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\(^8\) (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\(^9\) but has been reported to induce gastric outlet obstruction\(^6\) 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.

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**

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**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.\(^3\) Nevertheless, when clozapine is bioactivated (oxidized) to a nitroreum ion, it will induce neutrophil apoptosis at therapeutic levels.\(^2\) Most of the research regarding the mechanisms of agranulocytosis has focused on its effects on various hematopoietic cells. A recent
Letters to the Editors

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Mesenchymal stromal cells (MSCs) are nonhematopoietic stem cell lines, whereas clozapine without bioactivation was not cytotoxic. Mesenchymal stromal cells provide a specialized microenvironment for hematopoiesis that supports granulopoiesis and the development of other hematopoietic precursor cells. As toxicity of clozapine has been demonstrated in immortalized stromal cell lines, we investigated whether similar effects are seen on primary cultures of human bone marrow MSCs. We exposed MSCs to clozapine and its reactive metabolites that were generated by oxidation with horse radish peroxidase (HRP). Clozapine-induced neutropenia and agranulocytosis may have different etiological mechanisms. Milder cases of white cell dyscrasia may represent increased sensitivity to the reactive metabolite. 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. 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.

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 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.

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 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 + H2O2 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.
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.

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To the Editors:

Severe Bowel Ischemia Due to Clozapine With Complete Remission After Withdrawal

C 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. However, clozapine has proven to be more effective than other antipsychotic drugs against treatment-resistant schizophrenia and the negative symptoms of schizophrenia.8 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.9

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

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