Molecular dosimetry studies of smoking - induced carcinogenesis in target and surrogate tissues of humans

CHAPTER 9

SUMMARY AND CONCLUSION

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A large body of evidence has shown that tobacco smoking is involved in the etiology of several human cancers (1,2). Tobacco smoke carcinogenicity is largely ascribed to its DNA-reactive constituents (3). These compounds comprise a wide range of chemicals with specific structures, which enable them to bind covalently to DNA and form DNA adducts (3). Formation of DNA adducts is an event of potential significance in carcinogenesis because it may give rise to chromosomal aberrations, DNA strand breaks, oncogene activation and tumor suppressor gene inactivation (4-6). By definition, DNA adducts are the biologically effective dose markers of exposure to carcinogens. That is that they not only represent a prior exposure to carcinogens but they also imply a risk for cancer (5). Of course, the implication of DNA adducts in cancer is not straightforward. The fact is that other processes such as cellular proliferation along with, or separate from DNA adduct formation may modulate carcinogenesis (5,7). Therefore, interpretation of DNA adduct data in relation to cancer ought to be done cautiously. Theoretically, dosimetry of DNA adducts should be performed within the organs where tumor arises [target organs]. Practically, however, most target organs for tobacco-associated cancers are only invasively accessible (8,9). Also, the commonly used non-target organs show inconsistent surrogacy for the target ones (10,11). The latter is mainly due to the incomparability of exposure patterns in surrogate *versus* target organs. In addition, different cell compositions with varying biotransformational and DNA repair capacities in target and surrogate organs may also be responsible for such inconsistency (12,13).

In the present thesis, we explored the carcinogenicity of tobacco smoke in humans by dosimetry of DNA adducts in various target and surrogate matrices. Focusing on three major classes of DNA adduct-inducing agents, polycyclic aromatic hydrocarbons (PAH), aromatic amines and reactive oxygen species (ROS), we studied the representative DNA adducts, benzo[a]pyrene diol epoxide (BPDE) -DNA adducts, 4-aminobiohenyl (4-ABP) -DNA adducts and 8-oxo-7,8-dihydroguanine (8-oxo-Gua), respectively (14-17).

In chapter 2, we examined the validity of DNA adduct analysis in induced sputum (IS), a non-invasive derivative from the lower airway (18). We detected significantly higher levels of smoke-related DNA adducts in IS of smokers as compared to non-smokers by both versions of the ^{32}P-postlabeling assay, the nuclease P1 digestion and the butanol extraction methods. The similarity and correlation between the levels of adducts quantified by different enhancement methods confirm the previous findings by others indicating the PAH-derived nature of adducts in the lower respiratory tract (19-21). Technically, the NP1 digestion method degrades the C8-guanine-adducted nucleotides of aromatic amines and substantially enhances the N^2-guanine adducts, whereas the butanol extraction method enriches both types of adducts (22). Furthermore, the dose-dependency of smoke-related DNA adducts in IS suggests that this matrix can be used for molecular dosimetry of inhalatory carcinogens. Also, the comparability of our results to those obtained in bronchoalveolar lavage (BAL) cells, an already validated but invasively accessible matrix (23-25), implies that sputum induction may replace the BAL method for sampling airway.

In chapter 3, we validated DNA adduct dosimetry in IS and further, compared it with dosimetry of DNA adducts in peripheral blood lymphocytes (PBL) (26). First and foremost, DNA adduct analysis in IS produced similar results to what we had previously reported (18). Also, repeated measurement of DNA adducts in IS and PBL showed a consistency in the level of adducts in both matrices [over a three-week period]. Comparatively, adduct analysis in IS was more explicit than that in PBL both qualitatively and quantitatively. Moreover, there were some indications of the persistency of DNA adducts in PBL. For example, DNA adduct levels in PBL were dependent on the cumulative dose of exposure to tobacco smoke [pack years]. In addition, the levels of adducts in PBL did not drop as drastic as those in IS after smoking cessation. Altogether, it appears that dosimetry of DNA adducts in IS is a choice of preference for studying tobacco smoke carcinogenicity.

In chapter 4, we separately quantified aromatic amine- and PAH-DNA adducts in IS by immunohistochemistry of 4-ABP- and BPDE-DNA adducts, respectively (27). We found that the levels of both types of adducts were dose-dependently related to the current smoking intensity; however, the results were more pronounced for 4-ABP-DNA adducts. On the one hand, this shows the specificity of immunohistochemistry of 4-ABP-DNA adducts. On the other hand, it suggests that ubiquitous confounding exposure to PAH (28) may impact upon immunohistochemistry of BPDE-DNA adducts. Undoubtedly, the high levels of BPDE-DNA adducts in some of the non-smokers and the wider range of BPDE-DNA adducts as compared to 4-ABP-DNA adducts in non-smokers reinforce this view.

In chapter 5, we used DNA adduct dosimetry in IS and PBL together with ambient air monitoring for assessing non-smokers' exposure to environmental tobacco smoke (ETS). Our air monitoring data showed that spending an average period of time in a smoky pub results in relatively high exposure to ETS. Accordingly, we found reasonable enhancement in smoke-related DNA adducts in IS but not in PBL after the pub visit. Of most significance was the formation of BPDE-DNA adducts in IS of a few individuals at post-exposure time. Noteworthily, this adduct is formed at the mutational hotspots of lung cancer (6,29) for which ETS exposure is a known risk factor (30-33). Taken together, the results of this pilot study indicate that DNA adduct analysis in IS might potentially be used as an integral approach to assess ETS exposure as well as to study ETS-related cancers.

In chapter 6, we explored the relevance of markers of oxidative DNA damage/repair as well as antioxidative defense mechanisms for studying tobacco smoke carcinogenicity (34). Although it is a known fact that smoking induces oxidative stress, we found a down regulation of ROS-induced DNA damage (17) in smokers. We hypothesized that this phenomenon may occur as a result of adaptation of antioxidative defense and/or DNA repair systems in smokers. However, quantification of the antioxidative capacity of plasma and genotyping of a relevant antioxidant enzyme, glutathione *S*-transferase M1 (*GSTM1*) did not support this hypothesis. Also, measurement of the overall DNA repair activity and genotyping of a specific DNA repair enzyme, human 8-hydroxyl-2'-deoxyguanosine (8-OH-dG) - glycosylase/apurinic lyase (*hOGG1*) were not supportive of this hypothesis. Given the controversial results of other studies (35-41), we may consider that the herein-quantified markers are not specific and sensitive to show the subtle effects of smoking. For example, the low prevalence of *hOGG1* polymorphism may easily mask the impact of this genotype on DNA repair pathway in small-scale studies. It is worthy mentioning that in our study most

evaluated pathways were highly influenced by host co-factors. For instance, our multiple regression analysis revealed that the non-significant up regulation of plasma antioxidants scavenging capacity in smokers was mainly gender-related. Accordingly, adjustment of data for gender bridged the gap between smokers' and non-smokers' antioxidative capacity. Moreover, males who are under greater oxidative burden due to their higher metabolic rates (42), had lower level of oxidative DNA damage, which was explained by their elevated plasma antioxidants scavenging capacity. Altogether, it seems that the current markers of oxidative DNA damage/repair and antioxidative defense mechanisms need further validation before they can be used for studying tobacco smoke carcinogenicity in humans.

In chapter 7, we investigated the predictive value of DNA adducts for exposure to tobacco smoke and risk for oral cancer (43). Immunohistochemistry of PAH-DNA adducts in two different regions of the oral cavity with varying cancer susceptibility profiles (44-46) showed a dose-dependency of DNA adducts with regard to current smoking intensity. However, PAH-DNA adduct levels *in situ* did not correspond with the cancer proneness of the respective subsite. In fact, mouth floor cells, which are highly cancer susceptible had lower level of DNA adducts compared to buccal mucosa cells. Apparently, DNA adduct formation in the oral cavity is a consequence of exposure to tobacco smoke rather than a pre-requisite for developing oral cancer. Thus, dosimetry of DNA adducts in the oral cells can safely mirror the exposure to tobacco smoke. Yet, the complexity of the multi-stage process of carcinogenesis (5) makes it unpredictable by DNA adduct dosimetry *per se*.

In chapter 8, we quantified various smoke-associated markers to verify the efficacy of chemopreventive agent *N*-acetyl-L-cysteine (NAC) in humans. We assessed the effects of NAC on different biological endpoints because NAC has been shown to exert its effects through a variety of coordinated mechanisms (47-50). Accordingly, we found that NAC could modulate certain markers within specific matrices. For example, NAC significantly inhibited the formation of DNA adducts in the BAL cells but not in the oral mucosa or PBL. Also, we observed a dual effects of NAC [efficacy/inefficacy] within the individual matrices. For instance, the selectivity of NAC in inhibiting DNA adducts, boosting antioxidative capacity and preventing cytogenetic damages [micronuclei frequency] were independent of each other. Taken together, our results reaffirm the previous experimental data, which have shown a tissue specificity for uptake and efficacy of NAC (51-62). At the same time, they reiterate the importance of a multi-biomarker approach to study the underlying mechanisms of carcinogenesis.

Summarizing the herein-presented data and those from the literature, we may consider that dosimetry of PAH- and aromatic amine-DNA adducts in target and relevant surrogate matrices eg., induced sputum is a valuable means to study tobacco smoke carcinogenicity in humans. However, the relevance of ROS-induced DNA adducts and other oxidative stress-associated markers for such study remain to be seen. To better understand the smoking-induced carcinogenesis in humans and the roles that are played by chemical carcinogens in it, future large-scale research is needed. Such investigations should be conducted in a multi-disciplinary way to elucidate the impact of specific DNA adducts in target and validated surrogate matrices, along with genotyping/phenotyping of biotransformational and DNA repair enzymes.

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SAMENVATTING

In tabaksrook zijn meer dan 4000 chemische stoffen aanwezig waaronder 50 stoffen die mutageen en carcinogeen zijn gebleken in experimentele modellen en/of in de mens. Onder deze mutagene verbindingen bevinden zich de polycyclische aromatische koolwaterstoffen (PAK), aromatische amines en reactieve zuurstofverbindingen (ROS). Na blootstelling aan deze verbindingen zijn de volgende opeenvolgende stappen te onderscheiden; opname door het lichaam, biotransformatie in reactieve metabolieten, vorming van DNA interactie producten (DNA adducten), genetische veranderingen zoals oncogen-activatie en tumor-suppressorgen-inactivatie, en uiteindelijk de inductie van kwaadaardige tumoren. In het algemeen kan de tabaksrook gerelateerde carcinogene werking gevolgd worden door binnen deze keten van gebeurtenissen te monitoren, beginnend bij blootstelling aan tabaksrook tot de uiteindelijke ontwikkeling van tumoren. Deze benadering, ook wel biomonitoring genoemd, maakt gebruik van markers van blootstelling, markers van inwendige dosis, markers van moleculaire dosis, markers van preklinische effecten, markers van ziekte en markers van gevoeligheid. Markers die de gebeurtenissen aan het einde van deze keten weerspiegelen geven niet alleen een indruk van blootstelling maar tevens een indicatie van het risico. Idealiter zou biomonitoring plaats dienen te vinden in die organen waarin de tumor-vorming plaatsvindt (doelwitorganen). Echter, het is niet altijd mogelijk doelwitorganen zoals de long, te bestuderen vanwege de onmogelijkheid op routinematige basis weefsel hiervan te verkrijgen. Daarom wordt vaak gebruik gemaakt van surrogaatweefsels zoals witte bloedcellen. Een nadeel van het gebruik van surrogaatweefsel is, dat de gebeurtenissen hierin niet altijd representatief zijn voor hetgeen zich in het doelwitorgaan afspeelt. In onderhavige proefschrift is tabaksrook geïnduceerde carcinogeniteit bestudeerd in menselijk materiaal afkomstig van doelwit- en surrogaatweefsel door kwantificering van markers van moleculaire dosis zoals PAK-, aromatische amine- en ROS geïnduceerde DNA adducten.

Hoofdstuk 2 beschrijft een toepassing waarbij gebruik wordt gemaakt van geïnduceerd sputum, waarin zich cellen bevinden afkomstig van de lagere luchtwegen. Hoofdstuk 3 is een validatie studie waarbij DNA adduct metingen in geïnduceerd sputum vergeleken worden met die in perifere bloed lymfocyten. Hoofdstuk 4 beschrijft een studie waarin door immunohistochemische bepalingen in geïnduceerd sputum de relatieve bijdrage van PAK-DNA adducten en aromatische amine-DNA adducten met elkaar vergeleken wordt. Hoofdstuk 5 beschrijft een onderzoek waarin de relatie wordt bestudeerd van tabaksrook gerelateerde DNA adducten in geïnduceerd sputum en perifere bloed lymfocyten worden bestudeerd in relatie tot passieve blootstelling aan tabaksrook. In hoofdstuk 6 worden markers van oxidatieve DNA schade alsmede anti-oxidatieve verdedigingsmechanismen in rokers bestudeerd. In hoofdstuk 7 worden niveaus van DNA adducten vergeleken in verschillende locaties van de mondholte met verschillende gevoeligheidsprofielen voor kanker. Hoofdstuk 8 beschrijft een chemopreventieve studie met *N*-acetyl-L-cysteïne (NAC) in rokende vrijwilligers. In hoofdstuk 9 wordt tenslotte een overzicht gegeven van de uiteindelijke resultaten, samenvatting en aanbevelingen voor toekomstig onderzoek.

Geconcluderend kan worden dat biomonitoring van PAK- en aromatische amine-DNA adducten in doelwit- en relevant surrogaatweefsel, in het bijzonder geïnduceerd sputum, een waardevolle benadering is om tabaks-gerelateerde carcinogenese te bestuderen. Echter, de