Title:

Molecular changes and genetic susceptibility in relation to air pollution and environmental tobacco smoke: a case-control study in non-smokers nested in the epic investigation (GEN–AIR)

Objectives:

The overall aim is to quantitatively assess the effects of air pollution and Environmental Tobacco Smoke (ETS) on cancers of the lung, bladder, pharynx, and larynx in non-smokers in nine European countries (UK, Sweden, Denmark, Germany, The Netherlands, France, Spain, Italy and Greece). This will be done with a nested case-control design in the EPIC investigation (450,000 volunteers already recruited), by the means of biomarkers of exposure and susceptibility. Exposure assessment will be made by experts on the basis of the already available questionnaires plus objective information on air pollution in European cities.

In addition, we will measure cotinine in the blood for ETS exposure assessment, and we will assess the effect of dose and length of exposure to environmental pollutants on biological endpoints, such as DNA and hemoglobin adducts. Hemoglobin and WBC-DNA adducts formed by nitropyrenes, PAHs and other environmental contaminants (mainly due to traffic pollution and occupational exposures) will be measured. The adduct technology allows at least a partial separation of different sources of exposure (nitroarene adducts refer mainly to diesel exhaust, 4-aminobiphenyl-hemoglobin adducts to active or Environmental Tobacco Smoke).

We will study the dose-response relationship between adducts and disease according to genotypes for metabolism of the relevant carcinogens and DNA repair. We will also evaluate the relationships between cancer and mutations in relevant genes (p53, ras), as measured in the blood DNA according to a newly developed technique. A final goal is to evaluate the protective effect of fruit and vegetable consumption on the same cancers, in interaction with environmental exposures.

Scientific approach:

DESIGN

Nested control study

The present project represents the first attempt to measure the effects of air pollution and ETS on human health in a large longitudinal study (EPIC, European Prospective Investigation into Cancer and Nutrition), with a nested design, and with biological measures. The main advantage of the longitudinal study is that we will be able to measure adducts and other significant markers in blood samples collected several years before the onset of cancer and other diseases. Therefore, the measurements will not be influenced by early effects of the diseases themselves. In addition, the availability of 450,000 subjects (already recruited) will allow us to set up a nested case-cohort study with approximately 1,000 cases and 3,000 randomly chosen controls, with 80% power to detect a statistically significant relative risk of 1.4 in the highest vs. the lowest quintile of exposure.

EXPOSURE ASSESSMENT

Exposure assessment will be based on questionnaires (residence, Environmental Tobacco Smoke) and information on pollution levels in different European areas, available at the WHO Center in Bilthoven. Dr Krzyzanowski and Hoek (WHO) will be in charge of this part of the work.

BIOMARKERS OF EXPOSURE

Exposure to ETS will be determined by the measurement of nicotine and cotinine at the Mario Negri Institute, Milan (Dr Luisa Airoldi). The measurement of hemoglobin adducts will be performed at the same institution. The amount of hemoglobin available in EPIC is sufficiently large as to allow the measurement of adducts formed by 4-aminobiphenyl (a tracer of environmental tobacco smoke, ETS), benzo(a) pyrene (a tracer of ETS and automobile exhaust) and 1-nitropyrene
(a tracer for diesel exhaust). Several studies in Europe have shown that the levels of WBC-DNA adducts were higher among subjects heavily exposed to air pollutants, particularly during the summer. This observation has been made among police officers in Genoa, newspaper vendors exposed to urban traffic in Milan, and in residents in a highly industrialized area in United Kingdom. In all these cases the more exposed subjects had significant differences from those less exposed, with WBC-DNA adducts in the order of about 3 RALx10^-8 in the former (during the summer) and 1 in the latter. WBC-DNA adducts will be measured at the Centre for Cancer Study and Prevention in Firenze (M. Peluso) by 2P-postlabelling, according to a well-established methodology. Such WBC-DNA adducts have proved to be a reliable indicator of the total burden of aromatic genotoxicants adducted to DNA.

**RISK ASSESSMENT**

Genetically based metabolic susceptibility and DNA repair Genotype analyses on WBC-DNA for metabolic polymorphisms will be performed by a PCR-based method. The genotypes considered will be: N-acetyltransferase 1 and 2 (NAT1, NAT2), Glutathione-S-Transferase (GST) M1, GSTT1, GSTPi, CYP1A1 and other genes relevant to the metabolism of carcinogens. The choice of the relevant polymorphisms will be made on the basis of an extensive review of the literature (IARC Scientific Publication No. 148, 1999), that will be regularly updated. Metabolic polymorphisms will be investigated at IARC (Dr C. Malaveille), at the Aarhus University (Dr. H. Autrup) and at the Genetics Research Institute Dr S. Garte). Several polymorphisms for DNA repair will be investigated by PCR-based methods, including ERCC1, ERCC3, ERCC5, ERCC6, AGT (O6-alkylguanine-transferase) and other genes involved in Nucleotide Excision repair. Such analyses will be performed at the University of Torino (Dept. of Genetics, Dr. G. Matullo) and in Cambridge (Dr. A. Dunning).

**MARKERS OF EARLY RESPONSE**

Mutations in cancer genes as measured in blood DNAThe goal is to evaluate the relationships between cancer and mutations in relevant genes (p53, ras), as measured in the blood DNA according to a newly developed technique. Rationale: (a) to establish whether specific spectra of mutations are associated with the different types of cancers included in the analyses; (b) to establish whether any of these mutations are related to the exposures under investigation. This would be the first large-scale prospective study to measure somatic gene mutations in samples collected prospectively, years before the clinical onset of cancer. DNA mutations in plasma will be analyzed by P. Hainaut at IARC, Lyon.

**DOSE-RESPONSE MODELS**

Dose-response relationships are important to predict the effects of low-dose exposures. A linear relationship can be used to extrapolate from high to low levels of exposure with relative confidence. However, evidence of non-linear relationships should be considered seriously for the impact on predictions. A mathematician at the Institute for Scientific Interchange, Torino (Dr. Provenzale) will develop original novel models of analysis in order to study the time relationships between estimated external exposures, measurements of biomarkers of exposure (adducts), of susceptibility (DNA repair, metabolic polymorphisms), markers of early effect (DNA mutations in plasma), and outcome (cancer, COPD).