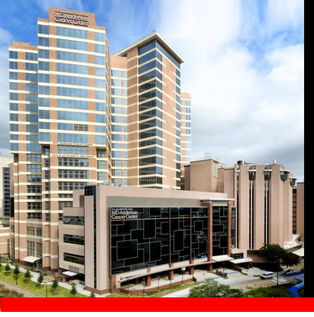

Substance P Receptor Antagonism Enhances Chemotherapeutic Responses in Triple Negative Breast Cancer

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Introduction

Triple negative breast cancer (TNBC), is characterized by aggressive clinical characteristics, poor prognosis, and high mortality rate within first 5 years [1, 2]. Doxorubicin (DOX) and Cisplatin (CIS), forms the basis of chemotherapy regimens for many cancers including TNBC [3-7]. However, both DOX and CIS often causes recurrence and chemoresistance and severe side-effects in TNBC. Novel therapeutic combinations are urgently needed to increase the efficacy of DOX and CIS and at the same time ablate or reduce toxicity and chemoresistance in TNBC. Substance P (SP), an 11-amino acid neuropeptide involved in pain transmission, acting via its high affinity receptor, neurokinin1 receptor (NK-1R), has shown to be mitogenic for human cancer cells in vitro [8]. NK1R is over expressed in TNBC cells [9]. In vitro studies have shown that SP prevents apoptosis of tumor cells and induces tumor cell migration [8]. We determined if NK1R antagonism can increase the efficacy of chemotherapy using human TNBC cell lines.

Methods

Cell Culture Human TNBC cell lines, MDA-MB 453, 468 and Sum 149, were a kind courtesy of Dr. Nato Ueno, Translational Breast Cancer Research, Department of Breast Medical Oncology, MD Anderson Cancer Center, Houston, TX. Sum 149 was cultured in RPMI, while all other cells were cultured in DMEM- containing 10% heat-inactivated fetal bovine serum, antibiotics (streptomycin and penicillin), an antifungal agent (amphotericin B) and were not passaged more than 4 wks continuously.
Viability Assay Cell viability was measured using MTT assay. Cells were plated in 96-well plates (10,000 cells/well); after 24 h, we treated triplicate wells with DOX/CIS/AP (with various conditions indicated in the figures; 0.003 µm to 100 µm). AP was treated 2 h before DOX/CIS treatment and continuing until termination. Control wells were treated with media alone. Following 72 h treatment, cell viability was determined. Each well was emptied, and MTT (1 mg/mL in medium containing 1% serum) was then added to each well and incubated at 37˚C for 2 h. Following which viable cells that contain NADPH-dependent oxidoreductase enzymes reduce the MTT to formazan. The insoluble formazan crystals were solubilized in an extraction buffer containing 20% sodium dodecyl sulfate and 50% dimethylformamide. The cells were incubated overnight with the extraction buffer at 37˚C, and OD was measured at 590 nm using a 96-well multi scanner. Data are represented as percentage viability related to untreated cells ± SEM.
Western blot (WB): WB was performed to determine levels of NK1R protein using rabbit anti-mouse NK1R antibody and actin antibody as loading control followed by quantification of bands using Image J as software.

TNBC cells express high levels of NK1R

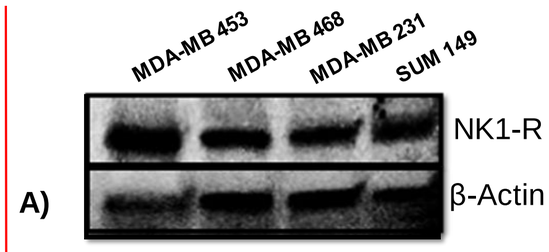

Figure 1. (A) NK1R expression as determined by western blotting in MDA-MB 453, 468 and Sum 149 cells.

NK1R Antagonism Enhances Efficacy of DOX in TNBC Cells.

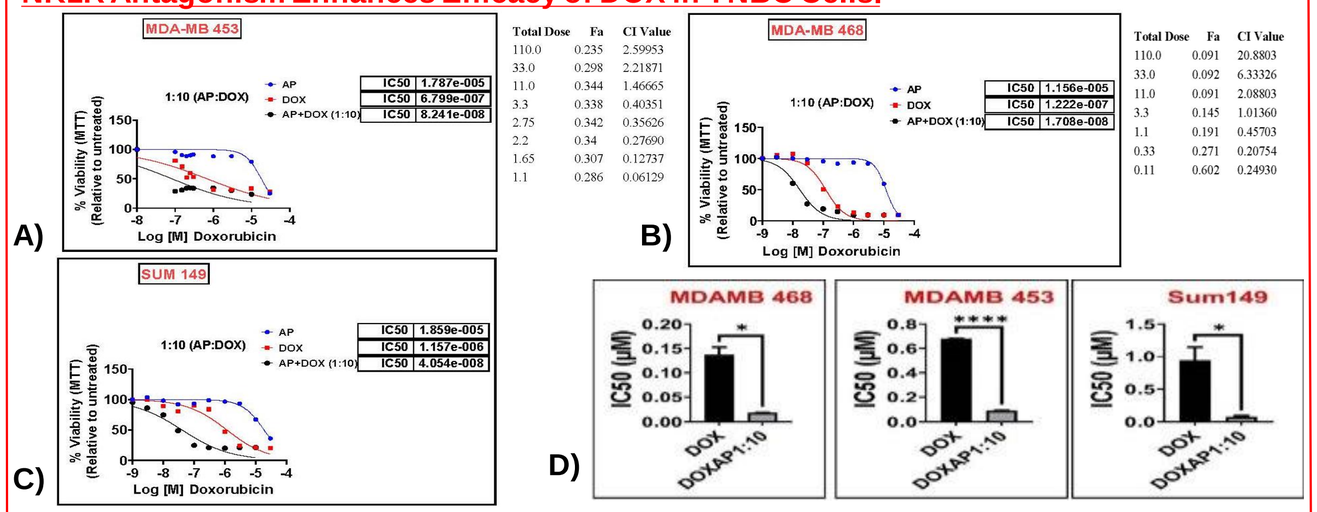

Figure 2. NK1R Antagonism causes enhancement of DOX-induced loss of viability in TNBC cells. Loss of viability induced by DOX in the presence and absence of AP in MDA-MB 453, 468 & Sum 149 cells (A–C). IC_{50} levels of DOX in presence and absence of AP in TNBC cells (D-F) (*, †, ‡, $p \leq 0.05$, ANOVA, $n = 2$).

NK1R Antagonism Enhances Efficacy of CIS in TNBC Cells.

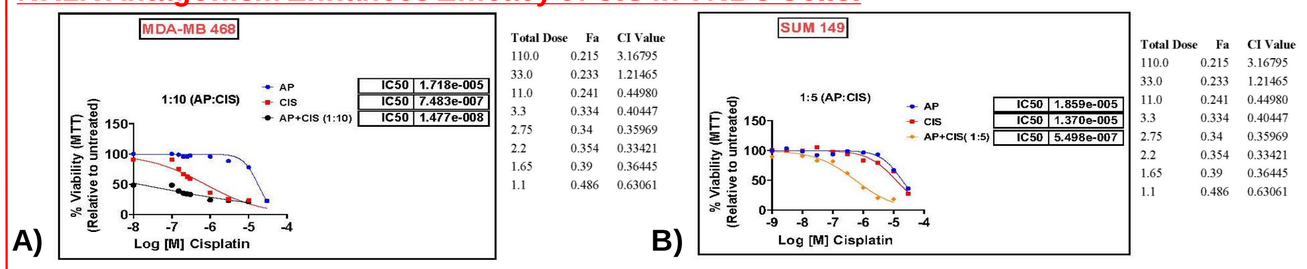

Figure 3. NK1R Antagonism causes enhancement of CIS-induced loss of viability in TNBC cells. Loss of viability induced by CIS in MDA-MB 468, and Sum 149 cells (A-B). IC_{50} levels of CIS in the presence and absence of aprepitant in TNBC cells (C-D) (*, †, ‡, $p \leq 0.05$, ANOVA, $n = 2$).

DOX and CIS IC_{50} Values by TNBC Cells

	MDA-MB 468		MDA-MB-453		Sum 149			Sum 149		MDA-MB 468
	Mean	SEM	Mean	SEM	Mean	SEM		Mean	SEM	Mean
AP	16.340	3.422	19.000	0.707	15.015	2.019	AP	19.295	0.50	17.180
DOX	0.137	0.015	0.940	0.210	0.680	0.001	CIS	10.859	2.01	0.748
AP:DOX (1:10)	0.018	0.001	0.066	0.021	0.088	0.006	AP:CIS (1:10)	1.010	0.29	0.014

Results

We determined by western blotting-high levels of SP receptor; NK1R protein expression in all 3 human TNBC cell lines that was tested; MDA-MB 453, 468 and Sum 149.
Most importantly, we determined that compared with DOX or CIS alone, treatment with DOX/CIS in the presence of aprepitant (a NK1R receptor antagonist) led to significantly enhanced loss of viability of TNBC cells (as determined by the MTT assay).
We further determined that compared with DOX or CIS alone, treatment with DOX/CIS in the presence of aprepitant increased levels of apoptosis of the TNBC cells (as determined by the TUNEL assay).

Discussion

DOX often causes recurrence and chemoresistance in TNBC [10, 11]. In addition, DOX has been known to induce cardiotoxic side effects such as electrocardiographic changes, decreased left ventricular ejection fraction values, and life-threatening heart failure or acute coronary syndromes in some patients [12].
Also, although cisplatin is highly effective as first-line therapy in TNBC, it can lead to chemoresistance and unwanted dire manifestations such as peripheral neuropathy, inhibited immune responses to infections, severe kidney damage, hearing loss, allergic reactions, hemorrhage, and gastrointestinal disorders [13, 14].
Our studies determined that combinations of NK1R antagonism with either of the 2 chemotherapeutic agents namely DOX or CIS resulted in greater efficacy compared to the chemotherapeutic agents by themselves in TNBC.

Conclusions

These studies implicate that NK1R - receptor antagonism in combination with DOX or CIS may possibly serve as novel, more efficacious and safer therapeutic options than existing therapies for TNBC.

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