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

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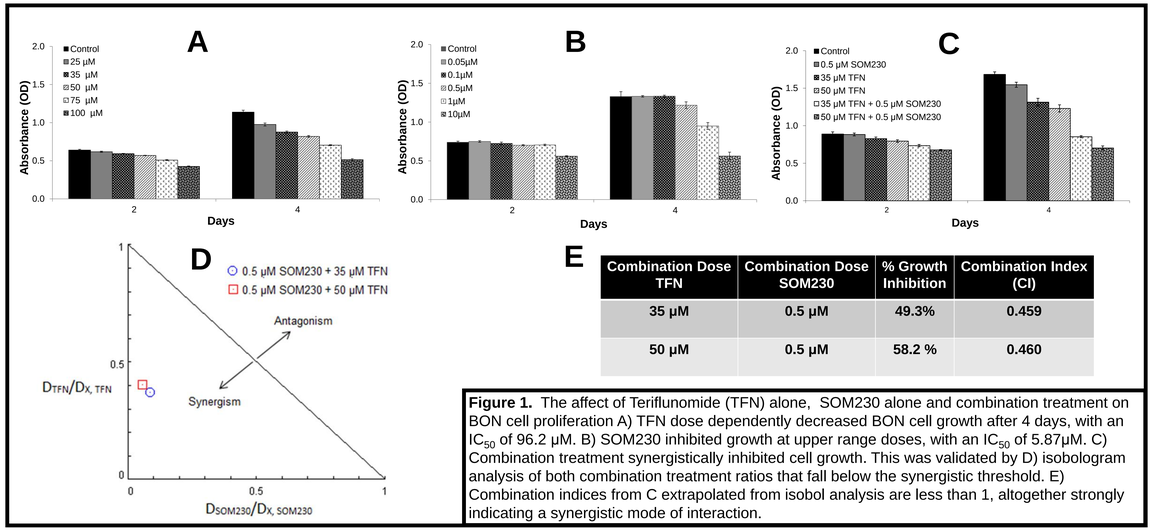
Introduction

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 methylthiazolyldiphenyl-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).



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Combination Dose TFN	Combination Dose SOM230	% Growth Inhibition	Combination Index (CI)
35 μM	0.5 μM	49.3%	0.459
50 μM	0.5 μM	58.2 %	0.460

Figure 1. The affect of Teriflunomide (TFN) alone, SOM230 alone and combination treatment on BON cell proliferation A) TFN dose dependently decreased BON cell growth after 4 days, with an IC_{50} of 96.2 μM. B) SOM230 inhibited growth at upper range doses, with an IC_{50} of 5.87μM. C) Combination treatment synergistically inhibited cell growth. This was validated by D) isobologram analysis of both combination treatment ratios that fall below the synergistic threshold. E) Combination indices from C extrapolated from isobol analysis are less than 1, altogether strongly indicating a synergistic mode of interaction.

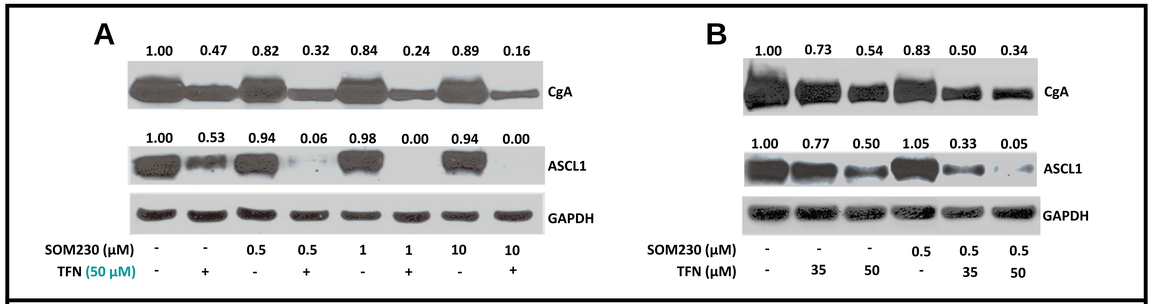

Figure 2. Combination therapy synergistically inhibits expression of CgA and ASCL1 in BON cells. A) While increasing doses of SOM230 alone only minimally affected CgA and ASCL1 expression levels, concomitant treatment with 50 μM of TFN potentiated SOM230 to cause up to 84% and 100% inhibition of CgA and ASCL1 respectively. B) The low dose combinations of TFN and SOM230 were shown to inhibit CgA and ASCL1 expression beyond the additive effect of either drug dosage alone. Both A and B present densitometric values as normalized to their respective GAPDH loading control and subsequently to the control densities.

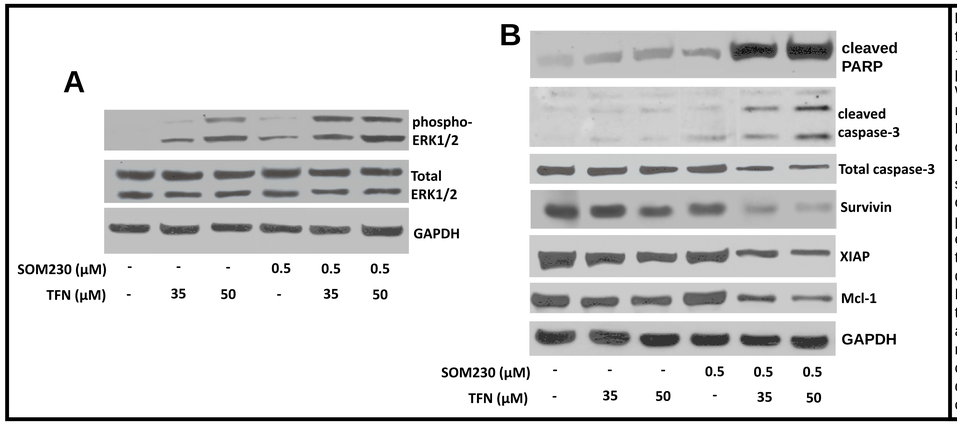

Figure 3. Combination therapy activates the Raf-1/MEK/ERK pathway and promotes apoptosis. A) While SOM230 alone only marginally activated the ERK1/2 pathway, combination treatment with TFN at the same synergistic low dose combinations increased phospho-ERK1/2 expression levels beyond the additive effect of either drug alone. B) Cleavage of PARP and caspase-3 and the inhibition of anti-apoptotic molecular markers following combination therapy demonstrates the induction of apoptosis.

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, combinatorial drug trials are warranted.

Acknowledgements

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