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The Evaluation of Thioridazine as a Hematopoietic Progenitor Cell Mobilizing Agent in Healthy Human Subjects

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Keywords

Thioridazine; CD34+ Hematopoietic Progenitor Cells; VLA-4; Stem Cell Transplantation

Adhesion molecules comprise a large class of proteins that control the trafficking of hematopoietic progenitor cells between the bone marrow compartment and peripheral blood. The normal trafficking of blood-forming cells is dependent upon the controlled regulation of CXCR4, LFA-1 and VLA-4-mediated binding and unbinding events involving stromal elements.^{1, 2} The mobilization of hematopoietic progenitor cells has been exploited for numerous medical conditions, including the use of erythropoietin for the harvest of autologous red cells transfusions, granulocyte colony stimulating factor (G-CSF) for the release of granulocytes to treat chemotherapy-induced myelosuppression, and plerixafor for the mobilization of CD34 + HPCs cells in autologous stem cell transplantation.³

Autologous and allogeneic stem cell transplantation remains an important therapeutic option for patients who require intensified chemotherapy for a variety of indications. Autologous stem cells were initially directly harvested from the bone marrow compartments of patients prior to receiving cytotoxic therapies. Advances in clinical therapy have allowed hematopoietic progenitor cells (HPCs) to be harvested from the peripheral blood. In human

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subjects, HPCs comprise a very small fraction of peripheral blood mononuclear cells (1 cell/1,000; or 0.1%). Such efforts have become vastly more efficient with the utilization of HPC chemical stimulants, termed mobilizing agents, which enhance the release of CD34+ HPCs from the bone marrow compartment into the peripheral blood for harvest.³⁻⁵ However, a number of problems related to the failure to mobilize sufficient numbers of CD34+ HPCs in donors or patients still exist,^{3, 5} necessitating further efforts to improve stem cell mobilization regimens to be short and maximally efficient. Repurposing (repositioning) of existing drugs can potentially provide one of the approaches to achieve this goal.⁶

Multiple hematological side effects have been reported to result from treatment with psychoactive phenothiazines. These reported toxicities include leucopenia, granulocytopenia, thrombocytopenia, agranulocytosis, and bone marrow aplasia.⁷⁻⁹ Until recently, the physiological mechanism causing these potentially life-threatening blood dyscrasias was unknown. However, recently we discovered that phenothiazines can almost instantaneously antagonize VLA-4 dependent cell adhesion and, after administration of thioridazine, HPCs could be rapidly mobilized into the peripheral blood in a murine model.¹⁰ We proposed that by interfering with VLA-4-mediated cell-cell adhesion involving HPCs in the bone marrow, phenothiazines might mobilize cells from the bone marrow niche. This phenomenon might help to explain the cellular mechanisms behind the frequent observation of dyserthropoeisis in patients who underwent treatment with thioridazine for psychosis.^{11, 12} Because the time-course for thioridazine-induced stem cell mobilization in human remains unknown, we hypothesized that thioridazine could be employed as a chemical means to mobilize CD34+ progenitor cells for peripheral blood collection. We thus designed an open-label, prospective, non-randomized feasibility clinical trial to assess the mobilizing capacity of thioridazine in healthy individuals.

Methods

Regulatory Compliance

The protocol was reviewed and approved by the local institutional review board. Written informed consent was obtained from all the participants (UNM HRPO #12-611), and the trial was received oversight from the UNM Cancer Center's Data Safety Monitor Board.

Dose Determination Strategy

The choice of the dose was dictated by the previously published pharmacokinetics and to protect the study subjects from rare serious adverse events.¹³ Thioridazine HCl is a piperidine phenothiazine that is rapidly and completely absorbed from the gastrointestinal tract, leading to maximum plasma concentrations within 2 to 4 hours. The plasma peak concentration (C_{max}) of thioridazine after a single oral dose of 50 mg reaches ~ 280 nM (C_{max} (nM) = 278 ± 132; half-life (hour), t_{1/2} = 6.5 ± 1.3; area under the concentration-time curve (nM*hour), AUC = 2270 ± 1148)¹⁷, and this concentration is at the onset of down-regulation of VLA-4-specific ligand binding *in vitro*¹⁰.

Typically, thioridazine dosing schedules range from 25 to 900 mg/d, and dose-limiting central neurotoxicities include sedation, headache and slowed cognition.¹⁴ As an important

safety consideration, thioridazine affects ventricular repolarization, prolonging the QTc interval in a dose-dependent fashion. Moreover, more than any other antipsychotic medication, thioridazine has been reported to cause *torsade de pointes*-type arrhythmias, syncope and sudden death.¹⁵ The dose-dependent adverse reaction of thioridazine may become clinically important in patients who are slow to metabolize the drug,¹⁶ or harbor any one of more than 10 genetic polymorphisms that lead to the long QTc syndrome.¹⁵ Because of these considerations, we chose to study one single 50 mg dose in our healthy volunteer subjects, as the serum drug concentrations obtained with this dose were safely tolerated by study subjects in other studies.¹⁴ The use of a higher dose or a different dosing schedules might be a valuable option in the future; however, because of safety concerns it is beyond the scope of this trial. In accordance with FDA regulations, an IND (115947) was obtained for the purposes of testing thioridazine as a CD34 cell-mobilizing agent.

Patient Selection and Study Design

We enrolled adult subjects who met the following criteria: Karnofsky score of 100%, use of adequate contraception during the study procedures, not on antibiotics to treat an underlying infection, and no history of cigarette smoking. All women were required to take a urine pregnancy test. Exclusion criteria included chemotherapy or radiation therapy within 4 weeks prior to enrollment in the study, an allergic reaction to thioridazine, concomitant use of phenytoin, known prolonged QTc syndrome, or any investigational agent within 28 days of study enrollment.

Subjects' eligibility was determined in screening visit when a complete physical examination was performed and a medical history was obtained. An ECG was done and baseline CBC with differential and LFTs prior to study enrollment. Subjects who met all eligibility criteria were scheduled for study visits. Peripheral blood was drawn at hour 0 on day 1 of the study. After receiving a single 50 mg dose of thioridazine, vital signs and peripheral blood samples were taken at 2, 4, 8 and 24 hours. Peripheral blood was analyzed for CBCs, each with a differential, and CD34 HPC absolute cell number at TriCore Reference Laboratories (Albuquerque, NM), using CLIA-approved, flow-based tests. Subjects returned to the clinic on the second day for the 24-hour post dose blood draw and follow-up questioning regarding any adverse events. All participants were contacted by telephone within two weeks of testing for any potential side effects.

Statistical analyses

The power analysis was based upon a prior analysis the baseline presence of CD34+ cells in peripheral blood, and on the results of 24 subjects' peripheral blood CD34+ cell counts after receiving plerixafor as a mobilizing agent.^{17, 18} To reach levels of statistically-significant elevation, we determined that at least an 11-fold increase in CD34+ cell count above baseline was needed in five or more study subjects. Based upon those previously observed means and standard deviations we determined that 80% power for a two-sided paired t-test of elevation was attainable with n = 6 subjects and average fold increase of at least 10.

We concentrated on changes from baseline to 8 hours in CD34 (percent of total WBC). The values we obtained were highly discrete (4 of 6 changes were exactly 0 as seen in the

Figure, so that instead of a paired t-test we used an exact Wilcoxon signed rank test (function `wilcox.exact` in the `exactRankTests` library in R). There was no statistically significant elevation ($p = 1.0$).

Results

A total of six patients were enrolled in the study, ages 24-43 years old.

All patients had normal CBC with differential at screening visit (table 1). Peripheral blood analyses did not show a significant change in baseline white cell count, cell differentials or in CD34 hematopoietic progenitor cell mobilization at 2, 4, 8 and 24 hours post dose (Figure). Analyses of CD34 + cell absolute cell counts showed no statistical difference between those identified at baseline versus any other time point within the 24 hour of administration. Analyses of percent changes in cell differentials, including the absolute phagocyte count (APC) and absolute lymphocyte count (ALC), as well as the CD34+ HPC cell count and percent values remained unchanged during the course of the study. All six patients experienced somnolence, two with Grade 0 events, and four with Grade 1 events, all of which had resumed to baseline within 24 hours of dosing. All subjects had a minor diminution of baseline blood pressure levels, consistent with previous reports. Thioridazine concentration of 200-300 nM is at the lower end of the range necessary to down-regulate VLA-4 mediated cell adhesion.¹⁰

Discussion

In an effort to extend the armamentarium of drugs that mobilize CD34+ hematopoietic progenitor cells, we undertook a feasibility study to assess if thioridazine might be further explored for this indication. We chose to use a single, 50 mg dose, as in a previous study of thioridazine pharmacokinetics, the mean peak concentration in healthy human volunteers was measured to be 278 ± 132 nM; 3 hours following an oral dose of medication, and was well tolerated by the study subjects.¹⁹ As in our study, the most common side effects in the study subjects were somnolence (Table). The level of allosteric interference needed to interfere with VLA-4 mediated cell-cell adhesion was predicted to be partially met at this serum concentration.

Very Late Antigen-4 is an adhesion protein that is ubiquitously expressed in all leukocytes, where it functions to mediate tight cell-cell binding through its counter-ligand VCAM-1. It plays important roles in mediating inflammatory responses to antigenic stimuli through its ability to create an immune synapse between various leukocytes. Because integrins represent attractive targets for treating cancer cell migration and leukocyte trafficking, we recently performed a screen to identify VLA-4 allosteric inhibitors and found that perphenazine, trifluoperazine, thioridazine, and several structurally related compounds exhibited properties of VLA-4 allosteric antagonists.¹⁰

We chose thioridazine for further study because it showed the highest potency among the studied compounds and caused hematopoietic progenitor cell mobilization in a mouse model,¹⁰ prompting the current study to ascertain whether thioridazine might mobilize CD34+ progenitor cells in healthy human subjects.

Several previous reports have identified the unique effects of phenothiazine drugs on morphological features of peripheral blood leukocytes. Others have reported on the appearance of “atypical circulating lymphocytes”,^{12, 20, 21} later described as “blast-type” cells in schizophrenic patients.²² It was reported that an increasing number of atypical lymphocytes coincided with the time that phenothiazine drugs were introduced in clinic, and before that time (1957) “normal blood pictures on schizophrenic patients” were recorded.¹² Authors also proposed that leukocyte “intermediate forms” can “escape” into the blood, in a process later termed “cell mobilization”. Our findings that several phenothiazine drugs antagonized VLA-4-dependent cell adhesion and increased the number of colony-forming units in mice provides a feasible explanation for these and other phenomena.¹¹ However, because schizophrenic patients are often treated for a prolonged time periods, it was necessary to determine whether a single dose of a phenothiazine could be used to mobilize HPCs in the short-term, consistent with the requirements for HPC harvest for autologous and allogeneic stem cell transplantation, donor collection and harvesting.

One of the significant factors that might explain the lack of thioridazine effect could be rapid drug metabolism. It has been reported that thioridazine undergoes S-oxidation in the thiazine ring in position 5, as well as aromatic hydroxylation, N-demethylation and N-oxidation. Cytochrome P450 2D6 is the major enzyme responsible for thioridazine metabolism (Reviewed by Daniel and colleagues²³). The plasma concentration kinetics of three major thioridazine metabolites after a single oral dose of 50 mg has been reported by others¹⁴. However, no information about the role of thioridazine metabolism in the regulation of integrin-dependent adhesion can be found in the literature.

Nevertheless, as shown by our previous studies, the dissociation of VLA-4-specific ligand occurs almost instantaneously after the addition of thioridazine or other structurally related compounds *in vitro*¹⁰. This suggests that an unmetabolized drug can be effective in VLA-4 inhibition and no additional enzymatically-dependent activation is required. However, as described for a number of drugs, it is also possible that certain thioridazine derivatives could be more active than the parent compound, specifically because thioridazine effects on VLA-4 do not require active cell signaling and thus, are not directly related to its major use as an anti-psychotic agent that depend on blocking of dopamine receptor D₂ activity¹⁰. Therefore, additional studies are needed to determine whether thioridazine metabolites participate in the regulation of VLA-4-dependent adhesion.

Recently, Plerixafor (trade name Mozobil, formerly AMD3100), an immunostimulant used in to multiply hematopoietic stem cells in cancer patients was approved by the FDA to be used in conjunction with G-CSF to mobilized CD34 + HPCs. Plerixafor was initially developed at the Johnson Matthey Technology Centre for potential use in the treatment of HIV because of its role in the blocking of CXCR4, a chemokine receptor which acts as a co-receptor for certain strains of HIV (along with the virus's main cellular receptor, CD4).²⁴ Development of this indication was terminated because of lacking oral availability and cardiac disturbances. Studies in pregnant animals also showed teratogenic effects. However, the drug was identified to mobilize CD34 + HPCs human subjects²⁵, but at high cost per patient. The average wholesale price of a single vial of plerixafor is \$7500. For a patient weighing 100 kg, a 4-day treatment course would be expected to cost ~ \$30,000.

In contrast to our results with thioridazine, the provision of single dose of plerixafor was shown to significantly increase CD34 + HPCs in healthy human subjects.⁴ We conclude that while thioridazine may lead to CD34 HPC mobilization over a long-term administration, possibly leading to hematopoietic abnormalities in patients with psychosis, in the short term it did not effectively mobilize HPCs for therapeutic utilization. VLA-4 allosteric inhibitory agents continue to attract interest as chemotherapy sensitizing agents for patients having with lymphoid neoplasms.²⁶ Should thioridazine be assessed as a chemotherapy-enhancing agent, we further recommend that study subjects be tested for not only for EKG variants, but also prolonged QTc genetic polymorphisms to assure their safety in the setting of a prospective clinical trial.²⁷

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References

1. Lapidot T, Dar A, Kollet O. How do stem cells find their way home? *Blood*. 2005; 106(6):1901–1910. [PubMed: 15890683]
2. Gazitt Y. Homing and mobilization of hematopoietic stem cells and hematopoietic cancer cells are mirror image processes, utilizing similar signaling pathways and occurring concurrently: circulating cancer cells constitute an ideal target for concurrent treatment with chemotherapy and antineoplastic antibodies. *Leukemia*. 2004; 18(1):1–10. [PubMed: 14574330]
3. Motabi IH, DiPersio JF. Advances in stem cell mobilization. *Blood reviews*. 2012; 26(6):267–278. [PubMed: 23068307]
4. Hubel K, Liles WC, Broxmeyer HE, et al. Leukocytosis and Mobilization of CD34+ Hematopoietic Progenitor Cells by AMD3100, a CXCR4 Antagonist. *Supportive cancer therapy*. 2004; 1(3):165–172. [PubMed: 18628138]
5. Vose JM, Ho AD, Coiffier B, et al. Advances in mobilization for the optimization of autologous stem cell transplantation. *Leukemia & lymphoma*. 2009; 50(9):1412–1421. [PubMed: 19603345]
6. Oprea TI, Bauman JE, Bologa CG, et al. Drug Repurposing from an Academic Perspective. *Drug discovery today Therapeutic strategies*. 2011; 8(3-4):61–69. [PubMed: 22368688]
7. Hall RL, Smith AG, Edwards JG. Haematological safety of antipsychotic drugs. Expert opinion on drug safety. 2003; 2(4):395–399. [PubMed: 12904095]
8. Sedky K, Lippmann S. Psychotropic medications and leukopenia. *Current drug targets*. 2006; 7(9): 1191–1194. [PubMed: 17017894]
9. Stubner S, Grohmann R, Engel R, et al. Blood dyscrasias induced by psychotropic drugs. *Pharmacopsychiatry*. 2004; 37(Suppl 1):S70–78. [PubMed: 15052517]
10. Chigaev A, Wu Y, Williams DB, Smagley Y, Sklar LA. Discovery of very late antigen-4 (VLA-4, alpha4beta1 integrin) allosteric antagonists. *The Journal of biological chemistry*. 2011; 286(7): 5455–5463. [PubMed: 21131351]
11. Chigaev A, Winter SS, Sklar LA. Is prolonged stem cell mobilization detrimental for hematopoiesis? Medical hypotheses. 2011; 77(6):1111–1113. [PubMed: 21963354]
12. Fieve RR, Blumenthal B, Little B. The relationship of atypical lymphocytes, phenothiazines, and schizophrenia. *Archives of general psychiatry*. 1966; 15(5):529–534. [PubMed: 5926600]
13. Singh S, Loke YK. Drug safety assessment in clinical trials: methodological challenges and opportunities. *Trials*. 2012; 13:138. [PubMed: 22906139]

14. Hartigan-Go K, Bateman DN, Nyberg G, Martensson E, Thomas SH. Concentration-related pharmacodynamic effects of thioridazine and its metabolites in humans. *Clinical pharmacology and therapeutics*. 1996; 60(5):543–553. [PubMed: 8941027]
15. Beach SR, Celano CM, Noseworthy PA, Januzzi JL, Huffman JC. QTc prolongation, torsades de pointes, and psychotropic medications. *Psychosomatics*. 2013; 54(1):1–13. [PubMed: 23295003]
16. von Bahr C, Movin G, Nordin C, et al. Plasma levels of thioridazine and metabolites are influenced by the debrisoquin hydroxylation phenotype. *Clinical pharmacology and therapeutics*. 1991; 49(3): 234–240. [PubMed: 2007317]
17. Hubel K, Liles WC, Broxmeyer HE, et al. Leukocytosis and Mobilization of CD34+ Hematopoietic Progenitor Cells by AMD3100, a CXCR4 Antagonist. *Support Cancer Ther*. 2004; 1(3):165–172. [PubMed: 18628138]
18. Holig K, Kramer M, Kroschinsky F, et al. Safety and efficacy of hematopoietic stem cell collection from mobilized peripheral blood in unrelated volunteers: 12 years of single-center experience in 3928 donors. *Blood*. 2009; 114(18):3757–3763. [PubMed: 19666868]
19. Hartigan-Go K, Bateman DN, Nyberg G, Martensson E, Thomas SH. Concentration-related pharmacodynamic effects of thioridazine and its metabolites in humans. *Clin Pharmacol Ther*. 1996; 60(5):543–553. [PubMed: 8941027]
20. Fessel WJ, Hirata-Hibi M. Abnormal Leukocytes in Schizophrenia. *Arch Gen Psychiatry*. 1963; 9:601–613. [PubMed: 14056842]
21. Vander Kamp H. Nuclear changes in the white blood cells of patients with schizophrenic reaction. A preliminary report. *Journal of neuropsychiatry*. 1962; 4:1–3. [PubMed: 13996207]
22. Kokai M, Morita Y, Fukuda H, Hatotani N. Immunophenotypic studies on atypical lymphocytes in psychiatric patients. *Psychiatry research*. 1998; 77(2):105–112. [PubMed: 9541146]
23. Daniel WA, Syrek M, Haduch A, Wojcikowski J. Pharmacokinetics and metabolism of thioridazine during co-administration of tricyclic antidepressants. *Br J Pharmacol*. 2000; 131(2): 287–295. [PubMed: 10991922]
24. De Clercq E, Yamamoto N, Pauwels R, et al. Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative JM3100. *Antimicrob Agents Chemother*. 1994; 38(4):668–674. [PubMed: 7913308]
25. Devine SM, Vij R, Rettig M, et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. *Blood*. 2008; 112(4):990–998. [PubMed: 18426988]
26. Shishido S, Bonig H, Kim YM. Role of integrin alpha4 in drug resistance of leukemia. *Frontiers in oncology*. 2014; 4:99. [PubMed: 24904821]
27. Pazoki R, de Jong JS, Marsman RF, et al. SNPs identified as modulators of ECG traits in the general population do not markedly affect ECG traits during acute myocardial infarction nor ventricular fibrillation risk in this condition. *PLoS One*. 2013; 8(2):e57216. [PubMed: 23437344]

Figure.

CD34 as percent of total WBC versus time since administration of thioridazine.

Measurements were taken at time of administration and at 2, 4, 8, and 24 hours post-administration. Each of the six panels of the graph represents one of the six subjects in the experiment.

Table 1

Patient Demographics, CBC results and highest-grade adverse events

Subject (age/Gender)	CD34+ Base %	CD34+ 8 hour %	Baseline WBC (APC/ALC)	8 hour WBC (APC/ALC)	24 hour WBC (APC/ALC)
Pt. 1 29/M White, Hispanic	0.05	0.05	6.4 (2.4/2.7)	7.1 (4.6/2.4)	5.3 (2.9/1.7)
Pt. 2 24/F White, Hispanic	0.05	0.04	4.1 (2.6/1.4)	5.5 (3.2/2.2)	4.2 (2.1/1.6)
Pt. 3 * 24/F White, Asian	0.04	0.03	6.9 (4.5/2.1)	6.3 (4.6/1.5)	5.5 (2.7/2.1)
Pt. 4 * 28/M White, Hispanic	0.04	0.06	5.6 (3.5/1.9)	6.8 (3.6/2.2)	5.7 (3.4/2.2)
Pt. 5 * 24/F White, Hispanic	0.08	0.08	8.3 (4.8/3.1)	6.1 (3.6/2.3)	6.1 (3.2/2.3)
Pt. 6 * 43/F White, Hispanic	0.04	0.04	6.4 (3.4/2.7)	7.1 (4.6/2.4)	5.5 (2.8/2.0)

All White Blood Count (WBC) hematological units expressed as 10^3 cells/ μ L

APC = absolute phagocyte count

ALC = absolute lymphocyte count

* Developed a Grade 1 somnolence adverse event, per definition of common terminology criteria for adverse events (CTCAE) version 4.0.