

## **Clozapine Induced Neutrophil Cytotoxicity in Rats**

Afshan Wasti, Rubina Ghani, Mehdi A. Manji, Nikhat Ahmed

Pathological Laboratories<sup>1</sup>, Neurochemistry Research Unit, Department of Biochemistry, University of Karachi<sup>2</sup>, Karachi.

### **Abstract**

**Objective:** The study was carried out to understand the pathogenesis of hematological dyscrasias and cytotoxicity following administration of both purified and commercially available form of Clozapine in an animal model.

**Methods:** The Albino Sprague Dawley rats (n=30) with an average weight of 180g were taken and divided into three groups. Haematological parameters including haemoglobin, haematocrit, RBC and differential counts, absolute indices, Red cell Distribution Width (RDW) and morphological features of RBCs by peripheral blood smear were performed by standard laboratory methods. Additionally Serum Iron Concentration (SIC), Total Iron Binding Capacity (TIBC) (Roche Ltd.) and the serum ferritin level (Randox Ltd.) were also determined in each group. All statistical analysis was performed using graph pad prism.

**Results:** Clozapine induced neutrophil toxicity was manifested in both experimental groups, with condensation and subsequent breakdown of chromatin material.

**Conclusion:** Our data, raised concerns about haematological safety and the potential mechanisms of neutrophil cytotoxicity related to the use of this drug (JPMA 56;62:2006).

### **Introduction**

Clozapine, a dibenzodiazepine is an atypical antipsychotic drug, which produces haematological side effects. Different epidemiological studies have shown that blood dyscrasias like leukopenia, neutropenia and thrombocytopenia (1-3% of patients); anaemia, leukocytosis and thrombocytosis (1% of patients) are associated with use of clozapine. This may represent a major problem for the management of treatment-resistant schizophrenic patients who do not respond to conventional or other atypical antipsychotics.<sup>1-4</sup>

An understanding of the pathogenesis of haematological dyscrasias is essential for the effective management of drug action. Haematological side effects of neuroleptic drugs occur infrequently but remain a potential cause of serious toxicity.<sup>5,6</sup> Unlike classical neuroleptic agents, clozapine is not associated with the development of acute extrapyramidal symptoms or tardive dyskinesias. Most side effects associated with clozapine are typical of antipsychotics in general, and are usually benign, tolerable, and manageable.<sup>7,8</sup>

The real problem of drug-induced neutrophil cytotoxicity is raised by the use of the atypical antipsychotic drug clozapine, which has been prescribed restrictedly, due to the incidence of drug-induced agranulocytosis in 1-2% of patients. The exact mechanism of this adverse effect is not yet known.<sup>9,10</sup> Many of these side effects of clozapine are observed early after treatment onset and are greatly reduced by dose adjustment.<sup>11</sup>

The aim of this study was to investigate the blood dyscrasias and cytotoxicity produced after long-term treatment with both purified and commercially available forms of clozapine. This was observed on the haematological indices in an animal model.

### **Material and Methods**

This study was conducted during June 2003 to January 2004 at both the Neurochemistry Research Unit, Department of Biochemistry, University of Karachi and the Mehdi A. Manji Pathological Laboratories in Karachi.

The Albino Sprague Dawley rats (n=30) with an average weight of 180g were divided into three groups. Group-1 (n=14) was treated with commercially available clozapine tablets (Novartis Co. Ltd.) while Group-2 (n=8) was administered purified (Sigma Co. Ltd.) form of clozapine (20mg/Kg) intramuscularly. The controls (n=8) were subjected to saline treatment for 21 consecutive days. The rats were decapitated 18 hours after the last dose of clozapine. Number of preparations were made in duplicate.

The haematological parameters including: haemoglobin, haematocrit, RBC counts, absolute indices including Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Cell Haemoglobin Content (MCHC), Red cell Distribution Width (RDW) and morphology and WBCs differential counts were performed by automated counter. Serum Iron Concentration (SIC), Total Iron Binding Capacity (TIBC) (Roche Ltd.) and the serum ferritin level (Randox Ltd.) were also determined.<sup>12-15</sup> The cell viability was performed to determine the numbers of apoptotic and

**Table 1. Comparison of Haematological indices following chronic Clozapine (both purified and tablet form) treatment in rats.**

Exp.Groups Parameters	Control			Clozapine Purified			Clozapine Tablets		
	Mean	SEM,	n	Mean	SEM,	n	Mean	SEM,	n
HB	11.725	1.05	8	11.350	0.631	8	11.18	0.208	14
RBC	5.733	0.259	6	5.966	0.128	6	5.68	0.085	14
PCV	33.075	1.72	8	28.875	1.655	8	29.25	0.464	14
MCV	54.425	0.462	8	53.100	0.494	8	51.51	0.584	14
MCH	19.05	1.045	8	20.875	0.219	8	19.72	0.455	14
MCHC	34.95	1.73	8	39.325	0.094	8	38.328	0.774	14
RDW	13.550	0.259	8	15.625	0.531	8	15.01	0.345	14

(Hb= haemoglobin g/dl, RBC= red cell count cu/mm, PCV= packed cell volume %, MCV= mean cell volume fl, MCH= mean cell haemoglobin pg, RDW= red cell distribution width %).

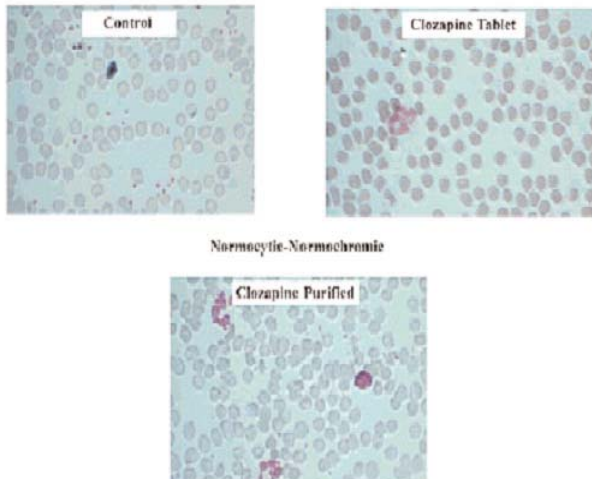


Figure 1. Screening strategy using RBC morphology after chronic Clozapine treatment (Both purified and tablet form with saline treated controls).

non-apoptotic cells by differential count. Differential diagnosis on morphologic examination of blood smear was also done.

All statistical analysis was performed using graph pad prism.

## Results

No characteristic change in RBCs morphology and haematological indices (Figure1 and Table 1) was observed subsequent to clozapine treatment. However, WBCs and neutrophil counts showed a marked increase ( $p<0.05$ ) with concurrent decrease in lymphocyte count in both treatment

**Table 2. Comparison of total and differential WBCs count following chronic Clozapine (both purified and tablet form) treatment in rats.**

Parameters	Clozapine Tablets % age Increase↑	Clozapine Purified or Decrease↓ ( $p<0.05$ )
White Blood Cells	30.46↑	54.9↑
Neutrophils	48.9↑	112.0↑
Lymphocytes	8.6↓	11.1↓

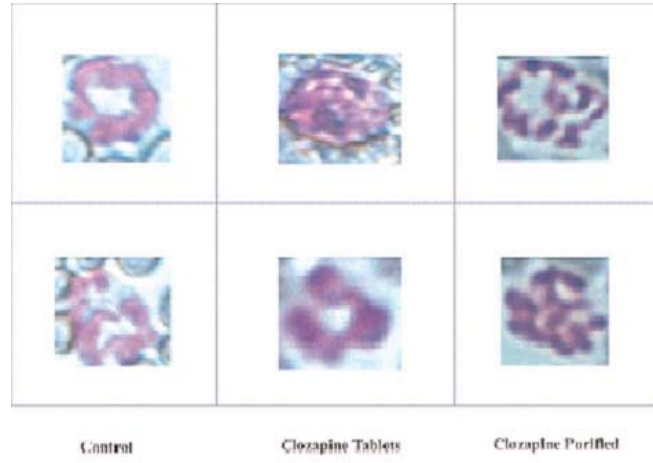


Figure 2. Toxic effect of Clozapine on neutrophils.

groups (Table 2).

The clozapine induced neutrophils toxicity was manifested in both the experimental groups with condensation and subsequent breakdown of chromatin material (Figure 2).

Group significance showed a marked decrease ( $p<0.0001$ ) in serum iron level, serum ferritin and transferrin saturation (%) with a concurrent increase in serum TIBC in both the experimental groups ( $n=30$ ) (Figure 3).

## Discussion

The effect of long-term clozapine treatment in the animal model on haematological indices showed slight alterations which could not be attributed to anaemia. The peripheral blood smear also did not show any characteristic effects of anaemia on RBC morphology (Figure 1) further confirming its absence. The alterations in iron profile after long-term clozapine treatment in both the experimental and control group indicated that the antipsychotic drug may affect the iron absorption and its binding affinity. The interaction, however between serum iron and antipsychotic drugs still remain unclear.<sup>16</sup>

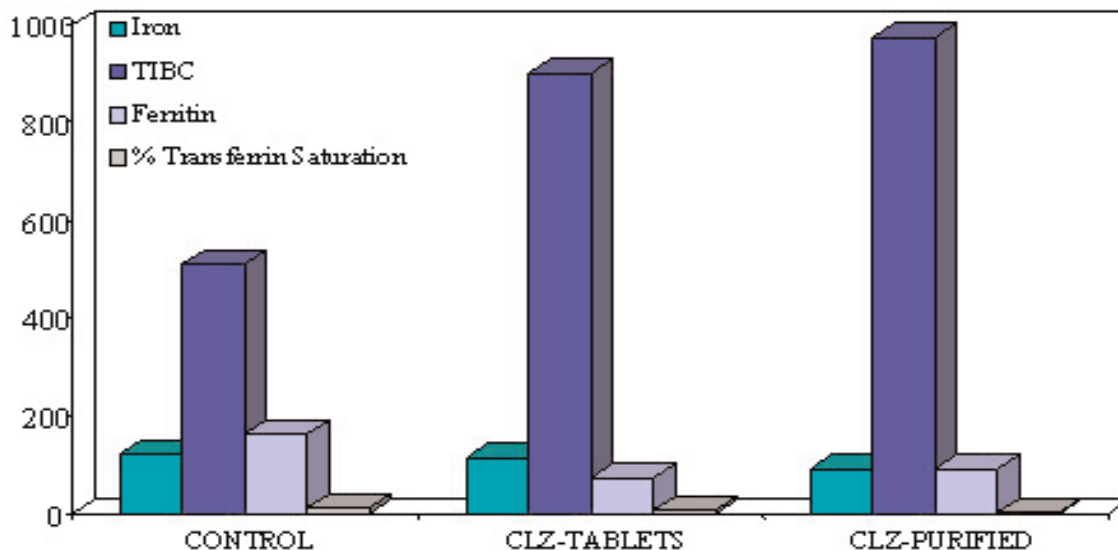


Figure 3. Comparison of Iron profile following chronic Clozapine (both purified and tablet form) treatment in rats. (Iron ug/dl, TIBC= total iron binding capacity ug/dl, % transferrin saturation, Ferritin ng/ml).

The effect of clozapine induced haematological dyscrasias was observed by measuring WBC toxicity. The marked increase in total WBC count, absolute neutrophil count and slightly decrease in lymphocyte count indicated the adverse effects. Clozapine reactive metabolites have been implicated in the toxicity but the mechanism by which this occurs is currently not known.<sup>17</sup> The condensation and subsequent breakdown of chromatin material observed in drug treated groups (Figure 2) may be suggestive of the fact that these cytotoxic metabolites may play an important role in the pathogenesis of clozapine induced neutrophils cytotoxicity.

Theoretically, it could be either due to the parent drug or to its stable metabolites; however, these do not seem to be toxic to peripheral or progenitor blood cells at therapeutic concentrations.<sup>18</sup> But such inactivation has been implicated in agranulocytosis associated with other drugs.<sup>19-20</sup>

### Conclusion

Our data, raised concerns about haematological safety and the potential mechanism of the drug-induced neutrophil cytotoxicity related with the chronic treatment of clozapine in an animal model. The sequential analysis based on the morphological and biochemical assessment suggests that clozapine is responsible for inducing neutrophil cytotoxicity. The possible hypothesis of the mechanism of drug-induced cytotoxicity is the involvement of reactive metabolites, which are formed by, or in close proximity to affect the blood cells. However, the therapeutic dosage range and the mechanism of clozapine-induced haematotoxicity are probably not due to direct toxicity of clozapine to the blood cells

or their precursors. The mechanism by which metabolites of clozapine causes Neutrophil toxicity still requires further investigation.

### Acknowledgment

The authors acknowledge the support of Prof. Dr. Anwar Ali Siddiqui, Associate Dean of Research, Juma Research Lab, Aga Khan University Hospital Karachi, for providing the facility of Microscopy used for the scanning of blood slides.

### References

1. Lubran MM. Hematologic side effects of drugs. *Ann. Clin. Lab Sci* 1989;19: 114-21.
2. Hampson M E. Clozapine induced thrombocytosis. *Br J Psychiatr* 2000;176:400.
3. Varsha Bhatt, Abdus Saleem. Review: Drug-induced Neutropenia-Pathophysiology, Clinical features and Management. *An Clin Lab Sci* 2004;34:131-7.
4. Ahn YM, Jeong SH, Jang HS, Koo YJ, Kang UG, Lee KY et al. Experience of maintaining clozapine medication in patients with red alert zone neutropenia: long-term follow-up results. *Int. Clin Psychopharm* 2004;19:97-101.
5. Fisher N, Baigent B. Treatment with clozapine. *BMJ* 1996;313:1262.
6. Patton WN, Duffull SB. Idiosyncratic drug-induced haematological abnormalities. Incidence, pathogenesis, management and avoidance. *Drug Safety* 1994;11:445-62.
7. Balon R, Berchou R, Zethelius M. Thrombocytopenia associated with chlorpromazine, haloperidol and thiothixene: a case report. *Can J Psychiatry* 1987;32:149-150.
8. Marlowe K. Thrombocytosis due to clozapine treatment: working towards an early marker for clozapine induced agranulocytosis. *B J Psychiatr* 2000;177:372-3.
9. Alvir JM, Lieberman JA, Schwimmer JL, Safferman AZ, Schaaf JA. Clozapine induced agranulocytosis: incidence and risk factors in the United States. *N Engl J Med* 1993;329:162-7.
10. Krupp P, Barnes P. Leponex associated granulocytopenia: a review of the situation. *Psychopharmacology* 1989;99:S118-21.

11. Frimat B, Gressier B, Odou P, Brunet C, Dine T, Luycky M, et al. Metabolism of clozapine by human neutrophils; evidence for a specific oxidation of clozapine by the myeloperoxidase system with inhibition of enzymatic chlorination cycle. *Fundam Clin Pharmacol* 1997;11:267-74.
  12. Oyewumi LK, Cernovsky ZZ, Freeman DJ, Streiner DL. Relation of blood counts during clozapine treatment to serum concentrations of clozapine and nor-clozapine. *Can J Psychiatry* 2002;47:257-61.
  13. Raphael SS. Practice of hematology. Lynch's Medical Laboratory Technology, Philadelphia, Saunder's Company 1983;pp. 696-7.
  14. Harries H, Shankland D, Henly R. Determination of serum transferrin by turbidity method. *Med Lab Sci* 1985;42:230-2.
  15. Williams H L. Estimation of serum iron. *Clin Chem* 1977;23:237-40.
  16. Ben -Shachar D, Youdim MB. Neuroleptic-induced super sensitivity and brain iron: iron deficiency and neuroleptic- induced dopamine D2 receptor super sensitivity. *J Neurochem* 1990;54:1136-41.
  17. Bessman JD, Feinstein DI. Quantitative anisocytosis as a discriminant between iron deficiency and thalassemia minor. *Blood* 1979;53:288-93.
  18. Williams DP, Munir Pirmohamed, Naisbitt DJ, Uetrechl JP, Park BK. Induction of metabolism dependent and independent neutrophil apoptosis by clozapine. *Mol Pharmacol* 2000;58:207-16.
  19. Gardner I, Zahid N, Crimmon DM, Uetrecht JP. A comparison of the oxidation of clozapine and olanzapine to reactive metabolites and the toxicity of these metabolites to human leukocytes. *Mol. Pharmacol* 1998;53:991-8.
  20. Uetrecht JP. Reactive metabolites and agranulocytosis. *Eur J Haematol* 1996;60:83-8.
-