The Synergistic Effect of Pasireotide and a Raf-1 Activating Agent in Carcinoids

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Introduction

Somatostatin analogs are the mainstay treatment for controlling tumor proliferation and hormone secretion in carcinoid patients. The new somatostatin analog Pasireotide (SOM230) may be more effective than others in its class, given its broader receptor spectrum and elevated binding affinity. Recent data suggest that ERK1/2 phosphorylation may potentiate the anti-tumor effects of somatostatin analogs in carcinoids. Additionally, ERK1/2 phosphorylation by Raf-1 activating agents has been shown to suppress biomarker expression in carcinoids. Thus, drugs that activate the Raf-1/MEK/ERK1/2 pathway may be synergistic with somatostatin analogs such as SOM230. Here, we investigate the effects of SOM230 in combination with Teriflunomide (TFN), a Raf-1 activator, in a human carcinoid cell line.

Methods

- Human GI carcinoid cells (BON) were incubated in either TFN (0-100µM), SOM230 (0-10µM) or a combination, for 96 hours. Cell growth was measured by methythiazolyldiphenyl-tetrazolium bromide (MTT) rapid colorimetric assay.
- Western blot analysis was performed for human achaete-scute complex-like 1 (ASCL1) and chromogranin A (CgA), and for pro-apoptotic markers.
- Combination index (CI) values and isobolograms were derived based on the Chou-Talalay method, and generated using Compusyn® software.
- Densitometric analysis of Western blotting results was done using Quantity One software v. 4.6.3 (Bio-Rad).

Results

Combination treatment with SOM230 and TFN reduced cell growth beyond the additive effect of either drug alone. Combination indices fell below 1, thus verifying synergy according to the Chou-Talalay CI scale. Treatment with either 35µM or 50µM of TFN alone dose dependently reduced ASCL1 and CgA expression, by less than 50%. Interestingly, SOM230 alone had little effect on biomarker expression. However, addition of low dose SOM230 following TFN further inhibited ASCL1 and CgA expression levels beyond the sum inhibitory effect of either drug alone. Combination of 0.5µM SOM230 and 50µM TFN reduced ASCL1 and CgA levels by 95% and 66% respectively, compared to controls. TFN also potentiated a range of SOM230 doses, and resulted in synergistic inhibition of ASCL1 and CgA expression. Combination treatment increased levels of phosphorylated ERK1/2, cleaved PARP and caspase-3, and reduced levels of total caspase-3, X-linked inhibitor of apoptosis (XIAP), survivin and Mcl-1, beyond the additive effect of either drug alone.

Conclusions

Combination treatment with SOM230 and TFN in BON carcinoid cells synergistically inhibits both cell growth and biomarker expression via the induction of apoptosis. Elevated Raf-1 activity following combination therapy may underlie the potent anti-tumorogenic effect consequent of synergistic interaction between the two drugs. Low dose combination therapy may accomplish symptomatic relief in carcinoid patients at low toxicity levels. As each drug has been evaluated independently in clinical trials, combinatory drug trials are warranted.

Acknowledgements

Funded by the Shapiro Research Award, Novartis® and the Howard Hughes Medical Institute.