DRUG METABOLISM

Molecular Approaches and Pharmacological Implications

GÉRARD SIEST

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Proceedings of the 9th European Workshop on Drug Metabolism held at Pont-à-Mousson, 11–15 June 1984

Editor

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PREFACE

A workshop is an opportunity for practicing science
an opportunity for discussion, for up-dating knowledge,
an opportunity to learn and to be stimultated by others.
These aims were achieved during the Ninth European Drug Metabolism Workshop, by way of eight symposia and sessions, three round table discussions and four poster sessions.

A workshop is an opportunity for friendship
450 scientists from 40 countries were gathered together for a week in the cloisters of the Abbaye des Prémontrés, Pont-à-Mousson, from June 11 to June 15, 1984.

We extend our warm thanks to the members of the Scientific Committee (D.D.BREIMER, J.W.BRIDGES, J.CALDWELL, S.GARATTINI, J.E.GIELEN, J.P.LEROUX, D.MANSUY, F.OESCH, H.SIES, B.TESTA, V.ULLRICH) who worked together with the staff of the Centre du Médicament at the University of Nancy.

Following this workshop, several participants requested a written copy and thus this book was brought about. The lectures were arranged in five chapters :

- Pharmacogenetics and metabolism in man
- Metabolism and toxicity
- Regulation at molecular, cellular and tissular levels
- Biotechology and new approaches
- Chemical structures and metabolic reactivity

We are most grateful to the authors for having prepared their papers promptly, thus ensuring rapid publication. Our thanks also go to Professor Marie Madeleine GALTEAU for her editorial work.

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PHARMACOGENETICS AND METABOLISM IN MAN

N-ACETYLATION PHARMACOGENETICS: NOTES AND COMMENTS ON ACETYLATOR STATUS, DRUG TOXICITY AND DISEASE

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ABSTRACT

Dose-dependent toxicities to arylamine and hydrazine drugs and other environmental chemicals are usually more common and more severe in genetically slow acetylators. Isoniazid-induced hepatitis and urinary bladder cancer resulting from occupational exposure to arylamines, such as beta-naphthylamine, are two disorders of particular interest because they illustrate how the acetylation polymorphism can alter the metabolic fate of these chemicals in more diverse and complex ways than is readily predicted. Pharmacogenetic studies in man and in animal models for the human acetylation polymorphism indicate that N-acetyl transfer occurs twice in the metabolism of isoniazid and of 2-aminofluorene. Both steps are subject to control by the hereditary polymorphism and both contribute to individual differences in toxicity from these chemicals.

KEYWORDS

Pharmacogenetics; N-acetylation; acetylator status; drug toxicity; arylamines; hydrazines; isoniazid-induced hepatitis; arylamine-induced bladder cancer; metabolic activation.

INTRODUCTION

The N-acetylating capacity of humans is essentially constant for an individual, but it varies remarkably from one person to another. The hereditary basis for these metabolic differences was elucidated some twenty years ago. This trait was among the first human pharmacogenetic traits to be identified. It was initially called the "Isoniazid Acetylation Polymorphism" because it was recognized in tuberculosis patients treated with isoniazid (INH), a new drug at that time. However, it is usually referred to now simply as the acetylation polymorphism since it affects the metabolism of many other drugs and environmental chemicals. Because of this trait, individuals can be identified as "rapid" or "slow" acetylators of these substances, and the acetylating capacity of human populations is bimodally distributed.

The acetylator status has pronounced effects on the metabolic fate of drugs such as INH, hydralazine, procainamide, dapsone, aminoglutethimide and various sulfonamides. It modulates the metabolism of some carcinogenic arylamines such as benzidine and beta-naphthylamine, also. In addition, the acetylator status can influence the fate of drugs which do not originally possess a free amino group, but may have one introduced metabolically. Sulfasalazine, nitrazepam, clonazepam and caffeine are examples of the latter drugs.

Biological differences in acetylating capacity have remarkable effects on the pharmacologic and toxicologic profiles of arylamines and hydrazines. The neurotoxicities which result from INH, the lupus erythematoses from hydralazine or procainamide, sulfasalazine-induced toxicity, and phenytoin toxicity associated with the interaction of phenytoin and INH are familiar, well-characterized disorders related to intake of some of these drugs. They are textbook examples of pharmacogenetic interest which illustrate the significance of acetylator status to clinical medicine. In these conditions, genetically slow acetylators usually have higher serum concentrations of the drug for any specified period of time after drug ingestion than rapid acetylators. As would be anticipated, these toxicities are dose-dependent, and there is general agreement that they are more common and more severe among slow acetylators.

Expression of a peculiar response to a drug or another environmental chemical is usually a highly complex event. It is likely to be a consequence of more than one characteristic of the host, and to be affected appreciably by extrinsic factors, too. It is influenced by the drug or chemical as well. Thus, an assessment of the contribution of any individual factor (such as acetylator status) to the toxic or therapeutic outcome is difficult, unless of course the factor has an overwhelming influence. For some disorders associated with prolonged intake of arylamine and hydrazine drugs, or with occupational exposure to similar chemical hazards, the relationship to acetylator status which is emerging is controversial. In some other conditions of interest, an association with acetylator status

affords a convenient and rational explanation for the peculiarity, but evidence is yet inadequate to establish a firm connection. Disorders for which the acetylator status has been claimed to be a predisposing element include INH-induced hepatitis, arylamine-induced urinary bladder cancer, spontaneous (idiopathic) lupus erythematosus, phenelzine toxicity, and hemolysis induced by sulfones and sulfonamides in glucose-6-phosphate deficiency. Isolated or anecdotal reports of a dependence on acetylator status have also appeared for several other diverse conditions. These include diabetes (Types I and II), breast cancer in women, INH-related induction of defluorination of flurane general anesthetics, INH-related perturbation of vitamin D metabolism, and Gilbert's syndrome (mild chronic unconjugated hyperbilirubinemia). A comprehensive review of these investigations is beyond the scope of the present discussion, but this has been presented elsewhere recently (Weber & Hein, 1984).

The toxicity of INH to liver, and of urinary bladder cancer from occupational exposure to arylamines such as beta-naphthylamine and benzidine, are of long standing interest to clinical, epidemiological and basic pharmacological investigators. These disorders are of particular interest within the context of this symposium because they illustrate how the metabolic fate of an arylamine or a hydrazine can be altered by a single genetic polymorphism in more diverse and complex ways than might be predicted. Thus, as a direct result of pharmacologic studies in man and in genetic animal models for the human acetylation polymorphism, we know that hereditary differences in the capacity of N-acetyl transfer act not only initially to convert INH, and arylamine carcinogens such as 2-aminofluorene (AF) to acetylated metabolites, but act again at later stages in their metabolism (Figure 1). In each instance, both steps are subject to the acetylation polymorphism. Furthermore, both steps appear to contribute significantly to the influence of acetylator status on toxicity, but to an extent not yet fully understood.

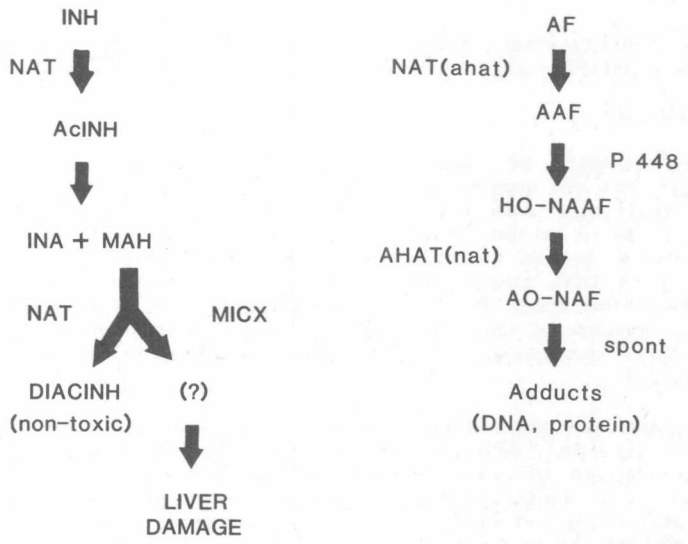

Fig. 1. Schematic diagram of the metabolic activation of isoniazid and 2-aminofluorene

INH-induced Hepatitis

This disorder has presented a challenging problem to clinical pharmacologists that has been investigated and debated at length for the past decade. Initially, Mitchell and coworkers suggested from epidemiological studies that INH might be more hepatotoxic for rapid than for slow acetylators (1976). They reasoned that rapid acetylators would produce monoacetylhydrazine (MAH) more rapidly from ingested INH than slow acetylators. They further suggested that MAH produced in man would be converted in greater amounts in rapid than in slow acetylators to potent reactive electrophiles that would bind covalently to hepatic tissue initiating necrosis. This hypothesis was questioned immediately by Ellard and coworkers on the grounds that MAH, like INH, was polymorphically acetylated because they had observed that rapid acetylators converted MAH to the relatively non-toxic metabolite, diacetylhydrazine (DIACINH), much more rapidly (more than 4 times faster) than slow acetylators. Thus, Ellard proposed that rapid acetylators should not be more susceptible to INH-induced liver damage because exposure to MAH was not dissimilar in the two phenotypes. Ellard's observations on MAH formation have received additional support from the human studies of Timbrell et al. (1977) and from our biochemical studies in the genetic rabbit model for the human acetylation polymorphism (Hein & Weber, 1982).

Beginning in the early 1970's, however, the role of acetylator status in INH hepatotoxicity had been examined in more than a dozen clinical studies, but the epidemiological data reported was complex and often conflicting. While some of the reports found rapid acetylators more susceptible, others found to the contrary that slow acetylators were more susceptible, and still others found no difference in susceptibility between the two acetylator phenotypes. Recently, Gurumurthy et al. (1984) have reported the results of a retrospective analysis summarizing the experience of the past 20 years in a series of controlled clinical trials at the Tuberculosis Research Center, Madras on the incidence of hepatitis accompanied by jaundice for 3000 patients treated with INH alone or in combination with other antituberculosis drugs. The overall incidence of liver damage measured in terms of clinically manifest jaundice among 1757 slow acetylators was 1.9% compared to 1.2% among 1238 rapid acetylators. The difference was not significant and Gurumurthy et al. concluded that the incidence of hepatotoxicity accompanied by jaundice was unrelated to acetylator status.

However, the analysis of the genetic basis for individual susceptibility to liver damage from INH has been carried farther. In a recent prospective study of the problem, Dickinson and coworkers (1981) described three distinct levels of risk: taking account of age among other risk factors, they found that slow acetylators older than 35 years have a very high risk (37%) compared to a moderate risk (13%) for rapid acetylators. They concluded that the risk vs benefit of INH therapy should be most carefully weighed in this readily identifiable group. Slow acetylators younger than 35 also were found to have a moderate risk (13%), and rapid acetylators younger than 35 have a relatively low risk (4%) of developing significant liver abnormality. In another recent study, Musch et al. (1982) found that the risk of hepatotoxicity measured by transaminase elevations was significantly greater among slow acetylators (46.6%) than among rapid acetylators (13.3%). Furthermore, Musch and colleagues found that the most severe signs of hepatotoxicity were confined to slow acetylators.

The observations of Gurumurthy et al. and of Dickinson et al. together with those of Musch et al. indicate that rapid acetylators are not especially predisposed to INH-induced liver disease; indeed, the opposite appears more likely. Their results provide a further point of importance - that the significance of acetylator status as a predictor of risk to this disorder is highly dependent on the index of liver damage chosen. That is, if one choses clinical jaundice as Gurumurthy et al. have done, "liver damage" is unrelated to acetylator status. However, if serum transaminase elevations are chosen, as the other investigators have done, "liver damage" is found to be dependent upon acetylator status. Clearly, failure to define such terms adequately may lead to loss of valuable information if not invalidation of conclusions reached.

Arylamine-induced Urinary Bladder Cancer

Occupational exposure to arylamines has been associated with the development of urinary bladder cancer for many years. Extensive studies in many laboratories have clearly demonstrated that metabolic factors are key determinants of species and tissue susceptibility to arylamine carcinogenesis. These studies have gone far toward identifying not only the metabolic pathways which characterize this process in different tissues, but also in determining the nature of the ultimate carcinogen(s) produced. Chief among the various enzymatic reactions which contribute to this process are N-hydroxylation and conjugation. N-Acetylation and N-deacetylation appear to be important modulators of this process also. Other reactions such as ring hydroxylation result in the production of less carcinogenic products.

Perhaps our knowledge of arylamine metabolism as it relates to metabolic activation of these chemicals is yet incomplete, but a consensus appears to have reached about the main events and the overall scheme of the activation process. Based upon a vast amount of information, Lower (1982) has recently presented a concise summary of the conceptual foundation of this process as it may occur in several target tissues. Thus, activation in liver and mammary gland vs that in urinary bladder is seen as proceeding by separate but interrelated pathways. One of these pathways results in the formation of the hepatocarcinogen, and possibly also the mammary carcinogen, while the other results in the formation of the bladder carcinogen. In the former pathway, an arylamine is N-acetylated by cytosolic, liver N-acetyltransferase (NAT). This is followed by enzymatic N-hydroxylation by a microsomal P1-450 (P-448) mixed function oxidase. The hydroxamic acid that is produced can be conjugated with sulfate to yield a potent hepatic carcinogen. It can be deacetylated by the arylhydroxamic acid acyltransferase (AHAT), which has been implicated in both liver and mammary gland cancer, to a reactive N-acetyoxyarylamine (AO-NAF in Fig. 1) that can react with nucleic acid in these target tissues. In the latter pathway, microsomal N-hydroxylation is followed by glucuronide conjugation to form the hydroxylamine-N-glucuronide, which breaks down spontaneously in the bladder to a reactive arylnitrenium ion that combines covalently with DNA.

It is now apparent that enzymatic acetyl transfer occurs twice in the arylamine activation process at points separated by N-hydroxylation (Fig. 1). Biochemical and genetic studies

performed in the rabbit model of the acetylation polymorphism strongly suggest that the acetyltransferase and the arylhydroxamic acid acyltransferase which catalyze these steps are either closely linked, or are identical enzymes (Glowinski et al., 1980). The question then is not whether acetyl transfer is implicated in the metabolic activation of carcinogenic arylamines, but whether these metabolic steps are sufficiently critical to affect the outcome in man.

The epidemiology of human arylamine-induced urinary bladder cancer is practially synonymous with that of industrial bladder cancer. The origins of epidemiological investigations can be accurately placed as starting with the report of Rehn, a German physician who reported in 1895 the unusual occurrence of urinary bladder cancer among fuchsin dye workers, and traced to the classic epidemiological investigation reported by Case and his coworkers in 1954. The study by Case et al. greatly advanced knowledge of occupational bladder cancer by showing that the risks of contracting this disorder were 30 times as great for the exposed dyestuff worker as for the general population. The latency period for bladder cancer development averaged 18 years, but varied widely from less than 5 years to more than 45 years. However, evidence that acetylator status may be a determinant of this disorder has only been accumulating for about ten years.

Six studies which provide information on acetylator status in bladder cancer patients have been reported. In the first of these, Wolf et al. (1980) examined the acetylator phenotype on a low risk population of urban bladder cancer patients in Denmark. They found an 13% excess ($p = 0.065$) of slow acetylators (46/71 = 64.8%) compared to a control population. This suggested that slow acetylators might be slightly more susceptible (odds ratio = 1.74) than rapid acetylators. Evans et al. (1983) examined the association of acetylator status to bladder cancer in a low risk English population and found a significant association with slow acetylation. However, studies of four other low risk populations failed to observe significant trends (Lower et al., 1979; Mommsen et al., 1982; Miller & Cosgriff, 1983; Woodhouse et al., 1982). Of particular interest is the study of Cartwright et al. (1982), who extended this work to high risk populations. These investigators have described a sample of 23 persons in Yorkshire, England, with documented exposure to benzidine from employment as chemical dye workers. A 40% excess ($p = 0.00005$) of slow acetylators was observed in this group (22/23 = 96%) compared to controls, a highly significant increase in susceptibility (odds ratio = 16.7) of slow acetylators to arylamine-induced bladder cancer. By further stratification of their data according to standard pathological criteria for classification of bladder tumors, Cartwright and coworkers also found an excess of slow acetylators among those with more invasive forms of bladder cancer. Mommsen et al. (1982) also observed that a preponderance of their patients with invasive disease were slow acetylators.

These data indicate a slight tendency toward an excess of slow acetylators among urban (low risk) bladder cancer populations although it does not always attain statistical significance. A larger and significant excess of slow acetylators appears to occur among bladder cancer populations exposed to specific arylamines. The latter conclusion, however, must be accepted cautiously since it rests on a single study and should be confirmed. Nevertheless, the observations as a whole are sufficiently provocative as to warrant further inquiry into the role of acetylator status as a susceptibility factor to human arylamine-induced cancer.

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