## RESEARCH ARTICLE

# Assessment of Cytotoxic Activities of 5 Cyclooxygenase Inhibitors Against Liver and Breast Cancers Cell Lines

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## Abstract

Cancer is a leading cause of death worldwide. The most common cancer type in women is breast cancer, whereas the predominant type in men is liver cancer. The major problem of cancer treatment is resistance of cancer cells to the existing anticancer agents. Therefore, new treatment options are needed. Cyclooxygenase (COX) is the enzyme in prostaglandin synthetic pathway that is frequently overexpressed in carcinoma and is associated with cancer progression, suggesting the possible exploiting its as promising targets by COX inhibitors, the non-steroidal anti-inflammatory drugs (NSAIDs). The study was performed to evaluate the cytotoxic activities of 5-fluorouracil (5-FU) and five NSAIDs against HEPG2 and HCC1937 cell lines using MTT assay. NSAIDs were found to significantly inhibit the viability of HEPG2 and HCC1937 cells. In addition, the combination of 5-FU and aspirin showed synergistic and additive activities in HEPG2 and HCC1937 cancer cells, respectively. On the other hand, the combination of 5-FU and celecoxib showed synergistic and antagonistic activities in HEPG2 and HCC 1937 cancer cells, respectively. The use of 5-FU in combination with celecoxib in breast cancer should be avoided due to drug interaction. This suggests that the selectivity of 5-FU and NSAIDs combination varies depending on the cancer cell types.

**Keywords:** Cyclooxygenase, breast cancer, liver cancer, NSAIDs, cytotoxicity

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## Introduction

Cancer incidence and mortality are rapidly growing worldwide. The cancer burden is increasing in economically developing countries according to population aging and growth, and adoption of cancer-associated lifestyle choices including smoking, physical inactivity, and diets. Liver cancer is the fifth most common cancer in men and the seventh in women. It is the third leading cause for cancer-related death in Asians.[1] Breast cancer is the leading cause of cancer death in female, accounting for 23% of the total new cancer cases and 14% of the total cancer deaths.[2] Recently, several reports indicated that mediators of inflammation such as prostaglandins (PGs), synthesized by cyclooxygenase (COX) enzymes, may play an important role in cancer disease.[3-5] Among inflammatory mediators, prostaglandin $E_2$ ($PGE_2$) plays an important role in cancer progression by several mechanisms. $PGE_2$ significantly promoted proliferation and survival of liver cancer cell by upregulating the expression of FUSE-binding protein 1[6] and survivin[7], and YB-1.[8] It also influenced the development and progression of breast cancer by regulation of aromatase[9] and EP4 receptor.[10]

COXs are rate-limiting enzymes in the formation of PGs from arachidonic acids. They exist in two isoforms, COX-1 and COX-2. The COX-1 is considered to be constitutively expressed, while COX-2 is highly inducible by various factors and is associated with tumorigenesis by enhancing angiogenesis[11], inhibiting apoptosis[12], promoting metastasis[13] and suppressing immune system.[14] COX-2 over-expression has been associated with tumor behavior and with prognosis in several cancers.[15] The selective inhibition of COX-2 activity in animal models showed a decrease of new blood vessel production in tumors, a decrease in new vessel formation, and an increase in tumor cell apoptosis.[16] Thus, COX-2 is a promising target for cancer chemotherapeutics.

Non-steroidal anti-inflammatory drugs (NSAIDs) consist of drugs that are used for reducing fever, pain, and inflammation because these drugs possess antipyretic, analgesic, and anti-inflammatory properties.[17] NSAIDs produce the therapeutic effects by decreasing the production of PGs through the inhibition of COX. These anti-inflammatory drugs have been shown to exhibit potent anti-tumor activity in a wide variety of human tumor types, such as colorectal, breast, and liver cancers.[18-20] The over-expression of COX-2 is associated with carcinogenesis, invasiveness, and metastasis of malignant tumors.[21] The roles of NSAIDs in preventing and treating tumors have been attracting broad attention in recent years because of their selective and specific inhibition of COX-2 activity.[22,23] In this study, *in vitro* cytotoxic activities of COX inhibitors (aspirin, celecoxib, ibuprofen, indomethacin and naproxen) and a traditional anticancer drug (5-fluorouracil or 5-FU), were investigated against hepatocarcinoma HEPG2 and breast cancer HCC1937 cells. In addition, the cytotoxic interactions between 5-FU and aspirin or celecoxib, including the chemosensitizing effect of aspirin/celecoxib on the activity of 5-FU were explored.

## Materials and Methods

### *Cell culture*

Liver cancer cell (HEPG2; Cell Line Service, Eppelheim, Germany) and breast cancer cell (HCC1937; ATCC® CRL2336™) were cultured in RPMI 1640 medium (Gibco, New York, USA) supplemented with 10% fetal bovine serum (Gibco, New York, USA), 100 units/mL penicillin and 100 mg/mL streptomycin in a humidified atmosphere with 5% $CO_2$ at 37°C.

### *Drugs and reagents*

The experimental drugs, 5-FU, aspirin, naproxen, and ibuprofen were purchased from Sigma Chemical (St. Louis, MO, USA). Indomethacin was purchased from Fluka (Fluka, Buchs, Switzerland) and celecoxib was purchased from Biovision (BioVision, CA, USA). Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide (MTT) were purchased from Sigma Chemical (St. Louis, MO, USA).

### *In vitro cytotoxicity of 5-FU, aspirin, indomethacin, ibuprofen, naproxen and celecoxib*

MTT assay was used to determine the effect of NSAIDs and 5-FU on the viability of HEPG2 and HCC1937 cells. The HEPG2 cells were seeded at $1\times10^4$ cells/well and the HCC1937 cells were seeded at $2\times10^4$ cells/well in 96-well plates, and incubated overnight at 37°C with 5% $CO_2$. The cells were treated with aspirin, indomethacin, ibuprofen, naproxen (10000, 5000, 2500, 1250, 625, 312.5, 156.25 and 78.13 μM); celecoxib (500, 250, 125, 62.5, 31.25, 15.63, 7.81 and 3.91 μM) or 5-FU (20000, 10000, 5000, 2500, 1250, 625, 312.5 and 156.25 μM) for 48 h. Then 20 μL MTT (5 mg/mL) was added and the plates were further incubated for additional 4 h. After removing the supernatant, DMSO (200 μL) was added to each well. Cell viability was determined by measuring the absorbance at 570 nm wavelength using a microplate reader (Thermo Scientific, Finland). Each assay was repeated three times and in triplicates. The growth inhibitory activities of the test drugs on each cancer cell line were calculated using the following formula:

$$\text{Inhibition rate (\%)} = \frac{\text{Average absorbance of experimental group}}{\text{Average absorbance of untreated group}} \times 100\%$$

The concentration of each drug that inhibits cell growth by 50% ($IC_{50}$) was determined by CalcuSyn version 1.1 (Biosoft, Cambridge, UK).

### *In vitro cytotoxicity of 5-FU/aspirin and 5-FU/celecoxib combinations*

The cytotoxicity of 5-FU when combine with aspirin or celecoxib were investigated at fixed ratios of 10:0, 7:3, 5:5, 3:7 and 0:10. In HCC1937 cells, the concentrations of 5-FU:aspirin were 20000:0, 14000:3000, 10000:5000, 6000:7000 and 0:10000 μM, and those of 5-FU:celecoxib were 20000:0, 14000:150, 10000:250, 6000:350 and 0:500 μM. For HEPG2 cells, the concentrations of 5-FU:aspirin were 5000:0, 3500:1500, 2500:2500, 1500:3500 and 0:5000 μM, and those of 5-FU:

celecoxib were 5000:0, 3500:150, 2500:250, 1500:350 and 0:500 μM. The $IC_{50}$ of each drug was obtained from each combination. The fractional inhibitory concentration (FIC) of each combination was calculated as the ratio of $IC_{50}$ of the drug combination and single drug following formula:

$$FIC = \frac{IC_{50} \text{ of drug combination}}{IC_{50} \text{ of a single drug}}$$

To interpret the interaction, the sum of the FIC (sum FIC) was calculated as ($IC_{50}$ of drug A in mixture/$IC_{50}$ of drug A alone) + ($IC_{50}$ of drug B in mixture/$IC_{50}$ of drug B alone), where A and B is a drug in combination. Sum FIC values indicate three types of cytotoxic interactions: synergism (sum FIC <1), addition (sum FIC =1), and antagonism (sum FIC >1).

***In vitro chemosensitizing effects of aspirin and celecoxib on 5-FU***

To investigate the chemosensitizing effect of aspirin and celecoxib, the cytotoxicity of 5-FU was performed by adding fixed concentrations of aspirin (1000 μM) or celecoxib (100 μM) to varying 5-FU concentrations. The reversal value was calculated from the ratio of $IC_{50}$ value of 5-FU with aspirin or celecoxib to $IC_{50}$ value of 5-FU alone.

## Results

***Effect of NSAIDs and 5-FU on cell viability***

The cytotoxic activities of COX-2 inhibitors, aspirin, naproxen, ibuprofen, indomethacin, celecoxib, and 5-FU on HEPG2 and HCC1937 cells were examined using MTT assay. The $IC_{50}$s of each drug on both cell lines were summarized in Table 1. NSAIDs and 5-FU were shown to inhibit the growth of HEPG2 and HCC1937 cells in a dose-dependent manner (Figure 1).

**Table 1.** Cytotoxic activities of aspirin, naproxen, ibuprofen, indomethacin, celecoxib, and 5-FU.

| NSAIDs | $IC_{50}$ (μM) | |
|---|---|---|
| | HEPG2 cells | HCC1937 cells |
| Aspirin | 3,625 | 1,893 |
| Naproxen | 2,731 | 8,558 |
| Ibuprofen | 374 | 1,957 |
| Indomethacin | 312 | 1,062 |
| Celecoxib | 127 | 259 |
| 5-FU | 568 | 8,720 |

***Cytotoxic activities of the combination of 5-FU and NSAIDs***

The 5-FU/aspirin and 5-FU/celecoxib combinations showed different activity on inhibition of the growth of two cancer cells (Figure 2). The medians (ranges) of sum FIC of 5-FU/aspirin against HEPG2 cells and HCC1937 cells were 0.72 (0.71-

0.73) and 0.91 (0.79-1.03), whereas those of 5-FU/celecoxib were 0.73 (0.54-0.92) and 2.14 (1.52-2.75), respectively. The results suggested that the combination of 5-FU and aspirin was selective to HEPG2 cells (synergism) and HCC1937 cells (additive) while the combination of 5-FU and celecoxib was selective to HEPG2 cells (synergism) but not in HCC1937 cells (antagonism).

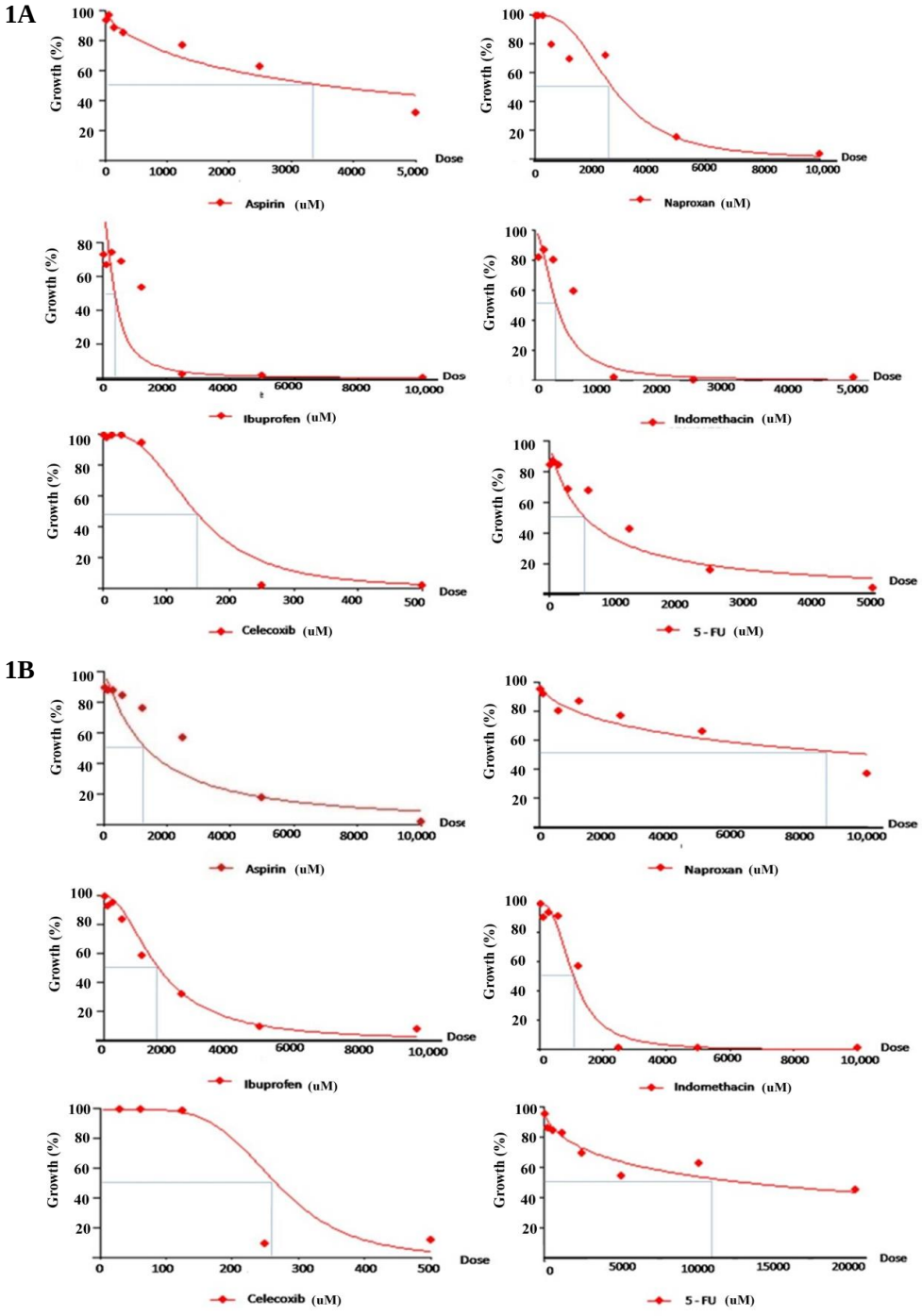


**Figure 1.** Concentration response curves of NSAIDs and 5-FU on HEPG2 (1A) and HCC1937 (1B) cell lines.

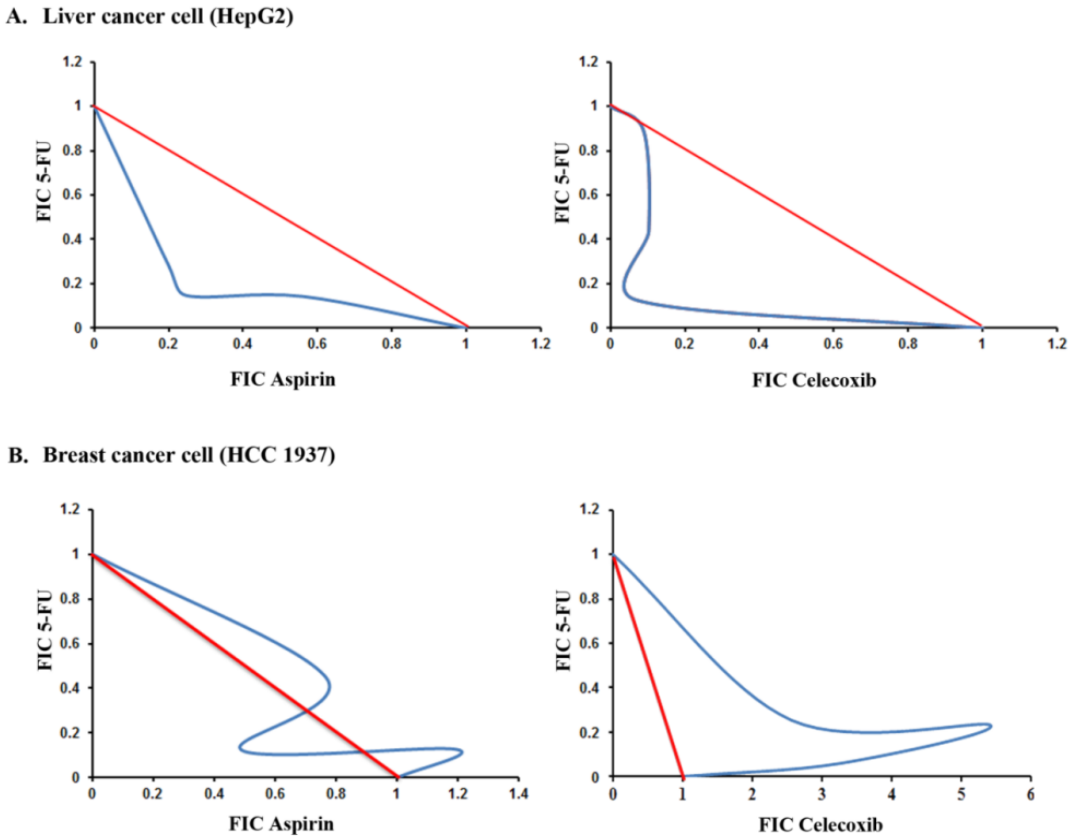


**Figure 2.** The isobologram plot of drug combinations, 5-FU/aspirin and 5-FU/celecoxib, on HEPG2 cells (A) and HCC1937 cells (B).

### *Effects of aspirin and celecoxib on chemosensitization of cancer cells treated with 5-FU*

The reversal or chemosensitizer ratios of celecoxib and 5-FU on HEPG2 and HCC1937 cells were 4.47 and 1.19, while those of aspirin and 5-FU on HEPG2 and HCC1937 cells were 1.94 and 2.75, respectively. These results indicated that celecoxib did not reverse 5-FU sensitivity on HCC1937 cells but had the chemosensitizing effect on HEPG2 cells whilst aspirin showed reversed 5-FU sensitivity on both HCC1937 and HEPG2 cells.

## Discussion and conclusion

Results obtained from the present study clearly indicated that all the five NSAIDs under investigation and 5-FU (standard drug) reduced the viability of HEPG2 and HCC1937 cells in a dose-dependent manner. The cytotoxicity of NSAIDs can be explained by inflammatory response in several cancers. Thus, drugs (such as NSAIDs) that inhibit inflammatory may be useful in cancer treatment. However, the $IC_{50}$ values of aspirin, naproxen, ibuprofen and indomethacin were high and varied among these two cell lines. It was noted that all NSAIDs was less

effective against HCC1937 cells compared to HEPG2 cells. This might be resulted from the differences in cancer cell characteristics.

Among five NSAIDS, celecoxib (a selective COX-2 inhibitor) had the most potent anticancer activity against both cells, with $IC_{50}$ values lower than 300 μM. This result was in accordance with the previous study showing that celecoxib inhibited proliferation of MCF-7 and MDA-MB-231 cells *in vitro* in a time- and dose-dependent manner.[24] However, in other studies, celecoxib did not affect tumor cell proliferation in primary adenocarcinomas and ductal carcinoma *in situ* of the breast *in vivo*, but prevented the development of breast cancer.[25-27] Numerous *in vivo* reports showed that treatment with celecoxib (500-1,500 mg/kg diet) in animal model with breast cancer markedly decreased the incidence, multiplicity, and tumor volume.[28,29] Apart from anti-inflammatory effect, celecoxib could possibly induce apoptosis, inhibit angiogenesis, and enhance cellular immune responses.[30]

The antitumor effect of 5-FU has been demonstrated to be due to the induction of apoptosis. Although 5-FU is widely used as anticancer drug, clinical applications have been limited due to drug resistance and low response rate when used as monotherapy. The combination therapies with other chemotherapeutic drugs or radiotherapy were used to enhance response rate and antitumor activity. In the present study, the combination of 5-FU with aspirin markedly inhibited HEPG2 cell proliferation compared to either drug alone, which is in consistent with the study in human colonic adenocarcinoma cells that demonstrated apoptotic activity.[31] The use of 5-FU in combination with celecoxib in HCC1937 cells should be avoided due to antagonistic interaction between both drugs. On the other hand, this drug combination had a synergic effect in HEPG2 cells, which is in accordant with the previous study in gastric cancer cells[32] and colorectal cancer cells.[33] This suggested that the selectivity of 5-FU and NSAIDs combination varies depending on the cancer cell types. In conclusion, the findings obtained from the current study suggested that NSAIDs could be developed as a potential chemotherapeutic candidate for treatment of liver and breast cancers.

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