NETUPITANT / PALONOSETRON (NEPA) INDUCES PERSISTENT NK_1 RECEPTOR INTERNALIZATION IN HEK-293 CELLS

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ABSTRACT

Introduction - Previous studies from our laboratory indicate that palonosetron induces 5-HT_3 receptor internalization and inhibits Substance P (SP)-mediated NK_1 receptor responses both *in vitro* and *in vivo,* likely as a result of inhibition of 5-HT_3/NK_1 receptor crosstalk. These results provided a tentative rationale for palonosetron's improved ability among 5-HT_3 receptor antagonists to prevent delayed emesis after emetogenic chemotherapy. More recently, using NG108-15 cells that express both the NK_1 and 5-HT_3 receptors, we have shown that palonosetron and netupitant trigger NK_1 receptor internalization in an additive manner and synergistically inhibit the SP NK_1 receptor response.

Objective - Characterize the contribution of each antagonist on NK_1 receptor internalization and determine the intracellular fate of NK_1 receptors following NEPA-induced internalization.

Methods –NEPA, netupitant and palonosetron were first incubated for 1 h with HEK-293 cells expressing only the NK_1 receptor. Antagonists were then removed and cells were allowed to recover for 6 hours. The extent of SP-triggered Ca^{2+} mobilization was used as a representation of NK_1 receptor levels at the surface.

Results - NEPA prevents SP-triggered Ca^{2+} mobilization in HEK-293 cells expressing the NK_1 receptor; however, this effect was only due to netupitant. Palonosetron alone did not prevent Ca^{2+} mobilization in the absence of 5-HT_3 receptors. Moreover, SP-mediated Ca^{2+} mobilization did not return 6 hours following NEPA removal.

Conclusion – The present studies, together with previous findings, suggest NEPA-triggered NK_1 receptor internalization. Further, receptor internalization is probably followed by receptor degradation rather than recycling. Receptor synthesis may be required before NK_1 receptor function is restored.

INTRODUCTION

APPROXIMATELY 25 – 30% OF PATIENTS UNDERGOING HIGHLY OR MODERATELY EMETOGENIC CHEMOTHERAPY STILL EXPERIENCE NAUSEA AND DELAYED EMESIS

A FIXED DOSE OF NETUPITANT AND PALONOSETRON HAS BEEN RECENTLY APPROVED BY THE FDA FOR THE PREVENTION OF CINV

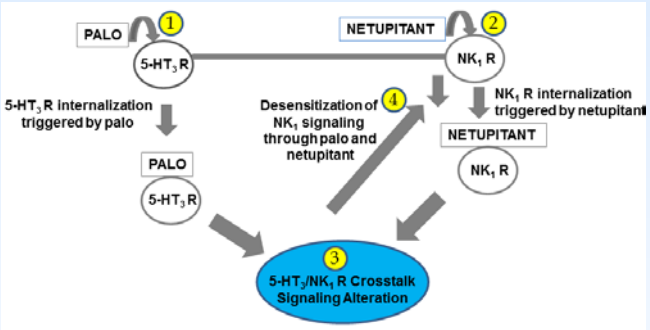
❑ Netupitant is a potent and selective NK_1 receptor antagonist [1]

❑ Palonosetron is the only 5-HT_3 receptor antagonist that has been found to be effective against both acute and delayed CINV [2]

❑ Mechanism of action of palonosetron against delayed emesis has been puzzling because it does not bind to the NK_1 receptor and is a selective antagonist for the 5-HT_3 receptor [3]

IS PALONOSETRON'S SUPPRESSION OF DELAYED EMESIS STILL DISTINCT OR OBSCURED WHEN USED IN COMBINATION WITH NK_1 RECEPTOR ANTAGONISTS?

❑ Recent studies using NG108-15 cells known to express both the 5-HT_3 and NK_1 receptors showed that netupitant and palonosetron exhibit a synergistic effect in the prevention of the NK_1 receptor response against its endogenous agonist, substance P (SP) [4]



Receptor internalization can be induced through direct binding of palonosetron to the 5-HT_3 receptor (1) and of netupitant to the NK_1 receptor (2). Internalization of either receptor could lead to alterations in receptor signaling crosstalk (3) that in turn could bring additional NK_1 receptor internalization and concomitant signal desensitization.

❑ Here, we explore the potential of netupitant and palonosetron to trigger NK_1 receptor internalization in HEK393 cells that only express the NK_1 receptor.

METHODS

SP-triggered Ca^{2+} mobilization was used to gauge NK_1 receptor internalization in HEK-293 cells expressing only the NK_1 receptor

1) Incubate cells with netupitant, palonosetron, NEPA or SP
2) Remove excess antagonist(s)/SP and allow dissociation followed by removal over different times (2.5 - 6 h)
3) Measure SP triggered Ca^{2+} mobilization

RESULTS

(1) NEPA AND NETUPITANT INHIBIT SP-TRIGGERED Ca^{2+} MOBILIZATION IN HEK-293 CELLS EXPRESSING NK_1 RECEPTORS WHILE PALONOSETRON DOES NOT.

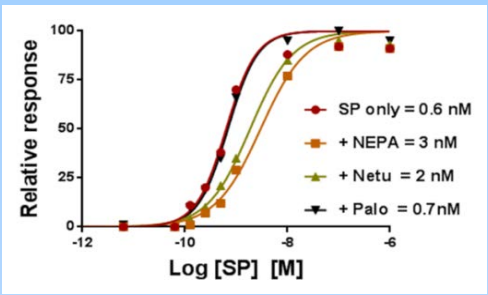


Fig 1 - Dependence of Ca^{2+} mobilization on SP concentration in HEK293 cells expressing the NK_1 receptor. Numbers listed correspond to EC_{50} values of SP under different conditions. Concentrations of antagonists used during incubation were 5-fold the corresponding K_d. Cells were allowed to recover for 2.5 h after removal of antagonists before measuring SP response.

(2) SP-MEDIATED Ca^{2+} MOBILIZATION WAS STILL SUPPRESSED 6 h FOLLOWING NEPA REMOVAL. SIMILAR RESULTS WERE OBSERVED AT 4 h.

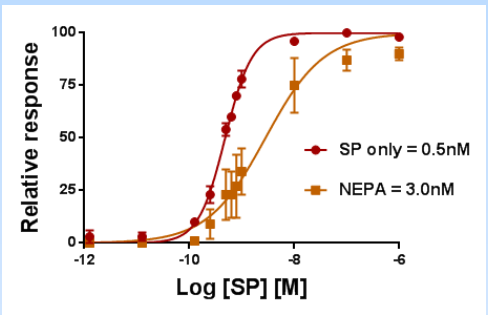
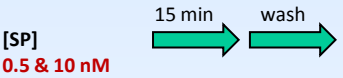
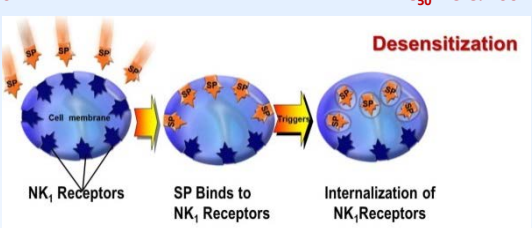


Fig 2 - Dependence of Ca^{2+} mobilization on SP concentration in HEK293 cells expressing the NK_1 receptor. Numbers listed correspond to EC_{50} values of SP ± NEPA. Conditions were the same as those mentioned in Fig 1 except cells were allowed to recover for 6 h after removal of antagonists before measuring the SP response.

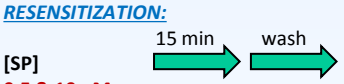
(3) SP TREATMENT OF HEK-293 CELLS EXPRESSING NK_1 RECEPTOR TRIGGERS RECEPTOR DESENSITIZATION FOLLOWED BY SENSITIZATION. NEPA TREATMENT ONLY TRIGGERS DESENSITIZATION.

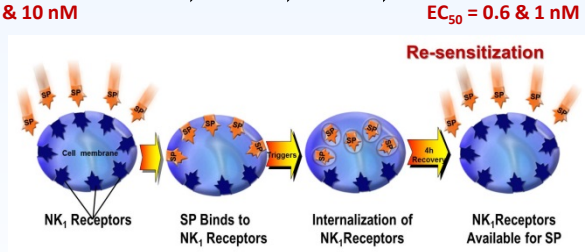
DESENSITIZATION:





RESENSITIZATION:





CONCLUSIONS

- ❑ **Lack of SP-triggered Ca^{2+} mobilization 6 h after exposure to NEPA is suggestive of receptors remaining inside cells rather than at the cell surface.**
- ❑ **Given the long time without recovery, receptor internalization following NEPA exposure is probably followed by receptor degradation rather than recycling. Receptor synthesis may be required before NK_1 receptor function is restored.**

REFERENCES

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2. *2014 Support Care Cancer 22(2), 469-77*
3. *1995 Br J Pharmacol 114, 851-9*
4. *2012 Eur J Pharmacol 689, 25-30*