MATERIALS AND METHODS

Luciferase Reporter Assays

HepG2 or HEKa-293 cells were transiently transfected with an expression vector for hGCSFR, hTPOR, hEPOR, mouse GCSFR or mutated receptors and either a STAT3-responsive or a STAT5-responsive luciferase reporter. Cells were treated with vehicle, rhGCSF or LG7455 for various times and luciferase activity was measured in a scintillation counter.

Radioligand-binding Experiments

UTP-hGCSFR and hGCSFR-H627N cells were incubated with [125I]rhGCSF (PerkinElmer) in the presence or absence of LG7455 or LG7455 for 2 h at room temperature. Cells were washed twice and the amount of radioactivity measured in a scintillation counter.

RESULTS

Activation of hGCSFR Function: LG7455 activates STAT3 and STAT5-responsive luciferase reporters in cells co-transfected with an hGCSFR expression vector. LG7455 was not active in the absence of transfected hGCSFR (not shown). Mean RLU (duplicates) ± SD

Cytokine Receptor Selectivity: LG7455 does not activate a STAT5-responsive luciferase reporter in HEKa-293 cells transfected with hTPOR or hEPOR expression vectors. Mean (triplicates) ± SD

Activity on Cell Growth and STAT Activation: LG7455 increases the viability of UTP-hGCSFR stable cells. It did not increase growth of TPO- or EPO-responsive UT-7 cells (data not shown). LG7455 increases the phosphorylation of STATs of STAT3. Mean (duplicates) ± SD

Induction of Granulocyte Differentiation: LG7455 induces the expression of the granulocyte-specific marker CD15 in CD34-positive, human bone marrow cells. LG7455 was active in the effect of 0.1 ng/ml hGCSF, comparable to a normal serum concentration of GCSF.

Species Selectivity: LG7455 activates STAT3 and STAT5 reporters in cells expressing human and cynomolgus monkey GCSFR, but not mouse, guinea pig or rabbit. This may be explained by differences in the amino acid sequence in the transmembrane domain among the species.

Summary

LG7455 is a novel small molecule human GCSF receptor agonist that activates GCSFR function. LG7455 activates STAT3 and STAT5 signal transduction and increases the viability of cells containing hGCSFR.

LG7455 promotes the differentiation of human bone marrow cells into granulocytes, significantly increasing expression of CD15.

LG7455 is dependent on the expression of human or monkey GCSFR.

A histidine in the hGCSFR transmembrane domain is needed for activity, similar to what has been found for small-molecule hTPOR agonists, such as eltrombopag.

LG7455 increases the binding of GCSF in a manner consistent with allosteric receptor modulation.

Conclusions

LG7455 is a novel small-molecule selective hGCSFR agonist that activates the receptor in a manner distinct from GCSF and similar to the mechanism of small-molecule hTPOR agonists.

Further optimization of the LG7455 structure series should lead to a new generation of orally-available small-molecule hGCSFR agonists for the treatment of neutropenia.