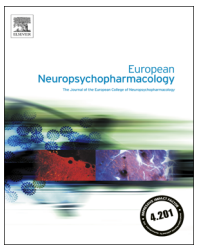
European Neuropsychopharmacology (▮▮▮▮) ▮, ▮▮▮–▮▮▮

www.elsevier.com/locate/euroneuro



The atypical antipsychotic clozapine selectively inhibits interleukin 8 (IL-8)-induced neutrophil chemotaxis

Marta Capannolo[a,1], Irene Fasciani[a,1], Stefania Romeo[a], Gabriella Aloisi[a], Mario Rossi[b], Pierangelo Bellio[a], Giuseppe Celenza[a], Benedetta Cinque[c], Maria Grazia Cifone[c], Marco Scarselli[d], Roberto Maggio[a,*]

[a]*Biotechnological and Applied Clinical Sciences Department, University of L'Aquila, 67100 L'Aquila, Italy*
[b]*Molecular Signaling Section, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, USA*
[c]*Department of Life, Health and Environment Sciences, University of L'Aquila, 67100 L'Aquila, Italy*
[d]*Department of Translational Research and New Technology in Medicine, University of Pisa, 56126 Pisa, Italy*

Received 7 May 2014; received in revised form 3 October 2014; accepted 8 December 2014

KEYWORDS
Clozapine;
Neutrophil chemotaxis;
Agranulocytosis;
Multi-drug resistance proteins;
Leukotriene B4

Abstract
Clozapine is the most effective antipsychotic to date, but its benefits are counterbalanced by the risk of severe hematological effects. In this study, we analyzed whether clozapine inhibits polymorphonuclear (PMN) leukocyte chemotaxis. We found that clozapine, within the therapeutic concentration range, potently and selectively inhibits PMN chemotaxis induced by interleukin 8 (IL-8), a chemokine inducing neutrophil migration. The effect was not due to its action at dopamine, serotonin and muscarinic receptors, or to a direct antagonism to IL-8 receptors. Furthermore, clozapine did not inhibit PMN chemotaxis by its presumed toxic mechanism. In fact, after an overnight incubation in cell culture, the drug did not increase the physiological PMN apoptosis. An interference of clozapine with the autocrine release of leukotriene B4 (LTB_4), a secondary chemoattractant secreted by neutrophils in response to the primary chemoattractant IL-8, was hypothesized. In agreement with this hypothesis, clozapine attenuated the IL-8-induced release of LTB_4 in PMNs. A series of experiments with an antagonist of the LTB_4 receptor, U75302, and an inhibitor of LTB_4 synthesis, zileuton, provided support to this conjecture. Intriguingly MK-571, an inhibitor of the multi-drug resistance protein MRP4, playing a pivotal role in effluxing LTB_4, completely blocked PMN chemotaxis induced by IL-8, but gave conflicting results when tested for its ability to reduce LTB_4 release, increasing LTB_4 efflux by itself but reducing the

*Correspondence to: Biotechnological and Applied Clinical Sciences Department, University of L'Aquila, Via Vetoio - Coppito 2 67100 L'Aquila, Italy. Tel.: +39 0862 433587; fax: +39 0862 433523.
E-mail address: roberto.maggio@univaq.it (R. Maggio).
[1]These two authors contributed equally to the work.

http://dx.doi.org/10.1016/j.euroneuro.2014.12.002

Please cite this article as: Capannolo, M., et al., The atypical antipsychotic clozapine selectively inhibits interleukin 8 (IL-8)-induced neutrophil chemotaxis. European Neuropsychopharmacology (2014), http://dx.doi.org/10.1016/j.euroneuro.2014.12.002

release when in combination with IL-8. The reduction of PMN chemotaxis due to clozapine could predispose patients to infections. Whether this effect is a prelude to clozapine agranulocytosis requires further investigation.

1. Introduction

Among atypical antipsychotic drugs, clozapine is the most effective in treating positive, negative, and cognitive symptoms in schizophrenia, particularly in patients resistant to other antipsychotics (Wenthur and Lindsley, 2013). The mechanism of action is multifactorial and based on its affinities for different receptors, including dopamine D_2 receptor (Meltzer, 2013). Unfortunately, benefits are counterbalanced by the risk of severe adverse events and primarily the risk of neutropenia/agranulocytosis (Nooijen et al., 2011). For this reason, the use of this drug is limited and patients on clozapine must be monitored on a regular basis with a full differential white blood cell count to prevent drug-induced neutropenia/agranulocytosis.

The mechanism underlining the hematological effect of clozapine is still unclear, and does not depend on its effect as an antagonist at dopamine receptors. The most accredited mechanism so far is the biotransformation of clozapine to a nitrenium cation catalyzed by the flavin-containing monooxygenase-3 system in leucocytes (Flanagan and Dunk, 2008; Jagadheesan and Mehrtens, 2007; Sikora et al., 2007), that in turn covalently binds to cellular proteins, thus causing toxicity by chemically modifying critical molecules within the neutrophils or their precursors. Yet, this mechanism is debated and a recent report suggests that unactivated clozapine may itself exert toxic effects (Lahdelma et al., 2010).

Surprisingly, while in literature there are hundreds of papers dealing with the mechanism of clozapine-induced agranulocytosis/neutropenia, not much work has been done to determine the effect of clozapine on neutrophils motility; so far, the only work that analyzed this effect is by Guaraldi et al. (1996). These authors found that clozapine did not alter human neutrophil chemotaxis stimulated by the N-formylated peptide fMet-Leu-Phe (fMLP) nor random migration of unstimulated cells.

Neutrophils are a type of polymorphonuclear (PMN) leukocytes and they are one of the major players during acute inflammation (Kolaczkowska and Kubes, 2013). They are typically the first leukocytes to be recruited to an inflammatory site and are capable of eliminating pathogens by multiple mechanisms.

Neutrophil chemotaxis is a highly organized mechanism that depends on the interplay of several mediators that are secreted both by the target cell and autocrinally and paracrinally by the neutrophil itself (Kolaczkowska and Kubes, 2013). Interleukin 8 (IL-8) is a potent chemotactic factor for neutrophils that is secreted by various cell types in response to a wide variety of stimuli, including proinflammatory cytokines, microbes and their products, and environmental changes such as hypoxia, reperfusion, and hyperoxia (Mukaida, 2003). IL-8 binds with similar affinity to two receptors belonging to the G protein-coupled receptor family, $CXCR_1$ and $CXCR_2$ (Holmes et al., 1991; Murphy and Tiffany, 1991). Although both are present in neutrophils, the main role in the their chemotaxis is played by $CXCR_1$ (Hammond et al., 1995).

IL-8 binding to $CXCR_1$ activates pertussis toxin-sensitive $G\alpha_i$ proteins leading to inhibition of adenylyl cyclase (Raghuwanshi et al., 2012). Besides, generated $G\beta\gamma$ subunits recruit and activate phosphatidylinositol 3-kinase-γ (PI3K-γ), which in turn generates phosphatidylinositol 3,4,5-trisphosphate (PIP_3) (Servant et al., 2000). PIP_3 activates protein kinase B (Akt) as well as GTPases, resulting in directed cell migration (Mukaida, 2003).

In this work, we found that clozapine potently and selectively inhibits IL-8-induced neutrophil chemotaxis within the therapeutic concentration range. Results indicated that the effect is not due to a direct antagonism of the drug on $CXCR_{1,2}$ receptors. A mechanism based on the alteration of the autocrine/paracrine activity of neutrophils was investigated and discussed.

2. Experimental procedures

2.1. Reagents

IL-8 was purchased from PeproTech (London, UK). Transwell filters were from Corning (Cambridge, MA). Cell culture reagents were from EuroClone S.p.A (Pero, Milan, Italy). U75302, LTB_4 and Ko143 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The other chemicals, were from Sigma-Aldrich.

2.2. L1.2 culture

L1.2 cells (an Abelson murine leukemia virus-transformed pre-B-cell) stably transfected with the human $CXCR_1$ receptor were maintained, as described by Wise et al. (2007), in suspension at 37°C with 5% CO_2 at a density of 1×10^6 cells per ml, in RPMI 1640 supplemented with FBS 10%, 2 mM L-glutamine, 100 U/ml penicillin, 0,1 mg/ml streptomycin sulfate, 1 mM sodium pyruvate, 10 mM Hepes buffer and 50 μM β-mercaptoethanol.

2.3. Isolation of PMNs

PMNs were obtained from buffy coats of citrate-phosphate-dextrose-treated blood from healthy medication-free volunteers through the courtesy of the Transfusion Medicine Service Laboratory, "San Salvatore" Hospital, L'Aquila, Italy. Human PMNs were separated to >95% purity by centrifugation through a Ficoll–Histopaque gradient, followed by 2% dextran sedimentation and lysis of contaminating red blood

Please cite this article as: Capannolo, M., et al., The atypical antipsychotic clozapine selectively inhibits interleukin 8 (IL-8)-induced neutrophil chemotaxis. European Neuropsychopharmacology (2014), http://dx.doi.org/10.1016/j.euroneuro.2014.12.002