Synthesis

Genotypic and phenotypic variability, individual susceptibility factors and industrial genotoxicants/neurotoxicants in occupational medicine

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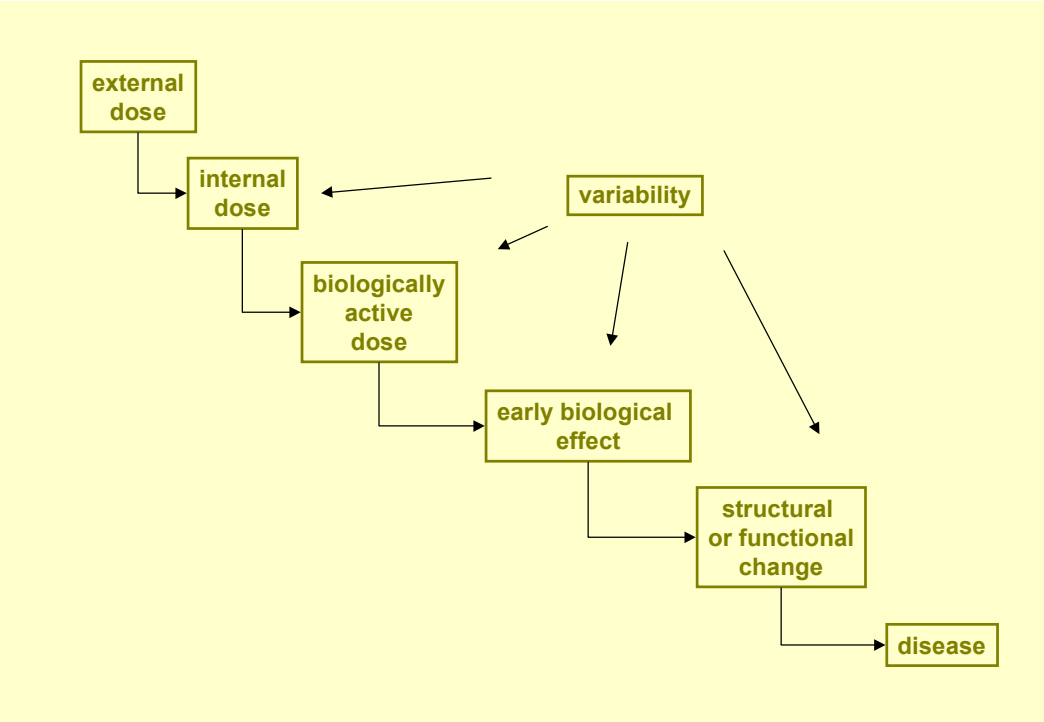
Introduction

Medical surveillance programmes for workers potentially exposed to hazardous toxicants at the workplace are based on the paradigm outlined below, and which summarises the sequence of events linking exposure to toxicants to the expression of a disease (occupational disease).



Three complementary approaches are generally distinguished: (1) *exposure monitoring* based on the use of parameters reflecting external dose, internal dose, biologically active dose and sometimes early reversible effects, (2) *effect monitoring* which explores the response of the organism to chemical exposure, ideally with early parameters predictive of a risk of disease, and (3) *susceptibility monitoring* which aims to take into account interindividual variability in the biodisposition of and response to xenobiotics. The latter aspect is, however, seldom implemented in occupational medicine because validated tools are not available, and there is no consensus about the acceptability of this approach.

During the last years, the sequencing of the human genome and the introduction of molecular biology techniques have introduced considerable progresses in almost all biomedical disciplines and have, in particular, allowed the development of new biomarkers of susceptibility (metabolising enzymes, DNA repair enzymes, cell cycle control genes, immune response genes, …). This perspective is, however, a serious cause of concern, in particular because it could lead to the possible exclusion of the most susceptible, the weakest workers from the working market. This issue is the subject of a passionate ethical debate in Europe

and the US, sometimes with very diverging views. In Belgium, when the present research programme was launched, the debate was not very well structured, and in view of the rapidly progressing technical developments, it was anticipated that these issues could not be overlooked longer. Should individual susceptibility testing be forbidden? Should individual susceptibility testing be implemented in occupational medicine? Should the implementation be controlled? How? What could be the benefit of these tests?

However, before to discuss these ethical issues, the following questions were considered:

1. *Are we ABLE to examine susceptibility factors? Are the current diagnostic tools sufficiently reliable and robust to be used on a large scale? Are tests to identify genotype or phenotype available in Belgium?*

2. *Is it potentially USEFUL to implement these tests? Provided that technical aspects are sufficiently mastered, what is the predictive value of these tests? What is their precision, accuracy? In which context could these tests provide a useful information?*

It was anticipated that only when a clear answer to these questions would be available it would be possible, based on solid scientific grounds, to discuss societal and ethical implications of susceptibility biomonitoring:

3. *If these tests are potentially useful, are they APPLICABLE in occupational medicine?*

When this scientific research programme was initiated, it was emphasised that the responsibility of scientists was to provide the necessary elements for a serene and well-founded discussion of this problematic. It was felt that it was inappropriate to discuss ethical implications of a test that was not available or for which the predictive value was not demonstrated.

Results

The strategy of the research programme is based on the **integration of newly developed biomarkers and interaction between basic and applied research**. This strategy proceeded in parallel with the identification of new biomarkers of individual susceptibility. The societal aspects constituted an important part of the project, with particular attention to the legal aspects.

Scientific aspects:

The close **interaction** and the **interdisciplinarity** of the involved teams allowed an integrated approach of the evaluation of exposure, effect and susceptibility biomarkers for the evaluation of occupational exposure to mutagens and opioid analgesics. Cross-fertilisation among laboratories was an important stimulating factor.

In a first step, the teams decided together about the methodologies to develop and validate, the populations to study, the questionnaires to prepare, the sampling schedules and the coordinating group. Advice was sought in the follow-up committee. In a second step, the different experimental studies were performed in the laboratories separately or in close collaboration (RUG/VUB, UCL/VUB, UCL/KUL/VUB). The final step involved integrated

statistical analysis of the combined data originating from the individual groups, and addressed the individual susceptibility to a given occupational situation.

The collaborative **study (KUL/UCL/VUB) on styrene** susceptibility combined a very broad range of different biomarkers. Special effort was made to develop accurate and sensitive quantification of styrene adducts, genotyping and phenotyping of relevant genes. The results indicate that chromosome/genome mutations are formed in workers exposed to low concentrations of styrene. Duration of exposure, age and smoking habits are important variables to consider in studies evaluating genotoxic effects on workers. Genotyping of metabolising and DNA repair enzymes are useful for the assessment of individual genotoxic risk to styrene. The *in vitro* DNA strand break phenotype might be a valuable method to estimate the repair capacity of workers.

As far as exposure to **cobalt dust (VUB/UCL)** is concerned, its mutagenic effects through the induction of oxidative damage were demonstrated *in vitro* in human lymphocytes and *in vivo* in rat lung cells as well. The evaluation of its carcinogenic potential was complemented with studies on induction of apoptosis and inhibition of DNA repair. Occupational exposure to cobalt-containing dust was performed in workers exposed to cobalt alone or to hard metal dust (WC-Co). No significant increase of genotoxic effects was found in workers exposed at the TLV-TWA (20µg/m^3). However multiple regression analysis indicated that workers who smoked and were exposed to hard metal dusts had elevated urinary 8-OHdG concentration and micronuclei values. It was concluded that this particular group of workers needs closer medical surveillance. In the same populations of workers and matched controls, genetic susceptibility was approached by the identification of individual capacity to remove oxidative damage and to repair DNA strand breakage. These results indicate that polymorphisms for genes which code for repair enzymes acting on oxidized bases (8-OHdG), single stranded DNA breaks (XRCC1) and double stranded DNA breaks (XRCC3) are involved in the variation of the extent of genotoxic effects induced in workers exposed to Co-containing dust. Interactions between these polymorphisms seem critical and therefore no single genotyping can be advised in particular. Moreover, as underlined in our previous study the interaction with smoking is a key factor, which is not surprising since smoking it-self can induce oxidative damage.
A prospective study on the predictivity of Glu69 polymorphism for lung parenchymal disease was performed by studying lung function parameters in workers from a cobalt producing plant and who were included in a respiratory monitoring programme between 1988 and 2001. 122 male workers with at least three lung function tests (FEV1, FVC) during the follow-up period were assessed. The main finding was that cobalt exposure contributed to FEV1, not FVC, decline over time, only in smokers. No influence of Glu69β polymorphism was detected. Although the amplitude of the additional decrement associated with exposure was relatively small (<20%) compared to the expected decline in a smoker, the results indicated that further efforts to reduce exposure and to encourage workers to quit smoking are still warranted.
The results obtained by the UCL-VUB contributed significantly to the **understanding of mechanisms of carcinogenesis** induced by cobalt dust, allowing possibly in the future a sound scientific approach of **individual susceptibility** for this type of occupational exposure.
Their complementary expertise (occupational toxicology and genotoxicity) led to the invitation to write together three **review papers** (Lison et al., 2000; Kirsch-Volders et al., 2002; De Boeck et al., 2003), to give several **talks** in Belgium and abroad on the assessment of genotoxicity in occupational exposure, and to participate to the **IARC working group** for the evaluation of cobalt-containing dust (2003).

As far as **ionising radiation (RUG/VUB/ULg)** is concerned, studies investigated the correlation between an enhanced chromosomal radiosensitivity, observed in a certain number of individuals (patients, workers) and the expression of DNA damage processing genes. These investigations can be considered only as a first stage in the understanding of **the molecular mechanisms behind radiosensitivity**. The detection of mutations, polymorphisms in DNA damage processing genes in individuals showing an enhanced *in vivo* radiosensitivity or *in vitro* chromosomal radiosensitivity is a very important issue for the future in the framework of **susceptibility to radiation of workers**. Although present studies have shown that chromosomal assays are reliable techniques for radiosensitivity assessment in population studies, further **optimisation** is necessary to improve their value for individual risk assessment. Multiple blood sampling will possibly increase the specificity of the assays.
Genotyping for several DNA repair genes **and phenotyping** for strand break repair were performed in workers from a nuclear power plant exposed to chronic low level ionising radiation. On the basis of these results, it is not possible to select a single genotype for prediction of the individual susceptibility to ionising radiation. A combination of the three genotypes, hOGG1, XRCC1 and XRCC3 polymorphisms, is advised. As an alternative or complement, the in vitro DNA strand break phenotype, which integrates several repair pathways, is recommended.

The study of occupational exposure to **opioids (KUL)** is the first in the field and is very successful with the development of adequate analytical methodologies. For the evaluation of inhalation exposure, appropriate sampling techniques and sampling strategies are commonly available. For dermal exposure to chemicals such standardised exposure assessment methodologies are still lacking and further **standardisation** is essential. Nevertheless, an explorative dermal exposure protocol was developed and successfully implemented. Spatial distribution of total body dermal fentanyl contamination was measured by means of dermal patch samplers placed on different anatomical regions of the body. Furthermore, a simple hand wiping protocol was found to be an adequate estimate parameter of total body dermal exposure. To monitor internal exposure, specific biomarkers can be used like the opioid levels and the concentration of their metabolites in urine. These urinary biomarkers were found to be highly correlated with total body fentanyl dermal exposure, while this correlation was significantly less pronounced in the case of inhalation exposure. The importance of different exposure pathways was explored and it was concluded that absorption through the skin could represent a major route of exposure. In these settings, the assessment of the workers' integrated individual opioid uptake through a biological monitoring strategy is essential. Moreover, fentanyl metabolism and clearance may be subject to inter-individual variability and selecting the appropriate biomarker of exposure could potentially provide additional information on the individual susceptibility of exposed workers. It is expected that this study would later serve **as a model** for the assessment of occupational exposure and individual workers' susceptibility for other pharmaceutical compounds having similar effects
The results obtained during this research programme are impressive. Their scientific quality is confirmed by the many joined publications in peer-reviewed journals. They cover the majority of the questions addressed at the start of the project. Methodologies are now made **available** to define genotypes for metabolic activation and DNA repair and to phenotype specific cellular functions. These methods are **useful** since their implementation contribute to refine the accuracy of biomarkers of exposure and the predictivity of the biomarkers of effect. Their **applicability** has further to be considered within the **ethico-legal** context.

Legal aspects:

Thanks to its **interdisciplinarity**, the project allowed a better understanding of the objectives and limits of the other disciplines concerned.

In addition to the reciprocal exchange of information, the integrated character of the interdisciplinarity within the network allowed a true exchange of points of view and a real confrontation of ideas between the various disciplines and even within them.

It also allowed a better comprehension of the **legal stakes** related to the question of the use of genetic testing in the framework of employment relations. These stakes were then placed in the wider context of the **prevention of, and compensation** for, occupational diseases. This allows us to highlight the strengths and weaknesses of our system in light of these stakes.

Conclusion

Several **genotyping** and **phenotyping** methodologies **were validated** by the collaborating teams to allow an adequate **prevention** of workers exposed to **mutagens/carcinogens and opioid analgesics** in the workplace. The laboratories demonstrated their **specificity** to detect interindividual variation for metabolisation of mutagens/carcinogens and repair of DNA lesions. They evaluated their responses in several industrial settings, but full understanding of the relationship between genotype and phenotype will require additional studies. The present results should therefore still be considered with **caution**.

Tests for genotyping and phenotyping have still **uncertain clinical validity** because of limited and potentially biased study populations, low genotype penetrance, variable expressivity, lack of understanding of phenotypic modifiers and multiple or ambiguous clinical endpoints. For gene variants associated with common diseases, low predictive value is expected as many other genetic and non-genetic factors also contribute to clinical outcomes. The clinical utility of a test depends also on the availability, safety and effectiveness of preventive or therapeutic measures offered to individuals with positive test results. Their application should be advised only as a complementary information for the normal **occupational surveillance**, and not for pre-screening of workers.

Both the scientific and legal aspects underlined the strengths of interdisciplinarity. This was essential for quality and feasibility of the project. And still more important, **the interaction between scientific and legal aspects provided a unique added value which made from good scientific questions an essential "demarche" for responsible science**.