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Doxorubicin-loaded Cholesterol-Glycopolymer Nanomicelles with Autophagy Activator Rapamycin for Synergistic Glioblastoma Cell Inhibition

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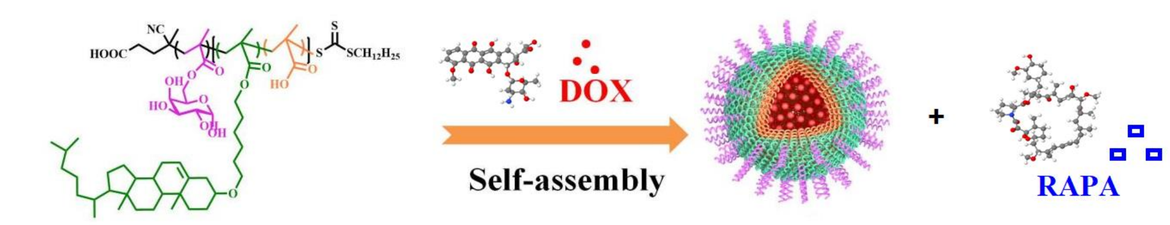
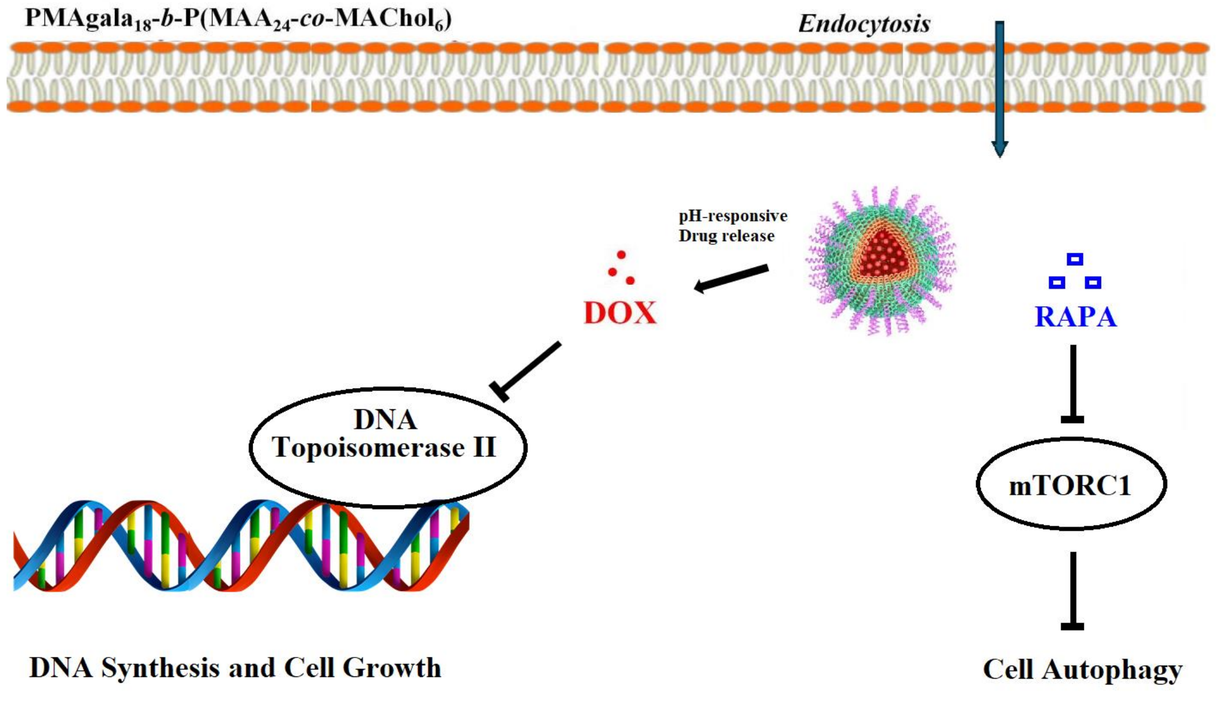
INTRODUCTION & AIM

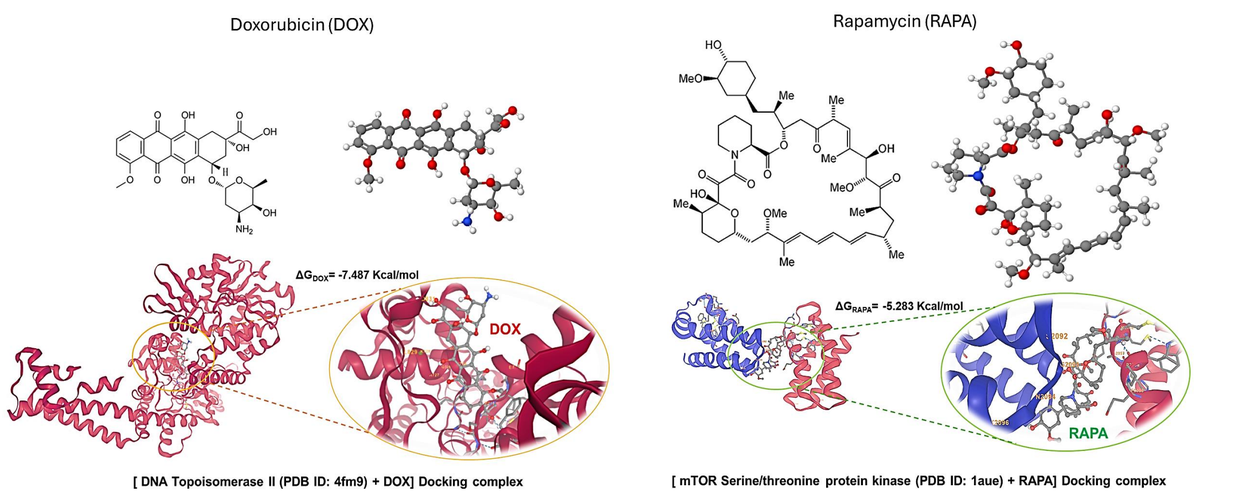
Many studies had disclosed that inducing autophagy could inhibit tumour growth by promoting degradation of oncogenic proteins and inducing autophagic/programmed cell death). Co-delivery of autophagy regulators with anticancer agents may enhance the cancer inhibition by integrating autophagic cell death and anticancer agent-induced cell death. Thus, further investigate the co-delivery of autophagy regulators and anticancer agents in drug delivery nanosystem is highly demanded.[1]

Our previous works disclosed that doxorubicin (DOX)-loaded cholesterol-based block copolymer and glycopolymer micelles could release the encapsulated DOX in weakly acidic lysosomal environment (pH 4-5) and efficiently inhibited the proliferation of several cancer cell lines [2,3]. To further understand the autophagy response of the DOX-loaded glycopolymer nanomicelles and develop a combination of "autophagy modulating + chemotherapy", in this study, an optimized cholesterol-based diblock glycopolymer PMAgala$_{18}$-*b*-P(MAA$_{24}$-*co*-MAChol$_6$) was synthesized and DOX-loaded glycopolymer micelles prepared, the physico-chemical Properties of these DOX-loaded micelles, including: Particle size, polydispersity (PDI), surface zeta potential, drug loading content (DLC, wt.%), drug loading efficiency (DLE, wt.%), number of loaded DOX , as well as relative colloidal stability (%), were investigated. The DOX-loaded glycopolymer nanomicelles have high DOX loading efficiency, caveolae-/clathrin-mediated endocytosis pathways, endosome-lysosome localization, and pH-responsive DOX release properties, and synergistic tumour cell inhibition effect with the co-delivery of rapamycin (RAPA, an autophagy activator) in Human Glioblastoma Carcinoma (H4) and H4-GFP-LC3 (stably over-expresses green fluorescent protein (GFP)-tagged microtubule-associated autophagy marker protein 1 light chain 3 (LC3) cancer cell lines. The findings indicated that we successfully developed a combinatorial strategy for co-delivery autophagy activator (RAPA)+ anticancer agent (DOX) for achieving synergistic drug delivery efficiency.





Scheme 1. Designation of the co-delivery system of DOX-loaded glycopolymer micelles + RAPA for achieving synergistic (blocking DNA synthesis + inducing cell autophagy) cancer cell inhibition



Scheme 2. Comparison of the molecular structures, optimized architectures of DOX and RAPA (top) and molecular docking with their bio-targets (Dox: Topoisomerase II; RAPA: mTOR serine/threonine protein kinase (by SWISSDOCK online software: https://www.swissdock.ch)

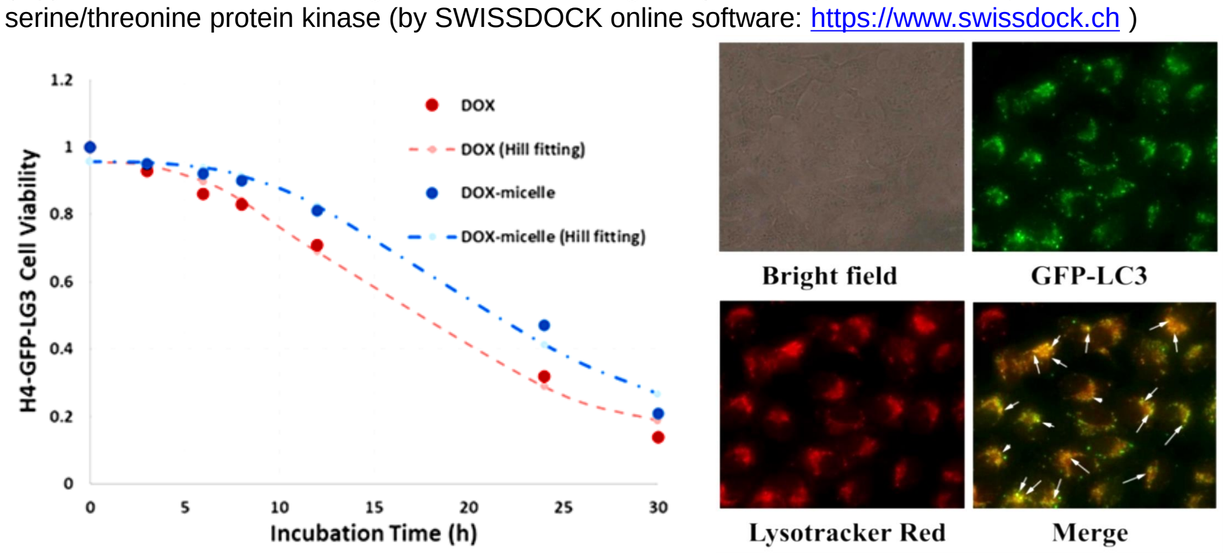


Figure 1.(a)Time-dependent H4-GFP-LG3 cell inhibition efficiency of free DOX and DOX-loaded glycopolymer micelles; (b) Visualization of RAPA-induced formation/aggregation of autophagy biomarker protein GFP-LC3 (green: GFP-LC3 induced by RAPA; red: lysosome labelled by lysotracker red; merge/orange: GFP-LC3 co-localized with lysosome), indicating RAPA is a highly efficient autophagy activator

The co-delivery effect of RAPA and DOX-loaded micelles could be analyzed by the Bliss independence index/score (ΔE) between the measured percentage of inhibition (E_{expe}) and predicted or calculated E_{pred} at certain concentrations (Bliss independent analysis was expressed as equation 1).

$$\Delta E = E_{expe} - E_{pred} = E_{expe} - (E_{RAPA} + E_{DOX} - E_{RAPA} * E_{DOX}) \quad \text{(eq. 1)}$$

Table 3. Bliss independent model analysis of the drug combination effect of DOX (free DOX and DOX-loaded micelles) and mTORC1 inhibitor RAPA

Two-drug combination	E_{RAPA}	E_{DOX}	E_{pred}	E_{expe}	ΔE	Effect
RAPA + DOX	0.066	0.608	0.634	0.702	0.068	Synergistic
RAPA + DOX-loaded micelles	0.066	0.508	0.540	0.699	0.159	Synergistic

RESULTS & DISCUSSION

Table 1. Properties of the DOX-loaded glycopolymer micelles

Particle size (nm), and (PDI)[a]	Zeta potential (mv)[a]	DLC (wt%) [b]	DLE (%) [b]	Number of DOX (/per polymer) [c]	Relative colloidal stability (%) [d]
115 ± 3, (0.107)	-21.6 ± 1.3	12.8	83.2	3.0	7.8

[a]The hydrodynamic particle size, polydispersity index (PDI) and zeta potential were measured by DLS at room temperature; [b] Drug loading contents (DLC) and drug loading efficiency (DLE) were calculated with a DOX feeding content of 15.0 wt%; [c] Number of DOX (/per polymer) was calculated using the formula: $M_{w\text{-}Polymer}$*DLC/$M_{w\text{-}DOX}$; $M_{w\text{-}Polymer}$ is the weight average molecular mass of polymer and $M_{w\text{-}DOX}$ is the molecular weight of DOX; [d] Relative colloidal stability (%) was calculated by the particle size change at 37ºC within 48 h in PBS buffer (pH 7.4, 10 mM) using the coefficient variation formula: (standard deviation/mean size)*100%

Table 2. Physicochemical properties of the DOX and RAPA molecules, related data was calculated using SwissADME online prediction software.

Molecule	Molecular weight	Lipophilicity (XLogP3)	Total polar surface area (TPSA, Å²)	Charged Group	Water solubility (LogS)	Permeability ($LogK_{skin}$, cm/s)
DOX	**543.50**	**1.27**	**206.07**	**NH_3^+**	**-3.91**	**-8.71**
RAPA	**914.17**	**6.02**	**195.43**	**-**	**-8.90**	**-7.60**

CONCLUSION

In this work, we prepared the DOX-loaded glycopolymer micelles, the physico-chemical Properties of these DOX-loaded micelles were investigated by various instruments. In Human Glioblastoma Carcinoma H4 and H4-GFP-LC3 cell lines, the co-incubation/co-delivery of MAgala18-b-P(MAA24-co-MAChol6)/DOX nanomicelles with autophagy activator RAPA could lead to an obvious enhanced tumor cell proliferation inhibitory effect, which might be due to synergistic effect of DOX (the Topoisomerase II inhibition and DNA intercalation) and RAPA (autophagy process activation and LC3-II protein degradation). Moreover, the synergistic effect of MAgala18-b-P(MAA24-co-MAChol6)/DOX nanomicelles and RAPA was further quantified by Bliss independent analysis. This work showed a preliminary study on the glycopolymer-based (DOX-RAPA) drug co-delivery nanosystem, which might inspire the future development of "anticancer agent-autophagy regulator co-delivery nanotherapeutics" toward clinical cancer chemotherapy.

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