Original Article

Antiproliferative Effect of Aspirin on Colorectal Cancer Cell Line

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ABSTRACT

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) such as Aspirin may have anticancer properties, and can be effective as a novel strategy for the treatment of colorectal cancer (CRC). The aim of this study was to assess the cytotoxic effects of Aspirin drug in CRC cell lines compared with Oxaliplatin drug *in vitro*.

Methods: Cell viability was assessed after treatment of SW742 and SW480 cells with Aspirin and Oxaliplatin by MTT assay, and the amount of IC50 was determined. Statistical analysis was performed through one-way ANOVA and Tukey multiple range analysis (SPSS 19.0 software ($P < 0.05$).

Results: Aspirin and Oxaliplatin considerably inhibited the growth of SW742 and SW480 cell lines. SW742 cell line was more sensitive to Aspirin than SW480 cell line. The cytotoxic effect of Oxaliplatin was higher than Aspirin in both cell lines.

Conclusions: This study demonstrated that both Aspirin and Oxaliplatin have cytotoxic effects on SW742 and SW480 cell lines in vitro. Thus, Aspirin may be considered as a therapeutic agent in CRC, however, further *in vivo* investigations are required to fully establish this effect.

Keywords: Aspirin, Colorectal Cancer, MTT Assay, Oxaliplatin.

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INTRODUCTION

Colorectal cancer (CRC) is known as most common gastrointestinal malignancies worldwide, nd is the fourth cause of cancer death [1]. In 2017, Statistical studies in the United States estimated 135,430 cases with CRC and 50,260 deaths due to the disease [2].

According to World Health Organization (WHO) reports, the incidence of CRC is increasing considerably in several regions, including Asia and Eastern Europe [3]. The rapid and growing trend of CRC is due to life style changes, smoking habitat decrease in physical activity and poor diets [4]. The most effective treatments for CRC, include chemotherapy, radiotherapy, and surgery. Various currently drugs used for chemotherapy in CRC include 5-fluorouracil alone or with leucovorin, capecitabine, oxaliplatin, irinotecan and other targeted agents [5]. Each of these drugs has side effects, with neuropathy and neutropenia being the main ones [6].

Despite the development of new surgical strategies and adjuvant therapies (chemotherapy and radiotherapy), little progress has been made in the alternative treatment of CRC [7]. Recently, non-steroidal anti-inflammatory drugs (NSAIDs) such as Aspirin have been considered as a promising and effective strategy for the inhibition and prevention of colon cancer cases [8].

Aspirin has been widely used as an analgesic, anti-inflammatory, and anti-platelet aggregation agent for decades [9]. It also prevents several diseases, such as myocardial infarction, ischemic stroke and is used in the management of patients with acute coronary syndromes [10]. Aspirin is containing acetyl salicylic acid. The salicylate group inhibits cyclin A2/CDK2, HMGB1 and NF-κB pathway. The acetyl group, via acetylation of serine residues, causes the inhibition of two isoforms, i.e., cyclooxygenases (COXs) [11]. COXs are major enzymes in converting arachidonic acid to prostaglandin H2, which generates other biologically active prostaglandins. These derivatives have impressive effects in many pathophysiological processes, such as angiogenesis, cell proliferation, cell migration, inflammatory responses and thrombosis in various tissues [12].

Since COX-2 isoform overexpression is observed in most CRC tissue and is associated with processes such as angiogenesis, inhibition of apoptosis, and metastasis of colon cancer cells, researcher have sought to assessment the effects of NSAIDs and selective COX-2 inhibitors (COXIBs) on CRC prevention and treatment [13].

Several epidemiological studies have demonstrated that Aspirin can be effective as a chemopreventive agent in several cancer types, including colorectal, esophageal, gastric, lung, breast and prostate cancers [14]. Also, regular and prolonged use of Aspirin, after the diagnosis of CRC, is associated with a significant reduction in patients' mortality [15]. The aim of this study was to

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determine the cytotoxic effects of Aspirin drug in colorectal cancer cell lines compared with Oxaliplatin *in vitro*.

MATERIAL AND METHOD

Chemicals

Aspirin, Oxaliplatin, Trypsin, Dimethyl sulfoxide (DMSO), Penicillin, 3-(4,5-dimethyl- 2-thiazolyl)-2,5-diphenyl-2-tetrazolium bromide (MTT) and streptomycin were purchased from Sigma (St. Louis, MO, USA). Fetal bovine serum (FBS) and DMEM medium was obtained from GIBCO Company (Germany).

Cell Culture and Drug Treatment

SW742 and SW480 cell lines were obtained from the cell bank of Pasteur Institute, Tehran, Iran. These cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated FBS, penicillin and streptomycin in 5% CO2 at 37°C. After extension of the culture to approximately 75%-80% confluence, the cells were passaged.

Determination of Cell Viability

Determination of cytotoxicity was performed by MTT colorimetric assay, defined as forming blue formazan by mitochondrial dehydrogenase found in normal cells. The cell suspension containing 1×10^4 cells per 100 µl of DMEM complete culture medium was added to plate wells, and was incubated in 5% CO2 at 37°C for 24 hours. The culture medium was then discarded, and the cells were washed with PBS once. Various concentrations of Aspirin (200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56 mmol/L) and Oxaliplatin (25, 12.5, 6.25, 3.12, 1.56, 0/78, 0/39, 0/19 µg/ml) were prepared, with 100 µl of each concentration added to wells in triplicate and incubated in 5% CO2 at 37°C for 24 hours. The next day, 10 µl of MTT (0/5 mg/mL PBS) was added to wells and incubated again at 37°C for 3 to 4 hours. Then, whole well contents were discarded and 100 µl of DMSO was added to wells and mixed by the shaker for 15 minutes. At the completion of the assay, cell viability was determined by the absorbance reading of each well at a wavelength of 570 nm by ELISA reader (Anthos, Australia). Toxicity level was assessed through the following formula [16]:

$$\text{Cytotoxicity} = 1 - \frac{\text{mean absorbance of toxicant}}{\text{mean absorbance of negative control}} \times 100$$

$$\text{Viability \%} = 100 - \text{Cytotoxicity \%}$$

Statistical Analysis

Data was defined as the mean ± standard deviation (SD). One-way ANOVA and Tukey multiple range analysis, regarding normal distribution, were used to assess the statistical significances of difference (P-values < 0.05). (SPSS 19.0 software Package, IBM Inc., Chicago IL, USA)

RESULTS

In this study, cytotoxic effects of Aspirin and Oxaliplatin on two cell lines were determined by MTT assay. The results demonstrated that Aspirin and Oxaliplatin considerably inhibited cell viability in the SW742 and SW480 cell lines in a dose-dependent manner.

Varying concentrations of Aspirin after 48 hours of incubation had different cytotoxic effects on SW742 and SW480 cell lines but, no inhibition activity was observed in the control group (Fig 1 and 2). The 50% growth inhibition concentration (IC50) of Aspirin in SW742 and SW480 cell lines following 48h of incubation was 5.597, 11.55 mmol/L, respectively (Table 1). The results indicated that SW742 cell line was more sensitive to Aspirin than SW480 cell line (Fig 3).

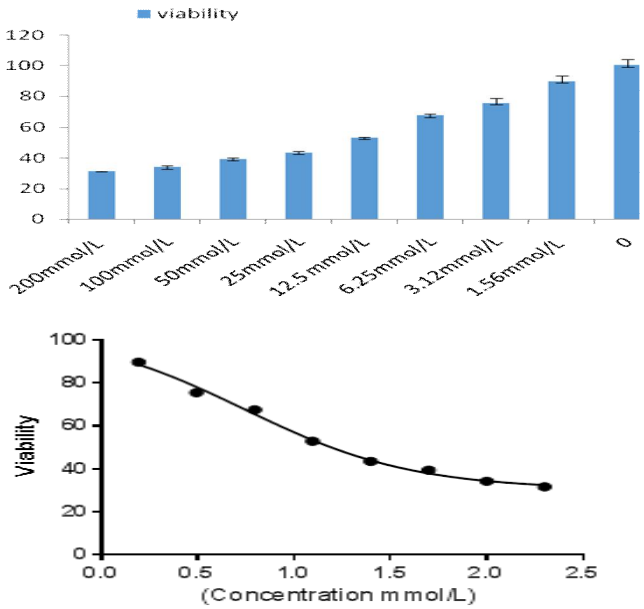

Figure 1. Effect of Aspirin (1.56- 200 mM) on cell viability of SW742 cell line.

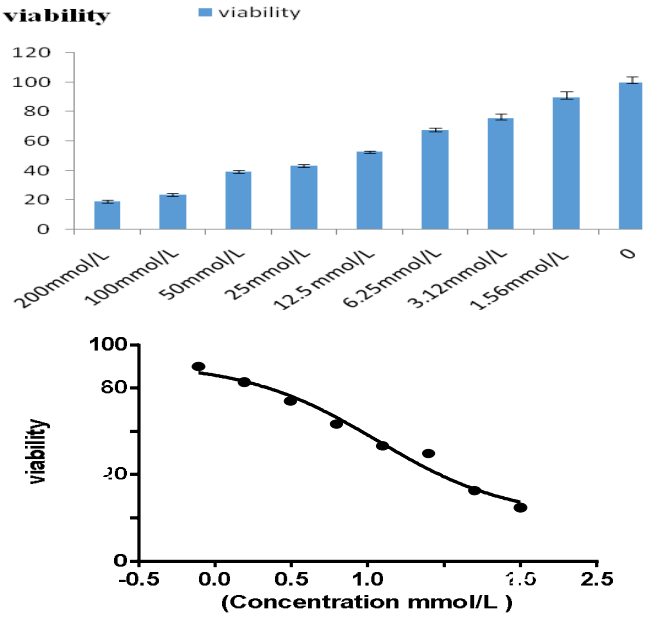

Figure 2. Effect of Aspirin (1.56- 200 mM) on cell viability of SW480 cell line.

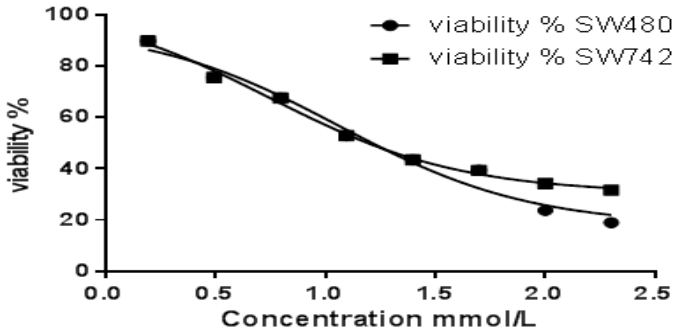

Figure 3. Viability of SW742 and SW480 cell lines to the treatment with Different concentrations of Aspirin.

Table 1. The IC50 values (mM) of Aspirin and Oxaliplatin in SW742 and SW480 cell lines after 48h.

		SW742	SW480
IC50	Aspirin	5.597	11.55
	Oxaliplatin	0/002	0/006

Further, the IC50 values (µg/ml) of Oxaliplatin drug in the SW742 and SW480 cell lines were determined (Fig 4 and 5) and it was evident that SW480 cell line was more sensitive to Oxaliplatin than SW742 (Fig 6). We found that Oxaliplatin cytotoxic effect was greater than Aspirin in both cell lines.

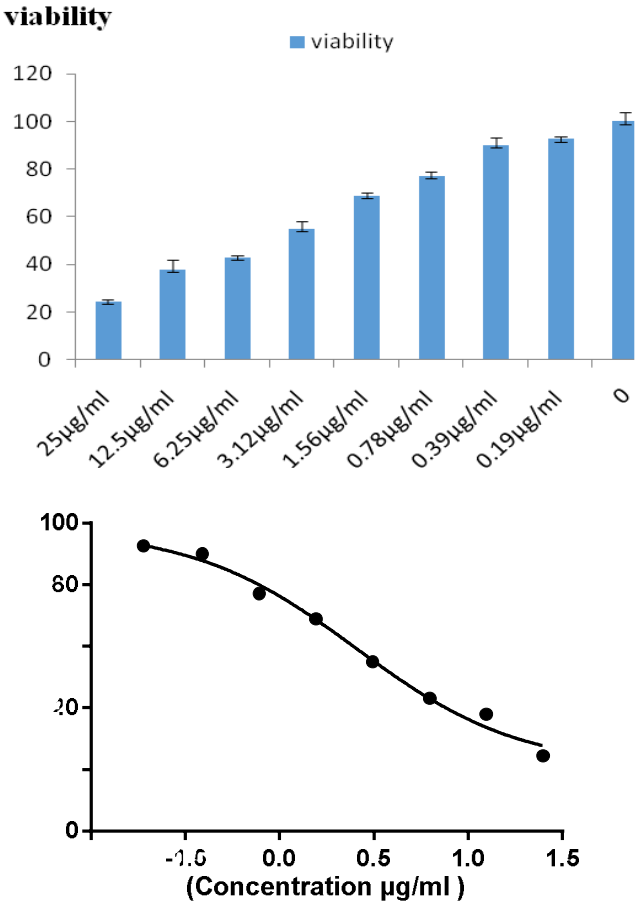

Figure 4. Effect of Oxaliplatin (0/19- 25 µg/ml) on cell viability of SW742 cell line.

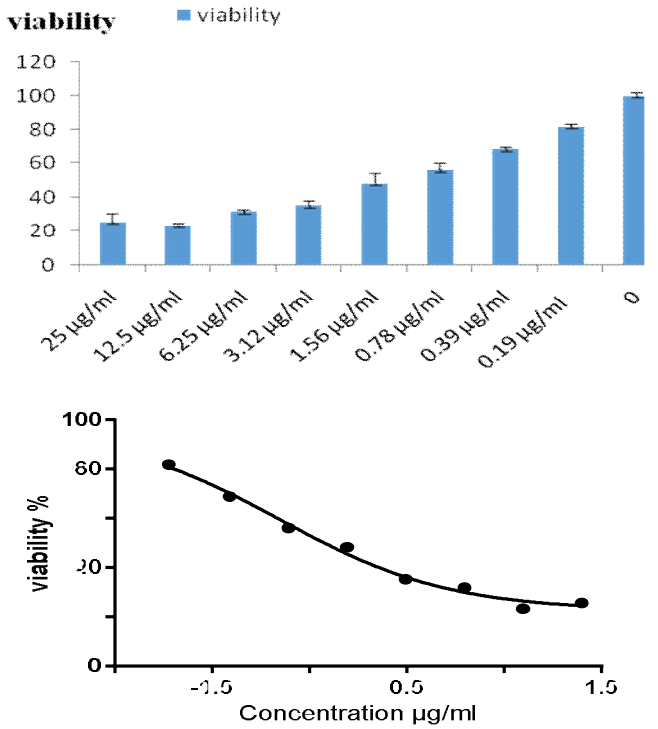

Figure 5. Effect of Oxaliplatin (0/19- 25 µg/ml) on cell viability of SW480 cell line.

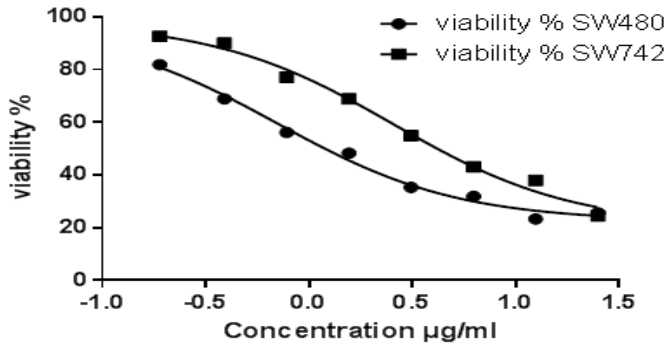

Figure 6. Viability of SW742 and SW480 cell lines after the treatment with varying concentrations of Oxaliplatin.

DISCUSSION

CRC remains a major public health problem worldwide. Regarding the complications of existing treatments, the need for the development of new methods is a fundamental necessity. Recently, Aspirin and other NSAIDs have attracted significant attention as compounds that might be of benefit in the chemoprevention of cancers.

Acetyl salicylic acid (known as Aspirin) has been used for relieving pain, inflammation, and fever for a long time [10]. Epidemiological studies in several trials have demonstrated that Aspirin has anti-cancer properties in addition to its anti-inflammatory properties. Besides inhibition of COX, Aspirin has additional properties, known as COX-independent mechanism [17]. Through this mechanism, Aspirin affects multiple intracellular pathway, angiogenesis, and apoptosis, which offers an important role in growth and progression of malignancies [12]. Epidemiological evidence has demonstrated that regular Aspirin intake

reduces the progression of various cancers, especially CRC [18].

This study reports that Aspirin and Oxaliplatin significantly reduce the viability of SW742 and SW480 cell lines. Based on the findings, Aspirin can inhibit proliferation in SW742 and SW480 cell lines, but SW742 cell line was more sensitive to this effect than the SW480 cell line. On the other hand, the sensitivity of the SW480 cell line is higher than that of Oxaliplatin. Furthermore, From the IC50 of Aspirin and Oxaliplatin, we found that Oxaliplatin cytotoxic effect was greater than Aspirin in both cell lines.

In a study by Ioannis et al [19], the effect of aspirin and bortezomib was investigated in such processes as proliferation and apoptosis on HCT116, HT-29, and CaCo2 cell lines in CRC. These authors reported that a combination of Aspirin and Bortezomib reduced cell proliferation and induced apoptosis in three different colorectal cell lines [19].

Another study by Ajay et al [17] determined the effects of Aspirin on the cellular growth, cell cycle, apoptosis and MMR protein levels incolon cancer cell lines and concluded that Aspirin through COX-independent mechanism caused an increase in MMR protein expression, induced apoptosis within 48 and 72h and arrested cell cycles in HCT116 cells.

Further, Yu et al [20] determined the effects of Aspirin on apoptosis, cell proliferation and invasion in a COX-2 negative CRC cell line. They demonstrated that Aspirin considerably inhibited the proliferation, increased apoptosis and decreased the invasive activity in SW480 cells.

The study by Subbegowda et al [21] Suggested that Aspirin caused cell cycle arrest and necrosis at high concentrations in vitro, but does not promote cell apoptosis [20].

CONCLUSION

The results of this and other studies strongly suggest that Aspirin demonstrates significant anti-proliferation effects in SW742 and SW480 cell lines in vitro, which is likely mediated via COX-independent mechanism. Therefore, Aspirin may be utilized as a novel strategy for the treatment of CRC. However, further investigations into the involved biological mechanisms are required to establish the precise mechanism of Aspirin effects on cancer cells.

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