The Mechanisms of Clozapine-Induced Neutropenia

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Despite its severe adverse effects, such as agranulocytosis, clozapine is the primary treatment for treatment-resistant schizophrenia. Clozapine is the gold standard for treatment-resistant schizophrenia. It was discovered over a half-century ago by researchers at Wander AG, a Swiss pharmaceutical company. After a decade, the use of clozapine began to fade away due to its alarming side effect but was re-introduced in the year 1989 for the treatment of refractory psychosis. Numerous comparative studies between clozapine and other antipsychotics have reported superior efficacy of clozapine in treatment-resistant schizophrenia.

clozapine

1. Introduction

Clozapine is the gold standard for treatment-resistant schizophrenia [1]. It was discovered over a half-century ago by researchers at Wander AG, a Swiss pharmaceutical company [2][3]. After a decade, the use of clozapine began to fade away due to its alarming side effect but was re-introduced in the year 1989 for the treatment of refractory psychosis [3]. Numerous comparative studies between clozapine and other antipsychotics have reported superior efficacy of clozapine in treatment-resistant schizophrenia [4][5].

Albeit its robust efficacy, clozapine is underutilized, with less than one-fifth of clozapine-eligible candidates receiving the treatment [2]. The use is limited due to various adverse effects, including agranulocytosis, a severe form of neutropenia characterized by neutrophil counts of less than 0.5×10^9/L [6]. The incidence of agranulocytosis varies across countries, ranging from 0.21 to 0.8% [6]. The mechanisms of clozapine-induced neutropenia and agranulocytosis are not well-understood but may be partly attributable to the selective effect of clozapine on stromal cells and neutrophils. When clozapine is bioactivated to cytotoxic metabolites, cell death is promoted secondary to oxidative stress and haptenation [7][8][9].

The establishment of a clozapine monitoring system allows for early detection of severe agranulocytosis [10], enabling timely intervention to be administered and thus reducing agranulocytosis incidence and mortality rates [11]. Although the established monitoring system has been successfully conducted for many years, the global pandemic of coronavirus disease 2019 (COVID-19) has forced significant adjustments to ensure clozapine treatment continuation while reducing COVID-19 exposure to patients. A consensus on clozapine was published to guide clinicians with focus on the frequency of ANC monitoring, clinical assessment, and dose changes [12]. In terms of ANC monitoring, the ANC frequency can be reduced to three monthly if the patient has been on clozapine

treatment for more than a year with no history of ANC of less than 2000/μL (or less than 1500/μL if the patient has history of benign ethnic neutropenia), and no practical or safe access to ANC testing [12].

2. The Mechanisms of Clozapine-Induced Neutropenia

Clozapine has been reported to exert initial bone marrow stimulation with subsequent risk of neutropenia, particularly between 6–18 weeks of treatment [13][14]. A neutrophil kinetic study demonstrated a gradual, but significant increase in blood neutrophil levels [14][15] with a concurrent increase in the granulocyte colony-stimulating factor (G-CSF) at three and six hours following clozapine administration [14]. These findings indicate kinetics of neutrophils are G-CSF-dependent. Interestingly, the clozapine effect is only restricted to neutrophils but not lymphocytes [14][15]. These findings were further supported by clinical findings that showed a transient elevation of neutrophil levels in a cohort of 100 patients in the first few weeks of clozapine treatment [16]. This transient elevation in the neutrophil count subsequently normalizes, however in a smaller cohort of patients, clozapine eventually induced neutropenia or agranulocytosis. The exact pathology underlying to this phenomenon is not fully elucidated, nonetheless several mechanisms have been proposed and a significant genetic predisposition has been observed.

One of the mechanisms that was initially proposed is related to the direct toxicity of clozapine metabolites to the bone marrow stromal cells, in particular the immature neutrophil subpopulation. It is worth noting that, clozapine in its natural form itself is cytotoxic, but only at supratherapeutic levels [9]. The metabolism of clozapine forms at least three different metabolites with distinct cytotoxicity levels (**Figure 1**). Clozapine *N*-oxide is a non-toxic metabolite, while *N*-desmethylclozapine is a less toxic metabolite than clozapine. Both are the products of a cytochrome P450 enzyme (CYP) metabolism, particularly *CYP3A4* and *CYP1A2* [17].

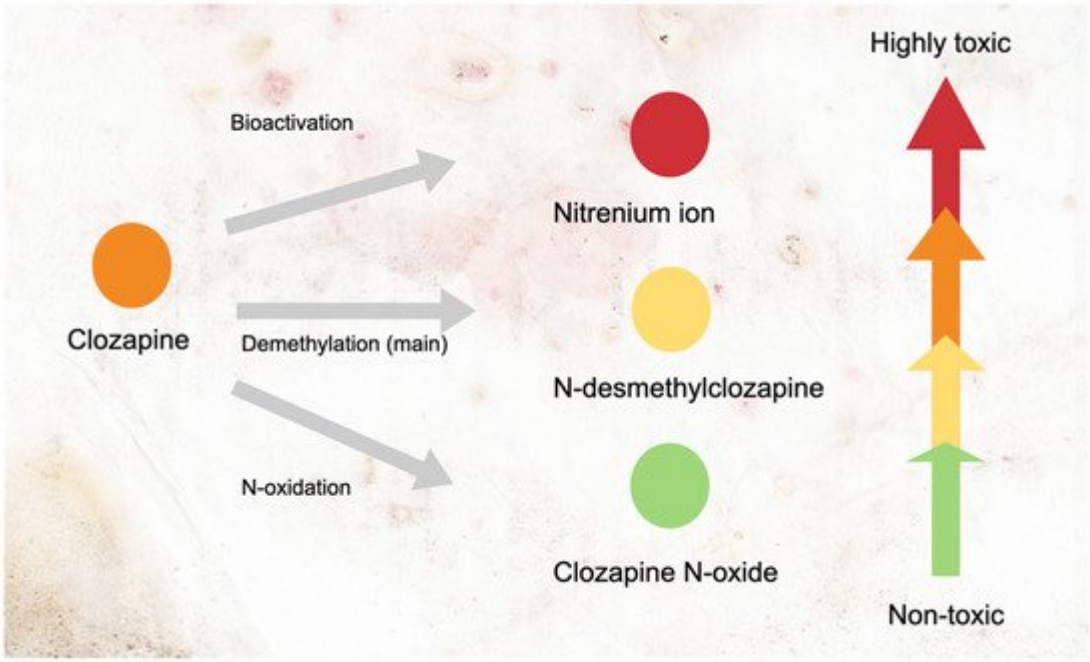

Figure 1. Metabolism of clozapine into metabolites and its cytotoxicity levels. Clozapine is primarily metabolized by cytochrome P450 (CYP) into desmethylclozapine. Another product of CYP metabolism is clozapine *N*-oxide, a non-toxic and inactive metabolite. Some clozapine is converted into nitrenium ions by hypochlorous acid (HOCl), the oxidant found in the activated neutrophils. The arrows with colors indicate the cytotoxicity levels of each compound in increasing order.

Nitrenium ion is a reactive and toxic metabolite of clozapine produced by the interaction of clozapine with hypochlorous acid (HOCl), a primary oxidant found in the activated neutrophils (**Figure 2**) [18]. The formation of nitrenium ion (**Figure 3**) can also be catalyzed by a combination of horseradish peroxidase and hydrogen peroxide (H_2O_2), as well as a combination of myeloperoxidase, H_2O_2, and chloride ion [8][9][18][19]. Interestingly, nitrenium ion formation is specific to clozapine. Utilizing the same metabolizing system (horseradish peroxidase and hydrogen peroxide) for other antipsychotics such as olanzapine and risperidone did not produce toxic metabolites [7]. Nitrenium ion has the ability to form a covalent bond with neutrophils, forming neutrophil haptenation which promotes apoptosis via tyrosine kinase activation [9][19].

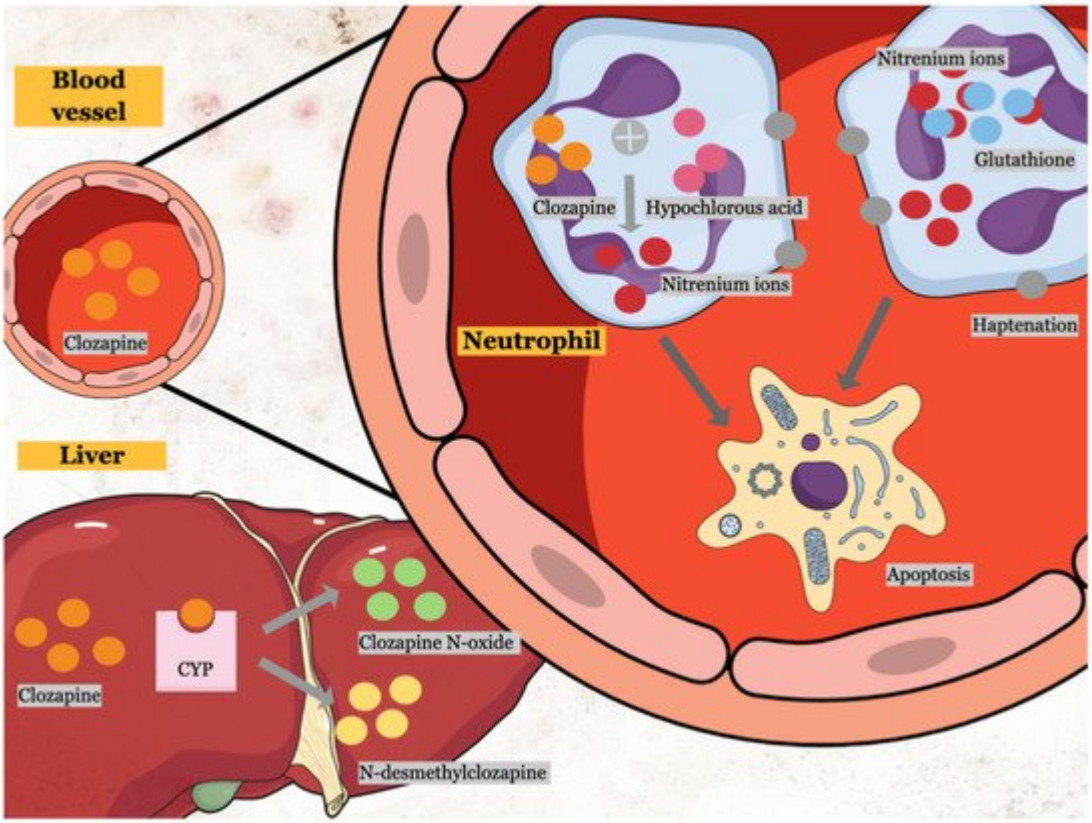

Figure 2. Mechanism of clozapine-induced neutrophil toxicity. Clozapine is metabolized by cytochrome P450 (CYP) into clozapine *N*-oxide and N-desmethylclozapine in the liver. Moreover, clozapine can react with hypochlorous acid in the neutrophils to form nitrenium ions, which later can form haptenation on the membrane surface, promoting apoptosis. Nitrenium ions can be detoxified by antioxidants such as glutathione (GSH) to form non-toxic GSH conjugates. Excessive use of GSH can lead to depletion, increasing cellular oxidative stress, resulting in cell death.

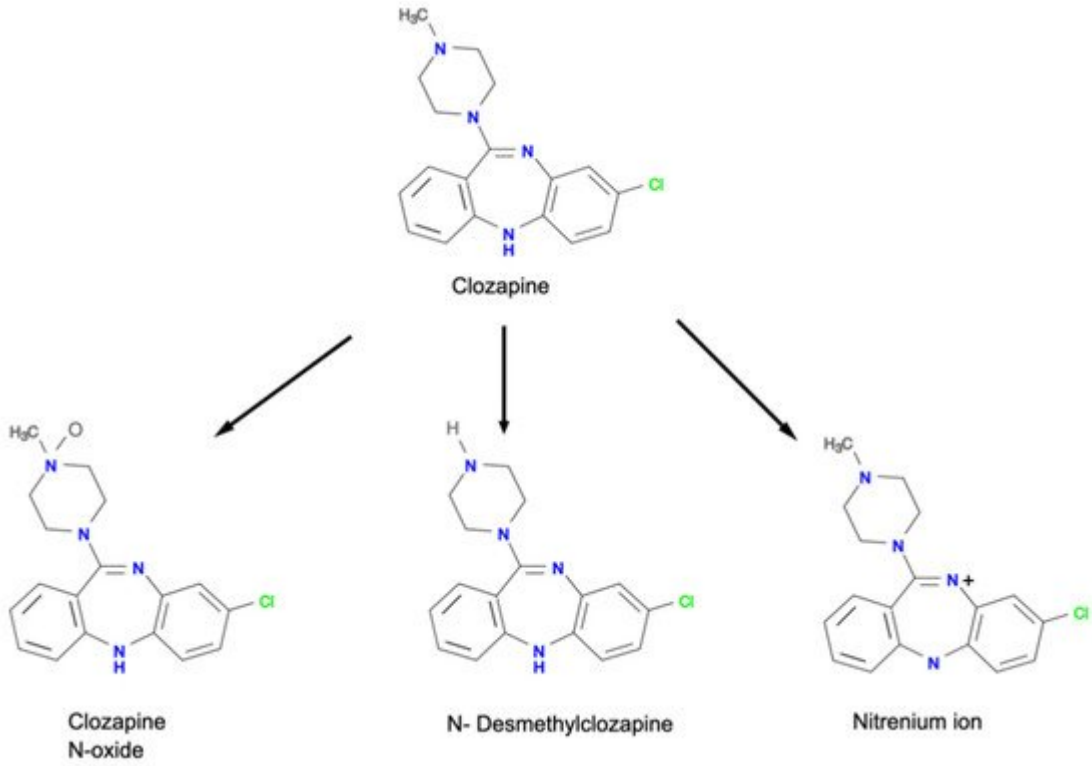

Figure 3. Chemical structures of clozapine and nitrenium ion (adapted from Pirmohamed and Park [20]).

Antioxidants play a pivotal role in the detoxification of toxic metabolites. Glutathione (GSH), an antioxidant, detoxifies nitrenium ions via conjugation [19]. Other exogenous antioxidants such as ascorbic acid, catalase, and N-acetylcysteine can also reduce clozapine-mediated cytotoxicity in stromal cells and neutrophils, further supported by the mechanism of neutropenia of the drug via oxidative stress [7][8].

Although the neutropenic effect of clozapine is consistently observed in in vitro studies, the prevalence of neutropenia and agranulocytosis in patients receiving clozapine is considerably low globally. The advancement of pharmacogenomics technologies has allowed the determination of genetic factors at molecular levels. Studies from few populations have reported significant associations of genetic polymorphisms in human leukocyte antigen (HLA), ABCB1 drug transporter, and glutathione S-transferase with clozapine-induced neutropenia [21][22]. Moreover, genetic polymorphisms in HLA, ABCB1, and NRH-quinone oxidoreductase 2 have been found to demonstrate significant associations with clozapine-induced agranulocytosis [21][22][23]. Additionally, another recent study also identified the *STARD9* and *UBAP2* gene variants in a cohort of patients with clozapine-induced neutropenia. *STARD9* is a mitotic kinesin, essential for pericentriolar matrix cohesion in mitosis. Depletion of *STARD9* promotes mitotic arrest secondary to fragmentation and dissociation of pericentriolar material, resulting in apoptosis in various cancer cells [24]. The *UBAP2* gene regulates ubiquitination, an essential process involved in cell proliferation and survival [25]. The combined effect of the gene variants and the direct toxic effect by the clozapine's metabolites promotes neutrophil toxicity [26]. However, given the statistical and methodological limitations, further studies are required to justify the role of genetic variants in clozapine-induced neutropenia and agranulocytosis.

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