

Urinary excretion of ϵ dA is not predictive of cancer development: A prospective nested case–control study

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Abstract

Human biomonitoring of the lipid peroxidation DNA modification 1,*N*⁶-ethenodeoxyadenosine (ϵ dA) excreted into urine is thought to be a potential marker for oxidative stress-related DNA damage and human cancer. We have tested this hypothesis in a prospective, nested case–control study. During the years 1984–1989, 24-h urines were collected from 1956 men in the Kuopio Ischaemic Heart Disease (KIHD) Risk Factor Study. ϵ dA concentrations were measured by LC-MS/MS in 24-h urine samples from 47 men with cancer diagnosed at follow up until 2001 and from 31 cancer free smoking-matched control subjects. Odds ratio for having higher than control median ϵ dA excretion rate and cancer, estimated by binary logistic regression, was 0.73 (95% CI 0.29–1.80, *p* = 0.49). In this study, the urinary excretion of ϵ dA provides no additional prediction of cancer development in males after controlling for smoking.

Keywords: Ethenodeoxyadenosine, lipid peroxidation, carcinogenesis, hypothesis; human urine

Introduction

Etheno (ϵ) modified DNA bases, such as ϵ dA, originating from known human carcinogens vinyl chloride or urethane, are also generated endogenously by reactions of DNA with products derived from lipid peroxidation and oxidative stress.[1] These DNA adducts have miscoding potential and specific repair pathways supporting the hypothesis that ϵ -DNA adducts play a causal relationship in carcinogenesis.[2] Furthermore, several studies have found elevated ϵ -DNA adduct levels in disorders known as risk factors for cancers.[3,4] However, data relating ϵ dA excretion to cancer development have not been published before. We tested directly the hypothesis that urinary ϵ dA excretion is predictive of cancer development in the KIDH cohort.

Methods

The Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) is an ongoing prospective population-based cohort study in middle-aged men from eastern Finland.[5] During the years 1984–1989, 1956 men were enrolled in the study. Follow-up in 1995 and in 2001 identified 82 cancer cases with 23 different 3-digit ICD-9 diagnoses. A few controls turned into cancer cases in the period 1995–2001 and no new controls were drawn for these. This results in an unbalanced number of controls of 75. The controls were matched according to municipality of residence, examination date, age and smoking. At the time of baseline examination between 1984 and 1989, 24-h urine samples were collected from 1956 subjects and aliquots stored at -20°C . A sufficient amount

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Table I. Baseline characteristics of the male subjects.

	Case	Control
Number	47	31
Smokers	22 (47%)	15 (47%)
Age (years)	54.9 (42.0–60.9)*	54.5 (42.0–60.9)*
Height (cm)	172.9 (161.2–183.3)*	173.1 (150.0–183.0)*
Body weight (kg)	82.5 (58.1–135.2)*	81.9 (53.7–117.6)*
Body mass index (kg/m ²)	27.5 (20.8–43.4)*	27.2 (20.0–37.1)*
Diuresis (ml/24-h)	1656 (780–2965)*	1507 (770–2600)*

* Average (range).

of urine, i.e. 3 ml, was available in 47 cases with 21 different ICD-9 diagnoses and 31 controls for analysis of ϵ dA by liquid chromatography tandem mass spectrometry, as described previously.[6] Baseline characteristics are shown in Table I. Excretion of ϵ dA was classified as “high” or “low” based on the median value of the control group and using this cut-off odds ratio for cancer risk was estimated by binary logistic regression with SPSS 12.0 for Windows.

Results

The ϵ dA excretion rates ranged from 2.9 to 98.5 pmol/24-h in all subjects, with urine concentrations ranging from 3.7 to 79.4 pmol/l urine. The difference in urine excretion between both groups was not statistically significant (32.4 ± 20.4 ; 29.2 ± 10.9 , $p > 0.05$). The excretion of ϵ dA was lower in smokers (28.6 ± 18.9) compared to non-smokers (33.3 ± 15.4), although not statistically significant ($p = 0.23$). Odds ratio for cancer risk by high–low ϵ dA excretion was 0.73 (95% CI 0.29–1.80, $p = 0.49$).

Discussion

Normal cellular respiration produces free oxygen radicals and oxidative stress occurs when production exceeds the anti-oxidant capacity. During oxidative stress DNA modifications can result from either direct reaction with the free oxygen radicals or their secondary lipid peroxidation products. Several distinct observations indicate that ϵ -adducts may be of importance in human carcinogenesis: (i) the finding of ϵ -adducts in tumor tissues, (ii) the highly miscoding nature of the lesions, (iii) the induction of the lesions with known carcinogens such as vinyl chloride, (iv) the finding of specific and efficient repair systems and (v) they originate from lipid peroxides produced during normal cellular respiration. The level of adducts observed in cellular DNA reflects the balance between adduct formation and repair. As a consequence of repair, adducts are excreted into the urine. Urinary excretion of oxidized DNA nucleosides was initially suggested at a biomarker of oxidative stress by Ames et al.[7] The hypothesis of the present study is based on the idea that a higher rate of adduct formation

would imply a higher risk of cancer by an increased risk for un-repaired lesions leading to a mutation. With time the risk of mutations in important genes like oncogenes and tumor suppressor genes would be increased. However, the results of this study do not support the hypothesis that urinary excretion of ϵ dA is a suitable biomarker for cancer risk.

Multiple other mutagenic products are produced during cellular respiration. It might very well be that the ϵ dA lesion represents such a small fraction of the total production of endogenously mutagens that even large changes would mean such a minute overall change that it would not be reflected in an increased cancer risk. The levels found support such a view. Etheno adducts are generally found in about 1 per 10^9 DNA bases, whereas 8-oxo-dG is found in 5 per 10^6 dG.

Another explanation for a negative finding in the present study could be a low range in excretion of ϵ dA in the population examined. However, there was a 2-fold difference in the average values of the “high” and “low” ϵ dA excretion, i.e. the two groups used for the statistical analysis: 18.4 and 43.4 pmol/24-h. The highest observed value was 34-fold higher than the lowest observed, also indicating a large range. Experimental animals exposed to high amounts of vinyl chloride or chloroethylene oxide revealed a approximately 30-fold increase of $N^2,3\text{-}\epsilon\text{Gua}$ levels in hepatocyte DNA[8] and a 50-fold increased urinary excretion of $1,N^6\text{-}\epsilon\text{Ade}$.[9] Bogdanffy et al. used a similar dose level (rats exposed to vinyl fluoride 6 h/day 5 days/week for 2 years), which resulted in 30% incidence of hemangiosarcomas,[10] a tumor not found in the present study. The range of ϵ dA excretion found in the present study appears of a sufficient magnitude and small differences cannot explain the negative finding.

A third explanation for our negative finding could relate to difference in organ adducts levels. Not much is known about the variation of adducts levels and mutation rate in single organs. The rate of adduct formation measured by urinary excretion represents an average whole body background production of ϵ -DNA adducts. The data presented in this paper cannot rule out that small organs could have particular high oxidative stress resulting in ϵ dA induced mutations and subsequent risk of carcinogenesis. On the other hand occupational exposure to vinyl chloride, known to induce ϵ dA adducts, is associated with an increased risk of hepatic angiosarcoma, but not of other cancers.[11] This indicates that in special circumstances, e.g. vinyl chloride exposure, ϵ -adducts can be linked to cancer risk. The lack of a general cancer risk from exposure to vinyl chloride supports our finding of lack of association between ϵ dA formation rate and cancer risk.

Cigarette smoking has been shown to be associated with increased oxidative damage and may provide a model for evaluating and validating methods for

quantification of oxidative stress. In the current and a prior study, [6] we found that smokers had lower urinary excretion of ϵ dA than non-smokers, although not significantly different. In contrast, Chen et al. found higher levels in smokers than in non-smoker of the ϵ -adducts; ϵ Ade, [12] ϵ Cyt [13] and ϵ dC. [14] Contradictory, in malignant and non-malignant lung tissue the DNA levels of ϵ dA and ϵ dC were about 50% lower in smokers, not reaching statistical significance. [15] This finding could be attributed to effective ϵ -DNA repair and explain the observed difference by Chen et al. [12–14] However, Speina et al. found no differences in ϵ Ade and ϵ Cyt levels between tumor and non-affected lung tissues in cancer patients, as well as their repair activities [16] and together these findings strongly points at no increase in this type of oxidative stress in smokers.

Several other DNA modifications from lipid peroxidation have been characterized. However, their relation to cancer development is unknown. Based on the fact that these lipid peroxidation products are found in levels as ϵ dA—show similar or less mispairing properties and are repaired by the same mechanisms—there is no evidence supporting a different role in predicting cancer development. However, it cannot be ruled out that the total amount of such lesions, other oxidative and reactive metabolite lesions or a special gene affinity for a given lesion could be the basis for a relationship to cancer development.

The etheno adducts to DNA have been hypothesized to be linked to cancer as a general mechanism. To test this hypothesis we examined all cancers irrespective of type and location. The odd's ratio for cancer development we observed was below 1 (0.73; 95% CI 0.29–1.80, $p = 0.49$) indicating no relationship between cancer development in general. Consequently, we conclude that even though there is a molecular biological possibility for a relation between lipid peroxidation product binding to DNA and cancer development in general, we found no evidence supporting this in men after controlling for smoking; future research must focus on single particular cancer types for establishing possible relations between etheno adduct formation and cancer development.

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for the ϵ dA measurements. Peter R. Hillestrøm and Henrik E. Poulsen wrote the report. All authors revised and approved the manuscript.

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