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SHORT COMMUNICATION

Inter-individual variation of smoking-related DNA adducts in lymphocytes–relationship to mRNA levels for CYP1A1 and DNA repair enzymes

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The measurement of DNA adducts is a useful indicator for environmental carcinogen exposure monitoring. To clarify the effect of metabolic activation and DNA repair system on the inter-individual variation of DNA adduct levels, aromatic DNA adducts and mRNA expression of metabolic and repair enzymes were measured in 43 human lymphocytes. Aromatic DNA adducts were measured by the nuclease P1 postlabelling method. The metabolic activation enzyme; cytochrome P4501A1 (CYPIA1), and the repair enzyme; excision repair cross complimenting gene (ERCC1), and the xeroderma pigmentosum C group cell gene (XPCC), mRNA expression were measured by the reverse transcription-PCR method. The mean adduct levels were 1.01 ± 0.49 in 43 subjects. There was a positive correlation between DNA adducts and CYP1A1 mRNA ($r = 0.33$, $p = 0.12$). DNA adduct levels had a positive correlation with ERCC1 ($r = 0.35$, $p = 0.03$) and a negative correlation with XPCC mRNA levels ($r = -0.28$, $p = 0.07$). We found Brinkman index, CYP1A1 genotypes, CYP1A1 mRNA and XPCC mRNA as a predictor for log DNA adduct levels in multivariate analysis. Metabolic activation and the repair system may explain the inter-individual variation of DNA adducts in lymphocytes.

Keywords: DNA adduct; repair.

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Introduction

Environmental carcinogens are enzymatically activated to form intermediates that can react with cellular DNA adducts. The measurement of DNA adducts is a useful indicator for environmental carcinogen exposure monitoring. There are several studies in which smoking-related DNA adducts were measured by the ^{32}P-postlabelling method in peripheral blood samples (Beach and Gupta 1992). However, most of the studies have shown a poor correlation between DNA adduct levels and smoking dose, because the inter-individual variation of adduct levels was very large (Hemminki 1995). The genetic polymorphisms of metabolic enzymes have been thought by several workers to clarify the inter-individual variation of DNA adduct levels (Ichiba *et al*. 1994, Hou *et al*. 1995, Rothman *et al*. 1995, Nielsen *et al*. 1996, Hemminki *et al*. 1997, Ichiba *et al*. 1997) but their results were not consistent. On the other hand, the DNA repair system was also thought to be an important factor for DNA adduct levels. Bulky aromatic DNA adducts were

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repaired by the nucleotide excision repair system. Excision repair has been mainly studied in xeroderma pigmentosum and the repair rate was shown to have very large inter-individual variation (Harris 1989). The relationship between repair system and DNA adduct levels were not so simple. High adduct levels can lead to high expression of repair enzyme. This would result in a positive correlation between adducts and repair enzyme expression. Alternatively, a high repair rate can decrease DNA adduct levels and would result in a negative correlation. There have been few studies which have analysed the effect of the repair system on DNA adduct levels in healthy human samples. Wei *et al.* (1995) said that the mRNA expression levels could be correlated with the levels of DNA adducts in human T-lymphocytes.

In this study, in order to explain the inter-individual variations of DNA adduct levels in lymphocytes, the metabolic activation enzyme and the repair enzyme mRNA expressions were measured among smokers and non-smokers, and compared with aromatic DNA adduct levels.

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Subjects and methods

The subjects were 43 healthy volunteers, 32 men and 11 women from our medical school students. Their mean age was 22.6 years. There were 16 smokers and 27 non-smokers. DNA and RNA were extracted from their peripheral blood lymphocytes. Aromatic DNA adducts were measured by the ^{32}P-postlabelling nuclease P1 method (Reddy and Randerath 1986). The results were given as a total number of adducts per 10^8 normal nucleotides. As metabolic activation enzyme, cytochrome P4501A1 (CYP1A1) mRNA expression was measured ($n=24$) by the reverse transcription-PCR (RT-PCR) method of Rumsby *et al.* (1996). As repair enzyme, excision repair cross complimenting gene (ERCC1) and xeroderma pigmentosum C group cell gene (XPCC) mRNA expressions were measured ($n=40$) by the method of Wei *et al.* (1995). The values were expressed as arbitrary densitometric units related to the β-actin gene. CYP1A1 Msp1 polymorphism of the 3′-flanking region was measured ($n=42$) by the PCR-restriction fragment length polymorphism method (Hayashi *et al.* 1992). Subjects were divided into three groups: m1/m1, m1/m2, and m2/m2. Unpaired *t*-test, Pearson's correlation and multiple regression analysis were used for statistical analysis. DNA adducts data were log transformed before statistical analysis.

Results and discussion

Table 1 shows analytical results. The mean adduct levels in smokers were slightly higher than those in non-smokers ($p=0.09$). But inter-individual variation was large (figure 1). CYP1A1, ERCC1 and XPCC mRNA expressions were not significantly different between smokers and non-smokers. There was a positive correlation between ERCC1 and XPCC mRNA expression ($n=40$, $r=0.41$, $p=0.008$). DNA adduct levels by the CYP1A1 genotypes, m1/m1, m1/m2 and m2/m2 were 1.04 ± 0.54, 0.96 ± 0.43, 1.60, respectively. There was no significant difference among the three genotypes.

Figure 2 shows the correlation between log DNA adducts and enzyme mRNA expressions. There was a positive correlation between log DNA adducts and CYP1A1 mRNA, but this was not significant ($n=24$, $r=0.33$, $p=0.12$). When subjects were divided into three groups according to CYP1A1 Msp1 genotypes, the correlation was 0.56 ($n=15$, $p=0.12$) in CYP1A1 m1/m2 genotypes. In m1/m1 genotypes, we could not get significant correlation. A significant positive correlation was found between log DNA adducts and CYP1A1 mRNA in CYP1A1 m1/m2 and m2/m2 genotypes only in smokers ($n=5$, $r=0.98$, $p=0.004$). Log DNA adduct levels had a significant positive correlation with ERCC1 ($n=40$, $r=0.35$, $p=0.03$) and a negative correlation with XPCC mRNA levels ($n=40$,

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Table 1. Analytical results of all subjects

	Smoker	Non-smoker	All
Number	16	27	43
No. of cigarettes/day	15 ± 11		
DNA adduct (/10^8)	1.13 ± 0.46	0.95 ± 0.49	1.01 ± 0.49
CYP1A1 mRNA[a]	1.06 ± 0.47	1.22 ± 0.65	1.14 ± 0.56
ERCC1 mRNA[a]	0.87 ± 0.20	0.79 ± 0.16	
XPCC mRNA[a]	0.53 ± 0.14	0.55 ± 0.14	
No. of Msp1 m1/m2	5 (32 %)	8 (31 %)	13 (31 %)
m1/m2	7 (44 %)	18 (69 %)	25 (60 %)
m2/m2	4 (25 %)	0	4 (10 %)

[a] The values are arbitrary densitometric units related to β-actin.

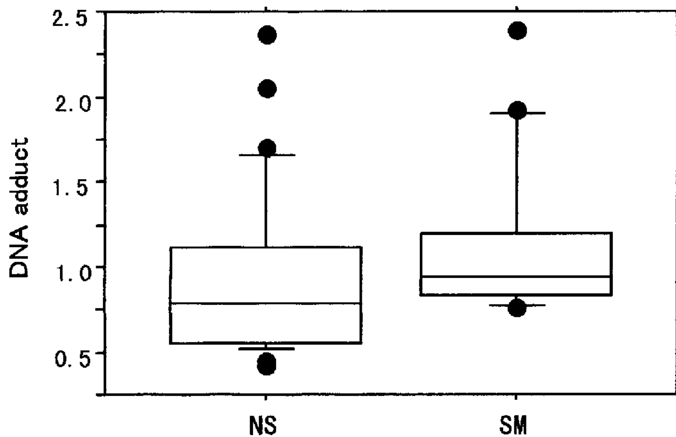

Figure 1. DNA adduct distribution in non-smokers (NS) and smokers (SM). Adduct levels were expressed number of adducts per 10^8 nucleotides. Boxes indicate 25th and 75th percentiles with median levels indicated with horizontal line. Outer horizontal lines indicate 10th and 90th percentiles. Small circles indicate data over 10th and 90th percentiles.

$r = -0.28$, $p = 0.07$). In non-smokers, log DNA adducts levels had a significantly positive correlation with ERCC1 mRNA levels ($n = 24$, $r = 0.63$, $p < 0.01$). In contrast, in smokers, we could not get a significant correlation between log DNA adducts and repair enzyme mRNA.

In this study we measured CYP1A1 mRNA expression and compared it with aromatic DNA adduct levels in lymphocytes. Phase 1 enzymes, such as cytochrome P4501A1, activate polycyclic aromatic hydrocarbons (PAH) contained in cigarette smoke. There have been several studies which have considered the effects of genetic polymorphism of CYP1A1 on DNA adduct levels. (Ichiba *et al.* 1994, Rothman *et al.* 1995, Hemminki *et al.* 1997, Ichiba *et al.* 1997). But their results were not consistent. There have been few studies that compared CYP1A1 mRNA expression with aromatic DNA adducts in lymphocytes. We expected that high expression of CYP1A1 would lead to high levels of DNA adduct. In this study we found a weak positive correlation between DNA adducts and CYP1A1 gene expression as we expected. Our results were consistent with those of Bartsch *et al.* (1995). They found that aryl hydrocarbon hydroxylase activity, CYP1A1 activity, and DNA adducts showed a positive correlation.

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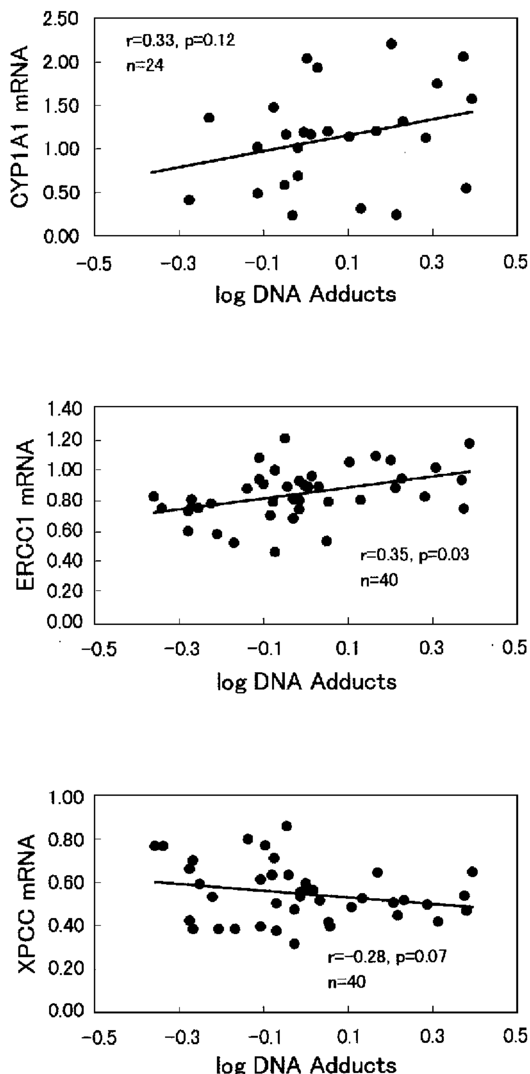

Figure 2. Relationship between DNA adducts and enzyme mRNA expressions of all subjects.

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Not only the activation system but also the DNA repair system has been thought to be an important factor for DNA adduct levels. Nucleotide excision repair is the main repair system for bulky aromatic DNA adducts (Sancar 1995). The nucleotide excision repair system was a complicated system with several enzymes. There have been few studies that measured repair enzyme expression and DNA adduct levels (Wei *et al*. 1995). In this study we measured two repair enzymes showing mRNA expression, ERCC1 and XPCC. We found that ERCC1 had a positive and XPCC had a negative correlation with DNA adducts. Why is there a positive correlation between adduct levels and ERCC1, while the correlation is negative with XPCC? Multiple regression analysis was carried out, taking into account gender, age, Brinkman index, CYP1A1 polymorphism and mRNA levels. Brinkman index, CYP1A1 genotypes, CYP1A1 mRNA, XPCC mRNA were found as predictors for

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log DNA adduct levels. Standard regression coefficient were 0.33 (CYP1A1 mRNA), –0.40 (XPCC mRNA), –0.28 (CYP1A1 genotype), and 0.42 (Brinkman index).

In conclusion, we found a positive effect of CYP1A1 mRNA and a negative effect of XPCC mRNA on DNA adduct levels. Metabolic activation and repair enzyme expression may explain inter-individual variation of DNA adducts in lymphocytes. In this study, the sample size was not large and only two repair enzymes were measured. We need to analyse other repair enzyme to explain inter-individual variation.

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