Plasma Clozapine and Desmethylclozapine Levels in Clozapine-Induced Agranulocytosis

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Clozapine may produce agranulocytosis in 1–2% of patients treated with it for 4 weeks or longer. Three mechanisms have been suggested: a direct toxic effect of metabolite of clozapine, an immunologic mechanism or a combination of both. N-desmethylclozapine, the major metabolite of clozapine, has been reported to be more toxic than clozapine itself (Gerson et al., 1994). In this study, plasma levels of clozapine and desmethylclozapine were measured in five patients who developed agranulocytosis. The levels of both parent compound and metabolite were within the range found in other patients and below the toxic range. If a toxic mechanism is involved in clozapine-induced agranulocytosis, an additional vulnerability factor must be important.

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KEY WORDS: *Clozapine; N-desmethylclozapine; Agranulocytosis; Schizophrenia*

Clozapine has been reported to be more effective than typical neuroleptic drugs for 30% to 60% of patients with neuroleptic-resistant schizophrenia (Kane et al. 1988; Meltzer et al. 1993). It is currently not recommended for use in patients with schizophrenia who respond to typical neuroleptic drugs because of a 1% to 2% risk of granulocytopenia, or agranulocytosis (Krupp and Barnes 1989; Alvir et al. 1993). These side effects of clozapine have been attributed to the destruction of white blood cell (WBC) precursors by an immune mechanism (Pisciotta et al. 1992), or a direct toxic effect of clozapine, or one of its metabolites (Gerson et al., 1994). With regard to the latter theory, it has been suggested that N-desmethylclozapine, the major clozapine metabolite, might be the cause of agranulocytosis because it is more toxic to WBC precursors than clozapine itself (Gerson and Meltzer 1992; Gerson et al. 1994). A free-radical metabolite of clozapine has also been suggested to be of importance for clozapine-induced agranulocytosis (Fischer et al. 1991). The purpose of this study was to measure plasma clozapine and desmethylclozapine levels in patients who developed agranulocytosis and to compare them with those who did not.

METHODS

Plasma levels of clozapine and desmethylclozapine were determined in five patients with schizophrenia (three males and two females) who developed agranulocytosis (WBC $\leq$ 500/mm^3). This was a consecutive series of patients in whom plasma clozapine levels were available. The duration of treatment with clozapine prior to the onset of agranulocytosis was 217–580 (232 $\pm$ SD 230) days. The dose of clozapine at the time agranulocytosis developed was 200–675 mg/day (mean 465 $\pm$ 185 mg/day). The mean age of the patients was 32.4 years $\pm$ 8.0 years. Four of the five (80%) patients who develop agranulocytosis were caucasian; one was black. Fifty-four of the 59 patients (34.9 $\pm$ 10.6 years old) who did not develop agranulocytosis were also Caucasian; the other four were black.

Two blood samples for determination of plasma levels of clozapine and its metabolite were obtained the

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Table 1. Plasma Clozapine and Norclozapine Levels in Schizophrenic Patients

Patient No. (Gender)	Clozapine Daily Dosage (mg)			Plasma Levels (ng/ml)	
	On Day of Onset of Reduced WBC	On Day of Sampling	Interval (days)	Clozapine	N-desmethylclozapine
1 (M)	600	600	0	433	270
2 (F)	200	200	0	237	106
3 (F)	450	450	1[a]	139	123
4 (M)	675	600	99[b]	218	189
5 (M)	400	350	156[b]	427	233

Interval between day of onset of reduced WBC and day of sampling (days).
[a] Samples drawn after the onset of reduced WBC.
[b] Samples drawn before the onset of reduced WBC.

day agranulocytosis was first noted, within 6 hr of the last dose; another was obtained the day after the discovery of agranulocytosis, 31 hrs after the last dose. Plasma was obtained day 99 and day 156 *before* agranulocytosis detection in two patients, 12 hr after the last dose. However, daily dosage at the times these two samples were drawn and the detection of agranulocytosis differed only by 75 mg and 50 mg, respectively. Plasma clozapine and desmethylclozapine levels were available from 59 patients who did not develop agranulocytosis (Hasegawa et al. 1993). These samples were obtained approximately 12 hours to 16 hours after the evening dose. The plasma concentrations of clozapine and desmethylclozapine was determined in duplicate by high pressure liquid chromatography in different assays (Hasegawa et al. 1993). The coefficient of variation was $\leqslant$ 6.8% (clozapine), and 6.7% (desmethylclozapine) within assays, $\leqslant$ 9.5% (clozapine) and 14.4% (desmethylclozapine) between assays.

RESULTS AND DISCUSSION

Plasma clozapine and desmethylclozapine level for the five subjects (two females, three females) who developed agranulocytosis are given in Table 1. The mean clozapine (291 $\pm$ SD 132 ng/ml) and desmethylclozapine (184 $\pm$ 70 ng/ml) concentrations are in the same range observed in 59 patients who did not develop granulocytopenia or agranulocytosis (371 $\pm$ 257 ng/ml (range 39 ng/ml to 939 ng/ml) and 274 $\pm$ 196 ng/ml (range 19 ng/ml to 885 ng/ml), respectively (Hasegawa et al. 1993). The clozapine and desmethylclozapine levels in the two groups were not significantly different. Although two samples for clozapine levels were taken long before the day of detection of reduced WBC count, both plasma clozapine and desmethylclozapine levels are significantly correlated with clozapine dose, and are fairly stable in most subjects over long periods at the same dose (Hasegawa et al. unpublished data; Perry et al. 1991).

The concentration (IC_{50} value) of desmethylclozapine toxic to the colony forming unit-granulocytomacrophage cells has been reported to be 2,500 ng/ml (Gerson and Meltzer 1993), a concentration 9-fold to 24-fold higher than the trough concentrations measured in the plasma of patients who developed agranulocytosis in this study; however, peak plasma clozapine concentrations following absorption of a 200 mg oral dose at steady-state have been estimated to be 2-fold higher than the trough levels (Cheng et al. 1988). Plasma desmethylclozapine levels are highly correlated with clozapine levels (Cheng et al. 1988) and should also be twice as high as trough levels, assuming there are no differences in clearance rates between clozapine and desmethylclozapine. It is, therefore, possible that plasma levels of desmethylclozapine in at least two of the individuals (patients one and five) might approach 25% [or higher] of the levels that cause toxicity to colony forming unit-granulocytomacrophage cells; however, trough levels of desmethylclozapine greater than 500 ng/ml were present in seven patients who did not develop agranulocytosis (Hasegawa et al. 1993) and have been measured in 45 other patients who did not develop agranulocytosis (Meltzer et al. unpublished data).

These considerations suggest that if desmethylclozapine is responsible for mediating clozapine-induced agranulocytosis through a direct toxic mechanism, it is necessary to postulate significant variation in vulnerability to its toxic effect in different patients. This might be intrinsic to granulocyte precursor cells, or related to an immunologic mechanism that contributed to agranulocytosis; thus, measuring trough plasma levels of clozapine or desmethylclozapine may not predict the development of agranulocytosis without additional information about vulnerability factors that have not yet been identified.

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