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# Effects of Diclofenac on 5-Fluorouracil Cytotoxicity in Colorectal Cancer (Caco-2) Cells

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***Abstract*** **–** Alongside surgery and chemotherapy in the treatment of colorectal cancer, combination therapy strategies are cited as a potential way to improve treatment outcomes. It has been delineated that non-steroidal anti-inflammatory drugs (NSAİDs) act as tumor suppressors in various cancer cells, induce apoptosis and increase the cytotoxic activity of some antineoplastic drugs. In the study, we aimed to determine the effects of diclofenac on cell viability in human colorectal cancer (caco-2) cell line, alone and in combination with 5-fluorouracil (5-FU), the backbone of chemotherapy. For this purpose, the effects of separate and combined applications of two drugs on the cell viability were investigated for 24 hours by dose-dependent MTT assay. Then, the effects of combinations of 5-FU (at $IC_{50}$ and $IC_{50}/2$ concentration) and wide concentrations of diclofenac (15.6-1000 µM) on the cell viability were evaluated for 24 hours. The $IC_{50}$ values of diclofenac and 5-FU were found to be 324.7 µM and 18.56 mM, respectively. The viability of cells treated with 5-FU (20 and 10 mM) decreased significantly with diclofenac doses of 250 µM and above, dose-dependently ($p<0.05$). In conclusion, diclofenac alone was also cytotoxic in caco-2 cells, and in combination with 5-FU, it can cause a decrease in cell viability with a synergistic effect. Our findings may provide the data for combination or alternative approaches, especially against colorectal cancer. Efficacy in chemotherapy should be evaluated with in vivo and clinical studies.

*Keywords – Cytotoxicity, Caco-2, 5-Fluorouracil, Diclofenac, MTT*

## I. INTRODUCTION

Cancer is among the global problems affecting public health and the economy. According to the current data of the GLOBOCAN database, colorectal cancer is the third most common type of cancer and the second leading cause of cancer-related death [1]. Surgery and chemotherapy, which are the optimum treatments for colorectal cancer, provide limited benefits in the recovery and survival of patients [2].

5-fluorouracil (5-FU), which forms the backbone of colorectal cancer chemotherapy, exerts its effect by inhibiting thymidylate synthase and forming cytotoxicity by joining the DNA and RNA of cancer cells. Drug combinations such as FOLFOX (5-FU and oxaliplatin) and FOLFIRI (5-FU, folinic acid and irinotecan) containing 5-FU are widely used in chemotherapy. However, drug-induced toxicity and drug resistance limit the treatment [3]. To improve treatment outcomes, it seems necessary to innovative treatment strategies such as combination therapy.

Frequent use of non-steroidal anti-inflammatory drugs (NSAIDs), which are well known for their analgesic, antipyretic and anti-inflammatory effects, has been associated with a reduced colorectal, breast, prostate and lung cancer risk, and it is suggested that it may play an effective role in

combined chemotherapy [4]. The mechanism underlying its anticancer activity has not been fully elucidated; however, it is thought to act as a tumor suppressor by inhibition of cyclooxygenase or non-COX or activation of the apoptotic pathway [5-7].

Diclofenac, a highly active and easily tolerated NSAID, inhibits both COX-1 and COX-2 enzymes. It also ensures that different mechanisms with the effect of prostaglandin synthesis inhibition may exist. Considering the multiple mechanisms of action on angiogenesis and the immune system, it is proposed that it may be promising in cancer treatment [8]. Based on this information, diclofenac was chosen as a potential combination agent to find solutions to the problems in colorectal cancer treatment. In the study, we aimed to determine, for the first time to our knowledge, the effects of diclofenac on cell viability, alone and in combination with 5-FU, in the human colorectal cancer (caco-2) cell line.

## II. Materials and Method

### *A. Chemicals*

5-Flourouracil was from Koçak Farma (Turkey). Diclofenac was from Deva (Turkey). Heat-inactivated Fetal bovine serum (FBS), MTT, trypan blue, trypsin–EDTA, and Dulbecco's phosphate-buffered saline (PBS) were from Sigma (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM) and penicillin-streptomycin were from (Thermo Fisher Scientific, Waltham, MA, USA).

### *B. Cell culture*

Caco-2 cells were acquired from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were maintained in DMEM containing high glucose (4500 mg/L), L-glutamine and sodium piruvate. The medium was supplemented with FBS (10%), and antibiotics (1%, penicillin-streptomycin) at 37°C, 5% $CO_2$.

### *C. Cytotoxicity assay*

Cell viability was determined by MTT assay [9, 10]. The caco-2 cells ($1 \times 10^4$ cells/well) were plated in triplicate. After the cells adhere, incubated with concentration of 5-FU or/and diclofenac for 24 h. The cells were counted with trypan blue dye. Culture medium containing PBS (1%) was used for negative control experiments. After treatment, 10 µL MTT (5 mg/mL in PBS) was added to each well for 4 h. 100 µL of DMSO was added and the absorbance of each sample was measured at 570 nm using a microplate reader (Epoch, Biotek, USA). The $IC_{50}$ was calculated from the dose-response inhibition curves obtained for diclofenac sodium and 5-FU using the GraphPad Prism software (GraphPad Software, San Diego, CA, USA).

### *D. Statistical analysis*

The results were represented as the mean ± standard deviation. All statistical analysis were performed by one-way analysis of variance (ANOVA) followed by by Fisher's least significant difference (LSD) test with GraphPad Prism 9.5.0. Statistical difference was accepted as $p < 0.05$.

## III. Results

### A. *Cytotoxic effects of diclofenac and 5-FU in caco-2 cells*

Diclofenac did not cause a significant cytotoxic effect at the concentrations of 15.6-125 µM, relative to the negative control; on the other hand, the cell viabilities were substantially reduced above 250 µM concentrations of diclofenac ($p<0.05$) for 24 h, in a dose-dependent manner. The $IC_{50}$ value of diclofenac sodium was 324.7 µM.

5-FU did not cause a significant cytotoxic effect at the concentration of 5 mM relative to the negative control; however, the cell viabilities were declined sharply above 10 µM concentrations of 5-FU ($p<0.05$) in a dose-dependent manner for 24 h. The $IC_{50}$ value of 5-fluorouracil was 18.56 mM for 24 h.

### B. *Effects of diclofenac on 5-FU cytotoxicity in caco-2 cells*

As shown in Fig 1 and 2, diclofenac did not change the $IC_{50}$ and $IC_{50}/2$ value of 5-FU (20 mM and 10 mM, approximately, respectively) at low concentrations (15.6-125 µM); however, the $IC_{50}$ and $IC_{50}/2$ value of 5-FU was drastically reduced at the higher concentrations (250 µM and above) of diclofenac, when compared to the negative control ($p<0.05$).

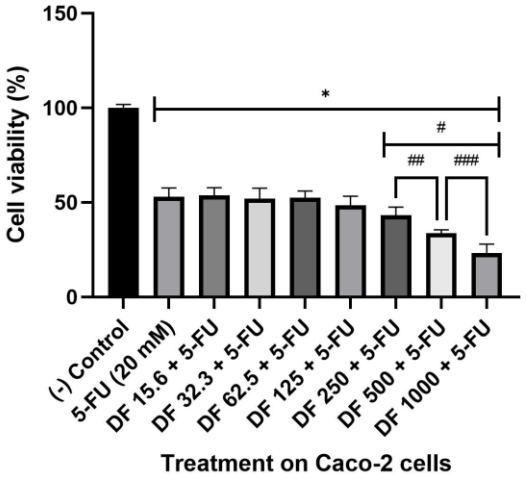


Fig 1. Effect of diclofenac on the cytotoxicity of 5-FU ($IC_{50}$, 20 mM, approx.) for 24 h on Caco-2 cells.

Results are represented as mean ± standard deviation. Cell viability was evaluated relative to the negative control. PBS was used as negative control. *p<0.05 compared with negative control. #p<0.05 compared with 5-FU (20 mM). ##p<0.05 compared with 250 and 500 µM concentrations of diclofenac in the combination groups. ###p<0.05 compared with 500 and 1000 µM concentrations of diclofenac in the combination groups. DF: diclofenac, 5-FU: 5-Fluorouracil.

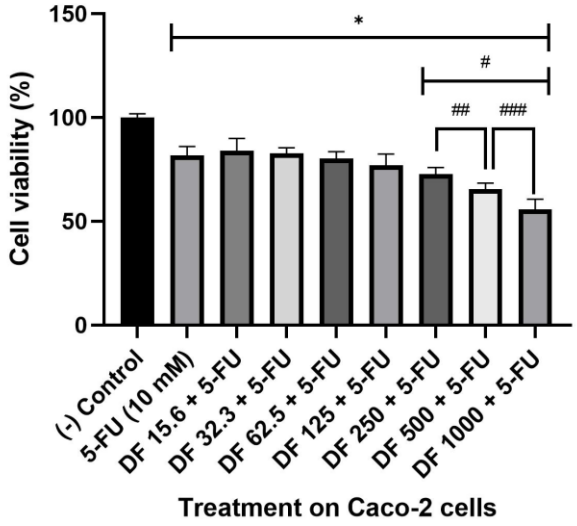


Fig 2. Effect of diclofenac on the cytotoxicity of 5-FU ($IC_{50}$/2, 10 mM, approx.) for 24 h on Caco-2 cells.

Results are represented as mean ± standard deviation. Cell viability was evaluated relative to the negative control. PBS was used as negative control. *p<0.05 compared with negative control. #p<0.05 compared with 5-FU (10 mM). ##p<0.05 compared with 250 and 500 µM concentrations of diclofenac in the combination groups. ###p<0.05 compared with 500 and 1000 µM concentrations of diclofenac in the combination groups. DF: diclofenac, 5-FU: 5-Fluorouracil.

## IV. DISCUSSION

Our results demonstrate that diclofenac has antiproliferative effect in caco-2 cell line for 24 hours of incubation. Moreover, diclofenac at relatively high doses (at concentrations of 250 µM and above) can potentiate the cytotoxic effect of 5-FU (at $IC_{50}$ and $IC_{50}$/2 concentrations). These findings indicate a synergistic anticancer effect of 5-FU and diclofenac on caco-2 cells.

Our findings are consistent with previous studies which reveal diclofenac-induced cytotoxicity in other colorectal cancer cells (HT29, HCT116 SW480) [11, 12]. It has been reported that different NSAIDs suppress tumor growth and metastasis of colon cancer in animal models and can be used as an adjuvant in colorectal cancer [13,14]. In consistent with our study, it was reported in a randomized clinical trial that the use of NSAIDs (celecoxib) increased the effectiveness of cytotoxic drug therapy [15]. In addition, it has been reported that the combined use of some NSAIDs (indomethacin, tolmethacin, acemethacin, mefenamic acid, etc.) in chemotherapy creates a synergistic effect in various human cancer cells [16, 17].

This study has some limitations. Considering the most commonly used chemotherapy drug combinations such as FOLFOX and FOLFIRI containing 5-FU, the effects of multiple drug combinations could have been evaluated by inclusion. In this study, not only examining the efficacy of diclofenac on 5-FU cytotoxicity in caco-2 cells, but also different possible cellular pathways such as apoptosis, cell cycle checkpoints and antioxidant defense system could be investigated. Our results, which reveal the supportive role of diclofenac in increasing the therapeutic efficacy of 5-FU in chemotherapy, are still preliminary, but it can be said that they bring new data to the relevant literature and offer promising findings. This study could be a pioneer for further research.

## V. CONCLUSION

As a result, it was revealed that diclofenac can increase the cytotoxicity of 5-FU at relatively high doses and show synergistic anticancer effects in caco-2 cells. It is predicted that diclofenac may play a crucial role against colorectal cancer cell development and progression. However, it needs to be supported by more extensive in vivo and clinical studies to confirm its therapeutic efficacy in combinational therapy.

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