Antitumor activity of EP80061, a small-glyco drug in preclinical studies

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INTRODUCTION
Heparan sulfate (HS) containing proteoglycans regulates the activity of many proteins (growth factors, cytokines, adhesion molecules) involved in pathologies like cancer, inflammation, cardiovascular, metabolic and neuro-degenerative diseases, as well as in viral infection.

HS mimicking oligosaccharides can interfere with protein/HS interactions and thus modulate the resulting biological effects. Endotis' platform for the development of anticancer "small-glyco drugs" aims at identifying HS-mimetic oligosaccharides with inhibitory activity on several growth factors and proteins involved in tumor growth and metastasis. A library of fully synthetic HS mimetics of all sizes containing various substitutions were evaluated in vitro for their affinity for these targets, as well as for their efficacy on cell proliferation, migration, endothelial tubule formation and heparanase inhibition.

Based on these assays, compounds exhibiting significant inhibitory activities were selected and their antitumoral properties was evaluated in vivo. This strategy led to EP80061 as a lead candidate for further developments of small-glyco drugs as anticancer compounds.

CONCLUSION & PERSPECTIVES
The data presented here showed that fully synthetic HS mimetics, mimicking natural HS, can interfere with the activity of several growth factors and chemokine involved in tumor growth and metastasis. They also showed that the introduction of chemical substitutions improved their inhibitory properties and led to target selectivity.

The optimization of the first series of EP compounds allowed the identification of potent inhibitors of in vitro tubules formation and heparanase activity. Moreover, the lead-candidate EP80061 demonstrated strong anti-tumoral and anti-metastatic effects in mouse and rat models.

We are now pursuing the optimization of EP compounds to improve their selectivity for anti-cancer targets and, to this aim, we are currently developing target-specific in vitro assays. We intend in the future, to open Endotis' platform to other therapeutic areas by developing "small-glyco drugs" selective for the HS-binding targets implicated in inflammation, neurodegenerative and infective pathologies.

FIRST OPTIMIZATION OF EP COMPOUNDS

ANGIOTIT ASSAYS

Material & Methods (TCS CellWorks)

- 24 and 96-well Angiograms (human angiogenesis model) were purchased from TCS CellWorks.
- Culture medium containing growth factors, reference inhibitors when appropriate, etc., supplemented with 10% FCS EP compound was replaced at day 1 (plate delivery), day 4 and day 7.

At day 10, anti-C031 ELISA and tube staining procedures were performed.

RESULTS

Several optimized EP compounds inhibited tubules formation, mimicking an anti-angiogenic activity. In particular, EP80061 promoted 22% inhibition of endothelial cells detection by ELISA and 48% inhibition of tubules staining. The number and size of tubules, as well as the number of junctions were significantly reduced by EP80061 (data not shown).

In these conditions, cell viability was superior to 95%.

In vivo RESULTS

FIRST SERIES OF EP COMPOUNDS

BIOACRE

Material & Methods

- Heparin was immobilized on a Biacore sensorchip.
- EP compounds (various doses) were co-incubated with a fixed concentration of the target (FGF-2, PDDF-B, VEGF-A, SDF-1) for 30 minutes.
- The mixture was then injected onto the streptatidin (control reference) and heparin surfaces.
- Percentages of inhibition were calculated from the binding of free heparin on target and IC50 were determined.

OLIGOSACCHARIDE SIZE-DEPENDENT EFFECT ON THE HS-MIMETIC AFFINITY FOR GROWTH FACTOR OR CHEMOKINE

Material & Methods

- Normal Human Dermal Fibroblasts were starved for 24 h in FGF-2-deprived medium containing only 50% of the recommended serum concentration.
- Cells were then stimulated with the corresponding growth factor for a 24 h period in presence of increasing concentrations of EP compounds (0; 0.1; 1; 10; 30 µM).
- The total number of cells was measured using the CellTiter 96 Aqueous One Solution Cell proliferation assay (Promega) and cell viability was assessed by the Trypan Blue method (+5%).

RESULTS

EP compounds promoted inhibition of FGF-2 and/or PDDF-B induced cell proliferation. Different level of inhibition efficacy and target selectivity were observed. None of the compounds inhibited VEGF-A-induced proliferation of Human Umbilical Vein Endothelial Cells data not shown.

HEPARANASE INHIBITORY ACTIVITY

Material & Methods (Chido Biosay)

- The assay is based on FRET technology and on the ability of heparanase to degrade HS.
- Briefly, an intact modified-HS substrate promotes energy transfer of fluorescence. When heparanase cleaves the substrate, the energy transfer is lost.

RESULTS

Optimized EP compounds displayed a relevant anti-heparanase activity.

**In vivo RESULTS USING EP80061**

**A473 SC TUMOR MODEL**

Material & Methods

- Tumor cell line: Human A473 adenosarcoma
- Animals: female Swiss nude mice (Charles river, France)
- Drug administration: bolus IP, daily from the day after cells injection
- Tumor induction: SC inoculation of 106 A473 cells to mice
- Reference substance: Avastin®

**RESULTS**

EP80061 and Avastin® displayed a significant antitumor activity in the SC A473 tumor bearing Swiss nude mice model.

**B16-F10 IV TUMOR MODEL**

Material & Methods

- Tumor cell line: murine B16-F10 melanoma
- Animals: female C57BL/6 mice (Charles river, France)
- Drug administration: bolus IV, simultaneously to cells injection
- Tumor induction: IV inoculation of 106 B16-F10 cells to mice
- At D14, mice were sacrificed. Lungs were fixed in Telypsioncic solution. Number of metastasis in lungs were counted.

**RESULTS**

EP80061 (10 mg/kg) induced a very potent anti-metastatic effect on B16-F10 disseminated tumor model in C57BL/6 mice.

**MAT 13762 IV TUMOR MODEL**

Material & Methods

- Tumor cell line: rat MAT 13762 mammary adenocarcinoma
- Animals: male Fischer 344 rats (Charles river, France)
- Drug administration: bolus IP, simultaneously to cells injection
- Tumor induction: IV inoculation of 105 MAT 13762 cells to rats
- At D14, rats sacrificed. Number of metastasis in lungs were counted.

**RESULTS**

EP80061 (10 mg/kg) induced a very potent anti-metastatic effect on MAT13762 disseminated tumor model in Fischer 344 rat.

**ADDITIONAL RESULTS**

Student test

Vehicle EP80061

EP80061

* p<0.05 ** p<0.01 *** p<0.001