Abstract
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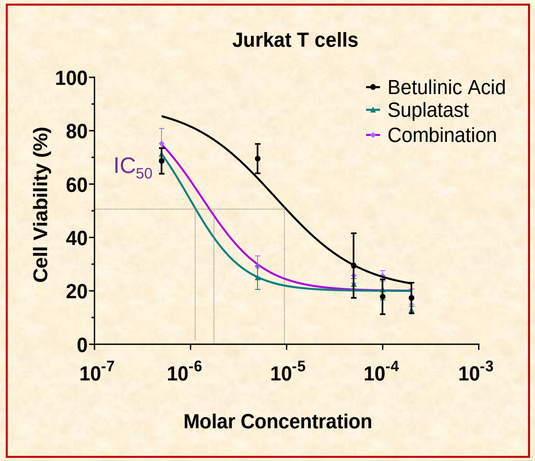
Betulinic Acid- A Sibling of Salicylic Acid or A Vehicle With Benefits

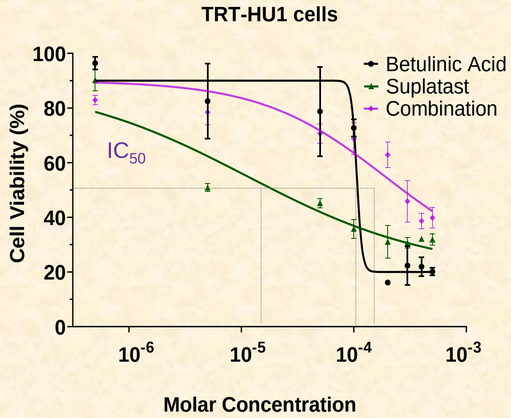
Pradeep Tyagi, Jonathan Beckel, Anthony Kanai, Anirban Ganguly
School of Medicine, University of Pittsburgh

Aspirin

Betulinic Acid
456.7 Daltons

Salicylic Acid
138.12 Daltons





Betulinic Acid Reduces Suplatast Related Toxicity on Urothelium

IC_{50}(μM)	Betulinic Acid	Suplatast	Combination
TRT-HU1 cells	106.32 ± 3.45μM	11.29 ± 5.66μM	**188.83 ± 2.89μM**
Jurkat T cells	7.67 ± 2.26μM	0.96 ± 0.33μM	1.31 ± 0.19μM
Fold Change (Selectivity for T cells)	13.85	11.71	**144.13**

BACKGROUND

- While inflammation is not uniformly detected in interstitial cystitis (IC), Hunner lesion guarantees the success of anti-inflammatory drug, cyclosporine in IC, but its narrow therapeutic index highlights the unmet need and a market for mild plant based anti-inflammatory drugs
- Prototype of non-steroidal anti-inflammatory (NSAIDs)- salicylic acid is also plant based, derived from bark of willow tree (L. Salix)
- For IC, however, risk-benefit ratio of NSAIDs is poor because the benefit of lower prostaglandins at the inflammatory site must be weighed against risk of ulcer from lower prostaglandins in stomach and in bladder mucosa[1]
- Indeed, the promise of prostaglandin analogs over NSAIDs in IC patients[2] drew us to the ancient wisdom of folk medicine
- Native Americans use the bark of white birch as medicine and the active moiety of *Betula alba*, Betulinic acid, even merited clinal testing as a topical ointment against melanoma (ALS 357) in a Phase I trial (NCT00701987)
- Since Betulinic acid induced apoptosis through changes in mitochondrial membrane potential and reactive oxygen species[3], Betulinic acid promises to exert an anti-inflammatory effect[4] while the self-assembly of the pentacyclic triterpenoid structure into different sizes and structures can entrap other potent anti-inflammatory drugs for synergistic action
- Towards that goal, we examined the selectivity of Betulinic Acid for immune cells over urothelium cells and the potential therapeutic advantage of Suplatast tosilate entrapped in Betulinic Acid nanospheres over free Suplatast tosilate[6]

METHODS

- Cell lines of immune cells and urothelium were incubated with empty or loaded Betulinic Acid nanospheres for 18h
- IC50 - concentration required for decreasing the viability by 50%- a right ward shift indexes a decrease in potency.

RESULTS

- Jurkat T- cells mirrored cancer cells in sensitivity to Betulinic acid with lower IC50 compared to TRT-HU1
- While TRT-HU1 cell line (42 h) for urothelium could tolerate much higher concentrations of Betulinic acid, faster doubling time of Jurkat T- cells (20 h) increases the demand for nutrients and Betulinic acid is mistaken as a nutrient by fast dividing cells
- A ten-fold difference in the cytotoxicity of Suplatast between immune and urothelial cells is intensified to 144-fold upon encapsulation of Suplatast tosilate into Betulinic acid nanospheres

INTERPRETATION

- Nutrient scarcity secondary to faster cell division triggers metabolic signals of pseudo-starvation and the induction of epigenetic changes re-routes other metabolic pathways (metabolic reprogramming) to fuel cell growth with alternative nutrients such as Betulinic acid[5]
- Being structurally similar to cholesterol[3-4] , Betulinic acid dupes starving immune and cancer cells for preferential accumulation in mitochondria to induce apoptosis through changes in mitochondrial membrane potential, produce reactive oxygen species, and the opening of mitochondrial permeability transition pores releases apogenic factors, activates caspases, and fragments DNA
- While anti-inflammatory action may not be as potent as salicylic acid but larger molecular size of Betulinic acid fosters self-assembly into nanospheres for encapsulation of Suplatast tosilate[6] to reduce its toxicity and offer opportunities for synergistic action after intravesical administration- “a vehicle with benefits".

CONCLUSIONS

- Findings corroborate the reported anti-inflammatory effect of Betulinic acid, stemming from its selective accumulation in mitochondria which confers metabolic signals of pseudo-starvation to faster dividing immune cells
- Betulinic acid could make value addition in down-regulating autoinflammatory responses including fibrosis associated with IC without incurring the toxicity of salicylic acid/ NSAIDs
- The self-assembling property of Betulinic acid is a bonus deserving of the moniker “a vehicle with benefits" for Suplatast tosilate.
- A similar response of fast replicating cancer and immune cells to Betulinic acid alludes to a shared metabolic vulnerability and explains the frequent application of anticancer drugs as anti-inflammatory agents: Methotrexate, BCG, Imatinib mesylate (Gleevec)[7].

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