



INVOLVEMENT OF TUBULIN ACETYLATION IN THE ANTIMOTILITY ACTIVITY OF THE TAXANES PACLITAXEL AND IDN 5390

K. Bonezzi ¹, B. North ², C. Manzotti ³, A. Riva ³, R. Giavazzi ¹, E. Verdin ², G. Taraboletti ¹

¹Department of Oncology, Mario Negri Institute for Pharmacological Research, Bergamo, Italy; ²University of California, San Francisco CA, USA; ³Indena S.p.A., Milan, Italy.

E-mail: taraboletti@marionegri.it

BACKGROUND

- ◆ Taxanes have antiangiogenic activity, due to inhibition of endothelial cell functions, including cell motility.
- ◆ The molecular mechanism of the antimotility activity of taxanes is unknown.
- ◆ From the screening of different taxanes we previously selected IDN 5390, an antiangiogenic seco taxane derivative that has potent antimotility activity and less cytotoxicity than paclitaxel.
- ◆ The α -subunit of tubulin is subjected to different post-translational modifications, including acetylation.
- ◆ Reversible acetylation of α -tubulin is associated with microtubule stability.

AIM OF THE STUDY

The aim of the study was to investigate the relationship between the ability of taxanes to promote α -tubulin acetylation and inhibit cell motility.

METHODOLOGY

◆ COMPOUNDS

IDN 5390 [13-(N-Boc-3-*i*-butylisoserinoyl)-C-7,8-*seco*-10-deacetylbaaccatin III] and paclitaxel were from Indena S.p.A. (Milan, Italy).

◆ CELLS

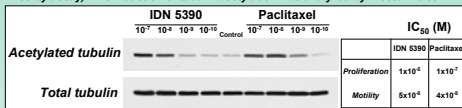
Endothelial cells (HUVECs), isolated from human umbilical cord veins. Human ovarian carcinoma cell lines 1A9 and HD-1A9 (cells overexpressing histone deacetylases; *Mol Cell* 11:437, 2003).

◆ EXPERIMENTAL PROCEDURE

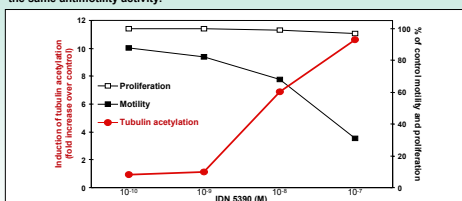
- Correlation of tubulin acetylation with the antimotility activity of taxanes. Endothelial cells and tumor cells were exposed to taxanes at the conditions (low concentrations and short times) that inhibited motility (Boyden chamber assay) but not proliferation. Tubulin acetylation was then analyzed by Western blot.
- To verify that perturbations of tubulin acetylation affected motility and the response to the antimotility activity of taxanes we used:
 - ◆ HD-1A9 cells;
 - ◆ inhibitor of histone deacetylases (spilitomicin).

IDN 5390 AND PACLITAXEL INDUCE TUBULIN ACETYLATION IN ENDOTHELIAL CELLS AT CONCENTRATIONS THAT INHIBIT MOTILITY BUT NOT PROLIFERATION

HUVEC were exposed to IDN 5390 and paclitaxel for 4 h (same exposure time as in the motility assay). The induction of tubulin acetylation was analyzed by Western blot.



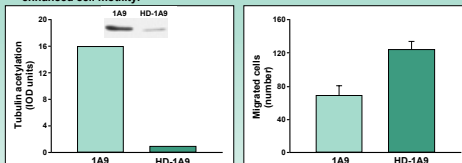
The effects of IDN 5390 and paclitaxel on tubulin acetylation are comparable, reflecting the same antimotility activity.



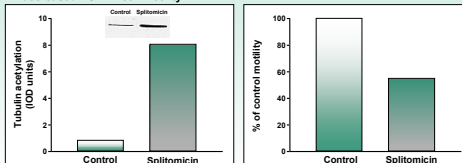
The concentrations of taxanes active in inducing tubulin acetylation are active in inhibiting endothelial cell motility but not proliferation.

MODULATION OF TUBULIN ACETYLATION AFFECTS THE MOTILITY BEHAVIOUR OF CELLS

A) Overexpression of histone deacetylase results in reduced tubulin acetylation and enhanced cell motility.

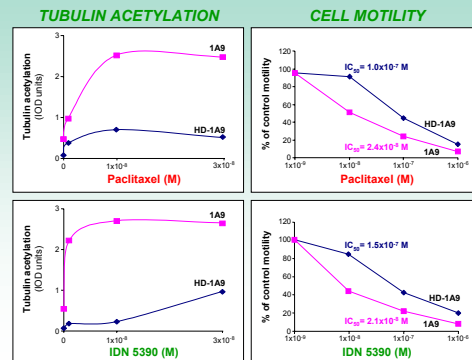


B) Inhibition of histone deacetylase activity results in increased tubulin acetylation and decreased HUVEC cell motility.



MODULATION OF TUBULIN ACETYLATION CAUSES A DECREASED SENSIBILITY TO THE ANTIMOTILITY EFFECTS OF TAXANES

Overexpression of histone deacetylase reduces the effects of paclitaxel and IDN 5390 on tubulin acetylation and cell motility.



SUMMARY OF RESULTS

- ◆ Taxanes induce acetylation of tubulin.
- ◆ Induction of tubulin acetylation by the taxanes paclitaxel and IDN 5390 is associated with inhibition of cell motility rather than proliferation, since it occurs at concentrations and exposure times active in inhibiting cell motility but not proliferation.
- ◆ Modulation of tubulin acetylation, through overexpression or inhibition of histone deacetylases, affects motility and the response to the antimotility activity of paclitaxel and IDN 5390.

CONCLUSION

Induction of tubulin acetylation is associated with the antimotility activity of taxanes.

AACR 97th Annual Meeting 2006 - April 1-5, 2006, Washington, DC