Carcinogenesis vol.29 no.5 pp.932–936, 2008
doi:10.1093/carcin/bgm286
Advance Access publication March 14, 2008

DNA adducts and cancer risk in prospective studies: a pooled analysis and a meta-analysis

Fabrizio Veglia[1], Steffen Loft[2], Giuseppe Matullo[1], Marco Peluso[3], Armelle Munnia[3], Frederica Perera[4], David H.Phillips[5], Deliang Tang[4], Herman Autrup[6], Ole Raaschou-Nielsen[7], Anne Tjønneland[7] and Paolo Vineis[1,8,*] for the Genair–EPIC investigators

[1]Life Sciences and Epidemiology Unit, ISI Foundation, Torino 10133, Italy, [2]Department of Environmental and Occupational Health, University of Copenhagen DK-1014, [3]Cancer Risk Factor Branch, Molecular Biology Laboratory, CSPO-Scientific Institute of Tuscany, Florence 50131, Italy, [4]Mailman School of Public Health, Columbia University, New York, NY 10032, USA, [5]Institute of Cancer Research, Brookes Lawley Building, Cotswold Road, Sutton SM2 5NG, UK, [6]Department of Environmental and Occupational Medicine, University of Aarhus, Aarhus DK-8000, Denmark, [7]Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen DK-2100, Denmark and [8]Department of Epidemiology and Public Health, Imperial College London, Norfolk Place, W2 1PG London, UK

*To whom correspondence should be addressed. Tel: +0044 20 75943372; Fax: +0044 20 75943196;
Email: p.vineis@imperial.ac.uk

Bulky DNA adducts are biomarkers of exposure to aromatic compounds and of the ability of the individual to metabolically activate carcinogens and to repair DNA damage. Their ability to predict cancer onset is uncertain. We have performed a pooled analysis of three prospective studies on cancer risk in which bulky DNA adducts have been measured in blood samples collected from healthy subjects (*N* = 1947; average follow-up 51–137 months). In addition, we have performed a meta-analysis by identifying all articles on the same subject published up to the end of 2006, including case–control studies. In the pooled analysis, a weakly statistically significant increase in the risk of lung cancer was apparent (14% per unit standard deviation change in adduct levels, 95% confidence interval 1–28%; using the weighted mean difference method, 0.15 SD, units higher adducts in cases than in controls). The association was evident only in current smokers and was absent in former smokers. Also the meta-analysis, which included both lung and bladder cancers, showed a statistically significant association in current smokers, whereas the results in never smokers were equivocal; in former smokers, no association was detected. The results of our pooled and meta-analyses suggest that bulky DNA adducts are associated with lung cancer arising in current smokers after a follow-up of several years.

Introduction

Bulky DNA adducts are markers of exposure to aromatic compounds and of the ability of the individual to metabolically activate carcinogens and to repair DNA damage (1). Experimental studies on animal models have highlighted the central role of DNA adduct formation in tumorigenesis (1). Polycyclic aromatic hydrocarbons (PAHs) are one of the major classes of carcinogens present in the environment capable of forming DNA adducts. Human studies show a clear dose–response relationship between occupational exposure to PAH and the levels of DNA adducts in lymphocytes of workers (2,3). Studies on the association between tobacco smoke and adducts have yielded inconsistent results (1,4,5). Some studies reported a negative correlation of DNA adducts with the consumption of fruit and vegetables and with the intake of flavonoids (6–10). A study undertaken in New York City after the events of 11 September 2001 found a direct relation between the amount of DNA adducts in umbilical cords of newborn children and proximity to the World Trade Center (11). When unrepaired, DNA adducts can cause mutations, including mutational hotspots in the p53 tumour suppressor gene and other genes, that may ultimately induce cancer formation.

High DNA adduct levels have been suggested to be predictive of lung cancer risk (12), consistent with the suspected role of PAHs in lung carcinogenesis (1). We present here the results of a pooled analysis of the three prospective studies currently available in which bulky DNA adducts have been measured in blood samples collected from healthy subjects. In addition, we have performed a meta-analysis including all articles on this subject published up to the end of 2006, including case–control studies.

Abbreviations: CI, confidence interval; GA, Genair; PAH, polycyclic aromatic hydrocarbon; RAL, relative adduct labelling; WMD, weighted mean differences.

Gerard Hoek, Michal Krzyzanowski, Luisa Airoldi, Alison Dunning, Seymour Garte, Pierre Hainaut, Christian Malaveille, Kim Overvad, Francoise Clavel-Chapelon, Jacob Linseisen, Heiner Boeing, Antonia Trichopoulou, Dimitrios Trichopoulos, Anna Kaladidi, Domenico Palli, Vittorio Krogh, Rosario Tumino, Salvatore Panico, H. Bas Bueno-De-Mesquita, Petra H. Peeters, Merethe Kumle, Carlos A. Gonzalez, Carmen Martinez, Miren Dorronsoro, Aurelio Barricarte, Carmen Navarro, J.Ramón Quiros, Goran Berglund, Lars Janzon, Bengt Jarvholm, Nicholas E Day, Tim J Key, Rodolfo Saracci, Rudolf Kaaks and Elio Riboli for the Genair–European Prospective Investigation into Cancer and Nutrition investigators.

Methods

Pooled analysis

We have identified three prospective longitudinal studies in which bulky DNA adducts were measured with similar techniques and incident lung cancer was considered as an outcome. We contacted the principal investigators of these studies and had access to the original data sets. Individual data on adduct levels, case/control status, age, gender, ethnicity, batch and smoking habits were collected. The studies are as follows: (i). Genair (GA), a nested case–control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort (current smokers and former smokers since <10 years excluded) (13); (ii) the Danish prospective Diet, Cancer and Health cohort study (DK), with a case–cohort design (14) and (iii) a nested case–control study within the Physicians' Health Study (US) (15). In each study, bulky DNA adducts were measured by the ^{32}P-post-labelling method, although in the GA and US studies enrichment of bulky adducts before labelling was achieved by means of nuclease P1 digestion, whereas butanol extraction was used in the DK study. In each study, subjects were enrolled after signing informed consent. Data sets were transferred to the ISI Foundation for analysis after being anonymized.

There were large differences in the mean levels of adducts in the three studies (~0.7 adducts per 10^8 bases in GA, 0.2 in the DK study, and ~7 in the US study). To overcome this, data were normalized after pooling assuming different measurement units in the different laboratories, according to the following formula:

$$\mathrm{RAL_{st}} = (\mathrm{RAL} - \mathrm{Mean}_{ic})/\mathrm{SD}_{ic},$$

where RAL = relative adduct labelling; Mean_{ic} and SD_{ic} = mean and standard deviation of the control group in the *i*th batch. Differences in mean values may be due to different experimental protocols and/or procedures for quantification; these factors are discussed later. The rationale for using normalized values and quartiles to standardize genetic pooled analysis has been put forward previously by several authors and this approach has become common practice (3,16,17).

Meta-analysis

The Medline database was searched for the period between 1993 and July 2006, with the aid of manual bibliography review. The studies to be included had to conform to the following criteria: (i) case–control or cohort studies comparing bulky DNA adduct levels in cancer patients and control subjects and (ii) separate comparisons for current, former and never smokers. We excluded specific adducts such as those formed by aflatoxin or cytostatic drugs. Nine studies were included that matched the selection criteria (7,13–15,18–22) for a total of 1028 cases and 1084 controls. Most studies were case–control

© The Author 2008. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oxfordjournals.org

Downloaded from http://carcin.oxfordjournals.org/ at University of North Texas on January 6, 2017

studies (only on lung and bladder cancer), and three were cohort studies (the same included in the pooled analysis).

Quality of the studies

We used three criteria to evaluate the quality of the studies and assigned scores on this basis (1 lowest, 3 highest; score 1 if information unavailable): A = population- or hospital-based study; B = response rate and C = blinding of procedures and consideration of confounders. Results of assessment are as follows: Popp (22) A2 B1 C2, average 1.7; Tang (18) A2 B3 C3, average 2.7; Hou (20) A3 B3 C3, average 3; Cheng (19) A1 B1 C2, average 1.3; Peluso (7) A2 B3 C2, average 2.3; Vulimiri (21) A2 B1 C2, average 1.7; Tang (15) A3 B3 C3, average 3; Peluso (13) A3 B3 C3, average 3 and Bak (14) A3 B3 C3, average 3. The three cohort studies included in the pooled analysis were in the highest qualitative score category.

Statistical analysis

In the pooled analysis, the adduct levels were standardized by dividing, within each study, means and standard deviations by the average of the control groups. Therefore, standardized means in the control groups were set to one in all the studies. In one article (21), the standard deviations were not reported explicitly and had to be computed from the results of the *t*-test. The pooled analysis was based on an unconditional logistic model after the normalization described above. Odds ratios and their 95% confidence intervals (CIs) were computed (i) by using normalized adducts as a continuous variable and (ii) for quartiles (reference category first quartile), after adjustment by sex, age, centre, batch and smoking habits (23).

In the meta-analysis, a slightly different method of standardization was used in order to provide results coherent with our previous meta-analysis (12): all means and standard deviations were divided, within each study, by the means of the control groups (12).

The meta-analysis was carried out using the RevMan 4.1 software, available through the Cochrane Library.

In both pooled and meta-analyses, we have computed standardized weighted mean differences (WMD) between cases and controls in each study and the overall WMD. For each WMD, we computed 95% CIs. Heterogeneity among studies was tested with Breslow–Day's test (23), and a random-effect model was used to account for interstudy variability (24). For the pooled analysis, a covariance analysis on standardized adduct levels, adjusting for study, smoking status, age, gender and for the interaction between gender and case/control status, was performed.

Results

Pooled analysis

Overall, 1200 subjects were included from the GA study, 500 from the DK study and 247 from the US study. The mean age at recruitment was 61 years in GA, 58 years in the DK and 62 years in the US study; the median length of follow-up was 51 months in the DK study, 72 months in GA and 137 months in the US study. One of the studies (13) enrolled only former or never smokers; therefore, it could not be included in the analysis of current smokers.

Table I shows the results for the association with lung cancer. A statistically significant association between adduct levels and the risk of lung cancer is apparent, with an increase of 14% per unit standard deviation change in adduct levels. The association appears stronger in women than in men but the difference is not significant in a heterogeneity test ($P = 0.14$). The association is apparent in current smokers, absent in former smokers and equivocal in never smokers. In the latter, the shape of the dose–response relationship is equivocal when risk is estimated by quartiles. Geometric means of adducts (RAL)

Table I. Odds ratios (ORs) for lung cancer in association with smoking habits, age, gender and standardized RAL (all estimates adjusted for the other variables, study centre and batch)

	n cases	*n* controls	OR	95% CI	*P* value
Never smokers	*73*	*632*	1.00		
Former smokers	*135*	*612*	1.98	1.44, 2.74	<0.0001
Current smokers	*238*	*254*	8.38	6.15, 11.41	<0.0001
Age			1.00	0.98, 1.02	0.95
Sex					
Male	*273*	*857*	1.00		
Female	*173*	*641*	0.96	0.85, 1.08	0.50
Interaction Ral × sex			0.91	0.81, 1.03	0.14
RAL continuous (increase by 1 SD)			1.14	1.01, 1.28	0.037
First quartile	*117*	*401*	1.00		
Second quartile	*111*	*357*	1.01	0.74, 1.37	0.98
Third quartile	*115*	*371*	1.26	0.93, 1.72	0.14
Fourth quartile	*103*	*369*	1.11	0.81, 1.53	0.51
Males					
RAL, continuous (increase by 1 SD)	*273*	*857*	1.04	0.90, 1.20	0.62
Females					
RAL continuous (increase by 1 SD)	*173*	*641*	1.25	1.03, 1.51	0.02
Never smokers					
RAL continuous (increase by 1 SD)	*73*	*632*	1.25	0.97, 1.60	0.088
First quartile	*9*	*176*	1.00		
Second quartile	*22*	*116*	3.35	1.53, 7.34	0.003
Third quartile	*24*	*167*	2.54	1.18, 5.48	0.02
Fourth quartile	*18*	*173*	1.83	0.82, 4.09	0.4
Former smokers					
RAL continuous (increase by 1 SD)	*135*	*612*	0.94	0.78, 1.13	0.49
First quartile	*36*	*148*	1.00		
Second quartile	*38*	*152*	1.05	0.63, 1.74	0.85
Third quartile	*29*	*163*	0.74	0.43, 1.26	0.27
Fourth quartile	*32*	*149*	0.91	0.54, 1.53	0.71
Current smokers					
RAL continuous (increase by 1 SD)	*238*	*254*	1.22	1.01, 1.47	0.04
First quartile	*72*	*77*	1.00		
Second quartile	*51*	*89*	0.60	0.38, 0.95	0.03
Third quartile	*62*	*41*	1.59	0.96, 2.62	0.07
Fourth quartile	*53*	*47*	1.12	0.68, 1.85	0.65

Downloaded from http://carcin.oxfordjournals.org/ at University of North Texas on January 6, 2017

were 0.20 in cases and 0.19 in controls in DK, 0.53 and 0.48 in GA, and 5.21 and 4.78, in the US study, respectively.

Figure 1 shows the WMD between cases and controls after standardization as described above. A statistically significant excess of adducts in cases over controls is apparent (+0.15 SD, units).

Meta-analysis

The results of the meta-analyses are reported in Figures 2–4, according to smoking habits (current, former, never smokers). For the meta-analysis, we used only weighted mean difference (WMD) and included also case–control studies, but the results for lung cancer are strictly comparable. Current smokers showed a statistically significant difference between cases and controls with cases having on average 73% higher levels of adducts than controls. Of nine studies included, six were consistent in reporting a difference, and all of them reached statistical significance. When only lung cancer was considered, the mean difference between cases and controls for current smokers was 0.79 (95% CI 0.33–1.25), the *P* value for heterogeneity was <0.00001 and the *P* value for association was 0.0007. No association between adduct levels and the case status was observed in former smokers (Figure 3), with complete consistency among investigations. When only lung cancer was considered, the mean difference between cases and controls for former smokers was −0.03 (95% CI −0.19 to 0.13), the *P* value for heterogeneity was 0.42 and the *P* value for association was 0.74. Results on never smokers were inconsistent; only two studies showed a statistically significant positive difference between cases and controls (one based on the measurement of adducts in the lung tissue and one based on measurements in white blood cells in bladder cancer cases and controls, ref. 18). Overall, the WMD for never smokers was 28%, not statistically significant and almost entirely attributable to the two studies mentioned above. When only lung cancer was considered, the mean difference between cases and controls for never smokers was 0.11 (95% CI −0.34 to 0.55), the *P* value for heterogeneity was <0.0001 and the *P* value for association was 0.64.

Sensitivity analysis

Excluding the papers with a quality score <2, we still had in Figure 2 four positive results out of five—the exception being Hou (20). We performed another sensitivity analysis including only studies on lung cancer and excluding the investigation that measured adducts in lung tissue. In this case, the standardized difference for RAL was 66% (95% CI, 18–115) for current smokers, −3% (95% CI, −19 to 13) for former smokers and –9% (95% CI, −35 to 17) for non-smokers, results similar to those obtained in the overall analysis.

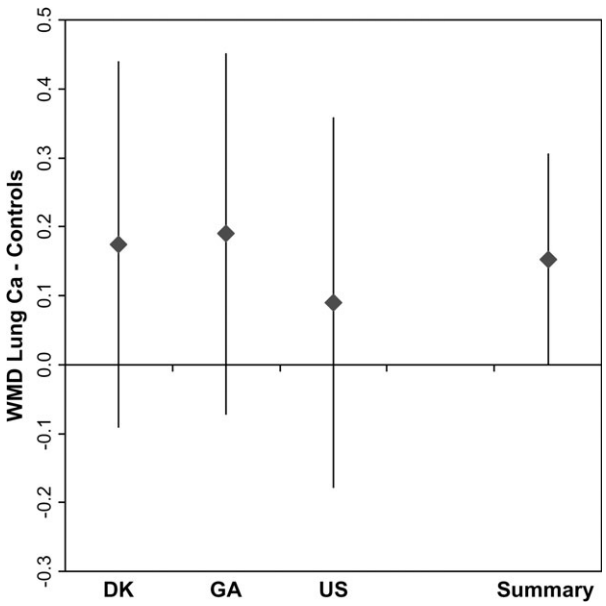

Fig. 1. Pooled analysis of studies on bulky DNA adducts and lung cancer. Diamonds show weighted mean difference between cases and controls and bars show their 95% CIs (random-effects model; analysis of covariance). DK, Danish cohort; US, US cohort.

Discussion

We have pooled the results of three prospective studies (the only ones to our knowledge) in which bulky DNA adducts were measured with a similar method and lung cancer incidence was used as an outcome. By analysing overall 1947 subjects, we have considerably increased statistical power of the individual studies, and we can reach firmer conclusions. The importance of these studies rests on the measurement of adducts in blood samples that were collected years before cancer onset (with a mean follow-up between 51 and 137 months), meaning that the adduct level measurements are supposed not to be influenced by metabolic changes associated with an already existing cancer. We found a weak association between DNA adduct levels and lung cancer, which was more obvious in current smokers. When adduct levels were subdivided into quartiles, the overall dose–response relationship was non-linear, with an increase in risk estimates followed by a levelling-off. The same pattern was evident in current smokers, whereas no dose–response was found in former smokers, and the pattern was extremely irregular in never smokers.

The apparent levelling-off of the dose–response relationship is puzzling. Although it could be the effect of an artefact, mechanistic hypotheses can also be put forward. In fact, a levelling-off in adduct levels in relation to external measurements of PAH has been observed in a meta-analysis on occupational exposure to PAH conducted by one of us (3). Also other investigations found a levelling-off of dose–response relationships (25,26). The level of active PAH derivatives available for adduction depends on several variables that include the rates of activation and deactivation and DNA repair. Bulky DNA adducts represent exposure to PAH and other aromatic compounds after the action of metabolizing enzymes (phases I and II); they are in steady state if exposure is constant. In addition, their levels also reflect the action of DNA repair enzymes. One hypothesis that was put forward in the paper mentioned above (3) was that the levelling-off could be related to a saturation of the enzymes involved in particular in the activation of PAH (phase I enzymes). Dose-dependent patterns in DNA repair could also be involved. The role of individual susceptibility related to DNA repair is indirectly suggested by the observation that the lymphocytes of cancer patients (and of their healthy relatives) show higher levels of DNA adducts when treated with electrophilic chemicals compared with lymphocytes of non-cancerous individuals (27). However, activation/deactivation and repair patterns can explain non-linearity in the relationship between external levels of exposure and adduct levels, although they cannot easily explain a levelling-off of cancer risk with increasing adduct levels. Finally, it should be considered that adducts in white blood cells only indirectly reflect adducts in the target organ.

For the meta-analysis, nine studies examining the association between cancer of the lung or of the bladder and the levels of bulky DNA adducts, according to smoking status, have been identified overall. A global 73% excess of adduct levels was found in cases compared with controls in current smokers (95% CI, 31–115%). No association between cancer status and DNA adduct levels was found among former smokers, whereas never smokers showed inconsistent results. These observations are based on much larger numbers than in the previous meta-analysis and are clearly in accordance with the findings of the pooled analysis.

There are some limitations to our model to be considered. In particular, the level of measurement error for bulky adducts is not well studied but seems to be high (coefficient of variation ∼20–30%; however, in the DK study the coefficient of variation was only 6% in replicate measurements in 500 samples). However, the effect of measurement error is to attenuate a relationship if error is evenly distributed in the comparison groups (28). Thus, measurement error

Downloaded from http://carcin.oxfordjournals.org/ at University of North Texas on January 6, 2017

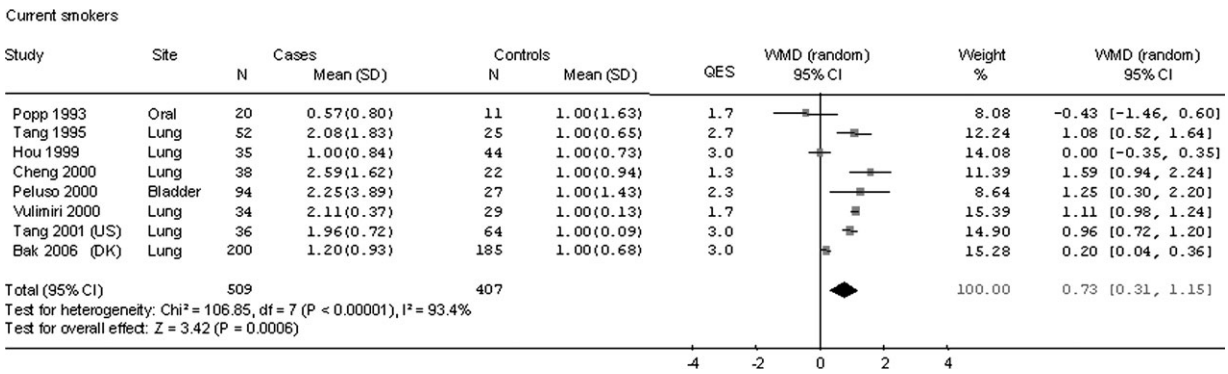

Fig. 2. Meta-analysis of studies on bulky DNA adducts and cancer, random-effect model, current smokers. WMD, weighed mean difference (random-effect model); DK, Danish cohort; US, US cohort; QES, quality evaluation score.

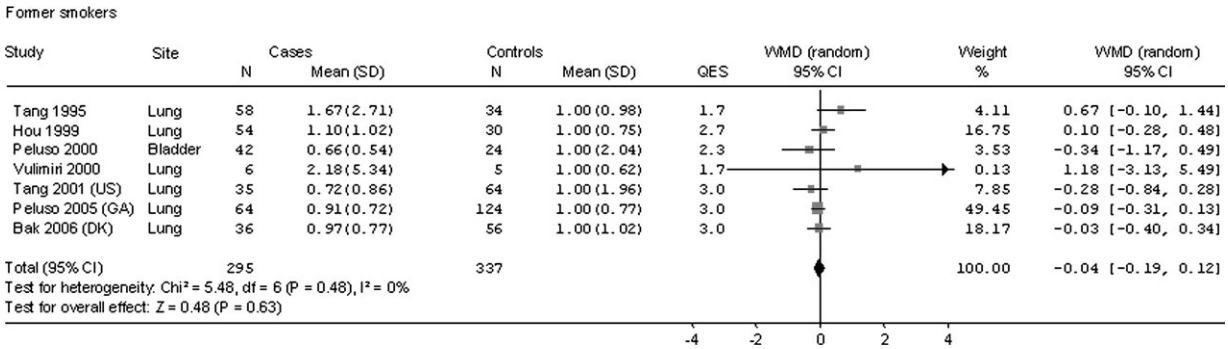

Fig. 3. Meta-analysis of studies on bulky DNA adducts and cancer, random-effect model, former smokers only. WMD, weighed mean difference (random-effect model); DK, Danish cohort; US, US cohort; QES, quality evaluation score.

is expected to blur existing associations rather than to reveal false associations.

An important observation that requires explanation is the presence of a clear association only in current smokers and an equivocal association in never smokers, both in the pooled and in the meta-analysis. 'Former smoker' was only episodically defined in the studies; heterogeneity in the definition might have blurred the association with adducts in former smokers. An alternative explanation is that only current smokers have the relevant exposure, and the difference between cases and controls is the expression of genetic predisposition to cancer, possibly related to the ability to repair DNA damage. Induction of phase II enzymes in smokers but not in non-smokers could also contribute to the interpretation of the differences between the two. In addition, the adduct concentration on the DNA is affected by the rate of DNA replication because the new strand does not have adducts. Individual differences in cell division therefore affect adduct levels. This may be of particular importance for adducts measured in leukocytes (including immune reactions and leukocytosis). Smokers have manifestations of chronic inflammation that justify leukocytosis and changes in leukocytes.

Publication bias could justify the findings if small positive studies have greater chances of being published than small negative studies. However, there is no evidence of an association between the study size and the results: negative studies tend to be as small as the positive ones (Figures 2–4).

As mentioned earlier, we have observed interstudy variation in the levels of adducts that were detected; variation was attenuated by normalizing the measures. Although an effort to compare and standardize laboratory procedures has been undertaken and published by a group of European researchers led by one of us (D.P.) (29), more standardized measurements are needed in future investigations. A comparison of the protocols of the three prospective studies reveals several differences that could account for the different values for adducts levels reported. In the GA study (13), adduct levels were calculated by relating the incorporation of radioactivity into adducts to the level of labelling of an aliquot of normal nucleotides removed from the DNA digest and labelled separately. In the US study (15), adduct levels were calculated by relating radioactivity incorporated into adducts to the specific activity of the [γ-^{32}P]adenosine triphosphate used, as determined by labelling a standard nucleotide on the same day. In the DK study (14), adduct levels were also computed from the specific activity of the [γ-^{32}P]adenosine triphosphate, but based on the value provided by the manufacturer; this value is always higher than that determined experimentally in the laboratory, which leads to a lower estimate of adduct levels. Other possible sources of interlaboratory variability are the choice of DNA digestion conditions, selection of sensitivity enhancement procedure [nuclease P1 digestion in two of the studies (13,15) and butanol extraction in the other (14)] and variations in the efficiency of the labelling reaction itself. Nevertheless, each study was conducted under internally consistent conditions, with a positive external control, such that comparisons between them can be made after normalizing the values.

An additional objection to our findings is that sound evidence about the fact that bulky DNA adducts represent mainly exposure to PAH is still lacking. In a recent study, the formation of bulky DNA adducts was examined using improved post-labelling procedures. Two different chromatography systems, e.g. high-urea or ammonium hydroxide systems, effective in the detection of aromatic DNA adducts were also employed to acquire more insights on the nature of the DNA adducts being measured by ^{32}P-post-labelling technique. A similar pattern and a similar average recovery of DNA adducts were obtained using urea or non-urea solvents, indicating that DNA adducts were likely induced by aromatic compounds, such as PAH and/or aromatic amines (30).

Downloaded from http://carcin.oxfordjournals.org/ at University of North Texas on January 6, 2017

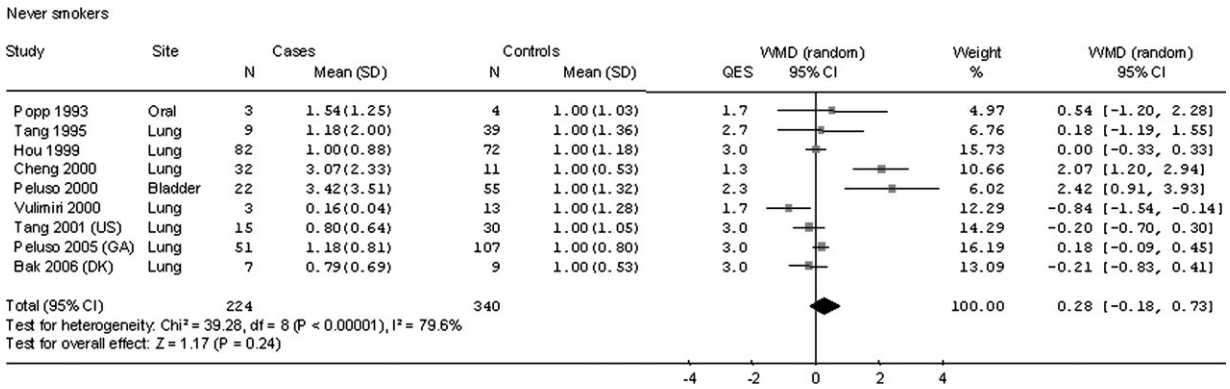

Fig. 4. Meta-analysis of studies on bulky DNA adducts and cancer, random-effect model, never smokers only. WMD, weighed mean difference (random-effect model); DK, Danish cohort; US, US cohort; QES, quality evaluation score.

In conclusion, despite some methodological limitations, our pooled and meta-analyses suggest that current smokers with high levels of adducts may have an increased risk of lung cancer.

Funding

European Union (FOOD-CT-2005-513943) (WP4-8) to Environmental Cancer Risk, nutrition and individual susceptibility Network of Excellence (EC CONTRACT Food-CT-2005-513943).

Acknowledgements

Conflict of Interest Statement: None declared.

References

1. Bartsch,H. (2000) Studies on biomarkers in cancer etiology and prevention: a summary and challenge of 20 years of interdisciplinary research. *Mutat. Res.*, **462**, 255–279.
2. Castano-Vinyals,G. *et al*. (2004) Biomarkers of exposure to polycyclic aromatic hydrocarbons from environmental air pollution. *Occup. Environ. Med.*, **61**, e12.
3. Peluso,M. *et al*. (2001) Analysis of 13(32)P-DNA postlabeling studies on occupational cohorts exposed to air pollution. *Am. J. Epidemiol.*, **153**, 546–558.
4. Godschalk,R.W. *et al*. (1998) Differences in aromatic-DNA adduct levels between alveolar macrophages and subpopulations of white blood cells from smokers. *Carcinogenesis*, **19**, 819–825.
5. Besaratinia,A. *et al*. (2000) Comparison between smoking-related DNA adduct analysis in induced sputum and peripheral blood lymphocytes. *Carcinogenesis*, **21**, 1335–1340.
6. Palli,D. *et al*. (2000) Diet, metabolic polymorphisms and DNA adducts: the EPIC-Italy cross-sectional study. *Int. J. Cancer.*, **87**, 444–451.
7. Peluso,M. *et al*. (2000) White blood cell DNA adducts and fruit and vegetable consumption in bladder cancer. *Carcinogenesis*, **21**, 183–187.
8. Mooney,L.A. *et al*. (1997) Contribution of genetic and nutritional factors to DNA damage in heavy smokers. *Carcinogenesis*, **18**, 503–509.
9. Hu,J.J. *et al*. (1996) Effects of dietary supplementation of alpha-tocopherol on plasma glutathione and DNA repair activities. *Cancer Epidemiol. Biomarkers Prev.*, **5**, 263–270.
10. Talaska,G. *et al*. (2006) Randomized controlled trial: effects of diet on DNA damage in heavy smokers. *Mutagenesis*, **21**, 179–183.
11. Perera,F.P. *et al*. (2005) Relationships among polycyclic aromatic hydrocarbon-DNA adducts, proximity to the World Trade Center, and effects on fetal growth. *Environ. Health Perspect.*, **113**, 1062–1067.
12. Veglia,F. *et al*. (2003) Bulky DNA adducts and risk of cancer: a meta-analysis. *Cancer Epidemiol. Biomarkers Prev.*, **12**, 157–160.
13. Peluso,M. *et al*. (2005) DNA adducts and lung cancer risk: a prospective study. *Cancer Res.*, **65**, 8042–8048.
14. Bak,H. *et al*. (2006) Bulky DNA adducts as risk indicator of lung cancer in a Danish case-cohort study. *Int. J. Cancer.*, **118**, 1618–1622.
15. Tang,D. *et al*. (2001) Association between carcinogen-DNA adducts in white blood cells and lung cancer risk in the physicians health study. *Cancer Res.*, **61**, 6708–6712.
16. Bonassi,S. *et al*. (2007) An increased micronucleus frequency in peripheral blood lymphocytes predicts the risk of cancer in humans. *Carcinogenesis*, **28**, 625–631.
17. Bonassi,S.M. *et al*. (1996) Is human exposure to styrene a cause of cytogenetic damage? A re-analysis of the available evidence. *Biomarkers*, **1**, 217–225.
18. Tang,D. *et al*. (1995) A molecular epidemiological case-control study of lung cancer. *Cancer Epidemiol. Biomarkers Prev.*, **4**, 341–346.
19. Cheng,Y.W. *et al*. (2000) DNA adduct level in lung tissue may act as a risk biomarker of lung cancer. *Eur. J. Cancer.*, **36**, 1381–1388.
20. Hou,S.M. *et al*. (1999) Hprt mutant frequency and aromatic DNA adduct level in non-smoking and smoking lung cancer patients and population controls. *Carcinogenesis*, **20**, 437–444.
21. Vulimiri,S.V. *et al*. (2000) Analysis of aromatic DNA adducts and 7,8-dihydro-8-oxo-2′-deoxyguanosine in lymphocyte DNA from a case-control study of lung cancer involving minority populations. *Mol. Carcinog.*, **27**, 34–46.
22. Popp,W. *et al*. (1993) DNA strand breakage and DNA adducts in lymphocytes of oral cancer patients. *Carcinogenesis*, **14**, 2251–2256.
23. Breslow,N.E. *et al*. (1980) Statistical Methods in Cancer Research. Volume I—The Analysis of Case-Control Studies. IARC Science Publications, Lyon, pp. 5–338.
24. DerSimonian,R. *et al*. (1986) Meta-analysis in clinical trials. *Control. Clin. Trials.*, **7**, 177–188.
25. Lewtas,J. *et al*. (1997) Air pollution exposure-DNA adduct dosimetry in humans and rodents: evidence for non-linearity at high doses. *Mutat. Res.*, **378**, 51–63.
26. Van Schooten,F.J. *et al*. (1997) 32P-postlabelling of aromatic DNA adducts in white blood cells and alveolar macrophages of smokers: saturation at high exposures. *Mutat. Res.*, **378**, 65–75.
27. Berwick,M. *et al*. (2000) Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J. Natl Cancer Inst.*, **92**, 874–897.
28. Copeland,K.T. *et al*. (1977) Bias due to misclassification in the estimation of relative risk. *Am. J. Epidemiol.*, **105**, 488–495.
29. Phillips,D.H. *et al*. (1999) Standardization and validation of DNA adduct postlabelling methods: report of interlaboratory trials and production of recommended protocols. *Mutagenesis*, **14**, 301–315.
30. Munnia,A. *et al*. (2007) 32P-Post-labelling method improvements for aromatic compound-related molecular epidemiology studies. *Mutagenesis*, **22**, 381–385.

Received September 25, 2007; revised November 30, 2007; accepted December 1, 2007

Downloaded from http://carcin.oxfordjournals.org/ at University of North Texas on January 6, 2017