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# REVERSIBLE METABOLISM OF CLOZAPINE AND CLOZAPINE N-OXIDE IN SCHIZOPHRENIC PATIENTS

WEN-HO CHANG[1], SHIH-KU LIN[1], HSIEN-YUAN LANE[1], FU-CHUAN WEI[2], WEI-HERNG HU[1], YW FRANCIS LAM[3], AND MICHAEL W. JANN[4]

[1]Laboratory of Biological Psychiatry, Taipei City Psychiatric Center, Taipei, Taiwan, [2]Hung-Chi Psychiatric Hospital, Taipei, Taiwan, [3]Dept. of Pharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA, [4]Depts. Of Pharmaceutical Sciences and Pharmacy Practice, Mercer University, Southern School of Pharmacy, Atlanta, GA, USA.

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## Abstract

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1. To characterize the interconversion process between clozapine and its metabolite clozapine N-oxide (CNO), eight healthy male schizophrenics were administered a single dose of clozapine or CNO in a randomized crossover manner.
2. Using a general pharmacokinetic model for the interconversion process, the mean total clearances of clozapine and CNO were 28.45 L/hr and 45.30 L/hr, respectively. These values were similar to the values obtained by the usual model-independent method of pharmacokinetic analysis.
3. When administered clozapine, mean CNO plasma concentrations of 17.7 $\pm$ 16.4 ng/ml were slightly lower than the other clozapine metabolite - desmethylclozapine (DCLOZ) plasma levels of 24.4 $\pm$ 8.6 ng/ml at the 12 hour time point. When CNO was administered, plasma concentrations at the 12 hour time point of clozapine were twice the amount of CNO (28.1 $\pm$ 8.9 ng/ml vs 14.4 $\pm$ 8.8 ng/ml).
4. DCLOZ plasma concentrations were detected in all patients upon clozapine administration. Upon CNO administration, only one patient had detectable plasma DCLOZ levels.
5. The interconversion process of clozapine and CNO could partially account for the wide interpatient variability reported for clozapine plasma concentrations in schizophrenic patients.

Key words: clozapine, clozapine N-oxide, desmethylclozapine, plasma concentrations, reversible metabolism.

Abbreviations: Area Under the Plasma Concentration Time Curve [AUC], Clearance [CL], Clozapine [CLZ], Clozapine N-oxide [CNO], Desmethylclozapine [DCLOZ], Exposure Enhancement [EE], Enterohepatic Cycling [EHC], Elimination rate constant [Kel], High Performance Liquid Chromatography [HPLC], Mean Residence Time [MRT], Recycling Factor [RF], fraction of dose conversion [RHO].

## Introduction

Clozapine is a typical neuroleptic drug used in the treatment of schizophrenia. Clozapine differs from other

typical neuroleptics. Tardive dyskinesia, a severe adverse side effect produced by typical neuroleptics has not been reported to occur with clozapine. Other extrapyramidal side effects appear to occur less frequently and are less severe in patients who receive clozapine compared to other typical neuroleptics. Also, clozapine was shown to improve positive and negative symptoms of schizophrenia in some patients who did not respond to typical neuroleptics. These refractory schizophrenic patients failed to respond to at least three structurally different neuroleptic agents prior to a clozapine trial. Clinical efficacy may be related to plasma clozapine concentrations and response rates have ranged from 30-100% with short-term therapy. During longer treatment, it was reported that 60% of patients who were unresponsive to previous neuroleptic therapy responded to clozapine (Wagstaff and Bryson, 1995). Despite these major advantages with clozapine, its widespread use has been abated due to the 1-2% incidence of agranulocytosis (Jann, 1991; Hasegawa et al, 1994). It has been recommended that in Taiwan and the United States that patients obtain a weekly complete blood count (CBC) with differential while receiving this drug. Preliminary studies comparing plasma concentrations of clozapine in Asians and Caucasians have reported higher concentrations in Asian given comparable clozapine dosages (Matsuda, 1996; Chong et al, 1997). However, comparison of clozapine metabolites have not yet been reported.

The therapeutic range of clozapine plasma concentrations remains to be firmly established (Jann et al, 1993; Wagstaff and Bryson, 1995). Perry et al (1991) measured clozapine and its desmethyl metabolite (desmethylclozapine) in refractory schizophrenics (N=29) and suggested a minimum threshold of 350 ng/ml of clozapine or a total of 450 ng/ml of clozapine plus desmethylclozapine was needed to achieve a therapeutic response. Similar findings were reported in refractory schizophrenics with plasma clozapine levels of 350 ng/ml to 420 ng/ml that distinguished responders from nonresponders (Potkin et al, 1994; Miller et al, 1994; Kronig et al, 1995). In a six week fixed dose 400 mg/day study with clozapine in refractory schizophrenic patients (N=61), it was reported that a minimum therapeutic response occurred in patients with plasma clozapine levels of 300 ng/ml. However, patients with plasma clozapine levels greater than 700 ng/ml had a diminished therapeutic response (Liu et al, 1996). The role of clozapine's metabolites in determining its clinical efficacy also remains to be investigated.

The disposition of clozapine in schizophrenic patients has been extensively evaluated in healthy volunteers and schizophrenic patients (Jann et al, 1993; Wagstaff and Bryson, 1995). The major metabolic pathways of clozapine involves the formation of two metabolites: desmethylclozapine and clozapine N-oxide shown in Fig 1. Both of these metabolites have been detected in plasma concentrations of patients treated with clozapine (Bondesson and Lindstrom, 1988). The desmethyl and N-oxide metabolite concentrations were lower than the parent drug and ranged from 20-30% and 10-50%, respectively. Two additional metabolite pathways of clozapine have been described where hydroxylated and protein-reactive metabolites are formed (Wagstaff and Bryson, 1995). Each of the four pathways involved with clozapine metabolism was suggested to be mediated by cytochrome P 450 (P450) isozyme systems. Interestingly, two of the metabolic pathways: the N-oxide and

the protein reactive metabolites are reported to be reversibly metabolized back to clozapine. The reaction with clozapine and clozapine n-oxide was preliminarily described with guinea pigs and man (Jann et al, 1994). Clozapine N-oxide 30 mg was administered to one patient and the area under the plasma level time curve (AUC) ratio for clozapine/clozapine N-oxide was reported to be 0.333. This finding would suggest that 1/3 of clozapine N-oxide could be converted back to clozapine in psychiatric patients. This study will attempt to characterize the reversible metabolic pathway of clozapine and clozapine N-oxide in schizophrenic patients.

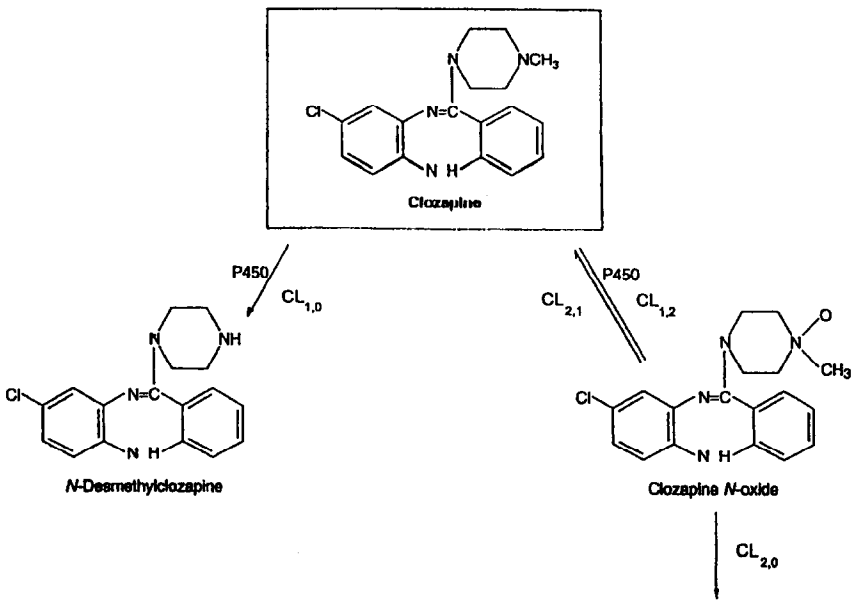


**Fig 1.** Disposition of clozapine and its metabolites: desmethylclozapine and clozapine N-oxide. Formation of these metabolites are influenced by the cytochrome P450 (P450) isozyme system. The parameters for the reversible metabolic process of clozapine and clozapine N-oxide are represented by the clearance ($CL_{1,2}$ and $CL_{2,1}$). Clozapine is converted to desmethylclozapine ($CL_{1,0}$) and clozapine N-oxide is further metabolized ($CL_{2,0}$).

## Methods

### Subjects

Eight male schizophrenic patients who met DSM-III-R criteria for schizophrenia aged 34-50 years (mean 43.0 years); weight 42.5-71 kg (mean 56.1 kg) were recruited into a randomized crossover study and given a single 100 mg dose of clozapine or clozapine N-oxide separated by a two week interval. Informed consent was obtained from all patients. The facility's Institutional Review Board approved the conduct of this study. Prior to drug administration, patients did not receive any oral neuroleptic medications for at least three weeks and depot injections for at least four months. Clozapine and clozapine N-oxide was obtained from Sandoz Pharmaceuticals (Switzerland). After an overnight fast, drugs were administered at 8:00 a.m. in powdered form wrapped in a piece of wrapped wafer.

**Assessments**

Blood samples were obtained before and after drug administration at 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 5, 6, 8, 12 and 24 hours. Clozapine, clozapine N-oxide, and desmethylclozapine were assayed by high performance liquid chromatography (HPLC) with UV detection (254 nM). The lower limit of detection was 10 ng/ml for clozapine, DCLOZ and CNO. The intra-assay and inter-assay coefficient of variation was <10% for all compounds (Weigmann and Hiemke, 1992; Chung et al, 1993).

**Pharmacokinetic and Data Analysis**

Pharmacokinetic parameters of clozapine, clozapine N-oxide and desmethylclozapine were determined for each patient by non-compartmental analysis. The area under the curve (AUC) was determined by log trapezoidal rule and extrapolated to infinity by dividing the last measured plasma concentration value by Kel. The terminal elimination rate constant is defined as Kel and is estimated by using at least the last three data points on the terminal log linear phase of the plasma concentration-time curve. Half-life was calculated by dividing the natural logarithm of 2 by Kel. The clearance (CL, L/hr) relative bioavailability was calculated by the equation CL/F = Dose/AUC, where F is the oral bioavailability of clozapine.

**Reversible Metabolism Model**

The interconversion model for clozapine (CLZ) and clozapine N-oxide (CNO) and their parameters were determined similar to the prednisone/prednisolone interconversion process shown in Fig 1 (Ebling and Jusko, 1986). The total clozapine clearance (L/hr) was calculated to be $CL_{1,2} + CL_{1,0}$ and the total clozapine N-oxide clearance (L/hr) to be $CL_{2,1} + CL_{2,0}$. The following individual clearances were determined by the following equation:

$$CL_{1,0} = \frac{D^{CLZ} \cdot AUC^{CNO}_{CNO} - D^{CNO} \cdot AUC^{CLZ}_{CNO}}{AUC^{CLZ}_{CLZ} \cdot AUC^{CNO}_{CNO} - AUC^{CLZ}_{CNO} \cdot AUC^{CNO}_{CLZ}}$$

$$CL_{2,0} = \frac{D^{CNO} \cdot AUC^{CLZ}_{CLZ} - D^{CLZ} \cdot AUC^{CNO}_{CLZ}}{AUC^{CLZ}_{CLZ} \cdot AUC^{CNO}_{CNO} - AUC^{CLZ}_{CNO} \cdot AUC^{CNO}_{CLZ}}$$

$$CL_{1,2} = \frac{D^{CNO} \cdot AUC^{CLZ}_{CNO}}{AUC^{CLZ}_{CLZ} \cdot AUC^{CNO}_{CNO} - AUC^{CLZ}_{CNO} \cdot AUC^{CNO}_{CLZ}}$$

$$CL_{2,1} = \frac{D^{CLZ} \cdot AUC^{CNO}_{CLZ}}{AUC^{CLZ}_{CLZ} \cdot AUC^{CNO}_{CNO} - AUC^{CLZ}_{CNO} \cdot AUC^{CNO}_{CLZ}}$$

where: $CL_{1,2}$ and $CL_{2,1}$ are the interconversion clearances, $CL_{1,0}$ and $CL_{2,0}$ are the clearances excluding the interconversion process of clozapine and clozapine N-oxide, respectively, from their central compartments. The subscript refers to the drug or metabolite measured in the plasma. The superscript refers to the drug or metabolite administered. D is the dose administered and AUC is the area under the plasma concentration time curve of the drug or metabolite. For example, $AUC^{CLZ}_{CNO}$ is the area under the plasma clozapine N-oxide concentration time curve when clozapine is administered.

The interconversion rates K p/m (p→m) and K m/p (p←m) were determined by:

$$K\ p/m = \frac{CL_{1,2} \cdot CL_{2,1}}{(CL_{2,1} \cdot CL_{2,0})^2} \qquad K\ m/p = \frac{CL_{1,2} \cdot CL_{2,1}}{(CL_{1,2} \cdot CL_{1,0})^2}$$

The terms $E_{eff\ 1,2}$ and $E_{eff\ 2,1}$ can be viewed as the efficiencies of $CL_{1,2}$ and $CL_{2,1}$ as true elimination pathways. These terms represent the probability of a molecule of converted drug or metabolite not being metabolized back to the original form and can be determined as:

$$E_{eff\ 1,2} = \frac{CL_{2,0}}{CL_{2,1} + CL_{2,0}} \qquad E_{eff\ 2,1} = \frac{CL_{1,0}}{CL_{1,2} + CL_{1,0}}$$

The RHO term is commonly used to describe the interconversion systems representing the fraction of the administered dose converted to the metabolite for the first time (Ebling and Jusko, 1986).

$$RHO\ p/m = \frac{CL_{1,2}}{CL_{1,2} + CL_{1,0}} \qquad RHO\ m/p = \frac{CL_{2,1}}{CL_{2,1} + CL_{2,0}}$$

The ratio of parent drug exposure in the presence or absence of back conversion represents the degree of conversation of exposure enhancement (EE) observed in reversible metabolism and can be calculated as follows:

$$EE = 1 + \frac{CL_{1,2} \cdot CL_{2,1}}{CL_{1,0} \cdot CL_{2,1} + CL_{2,0} \cdot CL_{1,2} + CL_{1,0} \cdot CL_{2,0}}$$

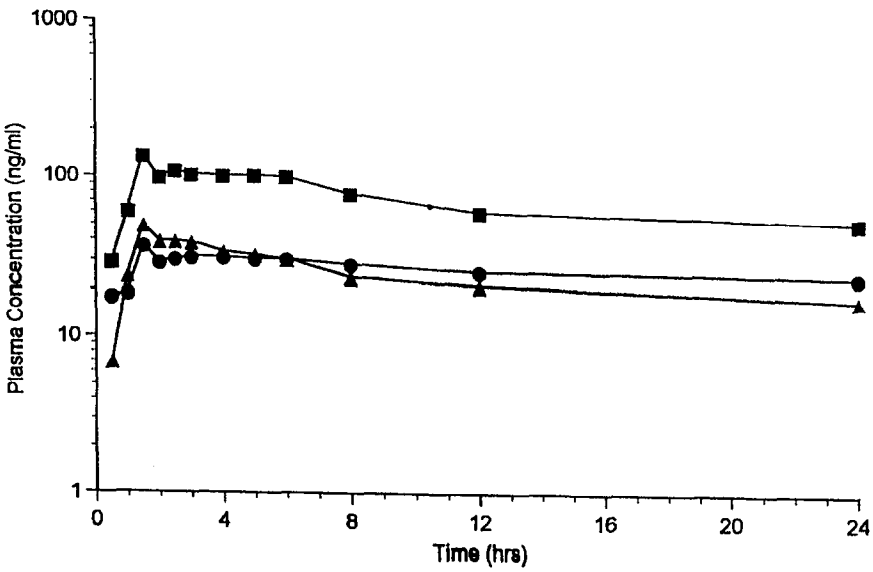


**Fig 2.** Plasma level time curves for clozapine (CLOZ, --■--), desmethylclozapine (DCLOZ, --●--), and clozapine N-oxide (CNO, --▲--) upon clozapine administration

## Results

### Clozapine and Metabolites Plasma Concentrations

The plasma level time curves for clozapine, clozapine N-oxide and desmethylclozapine when clozapine was administered to subjects with schizophrenia are featured in Fig 2. After clozapine was given, the lower lines represent the formation of clozapine N-oxide and desmethylclozapine which paralleled clozapine plasma levels. The plasma time curves for clozapine N-oxide and clozapine, when administered clozapine N-oxide, are presented in Fig 3. Again, the lower lines represent the formation of clozapine plasma concentrations, but desmethylclozapine was detected in only one patient. Although low amounts of clozapine plasma concentrations were detected quickly after clozapine N-oxide administration, clozapine plasma levels continued to increase up to 12 hours post-drug. At the 24 hour time period, plasma clozapine levels were slightly higher than clozapine N-oxide plasma concentrations.

### Pharmacokinetic Parameters of Clozapine and Its Metabolites

A summary of the pharmacokinetic parameters of clozapine and clozapine N-oxide are presented in Table 1. With both compounds, a wide interpatient variability was observed with clozapine, desmethylclozapine and clozapine N-oxide plasma concentrations. After clozapine administration, plasma concentrations of clozapine N-oxide and desmethylclozapine were detected in every patient. The mean AUC's of clozapine N-oxide and

desmethylclozapine were lower than clozapine and the mean AUC of clozapine N-oxide was slightly lower than the mean AUC of desmethylclozapine. The formation of clozapine N-oxide after clozapine administration occurred rapidly and almost as soon as clozapine plasma levels were detected. The maximal plasma concentrations (C max) of clozapine N-oxide were higher than desmethylclozapine. Plasma clozapine N-oxide levels were almost equivalent to desmethylclozapine plasma concentrations at 12 hours post-clozapine administration (C 12) and slightly less than at 24 hours (C 24). The remaining pharmacokinetic parameters of clozapine are shown in Table 1, whereas Ke, CL, and T½ could not be calculated for the metabolites.

The pharmacokinetic parameters of clozapine N-oxide (with clozapine N-oxide administration) are also presented in Table 1. The total mean AUC for clozapine when the N-oxide was administered was 1.96 times greater than the parent drug. The increased clozapine AUC indicates the continued drug accumulation and formation from the N-oxide compound. The time to maximal plasma concentrations (T max) of clozapine N-oxide occurred earlier (mean 2.1 hours) than clozapine (mean 3.4 hours) when given clozapine. Interestingly, the T max of clozapine when given the N-oxide agent occurred later with a mean time of 12.5 hours. The plasma concentrations of clozapine were greater than clozapine N-oxide at C 12 and C 24 hours post N-oxide administration. The remaining pharmacokinetic parameters of clozapine N-oxide were determined as the CL and Ke values of N-oxide were greater than clozapine by 60% and 45%, respectively. Correspondingly, a shorter elimination half-life of 57% was calculated for clozapine N-oxide compared to clozapine. Only one patient had detectable plasma concentrations of desmethylclozapine with only four data points shown in Fig 3.

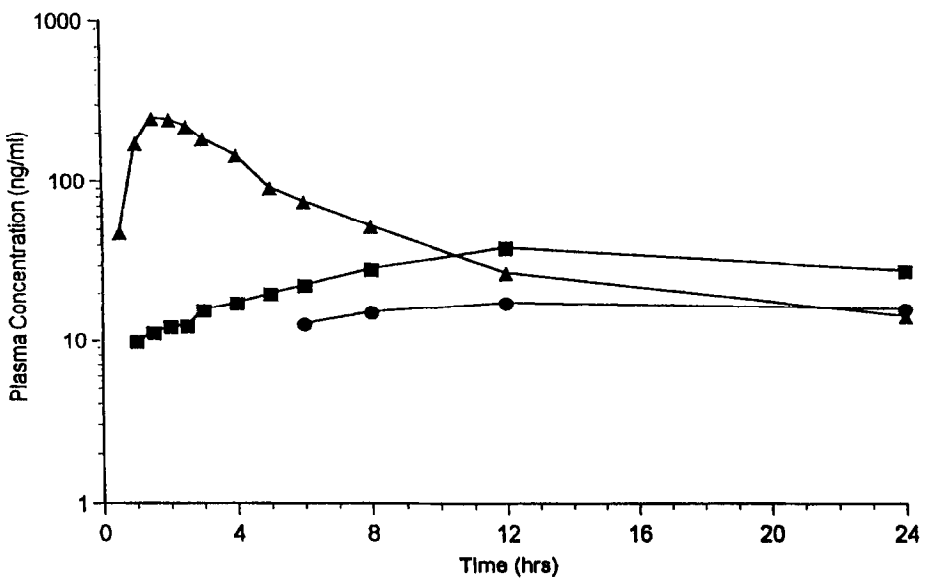


**Fig 3.** Plasma level time curves for clozapine N-oxide (CNO, --▲--) and clozapine (CLOZ, --■--), upon clozapine N-oxide administration. Only one patient had detectable desmethylclozapine (DCLOZ, --●--) plasma concentrations.

**Table 1**

**Summary (mean ± s.d.) Of Pharmacokinetic Parameters of Clozapine and Clozapine N-Oxide in Schizophrenic Patients**

| Parameter | Clozapine* | Clozapine N-Oxide | Desmethylclozapine | Clozapine N-Oxide** | Clozapine |
|---|---|---|---|---|---|
| AUC (ng) | 3028.4 | 1223.2 | 1741.6 | 1634.2 | 3209.4 |
| | ± 1234.2 | ± 962.3 | ± 576.4 | ± 467.2 | ± 1798.8 |
| T max (hr) | 3.4 | 3.1 | 4.4 | 2.1 | 12.5 |
| | ± 2.2 | ± 2.3 | ± 2.6 | ± 0.5 | ± 5.0 |
| C max (ng/ml) | 177.9 | 59.1 | 42.5 | 265.0 | 39.9 |
| | ± 128.8 | ± 41.3 | ± 17.3 | ± 177.5 | ± 14.0 |
| C 12 hr (ng/ml) | 60.2 | 21.6 | 26.0 | 27.2 | 38.9 |
| | ± 15.6 | ± 10.1 | ± 6.5 | ± 8.5 | ± 13.8 |
| C 24 hr (ng/ml) | 52.8 | 17.7 | 24.4 | 14.4 | 28.1 |
| | ± 29.7 | ± 16.4 | ± 8.6 | ± 8.8 | ± 8.9 |
| Ke (--) | 0.042 | ------- | ------- | 0.094 | -------- |
| | ± 0.008 | | | ± 0.052 | |
| T ½ (hr) | 17.3 | -------- | ---------- | 9.4 | -------- |
| | ± 4.1 | | | ± 4.6 | |
| CL (L/hr) | 35.7 | -------- | -------- | 59.9 | ---------- |
| | ± 14.4 | | | ± 14.8 | |

* Clozapine Administered, ** Clozapine N-Oxide Administered.

Pharmacokinetic parameters of desmethylclozapine from N-oxide administration could not be determined from this sparse data. The C 12 and C 24 plasma concentrations of clozapine N-oxide were almost identical when either clozapine or the N-oxide agent was given. However, clozapine plasma concentrations were 65% lower at the C 12 and 53% lower at the C 24 period when clozapine N-oxide was administered.

**Reversible Metabolism Parameters**

A summary of the interconversion parameters for clozapine and clozapine N-oxide are shown in Table 2. The mean total CL for clozapine ($CL_{1,0} + CL_{1,2}$) was 28.45 L/hr and the mean total CL for clozapine N-oxide ($CL_{2,0} + CL_{2,1}$) was 45.30 L/hr. The coefficient for Kd m/p was approximately 2.25 times greater than Kd p/m. The recycling factor (RF) appears to be very small. The mean RHO for value for m/p was 2.60 times greater than the mean RHO p/m value. The mean EE value was 1.06 with a narrow s.d. of 0.03 and represents the probability of a molecule of a converted drug metabolite not to be converted back to its original form. The terms $E_{eff\ 1,2}$ and $E_{eff\ 2,1}$ represent the efficiency of $CL_{1,2}$ and $CL_{2,1}$ as the true elimination pathways, respectively. The $E_{eff\ 1,2}$ and $E_{eff\ 2,1}$ values shown in Table 2 were almost equivalent. The oral administration route prevents the calculation of volume of distribution for clozapine N-oxide. Also, other variables such as mean residence time (MRT) and sojourn times could not be determined.

**Ratios of Metabolites/Clozapine AUC**

The mean AUC ratios of the metabolite/parent drug (clozapine, clozapine N-oxide and desmethylclozapine) are also presented in Table 2. When administered clozapine, the AUC ratios of clozapine N-oxide and desmethylclozapine were lower than 1.000, indicating a smaller amount of the metabolite being formed. Also, the mean AUC ratios for clozapine N-oxide/clozapine and desmethylclozapine/clozapine were almost similar. Conversely, when clozapine N-oxide was given, the AUC ratio of clozapine/clozapine N-oxide was greater than 1.000 and in fact, 2.223. This finding would indicate that the formation of clozapine continues to occur as clozapine N-oxide plasma concentrations decrease over time.

## Discussion

**Reversible Metabolic Clearances of Clozapine and Clozapine N-oxide**

This study demonstrates that reversible metabolism of clozapine and clozapine N-oxide metabolite occurs in schizophrenic patients. The clearance (CL) and elimination half-life of clozapine in these patients are similar to those from previous studies (Jann et al, 1993; Lin et al, 1994). This study also characterizes the pharmacokinetics of clozapine N-oxide in psychiatric patients. The disposition of clozapine N-oxide differs from clozapine with a higher CL and shorter elimination half-life shown in Table 1. Usually, metabolites have a longer elimination half-life compared to their parent compounds such as haloperidol and its reduced metabolite - reduced haloperidol (Jann et al, 1990). However, this may not occur with clozapine and clozapine N-oxide.

With the interconversion model, it appears that the major elimination route for clozapine and clozapine N-oxide is from the central compartment ($CL_{1,0}$ and $CL_{2,0}$).

From the interconversion variables calculated in Table 2 that indicate the amount of conversion from clozapine to clozapine N-oxide ($CL_{1,2}$), it is apparent that after a single dose, clozapine is converted to the N-oxide compound by approximately 18%, with a smaller amount metabolized back to clozapine. The total mean CL of clozapine ($CL_{1,0} + CL_{1,2}$) from Table 2 was 28.45 L/hr which was slightly lower (20%) than the mean value of 35.7 L/hr shown in Table 1. Both values of clozapine are within the CL pharmacokinetic parameters of previously reported studies (Jann et al, 1993; Lin et al, 1994). A similar finding occurred with clozapine N-oxide as the mean total CL ($CL_{2,0} + CL_{2,1}$) of 45.30 L/hr was also slightly lower (25%) than the mean value of 59.9 L/hr. Possible explanations for these slight differences include the wide interpatient variability observed with clozapine and clozapine N-oxide and potential contributions of other metabolic pathways such as hydroxylation (Wagstaff and Bryson, 1995).

**Table 2**
**Summary (mean ± s.d.) of the Reversible Metabolic Parameters of Clozapine and Clozapine N-Oxide**

| Parameter | Value |
|---|---|
| $CL_{1,0}$ | 24.09 ± 23.46 |
| $CL_{2,0}$ | 29.49 ± 2.37 |
| $CL_{1,2}$ | 4.36 ± 10.56 |
| $CL_{2,1}$ | 15.81 ± 85.00 |
| RHO (p/m) | 0.15 ± 0.09 |
| RHO (m/p) | 0.39 ± 0.11 |
| RF | 0.06 ± 0.05 |
| Kd (p/m) | 0.04 ± 0.03 |
| Kd (m/p) | 0.09 ± 0.07 |
| $E_{eff\ 1,2}$ | 0.65 ± 0.34 |
| $E_{eff\ 2,1}$ | 0.85 ± 0.44 |
| EE | 1.06 ± 0.03 |
| AUC CNO/AUC CLZ* | 0.421 ± 0.316 |
| AUC DCLOZ/AUC CLZ* | 0.637 ± 0.283 |
| AUC CLZ/AUC CNO** | 2.223 ± 1.543 |

AUC = Area under the plasma concentration time curve, CNO = Clozapine N-Oxide, CLZ = Clozapine, DCLOZ = Desmethylclozapine, * Clozapine administered, ** Clozapine N-Oxide administered.

**Reversible Metabolic Conversion Factors**

The other calculated variables from the interconversion process, RHO, EE, $E_{eff\ 1,2}$ and $E_{eff\ 2,1}$ further substantiate that based upon single dose administration, reversible metabolism does occur with clozapine and clozapine N-oxide. The appearance of clozapine N-oxide from clozapine administration was rapidly detected and plasma concentration paralleled clozapine plasma levels shown in Fig 1. With N-oxide administration, although clozapine plasma concentrations could also be rapidly detected, plasma clozapine concentrations continued to slowly increase shown in Fig 2. Interestingly, the $CL_{2,1}$, RHO (m/p), Kd (m/p) and $E_{eff\ 2,1}$ values are greater than their respective counter parts which could suggest that the conversion from clozapine N-oxide back to clozapine occurs at a greater rate than vice-versa. The interpretation of our data would suggest that the formation of clozapine N-oxide from clozapine administration occurs at a slower rate; however, once formed, the amount that undergoes a back conversion occurs rapidly.

The interconversion $CL_{1,2}$ and $CL_{2,1}$ display a wide standard deviation greater than the mean values shown in Table 2. Two patients had negative CL values calculated for $CL_{1,2}$ and $CL_{2,1}$. A negative drug CL could theoretically imply that the drug is accumulating and not being eliminated from the central or peripheral compartments. This phenomenon is observed with the continued formation of clozapine upon N-oxide administration. Further, the total AUC for clozapine when clozapine N-oxide was administered was greater by a factor of 2.223. Unfortunately, plasma samples were obtained only up to 24 hours post-drug administration. The 24 hour time period for sample collection was selected based upon the route dosing of clozapine where daily dosing is prescribed. The elimination half-life of clozapine and clozapine N-oxide are shorter than 24 hours, but an extended sampling of 72 to 96 hours would have been ideal and yielded more information on CL, AUC, and elimination half-life. Perhaps, with the additional sampling points, a decline in the clozapine plasma levels (with N-oxide administration) would be observed and the negative drug CL's not be calculated. Another factor that could produce a wide interpatient variability is enterohepatic recycling (EHC). EHC has been reported with fluphenazine and haloperidol (Forsman and Ohman, 1977; Midha et al, 1988), but has not yet been observed with clozapine.

**Clozapine N-Oxide and Desmethylclozapine Plasma Levels**

Plasma concentrations of clozapine N-oxide and desmethylclozapine were detected upon clozapine administration. Pharmacokinetic parameters of desmethylclozapine shown in Table 1 resembles data reported from previous studies (Lin et al, 1994). With clozapine administration, the C max, C12 and C 24 for clozapine was higher than clozapine N-oxide and desmethylclozapine. Upon N-oxide administration, clozapine was rapidly detected but the T max occurred much later at $12.5 \pm 5.0$ hours. Mean plasma clozapine levels were higher than the clozapine N-oxide at the C 12 and C 24 hour time periods. These findings further suggest that clozapine plasma concentrations are accumulating during this time period. Only one patient had detectable

plasma desmethylclozapine levels after clozapine N-oxide administration despite the high AUC determined for clozapine. This could suggest that another metabolic pathway could be responsible for the clozapine elimination once converted from clozapine N-oxide (Wagstaff and Bryson, 1995).

**Animal Data with Clozapine Metabolism**

Clozapine, desmethylclozapine and clozapine N-oxide were measured in rat plasma concentrations (Baldessarini et al, 1993). However, only clozapine brain concentrations were detected while the clozapine N-oxide remained undetectable. Desmethylclozapine brain concentrations were very low and undetectable at 24 hours post-dose. The pharmacologic activity of these two metabolites was reported to be lower than clozapine, based upon $^{3}$H-haloperidol binding affinity in rat striatal membranes, CNO was at least four times lower than clozapine (Schmutz, 1980). The back conversion on CNO to clozapine could partially explain the prolonged pharmacologic effects of clozapine. Reversible metabolism of clozapine and clozapine N-oxide was demonstrated to occur in guinea pigs with significant clozapine and clozapine N-oxide concentrations measured in the caudate and frontal cortex (Jann et al, 1994). The guinea pig model for the disposition of clozapine could be similar to that of haloperidol. Haloperidol's conversion to reduced haloperidol and its oxidization back to haloperidol was reported to occur in guinea pigs and not in rats (Korpi et al, 1985).

**Therapeutic Implications**

Reversible metabolism could influence the interpretation of therapeutic ranges for clozapine plasma concentrations. A therapeutic threshold of clozapine was described in patients with plasma levels that ranged from 350 ng/ml to 420 ng/ml (Perry et al, 1991; Potkin et al, 1994; Miller et al, 1994; Kronig et al, 1995). An optimal therapeutic range between 300 to 700 ng/ml was also described in psychiatric patients (Liu et al, 1996). Only one study suggested that the combined plasma concentrations of desmethylclozapine plus clozapine of 450 ng/ml had a therapeutic threshold (Perry et al, 1991). Various factors were reported to influence plasma clozapine concentrations which can contribute to the wide interpatient variability. These factors include: dose; gender; smoking; weight and age (Haring et al, 1989; Haring et al, 1990). The effects of these factors have not been determined with clozapine metabolites. Drug interactions could play an important role in the monitoring of clozapine plasma concentration. Fluoxetine (a potent P450 inhibitor of 2D6 and 3A4) was reported to inhibit the metabolism of clozapine and increase serum concentrations (Centorrino et al, 1994). Both DCLOZ and CNO plasma levels were also increased upon fluoxetine administration. With increased CNO plasma concentrations, this could partially account for the increased clozapine plasma levels. The effects of other serotonergic reuptake inhibitors upon CNO plasma levels have not been reported (Centorrino et al, 1996). The P450 isozymes responsible for the formation of various metabolites have not yet been fully characterized. However, P450 1A2 appears to be involved (Bertilsson et al, 1994). Fluvoxamine, a serotonergic reuptake inhibitor and a potent P450 1A2 inhibitor, was reported to significantly elevate plasma clozapine levels in psychiatric patients (Jerling

et al, 1994; Koponen et al, 1996). P450 2D6 appears to not be involved with the disposition of desmethylclozapine or clozapine N-oxide from clozapine (Fischer et al, 1992). In addition to these P450 isozymes, in-vitro studies have implicated the 3A4, 2C9 and 2C19 in clozapine's metabolism to these two metabolites (Fang et al, 1996).

The route monitoring of plasma clozapine concentrations can assist clinicians in therapeutic decisions regarding the use of clozapine in psychiatric patients. Clinicians have reported that low doses of clozapine given at steady-state conditions were a greater predictor of achieving therapeutic plasma concentrations than single dose administration (Oyewumi et al, 1995). With single dose administration, the equilibration between clozapine, clozapine N-oxide and desmethylclozapine pharmacokinetic compartments have not yet occurred which could explain their findings.

## Conclusion

This study represents initial estimates of the reversible metabolic process of clozapine and clozapine N-oxide based upon single dose administration. The role of reversible metabolism in drug disposition is underappreciated, usually due to the lack of the metabolite for administration. Although many other parameters need to be considered, the interconversion process can be partially characterized using this simple pharmacokinetic model. The significance of the other reversible pathway with the protein reactive metabolite remains to be determined. The significance of the interconversion process in the monitoring of plasma clozapine levels and it metabolites and clinical outcome need to be further evaluated.

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Inquiries and reprint requests should be addressed to:

Wen-Ho Chang, M.D.

Taipei City Psychiatric Center

309 Sung-Te Road

Taipei, Taiwan 10510, ROC