Metabolism of Clozapine† by Neutrophils
Possible Implications for Clozapine-Induced Agranulocytosis

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Summary
Many types of adverse drug reactions appear to involve reactive metabolites which, by their very nature, usually have short biological half-lives. Therefore, reactive metabolites formed by neutrophils, or neutrophil precursors in the bone marrow, would seem more likely to be responsible for drug-induced agranulocytosis than metabolites formed in the liver. We have found that several drugs associated with a relatively high incidence of drug-induced agranulocytosis are metabolised by activated neutrophils to chemically reactive metabolites. In preliminary experiments with clozapine, we found that clozapine was metabolised by neutrophils. It also reacted with hypochlorous acid, the principal oxidant generated by neutrophils, to form a reactive intermediate. This intermediate has a half-life of 1 minute in buffer, but reacts very rapidly with glutathione. We believe that this intermediate is a nitrenium ion. Such a metabolite could be responsible for clozapine-induced agranulocytosis, either by direct toxicity or through an immune-mediated mechanism.

1. Idiosyncratic Drug Reactions

Idiosyncratic drug reactions represent a significant medical problem because they are virtually impossible to prevent and can be life-threatening (Mathews 1984). They also pose difficulty for the development of new drugs because they are not detected by animal testing and are often not apparent until the drug has been released on the market (Bakke et al. 1984). Very little is known about the mechanism of these reactions.

The characteristics of idiosyncratic reactions suggest that they are not due to direct cytotoxicity. Specifically, the reaction does not occur in most people or in animals, no matter how high the dose of the drug (Park et al. 1987; Pohl et al. 1988; Uetrecht 1990). In addition, there is usually a delay of more than a week between starting the drug and the onset of the adverse reaction; however, rechallenge of a patient who has experienced an idiosyncratic drug reaction usually results in an immediate reaction.

One common type of idiosyncratic reaction is agranulocytosis. There is strong evidence that agranulocytosis due to aminophenazone (aminopyrine) is mediated by an antibody (Barrett et al. 1976; Goudemand et al. 1976; Moeschlin & Wagner 1952). This evidence includes the observation that serum from patients with acute aminophenazone-induced agranulocytosis caused comple
ment-dependent agglutination of normal neutrophils. One investigator went so far as to infuse blood from a patient into himself, and this caused an immediate drop in the recipient's neutrophil count. Even though the observed antibodies are directed against mature neutrophils, the bone marrow is affected in almost all cases of aminophenazone-induced agranulocytosis as well as in agranulocytosis caused by other drugs. Antibodies against neutrophils have also been found in many other cases of drug-induced agranulocytosis; however, in most cases there is no proof that these antibodies are pathogenic (Fibbe et al. 1986; Petz & Fudenberg 1975; Pisciotta 1978; Weitzman & Stossel 1978). In many other studies of drug-induced agranulocytosis, no evidence of antineutrophil antibodies was found, and most negative studies were probably never published.

In contrast to aminophenazone-induced agranulocytosis, the agranulocytosis associated with the use of chlorpromazine has more characteristics of direct cytotoxicity (Pisciotta 1969; Pisciotta et al. 1958). Specifically, the onset of agranulocytosis is more gradual, and the time-course is not accelerated on re-exposure. Pisciotta has shown that chlorpromazine is toxic to bone marrow cells and appears to be more toxic to bone marrow cells from patients who have had chlorpromazine-induced agranulocytosis (Pisciotta 1971).

During the last several decades, accumulated evidence has shown that many types of toxicity are due to reactive intermediates (Nelson 1982; Nelson & Pearson 1990). A few drugs such as penicillin and several of the anticancer agents are chemically reactive, but most reactive species arise from metabolism of the drug. Reactive intermediates can cause either direct cytotoxicity or immune-mediated reactions by acting as haptenes, as in penicillin-induced hypersensitivity (Parker 1982) or halothane-induced hepatic necrosis (Kenna et al. 1988). Most drug metabolism occurs in the liver; however, by their very nature, most reactive metabolites have very short biological half-lives and would not be expected to reach the bone marrow if formed elsewhere. It would seem that reactive intermediates formed by neutrophils or other cells in the bone marrow would be more likely to lead to agranulocytosis than reactive metabolites formed outside the bone marrow.

2. Drug Metabolism by Neutrophils

A major function of neutrophils and monocytes is the destruction of pathogenic organisms (Klebanoff & Clark 1978). When neutrophils encounter such organisms, they are activated, and there is a large increase in their oxygen uptake. This is referred to as a respiratory burst. The oxygen is converted to superoxide by the enzyme NADPH oxidase. The superoxide is in turn converted to hydrogen peroxide. Simultaneously, granules in the neutrophils release myeloperoxidase and several other agents. Hydrogen peroxide converts myeloperoxidase to its oxidised form called compound I (Harrison et al. 1980). Compound I is a strong oxidant, and it appears that it can metabolise drugs. Chloride, the major substrate of myeloperoxidase oxidised by compound I, is converted to hypochlorous acid (Weiss 1989) which is a strong oxidant and an active bactericidal agent used to kill bacteria in municipal water supplies.

Most of the superoxide formed by neutrophils is converted to hypochlorous acid. These systems are summarised in figure 1. In addition to mature neutrophils, some neutrophil precursors in the bone marrow are also capable of a significant respiratory burst and release myeloperoxidase on activation (Bainton et al. 1971; Zakhireh & Root 1979). We have demonstrated that many drugs associated with drug-induced agranulocytosis are oxidised by activated neutrophils to reactive metabolites by the combination of myeloperoxidase, hydrogen peroxide and chloride ion or simply by hypochlorous acid (Uetrecht 1990). Specifically, primary amines such as procainamide (Uetrecht et al. 1988a; Uetrecht & Zahid 1991), dapsone (Uetrecht et al. 1988b) and sulphamides (Cribb et al. 1990) are oxidised to hydroxylamines, nitroso metabolites and chloramines. The chloramines are not observed in the neutrophil incubations because they react very rapidly with the cells. In addition, propylthiouracil is metabolised by a series of reactions to a sulphonic acid, which is chemically reactive,