LTX-401 as a novel antitumor and immunotherapeutic agent in an experimental liver cancer model

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Background

Hepatocellular carcinoma (HCC) is a malignant disease characterized by few treatment options. Only in early stages of HCC are patients eligible for potentially curative surgical procedures such as resection and transplantation. Currently available diagnostic tools and treatments are not suitable for this purpose. Hence, HCC is a highly heterogeneous cancer and therapies designed to maximally influence the behavior of tumor antigens in an immunogenic tumor cell potentially induce durable clinical responses.

Structure-activity relationship studies on host defense peptides have allowed the design of molecular mimics of immune-stimulating ‘danger signals’ or DAMPs that may potentiate antitumor immunity. Owing to its amphiphatic nature, LTX-401 may affect the integrity of cancer cell membranes and induce rapid cell death resulting in necrosis, followed by the release of dominant-negative-stimulating danger signals or DAMPs thereby potentiating antitumor immunity.

FIG. 1: Schematic depiction of the abscopal tumor model. Animals were inoculated intravenously with either (A, B) 1 x 10⁶ or (C, D) 1 x 10⁷ JM1 tumor cells on day 0 followed by intrahepatic inoculation with 0.4 mg LTX-401 on two occasions (day 6 and 8) after tumor cell inoculation. (A) Partial abscopal response after 6 days or systemic response after 21 days. (B) Survival curves of animals treated with LTX-401. Animals rechallenged with LTX-401 once 3 weeks post-treatment and twice 5 weeks post-treatment. *p < 0.05.

Aim

This study was undertaken to investigate the applicability and efficacy of LTX-401 against both s.c. and orthotopic tumors in an immunocompetent rat model of HCC.

FIG. 2: Ultrastructural characteristics of LTX-401-induced cell death

Representative TEM micrographs of JM1 cells treated with 108 µM LTX-401 for 45 min. (A, B) Ultrastructural changes in untreated JM1 cells stimulated with 108 µM LTX-401 for 45 min. (C) Ultrastructural changes in untreated JM1 cells. (D) Bottom panel, mitochondrial morphology in untreated (A) compared to LTX-401-treated cells (A).

FIG. 3: JM1 cells release DAMPs and ROS when treated with LTX-401

(A) JM1 cells release HMGB1 from the lysate (L) to supernatant (S) after being treated with 271 μM LTX-401 for 45 min.

(B) JM1 cells release ROS after being treated with 271 μM LTX-401 for 45 min. Data are expressed as fold-change in ROS release relative to control.

(C) Opposite effects on mitochondrial morphology and respiratory function of LTX-401 treated JM1 cells compared to untreated JM1 cells. TEM micrographs of Cytochrome c-uptake experiment of untreated JM1 cells (108 µM LTX-401 for 45 min) and treated with LTX-401 for 45 min.

FIG. 4: Therapeutic efficacy of LTX-401 against subcutaneous JM1 tumors

Schematic depiction of the treatment schedule. Subcutaneous JM1 tumors were injected intratumorally with either (D) 0.3 or (B) 0.6 mg LTX-401 (alone) or (C) 0.6 mg LTX-401 intravenously for three consecutive days. (A) Subcutaneous tumors at day 18. (B) Survival curves of animals treated with LTX-401. Survival differences between LTX-401 and control animals were highly significant: p < 0.01.

(a) Schematic depiction of the treatment schedule. Subcutaneous JM1 tumors were injected intratumorally with either (C) 0.6 mg LTX-401 (alone) or (B) 0.6 mg LTX-401 and 0.6 mg interleukin-2 (IL-2) (alone) or (A) 0.6 mg LTX-401 and 0.6 mg IL-2 (co-administration).

(b) Schematic depiction of the abscopal tumor model. Animals were inoculated intravenously with either (C) 1 x 10⁶ or (B) 1 x 10⁷ JM1 tumor cells on day 0 followed by intravenous inoculation with 0.4 mg LTX-401 on two occasions (day 6 and 8) after tumor cell inoculation.

Conclusions

LTX-401:

• Intratumoral injection of LTX-401 was well tolerated, and no treatment-related deaths or systemic toxicities occurred.

• When inoculated subcutaneously and orthotopically, JM1 tumors, LTX-401 treatment resulted in a strong antitumor effect followed by complete tumor regression in the majority of animals.

• LTX-401 provides local tumor control followed by protection from immune responses and may be exploited as a co-formulated therapeutic agent in hepatocellular carcinoma.

References
