MN58b: an effective choline kinase inhibitor in the treatment of rat brain gliomas

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Introduction: Increased choline metabolism has been proposed as a hallmark of cancer, since high total choline (tCho) is consistently observed in both pre-clinical tumor models as well as in human tumors. Due to increased choline kinase (ChoK) activity, elevated phosphocholine (PC) is generally observed in proliferating cancer cells and in-situ tumors by MRS. Thus, inhibition of ChoK using specific ChoK inhibitors, such as MN58b1, appears to be a promising treatment strategy in tumors. We have recently developed a high yield, two-step synthesis of MN58b. In this study we tested the efficacy of MN58b in the treatment of intracranial F98 gliomas. The therapeutic effects were assessed using in-vitro assays as well as in-vivo using 1H MR Spectroscopy.

Materials and Methods: MTT assay: F98 rat glioma cells were plated in quadruplicate in 96-well plates at 7.5x10^4 cells/ml and incubated overnight. Culture medium was replaced with media containing varying concentrations of MN58b. After 24h, the medium was removed and 20µl of 5mg/ml Thiazolyl Blue Tetrazolium Bromide (MTT) in sterile PBS was added and the cells were incubated for 2h. 150µl DMSO was added and absorbance read at 550 nm.

ChoK Activity Assay: F98 rat glioma cells plated in 6-well dishes were treated with 0.5 µCi/ml of 3C-labeled choline for 1hr following addition of varying concentrations of MN58b. After 2hrs of treatment, cells were washed and fixed in 16% ice-cold trichloroacetic acid. Each sample was washed 3x in diethyl ether, lyophilized, and resuspended in water for thin layer chromatography separation (NaCl/CH3OH/H2O 50:70:0.5 and autoradiographed using a Fujifilm FLA-7000).

In-vivo Spectroscopy: In-vivo 1H MR spectra were obtained from the brains of six normal rats and five rats bearing an intracranial tumor. Three of the five tumor bearing animals were treated with MN58b (2mg/kg i.p. daily for 5 days) and MRS was repeated to evaluate the effects of treatment. In-vivo experiments were performed on a 9.4T horizontal bore scanner (Varian, Palo Alto, CA) equipped with 25 G/cm gradients. A 35 mm i.d. quadrature bi-axe surface coil (M2M, Cleveland, OH) was used. T2-weighted spin echo images were acquired (TR = 3000 ms, TE = 12.45 ms, NT = 128, SI = 4K and SW = 4000 Hz). Water suppression was performed using the VAPOR technique. An unsuppressed water spectrum was also acquired (8 averages) to compute metabolite to water ratios.

Results: The IC50 of MN58b was determined to be 20 µM for proliferation using the MTT assay and 3.2 µM for choline kinase activity as measured by the conversion of 3C-labeled Cho to PC using the ChoK assay. After 2hrs of treatment, cells were washed and fixative was used to prepare the samples for autoradiography.

Data quantification: *Ex-vivo* F98 cell extract MRS data were analyzed using MestReNova (Mestrelab Research) to look for changes in PC and GPC. *In-vivo* MRS data were analyzed using LC-model to measure concentration (arbitrary units (AU), relative to water) of the metabolites (tCho, lipid and lac) in untreated and treated brain tumors.

Table: Effects of MN58b on Cho, Lac and Lipid in F98 brain tumor.

<table>
<thead>
<tr>
<th>F98 Brain Tumor</th>
<th>tCho (mean±SEM)</th>
<th>Lac (mean±SEM)</th>
<th>Lipid (mean±SEM)</th>
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</thead>
<tbody>
<tr>
<td>Untreated (n=5)</td>
<td>1.15±0.35</td>
<td>3.62±1.61</td>
<td>3.28±1.41</td>
</tr>
<tr>
<td>Treated (n=3)</td>
<td>0.70±0.23</td>
<td>1.71±0.89</td>
<td>11.84±3.08</td>
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Fig. 1: *In-vitro* ChoK Activity of MN58b in F98 tumor cells.

Fig. 2: *In-vitro* NMR of F98 cell extracts. Sham treated cells show high PC (A). 10µM MN58b treated show high GPC and low PC (B). 20µM MN58b treated cells show further changes in GPC and PC (C).

Fig. 3: *In-vivo* MRS from untreated and MN58b treated F98 brain tumors. Untreated tumor (A) showing high tCho and low lip/lac. MN58b treatment resulted in reduced tCho and increased lip/lac level (B).

References:
