Abstract

**Background:** TTK is a dual-specificity protein kinase that is essential for the proper attachment of chromosomes to the mitotic spindle and for maintaining the spindle assembly checkpoint. TTK is overexpressed in cancer cells. Disruption of TTK function results in mitotic chromosome misalignment and activation of the spindle checkpoint. In an ongoing drug discovery program, we have developed a potent and selective inhibitor of TTK with favorable ADME/TK properties that inhibits the growth of cancer cells in culture and demonstrates anti-tumor activity in a mouse xenograft model.

**Materials and Methods:** Kinase activity was measured in biochemical assays by monitoring the incorporation of [3P] into protein substrates. Phosphorylated Smad2 was assayed by western blotting with antibodies against Smad2 and GAPDH.

**Results:** MPI-0479605 inhibited TTK kinase activity in vitro with an IC50 of 3.5 nM. This compound had moderate activity towards JNK (IC50 = 110 nM) and FER (IC50 = 250 nM) kinases and exhibited little or no activity against a panel of 32 other kinases, suggesting high specificity for TTK. MPI-0479605 induced apoptotic cell death in a dose-dependent manner in HCT116 cells and demonstrated cytotoxicity against a panel of other tumor cell lines. Furthermore, nocodazole-induced activation of the spindle assembly checkpoint and phosphorylation of Smad2 were confirmed in untreated cells, with MPI-0479605 in the presence of nocodazole. In vivo studies were conducted using nude mice bearing xenografts of HCT-116 colon cancer cells. Saline and MPI-0479605 resulted in plasma concentrations high enough to support in vivo antitumor studies. Finally, in HCT-116 tumor-bearing mice, daily treatment with 30 mg/kg MPI-0479605 resulted in a 49% tumor growth inhibition, whereas treatment with 150 mg/kg (4d × 24 d) caused a 75% tumor growth inhibition relative to vehicle-treated animals. The latter effect was comparable to treatment with 5-fluorouracil (100 mg/kg, weekly). Animals dosed with MPI-0479605 had less than a 15% reduction in body weight over the course of the study.

**Conclusions:** The small molecule MPI-0479605 potently inhibits TTK activity in vitro and demonstrates anti-tumor activity in a xenograft model.

**Summary and Conclusions**

1. **MPI-0479605 potently and selectively inhibits TTK activity and induces apoptosis.**
2. **Inhibition of TTK activity disrupts the spindle assembly checkpoint and impairs Smad2 phosphorylation.**
3. **MPI-0479605 exhibits good pharmacokinetic properties and demonstrates anti-tumor activity in a colon cancer xenograft model.**

These data support continued effort to identify TTK inhibitors for clinical development.

**References**