Department of Oncology[1]; Department of Clinical Laboratory[2]; Department of Thyroid and Breast Surgery[3], The First Affiliated Hospital of Xinxiang Medical University, Weihui, Henan Province, P.R. China

The anti-cancer effect of metoclopramide on triple-negative breast cancer cells

Yifan Chen[1,#], Min Zhang[1,#], Xinghao Ji[1], Jinjin Zhao[2], Shuang Qin[3], Yinghua Ji[1], Ruijuan Fan[1], Yanting Liu[1,*], Ping Lu[1,*]

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**Corresponding authors: Yanting Liu, Ping Lu, Department of Oncology, The First Affiliated Hospital of Xinxiang Medical University, Weihui, Henan Province, P.R. China*
453100 yanting.liu@foxmail.com(Y.L); lupingdoctor@126.com(P.L)
[#]These two authors contributed equally to this work.

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Triple-negative breast cancer (TNBC) is the most aggressive type of breast cancers. Chemotherapy is the most important therapeutic option for TNBC, and chemotherapy-induced nausea and vomiting (CINV) is inevitable. Metoclopramide is a good and cost-effective therapeutic option for chemotherapy-induced nausea and vomiting. However, it is not commonly used in breast cancer because it can increase serum prolactin levels by blocking dopamine D2 receptor. This study aimed at elucidating the effect of metoclopramide on triple-negative breast cancer, MDA-MB-231 cells were treated with various concentrations of metoclopramide, the cell proliferation was detected by MTT method, the apoptosis rate was detected by Annexin V/PI double staining method, the expression change of death-related protein was detected by Western Blot. We found that metoclopramide inhibits cell proliferation and induces cell apoptosis of MDA-MB-231 in a concentration-dependent manner, and the Bcl family was involved in this process.

1. Introduction

Breast cancer is one of the most common malignant tumors in women in the world, it still remains the second leading cause of cancer death among women, which seriously threatens women's health (Siegel et al. 2019). Among them, triple negative breast cancer (TNBC) is a refractory breast cancer with the worst prognosis among all breast cancer types (Sorlie et al. 2001). Currently, the main treatment of triple-negative breast cancer is still relying on chemotherapy. Nausea and vomiting caused by chemotherapy drugs is a common and daunting accompanying symptom, called chemotherapy-induced nausea and vomiting (CINV) (Dielenseger et al. 2019). CINV seriously affects the quality of life and the implementation and efficacy of chemotherapy.

For patients with triple-negative breast cancer, their chemotherapy regimens often lead to mild vomiting. According to NCCN guidelines, for mild vomiting chemotherapy drugs, drugs such as metoclopramide, ondansetron, palonosetron can be selected (Berger et al. 2017; Bevers et al. 2018). However, ondansetron and palonosetron are expensive, even more expensive than the price of chemotherapy medicine. As a cheap antiemetic drug, metoclopramide can effectively treat CINV without bringing economic burden to patients (LiverTox 2012). Unfortunately, metoclopramide can promote the secretion of prolactin (PRL) by inhibiting dopamine receptors, while previous studies have suggested that high serum prolactin levels can increase the risk of breast cancer (Tworoger et al. 2004; Tikk et al. 2014). Therefore, the application of metoclopramide in the antiemetic treatment of breast cancer patients is prohibited by the Pharmacopoeia of the People's Republic of China.

As research continues, it was found that the relationship between PRL and breast cancer is not exact (De Hert et al. 2016; Hachim et al. 2019; Lopez-Ozuna et al. 2019). Therefore, whether metoclopramide promotes the development of breast cancer remains doubtful. What's more, some research found that it's safe to be used in breast cancer patients (Roila et al. 1987).

In this study, we used metoclopramide to treat MDA-MB-231 cells. To our surprise, we found that metoclopramide can inhibit MDA-MB-231 cells in a concentration-dependent manner, and can promote the apoptosis of triple-negative breast cancer cells MDA-MB-231 by inhibiting the Bcl family. This study provides evidence for the application of metoclopramide in breast cancer patients.

2. Investigations and results

To determine the effects of metoclopramide on the triple-negative breast cancer cells, we first used the MTT method to detect the effect of different concentrations of metoclopramide (0, 0.5, 0.75, 1, 1.5, 2 mmol/L) on the proliferation of MDA-MB-231 cells after treatment for 24 h and 48 h. The results showed that compared with the control group, metoclopramide inhibited the cell viability of MDA-MB-231 in a concentration-dependent manner (Tables 1,2). Compared to the 24 h treatment group, the 48 h group had a better effect (Fig 1A,B). Then we used annexin v-FITC/pi apoptosis detection kit to detect the cell apoptosis, flow cytometry results showed that as the concentration of metoclopramide increased,

Table 1: MTT results of cytotoxicity after MDA-MB-231 cells treated by various concentrations of metoclopramide (0, 0.5, 0.75, 1, 1.5, 2 mmol/L) for 24 h

Wave range	OD of different group (Metoclopramide mmol/L)					
	0	0.5	0.75	1	1.5	2
570 nm	0.7704	0.5658	0.5497	0.4092	0.3054	0.4621
	0.7685	0.5831	0.4003	0.4346	0.4449	0.2831
	0.6345	0.6236	0.4207	0.3946	0.4065	0.2744
	0.7172	0.507	0.6338	0.629	0.3466	0.2353
	0.725	0.6027	0.4364	0.358	0.3755	0.3761
630 nm	0.1752	0.1432	0.1371	0.1102	0.092	0.1793
	0.1753	0.1448	0.1071	0.1277	0.1187	0.0886
	0.1535	0.1491	0.1144	0.1113	0.108	0.089
	0.1649	0.143	0.1539	0.158	0.0987	0.0794
	0.1691	0.145	0.1288	0.101	0.104	0.1034

Table 2: The MTT results of cytotoxicity after MDA-MB-231 cells treated by various concentrations of metoclopramide (0, 0.5, 0.75, 1, 1.5, 2 mmol/L) for 48 h

Wave range	OD of different group (Metoclopramide mmol/L)					
	0	0.5	0.75	1	1.5	2
570 nm	0.8885	0.6818	0.7757	0.5707	0.3287	0.0563
	0.9903	0.7965	0.7197	0.4936	0.5348	0.0694
	1.011	0.6965	0.6895	0.4149	0.3229	0.0641
	1.0346	0.858	0.576	0.5172	0.4374	0.2875
	1.0008	0.8388	0.5925	0.405	0.4069	0.2642
630 nm	0.1985	0.1677	0.1804	0.1491	0.1013	0.0447
	0.2143	0.1907	0.1809	0.1332	0.1394	0.046
	0.2188	0.1732	0.1759	0.1311	0.0976	0.0451
	0.2224	0.1943	0.144	0.1358	0.1233	0.0858
	0.217	0.1895	0.1452	0.1148	0.1093	0.0807

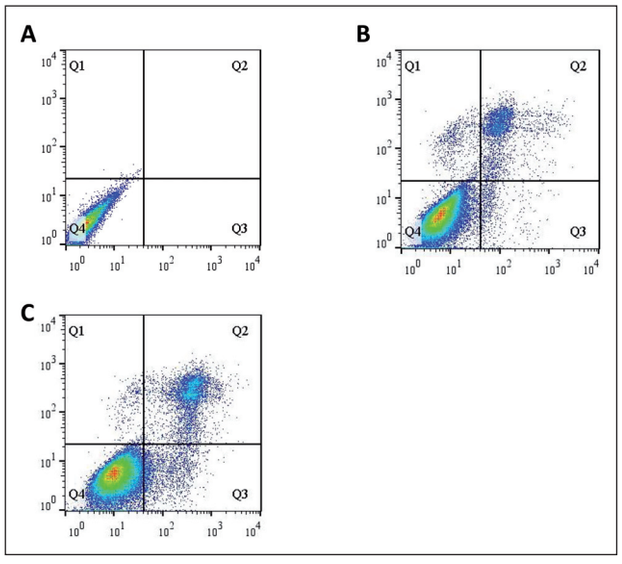

Fig. 1: Metoclopramide inhibits proliferation and promote apoptosis of triple-negative breast cancer cells. (A) The cell viability after treated with different concentrations of metoclopramide (0, 0.5, 0.75, 1, 1.5, 2 mmol/L) for 24 h; (B) The cell viability after treated with different concentrations of metoclopramide (0, 0.5, 0.75, 1, 1.5, 2 mmol/L) for 48 h; (C) The apoptosis rate after treated with different concentrations of metoclopramide (0, 0.75, 1mmol/L) for 48 h; (D) The Bcl family expression before and after treated with different concentrations of metoclopramide (0.75, 1 mmol/L).

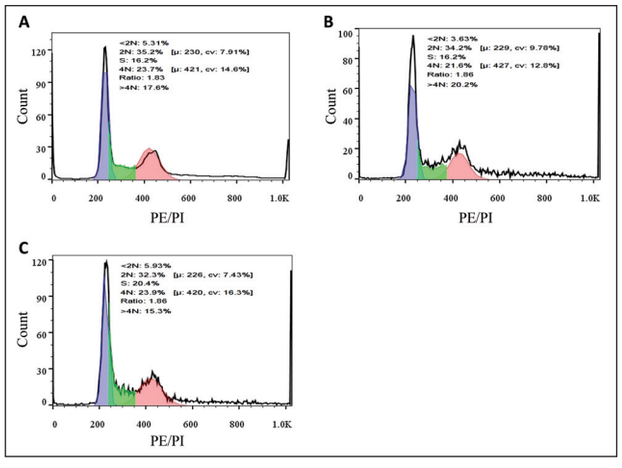

Fig. 2: The apoptosis results of MDA-MB-231 cells after treated by different concentrations of metoclopramide. (A) Flow cytometry results of the cell apoptosis without treatment; (B) Flow cytometry results of the cell apoptosis after treated by 0.75 mmol/L metoclopramide for 48 h; (C) Flow cytometry results of the cell apoptosis after treated by 1.0 mmol/L metoclopramide for 48 h.

the apoptosis rate of MDA-MB-231 cells increased significantly (Fig 1C, Fig. 2). In order to explore the mechanism of metoclopramide induced apoptosis, we used Western Blot to detect the Bcl family protein level in MDA-MB-231 cells before and after treatment with metoclopramide, results showed that compared to the control group, metoclopramide treatment for 48 h down-regulated the expression of Mcl-1, Bcl-XL, Bcl-2, and Bcl-W in a concentration-dependent manner (Fig. 1D). Flow cytometry results showed that metoclopramide had no effect on cell cycle of the MDA-MB-231 cells (Fig. 3), which indicates that the inhibition of proliferation is not induced by cell cycle blocking. In summary, we conlcude that metoclopramide inhibits proliferation and promotes apoptosis of triple-negative breast cancer cells *in vitro*.

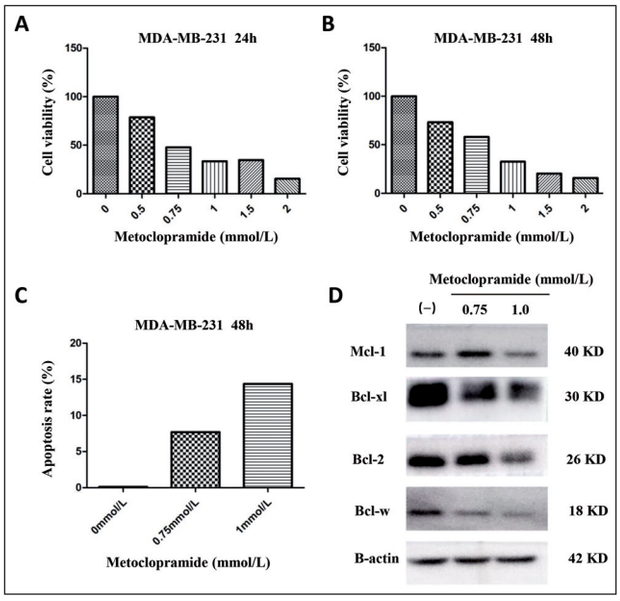

Fig. 3: The cell cycle after treated by different concentrations of metoclopramide. (A) Flow cytometry results of the cell cycle without treatment; (B) Flow cytometry results of the cell cycle after treated by 0.75 mmol/L metoclopramide for 24 h; (C) Flow cytometry results of the cell cycle after treated by 1.0 mmol/L metoclopramide for 24 h.

3. Discussion

Metoclopramide is a dopamine D2 receptor antagonist, which can act on the dopamine receptors in the chemical trigger zone (CTZ) to increase its threshold, it has a powerful central antiemetic effect. Dopamine D2 receptors are highly expressed in a variety of cancer tissues, including lung cancer, cervical cancer, breast cancer, and ovarian cancer. Studies have shown that dopamine D2 receptor antagonists have certain anti-tumor effects (Weissenrieder et al. 2019). In gynecological tumors, the high expression of D2 receptor is closely related to the progression of cervical cancer. After blocking the D2 receptor, the growth of cervical cancer cells is significantly inhibited (Mao et al. 2015). Dopamine receptor antagonists can even be used directly in the treatment of metastatic breast cancer and triple negative breast cancer (Park et al. 2016; Li et al. 2017). However, as a dopamine receptor antagonist, the effects of metoclopramide on breast cancer has not been reported. Here we found that metoclopramide inhibited the proliferation activity of MDA-MB-231 cells in a dose-dependent manner (Fig. 1A,B). Flow cytometry results showed that metoclopramide can promote the apoptosis of MDA-MB-231 cells *in vitro*, but have no effect on the cell cycle (Fig. 1C, Fig. 2). At the same time, the protein molecular changes were detected at the molecular level, and it was verified that metoclopramide can down-regulate the expression of anti-apoptotic proteins Bcl-2, Bcl-xl, Bcl-w, and Mcl-1 (Fig 1D), indicating that metoclopramide may take effect by inhibiting the Bcl family. Our study provides theoretical basis for the application of metoclopramide in breast cancer patients.

4. Experimental

4.1. Cell culture

The MDA-MB-231 cell line was purchased from China National Infrastructure of Cell Line Resource Sharing Service Platform (Beijing, China). The cell line was cultured in culture medium RPMI 1640 (Hyclone, Tianjin, China) containing 10% Fetal Bovine Serum (FBS; CLARK BIOSCIENCE, Richmond, VA, US), and 100 U/mL penicillin and 100 mg/L streptomycin (Solarbio Life Science, Beijing, China). Cells were cultured in 37 °C, 5% CO_2 cell culture incubator.

4.2. Cytotoxicity assay

The cytotoxicity of metoclopramide on MDA-MB-231 cells was tested with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Solarbio Life Science, Beijing, China) assay, metoclopramide was purchased from *Selleck* Chemicals (Shanghai, China). The MDA-MB-231 cells were seeded onto 96-well plates (6×10^3 cells/well) and exposed to various concentrations of metoclopramide (0, 0.5, 0.75, 1, 1.5, 2 mmol/L), then incubated in 37 °C, 5% CO_2 cell culture incubator for 24 h or 48 h. Then add MTT solution (5 mg/mL) into each well of cells, cultured for 4 h in 37 °C until formazan was constituted. Discard the medium and add 150 µl DMSO (Sigma Aldrich, MO, US), The optical density was measured at 570 nm and 630 nm using SpectraMax plus 384 microplate reader (Molecular Devices, CA, US). The cell viability was calculated by the following equation:
Cell viability (%) = [OD metoclopramide]/[OD (blank)] × 100%.

4.3. Annexin V-FITC and PI double staining assay

The MDA-MB-231 cells were seeded in a 6-well plate (8×10^5 / well) and incubated with various concentration (0, 0.75 and 1 mmol/L) of metoclopramide for 48 h. After treatment with metoclopramide, trypsin were used to dissociated the cells, after washed with PBS, cells were re-suspended in 300 µL of binding buffer (Beyotime, Shanghai, China), then the Annexin V-FITC and PI double staining was performed by Annexin V-FITC Apoptosis Detection kit (Beyotime, Shanghai, China), Cells were incubated in dark for 15 min, and the apoptosis of each group of cells was detected by flow cytometry (Calibur, BD Biosciences, CA, US).

4.4. Cell-cycle analysis

Cells were seeded in a 6-well (8×10^5 cells/well) and incubated with various concentration (0, 0.75 and 1 mmol/L) of metoclopramide for 24 h. After treatment, the cells were washed with PBS, then digested with trypsin without EDTA (Solarbio Life Science, Beijing, China). After the digestion, collected the cells and washed with pre-cooled PBS, then fixed with 70% absolute ethanol (-20°C, 24h), wash again with PBS, finally, 500 µL PI (Beyotime, Shanghai, China) was added and incubated in water bath at 37°C for 30 min. The DNA content was detected by flow cytometry (Calibur, BD Biosciences, CA, US). The data were analyzed by Cell Quest software (Becton Dickinson, Franklin Lakes, NJ) and FlowJo-V10. The percentage of cells in the G1 phase, the S phase, and the G2 phase was analyzed.

4.5. Western blot analysis

After treatment with various concentrations (0, 0.75 and 1 mmol/L) of metoclopramide for 48 h, cells were harvested and lysed in RIPA lysis buffer (Beyotime, Shanghai, China) with phosphatase inhibitor (Beyotime, Shanghai, China) for 15 min at 4 °C, cell debris was removed by microcentrifugation (4 °C, 12,000 rpm, 20 min) and then the protein concentration of supernatants was ascertained by BCA kit (Beyotime, Shanghai, China). Protein extraction solution (50 µg) was fractionated by SDS PAGE and transferred to a PVDF. After blocking with 5% BSA for 1 h, the membrane was reacted with primary antibodies at 4 °C overnight, primary antibodies including Mcl-1 (Cell Signaling technology, Shanghai, China), Bcl-2 (Cell Signaling technology, Shanghai, China), Bcl- w (Cell Signaling technology, Shanghai, China), Bcl-xl (Cell Signaling technology, Shanghai, China). Then the membrane was washed three times with TBS-T for 10 min, and incubated with secondary antibody (Anti-rabbit IgG; Cell Signaling technology, Shanghai, China) for 1 h at room temperature. Then the membranes were washed three times again with TBS-T for 10 min, the blots were developed using enhanced chemiluminescence detection agents (Merck Millipore, Beijing, China), and detected by Amersham Imager 600 (GE Healthcare, Pittsburgh, PA).

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Conflicts of interest: The authors declare that there are no conflicts of interest regarding this study.

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