other antacids. In the nonclozapine group, 9 (2.2%) were prescribed PPIs, 1 (0.3%) an H2 antagonist, and 5 (1.4%) were prescribed other antacids. Crude OR for receiving any antacid medication (clozapine, 18.6%; nonclozapine, 3.9%) was 5.2 (95% confidence interval, 2.8-9.6; $P < 0.0001$).

In the final multivariable model, use of antacids was associated with age ($P = 0.008$), prescription of a second antipsychotic ($P = 0.039$), laxative prescription ($P = 0.0002$), nonsteroidal anti-inflammatory drug prescription ($P = 0.0003$), and corticosteroid prescription ($P = 0.0001$). Gastroesophageal reflux is generally more common in whites[6] (who were overrepresented in the clozapine group), but ethnicity did not significantly influence frequency of use of antacids in our samples. No other factor was significantly associated with prescription of antacids. Accounting for confounders produced an adjusted OR of 3.4 (95% confidence interval, 1.7–6.8; $P = 0.0005$).

Thus, antacid prescription was significantly more prevalent in patients receiving clozapine than in those receiving nonclozapine SGAs. This observation extends our understanding of the previously reported association of clozapine with upper gastrointestinal symptoms.[1–4]

The reasons for increased prescribing of antacids in people taking clozapine are not clear. Clozapine seems to reduce gastric acid secretion[7] but has been reported to induce gastric outlet obstruction[8] and to impair esophageal function.[9] This impairment of esophageal peristalsis may be the cause of the frequently observed sialorrhea seen in people receiving clozapine.[10] The high use of anticholinergic agents to treat clozapine-associated sialorrhea (39.1% of subjects in this study) may also contribute to esophageal dysfunction: anticholinergic drugs have been linked to esophageal atony.[11]

Limitations of our method include the cross-sectional nature of data capture (thus making causation difficult to establish), that we did not account for some possible confounding variables that were not reliably recorded in our data sources (eg, smoking status), and that we did not clearly establish the reasons for antacid prescribing.

There are 3 important clinical implications of our findings: antacids seem to be frequently required in people taking clozapine, so clinicians should be aware of the increased likelihood of emergent upper gastrointestinal symptoms in these patients; the potential for interaction should be considered because omeprazole may reduce clozapine plasma levels[12]; and our findings suggest a possible link between the risk of fatal pneumonia in people prescribed clozapine[13] and the association of PPI use with an increased risk of pneumonia.[14]

In this cohort, antacid use was much more prevalent in those prescribed clozapine than in those prescribed other SGAs. It is likely that it was a result of an increased rate of gastroesophageal reflux symptoms in people taking clozapine.

AUTHOR DISCLOSURE INFORMATION

Professor Taylor has received consultancy fees, lecturing honoraria, and/or research funding from AstraZeneca, Janssen-Cilag, Servier, Sanofi-Aventis, Lundbeck, Bristol-Myers Squibb, Novartis, Eli Lilly, and Wyeth. Ms Olofinjana and Ms Rahimi have no conflicts of interest.

David Taylor, MSc, PhD MCMHP, MRPharmS
Pharmacy Department
South London and
Maudsley NHS Foundation Trust
and Department of Pharmaceutical Sciences
King's College
London, UK
David.Taylor@slam.nhs.uk

Olubanke Olofinjana, BPharm MSc, MRPharmS
Pharmacy Department
South London and
Maudsley NHS Foundation Trust
London, UK

Tamanna Rahimi, BSc
King's College School of Medicine
London, UK

REFERENCES

1. Mookhoek EJ, Meijs VM, Loonen AJ, et al. Dyspepsia in chronic psychiatric patients. *Pharmacopsychiatry.* 2005;38:125–127.
2. Baker RW, Chengappa KN. Gastroesophageal reflux as a possible result of clozapine treatment. *J Clin Psychiatry.* 1998;59:257.
3. Laker MK, Cookson JC. Reflux oesophagitis and clozapine. *Int Clin Psychopharmacol*. 1997;12:37–39.
4. John JP, Chengappa KN, Baker RW, et al. Assessment of changes in both weight and frequency of use of medications for the treatment of gastrointestinal symptoms among clozapine-treated patients. *Ann Clin Psychiatry.* 1995;7:119–125.
5. McEvoy JP, Lieberman JA, Stroup TS, et al. Effectiveness of clozapine versus olanzapine, quetiapine, and risperidone in patients with chronic schizophrenia who did not respond to prior atypical antipsychotic treatment. *Am J Psychiatry.* 2006;163:600–610.
6. Sharma P, Wani S, Romero Y, et al. Racial and geographic issues in gastroesophageal reflux disease. *Am J Gastroenterol*. 2008;103:2669–2680.
7. Glavin GB, Hall AM. Clozapine, a dopamine DA4 receptor antagonist, reduces gastric acid secretion and stress-induced gastric mucosal injury. *Life Sci*. 1994;54: L261–L264.
8. Schwartz BJ, Frisolone JA. A case report of clozapine-induced gastric outlet obstruction. *Am J Psychiatry.* 1993;150:1563.
9. McCarthy RH, Terkelsen KG. Esophageal dysfunction in two patients after clozapine treatment. *J Clin Psychopharmacol*. 1994;14:281–283.
10. Boyce HW, Bakheet MR. Sialorrhea: a review of a vexing, often unrecognized sign of oropharyngeal and esophageal disease. *J Clin Gastroenterol*. 2005;39:89–97.
11. Woodring JH, Martin CA, Keefer B. Esophageal atony and dilatation as a side effect of thiothixene and benztropine. *Hosp Community Psychiatry.* 1993;44:686–688.
12. Frick A, Kopitz J, Bergemann N. Omeprazole reduces clozapine plasma concentrations. A case report. *Pharmacopsychiatry.* 2003;36:121–123.
13. Taylor DM, Douglas-Hall P, Olofinjana B, et al. Reasons for discontinuing clozapine: matched, case-control comparison with risperidone long-acting injection. *Br J Psychiatry.* 2009;194:165–167.
14. Gulmez SE, Holm A, Frederiksen H, et al. Use of proton pump inhibitors and the risk of community-acquired pneumonia: a population-based case-control study. *Arch Intern Med*. 2007;167:950–955.

Clozapine Is Cytotoxic to Primary Cultures of Human Bone Marrow Mesenchymal Stromal Cells

To the Editors:

Clozapine is one of the most effective antipsychotic drugs, but its use is limited by a high incidence of agranulocytosis in 0.8% of patients.[1] The molecular mechanisms of clozapine-induced agranulocytosis are still poorly understood. Clozapine does not exhibit direct toxic effects to peripheral or progenitor blood cells at therapeutic concentrations.[1] Nevertheless, when clozapine is bioactivated (oxidized) to a nitrenium ion, it will induce neutrophil apoptosis at therapeutic levels.[2,3] Most of the research regarding the mechanisms of agranulocytosis has focused on its effects on various hematopoietic cells. A recent

report, however, demonstrated that bioactivated clozapine induced cell death in immortalized stromal cell lines, whereas clozapine without bioactivation was not cytotoxic.[4] Mesenchymal stromal cells (MSCs) are a nonhematopoietic stem cell population endowed with the capacity to generate osteoblasts, chondrocytes, adipocytes, and cells that regulate hematopoiesis.[5] The stroma provides a specialized microenvironment for hematopoiesis that supports granulopoiesis and the development of other hematopoietic precursor cells.[4–6] As toxicity of clozapine has been demonstrated in immortalized stromal cell lines,[4] we investigated whether similar effects are seen on primary cultures of human bone MSCs. We exposed MSCs to clozapine and its reactive metabolites that were generated by oxidation with horse radish peroxidase (HRP)–H_2O_2.[1,4]

Mesenchymal stromal cells were obtained from heparinized bone marrow aspirates of 5 healthy male volunteer donors, aged 21 to 29 years old, and one was 72 years old. The donors gave their written informed consent, and the study was approved by the ethics committee of Helsinki University Central Hospital, Finland. A previously described procedure was used to isolate the MSCs.[7] The detailed cell culture procedure can be obtained from the authors. The multilineage potential of the MSCs was tested by their ability to differentiate into osteoblasts and adipocytes as described.[5] Mesenchymal stromal cells from passages 4 to 5 and human fibroblast passages 8 and 17 were used for the experiments. The MSCs and the human fibroblasts were incubated with clozapine at a concentration of 10 μmol/L in the absence or presence of the oxidation system. Altogether, 3 U HRP/20,000 cells in a total volume of 100 μL in a 96-well cell culture plate were added, and the reaction started with 25 μmol/L H_2O_2, both diluted in phosphate-buffered saline as described.[1,4] Suitable concentrations of HRP and H_2O_2 for subsequent assays were determined in preliminary experiments (data not shown). The plates were incubated at 37°C and 5% CO_2 in air for 24 hours. The experiments were performed using 3 to 5 parallel wells per condition and the adenosine triphosphate (ATP) luciferase assay used to detect the cytotoxicity of the cell cultures. The assay procedure can be obtained from the authors upon request. The values given for stromal cells in the experiment comparing MSCs and human fibroblasts were the mean (SD) of 4 separate experiments and, for human fibroblasts, the mean (SD) of 3 experiments. The results of the individual experiments were combined and normalized to the values of the control MSCs and fibroblasts that were taken as 100%. The values comparing the effect of the bioactivation system were the mean of 2 experiments for MSCs and 1 experiment for human fibroblasts. The differences in means were analyzed by Student 2-tailed *t* test (2-sample equal variance), and statistical difference was compared with untreated cells as controls. A $P < 0.05$ was considered to indicate significance.

Mesenchymal stromal cells and fibroblasts were incubated with 10 μmol/L of clozapine for 24 hours, and cell viability was measured with the ATP luciferase assay. The MSCs were very sensitive ($P < 0.05$) to the toxic effects of 10 μmol/L clozapine (Fig. 1).

Interestingly, clozapine was not toxic to fibroblasts but rather appeared to stimulate their growth ($P = 0.006$). Moreover, unmodified clozapine at a concentration of 10 μmol/L was toxic to MSCs, whereas bioactivation with HRP + H_2O_2 nullified this toxicity. The difference was significant between untreated and clozapine-treated cells in the absence of bioactivation ($P = 0.006$). Treatment of MSCs with the oxidation system alone did not induce cytotoxic reaction ($P = 0.22$). Interestingly, oxidation counteracted the toxicity of clozapine because the difference between untreated cells and cells treated with bioactivated clozapine was not significant ($P = 0.50$). Clozapine (10 μmol/L) with or without bioactivation had no toxic effect on the fibroblasts. Without bioactivation, clozapine had a growth-stimulatory effect as compared with control cultures ($P = 0.03$). Bioactivation of clozapine seemed to cancel its growth-stimulatory effect on fibroblasts. Bioactivation alone stimulated fibroblast growth, but this effect nearly disappeared in combination with clozapine.

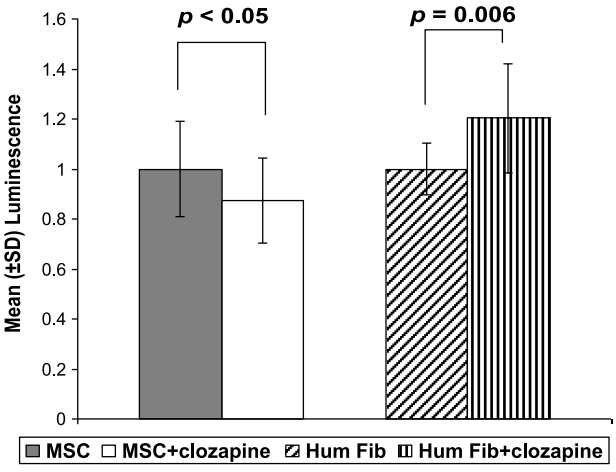

FIGURE 1. The effect of 10 μmol/L of clozapine on cultures of mesenchymal stromal cells (MSC) and of skin fibroblasts (Hum Fib). The cells were treated for 24 hours, and ATP content was measured by quantitative bioluminescence. The mean values (SD) are shown. The statistical differences between untreated cells and cells treated with clozapine are indicated.

DISCUSSION

Our results demonstrate that clozapine is cytotoxic to primary MSCs. Although bioactivation of clozapine has been claimed to play an important role in the development of clozapine-induced agranulocytosis, we were unable to find any additional toxicity of 10 μmol/L of bioactivated clozapine to primary bone MSCs. Our finding is supported by a study of Gardner et al[3] who reported that clozapine adducts did not induce myelotoxicity in rat bone marrow. The present findings differ from those of the study using immortalized human bone marrow MSC line, where clozapine was cytotoxic only after bioactivation.[4]

Clozapine-induced neutropenia and agranulocytosis may have different etiological mechanisms.[8] Milder cases of white blood cell dyscrasia may represent increased sensitivity to the reactive metabolite.[9] The more serious conditions and the fatal cases often occurring within the first 3 months of treatment may indicate a direct cytotoxicity toward the bone marrow MSCs. We were able to show toxic reaction toward mesenchymal stromal cells at a clozapine concentration of 10 μmol/L, which is slightly supratherapeutic, as 1 to 3 μmol/L corresponds to therapeutic levels in vivo.[2] Clozapine treatment typically extends from months to years. We hypothesize that the modest growth-inhibitory effects that we detected may be amplified in the bone

marrow of patients undergoing long-term therapy with clozapine. Mesenchymal stromal cells and fibroblasts could metabolize clozapine along different pathways and therefore accumulate toxic compounds differently. An alternative explanation could be that the uptake of clozapine by primary mesenchymal stromal cells may be more efficient.[10]

Our study has several limitations. The results are based on a small number of experiments, and also, there was considerable variation in the luminescence emitted by both cell types possibly reflecting the special nature of primary MSCs. Moreover, the MSCs of individual donors may differ in their sensitivity to clozapine, which could influence the results. We incubated the cells with clozapine for only 24 hours. The modest growth-inhibiting effects detected may be amplified in the bone marrow of patients undergoing long-term therapy with clozapine lasting typically months or even years. In addition, the onset of agranulocytosis is delayed. Furthermore, we did not study the effect of other atypical antipsychotics on bone marrow MSCs, and it is therefore not known whether stromal cell cytotoxic reaction is unique to clozapine.

In summary, we have demonstrated the specific sensitivity of cultured mesenchymal stromal cells to clozapine. Our results indicate that a direct cytotoxic effect on bone marrow MSCs is one possible mechanism by which clozapine induces agranulocytosis.

ACKNOWLEDGMENTS

The authors thank Tiiu Arumäe, Anna Wilenius, and Dr Johan Westberg from the Haartman Institute, University of Helsinki, and Davina Malviluoto from the Institute of Biomedicine, University of Helsinki, for technical assistance and Novartis Pharma for providing clozapine.

AUTHOR DISCLOSURE INFORMATION

Dr Lahdelma has received a research grant from the Finnish Medical Society Duodecim, H. Lundbeck Finland and from the University of Helsinki. She has also received compensation for speaking for H. Lundbeck and Novartis and participated as a clinical investigator in drug trials initiated by Lundbeck. Otherwise, the authors have no disclosures of competing interests to make.

Liisa Lahdelma, MD, PhD
Departments of Psychiatry and Pathology
Haartman Institute
University of Helsinki
Helsinki, Finland
liisa.lahdelma@kolumbus.fi

Sofia Oja, MSc
Department of Anatomy
Institute of Biomedicine
University of Helsinki
Helsinki, Finland

Matti Korhonen, MD, PhD
Unit for Pediatric Hematology and Oncology
Hospital for Children and Adolescents
Helsinki University Central Hospital
and Finnish Red Cross Blood Service
Helsinki, Finland

Leif C. Andersson, MD, PhD
Department of Pathology
Haartman Institute
University of Helsinki
and HUSLAB
Helsinki University Central Hospital
Helsinki, Finland

REFERENCES

1. Williams DP, Pirmohamed M, Naisbitt DJ, et al. Neutrophil cytotoxicity of the chemically reactive metabolite(s) of clozapine: possible role in agranulocytosis. *J Pharmacol Exp Ther*. 1997;283: 1375–1382.
2. Williams DP, Pirmohamed M, Naisbitt DJ, et al. Induction of metabolism-dependent and -independent neutrophil apoptosis by clozapine. *Mol Pharmacol*. 2000;58: 207–216.
3. Gardner I, Popovic M, Zahid N, et al. A comparison of the covalent binding of clozapine, procainamide, and vesnarinone to human neutrophils in vitro and rat tissues in vitro and in vivo. *Chem Res Toxicol*. 2005;18:1384–1394.
4. Pereira A, Dean B. Clozapine bioactivation induces dose-dependent, drug-specific toxicity of human bone marrow stromal cells: a potential in vitro system for the study of agranulocytosis. *Biochem Pharmacol*. 2006;72:783–793.
5. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999;284:143–147.
6. Guest I, Uetrecht J. Drugs that induce neutropenia/agranulocytosis may target specific components of the stromal cell extracellular matrix. *Med Hypotheses*. 1999;53:145–151.
7. Mandelin J, Hukkanen M, Li TF, et al. Human osteoblasts produce cathepsin K. *Bone*. 2006;38:769–777.
8. Flanagan RJ, Dunk L. Haematological toxicity of drugs used in psychiatry. *Hum Psychopharmacol*. 2008;23(suppl 1):27–41.
9. Uetrecht JP. Reactive metabolites and agranulocytosis. *Eur J Haematol Suppl*. 1996;60:83–88.
10. Bergemann N, Abu-Tair F, Aderjan R, et al. High clozapine concentrations in leukocytes in a patient who developed leukocytopenia. *Prog Neuropsychopharmacol Biol Psychiatry*. 2007;31:1068–1071.

Severe Bowel Ischemia Due to Clozapine With Complete Remission After Withdrawal

To the Editors:

Clozapine is a very efficient atypical antipsychotic whose use has decreased because of the risk of neutropenia and agranulocytosis, which makes clinical management complicated because of the need for hematologic monitoring.[1] However, clozapine has proven to be more effective than other antipsychotic drugs against treatment-resistant schizophrenia and the negative symptoms of schizophrenia.[2] The effectiveness of clozapine could be due to its dual action on serotonin and dopamine receptors; however, this same action could be responsible for the increased frequency of gastrointestinal effects related to hypomotility, including mild effects such as persistent constipation, and more severe effects, such as fecaloma, paralytic ileus, or, more rarely, very severe perfusion impairment leading to bowel ischemia and death.[3]

CASE REPORT

Our patient was a 34-year-old unemployed white male living with his parents in a small town on the outskirts of a large city in the center of Spain. Some months before the current episode, he had to quit his job as a systems engineer because of the severity of his symptoms. He had been diagnosed with paranoid schizophrenia 2 years earlier, and since then, he has been admitted to our psychiatric inpatient unit 4 times after suicide attempts (overdose of different antipsychotics and benzodiazepines in each case). During the same period, the patient also had to be hospitalized in our day unit. His schizophrenia was refractory to long-term treatment with different antipsychotics (olanzapine 20 mg/d, risperidone 4.5 mg/d, ziprasidone 120 mg/d), with a predominance of negative symptoms and progressive impairment. The patient was started on clozapine with a progressive increase in dosage to a maximum of 200 mg/d in the following 3 months until the current episode. No other antipsychotic drugs were administered during this period.

During his most recent hospital stay (November 2008) and after an episode of constipation lasting several days, the patient developed acute abdominal pain, hypotension, and hematemesis and had to be admitted to the general emergency room because of hemodynamic instability. An abdominal radiograph showed marked