COMMENTARY

Carcinogen metabolism in cultured human tissues and cells

Herman Autrup

Laboratory of Environmental Carcinogenesis, Fibiger Institute, Danish Cancer Society, Ndr. Frihavnsvej 70, DK-2100 Copenhagen Ø, Denmark

A large number of chemical carcinogens require metabolic activation before they are biologically active. The metabolism of a few of these compounds has been investigated in cultured human tissues and cells and the metabolism has been compared with the metabolism in organs from experimental animals in which the compounds induce cancer. Generally, only quantitative differences could be observed between animal and human tissues. The development of new methods to detect carcinogen-DNA adducts makes it feasible to study and compare the metabolism in human and animal tissues of an increasing number of potential human carcinogens. Furthermore, construction of cell lines, expressing the human forms of the carcinogen metabolizing enzymes, by biotechnology provides a new model to study the metabolism and to monitor for genetic markers in the same cells.

Introduction

More than 600 chemicals, groups of chemicals, industrial processes and occupational exposures have been evaluated by IARC for their carcinogenicity. For 196 chemicals there is insufficient evidence for carcinogenicity in humans, but studies in experimental animals and short term tests support the notion that these compounds are potential human health hazards (1). Additional support can be provided by comparative metabolism studies in target tissues and cells from experimental animals and man. Nearly half of the known carcinogens require metabolic activation before they can exert their mutagenic and carcinogenic activity. It has to be assumed that an animal carcinogen is more likely to also be a carcinogen in man if the metabolic pathway to the ultimate carcinogenic form is identical than if they differ. However, different pharmacokinetics have to be taken into account for human risk evaluation.

Most of the chemicals classified as carcinogens requiring metabolic activation belong to three major chemical classes, polycyclic aromatic hydrocarbons (PAH*), N-nitrosamines and aromatic amines. Polycyclic aromatic hydrocarbons are ubiquitous carcinogens and are present in e.g. automobile exhaust and cigarette smoke and are suspected to be some of the causative agents in lung cancer (2,3). Human exposure to carcinogenic N-nitrosamines may result from ingestion or inhalation of preformed compounds from the environment or from nitrosation of exogenous amino precursors in the body. The exposure to the in situ formed nitrosamines is associated with an increased risk of oesophageal cancer (4). Occupational exposure to aromatic amines has long been regarded as a determining factor in a high percentage of human bladder cancer cases. Furthermore, the higher incidence of bladder cancer among cigarette smokers may be a result of exposure to aromatic amines present in cigarette smoke (5–7).

Metabolism of chemical carcinogens in normal human cells

Studies on the metabolism of chemical carcinogens, especially PAH, have been performed in explant cultures and primary cultures of normal human epithelial tissues and liver (8,9). In addition, extracellular systems prepared from human tissues have been used for determination of activities of enzymes involved in the metabolism of carcinogens and as activation systems in both mammalian and bacterial mutation assays. One of the major limitations of the latter approach is that the active metabolite is formed outside the target cells for the biological activity. Furthermore, some enzyme activities may be lost in the isolation procedure. When hepatocytes and S9 mix isolated from hepatocytes were compared as external metabolizing systems, they were not equally effective and the effectiveness depended on the type of carcinogen (10). Furthermore, different benzo[a]pyrene (B[a]P) metabolite profile and DNA adduct pattern were observed when using subcellular and cellular preparations (11). Explant cultures and primary cultures have been used as activation systems in cell mediated mutagenesis (CM) assays using Chinese hamster V79 cells as the detector cell (Table I). The co-cultivation assay has one weakness in common with the subcellular activation systems, the active metabolites have to be transported into the target cells.

A number of metabolism studies have been conducted using carcinoma and hepatoma cell lines. However, in this commentary, I will limit my discussion to studies conducted with what are considered normal tissues or originating from normal tissues, although important information on the regulation of carcinogen metabolizing enzymes in human cells has been obtained by using the carcinoma and hepatoma cell lines.

One of the difficulties of using human tissues and cells in toxicological investigations is the availability of reproducible tissues. While the wide inter-individual variation in the metabolism of chemical carcinogens by human tissues may be of interest in evaluating risk of individuals, and in the study of genetic differences in metabolism of carcinogens (35), this variation is a complicating factor in reproducibility and interpretation of toxicological experiments. Furthermore, the enzyme activity expressed by the freshly obtained tissues or short-term cultured tissues is a function of the basal activity, and the exposure to various types of inducers of cytochrome P450 prior to the obtainment of the tissues. It is well known that cytochrome P450 activities are induced by dietary components, drugs, environmental pollutants and compounds in cigarette smoke. The level of arylhydrocarbon hydroxylase (AHH) activity is significantly higher in placentas from smoking mothers (36) and from mothers that had been exposed to polyhalogenated compounds (37). The effect of smoking on AHH activity in
pulmonary tissues has been shown to last 40–100 days after cessation of smoking (38).

Human lymphocytes have been used as both the target cells and the activating system. Heterocyclic amines induced chromosomal aberrations and sister chromatid exchanges (39) and tobacco-specific nitrosamine 4-(methyleneiminodiamine)-1-(3-pyridyl)-1-butanone (NNK) induced a significant number of sister chromatid exchanges (40) in the lymphocytes. Human pulmonary alveolar macrophages (PAM) have frequently been used as an activation system in the V79 co-cultivation mutagenesis test (41,42). Although PAMs are not considered a target cell for chemical carcinogenesis, the metabolism of carcinogens in these cells may play a role in lung carcinogenesis. The advantage of using PAMs is that these cells are less complicated to work with than other types of cells cultivated from the lung.

In recent years, tremendous progress has been made in the development of methods for maintaining and propagating human epithelial cells, e.g. immortalization with SV40 (43). These cells may provide a new opportunity to study the metabolism of carcinogens in human epithelial cells. However, the effect of the immortalization process of the expression of genes responsible for the metabolism of xenobiotics has not been investigated.

One of the disadvantages of using cell cultures to predict organ sensitivity is that specific cell types may be selected during the culture process and that the metabolizing activity in the selected cell type may not be representative for that particular organ. In the case of the lung tissue, different mixed function oxidase activity has been detected in Clara and alveolar type II cells with the highest activity in the Clara cells (44). In human ovary, the highest level of dimethylbenzantrachene (DMBA) hydroxylation was found in the granulosa cells, but the level of metabolic activity in the ovary was influenced by hormonal stimulation (45). High enzymatic activity in a particular cell type may indicate that this cell is more susceptible to the toxic and carcinogenic action of the chemical.

Cultured conditions may also influence the level of enzymatic activity in cells. The enzyme activities can be modulated by culture media compositions and supplementation with sera. For example, the level of calcium in the media influenced the metabolism of PAH in mouse keratinocytes (46) and it has been suggested that this effect is possibly due to Ca2+-induced differentiation. The AHH activity and enzyme inducibility was also dependent on cell growth in human epidermal cells (47). However, there was no correlation between the rate of incorporation of tritiated thymidine, as a measure of growth rate and B[alpha]P-adduct level in bronchial explants from the same donor. Addition of growth stimulatory factors to culture medium slightly increased the level of B[alpha]P metabolism in human bladder cells (H.Autrup and L.Dragsted, unpublished results). The quantitative level of metabolism was also found to be density dependent in human mammary epithelial cells (16). Primary cultures expressed enzymes involved in the metabolism of chemicals, but some of this activity was decreased during culture, e.g. cytochrome P450IA2 was only expressed in rat hepatocytes, when the cells were grown on a matrix of collagen (48).

### Table 1. Metabolism studies in cultured normal human tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Level</th>
<th>Biological endpoint</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder</td>
<td>C</td>
<td>M,D</td>
<td>12</td>
</tr>
<tr>
<td>Breast</td>
<td>E</td>
<td>M,D</td>
<td>13–15</td>
</tr>
<tr>
<td>Bronchus</td>
<td>C</td>
<td>M,D,CM</td>
<td>16–18</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>M,D,DR</td>
<td>12,19,20</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>M,CM,D</td>
<td>9,12,15</td>
</tr>
<tr>
<td>Colon</td>
<td>C</td>
<td>M,D</td>
<td>21</td>
</tr>
<tr>
<td>Duodenum</td>
<td>E</td>
<td>M,D</td>
<td>9</td>
</tr>
<tr>
<td>Endometrium</td>
<td>E</td>
<td>M,D</td>
<td>9</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>C</td>
<td>CM</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>M,D</td>
<td>9,23,24</td>
</tr>
<tr>
<td>Kidney</td>
<td>C</td>
<td>M,D</td>
<td>21</td>
</tr>
<tr>
<td>Oral mucosa</td>
<td>C</td>
<td>M,D</td>
<td>12</td>
</tr>
<tr>
<td>Liver</td>
<td>C</td>
<td>D,CM,BM,DR</td>
<td>25–30</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>D,M</td>
<td>23</td>
</tr>
<tr>
<td>Stomach</td>
<td>E</td>
<td>D,M</td>
<td>23</td>
</tr>
<tr>
<td>Skin</td>
<td>C</td>
<td>D</td>
<td>8,31–33</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>M,D</td>
<td>34</td>
</tr>
</tbody>
</table>

C, Cells; E, explants; M, metabolic profile; CM, cell mediated mutation assay; D, DNA-binding; BM, bacterial mutation assay; DR, DNA-repair.

### Metabolism of chemical carcinogens

Mixed function oxidases (MFO) are a class of enzymes involved in the biotransformation and oxidative activation of chemical carcinogens and other exogenous compounds. The main component of the MFO enzyme system is the cytochrome P450 isoenzymes. Many forms of cytochrome P450 have been isolated from both rodent and human tissues, and they have been classified into 17 families according to homologies of their amino acid sequences (49). The role of different cytochrome P450 in the metabolism of carcinogens has been evaluated using microsomal preparations from animals treated with inducers, using reconstituted systems with isolated P450 proteins, or by using cellular extracts from cells expressing specific forms of cytochrome as external activation systems (50,51). The relative composition of cytochrome P450, and the required NADPH cytochrome P450 reductase may be an important risk factor when determining organ toxicity. NADPH-cytochrome P450 reductase has been detected in most human extrahepatic tissues by immunohistochemical techniques. Considerable variation in the activity of NADPH cytochrome P450 reductase in different anatomical segments of the intestine was observed (52) and this difference might explain the different level of B[alpha]P-DNA adducts seen in the various segments of human colon (9). The presence of other enzymes is a prerequisite before metabolites generated by the cytochrome P450 enzyme system can exert their biological activity; for example, epoxide hydrolase was the rate limiting step in the metabolism of 7,12-DMBA to its ultimate carcinogenic form, the bay region diol epoxide (53).

Presently, very little is known about the expression of cytochrome P450 genes and other genes relevant for the metabolism of carcinogens in human target cells, e.g. the different lung cells.

In addition to acting as an extracellular activation system in the co-cultivation assay, the ability of human tissues to metabolize chemical carcinogens has been assessed by many different parameters (Table I). Identification and quantitation of metabolites released into the tissue culture media and quantitative and qualitative analyses of the carcinoeng-DNA adducts formed in the target cell DNA have commonly been used for the assessment of metabolic capabilities. Induction of DNA repair in the cultured tissues is an indirect measure for the formation of genotoxic metabolites by the metabolic system in the cells.
Table II. Chemical carcinogens metabolized by normal human tissue

<table>
<thead>
<tr>
<th>Carcinogen</th>
<th>Organ</th>
<th>Biological endpoint</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>UB,B,C,L</td>
<td>M,D,DR</td>
<td>14,23,28,54</td>
</tr>
<tr>
<td>Aromatic amines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Acetyl-aminofluorene</td>
<td>UB,L,S</td>
<td>M,D,DR</td>
<td>27,28,31,54,55</td>
</tr>
<tr>
<td>2-Naphthylamine</td>
<td>UB,L</td>
<td>D,DR</td>
<td>13,54</td>
</tr>
<tr>
<td>Other amines</td>
<td>L.C,UB</td>
<td>D,BM</td>
<td>9,13,26</td>
</tr>
<tr>
<td>Polycyclic aromatic hydrocarbons</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>UB,M,B,I,O,S</td>
<td>M,D,DR</td>
<td>12,17,18,28,31,54</td>
</tr>
<tr>
<td>7,12-DMBA</td>
<td>M,I,K</td>
<td>D,M,CM</td>
<td>8,17,21</td>
</tr>
<tr>
<td>Other PAH</td>
<td>B,I</td>
<td>D,M</td>
<td>12</td>
</tr>
<tr>
<td>N-nitrosamines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethylnitrosamine</td>
<td>B,I,L</td>
<td>D,CM,DR</td>
<td>8,28,54,56</td>
</tr>
<tr>
<td>Benzylnitrosamine</td>
<td>B,L,O,St</td>
<td>D,DR</td>
<td>9,24–25</td>
</tr>
<tr>
<td>Other nitrosamines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitro-PAH</td>
<td>B,I,L</td>
<td>D,DR</td>
<td>54,57,58</td>
</tr>
</tbody>
</table>

B. Bronchus; O. oesophagus; I. intestine; K. kidney; L. liver; M. mammary gland; S. skin; St. stomach; UB. urinary bladder.

**Metabolism of PAHs**

The metabolism of B[a]P and other PAH has been extensively studied in cultured human tissues (Table II). A weak association between the carcinogenic potency of the PAH in experimental animals and the level of PAH-binding to human bronchial DNA was established. The ultimate carcinogenic form has been identified as the PAH diol epoxide. Only B[a]P–DNA adducts have been unequivocally identified in human tissues. The major B[a]P–DNA adducts were the same in all cultured tissues and cells from humans and experimental animals (8) and the same metabolic profile was seen when rodent and human tissues were incubated with B[a]P (59).

The level of binding of B[a]P to DNA was higher in epithelial than fibroblastic cells obtained from the same individual and from different organs (12). Recently, Leadon et al. (18) reported that there was no association between B[a]P–DNA adducts and the cytotoxic response in human mammary cells. In contrast, an association between the number of thymidine glycol adducts induced by B[a]P or its metabolites and the cytotoxic response was observed.

No qualitative differences between the metabolism in human tissues and tissues from experimental animals could be established, but the binding level of B[a]P to DNA was higher in human than in rodent tissues (8).

**Metabolism of aromatic amines**

Aromatic amines can be hydroxylated at either the ring C-positions or at the exocyclic N-atom. The N-hydroxy aromatic amines are activated to a DNA binding metabolite by an acetyl CoA-dependent enzyme, while the C-hydroxylated compounds are considered detoxification products (60). The major carcinogen–DNA adducts have been identified as formed between the exocyclic N-atom of the amine and the C-8 position in guanine. The binding level of aromatic amines to DNA in bladder was insignificant, although these compounds are considered to be bladder carcinogens. It has been suggested that the metabolism in situ is unlikely to contribute to the carcinogenic effects of aromatic amines (13).

Protein pyrolysate products are a group of heteroaromatic amines that are highly mutagenic and potent systemic carcinogens in experimental animals. The metabolism of these compounds have been extensively studied in rodents, both in vivo and in vitro, but very little is known about the metabolism in human cells. The metabolism of 3-aminoo-1-methyl-5H-pyriddo(4,3)indole has been studied in explants of human colon (9). Information regarding the metabolism of protein pyrolysate products is both important and relevant, as this group of compounds are potential human carcinogens.

**Metabolism of N-nitrosamines**

Hydroxylation at the C-atom in the alpha position to the nitroso group is considered the major pathway in the activation of N-nitrosamines to their ultimate carcinogenic forms (61). The metabolism of both cyclic and acyclic nitrosamines has been investigated in both human and rat cultured tissues. High levels of metabolic activity, as measured by the level of binding of the ultimate carcinogen to cellular DNA, was found in the oesophagus and stomach, organs that are considered to be the major site for nitrosamine carcinogenesis. The metabolism was significantly higher in rat oesophageus than human, especially the metabolism of methylbenzyl nitrosamines, a compound that is used for experimental induction of oesophageal cancer in rat. Tobacco-specific nitrosamines, NNK and N'-nitrosornicotine (NNN), were substantially metabolized in both human bronchial and oesophageal tissues, but the metabolism in human tissues were less than in the corresponding rat tissues, as was the case with other nitrosamines (24).

**Metabolism of other carcinogens**

Aflatoxin B₁ (AFB), a mycotoxin produced by certain strains of the fungus *Aspergillus flavus*, is a very potent liver carcinogen in experimental animals. The ultimate carcinogen, AFB 8,9-epoxide that is formed by epoxidation, reacts mainly with the N-7 atom in guanine. This adduct is formed in DNA from all organs and in all species tested (64). The binding level of AFB to hepatocyte DNA was highest in cells from a susceptible species. The binding level in human hepatocytes was between that in hepatocytes isolated from male and female rats, susceptible and non-susceptible animals respectively (28).

Nitrated PAHs, found in diesel emission and environmental samples, are potent mutagens and are tumorigenic in experimental animals. The metabolic pathways to the ultimate carcinogens appear quite complex, and the DNA adduct is formed by covalent binding between the exocyclic N-atom and the C-8 position in guanine, similar to the adducts formed with aromatic amines. This type of adduct requires reduction of the nitro group. Studies in human fibroblasts suggest that the initial reduction to the nitroso-compound is the rate limiting step (63). Two DNA adducts were detected in lung and liver from rats treated with 6-nitrobenezopyrene and adducts with identical chromatographic properties were isolated from human bronchial explants treated with the same compound (58). Human bronchus also metabolized 6-nitrochrysene to a DNA binding metabolite (57). This information suggests that cultured tissues are able to reduce the nitro group to the nitroso group.

**Activation by other metabolic pathways**

In addition to the P450 associated activation that historically is considered the major pathway for activation of chemical carcinogens, the prostaglandin H-synthase dependent activation may be important (64). The role of this enzyme system in the metabolic activation of, e.g. benzo[a]pyrene-7,8-diol (65).
heterocyclic aromatic amine (66), aflatoxin B$_1$ (67) in animal cells has been demonstrated. The enzyme activity has been shown in extrahepatic human tissue microsomes (68). However, studies in human bronchus explants suggest that this pathway is of minor importance in the activation of B[a]P in this tissue (69).

**Conclusion**

Biotransformation of many cancer causing chemicals and other toxic compounds is required before the compounds attain their biological activity. Differences in metabolic capacity and in the stereospecificity of the metabolism may be important determinants in risk evaluation. Using explant cultures and primary cultures any difference between human and animals may be overshadowed by the great inter-individual variation observed among humans. Due to the difficulties obtaining 'reproducible' human tissues and the previously mentioned inter-individual variation, the value of results from experiments using human tissues in regulatory toxicological evaluation is limited. However, very important research information still has to be obtained, i.e. information on qualitative differences in metabolism between animals and man. Furthermore, information on the metabolism of many carcinogens in human tissues is still needed. Actually, only a few compounds have been investigated, but this has partly been due to the availability of radiolabelled carcinogens. Application of the 32P-postlabelling technique to detect and quantitate carcinogen - DNA adducts in human cells exposed to a carcinogen could partly overcome this problem (70). The chemical identity of the observed adducts may not be known, but the pattern can be compared with that in cultured animal tissues treated with the same carcinogen.

In recent years different forms of cytochrome P450 genes have been cloned, and the genes have been transferred into human and animal cells. The main emphasis has been on rodent P450. Chinese hamster V79 cell lines that stably express different rodent P450 have also been constructed and have been used for mutational analysis in the HPRT locus (71). A human lymphoblastoid cell line expressing a high level of P450IA1 has been transfected with the human cDNA for P450IIA2 and epoxide hydrolase. This constructed cell line was more sensitive to the mutagenicity of dimethylhydrazine and B[a]P than the parent cell line (72). A number of investigators are currently involved in the construction of cell lines expressing either rodent or human P450 to be used in toxicological testing. One of the advantages of these test systems is that the target cells express the metabolizing enzymes and that no external activation system is required.

New results using the cloned genes of specific forms of cytochrome P450 indicate differences between the animal and human forms of the P450 in their ability to convert a mutagen to a metabolite that is mutagenic in the Ames assay. Mouse P450IIA2 was 5- to 7-fold more active in the conversion of 2-acetylaminofluorene and benzo[a]pyrene-7,8-diol to its mutagenic metabolites (73). This information suggests that it is important to use cells expressing the human genes, if a quantitative risk evaluation has to be made.

These new assays give new information about the biotransformation of genotoxic agents by human metabolizing enzymes, but the information cannot be used to explain the difference in organ toxicity, as the P450 and associated enzymes are expressed to a different extent in different organs and cell types within the organs. Therefore, more traditional metabolism studies in explant and cell cultures of different human organs are still important.

Alternatively, the expression of the different enzymes involved in metabolism should be analysed in the different organs and in the various cell types.

**References**

23. Autrup, H., Harris, C.C., Wu, S.M., Bao, L.Y., Pei, X.F., Sun, T.T and


