Ann Med Res 2020;27(4):1188-92

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.02.179

Original Article

Metoclopramide increased cell proliferation in HepG2 cell line and sorafenib attenuated the effect

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Abstract

Aim: Metoclopramide is an antiemetic drug used for treating postoperative or chemotherapy-induced emesis. Sorafenib is a kinase inhibitor drug and is approved for the treatment of advanced primary liver cancer, renal cell carcinoma, thyroid cancer, and acute myeloid leukemia. Hepatocellular cancer is a common cause of cancer-related death and its treatment may require coadministration of an antiemetic medication. The study aims to investigate the effect of metoclopramide on hepatocellular cancer cell proliferation alone or in combination with sorafenib.

Material and Methods: Metoclopramide doses of 0.17 µM to 25 µM alone or in combination with sorafenib were administered to human hepatocellular cancer cell line, HepG2. Cell viability and proliferation test was used to determine the possible effects on proliferation. Hematoxylin and Eosin staining was performed to visualize the morphological effects of the treatments.

Results: Metoclopramide doses of 0.58 µM, 25µM increased cell proliferation when compared to the control group. Metoclopramide combination groups with 9.9 µM sorafenib were compared with control and sorafenib groups. Each combination group was comparable with the control group.

Conclusion: Metoclopramide increased proliferation in certain doses. Sorafenib inhibited the effect. Safety concerns about its use in hepatocellular cancer should be addressed in clinical trials.

Keywords: Hepatocellular cancer; metoclopramide; pharmacology; sorafenib

INTRODUCTION

Metoclopramide (MTC) is a dopamine receptor antagonist exerts muscarinic effects and is used as an antiemetic and prokinetic (1). Later, MTC was also shown to have a serotonergic role; acting as a 5-HT3 antagonist and a 5-HT4 agonist (2). MTC's effect beside dopamine antagonism lead to a newer group of antiemetic drugs, namely serotonergic antagonists (3). The mechanism of action of MTC may explain its clinical uses and the associated adverse drug effects. It is used in the treatment and prophylaxis of antineoplastic-induced and postoperative nausea and vomiting. Additionally, it is indicated as a prokinetic drug in gastroesophageal reflux or gastric motor diseases (4). Specific dopaminergic side effects include galactorrhea, tardive dyskinesia and an increase in aldosterone levels. MTC is prominently eliminated with urine and can be used in hepatic failure without dose adjustments. In edematous conditions, who are at risk of fluid overload like cirrhosis, drugs should be discontinued if adverse events or any symptoms appear.

Hepatocellular carcinoma (HCC) is the primary liver cancer and the leading cause of cancer-related deaths (5). Curative therapy is mainly surgical but most of the cases with HCC present an advanced disease that needs systemic pharmacotherapy (5). The first approved systemic treatment opportunity for advanced primary liver cancer, sorafenib, is a tyrosine kinase inhibitor. It was also approved for the treatment of renal cell carcinoma, thyroid cancer and acute myeloid leukemia (6,7). Tyrosine kinase inhibitors can cause gastrointestinal adverse effects, the most common of which is diarrhea. Sorafenib also causes nausea (2%) and vomiting (1%) which might be so severe that the existing therapy is modified (8). MTC is within the treatment opportunities both for postoperative nausea and for sorafenib induced emesis in advanced HCC (8,9).

Received: 28.02.2020 **Accepted:** 24.03.2020 **Available online:** 20.04.2020
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The study aims to investigate the effect of MTC on the viability of HCC cells. Additionally, the cell viability in response to the concomitant administration of MTC with sorafenib was explored.

MATERIAL and METHODS

Cell culture

HepG2 cells were seeded in 96-well plates and grown in *Dulbecco's modified Eagle's medium* (DMEM) supplemented with L-glutamine, 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 100 IU/ml penicillin and 100 µg/ml streptomycin overnight at 5% CO2 at 37°C. The next day, the cells were treated with drugs and incubated for 24 hours for 2,3-bis-(2-methoxy-4-nitro5-sulfophenyl)-2H-tetrazolium-5-carboxanilide salt (XTT) cell viability assay (Cat # 20-300-1000, Biological Industries, Israel) and Hematoxylin and Eosin (H&E) staining.

Drugs

MTC was purchased from Sigma Aldrich (Sigma-Aldrich, St. Louis, MO, USA, CAS Number: 364-62-5) and Sorafenib was purchased from LC Lab (LC Laboratories, Woburn, MA, USA, CAS Number: S-8599). Sorafenib was dissolved in dimethyl sulfoxide (Cat # A3672,0100, AppliChem, Germany) at a concentration of 10 mM and stored at -20°C. The dose of dimethyl sulfoxide used as solvent was a negligible dose for toxicity. Therefore, it has not been added to the study setup as a control group.

MTC was dissolved in distilled water at a concentration of 0.25 mM for storage at +4°C. Drugs were freshly diluted to designated concentrations with DMEM on the day of the experiment. To evaluate the effects on cell viability, MTC was administered at the doses from 25 µM to 0.17 µM with a dilution ratio of 2/7. Sorafenib was coadministered to evaluate the combined effect. Sorafenib dose was chosen to be 9.9 µM according to the study of Rangwala et al. in HepG2 cells (10).

Cell viability and proliferation assay

The human HCC cell line, HepG2 cells were seeded at a density of 30,000 cells per well in 96-well plates. After overnight incubation, drugs are administered as described above. XTT cell viability assay was performed after 24 hours of incubation with drugs according to the manufacturer's instructions. *Synergy Microplate Reader* was used to measure the optical density of the soluble product.

H&E staining

HepG2 cells were seeded on coverslips in 24 well plates and treated with MTC and/or sorafenib. After treatment, the cells were fixed with 4% formaldehyde and rinsed three times in phosphate-buffered saline. H&E staining was performed. Photographs were captured under a Zeiss Primovert light microscope (Jena, Germany)

Statistical analysis

Results were pooled from at least three independent experiments. All data are expressed as mean ± standard error of the means (SEM). Statistical evaluation was performed by one-way ANOVA test and multiple groups were compared by post-hoc Tukey analysis. GraphPad Prism V.8.01 (San Diego, CA, USA) is used for the statistics and graphs.

RESULTS

Treatment with MTC led to a trend of an increase in cell proliferation (Figure 1). The increase was significant at the doses of 0.58 µM and 25 µM when compared to the untreated control group ($F=[11,168]=5.575$. $\alpha=0.05$. $p=0.0079$ and $p=0.0089$, respectively). The combination groups demonstrated comparable viability percentages in comparison to the control groups. MTC doses of 0.17 µM and 25 µM in combination with sorafenib increased proliferation when compared to sorafenib treatment alone ($p=0.0038$ and $p=0.0119$, respectively). While MTC treatment alone resulted in higher cell viability at the doses of 0.58 µM and 25 µM in comparison to the control group, combination groups demonstrated no change at the respective doses which might point to an inhibition of the effect of MTC on cell proliferation by sorafenib.

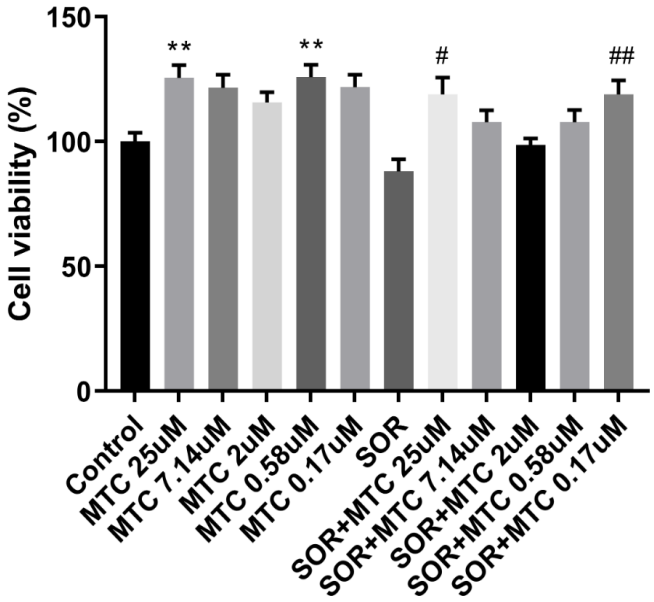

Figure 1. HepG2 cells were incubated with (i) MTC , (ii) sorafenib (SOR) (iii) combination of MTC with sorafenib for 24 h. The control group represents the untreated cells. Cell viability for each treatment is shown in comparison to the untreated control group. *shows a significant difference in cell viability in comparison to the untreated control group, # shows a significant difference in cell viability in comparison to the sorafenib treated group

In the control group, the typical multilayer appearance of HepG2 cells is observed (Figure 2A). Administration of MTC doses did not induce any morphological changes (Figure 2B-F). Sorafenib treatment resulted in a change in morphology (Figure 2G-L). Rounded cells (shown with arrows) were observed following treatment with sorafenib alone or in combination with MTC with H&E staining.

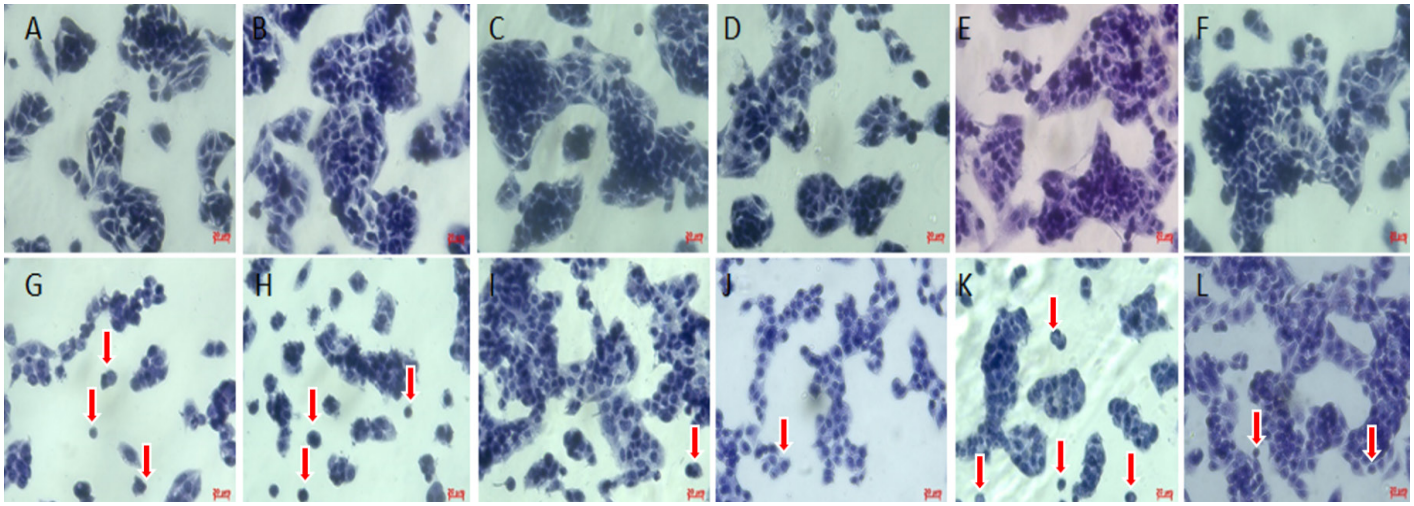

Figure 2. H & E staining was performed after incubation of HepG2 cells with (i) MTC, (ii) sorafenib (SOR) (iii) combination of MTC with sorafenib for 24 h A)CONT B) MTC 0.17 µM C) MTC 0.58 µM D) MTC 2 µM E)MTC 7.14 µM F)MTC 25 µM G) SOR H) SOR+ MTC 0.17 µM I) SOR+ MTC 0.58 µM J) SOR+ MTC 2 µM K) 7.14 µM L) SOR+ MTC 25 µM. Arrows indicate the rounded cells

DISCUSSION

Antiemetics and the associated possible effects on cancer cells is an old debate (11). Sorafenib is used for nearly a decade and since then pharmacotherapy has become an option in HCC. MTC has multifactorial effects on neuromodulators. It has effects on the dopaminergic system besides its muscarinic and serotonergic effects (1). The evidence for the effects on cell proliferation when used in combination with cancer drugs is scarce.

The effects of MTC on cell proliferation are conflicting in the literature. The studies reported that concomitant antineoplastic use determines the results. The cytotoxicity of cisplatin was decreased whereas the toxicity of epirubicin was increased when MTC was coadministered in a fibroblast and lung cancer cell line study (12). MTC was reported to induce cytotoxicity and to increase the effects of the ionizing radiation on human lung adenocarcinoma and virus-induced sarcoma (13). MTC within a 1-10 µM dose range was shown to inhibit DNA repair and induce DNA damage in human peripheral mononuclear leukocytes in a cell culture study (14). In contrary to these results, in an in vivo study, MTC administration for ten days was found to increase spontaneous spleen proliferation (15).

Dopamine is one of the neuromodulators in humans. It is also important in the peripheral tissues. MTC has an antidopaminergic effect. HepG2 cell proliferation was reported to be induced with dopaminergic stimulation (16). Cholinergic effects are also known to be evoked by MTC (1). Muscarinic acetylcholine receptor 1 was shown to promote the invasion of hepatocellular carcinoma cells *in vitro* (17). A possible role of this pathway in MTC related effects may be investigated in a future study.

Selective 5-HT3 serotonergic receptor antagonists, ondansetron and granisetron were investigated in a cell culture study in combination with antineoplastic medications. Granisetron alone had a cytotoxic effect and was found to enhance the estramustine and bleomycin-induced cytotoxicity, yet it did not demonstrate any interaction with the effect of epirubicin or cisplatin (11). In the same study, ondansetron did not change the cytotoxicity of the mentioned antineoplastic drugs (11). The absence of any proliferative effect in the study might prompt the effect of MTC was not caused by the 5-HT3 antagonism. The mentioned study showed the effects in a fibroblast and a lung cancer cell line which may not represent the hepatocellular cell line HepG2.

The clinical relevance of the study can be further discussed. The lowest dose of 0.17 µM in our study corresponds to the therapeutic range which is reported to be clinically efficient (1). The effect on proliferation does not seem to be dose-dependent.

Sorafenib attenuated the effect of MTC on proliferation. It may be interpreted as the effect of MTC is in a lesser degree of concern when it is administered with sorafenib. The sole use indications of MTC as post-operative emesis may be the issue in HCC. Moreover, there are some other off-label clinical uses that MTC is prescribed without sorafenib; for example, use in nursing mothers. The side effect of MTC, galactorrhea, becomes a purposed effect on breastfeeding difficulties. The antidopaminergic drug is known to be used as a galactagogue, a lactation stimulator in nursing mothers. MTC concentration in breast milk is near to mother plasma concentrations and achieve detectable concentrations in newborns plasma (4). American pediatric committee does not recommend MTC use due to the central nervous system effect in the mother and mentions the absence of long term safety data in the newborn (18). An experimental study in rats shows that MTC treatment of the mother results in an excessive proliferation index in the newborn liver tissue (19). MTC administration and hepatic proliferation are in a parallel

direction according to our study. MTC is not a safe drug in the pediatric age population and exerts serious adverse effects including acute dystonic reactions (20). Pediatric age group safety concerns and lack of long term safety data should be kept in mind and nursing mothers should be aided with nonpharmacological lactation counseling when possible.

MTC, a dopamine antagonist, increased proliferation at certain doses. This result may prompt the researchers to investigate the dopamine receptors to be a pharmacological target for HCC treatment. Despite the advantages, studies with immortalized cell lines have some limitations. The researchers might choose primary cell cultures wherever possible (21). The factors such as antibiotic use and genetic instability might be among the limitations of the studies with cell lines. Additionally, in vitro studies might do not reflect the in vivo drug response adequately.

CONCLUSION

Nevertheless, this work might suggest a potential proliferative role for MTC. Future in vivo or clinical studies are certainly required to further evaluate the use of MTC in HCC.

*The results of this study were partly presented in Maltepe University International Student Congress (MUISC) 2019. The project was supported by the Scientific-Technological Research Council of Turkey (TUBITAK, Project Number: 1919B011800621)

Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: TUBITAK
Ethical approval: Cell culture.

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