The amphiphatic B(2,2)-amino acid LTX-401 induces complete regression of experimental hepatocellular carcinoma

BRYNJAR MAUSETH, JI-HUA SHI, KETIL ANDRE CAMILO, Oystein Rekdal, Baldur Sveinbjørnsson and Pål Dag Line

Institute of Clinical Medicine, University of Oslo, Oslo, Norway
Division of Cancer, Surgery and Transplantation, Oslo University Hospital, Rikshospitalet, Oslo, Norway
Institute for Cancer Research, Department of Tumor Biology, Oslo University Hospital, Oslo, Norway
Department of Medical Biology, University of Tromsø, Tromsø, Norway
Lytix Biopharma, Oslo, Norway

Introduction

Hepatocellular carcinoma is the sixth most common cancer in the world and third most common cause of cancer mortality. While standard treatment includes surgical resection or transplantation, patients having unresectable tumors or widespread metastatic disease typically receive systemic chemotherapy. However, current pharmacological therapies have severe side effects and are seldom curative, thus illustrating the impending need to pursue new and improved therapeutic approaches.

The amphiphatic B(2,2)-amino acid LTX-401 is a novel cytolytic compound designed for intratumoral administration. Cancer cells treated with LTX-401 suffer irreversible damage to plasma membranes, resulting in cell death by necrosis and subsequent loss of intracellular content, including immune stimulating ‘danger signals’ or damage-associated molecular patterns molecules (DAMPs). Further in vivo studies have demonstrated potent anticancer effect against syngeneic mouse B16 melanomas, presumably by engagement of antimicrobial immune responses (Elie et al. 2016).

Aim

The aim of the present study was to investigate the in vivo mechanism of action by LTX-401 followed by an assessment of the antitumor immune responses (Eike et al. 2016).

Results

Table 1: LTX-401 displays high cytotoxic activity against a wide range of cancer cell lines and displays selectivity compared to human red blood cells.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>IC50 (µM) Human Red Blood Cells</th>
<th>IC50 (µM) LTX-401</th>
<th>Selectivity</th>
<th>IC50 (µM)</th>
<th>SD (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK-Mel-29</td>
<td>31.7 ± 2.3</td>
<td>3.3 ± 0.6</td>
<td>9.5</td>
<td>30.6 ± 3.9</td>
<td>0.6</td>
</tr>
<tr>
<td>HT-29</td>
<td>23.3 ± 1.5</td>
<td>3.3 ± 0.6</td>
<td>7.1</td>
<td>26.7 ± 2.9</td>
<td>0.6</td>
</tr>
<tr>
<td>BEL7402</td>
<td>35.4 ± 2.7</td>
<td>3.3 ± 0.6</td>
<td>10.7</td>
<td>30.6 ± 3.9</td>
<td>0.6</td>
</tr>
<tr>
<td>JM1</td>
<td>26.7 ± 2.9</td>
<td>3.3 ± 0.6</td>
<td>8.0</td>
<td>30.6 ± 3.9</td>
<td>0.6</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>30.6 ± 3.9</td>
<td>3.3 ± 0.6</td>
<td>9.2</td>
<td>30.6 ± 3.9</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*P<0.05, ns

Fig. 1 - LTX-401 rapidly induces cell death in JM1 cells

Fig. 2 - Treatment with LTX-401 leads to ultrastructural changes in JM1 cells

Fig. 3 - JM1 cells treated with LTX-401 release DAMPs, such as HMGB1, ATP and cytochrome C into the extracellular matrix.

By inducing necrotic cell death, cancer cells are rendered immunogenic due to the release of DAMPs and tumor antigens. Intratumoral treatment with LTX-401 induced complete regression of both subcutaneous and orthotopic B16 tumors followed by systemic protective immune responses, and thus represents a promising therapeutic approach against hepatocellular carcinoma.

Conclusion

• LTX-401 displays potent anticancer activity against several cancer cell lines with selectivity compared to human red blood cells

• By inducing necrotic cell death, cancer cells are rendered immunogenic due to the release of DAMPs and tumor antigens

• Intratumoral treatment with LTX-401 induced complete regression of both subcutaneous and intrapleural PT1 tumors followed by systemic protective immune responses, and thus represents a promising therapeutic approach against hepatocellular carcinoma

Table 2: in vivo efficacy of LTX-401

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Control</th>
<th>LTX-401 (36 µg)</th>
<th>LTX-401 (100 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous tumors</td>
<td>0/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Intratumoral treatment</td>
<td>0/5</td>
<td>3/3</td>
<td>3/3</td>
</tr>
</tbody>
</table>

*P<0.05

Fig. 4 - Tumor treatment schedule

Reference