

REVIEW PAPERS

THE OXIDATIVE PHENOTYPE AS A MARKER OF INDIVIDUAL PREDISPOSITION TO NEOPLASM INDUCED BY CHEMICAL CARCINOGENS

WALDEMAR LUTZ¹ AND WIESŁAW SUŁKOWSKI²

Department of Laboratory Diagnostics¹ and Otolaryngology and Audiology Division², The Nofer Institute of Occupational Medicine, Lodz, Poland

Key words: Cytochromes P-450. Aromatic hydrocarbons hydroxylase, gene expression. Debrisoquine. Antipyrine. Xenobiotic.

Abstract. The authors present a review of literature showing that the variations in xenobiotic oxidation phenotype, in the presence of cytochromes P-450, may be responsible, to a certain extent, for the varied individual susceptibility to neoplasm induced by chemical carcinogens. The evidence collected revealed that in the human population, one may distinguish individuals with high cytochrome activity and those in whom this reaction is low or even absent.

The search for the pharmacogenetic relationship between the oxidative metabolism of xenobiotics and neoplasm runs in two directions: that of defining a metabolic polymorphism accompanied by transcriptional activation of cytochrome P-450 genes and that of defining a constitutive expression of these genes. The assessment of the first polymorphism is most frequently done through a direct measurement of aromatic hydrocarbons hydroxylase activity in lymphocytes, or through an indirect measurement of its activity by adopting antipyrine as a marker drug. The definition of the other type of polymorphism is mostly based on the debrisoquine hydroxylation rate. However, attention should be drawn to the fact that the oxidation phenotype may play a significant part in the individual susceptibility to neoplasm induced by chemical carcinogens: other genetic predispositions (i.e. acetylation phenotypes) considerably affect the individual risk of cancer.

Not long ago it was thought that the carcinogenicity of chemical compounds encountered by human beings could be attributed to their chemical

Address reprint requests to W. Lutz, Department of Laboratory Diagnostics, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Lodz, Poland.



structure: Currently it is being associated not only with the chemical compounds' structure but also with their genetically determined metabolism in cells. Depending on which metabolic pathways dominate in the metabolism of a given compound, its carcinogenic properties may or may not emerge. A number of chemical compounds must be activated through an oxydative metabolism in the presence of cytochromes P-450 to become carcinogenic (23, 33).

Cytochromes P-450 are hemoproteins which catalyze molecular oxygen activation and are responsible for the penetration of the oxygen activated into molecules of varied chemical structures. This refers both to compounds synthesized by cells and to those derived from the environment. The majority of cytochromes P-450 isolated from human and animal tissues participate in oxydative processes of thousands of exogenic chemical compounds which penetrate organisms from the external environment i.e. environmental pollution, industrial chemical substances, substances present in natural food products and also in some drugs (5).

Information for the synthesis of a highly differentiated group of cytochromes P-450 is coded in genes which define the aminoacid sequence of their apoprotein. According to the level of similarity in DNA nucleotide sequence, genes encoding various apoproteins of cytochromes P-450 in mammals' cells, were categorized into eight gene families. Sub-families comprising 1 – 10 genes were then distinguished. All together they constitute one multigenetic super-family of cytochromes P-450. Gene families of cytochromes P-450 as well as some sub-families are distributed in various chromosomes. Probably they have been developed from one original gene through its duplication followed by mutation in individual gene loci (1, 39, 57).

Some chemical compounds i.e. phenobarbital or 3-methylcholantrene, induce synthesis of one or more forms of cytochrome P-450 corresponding to a given gene family: and that appears to be an interesting phenomenon. The induction process is associated with the transcription stimulation of messenger RNA (mRNA) synthetised gene, specific for a given cytochrome P-450. Cytochrome P-450 apoprotein increase in a cell is always preceded by an increased transcription period of the gene which is encoding that apoprotein. Induction, understood as an acceleration of gene transcription, may be considered as a mechanism of cell response to given chemical signals, e.g. xenobiotics or hormones. The acceleration of both xenobiotic oxidation and elimination of xenobiotics from an organism or the stimulation of the oxidation metabolism biologically important to endogenic substances, may be observed, depending on the form of cytochrome P-450 induced.

The development of active, genotoxic metabolic intermediates associated with the acceleration of xenobiotic oxidation appears to be an extremely important aspect of the synthetic induction of cytochrome P-450. Their collection in cell favours the development of gene mutations which consequently may initiate tumorogenic process (34, 42).

For many cytochromes P-450, present at oxidative xenobiotic metabolism, the emergence of inherited polymorphism has been indicated. In the human



population, one may distinguish persons with high, low or even non-existent activity of certain cytochromes P-450 (15).

The epidemiological survey revealed that the differentiation of oxidation metabolism phenotypes in the presence of cytochromes P-450 may be responsible, to a certain extent, for individual susceptibility to neoplasm induced by chemical carcinogens (16, 17, 49).

The pharmacogenetic relationship between xenobiotic oxidation metabolism and neoplasm has been investigated in two ways:

1. Metabolic polymorphism of transcriptional activation of genes encoding cytochrome P-450 of the P-450I family and participating in polycyclic aromatic hydrocarbons (PAH) oxidation. Recently, studies have been expanded to include the sub-family P-450IIE whose cytochromes are present in oxidation, e.g. nitrozoamine;
2. Metabolic polymorphism of constitutive expression of genes of cytochromes P-450 belonging to the sub-family P-450IID and participating in debrisoquine hydroxylation.

METABOLIC POLYMORPHISM OF TRANSCRIPTIONAL ACTIVATION OF CYTOCHROME P-450 GENES

Observing the hepatic metabolism of chemical carcinogens it was noted that the earlier administration of PAH to experimental animals considerably accelerates the metabolism of these carcinogens (6). Further investigations showed that the stimulation of carcinogen oxidation metabolism by PAH was accompanied by the synthetic induction of cytochrome P-450 in the liver (38). 3-methylcholanthrene was used as an inducer of cytochrome P-450 synthesis in the study described. Then it was found that the form of cytochrome P-450 induced by 3-methylcholanthrene differed from that induced by phenobarbital and that it was mainly responsible for the manifestation of carcinogenic properties of numerous chemical substances, e.g. benzo/a/pyrene (8). Cytochrome P-450 induced by 3-methylcholanthrene was named P-450IA1 according to the nomenclature proposed by Nebert et al. (39).

On investigating the properties of isosaphrole in hepatocyte metabolism, it was found that this compound induced synthesis of cytochrome P-450 with properties similar to cytochrome P-450IA1 (47). This form of cytochrome P-450 named cytochrome P-450IA2 was accompanied by the metabolic activation of such carcinogens as 2-acetylaminofluorene and aminobiphenyls (23).

The structure of genes CYP1A1 and CYP1A2, encoding apoproteins of cytochrome P-450IA1 and P-450IA2, is characteristic for mammalian genes i.e. they display a mosaic structure.

Genes consist of alternate sequences encoding links of the polypeptidic chain of apoprotein P-450 (exone) and non-coding sequences (introne). Non-coding sequences which precede the first exon perform a regulating function in gene expression (11).

The restriction analysis of the DNA genome of hybridised human and rodent cells showed that genes CYP1A1 and CYP1A2 are placed in humans in chromosome 15 (18).

The transcriptive activity of both genes increased remarkably when PAH was present in the cell. In response to 3-methylcholanthrene in hepatocytes, a quantitatively similar increase of mRNA was found in the apoprotein of both cytochromes P-450IA1 and P-450IA2. It was displayed almost one hour after administration of an inductor. The concentration of mRNA, specific for cytochrome P-450IA2, in a liver non-induced by PAH is much lower than mRNA of cytochrome P-450IA1 (24).

It is assumed that the mechanism regulating the expression of both genes, comprising a component sensitive to PAH, is similar, and perhaps even identical, while the component of the mechanism responsible for the constitutive expression of both genes varies (57).

The data on the stimulation of CYP1A1 gene expression by PAH, thus far, indicate that it is controlled by a genetic locus marked Ah because of its susceptibility to aromatic hydrocarbons (Aryl hydrocarbons).

Information for synthesis of intracellular protein receptors is encoded by locus Ah. The presence of the receptor is indispensable for obtaining a response from the cellular genome to PAH inducing activity (14). The gene (or genes) of the protein receptor is (are) placed in chromosomes other than genes CYP1A1 and CYP1A2 (42).

Synthetic induction of cytochrome P-450IA1 by PAH and the associated increase of cell oxidation ability measured as activity of aromatic hydrocarbons hydroxylation (AHH – Aryl Hydrocarbon Hydroxylase) are preceded by the creation of an inductor complex (e.g. 3-methylcholanthrene or 2,3,7,8-tetra-chlorodibenzo-p-dioxine – TCDD) with receptor protein.

The collection of complexes of inductor-receptor protein in nuclei is timed with acceleration of CYP1A1 gene transcription. The receptor protein binding PAH, i.e. benzo/a/pyren varied from that binding TCDD (42).

The investigation of the effect of PAH on AHH activity in cells from tissue culture, in conditions of temporary exposure to cycloheximid (protein synthesis inhibitor), revealed that the increase of AHH activity was significantly higher than that occurring after PAH induction without protein synthesis inhibitor (22). Further investigations showed that a so-called superinduction of AHH activity was associated with the accumulation of mRNA, specific for cytochrome P-450IA1, in the cell. That was preceded by the accelerated CYP1A1 gene transcription (57). The investigation revealed that the creation of the inductor – receptor protein complex, synthesized under the control of locus Ah was insufficient to obtain a maximum response expressed by a maximum period transcription of gene CYP1A1. It is assumed that two protein factors at least regulating transcription period of gene CYP1A1 exist in cells. The inductor – receptor protein complex stimulates the transcription process while a hitherto unknown protein factor inhibits the process (11).

Both protein factors regulating CYP1A1 gene transcription and affecting



the gene regulation zone are placed toward the end of 5' DNA chain from the start codon. In the isolated DNA fragment of genome 2.6 kilo base pair (kbp) long, preceding the start codon, three functional realms were found: the promoter, placed just before the start codon (towards the end of 5'); the inhibiting realm, 0.7–1.0 kbp distant from the promoter affected by the protein inhibiting factor and the realm activating CYP1A1 gene transcription after the attachment of inductor–receptor protein complex, 1.3–1.6 kbp distant from the promoter (57).

The detection of specific tissue differences as well as developmental differences in the expression of P-450 cytochrome genes in the P-450I family, indicated the presence of other proteins regulating the transcription activity of CYP1A1 and CYP1A2 genes (42).

Investigations into the polymorphism of transcription activation of CYP1A1 and CYP1A2 genes enjoy rather a long history. They were initiated in 1973 by Kellermann et al. (29). The authors tried to examine the range of AHH induction whose activity is associated with the expression of CYP1A1 gene.

The question as to how far the expression of the CYP1A2 gene is involved in measured AHH activity has not yet been answered. Observations have been made on human lymphocytes derived from tissue cultures and activated with mitogen. They revealed that in a population of 353 vegetarians under study, the range of AHH induction by 3-methylcholanthrene displayed a trimodal distribution. Three groups of individuals with slow, intermediate and fast AHH induction ability were distinguished. Suggestions on genetic control of AHH activity were later confirmed by Atlas et al. (3). The examinations were performed on lymphocytes derived from mono- and dizygotic twins. In monozygotic pairs, the range of AHH induction in lymphocytes was very similar, while in dizygotic pairs considerable discrepancies were noted.

Kellermann et al. (29) analysed in a subsequent study the range of AHH induction by 3-methylcholanthrene in lymphocytes sampled from persons with lung cancer, cancers of other organs and from healthy persons. The results of this examination demonstrated that persons with an intermediate phenotype of AHH activity are characterised by a risk of incidence of lung cancer 16 times greater and those with a fast phenotype of AHH activity 36 times greater than persons with a slow phenotype of AHH activity.

Although the application of lymphocytes in the analysis of AHH activity in humans encountered many methodological problems, numerous work have confirmed the cause-effect relationship between a fast phenotype of AHH induction and the increased incidence of lung cancer and cancer of other organs (37, 48, 54). However, several reports were issued which did not confirm the above-mentioned correlation. The trimodal distribution of induced AHH activity was also questioned (40). It should be stressed here that Kellermann et al. (29) based their hypothesis on a monogenic determination of AHH induction. As it appeared from later investigations into the value distribution of AHH induction in human populations, as well as from investigations into induction of molecular level (11), this phenomenon is characterised by multigenic determination.

In the individual human populations under study, a genetic polymorphism of AHH induction may occur in gene loci, both structural genes encoding the apoprotein of the cytochrome P-450I family and genes encoding proteins involved in the control of the transcription of those genes (11).

Applying the measurement of AHH activity in lymphocytes (stimulated or basic) as an index of individual PAH oxidation metabolism and related predisposition to pulmonary neoplasm, a number of research workers have called attention to the fact that the results of measuring this activity in lymphocytes derived from tissue cultures are incomparable. That is why Kellermann et al. (25, 28) applied the measurement of the antipyrine oxidation period to evaluate individual PAH oxidation. This drug is almost completely oxidised in the liver in the presence of three different cytochromes P-450. One of them is synthesized when controlled by a gene of the CYP1 family, and the other two when controlled by genes of the CYP2 sub-family (41, 44). Kellermann et al. (28) based the decision to adopt antipyrine as a marker drug (an index of PAH oxidation metabolism in the liver), on previous studies which had indicated a significant correlation ($r = 0.95$) between the antipyrine oxidation period (period of its elimination from the blood) and the range of AHH induction in lymphocytes sampled from the same persons in whom the antipyrine oxidation period had been determined (26, 27).

As was later confirmed by Breimar et al. (7), the period of antipyrine oxidation was mainly determined by the activity of cytochrome P-450 belonging to the P-450I family, hence those cytochromes P-450 which are present at PAH oxidation metabolism.

The question whether the range of AHH induction in the liver (assessed by the antipyrine test) is similar to the process in cells of other organs remains unanswered.

It is still not known whether conclusions on the interrelationship between activity and the incidence of respiratory cancer (of the upper airways) can be drawn from the assessment of liver oxidation enzyme activity. A similar comment can be made in regard to investigations into the relationship between AHH induction in lymphocytes and lung cancer and cancer of other organs. According to Pelkonen et al. (43), a superior cellular mechanism controlling the expression of genes of P-450I family may occur in human cells (also in animals). Recently, several papers have appeared suggesting that activation of cytochrome P-450 gene transcription is controlled, to a great extent, by the availability of heme derived from the pool of free cellular heme (45).

Ambre et al. (2) applied for the first time the antipyrine test in the study of oxidation metabolism of xenobiotics in the liver and its association with a predisposition to cancer of the upper airways. The authors determined antipyrine plasma clearance in persons with lung cancer and in persons with normal and pathological results of biochemical examinations and a liver function test. Patients with lung cancer and normal liver test results showed a higher antipyrine clearance rate and lower values of the biological half-life ($T_{1/2}$) of this drug, than patients with lung cancer and abnormal liver tests and healthy persons. Values of both antipyrine pharmacokinetic parameters in



persons with lung cancer and abnormal liver test results did not differ from the values observed in healthy persons.

In 1978, Kellermann et al. (28) suggested that the period of antipyrine regress in the blood (evaluation based on the determination of clearance rate or $T_{1/2}$) may constitute an indirect index of AHH activity and could be used for determining individual sensitivity to lung cancer. Investigations performed in lung cancer patients revealed that mean values $T_{1/2}$ AP in that group equal to 10.8 ± 36 hours within a range of between 3.8 and 20.6 hours were considerably lower than in healthy non-smokers (14.1 ± 2.3 hours within a range of between 7.7 and 18 hours).

The determination of the plasmatic clearance rate and antipyrine $T_{1/2}$ in healthy smokers revealed a significant increase in the clearance and shortening of the biological half-life of antipyrine. This observation proved that, in those persons, the activity of oxidative enzymes contributing to antipyrine metabolism increased in the liver. A similar increase of oxidative enzyme activity (determined as AHH activity) was found in lymphocytes of current smokers. It is assumed that the range of oxidative enzyme induction, defined as an increase of AHH activity, is caused by PAH contained in tobacco smoke and is genetically determined. Individuals responding to tobacco smoke with a considerable increase of AHH activity display a higher risk of cancer of the upper airways. A much smaller risk of such changes was observed in persons lacking, or with only a low increase of AHH activity (55).

Our own studies (34), in which the antipyrine test was applied as a direct index of AHH activity, showed that persons with larynx carcinoma displayed significantly lower values of antipyrine $T_{1/2}$ than non-smokers with other illnesses of the upper airways. The group of persons with larynx carcinoma was dominated by individuals with a fast oxidative phenotype of antipyrine; their number accounted for 47.7% of the whole population under study with larynx carcinoma. On the other hand, slow oxidants in this group constituted 15.9%. Among smokers suffering from other illnesses of the upper airways, intermediate oxidants predominated (43.7%), while fast and slow oxidants amounted to 37.5% and 18.8%, respectively. Another distribution of the antipyrine oxidation period was shown in non-smokers with other illnesses of the upper airways. Fast oxidants constituted 14.3%, slow 26.2% and intermediate 59.9%.

The studies provided evidence that antipyrine oxidants, namely persons displaying a special predisposition to neoplasm (according to suggestions made by Kellermann et al. 1978), undoubtedly constitute the highest percentage of persons with larynx carcinoma. A similar percentage (but slightly lower, however) was found in healthy smokers. This may support a hypothesis that persons of this group display a stronger predisposition to cancer of the upper airways.

When the antipyrine test was performed in persons with cancer of the urinary tract a distinctly low percentage of slow oxidants was found. It was twice as low as in the controls who smoked cigarettes (without the implications of the tumorigenic process) and almost three times lower than in the non-smoking control group.

From the review of the results of our own studies and the studies of other authors as to whether a relationship exists between the oxidative phenotype and increased incidence of cancer, it may be posited that belonging to the group of fast oxidants of antipyrine is one of the factors increasing the risk of cancer of the upper airways. In the case of urinary tract cancer, belonging to the group of fast oxidants does not seem to increase the risk although adherence to the group of slow oxidants may appear to be one of the reasons for a lower incidence of urinary tract cancer.

The evaluation of oxidative polymorphism related to cytochromes P-450 of the P-450IA sub-family has thus far been based on metabolic studies adopting, amongst others, antipyrine as a marker drug.

It is suggested now that the outcome of those studies be confirmed by the techniques of molecular biology, including restriction fragment length polymorphism analysis. Such studies have already been undertaken in relation to oxidative polymorphism associated with the transcriptive activation of genes encoding cytochromes of the P-450IIE family. Within this sub-family, at least two cytochromes P-450 have been distinguished and their synthesis induced by ethanol, imidazole, acetone, trichlorethylene and pirazole (52). The form of cytochrome P-450 displaying the largest range of induction, oxidizes aniline, alcohols and nitrozamine. The latter fact is of special significance since nitrozamines have for years been considered to be strong carcinogenic compounds. Preliminary observations revealed that the polymorphism in transcriptive activation of cytochrome P-450 genes of the P-450IIE family may have causal links with individual predisposition to certain types of cancer (50).

METABOLIC POLYMORPHISM OF CONSTITUTIVE EXPRESSION OF GENES OF CYTOCHROMES P-450

Studies of the debrisoquine oxidation period play a special part in the investigation into the metabolic polymorphism of constitutive expression of genes of cytochromes P-450 and in searching for an association between this process and cancer (58). This marker drug is oxidised in the liver in the presence of cytochrome P-450 into 4-hydroxylic derivative. Testing with the application of debrisoquine is relatively simple. The examinee was given *per os* 10 mg of debrisoquine (tablet) and then in a urine collection 8 hours later the concentrations of unchanged debrisoquine (D) and its 4-hydroxylic derivative (4-HD) were determined.

Individuals with a fast oxidation phenotype displayed a D/4-HD ratio below 1.9; in persons with an intermediate phenotype this ratio constituted 1.9–20.8 and those with a slow phenotype displayed a ratio over 20.8 (10).

The phenomenon of polymorphic 4-hydroxylation of debrisoquine was first described in 1977 by British research workers (36). Around the same time, German authors described the polymorphism of sparteine oxidation (9). Bearing in mind that the same cytochrome P-450 is present during the oxidation of debrisoquine and sparteine, some authors have referred to this as



the "debrisoquine" or "debrisoquine-sparteine polymorphism". Recent studies have revealed that two genes encoding the apoprotein of cytochrome P-450 in the P-450IID sub-family and one pseudogene are present in a human genome (13). CYP2D6 and CYP2D7 genes and the CYP2D8 pseudogene are placed in chromosome 22. They are contiguous and highly homologous in the sequence of nucleotides (13). It has not yet been decided whether both cytochromes P-450 (P-450IID6 and P-450IID7) contribute to the overall hydroxylation of debrisoquine and sparteine. For the time being, it is proposed that the debrisoquine-sparteine polymorphism be referred to as a genetic polymorphism of the CYP2D6 gene (51). Apart from the multiple genes encoding cytochrome P-450 in the P-450IID family, there are several mutant alleles in these genomic fragments which are responsible for the functional polymorphism. So far, the presence has been demonstrated of at least four mutations which may lead to the phenotype of debrisoquine slow oxidation (51). It is presumed that so-called slow oxidisers of debrisoquine display a recessive character. They constitute 5–10% of the human population and this index varies in different ethnic groups.

Such a phenotype did not arise only due to changes in the sequence of nucleotides contained in DNA fragments of the CYP2D6 gene of relevant exons, but also due to changes in the sequence of nucleotides producing introns. This results in the abnormal maturation of pre-mRNA for the P-450IID6 cytochrome (13). Because of that, additional nucleotides corresponding to intronic fragments of the DNA gene emerge, or a part of the nucleotides corresponding to exons is lost. The mRNA with inappropriate composition of nucleotides cannot be used as a matrix for the synthesis of the P-450IID6 apoprotein or may lead to synthesis of apoprotein with changed properties undetectable by immunological methods. It should be stressed that mutations of the nitrogen base in intronic fragments of the gene are not easily detected and they require the description of a complex sequence of the gene including exons, introns and flanking regions (21).

Restriction fragment length polymorphism analysis of genomic DNA, derived from slow and rapid oxidants of debrisoquine, revealed that the data obtained from the restriction analysis were not always consistent with metabolism based on the ratio of debrisoquine and its 4-hydroxylic derivative excreted through the urine (56). That requires a more thorough consideration of whether the distinction in the population under study of slow and rapid oxidants of debrisoquine based on metabolism was justified.

A potential relationship between the phenotype of debrisoquine oxidation (the expression of the CYP2D6 gene) and neoplasm has not, thus far, been formulated as a hypothesis, but is merely the outcome of an expanded research project seeking to reveal various aspects of the polymorphism of cytochrome P-450. A number of research workers have called attention to the fact that the cytochrome P-450IID sub-family, unlike the P-450IA sub-family, does not seem to contribute to the oxidative activation of potential carcinogens including those contained in tobacco smoke (59). In their opinion, searching for an association between the fast phenotype of debrisoquine oxidation and some



cancers is not fully justified. Responding to those comments Idle suggested (1989) that P-450IID6 is a considerably better dioxygenase (adds $-OOH$ to substrates) and that it is a monooxygenase (adds $-OH$ to substrate). However, it should be noticed here that, up till now, it has been neither confirmed nor denied that P-450IID6 contributes to the activation of carcinogens through dioxygenase.

The link between the debrisoquine oxidation phenotype and the incidence of cancer was first demonstrated by Idle et al. (20). They examined a group of Nigerians exposed to aflatoxin B_1 , a substance known for its carcinogenic properties, the incidence of liver cancer was higher in subjects displaying the rapid phenotype of debrisoquine oxidation. Initially this observation was confirmed by experimental studies. However, later studies refuted the relationship between debrisoquine oxidation phenotype and greater susceptibility to liver cancer. A predominating contribution of other factors unrelated to aflatoxin B_1 oxidation has been proved.

In 1984, Ayesh et al. (4) discussed the question of an association between debrisoquine oxidation phenotype and higher susceptibility to cancer, this time to lung cancer. The oxidation phenotype was defined in 479 persons, including 245 with lung cancer and 234 with obstructive lung diseases without implications of the tumorigenic process. All of the subjects had smoked in the past or were current smokers. Among those with lung cancer, those displaying the rapid phenotype of debrisoquine oxidation predominated, constituting 78.8%; those with the slow phenotype were 1.6% (4 persons). In the group with diseases without implications of the tumorigenic process, subjects with the rapid phenotype constituted 27.8% and those with the slow 9.0% (21 persons). According to Ayesh et al (4), the slow phenotype of debrisoquine oxidation decreases the risk of lung cancer in smokers. Similarly, a low percentage of subjects with the slow phenotype in a group of lung cancer patients was found by Law et al. (32). In their study of 104 persons with lung cancer, only two displayed the slow phenotype of debrisoquine oxidation.

A considerably lower percentage of slow oxidants among persons with bronchogenic carcinoma, although higher than in the British studies, was indicated by Roots et al. (46). Among 301 persons with bronchogenic carcinoma, slow oxidants constituted 7% (21 persons) while in the control group of 368 persons, slow oxidants constituted 10.9% (40 persons).

One interesting observation is that in a group of 38 bronchogenic carcinoma patients, none below 50 years of age displayed the slow phenotype of debrisoquine oxidation.

Analysing cancer of other organs, the same researchers found a significantly lower percentage of slow oxidants in the group of persons with gastric, larynx and bladder cancers. The comparison with groups of persons without the implications of the tumorigenic process is presented in Fig. 1. In the group of 184 persons with gastric cancer, the slow phenotype was displayed by 8 persons (4.2%). Remarkably low representation of slow oxidants was found in persons with gastric cancer of the intestinal type. Among the 90 subjects under study, only one person displayed the slow phenotype. In the case of the



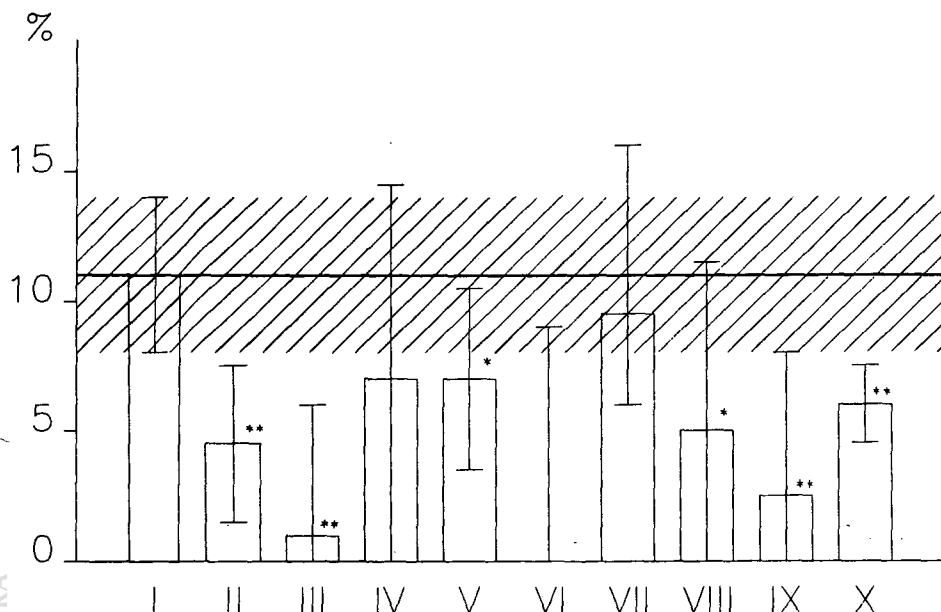
POOR
METABOLIZERS

Fig. 1. Percentage of slow oxidants in several selected types of cancer: I – reference group without implications of tumorigenic process; II – gastric cancer (n = 184); III – gastric cancer – intestinal type (n = 91); IV – gastric cancer – diffusive type (n = 74); V – bronchogenic carcinoma (n = 301); VI – bronchogenic carcinoma – persons over 50 years of age (n = 38); VII – pharynx cancer (n = 115); VIII – larynx cancer (n = 102); IX – bladder cancer (n = 82); X – total population under study with various types of cancer (n = 78). * – $p < 0.05$, ** – $p < 0.01$.

diffusive type of gastric cancer, 5 out of 69 persons displayed the slow phenotype. Among the remaining 17 examinees with mixed, or with unspecified gastric cancer, only two slow oxidants were found.

Bearing in mind the complexity of the genetic determination of cancer, Roots et al.(46) attempted to examine the frequency of the slow phenotype of debrisoquine oxidation in persons with bronchogenic carcinoma depending on their acetylation phenotypes. The studies showed that in the group of fast acetylators the percentage of slow oxidants was three times higher than in the group of slow acetylators. Gender appeared to be an important factor affecting the frequency of slow oxidants.

In females with bronchogenic carcinoma, the slow phenotype of debrisoquine oxidation was found only in fast acetylators. A reverse observation was made in males where slow oxidants were more common among slow acetylators (Fig. 2). The consequences of the differentiated susceptibility to neoplasm have not been clarified yet and further studies should be carried out.

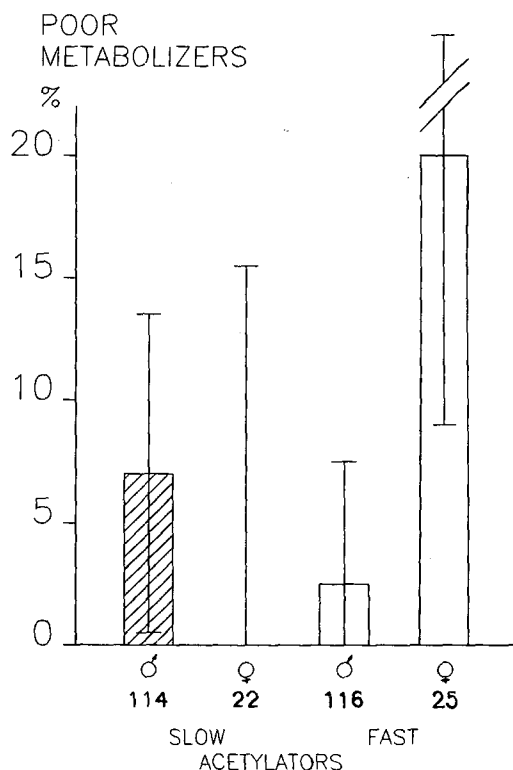


Fig. 2. Percentage of slow oxidants in the group of persons with bronchogenic carcinoma split according to sex and acetylation phenotypes.

Idle (19) suggests that, in the epidemiological survey, manifold causal factors should be searched for, due to the multifarious nature of lung cancer.

Therefore, if a relative risk associated only with the rapid phenotype constitutes in itself a risk of about 9, then, taking into account the histological sub-type of lung cancer and sex, in female fast oxidants suffering from microcellular lung cancer, the risk amounts to 22.4. In male fast oxidants exposed to PAH in the workplace, the relative risk increases to 39.2.

Apart from exposure to carcinogenic chemical factors in the workplace, a person's life-style, especially smoking increases the risk of cancer (53). Idle (19) suggests that in evaluating the risk of lung cancer, the carriers of HA-ras a 4 and GST-1 or GST1-2 alleles (GST-glutathion S transferase) should be also taken into consideration.

Co-occurrence of all the above mentioned factors in one person creates an especially high risk of lung cancer. In contrast, a non-smoker with a low phenotype, non-exposed to PAH and a carrier of GST1-0 and Ha-ras a 1, a 2 or a 3 alleles displays a very low risk of lung cancer.



REFERENCES

1. Adesnik M, Atchison M. Genes for cytochrome P-450 and their regulation. *CRC Crit Rev Biochem* 19: 247–305, 1986.
2. Ambre J, Graeff D, Bures F, Haupt D, Deason K. Antipyrine metabolism and bronchogenic carcinoma: *J Med* 8: 57–68, 1977.
3. Atlas SA, Vesell S, Nebert DW. Genetic control of interindividual variations in the inducibility of aryl hydrocarbon hydroxylase in cultured human lymphocytes. *Cancer Res*: 36, 4619–4630, 1976.
4. Ayesb R, Idle JR, Richtie JC, Crothers MJ, Hetzel MR. Metabolic oxidation phenotypes as markers for susceptibility to lung cancer. *Nature* 311: 169–170, 1984.
5. Boobis AR, Davies DS. Human's cytochrome P-450. *Xenobiotica* 14: 151–185, 1984.
6. Brown RR, Miller JA, Miller EG. The metabolism of methylated aminoazo dyes. 4. Dietary factors enhancing demethylation *in vitro*. *J Biol Chem* 209: 211–215, 1954.
7. Breimer DD, Schellens JHM, Soons PA. Assessment of *in vivo* oxidative drug metabolizing enzyme activity in man by applying a cocktail approach. Proceedings of the VII International Symposium on Microsomes and Drug Oxidations, pp. 1–9 Taylor and Francis, London, 1988.
8. Conney AH. Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons. GHA Clowes Memorial Lecture, *Cancer Res* 42: 4875–4917, 1982.
9. Eichelbaum M, Spannbrucker N, Steincke B, Dangler HJ. Defective N-oxidation of sparteine in man. A new pharmacogenetic defect. *Eur J Clin Pharmacol* 16: 183–187, 1979.
10. Gibson GG, Skett P. Introduction to drug metabolism. Chapman and Hall Ltd., pp. 55–84, London, New York, 1986.
11. Gonzalez FJ. The molecular biology of cytochrome P-450s. *Pharmacol Rev* 40: 243–288, 1989.
12. Gonzalez FJ, Jalswal AK, Nebert DW. P-450s: Evolution, regulation, and relationship to human cancer and pharmacogenetics. *Cold Spring Harbour Symp Quant Biol* 51: 879–884, 1987.
13. Gonzalez FJ, Skoda RC, Kimura S et al. Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature* 331: 442–446, 1988.
14. Greenlee WF, Neal RA. The Ah receptor. A biochemical and biological perspectives. In: *The Receptors*, Red M Conn, Academic Press, pp. 89–129 New York, 1985.
15. Guengerich FP, Umbenhauer DR, Churchill PF et al. Polymorphism of human cytochrome P-450. *Xenobiotica* 17: 311–316, 1987.



16. Hanke J, Indulski J. Biochemical indices of genetically determined predisposition to neoplasm. *Pol Tyg Lek* 42: 827–829, 1987 (in Polish).
17. Hanke J, Indulski J, Pawlak A. Genetic Issues in Toxicology and Occupational Medicine, pp. 102–105, PZWŁ, Warsaw, 1987 (in Polish).
18. Hildebrand CE, Gonzalez FJ, Mc Brode OW, Nebert DW. Assignment of the human 2,3,7,8-tetrachlorodibenzo-p-dioxin inducible cytochrome P-450 gene to chromosome 15. *Nucleic Acids Res* 13: 2009–2012, 1985.
19. Idle JR. Tobacco, host polymorphism and occupation as cooperative risk factors in human cancer. In: Consultation on genetic predisposition to toxic effects of chemicals, Krefeld, Federal Republic of Germany, 17–20 October, pp. 1–11, 1989.
20. Idle JR, Mahgoub A, Lancaster R, Smith RL. Some observations on the oxidation phenotype status of Nigerian patients presenting with cancer. *Cancer Lett* 11: 331–333, 1981.
21. Idle JR, Smith RL. The debrisoquine hydroxylation gene: a gene of multiple consequence. In: Proceedings: Second World Conference on Clinical Pharmacology and Therapeutics, eds: L Lemberger, Reidenberg M, Rockville, American Society of Pharmacology and Therapeutics, pp. 148–164, 1984.
22. Israel DI, Estolano MC, Galeazzi DR, Withlock JP. Superinduction of cytochrome P¹-450 gene transcription by inhibition of protein synthesis. *J Biol Chem* 260: 5648–5653, 1985.
23. Joanides C, Lum PY, Park DV. Cytochrome P-448 and the activation of toxic chemicals and carcinogens. *Xenobiotica* 14: 119–137, 1984.
24. Jones PBC, Galeazzi DR, Fisher JM, Withlock JP. Control of cytochrome P¹-450 gene expression by dioxin. *Science* 227: 1499–1502, 1985.
25. Kellermann G, Jett JR, Luyten-Kellermann M, Moses HL, Fontana RS. Variation of microsomal mixed function oxidase and human lung cancer. *Cancer* 45: 1438–1442, 1980.
26. Kellermann G, Luyten-Kellermann M, Horning MG, Stafford M. Elimination of antipyrine and benzo(a)pyrene metabolism in cultured human lymphocytes. *Clin Pharmacol Ther* 20: 72–78, 1976.
27. Kellermann G, Luyten-Kellermann M, Horning MG, Stafford M. Correlation of aryl hydrocarbon hydroxylase activity of human lymphocyte cultures and plasma elimination rates for antipyrine and phenylbutazone. *Drug Metab Dispos* 3: 47–52, 1975.
28. Kellermann G, Luyten-Kellermann M, Jett JR, Moses HL, Fontana RS. Aryl hydrocarbon hydroxylase in man and lung cancer. *Human Genet. Suppl* 1: 161–168, 1978.
29. Kellermann G, Luyten-Kellermann M, Shaw CR. Genetic variations of aryl hydrocarbon hydroxylase in human lymphocytes. *Am J Hum Genet* 25: 327–331, 1973.
30. Kellermann G, Shaw CR, Luyten-Kellermann M. Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *N Engl J Med* 298: 934–937, 1973.



31. Knudson AG. Hereditary cancer, oncogenes, and antioncogenes. *Cancer Res* 45: 143–143, 1985.
32. Law M, Hetzel MR, Idle JR. Debrisoquine metabolism and genetic predisposition to lung cancer. *Br J Cancer* 59: 686–687, 1989.
33. Lutz W. Microsomal cytochromes P-450 of liver cells and industrial and environmental xenobiotics. *Post Hig Med Dośw* 38: 451–480, 1984 (in Polish).
34. Lutz W, Sułkowski W, Jakubik C, Staniewicz A. Oxidative phenotype as a marker of susceptibility to larynx carcinoma. *Pol J Occup Med* 1: 312–321, 1988.
35. Lutz W, Szeszko A, Stankiewicz A. Does cytochrome P-450 — interrelated system of microsomal mono-oxygenases — protect an organism against toxicity of xenobiotics? *Pol Tyg Lek* 43: 795–798, 1988 (in Polish).
36. Mahgoub A, Idle JR, Dzings LC, Lancaster R, Smith RL. Polymorphic hydroxylation of debrisoquine in man. *Lancet* 1: 548–586, 1977.
37. Mc Lemoire TL, Martin R, Wray NP, Cantrell ET, Busbee DL. Reassessment of the relationship between aryl hydrocarbon hydroxylase and lung cancer, *Cancer* 48: 1438–1443, 1981.
38. Miller JA. Carcinogenesis by chemicals. An overview. *GHA Clowes Memorial Lecture, Cancer Res* 30: 559–576, 1970.
39. Nebert DW, Adesnik M, Coon MJ et al. The P-450 gene superfamily: Recommended nomenclature, *DNA* 6: 1–11, 1987.
40. Paigen B, Gurtsoo HL, Minowada J et al. Questionable relation of aryl hydrocarbon hydroxylase to lung cancer risk. *N Engl J Med* 297: 346–348, 1981.
41. Paulsen HE, Loft S. Antipyrine as a model drug to study hepatic drug-metabolizing capacity. *J Hepatol* 6: 374–382, 1988.
42. Pelkonen O, Lang M. Genetics of enzyme induction. In: *Enzyme Induction in Man*, eds EA Sotaniemi, O Pelkonen, Taylor and Francis, London, pp. 1–15, 1987.
43. Pelkonen O, Vahakangas K, Karki NT, Sotaniemi EA. Genetic and environmental regulation of aryl hydrocarbon hydroxylase in man: Studies with liver, lung, placenta and lymphocytes. *Toxicol Pathol* 12: 256–260, 1984.
44. Penno MB, Vesell ES. Monogenic control of variations in antipyrine metabolite formation. *J Clin Invest* 71: 1698–1709, 1983.
45. Ravishankar H, Padmanaban C. Regulation of cytochrome P-450 gene expression. Studies with a cloned probe. *J Biol Chem* 260: 1588–1592, 1985.
46. Roots I, Drakoulis N, Ploch M et al. Debrisoquine hydroxylation phenotype, acetylation phenotype, and ABO blood groups as genetic host factors of lung cancer risk. *Klin Wochenschrift* 66 (suppl XI): 87–97, 1988.



47. Ryan DE, Thomas PE, Levin W. Hepatic microsomal cytochrome from rats treated with izosafrole. *J Biol Chem* 255: 7941–7945, 1980.
48. Sartoris A, Cortesina G, Vitiello R et al. L'importanza della aryl hydrocarbon hydroxylase nella oncogenesi laringea. Dati preliminari. *Acta Otorhinol Ital* 5: 579–587, 1985.
49. Schulte PA, Halperin WE. Genetic screening and monitoring for workers. In: *Recent Advance in Occupational Health*, ed. JM. Harrington, Churchill Livingstone, Edinburgh, London, Melbourne, New York, pp. 135–154, 1987.
50. Seidergard J, Vorachek WR, Pero RW, Pearson WR. Hereditary differences in the expression of the human glutathione transferase active on transtilbene oxide are due to a gene deletion. *Proc Natl Acad Sci USA* 85: 7293–7297, 1988.
51. Skoda RC, Gonzalez FJ, Demierre A, Meyer UA. Two mutant alleles of the human cytochrome P-450 db1 gene (P-450C2D1) associated with genetically deficient metabolism of debrisoquine and other drugs. *Proc Natl Acad Sci USA* 85: 5240–5243, 1988.
52. Song BJ, Gelboin HV, Park SS, Yang CS, Gonzalez FJ. Complementary DNA and protein sequences of ethanol-inducible rat and human cytochrome P-450. *J Biol Chem* 261: 16689–16693, 1988.
53. Steenland K. Interaction between tobacco smoking and occupational exposures in the causation of lung cancer. *J Occup Med* 28: 110–118, 1986.
54. Trell E, Biorling G, Andreasson I, Korsgaard R, Mattiasson I. Carcinoma of the oral cavity in relation to aryl hydrocarbon hydrocarboxylase inducibility smoking and dental status. *Int J Oral Surg* 10: 93–99, 1981.
55. Vahakangas K, Pasanen M, Sotaniemi E, Pelkonen O. Drug metabolism in man: Life style factors: Smoking and alcohol. In: *Enzyme Induction in Man* eds: ES Sotaniemi, O Pelkonen, Taylor and Francis, London, pp. 1–10, 1987.
56. Yue OY, Bertilsson L, Dahl-Punsten ML, Sawe J, Sjoqvist F, Johansson I, Ingelman-Sundberg M. Dissociation between debrisoquine hydroxylation phenotype and genotype among Chinese. *Lancet* 2: 870, 1989.
57. Whitlock JP. The regulation of cytochrome P-450 gene expression. *Ann Rev Pharmacol Toxicol* 26: 333–369, 1986.
58. Wolf CR. Cytochrome P-450s: Polymorphic multigene families involved in carcinogen activation. *Trends Genet* 2: 209–236, 1988.
59. Zanger UM, Vilbois F, Hardwick JP, Mayer UA. Absence of hepatic cytochrome P-450 bufl1 causes genetically deficient debrisoquine oxidation in man. *Biochemistry* 27: 5447–5454, 1988.

Received for publication: 11th June, 1990.

Accepted for publication: 28th August, 1990.

