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# Selective *In vitro* Cytotoxic Effects of Piroxicam and Mefenamic Acid on Several Cancer Cells Lines

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**Abstract:** Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) are heterogenous group of compounds used to cure and prevent inflammation. It was demonstrated that NSAIDs has the ability to inhibit the viability of colon cancer cells *in vitro*. We investigated the effects of Piroxicam and mefenamic acid on the viability of 4 cancer cell lines in which 2 of them are colon cancer cell lines (HCT 116 and CaCo-2). Cell viability was determined using MTT assay. Both NSAIDs was observed to markedly decrease the cell viability of both cell lines (HCT 116 and CaCo-2). Piroxicam was statistically more cytotoxic towards the cancer cell lines when compared to mefenamic acid. However, the cytotoxic effect of NSAIDs was less potent on breast cancer cells (MCF-7) and liver cancer cells (Hep G2). In conclusion, piroxicam and mefenamic acid showed selective cytotoxic effects against colon cancer cells but not against liver or breast cancer cells.

**Key words:** Piroxicam, mefenamic acid, cancer, NSAIDs, HCT 116, CaCo-2

## INTRODUCTION

The pathogenesis of colon cancer, one of the commonest fatal malignancies in the developed countries, represents an important challenge for medical sciences. As the second leading cause of cancer deaths, colorectal cancer is a significant source of morbidity and mortality in the United States and other Western developed countries (Barnes and Lee, 1998). The appreciation of the role of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) in human colon cancer represents an important recent development. Several NSAIDs were demonstrated to decrease the incidence of and mortality from colon cancer (Thun *et al*., 1991).

Epidemiological studies have shown the association between long-term consumption of aspirin and a reduced risk of colon cancer (Thun *et al*., 1991; Weiss and Forman, 1996). Animal studies showed that NSAIDs such as indomethacin (Pollard and Luckert, 1981), sulindac (Moorghen *et al*., 1988) and piroxicam (Pollard and Luckert, 1989) reduce the number and size of carcinogen-induced colon tumors. The NSAID sulindac is the first pharmacologic agent demonstrated to induce regression of colonic polyps in Familial Adenomatous Polyposis (FAP) (Giardiello *et al*., 1993). Sulindac sulfone, a metabolite of sulindac, significantly inhibits the growth of colonic tumor cells *in vitro* and chemically induced colon cancer in animals (Piazza *et al*., 1997). However, sulindac failed to cause a complete regression of the polyps, suggesting that some polyps might be resistant (Duflois and Smalley, 1996).

Currently, there is an intense debate about the importance of Cyclooxygenase (COX) inhibition for the anti-colorectal cancer activity of NSAIDs. COX-2, up regulated in some colon adenomas and most colon carcinoma, appears to play an important role in colon carcinogenesis (Kutchera *et al*., 1996). It is generally, assumed that most, if not all, of the pharmacological effects of NSAIDs can be explained through their inhibitory effect on the COX enzymes. There is, however, significant evidence that COX-independent effects may also be operative (Abramson and Weissmann, 1989). However, the precise mechanism (s) by which NSAIDs exert their anti-neoplastic effects remains unclear. Why NSAIDs are anti-neoplastic prominently in the colon also remains unclear. Therefore, the aim of this present study was to evaluate the effects of 2 NSAIDs, piroxicam and mefenamic acid (Fig. 1) on several cancer cell lines; colon cancer (CaCo-2 and HCT 116), liver cancer (Hep G2) and breast cancer (MCF-7).

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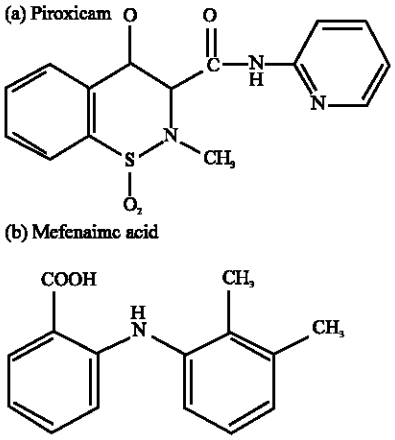


Fig. 1: Chemical structures of piroxicam and mefenamic acid

## MATERIALS AND METHODS

**Preparations, harvesting and plating of cells:** Piroxicam and Mefenamic Acid (Sigma Chemicals, UK) were dissolved in 0.1% Dimethyl Sulfoxide (DMSO) to give a concentration of 100 mM stock solution. A serial dilution of the stock solution with Dulbecco's modified Eagle's medium (DMEM; Life Technologies, USA) or RPMI 1640 culture medium was performed to give a final concentration of 200 μM. HCT 116, CaCo-2, MCF-7 and Hep G2 cell lines were obtained from the American Type Cultures Collection (ATCC, Rockville, MD) were cultured in the following growth medium as recommended by ATCC; RPMI 1640 (HCT 116 and CaCo-2) and DMEM (Hep G2 and MCF-7). All media were supplemented with 10% Fetal Calf Serum (FCS; Sigma Chemicals, UK). Culture flask with 90-100% confluency of cells was chosen to be harvested. Cells were detached from the culture flask by trypsinization. The concentration of cells was determined using Trypan Blue (Sigma Chemicals, UK) exclusion (Somchit *et al*., 2002). Total 100 μL of medium with $1\times10^5$ cells was pipetted into each well of the 96-well microtiter plate. The plate was incubated overnight at 37°C with 5% $CO_2$ in air prior to treatment.

**Dosing and cell viability testing:** Approximately 24 h of incubation, 200 μL drug solution of 200 μM concentration, was plated in each well in the first row of the plate. Total 100 μL of fresh medium was added to the rest of the wells. A 2-fold dilution was performed. Another 100 μL of medium was added giving the final concentration of 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 μM of drugs in wells of the respective rows. The plate was then incubated for 3 days under the same condition as mentioned above.

Cell viability was determined using 3- (4, 5-dimethylthiazol -2 -yl) -2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, after 3 days of incubation, 20 μL of MTT (5 mg $mL^{-1}$) was added to each well. The plate was then again incubated for further 4 h to allow reduction of MTT. Subsequently, the medium consisting MTT was aspirated from the wells carefully. About 100 μL of DMSO was added to the wells to dissolve the formazan crystals. The plate was then incubated for another 15 min. The cell viability was determined by the optical density reading of formazan solution using the ELX 800 ELISA machine.

**Statistical analysis:** Data are expressed as mean±SD of 4 separate experiments. Statistical significance was defined at $p<0.05$ using analysis of variance. Significant treatment means were subjected to Tukey post test.

## RESULTS AND DISCUSSION

Table 1 shows the effects of NSAID piroxicam on the viability of cancer cell lines at different drug concentrations. Piroxicam showed a markedly cytotoxic effect on HCT 116 and CaCo-2 cells. It was clearly observed that both affected the cell viability by reducing it up to almost 40%. Statistically, lower cell viability when compared to controls was observed at 12.5 mM for Caco2, 3.125 mM for HCT 116, 100 mM for Hep G2 and 50 mM for MCF7 cells. Piroxicam was not effective in killing liver cancer cells (Hep G2) and breast cancer cells (MCF-7).

Table 2 reveals mefenamic acid effects on the 4 cancer cell lines. Mefenamic acid caused mild cytotoxic effects on both colon cencer cells where the viability was approximately 75%. Hep G2 and MCF-7 cells were resistant to mefenamic acid where their viability at 100 mM drug concentration were 89.39±0.76 and 90.79±1.32, respectively.

It was evident that NSAIDs selectively kill human colorectal cancer cells *in vitro*. Both piroxicam and mefenamic acid exerted cytotoxic effects on HCT 116 and CaCo-2 cells *in vitro* in a dose-dependent manner. Piroxicam was more potent in killing these colon cancer cells. Smith *et al*. (2000) reported that NSAIDs inhibit proliferation and induce apoptosis in human colorectal cancer cells *in vitro*. Furthermore, NSAIDs which are structurally unrelated (aspirin, indomethacin, naproxen and piroxicam) have shown to exert anti-proliferative effect on HCT-29 colon adenocarcinoma cells *in vitro* in a time- and concentration-dependent manner.

As for the colon cancer cell lines, the NSAIDs effect on cell viability were pronouncedly shown. HCT 116 cells showed more cells regression compared to CaCo-2 cells.

Table 1: Viability of cancer cell lines treated with various concentrations of piroxicam

| | Percentage viability of cells | | | |
|---|---|---|---|---|
| Piroxicam (mM) | CaCo-2 | HCT 116 | Hep G2 | MCF 7 |
| 0 | 100.27±3.21$^{ax}$ | 104.32±4.17$^{ax}$ | 102.20±6.36$^{ax}$ | 104.27±3.72$^{ax}$ |
| 1.5625 | 99.64±5.42$^{ax}$ | 104.16±1.24$^{ax}$ | 104.19±8.24$^{ax}$ | 106.46±2.00$^{ax}$ |
| 3.125 | 100.62±8.37$^{ax}$ | 96.61±3.92$^{bx}$ | 103.34±0.39$^{ax}$ | 104.51±5.63$^{ax}$ |
| 6.25 | 92.82±9.04$^{ax}$ | 76.55±3.62$^{cy}$ | 105.36±9.51$^{ax}$ | 101.75±1.81$^{ax}$ |
| 12.5 | 94.94±1.33$^{bx}$ | 72.55±3.47$^{cy}$ | 104.61±5.61$^{ax}$ | 101.69±8.18$^{ax}$ |
| 25 | 89.41±6.83$^{cy}$ | 72.56±3.53$^{cz}$ | 103.32±0.46$^{ax}$ | 86.76±9.99$^{ay}$ |
| 50 | 71.10±3.16$^{dy}$ | 58.10±3.58$^{dz}$ | 102.55±2.53$^{ax}$ | 86.45±3.68$^{by}$ |
| 100 | 56.46±9.66$^{ey}$ | 42.76±1.99$^{ex}$ | 94.61±4.42$^{by}$ | 80.56±3.89$^{bx}$ |

Table 2: Viability of cancer cell lines treated with various concentrations of mefenamic acid

| | Percentage viability of cells | | | |
|---|---|---|---|---|
| Mefenamic acid (mM) | CaCo-2 | HCT 116 | Hep G2 | MCF 7 |
| 0 | 102.20±6.17$^{ax}$ | 101.46±4.71$^{ax}$ | 103.90±5.26$^{ax}$ | 100.21±4.16$^{ax}$ |
| 1.5625 | 103.26±5.76$^{ax}$ | 100.85±4.90$^{ax}$ | 107.87±7.22$^{ax}$ | 102.85±1.59$^{ax}$ |
| 3.125 | 103.91±3.92$^{ax}$ | 93.70±3.32$^{ay}$ | 106.56±4.02$^{ax}$ | 103.93±1.43$^{ax}$ |
| 6.25 | 102.89±9.35$^{ax}$ | 71.10±4.38$^{by}$ | 107.99±2.68$^{ax}$ | 101.00±5.79$^{ax}$ |
| 12.5 | 105.18±6.13$^{ax}$ | 76.39±3.01$^{by}$ | 108.46±2.98$^{ax}$ | 103.24±1.26$^{ax}$ |
| 25 | 100.12±6.84$^{ax}$ | 70.41±1.14$^{cy}$ | 106.42±0.62$^{ax}$ | 100.98±5.07$^{ax}$ |
| 50 | 86.79±1.61$^{bx}$ | 69.90±0.77$^{cy}$ | 91.72±7.32$^{ax}$ | 87.91±3.55$^{bx}$ |
| 100 | 76.72±1.22$^{cy}$ | 72.47±0.92$^{bcz}$ | 89.39±0.76$^{bx}$ | 90.79±1.32$^{bx}$ |

(n = 4/group from four separate experiments). $^{a-e}$Means with different superscript differ significantly ($p<0.05$) in the same column; $^{x-z}$Means with different superscript differ significantly ($p<0.05$) in the same row

Piroxicam exerts its cytotoxic effect higher than mefenamic acid even at low doses of 3.125 mM. Nevertheless, their ability in decreasing the cells' survival was irrefutable. It was previously demonstrated that piroxicam and mefenamic acid were able to inhibit cell proliferation (Quesada *et al*., 1998; Weiss *et al*., 2001). Moreover, piroxicam was shown not only to inhibit the number but also the size of gastrointestinal adenomas (Quesada *et al*., 1998).

NSAIDs have been suggested to have a chemopreventive effect against colon carcinoma. By using Azoxymethane (AOM)-induced aberrant crypt foci, Piroxicam was demonstrated not only to cause regression of aberrant crypt foci, but also to prevent, both of which were associated with the prevention of colon tumors (Pereira *et al*., 1996).

While, NSAIDs have been shown to exert preventive effects against the development of colonic tumors in human and in chemically-induced tumors in animal models, the mechanism (s) involved in this phenomenon is still unclear. One potential mechanism for this chemopreventive effect lays in the ability of aspirin and other NSAIDs to inhibit Prostaglandin (PG) biosynthesis (Meade *et al*., 1993). However, it remains unclear whether or not individual NSAIDs act by COX-2 inhibition. It was demonstrated that Indomethacin (a non-selective COX inhibitor) (Smith *et al*., 2000) and Sulindac (Waddell, 1998) still had the ability to affect the viability of colon cancer cell lines, regardless of COX-2 expression nor RNA for synthesis of such enzymes. Thereby, it is concluded that NSAIDs have cytotoxic effects on colon cancer cells and that they act on human colorectal cancer cells via unknown mechanism/s.

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