METABOLISM OF DRUGS BY LEUKOCYTES

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SUMMARY

Neutrophils and monocytes can metabolize drugs to reactive metabolites, especially those drugs that have nitrogen or sulfur in a low oxidation state. The major system involved in this oxidation is the combination of NADPH oxidase and myeloperoxidase which generates HOCl. Although this system is unlikely to be quantitatively important, i.e. it is unlikely to have a significant effect on the pharmacokinetics of a drug, the reactive metabolites produced appear to have significant biological effects. Reactive metabolites, by their very nature, have short half-lives, and most of their effects will be exerted on the cells that formed them. Therefore, they are likely to be important for adverse reactions that involve leukocytes, such as agranulocytosis and immune-mediated reactions. However, the mechanism of these reactions is unknown and evidence for the association of leukocyte-derived reactive metabolites with such reactions is circumstantial at present. There is also circumstantial evidence to link the formation of such reactive metabolites to the antiinflammatory effects of some drugs. Possible mechanisms include the scavenging of other reactive species or inhibition of cells, especially neutrophils and macrophages, involved in inflammation. The oxidation of drugs by leukocytes requires activation of the cells; therefore, infection or other inflammatory conditions that activate leukocytes may represent one of the risk factors for idiosyncratic drug reactions.

1. INTRODUCTION

Many of the pharmacological properties of drugs, both beneficial and toxic, are due to metabolites of the drug rather than to the parent drug. The major site of drug metabolism is the liver. The major hepatic enzyme responsible for oxidative metabolism is the cytochrome P450 system. However, drug metabolism also occurs in other organs and can be mediated by other enzymes. These other sites and enzymes are likely to be most important when the metabolite has a short half-life so that the metabolite does not enter the circulation. Adverse reactions, in many cases, are likely due to reactive metabolites with short half-lives /1/. Many adverse reactions involve leukocytes, either because they are the target of toxicity as in agranulocytosis, or because they involve the immune system /2,3/. Some therapeutic effects of drugs, such as

antiinflammatory effects, also involve leukocytes and may involve the formation of reactive metabolites /4,5/.

Leukocytes are composed of lymphocytes, monocytes and polymorphonuclear leukocytes, mainly neutrophils. Although several types of leukocytes contain small amounts of cytochrome P450, the quantity present is unlikely to lead to a significant amount of drug metabolism /6/. The products of metabolism of drugs by cytochrome P450 in leukocytes are likely to be similar to those which occur in other organs, and since the metabolism appears to be quantitatively insignificant, it will not be discussed further. Leukocytes also contain significant concentrations of cyclooxygenase /7-9/ which can oxidize some drugs /10,11/. In addition, rodent monocytes and neutrophils contain nitric oxide synthase and nitric oxide can be converted to very strong oxidants such as peroxynitrite /12/. However, it appears that the corresponding human cells contain little or no nitric oxide synthase /13/. The major enzyme leading to the production of an oxidant in neutrophils and monocytes is NADPH oxidase, which converts oxygen to superoxide that is in turn converted to hydrogen peroxide /14,15/. Neutrophils and monocytes also contain myeloperoxidase (MPO) which is oxidized by hydrogen peroxide to an oxidized form of the enzyme called compound I. Compound I can probably oxidize drugs, but the major substrate is chloride which is converted to hypochlorous acid /16/. Hypochlorous acid is a very strong oxidant and chlorinating agent. Eosinophils, the second most common granulocyte after neutrophils, also contain NADPH oxidase and a similar peroxidase called eosinophil peroxidase. Eosinophil peroxidase has a much higher affinity for bromide ion than chloride ion, and even though the physiological concentration of bromide ion is much lower than chloride ion, it effectively competes with chloride ion for oxidation to hypobromous acid /17/. In summary, it appears that the major enzyme system responsible for oxidation of xenobiotics in neutrophils and monocytes is NADPH oxidase/myeloperoxidase although prostaglandin synthase may make a significant contribution in certain circumstances. Prostaglandin synthase and myeloperoxidase are peroxidases; therefore, these reactions can be viewed as either peroxidase-mediated or hypochlorite-mediated. Phase II metabolism, such as acetylation, can also occur in leukocytes, but phase II metabolism in leukocytes is beyond the scope of this review.

Unlike cytochrome P450, which is capable of oxidizing almost any substrate if it binds to the enzyme, the $MPO/H_2O_2/Cl^-$ and

prostaglandin synthase systems are only able to oxidize substrates that are relatively easy to oxidize, especially those containing a nitrogen or sulfur in a low oxidation state. Examples include arylamines and sulfhydryl groups. Drugs containing such functional groups are associated with a relatively high incidence of adverse drug reactions, especially agranulocytosis /2,3/. In addition, such drugs often have antiinflammatory properties.

2. SPECIFIC METABOLIC PATHWAYS

2.1 Primary arylamines

Despite the observation that primary arylamines are associated with a relatively high incidence of adverse drug reactions, there are several drugs which contain a primary arylamine functional group. They include procainamide, dapsone, sulfonamide antibiotics and aminoglutethimide. Some other drugs are metabolized to a significant degree to arylamines. This group includes practolol, acebutolol and chloramphenicol.

Procainamide is associated with a very high incidence of a lupus-like syndrome, about 20% /18-21/, and also agranulocytosis /22-24/. Dapsone and sulfamethoxazole are associated with methemoglobinemia, hemolytic anemia, agranulocytosis and generalized hypersensitivity reactions /25-29/. Aminoglutethimide is associated with agranulocytosis, hypothyroidism and generalized hypersensitivity reactions /30-32/. Practolol is no longer used because it was associated with an unusual autoimmune oculocutaneous syndrome /33-36/; acebutolol is associated with lupus /37,38/ and chloramphenicol with aplastic anemia /39,40/.

Procainamide is an arylamine which is oxidized in the liver to a hydroxylamine /41/, but significant concentrations do not appear to escape the liver and that is presumably why procainamide is not associated with hemolytic anemia and methemoglobinemia which are due to hydroxylamine metabolites /28/. Procainamide is also oxidized by activated neutrophils and monocytes to the hydroxylamine /42/, and, in addition, neutrophil/monocyte-derived HOCl chlorinates the arylamine to a chloramine /43/. In contrast, significant oxidation was not observed with lymphocytes or platelets /44/. The same products were produced by the combination of $MPO/H_2O_2/Cl^-$. Inhibitors of

prostaglandin synthase did not inhibit oxidation of procainamide by neutrophils.

The hydroxylamine of procainamide is not a very strong electrophile but it is readily oxidized further, either enzymatically or simply by oxygen, to a more electrophilic nitroso derivative /45/. The nitroso derivative reacts rapidly with glutathione and other sulfhydryl containing nucleophiles to form sulfinamide derivatives. Another pathway that leads to a more electrophilic derivative of the hydroxylamine is O-acetylation (unpublished observation). The resulting acetoxy-derivative is more reactive because acetate is a better leaving group than hydroxide ion; however, its reaction with glutathione is slow. The N-chloro-derivative is more reactive; however, its major reaction with glutathione is to form oxidized glutathione, presumably by transferring chlorine to glutathione to form a sulfenyl chloride which would react with another glutathione molecule to form oxidized glutathione. N-Chloroprocainamide also spontaneously rearranges to 2-chloroprocainamide /46/.

Dapsone and sulfamethoxazole are also oxidized by the liver to hydroxylamines, but unlike procainamide, these hydroxylamines do reach the circulation and can be detected in the urine /47,48/. These hydroxylamines are also formed by activated neutrophils /49/; however, the sulfone group is even more electron-withdrawing than the amide group on procainamide and the hydroxylamine and acetoxy derivatives are less reactive. This is presumably because the transition state of the reaction has nitrenium ion character which would be inhibited by electron withdrawing groups. Likewise, HOCl leads to N-chlorination, and because of the greater electron withdrawing effect of the sulfone groups, rearrangement of the N-chloro-derivatives is slower /43/. This is in contrast to hydroxylamines and acetoxy derivatives of carcinogens, such as aminobiphenyl /50/, which are much more reactive, and it may be impossible to isolate the N-chloro-derivative. The hydroxylamine of arylamines such as aminoglutethimide is likely to be more like that of aminobiphenyl; however, its oxidation by activated leukocytes has not been elucidated. A summary of the leukocyte-mediated metabolic pathways possible for primary arylamines is presented in Figure 1.

2.2 Other arylamines

Clozapine represents an important advance in the treatment of schizophrenia, both because it is very effective and also because it is

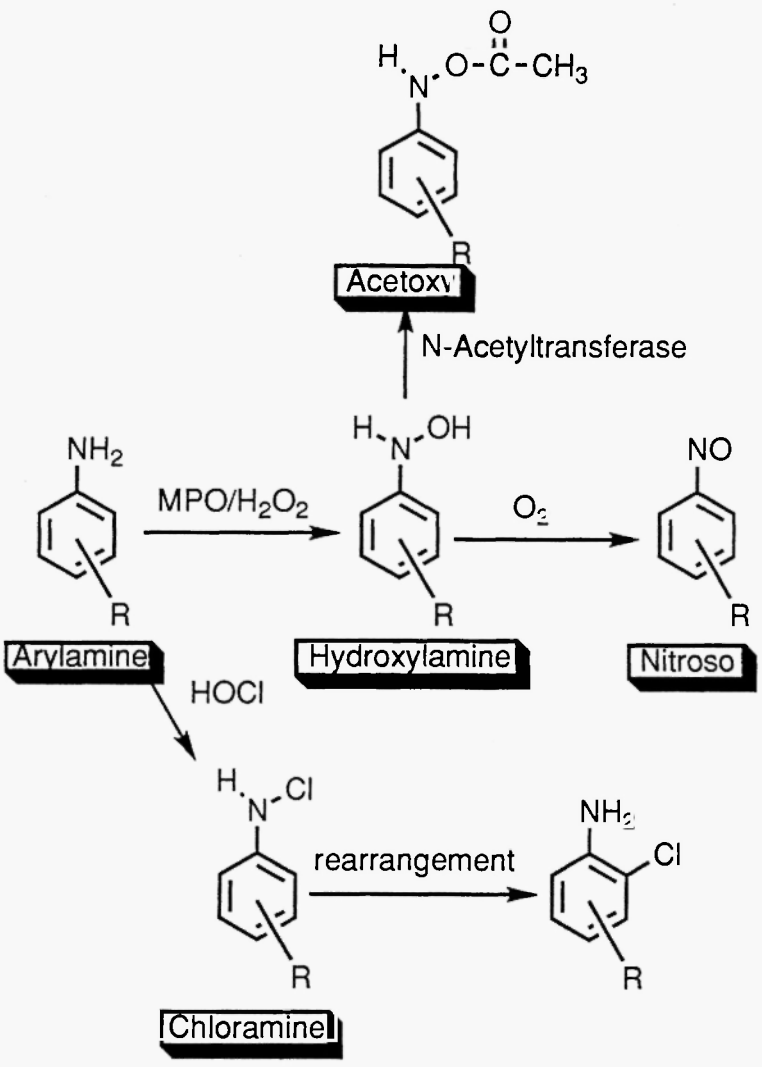

Fig. 1: Metabolic pathways leading to reactive metabolites of arylamines.

not associated with extrapyramidal side effects which often limit the use of other neuroleptics. However, clozapine was found to be associated with a 1-2% incidence of agranulocytosis which has severely limited its use /51-53/. Clozapine is an arylamine which is oxidized by HOCl to a reactive intermediate that can be trapped by glutathione /54/. The reaction appears to involve N-chlorination followed by loss of chloride ion to form a highly delocalized nitrenium ion with a surprisingly long half-life. This nitrenium ion appears to be toxic to neutrophils from patients with a history of clozapine-induced agranulocytosis (unpublished observation).

Diclofenac, which is the only common non-steroidal anti-inflammatory drug (NSAID) that is an arylamine, appears to be associated with a slightly higher incidence of agranulocytosis than other NSAIDs /55/. It has been reported to be oxidized by the myeloperoxidase system to dihydroxyazobenzene, but it is difficult to speculate by what pathway such a product would be formed /56/.

2.3 Arylamines capable of forming a quinone-like structure

Vesnarinone is a new drug that appears to significantly decrease mortality in patients with severe congestive heart disease /57/. However, it is associated with a 1-2% incidence of agranulocytosis. Although metabolism of this drug in the liver is slow /58/, it is metabolized to reactive metabolites by activated neutrophils, and at least *in vitro*, this leads to almost 5% of the drug covalently binding to the cells /59,60/. The pathway leading to the reactive metabolites starts with N-chlorination of the arylamine nitrogen followed by loss of HCl across the aromatic ring to form an iminoquinone, as shown in Figure 2. This hydrolyzes to another reactive intermediate with a structure similar to the reactive metabolite of acetaminophen. These reactive metabolites can also be trapped by glutathione. We believe that these reactive metabolites are likely to be responsible for vesnarinone-induced agranulocytosis.

Amodiaquine is an antimalarial drug whose use is associated with a relatively high incidence of agranulocytosis, and it is also oxidized to a reactive iminoquinone intermediate by activated neutrophils /61/. Patients with amodiaquine-induced agranulocytosis are reported to have antibodies against amodiaquine-modified neutrophils /62/.

Another drug with a hetero-atom *para* to an arylamine is 5-aminosalicylic acid. This drug is used for the treatment of inflammatory bowel disease and its activity has been postulated to be due to its

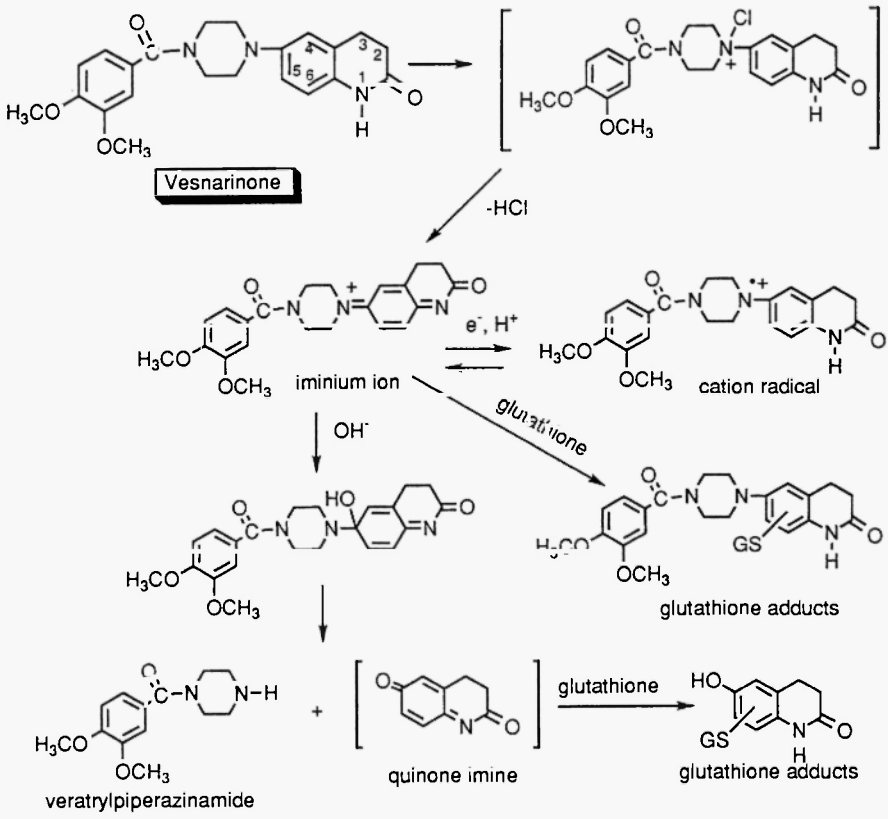

Fig. 2: Proposed mechanism for oxidation of vesnarinone by leukocytes.

inhibition of neutrophil function which appears to play a prominent role in the pathogenesis of inflammatory bowel disease /63,64/. We have found that 5-aminosalicylic acid is oxidized by HOCl to a quinone-imine, presumably through a N-chloro-intermediate (unpublished observation). The quinoneimine hydrolyzes to a quinone, and both intermediates can be trapped with glutathione. In general, the presence of a hetero-atom *para* to an aromatic amino group that can facilitate loss of chloride from a chloramine intermediate will likely lead to a reactive quinone-like structure.

2.4 Other compounds with an easily oxidized nitrogen

The first drug to be recognized to cause agranulocytosis was aminopyrine /65-68/. It contains a dimethylamino group which is readily chlorinated. It has been reported that reaction of aminopyrine with HOCl leads to a cation radical /69/. It was proposed that this was due to loss of a chlorine free radical from the N-chloro species. We have produced strong evidence that, in fact, the mechanism involves loss of a chloride ion to form a reactive dication which can react with another molecule of aminopyrine to form two molecules of cation radical (unpublished observation). Such a reactive intermediate could be responsible for aminopyrine-induced agranulocytosis.

Hydralazine is a hydrazine used for the treatment of hypertension. It is associated with a high incidence of drug-induced lupus /70-72/. It is oxidized by activated neutrophils or HOCl to a reactive metabolite, probably a diazonium ion, that results in covalent binding to the neutrophils /73,74/. Likewise, isoniazid is a hydrazide, also associated with drug-induced lupus, that is also oxidized by activated neutrophils to a reactive metabolite /75/.

2.5 Sulfur-containing functional groups

Drugs with sulfur in a low oxidation state are easily oxidized and are generally oxidized by peroxidases. Such drugs include propylthiouracil, penicillamine and captopril. The therapeutic use of propylthiouracil is for the treatment of hyperthyroidism. It inhibits thyroid peroxidase which is similar to myeloperoxidase /76/. The major serious adverse reaction associated with its use is agranulocytosis and it is also associated with drug-induced lupus /77-80/. It also induces a lupus-like syndrome in a high proportion of cats treated with the drug /81/. We have demonstrated that it is oxidized through a series of intermediates to a reactive sulfonic acid /82/. Several of the intermediates are also likely to be reactive, especially the first intermediate, which is assumed to be a sulfenyl chloride. This pathway is shown in Figure 3. Such intermediates could be responsible for both the therapeutic effects of the drug and the adverse reactions associated with its use.

Penicillamine and captopril are also associated with a variety of autoimmune-type adverse reactions, including myasthenia gravis for penicillamine /83-86/. The drugs are readily oxidized and form mixed disulfides with protein /87/. Although their metabolism by activated

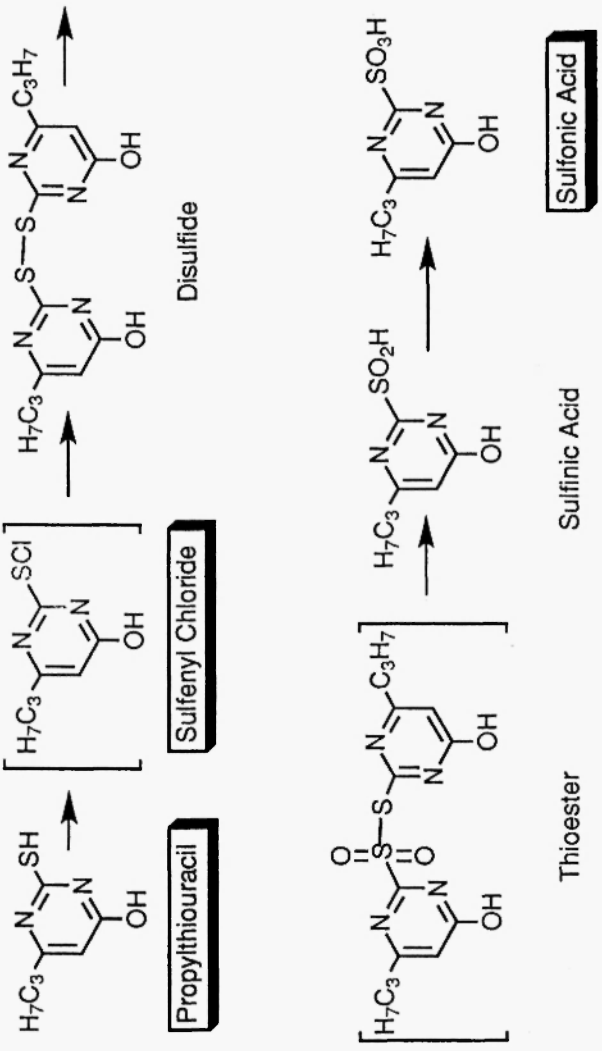

Fig. 3: Metabolism of propylthiouracil by leukocytes.

leukocytes has not been reported, it is reasonable to assume that oxidation by leukocyte-derived HOCl would lead to a reactive sulfenyl chloride and this would react with other protein sulfhydryl groups.

2.6 Oxidation of carbon

Although oxidation of carbon is in general much more difficult than oxidation of nitrogen- and sulfur-containing compounds, and oxidation by peroxidases at carbon is rare, there are a few cases in which oxidation of carbon does occur. One example is phenylbutazone. The carbon that is oxidized is flanked by two carbonyl groups which make the hydrogen on the carbon acidic. It is oxidized by peroxidases to a chlorinated derivative, an alcohol and a hydroperoxide /88/. Phenylbutazone is also associated with a relatively high incidence of agranulocytosis and aplastic anemia /89,90/.

Carbamazepine is associated with a relatively high incidence of generalized idiosyncratic drug reactions as well as agranulocytosis /91,92/. It is oxidized by activated neutrophils or HOCl to a reactive carbonium ion which very rapidly rearranges with contraction of the 7-membered ring and subsequent loss of the exocyclic carbon as shown in Figure 4 /93/. Covalent binding of carbamazepine to activated neutrophils was also observed.

The concern with diethylstilbestrol is breast cancer as well as abnormalities and cancer in children of mothers who took the drug during pregnancy. There is speculation that these effects may be related to the formation of a reactive metabolite /94/. Activated neutrophils or hypochlorous acid were shown to activate diethylstilbestrol to a metabolite which covalently binds to the cells /95/. The mechanism of the oxidation and the identity of the reactive intermediate are unknown and could involve either oxidation of the phenolic group or the carbon-carbon double bond. Even the oxidant is in question because covalent binding was not inhibited by azide which inhibits myeloperoxidase.

3. MECHANISMS BY WHICH LEUKOCYTE-GENERATED REACTIVE METABOLITES MAY LEAD TO ADVERSE DRUG REACTIONS

3.1 Involvement of reactive metabolites

The mechanisms by which drugs cause idiosyncratic drug reactions are unknown. In general, drugs which are readily metabolized to

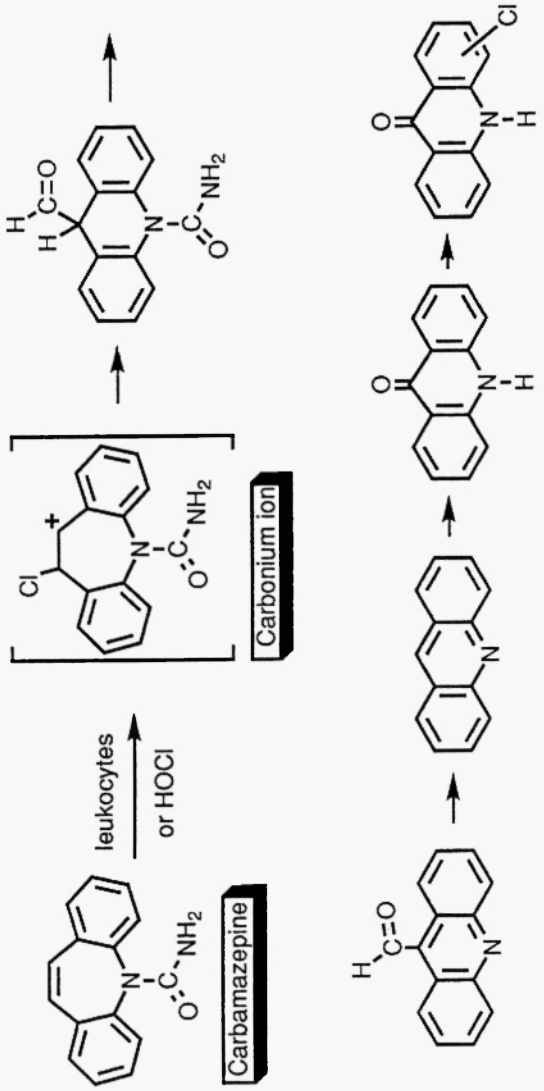

Fig. 4: Proposed mechanism for oxidation of carbamazepine by leukocytes.

reactive metabolites by activated neutrophils are associated with a relatively high incidence of agranulocytosis and other idiosyncratic drug reactions /2,3/. This makes it appealing to hypothesize that reactive metabolites are involved in many or most idiosyncratic drug reactions, and this hypothesis has been prevalent in the study of adverse reactions. However, there are drugs associated with idiosyncratic reactions for which it is difficult to hypothesize a reactive metabolite, and there are very few examples in which there is compelling evidence for the involvement of a reactive metabolite in the mechanism of an idiosyncratic drug reaction. The best example for which there is good evidence for the involvement of a reactive metabolite is halothane-induced hepatitis. Halothane is metabolized in all patients to a reactive trifluoroacetyl halide. Patients with halothane-induced hepatotoxicity have antibodies against trifluoroacetylated protein while other patients treated with halothane do not /96,97/. Although some risk factors are known, such as multiple exposure and obesity, it is not clear why some patients develop an immune response to the trifluoroacetyl halide-modified protein and others do not.

3.2 Involvement of the immune system

The finding of antibodies in halothane-induced hepatitis that correlate with disease provides relatively strong evidence for involvement of the immune system. The idiosyncratic nature of this type of adverse reaction also suggests involvement of the immune system. The association of idiosyncratic drug reactions with reactive metabolites is natural if the reaction is immune-mediated because a major mechanism by which small molecules can induce an immune response is by irreversibly binding to a macromolecule and acting as a hapten. In general small molecules are not immunogenic but they can modify proteins and other macromolecules and make them immunogenic /98-100/. Yet in most idiosyncratic drug reactions, drug-dependent antibodies are not found and involvement of the immune system is rarely proven. Other tests, such as the lymphocyte transformation test, that should be useful in the diagnosis of immune-mediated reactions, have, with a few exceptions, been disappointing. Such failures could be due to technical difficulties, such as failure to use or generate reactive metabolites of the drug. Some studies have successfully used urine from patients as a source of metabolites /101/. It is surprising that such a technique would be successful because most reactive metabolites that could act as haptens are unlikely to be

sufficiently stable to allow urine, which is often not even fresh, to be used as a source of reactive metabolite.

In the area of drug-induced agranulocytosis, there is relatively strong evidence that aminopyrine-induced agranulocytosis is due to drug-dependent antibodies /68,102,103/. In addition, the clinical course of the reaction is consistent with an antibody-mediated reaction. There is usually a delay of a week or more between starting aminopyrine and the development of agranulocytosis. In contrast, on subsequent exposure to the drug fever and neutropenia occur within a few hours. This argues for destruction of mature neutrophils, since if only neutrophil precursors were involved, it would take longer before their destruction would be reflected by an absence of circulating neutrophils. Several investigators have reported evidence for drug-dependent antibody-mediated neutropenia /62, 104-107/.

In contrast to aminopyrine-induced agranulocytosis, agranulocytosis associated with drugs such as clozapine and vesnarinone is significantly different. Although there is one report of evidence for antineutrophil antibodies in clozapine-induced agranulocytosis /108/, the evidence is not strong, and we have not been able to detect convincing evidence of antineutrophil antibodies even when we used reactive metabolites of the drugs to modify neutrophils. In addition, although reexposure of patients with a history of clozapine-induced agranulocytosis led to reoccurrence of agranulocytosis, the lag between restarting the drug and the development of agranulocytosis was, on average, 14 weeks /109/. Although the target of clozapine-induced agranulocytosis is the bone marrow and there will be a delay of a week or two before the absence of neutrophil precursors will lead to an absence of circulating neutrophils, the observed delay is much too long to be typical of an amnestic response of the immune system. We have found that the reactive intermediate generated by the reaction of clozapine with hypochlorous acid is much more toxic to neutrophils and neutrophil precursors from a patient with clozapine-induced agranulocytosis than to cells from controls; however, it will require the study of many more patients before we will know whether this is a consistent finding. In addition, we have no idea what the basis for this difference in sensitivity to the reactive metabolite might be because the characteristics of the reaction are not what would be expected for any simple mechanism. It is probably wise not to limit investigation into the mechanisms of idiosyncratic drug reactions to immune-mediated mechanisms.

4. INVOLVEMENT OF LEUKOCYTE-GENERATED METABOLITES IN THE TREATMENT OF INFLAMMATION

Several of the drugs that are associated with drug-induced agranulocytosis also have antiinflammatory properties, especially in diseases in which the inflammation is believed to be due to neutrophils. For example, dapsone is very effective for the treatment of dermatitis herpetiformis which is a disease of unknown etiology in which neutrophils infiltrate the skin and cause vesicles and severe itching /29,110,111/. Sulfapyridine is also effective in this disease. Dapsone also has activity in the treatment of Brown Recluse spider bites /112/ and rheumatoid arthritis /113,114/. More recently evidence was found that it may decrease the incidence of Alzheimer's disease in patients chronically treated with the drug /115/. The hypothesis that arose to explain this possible association is that Alzheimer's disease is due to destruction of neurons by microglial cells which are analogous to macrophages found in the rest of the body. Inhibition of these cells in a manner similar to that observed in dermatitis herpetiformis would therefore have a protective effect. The use of other antiinflammatory drugs, such as indomethacin, was also reported to decrease the incidence of Alzheimer's disease.

The exact mechanism by which dapsone and sulfonamides exert their effects on neutrophils is not known. Stendahl first reported that dapsone inhibited myeloperoxidase but had no effect on neutrophil migration /116/. This inhibition appears to be due to reduction of the oxidized form of the enzyme, compound I, to an inactive form of the enzyme, compound II /117/. Later it was reported that dapsone could affect neutrophil migration, but it was dependent upon which chemotactic agent was used /118/. It was also reported that dapsone inhibits neutrophil adherence /119,120/.

Sulfasalazine has been used for the treatment of inflammatory bowel disease for many years and more recently it has been shown to have therapeutic effects in rheumatoid arthritis and ankylosing spondylitis /121,122/. It was found to be metabolized in the gut to sulfapyridine and 5-aminosalicylic acid. The major therapeutic effect on inflammatory bowel disease appears to depend on the 5-aminosalicylic acid although sulfapyridine may also make a contribution /123,124/. 5-Aminosalicylic acid is now often used by itself for the treatment of inflammatory bowel disease because its use is associated with a lower incidence of toxicity than sulfasalazine. Neutrophils are believed to play a major role in the pathology of inflammatory bowel disease and

5-aminosalicylic acid is a scavenger of the oxidants generated by activated neutrophils /125/.

Propylthiouracil is used to inhibit the synthesis of thyroxine in patients who have hyperthyroidism. Thyroxine is synthesized by thyroid peroxidase which is a peroxidase similar to myeloperoxidase. Propylthiouracil is oxidized to reactive metabolites by both peroxidases and this metabolism may be involved in the inhibition of the peroxidases, either because it is a competitive inhibitor of the enzyme or because the reactive metabolites formed inhibit the enzyme /126,127/. Propylthiouracil is also known to inhibit reactive oxygen production by neutrophils /128/. Propylthiouracil was found to increase survival in patients with alcoholic hepatitis /129/ which is characterized by a hepatic infiltrate of neutrophils, and it also has a protective effect in acetaminophen-induced hepatotoxicity in which part of the mechanism appeared to be to prevent the inflammatory response /130/. Therefore metabolism of propylthiouracil by neutrophils may be related to both toxic and therapeutic effects of the drug.

Phenylbutazone is used primarily as a non-steroidal anti-inflammatory drug, and, as mentioned earlier, it is oxidized by the myeloperoxidase system to several metabolites. It also inhibits myeloperoxidase, at least in part, due to its ability to convert compound I of myeloperoxidase to the inactive form, compound II /117/. The activity of many other drugs, such as that of penicillamine against arthritis, may also involve oxidation by activated leukocytes.

Based on such evidence, it is reasonable to hypothesize that reactive metabolites generated by leukocytes can have anti-inflammatory properties and also lead to some types of toxicity. The challenge will be to test this hypothesis, and, where it is found to be correct, to determine the mechanism by which these reactive metabolites exert their effects.

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