

Carcinogen metabolism and individual susceptibility

by Olavi Pelkonen, MD¹

PELKONEN O. Carcinogen metabolism and individual susceptibility. *Scand J Work Environ Health* 1992;18 Suppl 1:17–21. Many chemical carcinogens need activation by drug metabolizing enzymes, principally cytochrome P450 enzymes, to become capable of binding to deoxyribonucleic acid and initiating the carcinogenic process. The activity and inducibility of drug metabolizing enzymes are regulated by interplay between genetic, host, and environmental factors. Consequently, individual differences in cancer susceptibility might be explained somewhat by genetic differences in metabolic activation. Three examples, the induction of aryl hydrocarbon (benzo[a]pyrene) hydroxylase by polycyclic aromatic hydrocarbons, polymorphic debrisoquine/sparteine oxidation, and polymorphic acetylation, are briefly reviewed. Despite useful animal models and promising early human findings, no consensus has been reached about the significance of genetically based differences in drug metabolism in cancer etiology. It is expected that, with the use of molecular biological methods, the genetic background of study subjects can be investigated without bias caused by the disease, age, treatment, or other factors which have plagued investigations thus far.

Key terms: acetylation polymorphism, aryl hydrocarbon hydroxylase, cytochrome P450 enzymes, debrisoquine/sparteine oxidation polymorphism, inducibility.

Most chemical carcinogens need metabolic activation to be able to bind to deoxyribonucleic acid (DNA) and initiate the carcinogenic process (1). Metabolic activation is catalyzed by the so-called drug- or xenobiotic-metabolizing enzymes, which are located in the liver and other tissues (2, 3). The nature, tissue specificity, and regulation of these enzymes have been reviewed in great detail recently (4, 5). In this report, I try to summarize what is known about the regulation and interindividual variation, on the basis of hereditary factors, of the enzymes activating chemical carcinogens and to what extent the variability is associated with differential susceptibility to chemical carcinogens.

Host conditions "sensitizing" to chemical carcinogens

On the basis of several animal experiments, it is known that the carcinogenicity of a given chemical can be modified by the genetic background of the experimental subject, nongenetic host factors, and a multitude of environmental factors. In humans, the identification of both endogenous and exogenous risk factors have led to a search for "markers" which would reveal individual differences in the risk to contract a specific cancer. Much effort has been directed towards the investigation of familial factors, which presumably

in many cases can be equated with genetic background. A recent book was dedicated to this subject (6). For example, with respect to human cancers associated primarily with chemical exposures, host susceptibility to lung cancer has received considerable attention. Several epidemiologic studies have revealed a familial component in lung cancer which renders persons susceptible to a several-fold risk of contracting lung cancer (6, 7). The nature of this familial factor is not known at present, although speculations range from specific oncogenes to differences in the activation of carcinogens.

Interindividual variability in carcinogen metabolism

The interindividual variability of drug-metabolizing enzymes in human tissues has been a topic of recent reviews (8, 9). It is sufficient to say in this context that it is not rare to see a 30- or even 100-fold variation in the activity of almost any drug-metabolizing enzyme. It is possible that part of the variation in *in vitro* measurements is due to technical problems inherent in human studies (eg, tissue storage, variable sources, etc), but on the basis of *in vivo* drug elimination studies it is safe to say that several tenfold differences are more a rule than an exception. Part of this variation is certainly due to environmental factors and nongenetic host factors, which may fluctuate in a given person from time to time. However, part of the variation is certainly due to genetic factors, which may be of considerable importance in determining an individual's ability to activate and detoxify chemical carcinogens, the result being differences in susceptibility and risk.

¹ Department of Pharmacology and Toxicology, University of Oulu, Oulu, Finland.

Reprint requests to: Dr O Pelkonen, Department of Pharmacology and Toxicology, University of Oulu, SF-90220 Oulu, Finland.

Genetics of metabolism

Although long-lasting environmental or host-mediated factors may be of importance in carcinogenesis, in this report I am interested in genetic determinants of carcinogen metabolism, and in the possible consequences as to the cancer susceptibility of genetically determined metabolic differences. It is of interest to look at what is known about human P450 isozymes, their ability to metabolize common carcinogens, and their regulation (table 1). It is clear that many of these "major" isozymes are regulated primarily by environmental factors; therefore their activities are expected to fluctuate from time to time when environmental conditions change. An illustrative example is P450 1A2, which is shown to be able to activate several potential carcinogens. This isozyme is expressed in the liver, but not in extrahepatic tissues. The isozyme is at least partially under the control of the Ah locus, but also other regulatory factors responsive to the environment are operative. Consequently, it is not known to what extent changes in activities associated with P450 1A2 could explain differential sensitivity to carcinogens. It seems that very little is known about the genetic regulation of those human P450 isozymes which have been shown to activate most chemical carcinogens, and consequently the role of these isozymes in the differential susceptibility of humans to carcinogens is difficult to study. However, there are at least two or three isozymes that are candidates for primarily genetic regulation. The situation is clear with P450 2D6 and P450 2C9, both of which represent well-known genetic polymorphisms, namely, debrisoquine/sparteine oxidation and mephenytoin hydroxylation, respectively. The situation is less clear with 1A1, an isozyme also called (not totally correctly) aryl hydrocarbon hydroxylase (AHH) and implicated in the activation of benzo[a]pyrene and other polycyclic aromatic hydrocarbons (PAH). Its inducibility is thought to be regulated by the so-called Ah locus, in analogy with inbred strains of mice, which are either responsive or nonresponsive to induction by PAH (12). Consequently, it may be useful to look at the studies aimed at revealing the as-

sociations, if any, of these polymorphisms with cancer susceptibility.

Aryl hydrocarbon hydroxylase and lung cancer

Considerable effort has been put into studies on the possible association between metabolic activation and lung cancer. (See reference 7.) The rationale behind these attempts is clear. First, lung cancer is the most common cancer, and the majority of cases is caused by cigarette smoking (ie, most probably by chemical carcinogens). Second, there is some evidence from epidemiologic studies that there is indeed a familial component in the etiology of this disease. Third, there is a genetic mouse model in which single-gene differences in the activation of PAH are associated with differences in susceptibility to chemically induced cancer, including lung cancer (12). Thus it is no wonder that several case-referent studies have been performed since 1973. A critical compilation and analysis of these studies has been published recently (7), and in this report it is enough to summarize this analysis (table 2). The overall conclusion is that the evidence taken collectively does not convincingly demonstrate an association between the inducibility of AHH activity and susceptibility to lung cancer induced by cigarette smoke.

Because the lymphocyte assay for determining Ah locus status can be affected by host and environmental conditions (see table 2), attempts to develop more reliable methods have been undertaken. Two restriction fragment length polymorphisms of the P450 1A1 gene have been studied in lung cancer patients. In a study of Kawajiri et al (15), the frequency of the homozygous rare allele of the gene identified by the MspI restriction enzyme was about threefold higher in patients. On the other hand, Haugen et al (16) did not find any correlation between the PstI polymorphism (in strong linkage disequilibrium with the MspI polymorphism) and lung cancer. It may be appropriate to say that nothing is known about the association of

Table 1. Metabolism of some chemical carcinogens by "major" human P450 isozymes.^a

Isozyme ^b	Carcinogen	Remarks
1A1 (c)	Benzo[a]pyrene, polycyclic aromatic hydrocarbons	Inducibility controlled by the Ah locus: present in lungs and other extrahepatic tissues
1A2 (d)	Numerous substances	Inducibility controlled by the Ah locus: present only in the liver
2A6 (Coh)	Aflatoxin B ₁ , diethyl-nitrosamine	Regulation unknown
2C9 (mep)	?	Polymorphic regulation
2D6 (db1)	?	Polymorphic regulation (see the text)
2E1 (j)	Nitrosamines, benzene	Regulation unknown
3A4 (p)	Benzo[a]pyrene, ATB1	Mainly nongenetic

^a The data in this table are based on the review articles of Guengerich (5, 10), Gonzalez et al (3), and Gonzalez (4).

^b For P450 nomenclature see Nebert et al (11).

Table 2. The Ah locus and lung cancer in man: problems and caveats.^a

	Remarks
Rationale (clear and plausible)	Familial component in etiology Metabolically produced mutagenic and carcinogenic metabolites Increased production after exposure to cigarette smoke, polycyclic aromatic hydrocarbons and other carcinogens
Methodology (less than satisfactory)	Fluorescence assay; no active metabolites Radiometric assay; no identified metabolites Reactive metabolite assay; difficult Tissue; validity to use lymphocytes questioned
Genetic control (far from established in man)	Mice: Ah locus model established Human: conflicting results in several studies
Environmental factors (still far from clear for humans)	Hepatic; mouse model thoroughly researched Extrahepatic; mouse model fairly established; in humans, "target" bronchial activity — little relevant knowledge (see, eg, reference 13)
Evidence for association	Animal studies Both positive and negative (but basically all explained by pharmacokinetic and other factors, see reference 14) Human studies (equivocal; see reference 7) 20 case-referent studies 10 positive 8 no association 2 negative
Potential biases (see reference 7)	Age Cancer Other illness Smoking Treatment (medication, etc)
New methodology	Restriction fragment length polymorphism Polymerase chain reaction

^a For a thorough analysis, see Law (7). Some crucial references can be found in the text.

either of these polymorphisms with the phenotypic inducibility of AHH activity in humans. Certainly further studies are required before any definite conclusions can be drawn.

Antipyrine elimination and lung cancer

Because of many practical problems in using lymphocytes to measure AHH inducibility, attempts to find substances which could be used in vivo and could give information about the status at the Ah locus have been repeatedly made. Early studies indicated that antipyrine elimination and the production of its three metabolites would correlate with AHH inducibility, and thus it would be possible to use antipyrine as a noninvasive measure for a person's metabolic status. (See reference 7.) However, these early hopes seem to be unfounded. First, antipyrine elimination is affected by several host factors and environmental conditions, and it is practically impossible to determine any genetic component on an individual basis. Second, the antipyrine test is susceptible to all of the biases and reservation that plague the measurement of AHH inducibility in lymphocytes. (See table 2.) Consequently it is no wonder that in actual studies addressed to the association between antipyrine elimination and lung cancer no association has usually been found. (See reference 7.)

Debrisoquine/sparteine oxidation polymorphism and lung cancer

The debrisoquine/sparteine oxidation polymorphism is the best characterized pharmacogenetic condition affecting a P450 isozyme in humans (17). The pharmacological and toxicologic significance of this trait has amply been shown; people with poor metabolism, which represent about 5–10 % of the Caucasian population, are more susceptible to side effects and toxicity during therapy with, for example, tricyclic antidepressants and some neuroleptics (18). The possibility that this trait is associated with lung cancer was raised by a study by Ayesh et al (19), in which the authors demonstrated the relative scarcity of the poor metabolizer phenotype among lung cancer patients. Although there was no plausible biological background to explain this apparent association, several other studies have followed with mixed results. (See references 7 and 21 and the references therein.) The phenotyping method which uses debrisoquine is rather reliable because environmental factors are not likely to interfere with the results, but there is always a possibility that interacting drugs and still unrevealed host factors may result in incorrect phenotyping. Recently, specific and reliable methods for genotyping on the basis of polymerase chain reaction have been published (20, 21), and it is expected that definite studies employing these unequivocal genotyping assays will be

published soon. Whether they are able to settle the current dispute remains to be seen. Until then, it is only possible to list two current hypotheses to explain the apparently positive association, namely, an unknown carcinogen activated by the P450 2D6 isozyme or a linkage disequilibrium between the debrisoquine locus and a postulated "oncogene" locus conferring susceptibility to lung cancer by cigarette smoking.

The role of debrisoquine/sparteine polymorphism as a marker in other types of cancer has also been studied. A lower proportion of the poor metabolizer phenotype, as well as a shift towards a lower metabolic ratio (indicating more extensive hydroxylation), among patients with liver or gastrointestinal cancer has been observed for Nigerian subjects (22). Discrepant results have been observed for subjects with bladder (23) or breast cancer. However, even in those studies showing a positive association, some factors potentially resulting in bias were not controlled.

Acetylation polymorphism and bladder cancer

Polymorphic acetylation, spontaneous diseases, and drug therapy have been dealt with in recent reviews (24, 25). There are several reasons to expect an association between occupationally induced bladder cancer and N-acetylation polymorphism (26). First of all, because acetylation is important for several aromatic amines (eg, β -naphthylamine) known to be bladder carcinogens, slow detoxication through this pathway results in an increase in alternative metabolic activation reactions, presumably N-oxidation. Second, there is a considerable body of evidence that reactive metabolites of bladder carcinogens are indeed responsible for the initiation of this cancer. Phenotyping slow and rapid metabolizers is relatively reliable with several possible probe substances, although occasional "intermediate" acetylators are encountered. Furthermore, this trait is not appreciably affected by host or environmental factors. Nevertheless, potential biases are always suspected because the phenotype is determined by the elimination rate or the appearance or ratio of a metabolite.

As expected, a preponderance of slow acetylators among occupational bladder cancer cases has been observed in several case-referent studies. (See reference 24 and the references therein.) However, no such association has been found with respect to "spontaneous" or nonoccupational bladder cancer in most reliable studies, although there are also findings to the contrary. (See reference 23 and the references therein.) Thus it seems that there are probably no "hidden" bladder carcinogens in the general environment, perhaps with the exception of those present in cigarette smoke, a known predisposing factor of bladder carcinogenesis. The elucidation of the molecular biology of N-acetylation polymorphism and the availability of

new probes for genotyping will certainly be useful in future studies.

An early suggestion of an association between rapid acetylation phenotype and breast cancer risk has not been confirmed in later studies (27).

Significance of associations

As briefly reviewed in this report, several associations between cancer susceptibility and genetically controlled metabolic activities have been demonstrated. However, equally numerous negative studies have been published, and currently it is almost impossible to decide how significant the genetic traits are in cancer sensitivity. Considering the multifactorial and multiphasic nature of chemical carcinogenesis, it may be too naive to expect strong correlations between metabolism and cancer susceptibility. Metabolic trait, if a risk factor at all (it may well be that later stages of carcinogenesis are much more important in the final outcome than initiation), may contribute only in a very modest way to the development of any specific cancer, and this minor contribution remains relatively weak or even unnoticed even in properly designed analytical and molecular epidemiologic studies.

Future trends

Although properly designed epidemiologic studies remain the final arbiter of the present problem, recent advances in molecular biology and new molecular probes (restriction fragment length polymorphism and polymerase chain reaction) certainly enhance the possibility to study genetic background without the interference of host or environmental influences. Nevertheless, it is unlikely that even these new probes could totally replace assays based on the detection of activity, binding, and kinetics. In this area, perhaps a wider view is required. For example, in most studies only a single enzyme or pathway is under investigation, and it would be wise to consider alternative routes of metabolism. Although it may be too simplistic to assume that there is only one activating and one inactivating pathway, it may be useful to try to incorporate wider metabolic aspects into a single study. Metabolic and kinetic studies are required for an understanding of the biology of cancer, because even a good genetic probe showing an association with cancer risk may leave the researcher in darkness, as is the situation now with debrisoquine/sparteine oxidation polymorphism.

Acknowledgments

The useful comments of Dr K Vähäkangas are gratefully acknowledged. This review was written within the framework of the COST B1 Action Project.

References

1. Conney AH. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polyaromatic hydrocarbons. *Cancer Res* 1982;42:4875—919.
2. Pelkonen O, Nebert DW. Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. *Pharmacol Rev* 1982;34:189—222.
3. Gonzalez FJ, Jaiswal AK, Nebert DW. P-450 genes: evolution, regulation, and relationship to human cancer and pharmacogenetics. *Cold Spring Harbor Symp Quant Biol* 1986;51:879—90.
4. Gonzalez FJ. Molecular genetics of the P-450 superfamily. *Pharmacol Ther* 1990;45:1—38.
5. Guengerich FP. Characterization of human microsomal cytochrome P-450 enzymes. *Annu Rev Pharmacol Toxicol* 1989;29:241—6.
6. Lynch HT, Hirayama T, ed. Genetic epidemiology of cancer. Boca Raton, FL: CRC Press, 1989.
7. Law MR. Genetic predisposition to lung cancer. *Br J Cancer* 1990;61:195—206.
8. Vähäkangas K, Pelkonen O. Host variations in carcinogen metabolism and repair. In: Lynch HT, Hirayama T, ed. Genetic epidemiology of cancer. Boca Raton, FL: CRC Press, 1989:35—54.
9. Harris CC. Interindividual variation among humans in carcinogen metabolism: DNA adduct formation and DNA repair. *Carcinogenesis* 1989;10:1563—6.
10. Guengerich FP. Roles of cytochrome P450 enzymes in chemical carcinogenesis and cancer chemotherapy. *Cancer Res* 1988;48:2946—54.
11. Nebert DW, Nelson DR, Adesnik M, Coon MR, Estabrook RW, Gonzales FJ, et al. The P450 superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* 1989;8:1—13.
12. Nebert DW. The Ah locus: genetic differences in toxicity, cancer, mutation, and birth defects. *Crit Rev Toxicol* 1989;20:153—74.
13. Petruzzelli S, Camus A-M, Carrozzi L, Chelarducci L, Rindi M, Menconi G, et al. Long-lasting effects of tobacco smoking on pulmonary drug-metabolizing enzymes: a case-control study on lung cancer patients. *Cancer Res* 1988;48:4695—700.
14. Okey AB. Enzyme induction in the cytochrome P-450 system. *Pharmacol Ther* 1990;45:241—98.
15. Kawajiri K, Nakachi K, Imai K, Yoshii A, Shinoda N, Watanabe J. Identification of genetically high risk individuals to lung cancer by DNA polymorphisms of the cytochrome P450IA1 gene. *FEBS Lett* 1990;263:131—3.
16. Haugen A, Willey J, Borresen AL, Tefre T. PstI polymorphism at the human P1450 gene on chromosome 15. *Nucleic Acid Res* 1990;18:3114.
17. Meyer UA, Skoda RC, Zanger UM. The genetic polymorphism of debrisoquine/sparteine metabolism — molecular mechanisms. *Pharmacol Ther* 1990;46:297—308.
18. Brosen K, Gram LF. Clinical significance of the sparteine/debrisoquine oxidation polymorphism. *Eur J Clin Pharmacol* 1989;36:537—47.
19. Ayesh R, Idle JR, Ritchie JF, Crothers MJ, Hetzel MR. Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. *Nature* 1984;312:169—70.
20. Heim M, Meyer UA. Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. *Lancet* 1990;336:529—32.
21. Gough AC, Miles JS, Spurr NK, Moss JE, Gaedigk A, Eichelbaum M, Wolf CR. Identification of the primary gene defect at the cytochrome P450 CYP2D locus. *Nature* 1990;347:773—5.
22. Idle JR, Mahgoub A, Sloan TP, Smith RL, Mbanefo CO, Bababunmi EA. Some observations on the oxidation phenotype status of Nigerian patients presenting with cancer. *Cancer Lett* 1981;11:331—8.
23. Horai Y, Fujita K, Ishizaki T. Genetically determined N-acetylation and oxidation capacities in Japanese patients with non-occupational urinary bladder cancer. *Eur J Clin Pharmacol* 1989;37:581—7.
24. Evans DAP. Survey of the human acetylator polymorphism in spontaneous disorders. *J Med Genet* 1984;21:243—53.
25. Weber WW. The acetylator genes and drug response. New York, NY: Oxford University Press, 1987.
26. Hein DW. Acetylator genotype and arylamine-induced carcinogenesis. *Biochim Biophys Acta* 1988;948:37—66.
27. Webster DJT, Flook D, Jenkins J, Hutchings A, Routledge PA. Drug acetylation in breast cancer. *Br J Cancer* 1990;60:236—7.