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TOBACCO AND CANCER

Perspectives in Preventive Research

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Perspectives in Preventive Research

Proceedings of the Workshop of the
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Foreword

The European Organization for Cooperation in Cancer Prevention Studies (ECP) was established in 1981 to promote collaboration between scientists working in the various European countries on cancer causation and prevention. The work of ECP therefore complements but does not overlap with that of EORTC (which was solely concerned with treatment).

In order to achieve this aim, various working groups - to deal with specific cancers or aspects of cancer aetiology, and to explore the opportunities for advances on a cooperative European basis - were established. It was also decided to hold annual symposia to draw general attention to a field in which there seemed to be many opportunities for progress in matters of prevention.

At present, seven working groups are active in fields such as tobacco and cancer, cancer of the breast, colorectal cancer, diet and cancer, hormones and sexual factors and cancer, AIDS virus and cancer and, finally, public information in the field of cancer prevention.

ECP has set up an annual symposium series, the symposia being organized by the working groups in turn. These symposia have been devoted to themes of high priority to cancer prevention: 'Tobacco and Cancer' (1983), 'Hormones and Sexual Factors in Human Cancer Aetiology' (1984), 'Diet and Human Carcinogenesis' (1985), 'Concepts and Theories in Carcinogenesis' (1986), 'Preventive Strategies for Cancer related to Immune Deficiencies' (1987), and 'Gastric Carcinogenesis' (1988).

During the 1987 meeting of the Scientific Committee the importance of the new aspects of the tobacco/cancer relation was emphasized.

While the link between tobacco and human cancer was well established as early as 1950 by DOLL and HILL, and while it was later recognized that about 30% of all deaths caused by cancer were related to tobacco, attention in recent years has also been focused on the effects on health of passive smoking (i.e. the involuntary inhaling of tobacco and of tobacco chewing). There is no longer any doubt that tobacco smoke is by far the most widespread carcinogenic agent in our environment. By cutting tobacco consumption we could effectively reduce cancer deaths throughout the world. It is the reason why ECP decided to organize a workshop to reviewing recent data on new questions raised by this relation between tobacco and human cancer and to establishing priorities for preventive research.

With that aim, experts were assembled at a workshop in Brussels, 29–30 September 1988 and this volume represents the outcome of that workshop.

We are indebted to the speakers for their participation in the workshop and for prompt submission of their manuscripts.

We are grateful to the sponsor of the workshop, the 'Europe against Cancer' of the European Communities. We also want to express our gratitude to 'L'Association contre le Cancer' (Belgium) which supports the publication of this book.

Our appreciation also goes to Mrs C. Cattoir and Ms M.C. Gueur for the organization of the workshop and for their secretarial assistance.

A.P. Maskens

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A.P. Maskens et al., editors

INTRODUCTION

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Many scientists seem to share the view today that research in the field of tobacco related carcinogenesis is no longer required as the causal link between tobacco smoking and cancer of various human organs has been clearly established. One should concentrate, they say, on preventive action : information, education, legislation.

ECP clearly and entirely supports such preventive efforts, and more especially those undertaken by WHO, by the EEC "Europe against Cancer" programme, and by several active national leagues against cancer in Europe.

Yet, the tobacco/cancer problem is so important, and the perspective to actually ban tobacco from our societies is so remote in the present context, that the Scientific Committee of ECP expressed the view that research into this field was to remain an important priority.

The question, however, of whether tobacco causes cancer in humans no longer needs to be addressed, considering the bulk of the existing evidence. Perhaps do we have to pay attention to more specific questions with direct consequences on potential preventive action. For instance, while legal action has now been taken in several countries to regulate tar contents of tobacco, should not other categories of powerful carcinogens be submitted to similar controls ? In that respect, proper evaluation of the relative importance of nitrosamines is worth being given proper attention.

Another research field of major practical interest is concerned with the pharmacological and neurophysiological mechanisms of the addictive effects of tobacco smoking. Why is it so difficult for individuals to quit smoking ? How to better help them quit ?

As individuals can become addicted, so do societies. How is it that, whatever the weight of the existing evidence, the tobacco

industry is unable to admit the carcinogenicity of tobacco smoke ?

Here, the nature of the addiction is economic and social, and here too, specific questions need to be asked. What is the extent of the independence of governments, considering their income from tobacco taxes, the action of agricultural lobbies, and the enormous financial power of industrial lobbies ?

Is the progress to be found from a continuous fight between health professionals and industry, or should we not study the mechanisms of tobacco dependence of our societies so as to improve our chances of finding viable solutions at that level as well ? The implacable rules that govern international trade and economic wars are probably as relevant to human tobacco carcinogenesis as are the properties of tar constituents.

Thus, the cancer specialist will find in this volume several papers devoted to less usual topics in this field. Our hope is that the effort of the editors in organizing the workshop will be rewarded by a renewed interest in collaborative research in this important field of disease prevention.

NITROSAMINES

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Tobacco and cancer. Perspectives in preventive research.
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TISSUE-SPECIFICITY OF N-NITROSAMINE-METABOLISM IN EXPERIMENTAL ANIMALS: STUDIES ON SOME N-NITROSAMINES PRESENT IN TOBACCO AND TOBACCO SMOKE

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INTRODUCTION

Several N-nitrosamines have been detected in tobacco and tobacco smoke. These compounds are formed during tobacco processing and during tobacco smoking from amines reacting with nitrite or nitrogen oxides. The N-nitrosamines derived from the tobacco-alkaloides (tobacco-specific N-nitrosamines) are the most prevalent ones in tobacco and tobacco smoke, but many other N-nitrosamines may also be present (1).

When given to animals, most N-nitrosamines exhibit strong carcinogenic effects (2). The comparatively high concentrations of N-nitrosamines which are present in tobacco and tobacco smoke indicate that they may play a role in the tobacco-related cancers in man.

The N-nitrosamines are stable under physiological conditions and it is generally accepted that the tumourigenesis by these substances results from metabolic activation to metabolites, which react with DNA and other cellular macromolecules (2). For most N-nitrosamines, the initial and rate-limiting step in the activation process is considered to be an enzymatic oxidation on an α-carbon, which yields an unstable α-hydroxylated N-nitrosamine. This substance decomposes spontaneously to generate the electrophilic metabolites, which react with nucleophilic cellular constituents including DNA (2). The α-carbon hydroxylation pathways appear to be catalyzed by cytochrome P-450 enzymes (3,4). The bioactivation of the N-nitrosamines in various tissues should therefore correlate with the presence of cytochrome P-450.

The carcinogenic effects of the N-nitrosamines show remarkable organ specificities. The mechanisms underlying the neoplastic transformations of certain cell types are not known in detail, but high capacity for N-nitrosamine metabolism may be one factor of great importance.

In a series of studies we have examined the disposition of N-nitrosamines in the tissues of experimental animals. A major aim in the investigations has been the tracing of N-nitrosamine-metabolizing tissues.

In the present paper, a review is given of results obtained in our studies. Two tobacco-specific N-nitrosamines - 4-(N-nitroso-methylamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosonor-nicotine (NNN) - have been examined (5-11). These appear to be the most important tobacco-specific N-nitrosamines because of their strong carcinogenicity. N-nitrosodimethylamine, N-nitroso-diethylamine, N-nitrosodibutylamine, N-nitrosopyrrolidine and N-nitrosomorpholine are volatile N-nitrosamines which may be present in tobacco and tobacco smoke, and these substances have also been included in our experiments (12-19). In addition, we have examined N-nitrosodiethanolamine - a non-volatile N-nitrosamine which has been identified in tobacco products and tobacco smoke (20).

EXPERIMENTAL DESIGN

The initial step in the experiments was usually a whole-body autoradiographic study using a ^{3}H- or ^{14}C-labelled N-nitrosamine. This method allows an unbiassed screening of the labelling of all tissues of the body. For volatile N-nitrosamines, low-temperature autoradiography and autoradiography with dried tape-sections were used to distinguish the tissue-distribution of the volatile non-metabolized N-nitrosamines from the distribution of non-volatile metabolites. By extraction of the tape-sections with trichloro-acetic acid and organic solvents before the autoradiography it was possible to localize metabolites which were firmly bound to the tissues. Microautoradiography was used to examine in detail the cellular localization of metabolites.

The autoradiographic studies formed the basis for <u>in vitro</u>-experiments in which the capacity of various tissues to metab-olize the N-nitrosamines was examined.

TISSUE-DISTRIBUTION OF THE N-NITROSAMINES

Low-temperature autoradiography with volatile N-nitrosamines, such as N-nitrosodimethylamine, N-nitrosodiethylamine, N-nitroso-dibutylamine, N-nitrosopyrrolidine and N-nitrosomorpholine, showed an even labelling of the tissues of the body at short in-

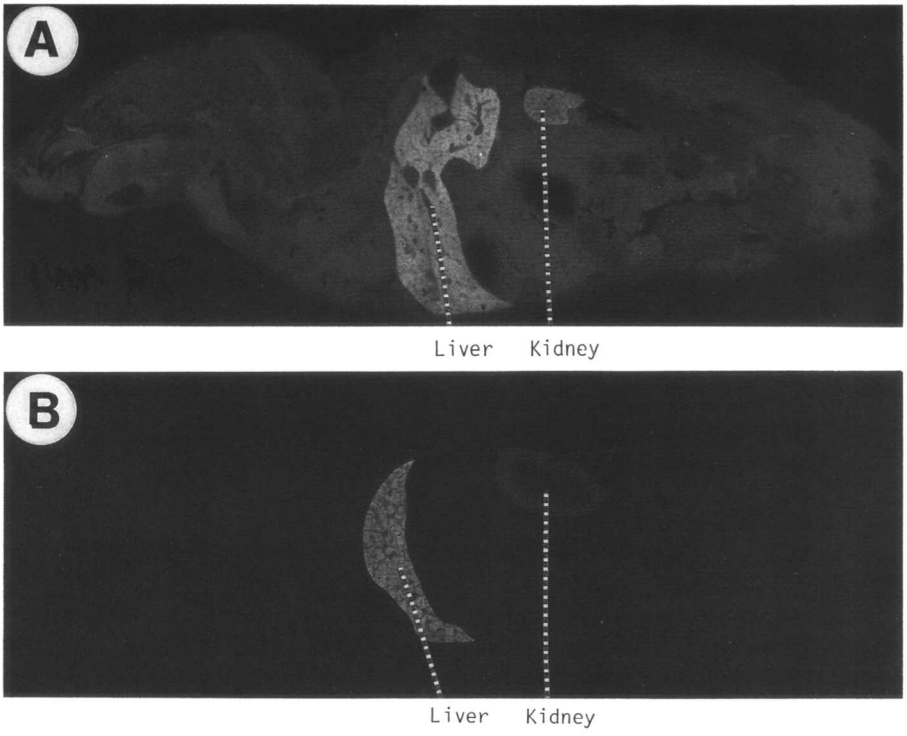

Figure 1. Whole-body autoradiograms of a C57Bl-mouse killed 1 min. after an i.v. injection of ^{14}C-N-nitrosodimethylamine. (A) has been obtained by low-temperature whole-body autoradiography by exposure of a saw-hemisection at -80°C. (B) has been obtained by autoradiography of a dried tape-section at -20°C. In (A) there is an even 'background-radioactivity' in most tissues; a high radioactivity is present in the liver and a considerable labelling is also present in the kidney. In (B) non-volatile radioactivity is still present in the liver, and to a lower extent in the kidney, whereas the volatile radioactivity in the other tissues has evaporated. These data indicate a homogeneous distribution of the non-metabolized (volatile) ^{14}C-N-nitrosodimethylamine in the body and a rapid formation of (non-volatile) metabolites in the liver, and to a lower extent also in the kidney (from ref. No. 12).

tervals after the administrations (12-19) (Fig. 1). This indicates an ability of these substances to freely pass the cellular membranes and distribute in the extra- and intracellular tissue-water. N-nitrosodiethanolamine, which is a polar N-nitrosamine, appeared to penetrate the blood-brain barrier into the central nervous system with some difficulty, although cellular membranes of other tissues seemed to be freely permeable for this substance (20). NNN and NNK were also distributed throughout the whole bo-

dy, but since they are weak bases they will be trapped in areas with low pH, such as the lumen of the stomach (5,6,8-11). NNN and NNK were also localized in melanin-containing tissues, such as melanin of the eyes and the skin (5,9-11). Melanin is a polymer which is rich in free carboxyl groups and binds electrostatically basic compounds, such as NNN and NNK (21). In addition, NNN and NNK were found to accumulate in some exocrine glands such as salivary and lacrimal glands, Harder's gland and preputial glands (5,6,8,11). It is possible that this results from ionic association between these N-nitrosamines and negatively charged constituents (e.g. acid mucopolysaccharides) in these tissues. There are no indications in the literature of NNN- or NNK-related tumours in the tissues accumulating high concentrations of these substances.

As mentioned previously, the carcinogenicity of the N-nitrosamines is characterized by a marked tissue-specificity. The observation that the non-metabolized N-nitrosamines are distributed throughout the body with similar concentrations in most tissues indicates that the unchanged compounds are rather inoccuous. It is also apparent that the organ-specific carcinogenicity is not related to a preferential accumulation of the non-metabolized N-nitrosamines in the target tissues.

TISSUE-LOCALIZATION OF N-NITROSAMINE METABOLITES AND TISSUE-SPECIFICITY OF N-NITROSAMINE METABOLISM

The whole-body autoradiography showed a rapid localization of metabolites in specific tissues after the administration of the ^{14}C-labelled N-nitrosamines.

The structures accumulating metabolites were always found among a spectrum of tissues. These included the epithelial linings and some glands in the upper respiratory and alimentary tracts, and in addition the liver and the kidney. In the respiratory tract, structures which were found to be labelled were the nasal olfactory and respiratory mucosa, Bowman's glands in the nasal olfactory area, serous glands in the nasal respiratory area, the lateral nasal gland (Steno's gland), the epithelium of the naso-pharyngeal duct, the pharyngeal and tracheal mucosa and the mucosa of bronchi and bronchioles. In the alimentary tract, structures which were found to be labelled included the oral mucosa (tongue, cheeks, palate, gingiva), the laryngeal and oeso-

phageal mucosa and, in a few instances, salivary glands. The number of structures, among the spectrum of tissues mentioned above, which were labelled varied for the different N-nitrosamines. Also, for the same N-nitrosamine variations in the spectrum of tissues which was labelled could sometimes be observed between different animal species.

When the tissues were tested for N-nitrosamine-metabolizing capacity *in vitro*, it was found that the ability of a tissue to metabolize an N-nitrosamine almost invariably correlated to a capacity of the same tissue to accumulate N-nitrosamine-metabolites *in vivo*. These data provide strong evidence that the distribution patterns for the metabolites after the N-nitrosamine administrations are depending on local biotransformations in the various tissues.

A more detailed discussion about the tissue-specificity of the metabolism of the N-nitrosamines and the correlation between this metabolism and the carcinogenic effects of the N-nitrosamines will be given below.

The liver, being the major site of metabolism of xenobiotics, exhibited a marked ability to form and accumulate metabolites from most of the studied N-nitrosamines. After the administration of ^{14}C-N-nitrosodimethylamine to mice, the labelling of the liver was very dominating (12) (Fig. 1). However, administration of the structurally related ^{14}C-N-nitrosodiethylamine resulted in an accumulation of metabolites in several extrahepatic tissues in the mice (14). In Syrian golden hamsters given ^{14}C-N-nitrosodiethylamine there was also a labelling of extrahepatic tissues (17) (Fig. 5). However, whereas in the mice a marked *in vivo* accumulation of metabolites and *in vitro* capacity to metabolize the ^{14}C-N-nitrosodiethylamine was observed both in the upper respiratory and alimentary tracts, the study in the hamsters showed that these events were confined to the respiratory tract. Studies in Sprague-Dawley rats (unpublished) have shown that metabolites from ^{14}C-N-nitrosodimethylamine are localized specifically in the liver, whereas for ^{14}C-N-nitrosodiethylamine both the upper alimentary and respiratory tracts are labelled (Fig. 2). Our results correlate with carcinogenicity data: N-nitrosodimethylamine is a potent hepatocarcinogen in all species examined; N-nitrosodiethylamine induces tumours, in addition to the liver, in the upper alimentary and respiratory tracts in mice and rats, whereas