Combination Therapy with Captopril and Temozolomide Inhibits Matrix Metalloproteinase-2, Reduces Cell Migration, and Extends Survival In An Intracranial Gliosarcoma Model

The Johns Hopkins Department of Neurosurgery

Leon Pinheiro, Alexander Perdomo-Pantoja, Joshua Casaos, Sakib Huq, Iddo Paldor, Veronica Vigilar, Antonella Mangraviti, Yuan Wang, Timothy F. Witham, Henry Brem, and Betty Tyler
Disclosures

• None relevant to this study.
Background

- MMP-2 break downs elements of the ECM and is associated with glioma cell migration [1, 2].
- Upregulation of MMP-2 in human glioma has been correlated with tumor aggressiveness and prognosis [3].
- Evidence suggests that captopril might inhibit MMP-2 (Zn\(^{2+}\) chelator) in a dose-dependent manner [4].

Objective

• To evaluate the effects of captopril on MMP-2 expression in gliosarcoma cells *in vitro* and on survival in an *in vivo* murine intracranial gliosarcoma model.
Methods

• For survival, F344 rats were implanted intracranially with 9L gliosarcoma tumor fragments, and were then divided into groups:
  – [1] Control, receiving no treatment
  – [2] Oral captopril, 30 mg/kg/daily on Days 5-14
  – [3] Oral Temozolomide (TMZ), 50 mg/kg on Days 5-9

• For immunohistochemistry, on day 0, additional F344 rats were implanted intracranially with 9L gliosarcoma cells and divided into groups:
  – [1] Control
  – Rats from each group were sacrificed at days 6, 8, and 11.

• To assess MMP-2 protein level, cultured 9L gliosarcoma cells were treated with either 100, 150, or 200 µg/mL of captopril or ultrapure water as the vehicle, and protein expression was assessed via Western blots at 24, 48, 72 and 96 hours.
Results

• Captopril treatment results in decreased MMP-2 expression.

• Captopril was associated with significantly decreased cell migration via scratch wound assay.
Results

- Increased survival of captopril treated rats implanted with 9L gliosarcoma

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Median Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14 days</td>
</tr>
<tr>
<td>Captopril</td>
<td>16 days</td>
</tr>
<tr>
<td>TMZ</td>
<td>23 days</td>
</tr>
<tr>
<td>Captopril and TMZ</td>
<td>27.5 days</td>
</tr>
</tbody>
</table>

(p = .001 for Captopril vs. Control, p < .001 for TMZ vs. Control, p = .01 for TMZ vs. Captopril and TMZ)
Results

- Decreased MMP-2 expression in tumor tissue after captopril treatment

![Image of immunohistochemistry analysis](image-url)

### Summary of Immunohistochemistry Analysis

<table>
<thead>
<tr>
<th>Time-points</th>
<th>Day 6</th>
<th>Day 8</th>
<th>Day 11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Captopril</td>
<td>Control</td>
</tr>
<tr>
<td>AVG # of cells/sample</td>
<td>1470.87</td>
<td>1937.5</td>
<td>2174.62</td>
</tr>
<tr>
<td>Rate of MMP-2 positive cells</td>
<td>0.510</td>
<td>0.530</td>
<td>0.545</td>
</tr>
<tr>
<td>SD of rate of MMP-2 positive cells</td>
<td>0.157</td>
<td>0.122</td>
<td>0.137</td>
</tr>
</tbody>
</table>

*p-value*:
- Day 6: 0.34
- Day 8: 0.04
- Day 11: 0.002

Statistically significant values are written in **bold**.

Abbreviations: AVG=Average, MMP-2=Metalloprotease-2, SD=Standard Deviation
Potential Mechanisms and Involved Pathways

Conclusions

- **Captopril** as adjuvant therapy to TMZ *increased survival* in a rat intracranial gliosarcoma model.
- A *reduction in gliosarcoma cellular migration* with captopril treatment was demonstrated *in vitro*, as well as reduction in *MMP-2 expression* *in vitro and vivo* in brain tumor tissue of rats.
- Captopril may serve as a *potential adjuvant to TMZ* therapy.
- Further *investigation to elucidate the mechanism* underpinning the adjuvant/additive effect of captopril for the treatment of GBM.
Thanks

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Leon Pinheiro
Joshua Casaos
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