# Detection of Carcinogen–DNA Adducts in Human Fetal Tissues by the $^{32}$P-Postlabeling Procedure

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Tobacco smoke contains a number of genotoxic compounds that are metabolized to their biologically active forms that subsequently react with cellular DNA to form covalently bound carcinogen–DNA adducts. Several analytical procedures have been developed to detect these adducts in human tissues. Using the nuclease P1-enhanced $^{32}$P-postlabeling procedure for bulky adducts, we have detected at least 24 adducts in DNA isolated from placenta and umbilical cord DNA. Adducts were detected in both smokers and nonsmokers, but the relative adduct level (RAL) was significantly higher in smokers (42.8, 8 cases) than in nonsmokers (19.7, 11 cases). The origin of the adducts in nonsmokers remains unknown. The adduct levels in artery DNA were significantly lower than in the vein and the placenta, and a paired nonparametric analysis showed a significant association between the adduct levels in the three tissues. Our results show a maternal transfer of carcinogens present in cigarette smoke to fetal tissues and show that the tissues can metabolize the carcinogens to their DNA binding metabolites. The presence of adducts in fetal tissues may be indicative of genomic damage and may predispose the individual for the development of a serious disease later in life.

## Introduction

Epidemiological and laboratory investigations have shown that environmental carcinogens, including the ones found in tobacco smoke, play an important role in the etiology of cancer [e.g., cancer of the lung, larynx, oral cavity, and bladder (1)]. Recently, it has been proposed that chemical carcinogens may also be involved in the development of various forms of cardiovascular disease (2,3). In addition, smoking tobacco products during pregnancy is associated with an increased risk to the unborn child, resulting in preterm delivery, decreased birth weight, stillbirth, and a number of serious congenital malformations (4–6).

Chemical carcinogens are metabolized in a number of human tissues and cells (7,8), and smoking related carcinogen–DNA adducts have been detected in human tissue (9–11). The adduct levels in lung tissue correlate with the number of cigarettes smoked, and these adducts are retained for a relatively long period after cessation of smoking (9). Exposure to chemical carcinogens during pregnancy leads to *in vivo* formation of carcinogen–DNA adducts not only in the placenta, but also in the lungs and livers of aborted fetuses (5,12,13).

We report that covalently bound DNA adducts, as assessed by $^{32}$P-postlabeling, are present in the placenta as well as in umbilical cord vasculature obtained from otherwise healthy smoking and nonsmoking donors. Adduct levels were found to be significantly higher in smokers as compared to nonsmokers. Maternal adduct levels were significantly higher than fetal levels, and marginally significant differences were obtained between adduct levels in the three tissues when smokers and nonsmokers were compared.

## Materials and Methods

*Donors.* Nineteen completely healthy women were selected, all of whom had had an uncomplicated pregnancy and delivery and who had given birth to healthy, full-term children without any abnormalities and with an Apgar score of 10/10. Prior to the collection, all the women gave informed consent according to the Helsinki II declaration. According to the questionnaire, 11 of these women were nonsmokers, including 3 who had ceased smoking more than a year before pregnancy. The smoking group comprised eight women, of whom two said that they smoked less than 10 cigarettes daily.

*Isolation of Tissues and DNA.* Tissue blocks were obtained from placenta and umbilical cord within 4 hr after delivery, and cord artery and vein were carefully dissected. All samples were coded before DNA isolation and postlabeling in order to perform a blind analysis of the samples. DNA was isolated from the tissues essentially as described (4). The endothelium of the um-

bilical cord vessels was not removed from the basement membrane before DNA isolation. The concentration and quality of the DNA were estimated by spectrophotometry at 260 and 280 nm. Following quantification, purified DNA was frozen and stored at −20°C until use.

*32P-Postlabeling Assay.* DNA was enzymatically degraded to deoxynucleoside 3'-monophosphates by incubation with a mixture of micrococcal nuclease (2U/μL) and spleen phosphodiesterase (2 μg/μL) for 16 hr (15). Nuclease P1 (0.3 μg/μg DNA) was used to remove nonmodified nucleosides from the suspension before postlabeling (16). The carcinogen-modified nucleosides were transformed to their 5'-32P-labeled deoxynucleotide 3',5'-bisphosphate by T4 polynucleotide kinase (3 U/4 μg DNA) and freshly made [γ-32P]ATP (75 μCi/4 μg DNA) (17). The samples were incubated for 30 min at 37°C with potato apyrase (10 mU/μg DNA) to remove unreacted [γ-32P]ATP. The samples were then separated on PEI-cellulose TLC plates (10 × 20 cm).

The plate was autoradiographed and the spots were quantified by Cerenkov counting. Relative adduct level (RAL) values were calculated as described (16) and expressed as number of base modifications per $10^9$ nucleotides.

***Statistical Methods.*** For most statistical analyses, an unpaired, one-tailed Student's *t*-test was applied, using a significance level of 5%. For comparison of adduct levels between placenta, umbilical cord vein, and artery, Duncan's multiple rank test was used.

# Results

## Identification of Carcinogen–DNA Adducts

Covalently bound carcinogen–DNA adducts were detected in tissues from both smokers and nonsmokers. A total of 24 different adducts was detected. No definitive correlation was found between specific adduct spots and smoking status. Because the amount of radioactivity in some areas of the autoradiographs was very high, we were unable to separate these areas into individual spots. These were, therefore, counted as one single spot and given a spot number of their own, even though spot numbers already were assigned to areas of the chromatogram occupied by the composite spot. This may have led to discrepancies when specific smoking-related spots were evaluated. The presence of covalently bound DNA adducts in one tissue did not necessarily correlate with the presence of the same adduct in the other tissues from the same donor.

## Relative Adduct Levels

Large interindividual variation was observed in total adduct levels (RAL value) in tissues from both smokers and nonsmokers. Smokers had higher adduct levels as compared to nonsmokers. RAL values in smokers regardless of tissue, compared with nonsmokers (Table 1) were significantly different ($p = 0.021$) using an unpaired Student's *t*-test, and the adduct level in maternal tissue was significantly higher ($p = 0.030$) than in fetal tissue. No statistical difference was observed when total fetal adduct levels between smokers and nonsmokers were compared ($p = 0.089$).

The mean value of RAL in placenta from smokers and nonsmokers was equivalent to that in the corresponding um-

Table 1. Mean adduct levels in human placenta and umbilical cord vessels.

| Smoking status | Tissue | RAL value × $10^9$ nucleotides[a] | Sample size | *p*-Value[b] |
|---|---|---|---|---|
| Nonsmoker | Total[c] | 19.7 ± 4.2 | 29 | |
| | | | | 0.021 |
| Smoker | Total[c] | 42.8 ± 11.5 | 22 | |
| Both | Fetal | 21.8 ± 6.1 | 33 | |
| | | | | 0.030 |
| Both | Maternal[d] | 44.0 ± 11.0 | 18 | |
| Nonsmoker | Fetal | 14.7 ± 5.1 | 19 | |
| | | | | 0.089 |
| Smoker | Fetal | 31.4 ± 12.4 | 14 | |
| Both | Placenta | 44.0 ± 11.0 | 18 | A[e] |
| Both | Vein | 30.1 ± 4.9 | 16 | A,B[e] |
| Both | Artery | 14.0 ± 11.2 | 17 | B[e] |
| Nonsmoker | Placenta | 29.2 ± 6.8 | 10 | |
| | | | | 0.067 |
| Smoker | Placenta | 62.6 ± 22.3 | 8 | |
| Nonsmoker | Vein | 7.3 ± 2.3 | 10 | |
| | | | | 0.054 |
| Smoker | Vein | 23.5 ± 11.0 | 7 | |
| Nonsmoker | Artery | 22.9 ± 10.0 | 9 | |
| | | | | 0.244 |
| Smoker | Artery | 39.4 ± 23.0 | 7 | |

RAL, relative adduct level.
[a]RAL value ± SE of the mean.
[b]Unpaired, one-tailed Student's *t*-test except where noted.
[c]Placenta, vein, and artery tissues were combined.
[d]Maternal tissue is defined as placenta, athough this is a mixed tissue consisting of both maternal and fetal tissues.
[e]Statistical analysis was done with Duncan's multiple range test.

bilical cord artery, whereas the level in umbilical cord vein was significantly lower than the mean RAL value in placenta. Furthermore, comparison between smoking status of the mother and mean RAL values in placenta, umbilical cord vein, and artery showed that the levels tended to be higher in smokers than in nonsmokers. This observation, however, was only marginally significant for placenta and vein ($p = 0.067$ and $p = 0.059$, respectively).

# Discussion

Smoking-related DNA adducts have been demonstrated in placental tissues (5,12,18). Using the 32P-postlabeling technique, carcinogen–DNA adducts have been detected in umbilical cord vein and artery in addition to placenta from both nonsmoking and smoking women. The adducts in nonsmokers must be ascribed to chemical compounds present in the general environment. The tissue donors lived in a highly populated area of Copenhagen. An alternative explanation for these background spots in nonsmoking donors could be that these adducts originate from intrinsic factors (19) or are related to age or dietary products (20). Third, the presence of background adduct spots in nonsmokers may be indicative of passive smoking, but information on exposure to environmental tobacco smoke was not obtained. The observation that covalently bound DNA adducts were present not only in the placenta but also in umbilical cord vein and artery, two tissues of solely fetal origin, indicates that the fetus is transplacentally exposed to genotoxic compounds found in the general environment. We have not been able to show any correlation between distinct adduct spots and maternal smoking, similar to other investigators.

Mean adduct levels in tissues from smokers were significantly higher than in tissues from nonsmokers. Large interindividual variation in the relative adduct level was observed within both the smoker and nonsmoker group. For smokers, the number of cigarettes smoked per day may have an effect as seen in human lung (10). More than a 70-fold variation in the level of aryl hydrocarbon hydroxylase activity was observed in placental tissues. This combined with differences in DNA repair capabilities could influence adduct levels (9).

The mean adduct level in maternal tissues was significantly higher than the level in fetal tissue. To reach fetal tissues, compounds must diffuse across the placental barrier, which may retain several compounds due to differences in hydrophobicity. Comparing the mean nonsmoking maternal adduct level with mean nonsmoking fetal tissue shows that there is a marginally significant higher level in maternal tissue, whereas the difference in tissues from smokers was obliterated. This may indicate that the placenta of nonsmoking donors metabolizes the relatively low levels of chemical carcinogens present, turning them into DNA binding forms, which mostly react with placental DNA. In smokers, however, the amount of chemical substances exceeds the metabolizing capacity of the placenta, and procarcinogens or metabolites are transferred to the fetus resulting in adduct levels comparable to that of maternal tissue.

The observation that the mean adduct level in placenta and fetal umbilical cord vein DNA is marginally increased in smokers is an interesting finding, because this substantiates that transplacental exposure to chemical carcinogens occurs in humans. However, no statistical difference between average adduct levels in umbilical cord artery from smokers and nonsmokers was observed, although the trend indicates that such a correlation might exist.

Several animal studies have shown that transplacental exposure to chemical carcinogens may lead to an increased risk of developing cancer later in life. The finding that adducts are formed *in utero* in otherwise normal human fetuses, not only as a consequence of maternal smoking, but also as a consequence of background exposures to genotoxic compounds in the environment is of concern because the DNA damage may result in an initiated cancer cell.

# Conclusions

It can be concluded that carcinogen–DNA adducts are formed in human placenta and in umbilical cord artery and vein as a consequence of transplacental exposure to genotoxic compounds. The formation of adducts in these tissues seems to correlate to maternal smoking during pregnancy, although adducts are formed to a certain extent in nonsmokers. The latter is probably due to environmental exposures from other sources. Adduct levels in tissues obtained from smokers were marginally higher than in tissues from nonsmokers. Fetal metabolism seems to play a role in the transformation of genotoxic compounds into reactive metabolites because it was observed that the level of adducts in umbilical cord artery was of the same magnitude as that found in the placenta, whereas mean adduct levels in the umbilical cord vein were significantly lower than in placenta.

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