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Design of Polymer-Drug Conjugates with a ROCK inhibitor as Therapeutic Strategy for Advanced Breast Cancer

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INTRODUCTION

Breast cancer (BC) accounts for 21% of all female cancer deaths in Europe, suffers a mortality rate of ~31 per 100,000, and is considered the primary cause of death for women aged 35-64 years [1]. A breast cancer subtype known as triple negative breast cancer (TNBC) affects between 10-20% of patients, suffers from overall poor prognosis, as TNBC does not respond to hormonal or HER2 targeting therapies, and is more likely to be invasive. The overall survival rate has vastly improved in recent times due to early detection and the development of improved treatment approaches, but the metastatic disease remains mostly incurable [2]. Anti-cancer research has provided little progress towards improved survival rates for patients with metastatic disease due, in part, to the complex and heterogeneous nature of the disease. However, the field of nanomedicine has developed promising approaches for disease treatment, diagnosis, and monitoring, with the potential to significantly improve cancer treatment [3]. Our lab employs the polymer-drug conjugates (PDCs) as a means to enhance drug solubility, increase plasma half-life, induce fewer secondary/side effects, and suffer from lower renal excretion [4]. Early clinical trials involving PDCs have demonstrated activity in chemotherapy-refractory patients and have significantly reduced drug-related toxicity [5]. Rho Kinase (ROCK) inhibitors are promising drugs for various clinical applications [6] and can inhibit tumor cell motility and metastasis, modify tumor cell morphology, and suppress anchorage-independent growth, thereby making them ideal drugs for metastatic breast cancer treatment [7, 8].

OBJECTIVE

Our overall aim is to design a new family of PDCs to treat metastatic breast cancer. In this communication, we present a first approach: the synthesis of a ROCK inhibitor conjugated via different linker modules to a multifunctional and biodegradable polymer (poly-L-glutamic acid or PGA) and its evaluation in TNBC cell lines.

METHODOLOGY

Polyglutamate conjugates were synthesized employing direct coupling of PGA and amino-functionalized ROCK inhibitor derivatives. All conjugates were fully characterized (NMR, GPC, UV-Vis, HPLC, DLS) and kinetics of release were studied in conditions mimicking intracellular conditions. 4T1 and MDA-MB-231 cell lines were seeded at 5,000 cell/well and 7,500 cell/well, respectively, and allowed to adhere for 24 hours (h). Then, the medium was replaced by fresh complete medium containing the free drug or the conjugates at different concentrations. After 72 h, cell viability was measured via the MTS assay, and subsequent cell cycle, apoptosis, oxidative stress, protein expression, and migration assays were carried out with IC50 values for all tested compounds. Three independent assays were performed in all cases.

RESULTS AND DISCUSSION

We conjugated the Rock inhibitor to PGA via biodegradable linkers (amide, carbamate, and a self-immolative linker consisting of an ethylene disulfide-carbamate) to confer optimized drug release kinetics. Conjugates exhibited comparable physicochemical properties (4.5-5 nm in diameter with zeta potential from -19 to -23 mV), total loading (11.5-13.7 %) and free drug content (below 0.1%). Release studies demonstrated that the self-immolative linker provided the fastest drug release (almost 80% for the first 4 hours), while the carbamate and amide linkers displayed relatively less activity (5 % and 1 % in 24 hours, respectively).

We performed cell toxicity studies with free and conjugated forms of the ROCK inhibitor in different TNBC cell lines, confirming the importance of appropriate rational design to for optimal biological activity. We assessed the biological effect of the more successful conjugate in MDA-MB-231 cells, where the conjugate displayed 7-fold greater toxicity when compared to the free drug. We also examined the mechanism of action, finding that ROCK inhibitors induced a higher level of oxidative stress after conjugation, while Western Blot analysis demonstrated that conjugation induced a more significant inhibition of ROCKII in comparison with free drugs. Migration assays also demonstrated a more pronounced inhibition by the conjugate ROCK inhibitor, indicative of greater anti-metastatic activity.

CONCLUSION

In this communication, we successfully synthesized and characterized a new PDC, a PGA-conjugated ROCK inhibitor, and demonstrated how rational design can lead to improved drug activity *in vitro* in a TNBC cell line.

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