DISCOVERY OF A SMALL, NON-PEPTIDYL MIMIC OF GRANULOCYTE COLONY-STIMULATING FACTOR

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1 INTRODUCTION

Granulocyte colony-stimulating factor (G-CSF) is a 21 kDa hematopoietic cytokine secreted by bone marrow stroma cells, macrophages, fibroblasts and endothelial cells. Recombinant human G-CSF, available in both glycosylated and non-glycosylated forms, has become an important therapeutic agent for the treatment of a variety of human neutropenias, including those resulting from chemotherapy, congenital defects and bone marrow transplantation.1 Genetically engineered G-CSF, like any other recombinant growth factors, must be administered either subcutaneously or intravenously. Although other agents have been shown to activate cytokine receptors by oligomerization,2 no smallmolecule cytokine mimics with potential for oral delivery have yet been reported.

2 METHOD AND RESULTS

**2.1 Identification of a Suitable G-CSF Mimic**

An assay was designed to identify non-peptidyl compounds that activate the G-CSF receptor based on activation of STATs, which are known to play a central role in the GCSF-mediated responses. From the drug resistant clones responsive to G-CSF, a single clone, which exhibited 20-fold induction of luciferase activity by G-CSF and the same pattern of JAK and STAT activation as the parental cells, was selected to screen a library of synthetic organic compounds. For the screen, the cells were incubated for 2.5 hours with individual compounds at a concentration of 10 μM in a 96 well plate format. Compound SB-247464 (Figure 1) was identified as a hit in the assay and showed a dose–response effect with a maximum efficacy of 30% that of G-CSF at 1 µM.



**Figure 1** *Structure of SB-247464.*

As expected, SB-247464 induced activation of G-CSF signal transduction pathways, the efficacy being *ca.* 25–50% that of G-CSF, consistent with data from the luciferase assay.

**2.2 Assessment of Activity of SB-247464**

To assess SB-247464 in supporting the proliferation and differentiation of cells of the granulocytic lineage, colony-forming unit-granulocyte (CFU-G) assays from murine bone marrow were performed. SB-247464 stimulated the production of granulocytic colonies, with an efficacy 20–80% of that of G-CSF at 0.3–3 μM; the colonies appeared uniformly smaller than those promoted by G-CSF, but were consistently larger than 30 cells. Likewise, SB-247464 was able to mimic the activity of G-CSF *in vivo* (Figure 2): subcutaneous administration twice a day to normal mice caused a dose-dependent increase in peripheral blood neutrophils after 4 days. Efficacy at 30 mg/kg was comparable to that of 50 μg kg–1 of G-CSF, elevating the neutrophil counts to *ca.* 400% over baseline. The magnitude of the increase was equivalent to that effected by administration of 5–30 μg kg–1 day–1 of G-CSF to normal or neutropenic humans. Table 1 shows examples.



**Figure 2** *Granulopoietic activity in vivo;* \* *indicate neutrophil counts.*

**Table 1** *Neutrophil count and granulopoietic activity.*

|  |  |  |
| --- | --- | --- |
| *Neutrophil count* | *Granulopoietic activity* | *Data* |
| 1 | 0.1 | Yes |
| 2 | 0.2 | No |
| 3 | 0.3 | No |
| 4 | 0.4 | – |
| 5 | 0.5 | Yes |

3 CONCLUSION

The identification of SB-247464 as a G-CSF mimetic provides proof of principle for drug discovery using JAK/STAT-based assays, and shows for the first time that a small nonpeptidyl molecule can trigger the selective activation of a cytokine receptor. These findings may lead to the development of orally available G-CSF mimics for use in the treatment of neutropenia.

**References**

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