In vitro cytotoxic activities of the membrane active nonapeptide LTX-315 (Oncopore™) against human melanoma cells

**Abstract #2589**

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**Background**

Cationic antimicrobial peptides (CAPs) are a diverse group of highly conserved peptides found in virtually all species of life as a part of the innate immune system. In addition to the bactericidal effects, CAPs have been shown to have antitumor activity[1-4]. LTX-315 is an anti-cancer nonamer peptide derived from bovine lactoferricin (Fig 1). Experimental studies in animal models have shown that intratumoral treatment with LTX-315 induces complete regression and systemic protective immune responses [5]. Tumor cells treated with LTX-315 are rapidly killed by a lytic mode of action of life as a part of the innate immune system. In addition of highly conserved peptides found in virtually all species of life as a part of the innate immune system. In addition of highly conserved peptides found in virtually all species of life as a part of the innate immune system. In addition.

**Aim**

To investigate the mode of action underlying the cytotoxic activity of LTX-315 against a human melanoma cell line LTX-315.

**Results**

- **Fig. 2 - In vitro kinetics of LTX-315**
  - Shows the in vitro kinetics of LTX-315 against a human melanoma cell line A375 after treatment with LTX-315.

- **Fig. 3 - The cancer cells are killed by a lytic mode of action**
  - Bright field confocal images of A375 cells treated with 17 µM LTX-315.

- **Fig. 4 - LTX-315 internalizes and associates with mitochondria**
  - Fluorescence-labeled peptide was associated with mitochondria (Fig 4) with a subsequent disintegration of the mitochondrial membrane (Fig 5) shown at ultrastructural level (Fig 6).

- **Fig. 5 - LTX-315 induces disintegration of mitochondria**
  - A375 cells were labeled with Mitotracker (red) and nucleus stained with DAPI (blue).

- **Fig. 6 - LTX-315 induces disintegration of mitochondria**
  - A375 cells were treated with 17µM LTX-315 for 60min and analyzed by transmission electron microscopy (TEM). Arrows: mitochondria.

- **Fig. 7 - LTX-315 treatment induces cytochrome-C release**
  - Cytochrome-C release in the supernatant after LTX-315 treatment (35µM) of A375 cell at designated time points (5, 15, 45 min) was determined by ELISA assay.

- **Fig. 8 - LTX-315 induces reactive oxygen species (ROS)**
  - ROS generation following LTX-315 treatment was measured by fluorometric assay.

- **Fig. 9 - Extracellular ATP levels following LTX-315 treatment**
  - Extracellular ATP levels following LTX-315 treatment (35uM) of A375 cell at designated time points (5, 15, 45 min) was determined by luciferase bioluminescence.

- **Fig. 10 - LTX-315 induces release of HMGB1**
  - LTX-315 treatment induces release of HMGB1.

**Conclusions**

These findings demonstrate that LTX-315 has a membrane perturbing effect that results in the release of a number of danger signal molecules (DAMPS). It’s effects against both the cell membrane and the mitochondria membrane may explain LTX-315’s ability to induce complete regression and long term protective immune responses in a number of experimental models (see poster 19 and 20).

**Referanser**


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