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Schiff base-Poloxamer P85 combination demonstrates chemotherapeutic effect on prostate cancer cells *in vitro*

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ABSTRACT

Prostate cancer is a multistep and complicated cancer type that is regulated by androgens at the cellular level and remains the second commonest cause of death among men. Discovery and development of novel chemotherapeutic agents enabling rapid tumor cell death with minimal toxic effects to healthy tissues might greatly improve the safety of chemotherapy.

The present study evaluates the anti-cancer activity of a novel heterodinuclear copper(II)Mn(II) complex (Schiff base) in combination with poly(ethylene oxide) and poly(propylene oxide) block copolymer (Pluronic) P85. We used assays for cell proliferation, apoptosis, cell migration and invasion, DNA binding and cleavage to elucidate the molecular mechanisms of action, in addition to the anti-inflammatory potency of the new combination. The combined treatment of Schiff base and P85 lead to a remarkable anti-cancer effect on prostate cancer cell lines. Cell proliferation was inhibited in Schiff base-P85 treatment. The activity of this formulation is on DNA binding and cleavage and prevents inflammation in *in vitro* conditions. This is the first study presenting the anti-cancer activity of the present Schiff base derivative and its combination with P85 to treat prostate cancer *in vitro*.

1. Introduction

Prostate cancer is a multistep and complicated cancer type which is characterized by hormone regulation at the molecular and cellular level, and is the most common cancer with a high prevalence [1,2]. Although androgen deprivation therapy is used as a first line treatment option for several years, a concomitant chemotherapeutic treatment is often required for a successful treatment outcome [3]. Therefore, the improvement of current therapeutic approaches, screening of new drugs and identification of new agents is essential for the treatment of advanced prostate cancer. The introduction of many novel targeted therapeutics into clinical practice is a rapidly growing field in medical oncology. Cancer chemotherapy involves targeting cancer cells with cytotoxic agents. Chemotherapy has been considered as a solution for the treatment of many cancers, resulting in destruction of malignant cells, while having a nonspecific toxicity on all other cell types. The current chemotherapeutic approach for prostate cancer is to use single or combined chemotherapeutic agents to increase survival rates for hormone refractory disease [4]. These therapeutic strategies are able to devastate the tumor but are often ineffective on advanced prostate cancer. Although the combination of the chemotherapeutic agents is considered to be effective against prostate cancer, there is currently no reported data regarding the increased survival rates or improved patients life quality [5]. Developments of new chemotherapeutic agents that enable the inhibition of prostate cancer progression are the aim of interest. Moreover, the impact of cytotoxic chemotherapy is not limited to the cancer tissue. Therefore, future treatments will include cytotoxic tools that preferentially target tumor cells.

Pluronics as an interesting class of polymeric materials arranged in a triblock structure consisting of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) units. The number of these hydrophilic and hydrophobic units determines the micelle characteristics and diameter of micelles carrying drug molecules

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within the core [6]. Pluronic P85-based formulations of different chemotherapeutics have been evaluated for anticancer activity *in vitro* for resistant cancer cells in many studies. The inhibitory role of P85 on multidrug resistant development was demonstrated by a previous study in combination with the well-known anticancer agent doxorubicin [7]. The toxicity of a broad range of pharmaceuticals has been increased in monolayer cell culture of Bovine Brain Microvascular Endothelial Cells (BBMEC) and Caco-2 cells when combined with P85 [8]. The sensitizing effect of P85 has been evaluated for carboplatin on colorectal cancer cells *in vitro* in a previous study conducted with three different Pluronic block copolymers; L61, P85 and F127 [9]. Because P85 molecules are able to integrate into cell membranes and decrease Pgp ATPase activity both by inhibition and ATP depletion [10], the use of P85 in combination with chemotherapeutic drugs could be a powerful solution to overcome drug resistance in cancer cells. Alternative drug molecules and their P85 combinations could be viable candidates to control cancer cell growth. Schiff bases derived from the condensation reaction of a primary amine and carbonyl compounds are able to generate highly stable complexes with metal ions, which make them attractive sources for biological applications [11].

Different Schiff base derivative compounds in the literature were reported to be anti-inflammatory [12], antifungal [13], antimicrobial [14] and antihypertensive [15]. In addition to their comprehensive biological activities, the anticancer activity of different Schiff base derivatives was reported in the previous studies [16–18]. In the current study, a novel Schiff base derivative heterodinuclear copper(II)Mn(II) was combined with P85 to enhance anticancer activity on prostate cancer *in vitro*. Schiff base have exerted remarkable anti-cancer activity when combined with the P85 against prostate cancer in vivo. Moreover, combination of Schiff base and P85 have been tolerated well by mice without exerting toxicity for the selected drug dose. A 62% success was obtained in drug applied animals compared to baseline [18].

The challenge for the future prospects of this study is to discover a targeted and cost effective chemotherapeutic drug with specific toxicity for prostate cancer cells. Because the signaling pathways that are involved in cancer initiation and cell death are different, the mechanism of the chemotherapeutic agents should be identified. The purpose of the current study is to determine the mechanism of newly developed drug combination on prostate cancer cells. We report the activity of a new chemotherapeutic drug for prostate cancer in vitro.

2. Materials and methods

2.1. Preperation of drug combination

Heterodinuclear copper(II)Mn(II) complex (Schiff base) was synthesized and characterized by our group as described previously [19]. Schematic representation of the complex is given

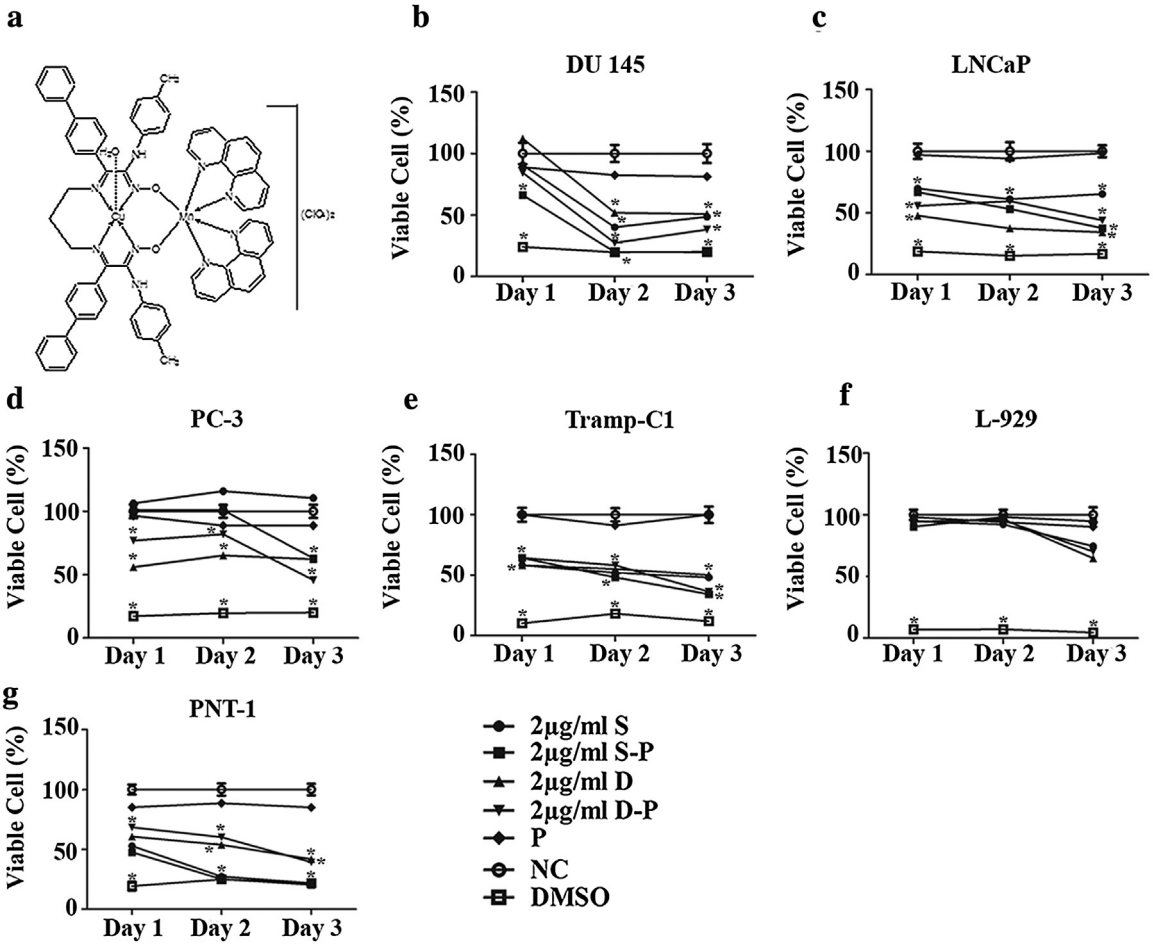

Fig. 1. (a) Schematic representation of novel Schiff base derivative (Heterodinuclear copper(II)Mn(II) complex). (b) Effect of 2 μg/ml Schiff Base, Docetaxel and their combination with P85 on the cell viability of DU 145, (c) LNCaP, (d) PC-3, (e) Tramp-C1, (f) L-929, (g) PNT-1 cells.
Abbreviations: P: Pluronic P85 (0.05% w/v), NC: Negative Control (Growth medium), S: Schiff base (2 μg/ml), D: Docetaxel (2 μg/ml), SP: Schiff base-P85 combination, DP: Docetaxel-P85 combination, DMSO:20% DMSO as positive control. $^{*}P < 0.05$. Notes: Results were analyzed by one-way ANOVA and Tukey's posttest.