The Effects of Ibuprofen Cytoxic Dose on caspase-3, -8 and -9 Activity level in cervical cancer (Hela) cells

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ABSTRACT

BACKGROUND AND OBJECTIVE: Recent studies have shown that ibuprofen has been implicated in the destruction and death of cells and tissues and can have an anti-cancer effect on cervical cells, although the mechanism of this effect is not well known in cellular and molecular terms. Accordingly, the aim of this study was to investigate the effect of cytotoxic concentration of ibuprofen on the activity of caspases -3−, 8 and -9 in cervical cancer (Hela) cells.

METHODS: In this experimental-laboratory study, Hela cells were prepared from Pasteur Institute Cell Bank and were divided into the control group and groups exposed to 0.01, 0.1, 1 and 10 mg/ml of Ibuprofen. Viability of cells was measured by MTT assay. The activity level of caspases-3, -8 and -9 was assessed by colorimetric method.

FINDINGS: The viability decreased significantly in cervical cancer cells exposed to 0.1(76%), 1(64%) and 10(15%) mg/ml of ibuprofen compared to control group (100% $p<0.05$, $p<0.001$ and $p<0.001$ respectively). The caspases-3, -8 and -9 activity level increased significantly in cervical cancer cells exposed to IC50 dose of ibuprofen compared with control group ($p<0.001$, $p<0.001$ and $p<0.01$ respectively).

CONCLUSION: The results of present study showed that ibuprofen is able to reduce the viability of cervical cancer cells in a dose-dependent pathway, and this pathway is induced by activating of caspases-3, -8 and -9.

KEY WORDS: *Ibuprofen, Caspase-3, Caspase-8, Caspase-9, Cervical Cancer.*

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Introduction

Cervical cancer is a cancer of the cervix. Early in the disease, usually no symptoms are seen. Subsequent symptoms may include abnormal vaginal bleeding, pelvic pain, or pain during intercourse (1). Cervical cancer is one of the most common malignancies in women. It has the second highest incidence after breast cancer. It is estimated that around 1.4 million women worldwide have cervical cancer (2).

Reports have suggested that ibuprofen can affect cancer. Ibuprofen can act as a preventive agent in pancreatic cancer or as a reducing risk factor for colorectal cancer (3). Ibuprofen improves reproductive system cancer and can inhibit ovarian adenocarcinoma growth in 33% (4) and also affects cervical cancer cells (5). Scientific reports have shown that ibuprofen is involved in the destruction and death of cells and tissues. In a study performed on mice with streptococcal infection, it was shown that ibuprofen can cause tissue damage (6). According to studies done by ibuprofen, it is effective in apoptosis of some cells (7). In fact, ibuprofen can induce apoptosis via caspase-dependent pathway (8).

In contrast, some studies have shown that ibuprofen has no significant association with cancer (9) or may in some cases cause or exacerbate cancer. In this regard, research results indicate that ibuprofen can increase the risk of death in patients with cervical cancer (10). Inhibition of caspases by NSAIDs also prevents cell death (11), which can exacerbate cancer. In one study that examined the effect of regular aspirin use on reducing the risk of cancer caused by chronic inflammation, no significant association was found between aspirin use and the level of inflammatory markers (12). Considering the significant incidence of cervical cancer in the world (13) and Iran (14), as well as the physical, psychological, social and economic consequences of cervical cancer (15), as well as the contradictory results in the field of research(4,5,9,10,16) and the limitations of previous studies regarding the effect of ibuprofen on the function of caspases in cervical cancer cells, this study aimed to investigate the effects of cytotoxic concentration of ibuprofen on caspase 3, 8 and 9 activity in cervical cancer cells (Hela).

Methods

In this experimental-laboratory study, ibuprofen from Aboureihan Pharmaceutical Company and cervical cancer cells from the Pasteur Institute cell bank were prepared and kept in standard conditions. Ibuprofen was completely dissolved in 100% dimethyl sulfoxide (DMSO) at various concentrations and stored in a dark vial at -20 ° C after passing through a syringe filter. Cells were maintained in serum-free keratinocyte medium containing penicillin (50 units/ml) and streptomycin (50 g/ml). Cells were grown in complete culture medium under 95% air and 5% carbon dioxide at 37 °C.

HeLa cells were divided into control and ibuprofen recipients but the control group did not receive any treatment, and MTT staining was used to evaluate the cytotoxic effect of ibuprofen on HeLa cells. For this purpose, cells were implanted in 96 well plates containing 3.3 * 104 cells / cm3 and incubated for 24 h. After 24 h, fresh medium containing ibuprofen was added at different concentrations and cells were incubated for 24 h.

In each well, different concentrations of the drug were applied three times. At the end of the exposure time, the culture medium was removed from the wells containing the cells and 200 μl of fresh medium was added to each well and then allowed to grow for another 24 h. At the end of growth time per well, 200 μl of fresh medium and 50 μl of MTT was added. Plates were then wrapped in aluminum foil and incubated in humid environment at 37 °C for 4 to 8 hours. The medium containing MTT was removed from the wells and the residual formazan crystalline from MTT was dissolved by adding 200 μL DMSO and 25 μL glycine buffer to each well. The absorbance was read immediately at 570 wavelengths. Wells containing medium and MTT and cell-free were considered as Blank. All experiments were repeated three times.

The activity of caspases 3, 8 and 9 was determined using the ApoTarget colorimetric laboratory kit (Abnova, Taiwan) according to the manufacturer's instructions. In summary, apoptosis was induced in HeLa cancer cells using ibuprofen and cultured in the control group at the same time. Cells were counted and plated at a density of 3-5 × 106 cells in each sample and subsequently "reconstituted in 50 μL of cold cell lysis buffer" and incubated on ice for 10 minutes and incubated for one minute and were centrifuged using microcentrifuges. The supernatant (cytosol extract) was added to a fresh tube and placed on ice. Each cytosolic extract was diluted to 50-200 μg of protein in 50 μl of cell lysis buffer. The number of samples was determined for measurement and sufficient reaction

buffer was added. 50 μL of the reaction buffer was added to each sample. Then 5 μL of 4 mM substrate was added and incubated for 1-2 h at 37 °C. Samples were kept in the dark during incubation. Finally, the samples were read in a microplate reader at 400 nm or 405 nm and the activity of caspases 3, 8 and 9 was determined by direct comparison with the control. For statistical analysis, using the Kolmogorov-Smirnov normal distribution of data was assessed and after the attainment of normal distribution of data, information related to the cytotoxic effect of testosterone were analyzed using analysis of variance (ANOVA) and Tukey post hoc test. In addition, caspase activity data were analyzed using Student's t-test in SPSS 20 software and $p < 0.05$ was considered significant.

Results

Cervical cell viability was not significantly decreased in the ibuprofen group at the concentration of 0.01 mg/ml compared to the control group. Cervical cell viability significantly decreased in the ibuprofen group at concentrations of 0.1, 1 and 10 mg/ml compared to the control group ($p < 0.05$, $p < 0.001$ and $p < 0.001$, respectively). Increasing the concentration of ibuprofen also decreased cell viability and appears to be dose dependent. The highest decrease in viability was observed in cervical cancer cells exposed to 10 mg / 1 (Fig 1). Caspase 3, 8 and 9 activities in cervical cancer cells exposed to IC50 dose of ibuprofen significantly increased compared to the control group ($p < 0.001$, $p < 0.001$ and $p < 0.01$, respectively) (Fig 2).
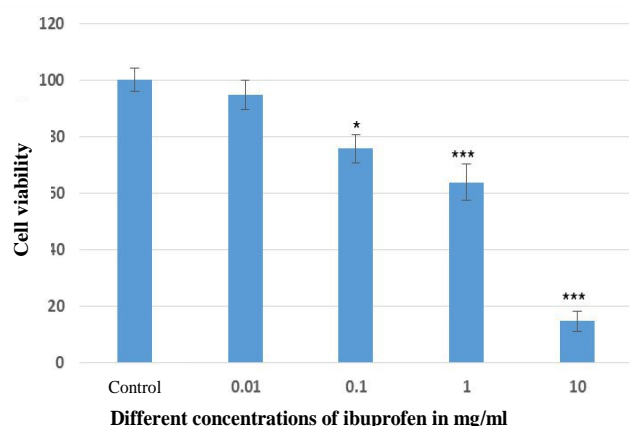

Figure 1. Comparison of the toxicity effect of different concentrations of ibuprofen in mg/ml on HeLa cell line compared to the control group. * And * indicate significant difference with control group with $p < 0.05$ and $p < 0.001$, respectively**
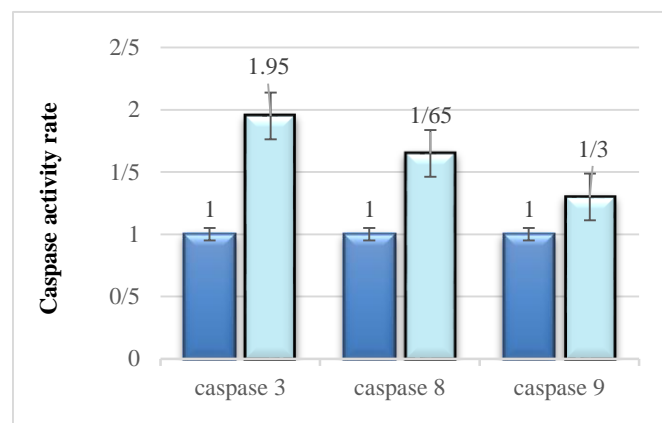

Figure 2 shows the percentage of caspase 3, 8 and 9 activities in cervical cells Indicates the effect of IC50 dose of ibuprofen on the percentage of caspase 9,8,3 activity compared with the control group (no drug exposure). According to statistical analysis, the IC50 dose of ibuprofen resulted in significant increase in the group receiving the drug compared to the control group ($p < 0.001$, $p < 0.001$ and $p < 0.05$, respectively).

Discussion

The results of this study indicate that ibuprofen can have cytotoxic effects on cervical cancer cells and this effect is mediated by increased caspase 3, 8 and 9 activity and induction of apoptosis. In line with this finding, other studies have shown that ibuprofen can induce apoptosis through the caspase pathway in cancer cells. Preventive role of NSAIDs has been shown in various types of malignant tumors. As an example, it has been shown that 2 mM ibuprofen can induce apoptosis in colon cancer by inducing apoptosis.

In fact, ibuprofen induces apoptosis by increasing expression of (DR5) death receptor 5 via the caspase-dependent pathway. (17). Research results indicate that the use of NSAIDs may reduce the risk of reproductive tract cancer (18).

According to research, NSAIDs induce apoptosis; in fact, several different types of NSAIDs have the ability to induce apoptosis, but ibuprofen is one of the NSAIDs that has high potency to induce apoptosis during different stages of the cell cycle (19). In a study on gastric cancer cells, it was shown that ibuprofen dose-dependently (at doses of 100,200,300,400,500 uM) induce cell death, which is consistent with previous findings investigated the effect of ibuprofen on gall bladder, prostate, breast, kidney and ovarian cancer cells. This study showed that ibuprofen induces apoptosis in cancer cells by activating caspase (7). In contrast, some research findings suggest that ibuprofen can induce cancer or proliferate cancer cells. Ibuprofen

inhibits COX binding to the enzyme and results in reduced prostaglandin production, resulting in angiogenesis and tumor growth in endometrial tumors. Studies have shown that ibuprofen use may increase the risk of death in patients with cervical cancer (10). In addition, it has been shown that catalyze inhibition of caspases via NSAIDs results in preventing cell death. It was also shown that when the cell was exposed to 100 μM concentration of NSAIDs, caspase activity decreases (11), which can exacerbate cancer. The possible mechanism of the effect of ibuprofen on cervical cancer cells may be due to the presence of ibuprofen receptors in cancer cell membrane (17). In this experiment, ibuprofen seems to increase caspase activity by binding to the membrane of cervical cancer cells and this leads to apoptosis in cervical cancer cells (20). Present study was conducted to investigate the effects of ibuprofen on activity of caspases 3, 8 and 9

and it is highly recommended further cellular and molecular studies regarding the Ibuprofen effects on apoptotic and anti-apoptotic genes, and also use of fluorescent staining techniques and electron microscopy to study the precise effects of ibuprofen on cervical cancer cells.

The results of the study showed that ibuprofen is capable of decreasing the viability of cervical cancer cells in a dose-dependent pathway by activation of caspases 3, 8 and 9. The findings of this study can be used as a basis in applied pharmaceutical research.

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