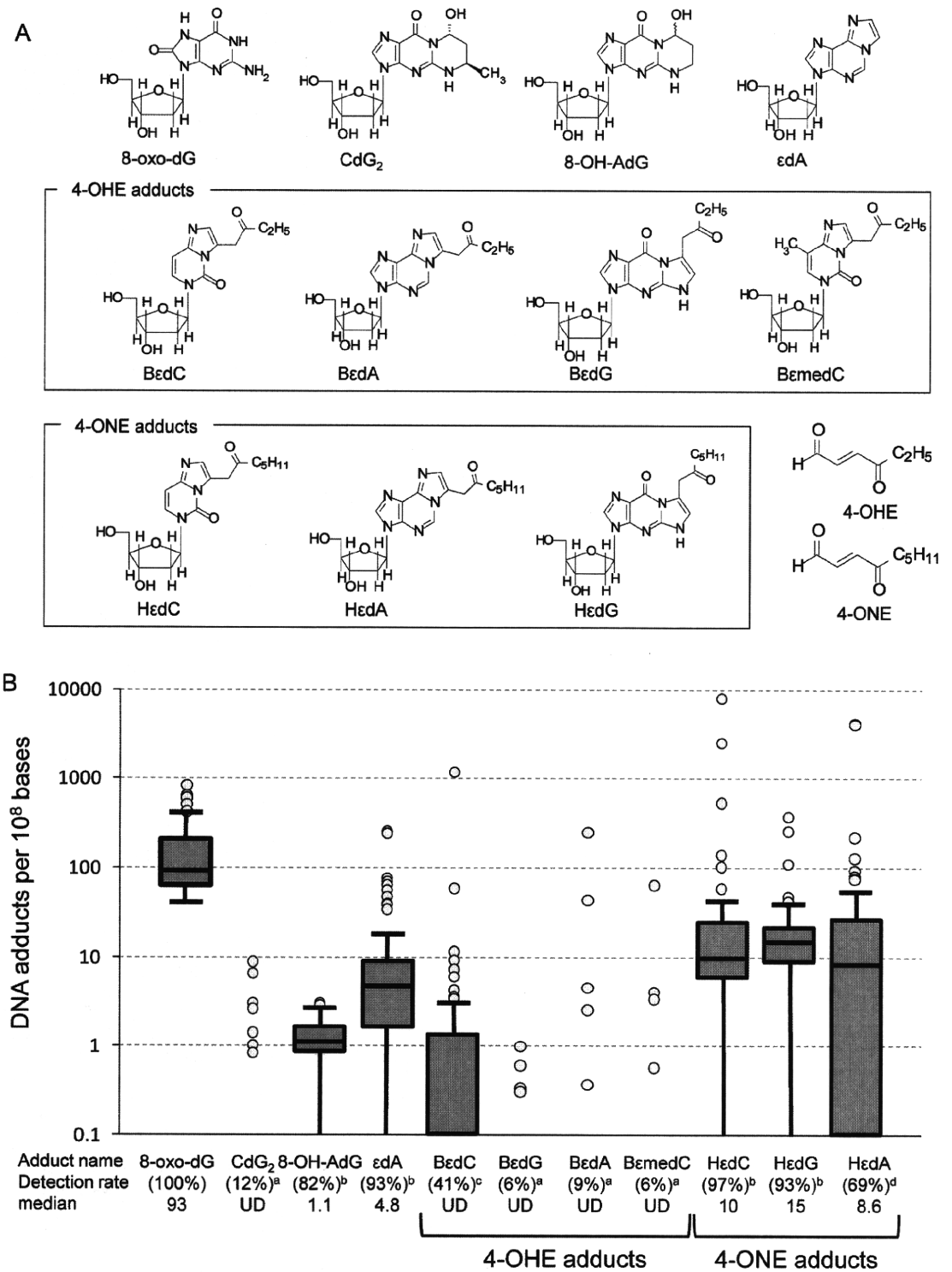


**Figure 2.** Level of LPO-induced DNA adducts in human tissues. (A) Chemical structure of DNA adducts detected in human tissues and the chemical structure of 4-OHE and 4-ONE. (B) Box-whisker plot of the levels of DNA adducts detected in human autopsy tissues, including the colon, liver, lung, pancreas, spleen, kidney, heart, and small intestine ($n = 68$). The boxes indicate the 75th percentile, the median, and the 25th percentile. The ends of the whiskers indicate the minimum and maximum data values unless outliers are present, in which case the whiskers extend to a maximum of 1.5 times the interquartile range. Circles above the whisker indicate outliers. Although crotonaldehyde-induced $CdG_1$ and acrolein-induced 6-OH-$AdG_1$ and 6-OH-$AdG_2$ were also monitored, we could not detect those adducts. Detected rate and median are shown under each DNA adduct. UD: under the detection limit. a, 75th percentile was UD; b, minimum was UD; c, median was UD; d, 25th percentile was UD.

indicating that DNA adducts induced by 4-ONE and 4-OHE are often formed in human lungs.

**Detection of 4-ONE- and 4-OHE-Induced DNA Adducts in Human Autopsy Tissues.** To elucidate whether the levels of

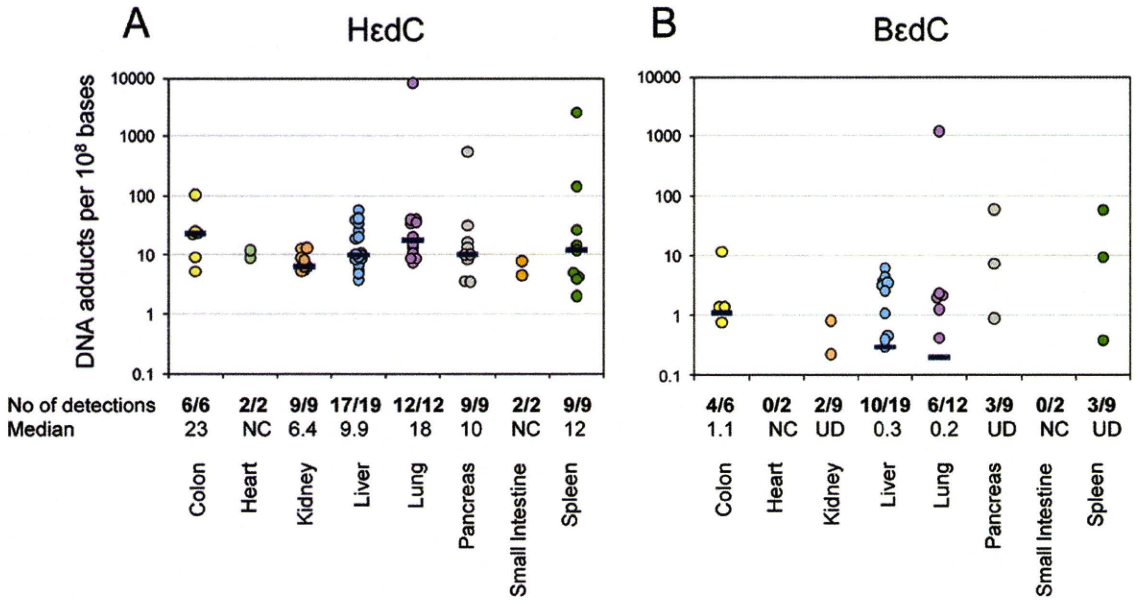


**Figure 3.** DNA adduct levels of HεdC and BεdC detected in various human autopsy tissues. Data from the DNA of 6 colons, 2 hearts, 9 kidneys, 19 livers, 12 lungs, 9 pancreases, 2 small intestines, and 9 spleens were plotted as circles, and the blue bars indicate the median values. NC: not calculated because the sample number was only 2. UD: median was under the detection limit.

4-ONE- and 4-OHE-related DNA adducts are comparable to those of other DNA adducts frequently found in human tissues, we measured the levels of various DNA adducts by using LC-MS/MS in 68 human autopsy specimens obtained from 26 persons, including samples of colon ($n$ = 6), liver ($n$ = 19), lung ($n$ = 12), pancreas ($n$ = 9), spleen ($n$ = 9), kidney ($n$ = 9), heart ($n$ = 2), and small intestine ($n$ = 2). The approximate detection limit of the DNA adducts (in the case that 50 $\mu$g of DNA was digested and 40% of the portion was injected to the LC/MS/MS) were as follows: 8-oxodG (1.65 adduct per $10^8$ bases), εdA (0.17), $CdG_1$ and $CdG_2$ (0.17), 8-OH-AdG (0.05), 6-OH-$AdG_1$ and 6-OH-$AdG_2$ (0.08), HεdC (0.33), HεdA (1.65), HεdG (1.65), BεdC (0.17), BεmedC (0.17), and BεdA (0.83) and BεdG (0.83) (Supporting Information Figures S-2 and S-3, Table S-8), and the calibration curves of each DNA adducts are shown in Supporting Information, Figure S-4. We could detect the target DNA adducts in several human tissue samples (the representative chromatographs are shown in Supporting Information, Figures S-5, S-6, and S-7). The results revealed that the levels of target DNA adducts varied considerably among individuals or organs (Figure 2 and Supporting Information, Table S-8). Figure 2 shows the DNA adduct levels of the oxidative lesion 8-oxodG as well as the LPO-related lesions $CdG_2$, 8-OH-AdG, εdA, BεdC, BεdG, BεdA, BεmedC, HεdC, HεdG, and HεdA. 8-OxodG was detected in all autopsy tissues, and high detection rates were also found for εdA (93%) and 8-OH-AdG (82%). 4-ONE-related DNA adducts were also frequently detected in various tissue samples: total detection rates for HεdC, HεdG, and HεdA were 97%, 93%, and 63%, respectively. 4-OHE-related BεdC, having a total detection rate of 41%, was commonly found in the colon, liver, and lung, with detection rates higher than 50%. However, the other 4-OHE-related adducts, BεdG, BεdA, and BεmedC, showed lower detection rates of 6%, 9%, and 6%, respectively. The detection rate of the crotonaldehyde-derived DNA adduct $CdG_2$ was 12%. Although crotonaldehyde-induced $CdG_1$ and acrolein-induced 6-OH-$AdG_1$ and 6-OH-$AdG_2$ were also monitored, we could not detect those adducts in any sample. The level of each DNA adduct per $10^8$ bases ranged as follows: 8-oxo-dG, 41.6−837 (median 93.2); $CdG_2$, not detected (ND) to 8.98 (median was under the detection limit); 8-OH-AdG, ND to 3.04 (median 1.14); εdA, ND to 259 (median 4.83); BεdC, ND to 1186 (median was under the detection limit); BεdG, ND to 0.99 (median was under the detection limit; BεdA, ND to 254 (median was under the detection limit); BεmedC, ND to 63.8 (median was under the detection limit); HεdC, ND-8204 (median 10.3); HεdG, ND to 377 (median 15.0); and HεdA, ND to 4186 (median 8.63).

**Adduct Levels of HεdC and BεdC in Different Organs.** As shown in Figure 3, DNA adduct levels of HεdC and BεdC range broadly in different organs. HεdC was detected in all tissue samples except for two liver specimens, whereas BεdC was detected in the colon, kidney, liver, lung, spleen, and pancreas. The median level of HεdC in different organs ranged from 6.4 (kidney) to 23 (colon) adducts per $10^8$ bases, whereas the median of BεdC was 1 or 2 orders of magnitude lower. However, an extremely high level of HεdC (more than 100 adducts per $10^8$ bases) was found in one colon, one lung, one pancreas and two spleen DNA samples, all from different individuals. Also, an extremely high level of BεdC was observed in one lung DNA sample, the same one that showed a high HεdC level as described above. The results suggest that 4-ONE- and 4-OHE-related DNA adducts are widely distributed in various tissues.

Figure 4 shows the correlations of BεdC, εdA, and 8-oxodG with HεdC in human tissue autopsy samples. The DNA adduct level of HεdC was strongly correlated to LPO-induced BεdC ($R^2$ = 0.94) and εdA ($R^2$ = 0.70), but no correlation could be seen between HεdC and the oxidative damage-related lesion 8-oxodG ($R^2$ = 0.02).

## Discussion

In this study, we clearly demonstrated that DNA adducts derived from 4-ONE and 4-OHE occur commonly in human tissues. The levels of the 4-ONE-related DNA adducts HεdC, HεdA, and HεdG in human tissue samples were similar to each other (Supporting Information, Figure S-9), and their median values were 2- to 3-fold higher than that of εdA. However, the 4-OHE-related adducts BεdC, BεmedC, BεdA, and BεdG were detected at lower levels and frequencies; in most samples, their

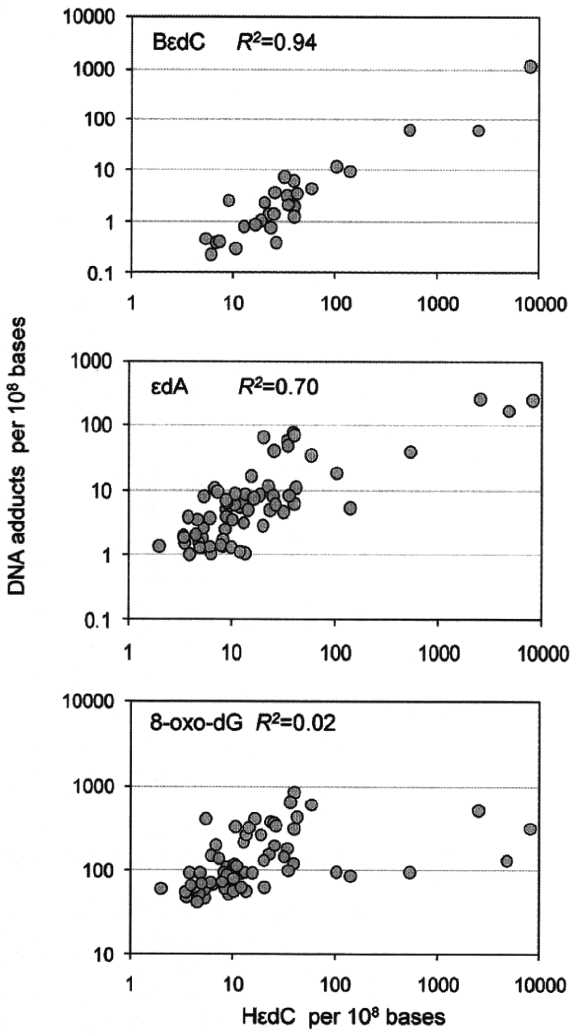


**Figure 4.** Correlations among DNA adduct levels of HεdC vs BεdC, εdA, and 8-oxodG. $R^2$: coefficient of determination. For the $R^2$ calculation, not detected data was treated as 0.

levels were similar to that of crotonaldehyde-derived $CdG_2$ or acrolein-derived 8-OH-AdG. Importantly, in some cases, the levels of these 4-ONE- and 4-OHE-derived DNA adducts were comparable to or even higher than that of the most abundant DNA adduct, 8-oxo-dG. Thus, these recently recognized DNA adducts may be an important source of somatic mutations and could significantly contribute to cancer formation in humans.

The tissues adjacent to those taken for adductome analysis were microscopically examined for the absence of tumor cells. The histological findings varied in terms of inflammation, not otherwise specified. Details of histological characteristics and their relationship to the DNA adducts level are under investigation.

Mutagenic properties of HεdC have been demonstrated in mammalian cell lines and *Escherichia coli* (*26, 27*). Pollack et al. (*26*) reported that in human cell lines HεdC blocked DNA synthesis and also miscoded markedly during the replication of a shuttle vector site-specifically modified with HεdC. The miscoding frequency was higher than 90%, and dT and dA were preferentially inserted opposite the lesion in human cells. HεdC was also shown to be genotoxic in a similar host-vector system consisting of mouse fibroblasts and a replicating plasmid bearing a site-specific HεdC (*25*). Moreover, the results indicated that the Y family DNA polymerases $\eta$, $\kappa$, and $\iota$ preferentially catalyzed the insertion of dT opposite HεdC, whereas an unidentified DNA polymerase was suggested to catalyze the insertion of dA opposite HεdC (*27*). Information about the potential mutagenic properties of the other 4-ONE- and 4-OHE-derived DNA adducts found in human autopsy tissues is still unavailable; thus, further studies concerning the mutagenicity and DNA repair pathways of these newly identified DNA adducts are necessary.

Human tissues could be exposed to 4-ONE and 4-OHE endogenously and exogenously. The endogenous formation of 4-ONE and 4-OHE is via the oxidation of $\omega$6- and $\omega$3-PUFAs in tissues. Because all bodily tissues contain both $\omega$6- and $\omega$3-PUFAs, 4-ONE and 4-OHE could be produced simultaneously under oxidative stress conditions. The near-perfect correlation between the levels of HεdC and BεdC ($R^2$ = 0.94) shown in Figure 4 strongly suggests that there is endogenous and simultaneous formation of 4-ONE- and 4-OHE-derived DNA adducts. According to the slope of the regression curve, the level of HεdC was about 7 times greater than that of BεdC. This also supports the endogenous-formation hypothesis because in all tissues except the brain, the total concentration of $\omega$6- PUFAs is several times higher than that of $\omega$3-PUFAs (*28, 29*).

However, no correlation was observed between the level of HεdC and the level of the oxidative DNA lesion 8-oxo-dG (Figure 4). This discrepancy may be explained by the contribution of enzymatic formation pathways to 4-ONE. For example, Blair's group demonstrated that overexpression of cyclooxygenase-2 (COX-2) increased the level of 4-ONE-derived DNA adducts in both rat intestinal epithelial cells (*30*) and the small intestine of C57BL/6J $APC^{min}$ mice (*31*). COX-2 is an enzyme that is responsible for the formation of the important biological mediator prostaglandin H2. COX-2 can also convert arachidonic acid into 15(*S*)-hydroperoxy-5*Z*,8*Z*,11*Z*,13*E*-eicosatetraenoic acid (15-HPETE), which undergoes homolytic decomposition to the DNA-reactive bifunctional electrophiles 4-hydroperoxy-2(*E*)-nonenal (HPNE), 4,5-epoxy-2(*E*)-decenal (EDE), 4-HNE, and 4-ONE (*31*). 4-ONE is also produced enzymatically from arachidonic acid by the 5-lipoxygenase (5-LO)-related pathway (*32*). 5-LO is an enzyme that is responsible for the formation of leukotriene $A_4$. The precursor of leukotriene $A_4$, 5(*S*)-hydroperoxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid (5(*S*)-HPETE), generated from arachidonic acid by 5-LO, decomposes to form 4-ONE and HPNE (*32*). The considerably good correlation between the DNA adduct levels of HεdC and εdA, as described in Figure 4 ($R^2$ = 0.70), also suggests the involvement of this metabolic pathway, because εdA is known to be produced by HPNE and EDE (*31*). If 4-OHE is also produced enzymatically from abundant $\omega$3-PUFAs such as docosahexaenoic acid, this would help to explain why the level of BεdC nearly perfectly correlates with the level of HεdC but the level of 8-oxo-dG does not. Further study is needed to elucidate this point.

The exogenous sources of 4-ONE and 4-OHE are foods and cooking vapor. Kasai and Kawai reported that several types of cooked fishes and cooking oils contain 4-OHE in the range of a few to tens of micrograms per gram (*21*). They further reported that the cooking vapor emitted during fish broiling also contains 4-OHE (*21*). In an animal experiment, orally administered 4-OHE resulted in the formation of BεdC, BεdG, and BεmedC in cells of the gastrointestinal tract, but no increase in the level of DNA adducts was observed in the liver and kidney (*19*), indicating that, except for the gastrointestinal tract, the oral route is probably not a significant source of 4-OHE. However, the

impact of cooking vapor in terms of the formation of DNA adducts in pulmonary tissues remains to be resolved.

In conclusion, DNA adducts caused by 4-ONE and 4-OHE are ubiquitous in various human tissues, and even predominant in some cases. It is very likely that these DNA adducts cause somatic mutations and cancers, contribute to aging, and have other adverse effects related to DNA damage. Further studies of their exposure routes and biological properties should be carried out to elucidate the impact of these DNA lesions on human health.

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**Supporting Information Available:** Properties of the patients; sensitivity of LC/MS/MS analysis for each DNA adduct (1 and 2); calibration curves of each DNA adduct; representative chromatographs of DNA adducts, 4-OHE-derived DNA adducts, and 4-ONE-derived DNA adducts in human spleen DNA; DNA adducts level in human tissues; and correlations among the 4-ONE-derived DNA adduct level of HεdC vs HεdA (A) and HεdG (B). This material is available free of charge via the Internet at http://pubs.acs.org.

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