Ursolic acid nanocrystals as a MGMT inhibitor in the treatment of glioblastoma

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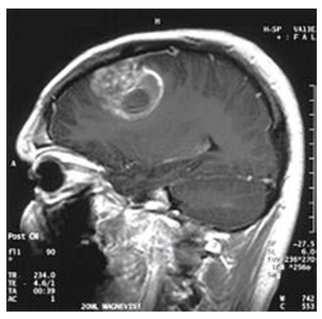
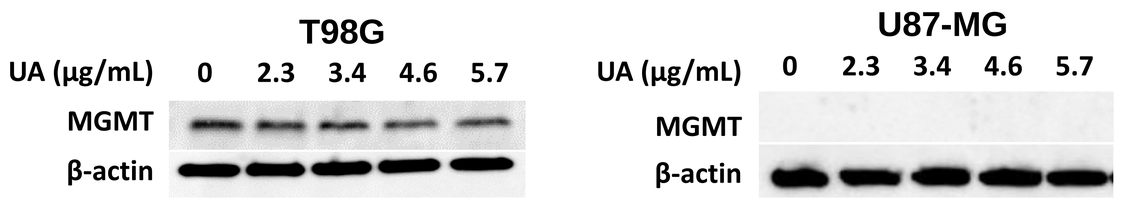
Introduction

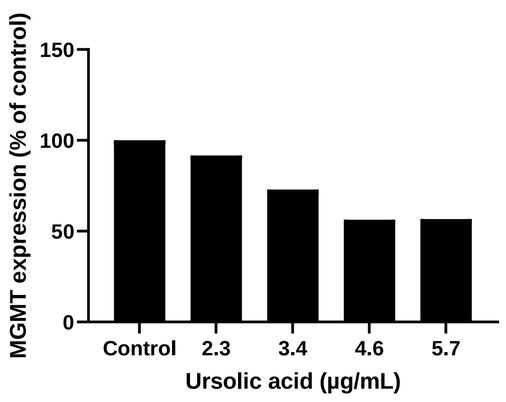


Glioblastoma (GBM) is the glioma with the highest grade (IV) according to the World health Organization [1]. Despite it being a rather rare condition (6 cases for 100,000) [2], the survival prognosis is very low. The overall median survival is of only 15 months after treatment initiation [3]. The chemotherapy agent used in the gold standard treatment (Stupp protocol) is temozolomide (TMZ) : an alkylating agent. TMZ adds methyl adducts on the ^{6}O position of guanines [4,5]. The ^{6}O-methylguanine-DNA-methyltransferase (MGMT) is a highly conserved protein involved in DNA reparation by removing alkyl groups from the ^{6}O position of guanines [6]. The mechanism of action of MGMT thus impairs the efficacy of a TMZ treatment and the outcome of the Stupp protocol is then dependent on the expression of MGMT. The median survival of patients with a methylated MGMT promotor (low MGMT expression) treated with TMZ is higher than for patients with a non-methylated MGMT promotor (high MGMT expression) [7,8]. The strategy used in this study is to use a MGMT inhibitor, ursolic acid (UA) to restore TMZ efficacy for patients with non-methylated MGMT promotor. The capacity of UA to inhibit the expression of MGMT has been studied and UA has been formulated in nanocrystals (NCs) for wich their destabilization and internalization has been observed using FRET (Fluorescence Resonance Energy Transfer) inducing dyes.

1. MGMT inhibition using ursolic acid

T98G (MGMT-positive) and U87-MG (MGMT-negative) cells were treated with different UA concentrations for 48h and MGMT protein expression was observed via Western Blot. MGMT protein expression was semi-quantified for T98G cells only.

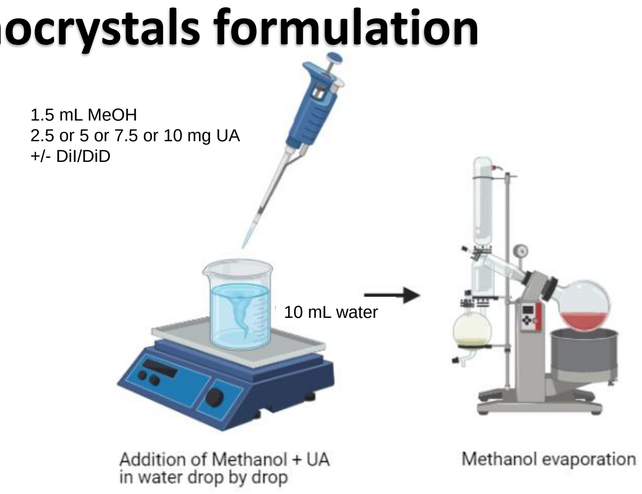


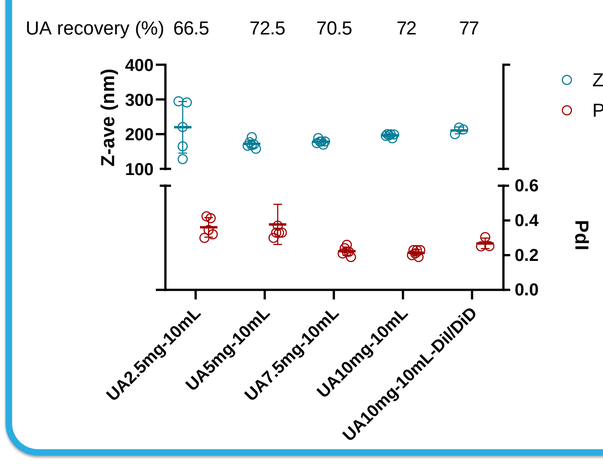


→ UA seems to inhibit MGMT expression in MGMT-positive cells

2. Ursolic acid nanocrystals formulation

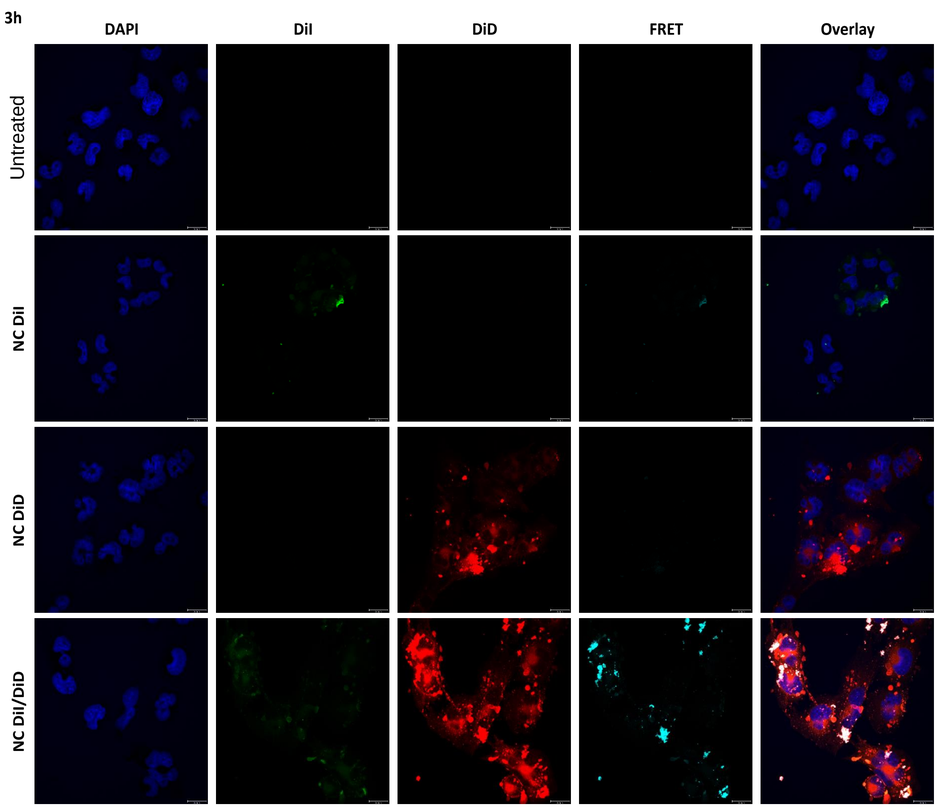
UA NCs have been formulated using a antisolvent crystallization method. The non-fluorescent NCs were then characterized by Dynamic Light Scattering. UA formulation yields of NC suspensions was quantified by Ultra Performance Liquid Chromatography.





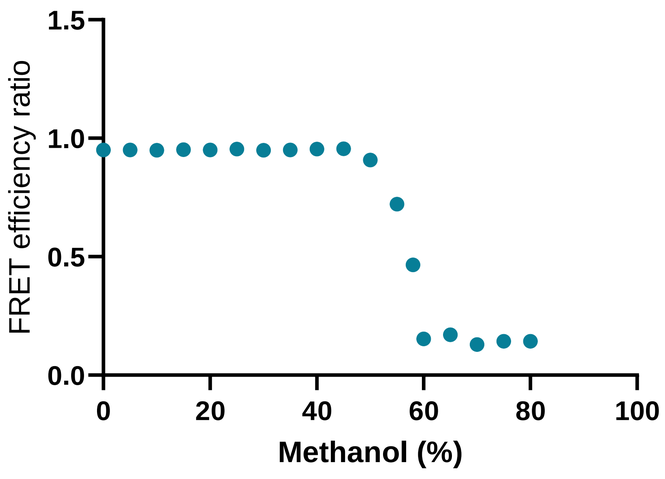
→ UA NCs formulation was successful and NCs are approximately 200 nm wide, independently of the formulation conditions

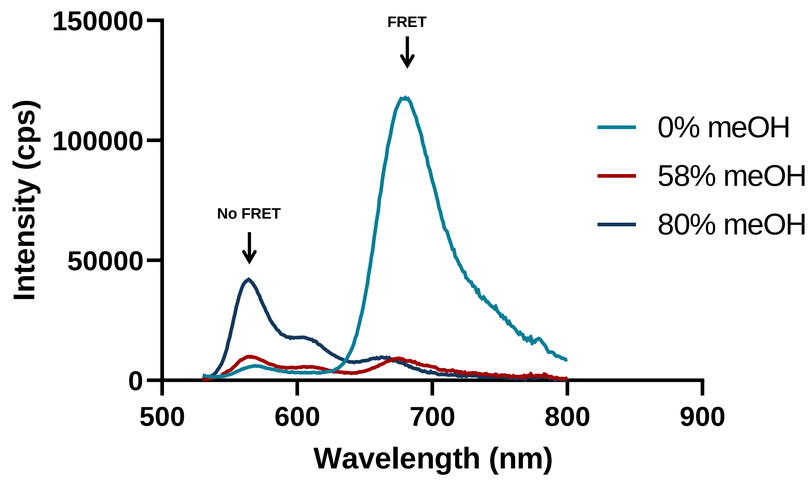
3. Ursolic acid nanocrystals internalization and destabilization



T98G cells were incubated with either UA NCs containing only DiD, only DiI or both for 3h, fixed, dyed with DAPI and observed by confocal microscopy

UA NCs containing DiI and DiD were analysed after dilution in different proportions of methanol with spectrofluorometry at a 520nm excitation. The FRET efficiency ratio was calculated with the following formula : FRET efficiency ratio = $I_{680nm}/(I_{569nm}+I_{680nm})$ where 569nm corresponds to the maximum emission of the donor (DiI) and 680nm corresponds to the maximum emission of the acceptor (DiD).





→ UA NCs are able to hold a FRET signal and this signal disappears when the NCs are destabilized. The FRET signal allowed visualization of internalization of intact UA NCs in T98G cells after only 3 hours.

Conclusion

UA was effective at reducing the expression of MGMT in MGMT-positive cells (T98G). UA was then formulated into 200 nm nanocrystals using an antisolvent crystallization method with a recovery of approximately 70% regardless of the formulation conditions. FRET inducing dyes (DiI and DiD) were successfully added to the UA NCs formulation. The destabilization of the NCs was observed by a loss of FRET efficiency with dilutions in higher percentages of methanol. After 3 hours of contact with MGMT-positive cells, UA NCs were internalized and still intact. MGMT inhibition with UA NCs and their destabilization will be further studied *in cellulo* as well as the improvement of efficacy of TMZ with the UA NCs.

References:
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Acknowledgements: