual, the number of ADLs showed a 2-fold interindividual variability that was not statistically significant (\( P = 0.56 \), Kruskal-Wallis test; Fig. 4B). The median value for each patients ranged from 5.75 to 7.90 ADLs per 10^6 nucleotides.

**Discussion**

We report here, for the first time, a comparison of two types of endogenous DNA damage in different human tissues and in different individuals. Etheno adducts have been measured in surgery samples from liver (11), colon (12), and lung (25). These data are summarized in Fig. 5, together with our present data on autopsy samples. For the sake of clarity, only the minimal, median and maximal values have been represented for each data set. Although there are some differences in the maximal values, the median values are very similar, ranging from 3.1 to 17 ADLs per 10^6 nucleotides (median values from 5.75 to 7.90 ADLs per 10^6 nucleotides). The cerebellum had the lowest number: 2.80 to 11.3 ADLs per 10^6 nucleotides (median value, 3.70).

Concerning ADLs, comparable data are available for the liver only. In autopsy liver samples, Nakamura and Swenberg indicated that the level of ADLs should not change dramatically in brain DNA up to 24 h after death. Concerning ADLs, comparable data are available for the liver only. In autopsy liver samples, Nakamura and Swenberg...
and H9280 as docosahexaenoic acid and arachidonic acid, that are easily levels of ADLs (Fig. 4A).

In this study, we observed similar amounts of endogenous DNA damage in different tissues, with some exceptions. The cerebellum contained less DNA damage (both etheno adducts and ADLs) than the other tissues examined. The levels of the etheno adducts, 8A and 8C, were higher in the gray and white matter of the cerebrum in approximately half of the samples. Higher levels of 8A and 8C have been observed previously in rat brain than in other tissues, including liver, lung, and kidney (28). ADLs were more frequent in colon tissue, which is consistent with a previous study showing higher levels of ADLs in colon DNA than in liver, lung, and kidney DNA of rats (27). In the same study, high levels of ADLs were found in whole brain DNA of rats; however, more recent studies using the same procedures that were used in this study did not find any difference between brain and other tissues.5 In human cerebrum, the gray matter, but not the white matter, contains slightly higher levels of ADLs (Fig. 4A).

The brain is highly sensitive to oxidative imbalance as a result of its high oxygen consumption rate, high content of transition metals and ascorbate (which together act as potent prooxidants), and the relative paucity of antioxidant enzymes compared with other tissues (29). Brain tissue is more lipid-rich than most other tissues and contains high levels of PUFAs, such as docosahexaenoic acid and arachidonic acid, that are easily peroxidized (30). Arachidonic acid, an ω-6 PUFA, is thought to be the major source of HNE and of HNE-DNA adducts in mammalian cells (31, 32). Consequently, the brain is more susceptible to oxidative damage and LPO than other organs (33). A study on rat tissues has shown higher levels of aldehydes (thiobarbituric acid reactive substances) in brain as compared with the other tissues, including liver, lung, kidney (34). Our present data showing higher amounts of 8A and 8C in gray and white matter of the cerebrum further suggest that the rate of LPO is relatively high in the cerebrum. In contrast, the cerebellum exhibited lower levels of etheno adducts. This is in keeping with studies in rats showing that the gray matter of the cerebrum is more susceptible to lipid peroxide formation than the cerebellum (35, 36) and that background levels of malondialdehyde are higher in gray matter of the cerebrum than the cerebellum (37).

Comparison of the median values (obtained from six to eight different tissues from each individual) shows an interindividual variability of 4-fold for the amounts of 8A (Fig. 1B) and 8C (Fig. 2B) and of 2-fold for the level of ADLs (Fig. 4B). This may reflect interindividual variations in DNA repair activities, which were not determined in the present study. Endogenous levels of these etheno adducts in humans may be controlled, in part, by the activity of two DNA N-glycosylases, the alkylpurine DNA, N-glycosylase and the mismatch-specific thymine DNA N-glycosylase (25). The measured levels of etheno DNA adducts suggest that individuals a, b, c, and d might have been relatively deficient for the repair of 8C (Fig. 2B) and patients a and b (and to a lesser extent c and d) for the repair of 8A (Fig. 1B).

There was no correlation between the levels of endogenous DNA damage and the age or sex of the patients or the postmortem interval (Table 1). All of the individuals examined in this study suffered from common illnesses associated with an increased oxidative stress/LPO, such as cancer (38), atherosclerosis (39), diabetes (40), liver cirrhosis [see Fiorelli et al. (41) for alcoholic cirrhosis and Jain et al. (42) for hepatitis C virus-induced cirrhosis), liver steatosis, chronic obstructive pulmonary disease, and other lung diseases (43, 44), acute respiratory distress syndrome (45), sepsis (46), intestinal ischemia (47), renal insufficiency (48). Patient k had been exposed to asbestos, which is known to induce oxidative stress. However, in most cases, there was no correlation between the clinical autopsy record of the patient and high levels of etheno adducts or ADLs in the affected tissue. Possible exceptions are discussed below.

With high levels of 8A and 8C in gray matter of the cerebrum, patient b had a metastatic melanoma and received palliative irradiation of the brain (five doses of 4 Gy each). He died 2 months later. It has been reported that γ-irradiation of DNA in vitro leads to the formation of 3′-phosphoglycolaldehyde residues in DNA, which react in situ with nucleobases, yielding etheno adducts (49). This could be an alternative pathway for the formation of ethenobases in vivo. Although etheno adducts have been shown to be cleaved from DNA in vitro through the base excision repair pathway (50), several studies in vivo suggest that they are relatively persistent in DNA under certain circumstances (24, 51, 52).

High levels of 8C were found in gray matter of the cerebrum from patient c, who suffered from a subdural hematoma, a condition that could possibly lead to an increased oxidative stress in the brain. Experimental traumatic injury to the brain of rodents leads to increased LPO (53) and increased levels of HNE (54) in the brain. Patient c also cumulated several diseases associated with increased oxidative stress/LPO: atherosclerosis, diabetes, pneumonia, and renal insufficiency. In a rat model, chronic renal failure resulted in an increased oxidative stress in the gray matter of the cerebrum (55). There was no obvious factor in the clinical data that could explain the high amounts of etheno adducts detected in the brain white matter of patients a, b, and e. These patients suffered from liver cirrhosis (a, d) or from peripheral arterial occlusive disease (e), conditions that may be associated with systemic oxidative stress/LPO. In addition, patients a and d may have had a lower capacity for repairing 8C, as suggested by Fig. 2B.

Patient d, who was diagnosed with a colon adenoma, had relatively high levels of 8A and 8C in DNA from colon tissue, but not from colon mucosa. Increased levels of 8A and 8C have been detected in colonic epithelia (polyps) of familial adenomatous polyposis patients and in colon tissue proximal to colon carcinoma (12). It should be noted that, in the eight patients examined in the present study, we found, on the average, slightly higher (1.5-fold) levels of 8A and 8C in colon tissue (i.e., including the epithelial and muscular layers) as compared with colon mucosa. M1G, the major DNA adduct of malondialdehyde (another reactive aldehyde produced by LPO), is increased in the colon mucosa from patients with colon adenoma (56). However, the level of M1G in colorectal mucosa is also affected by diet (56). Dietary habits were not recorded for the present study, but there is the possibility that different dietary habits might explain why high amounts of 8A and 8C were found in colon DNA from patient d but not from patient e, who also suffered from colon adenoma.

Two patients, a and d, who suffered from liver cirrhosis, exhibited slightly elevated levels of 8C in their hepatic DNA. Liver cirrhosis (either of alcoholic or viral origin) is associated with stimulation of oxidative stress/LPO (41, 42), which could be a cause for these elevated levels of etheno DNA adducts. However, in a previous analysis of three liver samples from

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5 Asakura, S. and Swenberg, J. A. manuscript in preparation.
cirrhotic patients, no increase in the $\text{eC}/\text{cC}$ ratio was found, compared with normal hepatic tissue; one cirrhotic liver sample contained a higher level of $\text{eA}$ than the two others and than the normal liver samples (6). More recently, Nair et al. detected increased amounts of $\text{eA}$ in liver parenchyma from cirrhotic individuals.\(^6\) Administration of ethanol to rats induced the formation of both $\text{eA}$ and $\text{eC}$ in liver DNA (16).

There is no known factor in the clinical data that could be associated with the high levels of etheno adducts measured in liver DNA of individual b and in lung DNA of individual a. As suggested above, lower DNA repair activities might be involved in part.

Patient h had very high levels of ADLs in colon DNA, but was not diagnosed with any disease of the colon. Other cases of slightly elevated levels of ADLs (Fig. 3A) cannot be correlated with the clinical data, except the gray matter of the cerebrum of patient b and lung of individual l. Patient b, who also had high levels of $\text{eC}$ in the gray matter of the cerebrum (see above), suffered from a melanoma metastatic to the brain and had received radiotherapy before death. Patient l had acute alveolar injury of the lungs.

To conclude, these data demonstrate tissue-specific variations in the steady-state levels of endogenous DNA damage, both for etheno adducts and ADLs. In addition, interindividual differences in background amounts of etheno adducts may be determined, in part, by DNA repair activities. As expected, there was a strong correlation between levels of $\text{eA}$ and levels of $\text{eC}$, two DNA lesions that arise through a common pathway, LPO (Fig. 3). The mean ratio of $\text{eA}/\text{eC}$ was 0.49, which is similar to the ratio of 0.50 measured in DNA treated in vitro with 4-hydroxy-2,3-epoxynoralan (data not shown). No correlation was found in any of the tissues examined between amounts of ethenobases and amounts of ADLs.

Acknowledgments
The authors thank the excellent technical assistance of G. Brun and A-M. Campus.

References
5. Nath, R. G., and Chuong, F. L. Detection of exocyclic 1,N²-propanodeoxy- 

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15. Nair, J., Stone, H., Nagao, M., Barbin, A., and Bartsch, H. Copper-dependent formation of miscoding etheno-DNA adducts in the liver of Long Evans cinna-

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