Interplay of Factors Leading to Adverse Drug Reactions in the Liver, A Personal Viewpoint

FENTON SCHAFFNER
Mount Sinai School of Medicine of The City University of New York
Received January 26, 1977

Adverse drug reactions in liver involve formation of a reactive metabolic intermediary of the drug, binding of the intermediary to macromolecules in the cell, notably proteins in the plasma membrane, immunological response to these altered proteins and attack against hepatocytes bearing these altered proteins by immune mechanisms. At each step in this complex process many factors act to enhance or depress drug metabolism, metabolite disposition, macromolecular binding, neoantigen formation, and the cell mediated and humoral immune attack. The extent and direction of each step may be dose dependent but the complexity of the overall mechanism is so immense that predictability of hepatic drug reactions is unlikely in most instances.

Adverse drug reactions involving the liver were considered originally to be "idiosyncratic" in nature without further defining this term. When allergy became understood as an immunologic phenomenon, the reactions were felt to be the result of hypersensitivity with drugs acting as haptens. As more were seen, the reactions were classified into direct toxicity, cholestasis and viral hepatitis-like [1]. The first was described as the result of dose related injurious action of the drug leading to cell death, the second as the result of impairment of function of the bile secretory apparatus of the hepatocyte, rather than interference with bile flow by ductular obstruction, and the third, as the result of a hypersensitivity reaction. The details of metabolism for many drugs have been elucidated now and intermediary metabolites formed in the hepatocyte have been blamed for injury rather than the drug itself [2]. The purpose of this paper is to examine aspects of both metabolism and immune responsiveness and to hypothesize how a complex interplay of several factors is probably necessary to produce many of the adverse reactions in the liver.

THE METABOLIC FATE OF DRUGS

A drug given by mouth is carried to the liver via the portal vein bound to serum proteins, most often albumin. The transfer across the hepatocellular plasma membrane probably requires polysaccharide binding sites on the external surface attached to integral membrane proteins of the hepatocytes. Whether the drug is taken into the cell by endocytosis, by a change in the shape or position of the membrane protein or by dissolution in the lipid bilayer of the membrane is unknown. Once inside the cell the drug is eventually bound to a heme containing cytoplasmic cytochrome in the smooth endoplasmic reticulum. At least two [3] and perhaps many [4] cytochromes are present. The one metabolizing mainly aliphatic compounds is called cytochrome P-450 because its maximal absorbance of light in carbon monoxide atmospheres

489

1This article is the eleventh in a series entitled, "Seminars on Liver Disease," that have been presented as part of the Training Program in Liver Disease at the Veterans Administration Hospital, West Haven, Connecticut. Dr. Harold O. Conn, Professor of Medicine, Yale University School of Medicine and Director of the Training Program in Liver Disease, is guest editor.

Please address reprint requests to: Fenton Schaffner, M.D., Chief, Division of Liver Diseases, Department of Medicine, Mount Sinai School of Medicine of CUNY, New York, NY 10029

Copyright © 1977 by The Yale Journal of Biology and Medicine, Inc.
All rights of reproduction in any form reserved.
occurs at 450 nanometers. The one which metabolizes mainly aromatic compounds is called P₄ 450 or P-448 and is associated with aryl hydrocarbon hydroxylase. These cytochromes in the drug metabolizing enzyme system can be induced by the drug causing a reaction or by other drugs or chemicals [5,6]. The drug can be oxidized or deacylated by the cytochrome to the form of the drug that is active pharmacologically [7]. Then it is further oxidized and conjugated with glucuronide or if a large amount is present with glutathione. Occasionally the drug moves to the soluble fraction of the cytoplasm where sulfatation or acetylation can occur.

Oxidation to Reactive Intermediaries

The initial metabolic step involves oxidation at one carbon atom or one double bond with formation of an oxidized intermediary and water. The cytochrome P-450 requires molecular oxygen and the enzyme system has been called a “mixed function oxidase.” The intermediary which forms in the initial stages of oxidation is an epoxide, a superoxide or a free radical [8,9]. It usually has a short half life but it can escape further metabolism. The metabolite is very reactive and can bind to cell macromolecules like protein, RNA and DNA. Some of the macromolecules are structural components, some are enzymes and some are informational in nature. The intermediary metabolites of a given drug probably bind preferentially to specific sites on a given macromolecule because toxic reactions are reproducible even in different species. The effect of such binding depends on the rate of formation of the intermediary as well as on the type of macromolecule bound. The intermediary free radical formed from carbon tetrachloride, for instance, destroys the lipoprotein membrane of the endoplasmic reticulum. The acute centrolobular hepatic necrosis following tumor chemotherapy with such drugs as mithramycin [10] or mitomycin C [11] or from overdose of acetaminophen [12] probably results from metabolite binding to macromolecules, the last as suggested by Mitchell et al [7,9]. The rate of formation of metabolites in turn is in part genetically controlled [13,14] but also individual factors play a role. The amount of enzyme depends directly on the state of protein nutrition [15] and on how much it is induced by environmental pollutants [16] or by preceding drug therapy [6]. Its state of reactivity can be influenced by competition for metabolism by endogenous substances or by other drugs or by hepatocellular injury from disease.

Disposition of Intermediaries

Normally intermediaries are moved by continuation of the oxidative process with the help of enzymes such as epoxide hydrase [17] until a more stable and polar intermediate is formed. This intermediate is then conjugated with a polar compound making the original drug, which was usually non-polar, polar and readily excreted into the urine or bile depending upon its molecular weight. When more intermediary is formed than the normal pathway can handle a back up mechanism may be available in that the excess is scavenged by secondary and nonspecific pathways. These involve combining with glutathione or with vitamin E and probably other pathways. The former combinations can be further metabolized to form mercaptides which are secreted in bile or urine. Little is known of the role of these pathways in drug metabolism, let alone in adverse drug reactions and therefore much of what can be said is speculative. Indeed the subject of glutathione in the liver was the subject of a workshop held recently (Arias IM, Jakoby WB: Glutathione: Acetabolism and Function. New York: Raven Press, 1975, p 377). The rate of degradation of escaped intermediaries depends on the activity of the epoxide hydrase, on the availability of substrate for conjugation and on the amount of glutathione or pantothenic acid
available for scavenging. Induction of metabolic enzyme activity by preceding drug administration or by environmental factors may accelerate formation of intermediaries on one hand and their removal through conjugation on the other. Competition for conjugation can also cause release of more reactive intermediaries. The effects of acetamenophen overdosage may exhaust the primary conjugation pathway and the supply of glutathione, thereby permitting the excess reactive intermediary to bind to vital cell macromolecules. This consideration has led to the prophylactic and therapeutic use of sulfur containing amino acids or analogues [18].

ALTERATIONS IN HEPATOCYTIC PLASMA MEMBRANES

The plasma membrane is a lipid bilayer with external and internal surfaces consisting of the hydrophilic ionic portion of the fatty acids and phospholipids, and a liquid hydrophobic central portion [19,20] (see these references for specific drug effects mentioned in what follows). Protein molecules are scattered throughout the bilayer. These proteins called integral membrane proteins are necessary to maintain the membrane. They have hydrophilic portions which protrude above the membrane surface or below it into the cytoplasm or in both directions and hydrophobic portions held in the lipid center of the membrane bilayer. Carbohydrate moieties attached to the hydrophilic portion of the integral membrane protein protruding above the cell surface are specific receptors which bind hormones, antibodies and other substances that enter the cell or otherwise influence the interior of the cell. The distribution of the protein molecules in the membrane varies depending on the substances that bind to their receptor sites, the physical contacts of the cell and on cytoplasmic skeletal elements. The last are rigid microtubules anchoring membrane proteins or contractile microfilaments moving them about in the membrane. Drugs can influence the cytoskeletal structure associated with the plasma membrane and its control in many ways. Some substances cause aggregation of integral membrane proteins to form small clusters or larger caps which then enter the cell by endocytosis. [20]. This clustering can be prevented by drugs which injure cellular function in a nonspecific fashion, but it also can be prevented by drugs like colchicine or vinblastine which depolymerize the protein tubulin of the microtubules or like cytochalasin B which disrupts microfilaments. Some drugs enhance aggregation of membrane protein like the tertiary amine anesthetics [20]. Probably many drugs or their metabolites influence the cytoskeletal structure by directly affecting microtubules and microfilaments or by nonspecifically interfering with cellular function. Furthermore interactions of drugs may alter the cell membrane in complex ways so that membrane proteins are internalized or shed. The process of shedding exposes hitherto "unseen" portions of the membrane proteins to the immune system of the body thereby stimulating an immune response. Finally reactive intermediary metabolites of drugs may bind to specific proteins in the plasma membranes like those that are part of the HLA antigen complex. The patient's immune surveillance mechanism may therefore not recognize this altered protein as self and attack the entire cell injuring it or rejecting it. Thus drugs taken up and metabolized by hepatocytes not only alter the internal composition of these cells but also their surfaces. As yet unknown factors govern the rates of synthesis, polymerization and orientation of the cytoskeletal proteins tubulin of microtubules and actin and myosin of microfilaments.

IMMUNOLOGICAL RESPONSE TO PLASMA MEMBRANE ALTERATIONS

The presence of abnormal membrane proteins or the exposure of shed integral membrane proteins or the more easily detached nonessential peripheral membrane proteins may induce humoral antibody formation as well as a cell mediated immune
response. Such mechanisms have been proposed for hepatitis B [21, 22] and they may also be suggested to be acting in the drug induced viral hepatitis-like reactions occurring after halothane [23], methyldopa [24] or isoniazid [25]. Indeed a similar pathogenesis may explain the similar clinical and pathological picture of viral and drug induced hepatitis. In the case of methyldopa, a superoxide anion has been said to be responsible [26]. The nature and intensity of this response depends on the rate of release of the surface proteins, their uptake by macrophages or leukocytes or binding to the surfaces of lymphocytes, their antigenicity and the reactivity of the host immune system. Plasma membrane fragments, such as those that may be released by shedding of cytoplasm from an injured cell, are ingested by Kupffer cells and leukocytes setting up foci of inflammation [27]. Drugs or their metabolites may influence many steps in this complex process by determining the rate at which protein is made available to act as an antigen, the phagocytic ability of macrophages and their lysosomal digestive function, the surface binding of lymphocytes, the rate of antibody formation and the rate of production of specifically cloned lymphocytes and finally the availability of receptor sites on the hepatocellular surface for immune reactions. Drugs even compete with their metabolites in some of these processes. Hepatocellular injury may play an adjuvant role enhancing immune responsiveness on one hand and a suppressing role on the other by slowing drug metabolism or reducing available receptor sites. The hepatocellular injury also stimulates collagen production and deposition. The fibrosis may play a role in the development of chronic hepatitis first described after long term use of the laxative, oxyphenisatin [28] and now even recognized after prolonged aspirin ingestion [29]. The pericellular fibrosis along with the development of a complete basement membrane may constitute a physical barrier which can interfere with blood-liver exchange of nutrients and oxygen [30]. This barrier may also be protective by keeping antibody molecules and killer lymphocytes away from the hepatocellular surface.

**OPPOSING FORCES IN ADVERSE REACTIONS**

Opposing forces are active at each step of drug uptake, metabolism and disposition of metabolites as well as at each step of formation of neoantigens and response of the body to these proteins (Table 1). Genetic factors play a major role in all of these steps but, except for slow and fast acetylators of some drugs like isoniazid and for congenital defects in immune responsiveness, little is known about genetic control [14,31]. The rate of the initial step in metabolism can be accelerated by induction of the responsible enzymes by environmental pollutants like pesticides [32] or polychlorinated biphenyls [33], other drugs or even the drug in question. Liver cell injury can reduce the rate of drug metabolism as can any viral infections which induce interferon. Indeed all natural or synthetic interferon inducers drastically reduce the rate of drug metabolism by decreasing the activity of cytochrome P-450 [34]. The rate can be decreased by protein malnutrition and by competition from endogenous or the same exogenous factors just mentioned. Similarly the rate of disposition of the reactive intermediary metabolite via the normal detoxification pathways can be accelerated by induction and slowed by competition. The rate of repair of macromolecules altered by drug metabolite binding varies under different physiological and pharmacological circumstances. The binding sites on the cell macromolecules may also be competitively blocked by other substances or they may be altered by changes in macromolecular conformation because the cytoskeleton is altered by drugs of cellular injury. Organs other than the liver may also participate in the metabolism and disposition of some drugs and the rates, pathways and organs involved in drug
| Steps                              | Anatomic Sites or Process                      | Enhancing Factors                                      | Depressing Factors                                                                 |
|-----------------------------------|-----------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------------------------------|
| Hepatic uptake                    | Disse space                                   | Rapid GI absorption or parenteral administration       | Malabsorption, impaired circulation, reduced carrier proteins in serum, Disse space filled with edema or fibrosis, receptors or carriers blocked by endogenous substances or other drugs |
|                                   | Plasma membrane receptors                     | Induced receptors or carrier proteins                  |                                                                                   |
|                                   | Cytoplasmic carrier proteins                  |                                                        |                                                                                   |
| Oxidation with intermediary formation | Microsomal cytochrome P-450                   | Induction by other drugs or environmental pollution    | Competition by other drugs or endogenous substrates, protein malnutrition, any hepatocellular injury, interferon inducers |
| Disposition of metabolites        | Microsomal conjugation                        | Induction, congenitally rapid metabolism                | Competition, depletion of conjugating molecules because of dietary enzyme deficiency. Congenitally slow metabolism |
|                                   | Glutathione binding with mercaptide formation |                                                        |                                                                                   |
|                                   | Macromolecular binding                        | Excess supply of metabolite                           | Destruction by metabolite of binding sites (direct toxicity leading to necrosis)    |
| Neoantigen formation              | DNA or RNA binding leading to synthesis of new protein | Reduced repair processes                              | Induction of repair enzymes                                                       |
|                                   | Binding to cellular protein directly          | Appropriate receptor sites                             | Lack or blockade of receptor sites                                                |
|                                   | Binding to plasma membrane protein, especially HLA antigens |                                                        |                                                                                   |
| Immune attack                     | Macrophages, T and B lymphocytes, K cells, hepatocellular plasma membrane | Previous sensitization                                  | Immunosuppressive drugs or disease, congenital immunologic defects                |
|                                   |                                               | Adjuvant effect of liver cell injury                   |                                                                                   |
metabolism vary from species to species so that extrapolation of experimental studies to man is possible only if metabolism is similar [35].

**PREDICTABILITY OF DRUG REACTIONS**

Thus to produce cellular injury a drug and its metabolites must wind their way through a devious pathway filled with detours and obstacles as well as rapid slides. Only with the direct toxins are the pathways simple enough to quantitate so that dose relations can be established. With most drugs that cause adverse reactions, predictability itself is not yet possible. Dose relationships probably exist in individual cases and at each step, but these are lost in the complexity of metabolic reactions some of which amplify and others diminish the effect of the drug in question. The multiplicity of effects at each step also makes prevention a distant goal.

**REFERENCES**

1. Perez V, Schaffner F, Popper H: Hepatic drug reactions. In Progress in Liver Diseases, vol. 4. Edited by H Popper and F Schaffner. New York: Grune and Stratton, 1972, pp 597–625
2. Gillette JR: A perspective on the role of chemically reactive metabolites of foreign compounds in toxicity. I. Correlation of changes in covalent binding of reactive metabolites with changes in the incidence and severity of toxicity. Biochem Pharmacol 23:2785–2794, 1974
3. Shoeman DW, Chaplin MD, Mannering GJ: Induction of drug metabolism. 3. Further evidence for the formation of a new P-450 hemoprotein after treatment of rats with 3-methylcholanthrene. Molec Pharmacol 5:412–419, 1969
4. Thomas PE, Lu AYH, Ryan D, et al: Immunological evidence for six forms of rat liver cytochrome P-450 obtained using antibodies against purified rat liver cytochromes P-450 and P-448. Molec Pharmacol 12:746–758, 1976
5. Remmer H, Schenkman JB, Estabrook RW, et al: Drug interaction with hepatic microsomal cytochrome. Molec Pharmacol 3:113–123, 1967
6. Conney AH: Pharmacological implications of microsomal enzyme induction. Pharmacol Rev 19:317–366, 1967
7. Mitchell JR, Nelson SD, Thorgeirsson SS, et al: Metabolic activation: Biochemical basis for many drug-induced liver injuries. In Progress in Liver Diseases, vol 5. Edited by H Popper and F Schaffner. New York: Grune and Stratton, 1976, pp 259–279
8. Gillette JR, Mitchell JR, Brodie BB: Biochemical basis for drug toxicity. Ann Rev Pharmacol 14:271–288, 1974
9. Mitchell JR, Jollow DJ: Metabolic activation of drugs to toxic substances. Gastroenterology 68:392–410, 1975
10. Ream NW, Perla CP, Wolter J, et al: Mithramycin therapy in disseminated germinal testicular cancer. JAMA 204:1030–1036, 1968
11. Moertel CG, Rectemier RJ, Hahn RG: Mitomycin C therapy in advanced gastrointestinal cancer. JAMA 204:1045–1048, 1968
12. Davidson DGD, Eastham WN: Acute liver necrosis following overdose of paracetamol. Brit Med J 7:497–499, 1966
13. Kalow W, Inaba T: Genetic factors in hepatic drug oxidations. In Progress in Liver Diseases, vol 5. Edited by H Popper and F Schaffner. New York: Grune and Stratton, 1976, pp 246–258
14. Nebert DW, Felton JS: Importance of genetic factors influencing the metabolism of foreign compounds. Fed Proc 35:1133–1141, 1976
15. McLean AEM, Day PA: The effect of diet on the toxicity of paracetamol and the safety of paracetamol-methionine mixtures. Biochem Pharmacol 25:37–42, 1975
16. Gillette JR: Environmental factors in drug metabolism. Fed Proc 35:1142–1147, 1976
17. Oesch F, Tohenen H, Fahrlaender H: Epoxide hydrase in human liver biopsy specimens: Assay and properties. Biochem Pharmacol 23:1307–1317, 1974
18. Mitchell JR, Thorgeirsson SS, Potter WZ, et al: Acetaminophen induced hepatic injury: Protective role of glutathione in man and rationale for therapy. Clin Pharmacol Therap 16:676–684, 1974
19. Singer SJ, Nicolson GL: The fluid mosaic model of the structure of cell membranes. Science 175:720–731, 1972
20. Nicolson GL, Poste G: The cancer cell: Dynamic aspects and modifications in cell-surface organization. New Engl J Med 295:197–203, 253–258, 1976
21. Guad F, Bianchi L, Sonnabend W, et al: Pattern of core and surface expression in liver tissue reflects state of specific immune response in hepatitis B. Lab Invest 32:1–9, 1975
22. Edgington TS, Chisari FV: Immunological aspects of virus B infection. Am J Med Sci 270:213–227, 1975
23. Schaffner F: Halothane hepatitis. In Controversies in Medicine, vol 2. Edited by FJ Ingelfinger, RV Ebert, M Finland, et al. Philadelphia: Saunders, 1974, pp 565–579
24. Black M, Mitchell JR, Zimmerman HJ, et al: Isoniazid-associated hepatitis in 114 patients. Gastroenterology 69:289–302, 1975
25. Maddrey WC, Boitnoff JK: Severe hepatitis from methyldopa. Gastroenterology 68:351–360, 1975
26. Dybing E, Nelson SD, Mitchell JR, et al: Oxidation of α-methyldopa and other catechols by cytochrome P-450-generated superoxide anion: Possible mechanism of methyldopa hepatitis. Molec Pharmacol 12:911–920, 1976
27. Glaumann H, Trump BF: Lysosomal degradation of cell organelles. IV. Heterophagocytosis and acute inflammation in liver after intravenous infection of liver-cell plasma membranes. Exp Molec Pathol 25:371–389, 1976
28. Reynolds TB, Peters RL, Yamada S: Chronic active and lupoid hepatitis caused by a laxative, oxyphenisatin. New Engl J Med 285:813–820, 1971
29. Seaman WE, Ishak KG, Plotz PH: Aspirin-induced hepatotoxicity in patients with systemic lupus erythematosus. Ann Intern Med 80:1–8, 1974
30. Schaffner F, Popper H: Capillarization of hepatic sinusoids in man. Gastroenterology 44:239–242, 1964
31. Vesell ES, Lang CM, White WJ, et al: Environmental and genetic factors affecting the response of laboratory animals to drugs. Fed Proc 35:1125–1132, 1976
32. Hunter J, Maxwell JD, Stewart DA, et al: Increased hepatic microsomal enzyme activity from occupational exposure to certain organochlorine pesticides. Nature 237:399–401, 1972
33. Allen JR: Response of the nonhuman primate to polychlorinated biphenyl exposure. Fed Proc 34:1675–1679, 1975
34. Renton KW, Manering GJ: Depression of the hepatic cytochrome P-450 mono-oxygenase system by administered tilorone [2, 7-bis [2-(diethylamino) ethoxy] fluoren-9-one dichlorid]. Drug Metab Dispos 4:223–231, 1976
35. Dybing E: Organ and species differences in microsomal activation of methyldopa. Drug Metab Dispos 4:513–516, 1976

Fenton Schaffner, M.D.
George Baehr Professor and Chief
Division of Liver Diseases
Department of Medicine
Mount Sinai School of Medicine of The City University of New York
New York, New York 10029