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COMMENTARY

PHARMACOGENETICS

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DEFINITIONS AND HISTORY

Pharmacogenetics deals with those variations in response to drugs that are under hereditary control. As applied to man, pharmacogenetics is a relatively new field; not until the 1950's were sufficiently large populations of human subjects exposed to enough therapeutic agents to establish that certain genetic constitutions are exceedingly susceptible to drugs. Although the initial examples in man concerned drug sensitivity, more recently discovered pharmacogenetic entities include drug resistance. In 1957, Motulsky [1] emphasized that certain forms of drug toxicity in man result from hereditary disorders manifested by structural alterations in specific proteins that degrade drugs or their metabolites. Vogel [2] coined the term "pharmacogenetics" in 1959, and Kalow [3] wrote a seminal monograph on the subject in 1962. Several reviews on various aspects of the discipline have been published recently [4–7].

With respect to experimental studies in laboratory animals, pharmacologists have long been aware of genetically controlled variability among strains and species in response to drugs [8–13]. However, until recently, pharmacologists focused their attention mainly on the extensive range of environmental factors responsible for altered reactions to experimental agents. A key theme in research on variations in drug response has been the attempt to separate genetic from environmental factors. In many instances such separation is possible, but differences in drug response can be caused by environmental factors superimposed upon genetic factors. In considering the distinction between genetic and environmental factors, one should recall that the most celebrated concept of genetic control, as developed by Jacob and Monod [14, 15], specifies an interaction of a genetic product with another compound, presumably environmentally controlled, to produce a new substance that regulates the activity of a structural gene by binding to an adjacent operator gene. It is important to emphasize that variations in drug response, whether controlled by genes, environment, or both, can occur at sites of absorption, distribution, protein binding, target receptors and excretion, as well as at locations of drug biotransformation in hepatic and extrahepatic locations. Furthermore, variations in drug response can arise from genetic or environmental factors operating independently on several of these discrete processes.

PHARMACOGENETIC VARIANTS EXHIBITING MENDELIAN INHERITANCE

Genetic differences due to the action of a single mutant gene responsible for the production of a single aberrant protein affect the disposition of and the response to certain drugs. In man, the 13 entities described in Table 1 show the relatively few instances presently known in which a particular gene acting at a single locus on a chromosome produced an abnormal drug response.

Each of the pharmacogenetic entities shown in Table 1 behaves as a single factor probably resulting from a single point mutation affecting a single protein, the gene product. This aberrant gene is transmitted in classical Mendelian fashion. At some of these loci multiple mutant genes, called alleles, have been detected. The pharmacogenetic entities listed in Table 1 are inborn errors of metabolism affecting proteins intimately involved in drug metabolism or response. Several are extremely rare, like most inborn errors of metabolism; bishydroxycoumarin sensitivity, deficient parahydroxylation of diphenylhydantoin, and warfarin resistance have been reported thus far in only one or two families. However, more extensive population screening could reveal that these pharmacogenetic conditions are much more common than currently believed. In any event, other conditions listed in Table 1, such as G-6-PD deficiency and slow inactivation of isoniazid, occur commonly; in certain populations the gene frequency of the "aberrant" allele is higher than that of the "normal" allele [16]. Problems of drug toxicity arise when an individual with a mutant enzyme receives only a single type of drug. However, in G-6-PD deficiency, hemolysis follows the administration of many commonly used drugs. In the mutant form encountered most frequently in the United States, the resultant hemolysis is mild and self-limited, even in the face of continued drug administration.

Conditions listed in Table 1 probably do not constitute a major threat of drug toxicity to many individuals. However, since the early 1950's, it has been recognized that large inter-individual differences exist in rates of plasma decay of commonly used drugs. For example, plasma clearance of such commonly used drugs as phenylbutazone [17], antipyrine [18] and bishydroxycoumarin [19] exhibits 3- to 10-fold variations among individuals. The magnitude of the inter-individual differences in pharmacokinetics, observed after

Table 1. Pharmacogenetic conditions with putative aberrant enzyme, mode of inheritance, frequency, and drugs that can elicit the signs and symptoms of the disorder

Name of condition	Aberrant enzyme and location	Mode of inheritance	Frequency	Drugs that produce the abnormal response
Genetic conditions probably transmitted as single factors altering the way the body acts on drugs (altered drug metabolism)				
Acatalasia	Catalase in erythrocytes	Autosomal recessive	Mainly in Japan and Switzerland, reaching 1 per cent in certain small areas of Japan	Hydrogen peroxide
Slow inactivation of isoniazid	Isoniazid acetylase in liver	Autosomal recessive	Approximately 50 per cent of U.S.A. population	Isoniazid, sulfamethazine, sulfamaprine, phenelzine, dapsone, hydralazine, procainamide
Suxamethonium sensitivity or atypical pseudocholinesterase	Pseudocholinesterase in plasma	Autosomal recessive	Several aberrant alleles; most common disorder occurs 1 in 2500	Suxamethonium or succinylcholine
Diphenylhydantoin toxicity due to deficient parahydroxylation	? Mixed function oxidase in liver microsomes that parahydroxylates diphenylhydantoin	Autosomal or X-linked dominant	Only one small pedigree	Diphenylhydantoin
Bishydroxycoumarin sensitivity	? Mixed function oxidase in liver microsomes that hydroxylates bishydroxycoumarin	Unknown	Only one small pedigree	Bishydroxycoumarin
Acetophenetidin-induced methemoglobinemia	? Mixed function oxidase in liver microsomes that de-ethylates acetophenetidin	Autosomal recessive	Only one small pedigree	Acetophenetidin
Genetic conditions probably transmitted as single factors altering the way drugs act on the body				
Warfarin resistance	? Altered receptor or enzyme in liver with increased affinity for vitamin K	Autosomal dominant	Two large pedigrees	Warfarin
Glucose 6-phosphate dehydrogenase deficiency, favism or drug-induced hemolytic anemia	Glucose 6-phosphate dehydrogenase	X-linked incomplete codominant	Approximately 100,000,000 affected in world; occurs in high frequency where malaria is endemic; 80 biochemically distinct mutations	A variety of analgesics [acetanilide, acetylsalicylic acid, acetophenetidin (phenacetin), antipyrine, aminopyrine (Pyramidon)], sulfonamides and sulfones [sulfanilamide, sulfapyridine, N_2-acetylsulfanilamide, sulfacetamide sulfisoxazole (Gantrisin), thiazolsulfone, salicylazosulfapyridine (Azulfidine), sulfoxone, sulfamethoxypyridazine (Kynex)], antimalarials [primaquine, pamaquine, pentaquine, quinacrine (Atabrine)], non-sulfonamide antibacterial agents [furazolidone, nitrofurantoin (Furadantin), chloramphenicol, *p*-aminosalicylic acid], and miscellaneous drugs [naphthalene, vitamin K probenecid, trinitrotoluene, methylene blue, dimercaprol (BAL), phenylhydrazine, quinine, quinidine]
Drug-sensitive hemoglobins Hemoglobin Zurich	Arginine substitution for histidine at the 63rd position of the β-chain of hemoglobin	Autosomal dominant	Two small pedigrees	Sulfonamides
Hemoglobin H	Hemoglobin composed of four β-chains	Autosomal recessive	Approximately 1 in 300 births in Bangkok	Same drugs as listed above for G-6-PD deficiency
Inability to taste phenylthiourea or phenylthiocarbamide	Unknown	Autosomal recessive	Approximately 30 per cent of Caucasians	Drugs containing the N- C=S group such as phenylthiourea, methyl and propylthiouracil
Glaucoma due to abnormal response of intraocular pressure to steroids	Unknown	Autosomal recessive	Approximately 5 per cent of U.S.A. population	Corticosteroids
Malignant hyperthermia with muscular rigidity	Unknown	Autosomal dominant	Approximately 1 in 20,000 anesthesized patients	Various anesthetics, especially halothane
Methemoglobin reductase deficiency	Methemoglobin reductase	Autosomal recessive heterozygous carriers affected	Approximately 1 in 100 are heterozygous carriers	Same drugs as listed above for G-6-PD deficiency

administration either of a single oral dose of a drug or of multiple doses when steady state drug concentrations are attained, far exceeds the normal ranges for many routine laboratory values. These large inter-individual variations do constitute a major therapeutic danger to certain patients in a population receiving drugs on dosage schedules based almost exclusively on body wt. For example, the patient who clears a drug rapidly from the body would require more of the drug to achieve a therapeutic effect than the patient of intermediate clearance capacity. However, toxicity could result from the usual dose of a drug given to a patient with little ability to remove the drug. After chronic administration of a drug to this individual, accumulation of the drug occurs, the extent of accumulation being determined by the metabolic clearance rate of the drug. To avoid extremes of drug toxocity and failure to derive therapeutic benefit, individualization of drug doses by measurement of drug concentrations in the body has been advocated [20]. However, before such blood drug measurements would be practical, it is necessary to establish for each compound those blood concentrations that are ineffective, therapeutic and toxic both in normal subjects and in patients with disease states that might alter the kinetics of interaction between the drug and receptor sites. It was previously assumed in discussion of this subject that receptor sites show little inter-individual variation not only in health but also in disease; the latter portion of the assumption is now being challenged [20]. Measurements of drug concentrations in blood are highly desirable theoretically and in some cases have been demonstrated to be useful therapeutically; no substitute for them is now generally available. However, there are several potential pitfalls in their interpretation [20].

CONTINUOUS VARIABILITY AND POLYGENIC INHERITANCE

Certain measured traits, such as intelligence quotient, blood pressure and height, come under the heading of continuous variability. Such variability can be produced by both genetic and environmental factors. Hereditary control over the trait in question is maintained by genes situated at two or more different positions (loci) on a chromosome or chromosomes. When populations are tested for a trait controlled by genes at multiple loci, the resulting distribution curve is unimodal and Gaussian. The relative contributions of genetic and environmental factors to continuous variability can be determined from analyses of twins, families or even populations.

In contrast to the unimodal distribution curves produced by multiple factors, discontinuous curves of population response to a drug are obtained from disorders transmitted as Mendelian dominants or recessives. These discontinuous curves, produced by genes at a single locus on the chromosome, are more easily analyzed because each discrete portion of the curve generally corresponds to a different phenotype. In other words, the different genes present at a single genetic locus and their corresponding phenotypes segregate both in the pedigree and in the distribution curve. All the pharmacogenetic conditions listed in Table 1 yield polymodal, discontinuous distribution curves.

One practical reason for distinguishing between the relative contributions of genetic and environmental influence over variation is that environmental factors can often be controlled, whereas genetically controlled components of variation are more stable, reproducible and hence more easily taken into account in the administration of drugs. Much drug therapy today remains ineffective or only partially effective because of our failure to consider genetic mechanisms that are intimately involved in inter-individual variations in response to drugs.

INTER-INDIVIDUAL VARIATIONS IN RATE OF DRUG ELIMINATION FROM HUMAN PLASMA

In addition to genetic factors, numerous environmental factors also contribute to unimodal distribution curves produced when large populations are examined for characters or traits controlled by multifactorial mechanisms. The response of rodents to drugs can be altered by exposure to inducing agents, degree of health or illness, hormonal or nutritional status, cleanliness of litter, painful stimuli, ambient temperature, degree of crowding, time of day of drug administration, and type of bedding [21–25]. Thus, when we approach the question of the relative contributions of genetic and environmental factors to large inter-individual variations in clearance of commonly used drugs from human subjects, we can anticipate a large component of the variability to be environmental.

Twin studies are a particularly useful tool to examine the question of the relative contributions of genetic and environmental factors to large inter-individual variations in drug clearance. With the assumption that no greater differences in environment prevail for identical than for fraternal adult twins living in separate households in a large city, we can conclude that the genetic contributions to large inter-individual differences in rates of drug clearance from plasma would be small if the response of identical twins to a drug were just as variable as that of fraternal twins. On the other hand, if intra-twin differences in monozygotic twins were much less than those of fraternal twins, then the contribution of genetic factors to variability would be expected to be large. The results of pharmacokinetic examinations of all drugs thus far investigated by means of twin studies—ethanol [26], bishydroxycoumarin [27], phenylbutazone [28], antipyrine [29], diphenylhydantoin [30], halothane [31], nortriptyline [32] and isoniazid [33]—reveal much greater similarity between identical than between fraternal twins. The data from such a twin study are shown in Fig. 1.

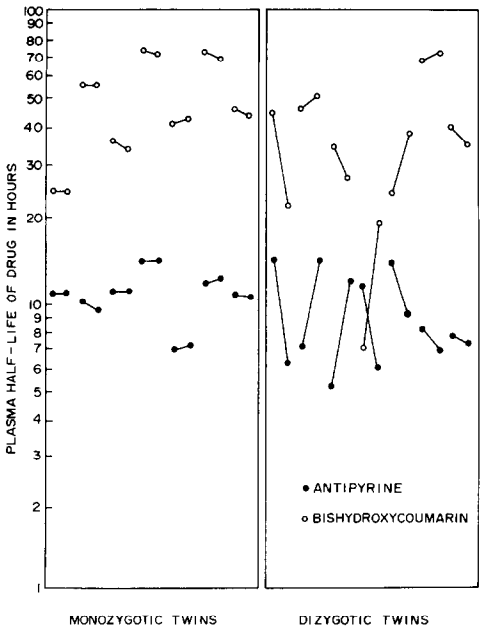

Fig. 1. Plasma half-lives of bishydroxycoumarin and antipyrine were measured separately at an interval of more than 6 months in healthy monozygotic (identical) and dizygotic (fraternal) twins. A solid line joins the values for each set of twins for each drug. Note that intra-twin differences in the plasma half-life of both bishydroxycoumarin and antipyrine are smaller in monozygotic and in dizygotic twins. Data are from the work of Vesell and Page [27, 29].

From a genetic point of view, family studies are more definitive than twin studies for establishing the modes of inheritance. However, family studies on the rates of drug metabolism in three generations are subject to errors introduced by the fact that drug metabolism varies with age, sex and environment. The latter may be quite different within a family for young children, parents and grandparents. It is of interest, however, that in spite of these methodological differences between twin and family studies, the results are in close agreement.

Three independent family studies, which were performed in the United States with bishydroxycoumarin [34], in England with phenylbutazone [35] and in Sweden with nortriptyline [36], disclosed predominantly genetic control over large inter-individual variations in the plasma pharmacokinetics of these drugs. Furthermore, these family studies suggested polygenic control, in that the mean pharmacokinetic value for the sibs fell midway between the values of the parents. Polygenic control was also suggested by the unimodal distribution curves obtained by plotting data from twin studies shown in Fig. 1.

GENETIC DISORDERS WITH ALTERED DRUG SENSITIVITY

Many inborn errors of metabolism are associated with abnormal responses to drugs, even though the underlying genetic lesion does not involve a gene product directly concerned with drug absorption, distribution, biotransformation, receptor site interaction or excretion. Nonetheless, because of these altered drug responses, patients with these genetic diseases can often avoid drug toxicity if exposure to certain drugs is modified or eliminated.

In gout, three discrete types of aberrant effects of drugs have been described:

(1) Acute attacks of gout may be induced by alcohol ingestion. Nicotinamide adenine dinucleotide is reduced during ethanol metabolism, thereby favoring lactate formation from pyruvate in amounts sufficient to impair renal elimination of uric acid [37].

(2) Diuretic agents, such as chlorothiazide or furosemide, cause hyperuricemia by reducing renal excretion of uric acid [38]. Pre-existing hyperuricemia is enhanced by these drugs, and the risk of diuretic-induced gout is increased in genetically predisposed individuals.

(3) Allopurinol limits uric acid production by two different mechanisms. It decreases the conversion of hypoxanthine and xanthine to uric acid, and it also inhibits the synthesis of purines. The second effect is absent in 0·5 per cent of gouty patients in whom the "purine salvage enzyme," hypoxanthine-guanine phosphoribosyltransferase (HGPRT), is partially deficient [39]. In these patients, allopurinol ribonucleotide is not formed; thus purine synthesis is not inhibited and xanthine oxidase is subjected to a higher concentration of allopurinol. As a result, these patients may form xanthine renal calculi.

A few other drugs are also biotransformed by HGPRT into ribonucleotides. For pharmacologic activity, the antineoplastic agent 6-mercaptopurine must be converted *in vivo* by HGPRT to its ribonucleotide. The immunosuppressive agent azathioprine is initially metabolized to 6-mercaptopurine which is then activated by HGPRT. Thus, HGPRT-deficient patients are resistant to the therapeutic actions of several drugs but are particularly sensitive to toxicity after chronic administration due to accumulation of the parent drug.

Hepatic porphyrias constitute several genetically distinct disorders involving the metabolic pathway of porphyrins and heme biosynthesis in the liver. One feature of the hepatic porphyrias is overproduction of the rate-limiting enzyme δ-aminolevulinic acid (ALA) synthetase in the liver. Several drugs that induce hepatic ALA synthetase may precipitate clinical exacerbations when given in therapeutic doses to patients in remission or in latent stages of hereditary hepatic porphyrias. These drugs include barbiturates, sulfonamides, griseofulvin, estrogens including those used in contraception, some anticonvulsants and tranquilizers,

and possibly general anesthetics, ethanol and chloroquine. This effect is not invariable, however, and patients with latent porphyria have been known to receive barbiturates or other drugs without developing clinical symptoms [40–42]. Gilbert's syndrome represents a common form of chronic, mild, non-incapacitating hyperbilirubinemia. The excess bilirubin is entirely in the unconjugated form, and there is no serious liver dysfunction. Gilbert's syndrome is often accompanied by a moderate reduction in activity of hepatic glucuronyltransferase [43], but defects of bilirubin uptake have also been implicated, suggesting heterogeneous causation. Alcohol, cholecystographic agents and, to some extent, the estrogens in oral contraceptives may increase plasma levels of bilirubin and produce jaundice.

THERAPY OF GENETIC DISEASE— NEW ASPECTS OF PHARMACOGENETICS

A fascinating aspect of pharmacogenetics deals with the therapy of genetic lesions. Once an inborn error of metabolism is sufficiently defined to permit isolation of the primary gene product, theoretically it should be possible to relieve the metabolic block by administration of the purified protein obtained from a normal source. Because enzymes are highly efficient catalysts, a very small amount of enzyme could theoretically reverse the toxicity arising from such genetic defects.

Alternatively, the need for replacement would be obviated if organ transplants could supply normal enzyme to genetically affected subjects. A major problem with an exogenously administered enzyme is that such proteins may experience difficulty in traversing cell membranes to reach the cytoplasm or intracellular organelles where they act. Thus, treatment of metachromatic leukodystrophy with arylsulfatase A has been unsuccessful [44]. Another approach involves passing a patient's plasma through columns or over sheets of stably bound enzyme to reduce the level of a toxic substrate, thereby favoring the removal of intracellular toxic substrates through re-equilibration [45].

Another approach to enzyme replacement is suggested by the observation that, for certain mucopolysaccharidoses, mixed cultures of cells from two genetically distinct forms of the disease grow normally [46–48]. Some molecule, probably an enzyme, passes between contiguous cells, reducing mucopolysaccharide accumulation. It seems reasonable to expect that such a molecule, purified and given to patients, might produce therapeutic benefit in certain mucopolysaccharidoses.

Finally, primary genetic material in the form of DNA or RNA has been provided to human mutant cells in culture to induce production of a normal enzyme. Merril and Geier [49] infected human galactosemic cells with transducing bacteriophage that contained the enzyme deficient in galactosemia, galactose-1-phosphate uridyl transferase. The bacteriophage initiated transferase synthesis within the galactosemic cells. Previously, experiments with viral-transformed cells suggested that foreign DNA could be successfully integrated and expressed within a cell line [50–53]. Munyon *et al.* [51] transformed thymidine kinase-deficient L cells growing in culture by infecting them with ultraviolet-irradiated herpes simplex virus. Recently, Cohen *et al.* [54] succeeded in inserting genetic material from one strain of *Escherichia coli* into another strain, and from *Staphylococcus aureus* into *E. coli* [55] with maintenance of function of the transplanted DNA in the new host. These techniques may be employed to reduce bacterial resistance to certain antibiotics. Although several major questions must be resolved before such techniques could be tried in man, these experiments offer hope for eventual success in the therapy of genetic disease. At present, severe genetic diseases are usually not amenable to effective therapy. Advances in prenatal diagnosis by means of amniocentesis have resulted in therapeutic abortions [56–62], rather than in early initiation of effective treatment. Hopefully, genetic therapy may become available in the not too distant future through development of some of the approaches outlined here.

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