Summary

Temozolomide-fatty acid conjugates for glioblastoma multiforme: in vitro and in vivo evaluation

Glioblastoma multiforme (GBM) is one of the most lethal brain tumors, posing a dismal prognosis and limited treatment options. Temozolomide (TMZ) has emerged as a first-line chemotherapeutic agent for GBM, but its effectiveness is hampered by various limitations such as its short half-life, rapid metabolism, less than 1% brain bioavailability, resistance mechanisms like methyl guanine methyl transferase (MGMT)-based chemoresistance, and associated hematological toxicities due to high dose. To overcome these limitations,various strategies were adopted such as nanotechnology-based systems. However, the intricate physicochemical properties such as the hydrophilic behavior of TMZ make it a challenging candidate for loading into these nanocarriers.

In our recent research work, a novel approach was used to overcome these challenges of TMZas an anticancer agent for GBM. We conjugated TMZ with different fatty acids, i.e., linoleic acid (LA), oleic acid (OA), and palmitic acid (PA), to obtain TMZ-fatty acid conjugates which are comparatively hydrophobic, less prone to degradation, and potent. The fatty acid conjugated TMZ could impart immense potential such as fatty acids reducing resistance (via MGMT depletion) and giving a synergistic effect through their anticancer activity.TMZ-fatty acids conjugate loaded nanocarrier could show stability over time and improve the circulation time to facilitate patient compliance. Due to their nano size, these carriers could potentially cross various barriers to reach glioma cells such as the blood-brain barrier.

The fatty acid conjugates of temozolomide were prepared using multistep reactions. Briefly, on one side Temozolomide was converted to temozolomide acid, and on another side, the fatty acids were converted to fatty acid hydrazide. Lastly, both intermediates were conjugated using EDC/Hobt amide coupling chemistry. The conjugates were characterized using ^{1}H NMR and mass spectrometry followed by purity analysis using RP-HPLC.The synthesized fatty acid conjugates were evaluated for their activity using glioma cancer cell lines i.e. C_6 and U87-MG. Cell viability assay was performed for the evaluation of anticancer activity followed by apoptosis assay. Further, western blotting was performed to evaluate the MGMT expression, which plays a

significant role in TMZ resistance. In-vivo efficacy was evaluated in C_6 induced orthotopic glioma model.

The temozolomide-fatty acid conjugateswere successfully synthesized and characterized using mass spectrometry and 1 H NMR analysis. The purity of the conjugates was found to be > 90 %. Further, the TMZ-OA, TMZ-LA, and,TMZ-PA showed IC_{50} of 101.4uM, 67.97uM, and 672.04 uM respectively, w.r.t free TMZ, which was having IC_{50} of 1098 µM in C_6 cell line. Similarly, IC_{50} of TMZ-OA, TMZ-LA, and, TMZ-PA in U87-MG cells were 428.25uM, 366.42uM,and 413.69 uM respectively. As per the apoptosis data, the TMZ-OA, TMZ-LA, and TMZ-PA showed 81.68%, 52.2%, and 82.78% of apoptosis in C_6 cells. Similarly, in U87-MG cells the TMZ-OA, TMZ-LA, and TMZ-PA showed 52.44%, 60.82%, and 56.93% apoptosis. Interestingly, after TMZ-OA, TMZ-LA, and TMZ-PA treatment the MGMT expression gets reduced (as per the western blot data) in MGMT-expressing cell lines.

Further, as per in vivo efficacy study in the orthotopic glioma model the TMZ-OA conjugate provides better outcomes in terms of the overall health of the animals, survival rate, and tumor volume in the histological evaluation of the brain sections.

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