

The Effects of Diclofenac on Proliferation of L929 Tumor Cell Line in Cell culture

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Abstract: Diclofenac is a nonsteroidal anti-inflammatory drug used to treat pain and inflammation. This study was exerted to determine the effects of diclofenac on viability of L929 cell lines in cell culture. In this laboratory experimental study, L929 cell lines were exposed to 10mg/ml, 1mg/ml, 0.1mg/ml, 0.01mg/ml and 0.001mg/ml of diclofenac in cell culture. After 48 hours, the viability of cell lines was examined with MTT assay. The data was analyzed using ANOVA. Our findings show that viability of L929 cell lines decreased based on dose dependent pattern in response to exposure to different doses of diclofenac. There was lowest viability of L929 cells in cells exposed to 10mg/ml of diclofenac ($P < 0.001$). Our findings show that diclofenac has anti-proliferative effects against L929 cells.

Keywords: Diclofenac, Viability, L929.

1. Introduction

Cancer is the anomalous growth of cells in our bodies that can lead to death. Cancer cells usually attack and destroy normal cells. These cells are born due to imbalance in the body and by correcting this unevenness; the cancer may be treated [1]. Every year, millions of people are diagnosed with cancer, leading to death. Consistent with the American Cancer Society, deaths arising from cancer constitute 2-3% of the annual deaths recorded worldwide [2]. To form a primary tumor, the cancer cells should master the remodeling of the cancerous and adjacent tissue attracting blood vessels and transforming the surrounding tissue, to promote tumor growth and local invasion. Finally at organism level, cancer affects global functions of organism, evading its immune defense, utilizing the organism's blood circuitry to spread and metastasize, and inducing systemic metabolic alteration such as cachexia [3]. It is this final systemic state of the diseases that ultimately results in patient death [4]. Inflammation is highly related to both carcinogenesis and to progression of tumor growth [5]. Diclofenac, one of the oldest NSALDs has been in use since 1976 [6]. The studies show that there is relationship between diclofenac treatment and tumor size [7], [8]. The aim of this study was to investigate the effects of diclofenac on proliferation of L9292 cell line in cell culture.

2. Material And Methods

2.1 Diclofenac Preparation

Diclofenac was prepared as powder and different concentrations of diclofenac (10mg/ml, 1mg/ml, 0.1mg/ml, 0.01mg/ml and 0.001mg/ml of diclofenac) were used in our study.

2.2 Protocol of Study

We used MTT assay in this work to determine the effects of diclofenac on L929 cells viability in cell culture. The mouse fibroblasts which were used in the experiment are manufactured frozen mouse fibroblasts L929. The cells were dissolved in the water bath on the temperature of 37⁰ C and then washed up by a heated minimal essential medium, supplemented with 10% fetal calf serum and 1% penicillin, streptomycin and neomycin in order to completely remove the cryoprotective DMSO-dimethyl sulfoxide. Cells were placed in to flasks with the cell medium (MEM+ 10% foetal calf serum + 1% penicillin, streptomycin, and neomycin) and left in the incubator on 37⁰ C and 5% CO₂. The cells were microscopically monitored every 24 hours changing the medium. When the cells in the flasks multiplied and conflated and when the absence of any bacteria or fungus was determined the splitting was initiated. The medium was taken out and the cells were washed with PBS which does not contain Ca²⁺/Mg²⁺. Then trypsin EDTA was added 1 ml per 25 cm². The flasks were slightly shaken and put into the incubator for 10 minutes. After that the cells were microscopically watched, in order to make sure that they had split from the base and that they were floating. Then the cells were suspended with a small quantity of cell medium in order to activate trypsin, it was taken 100-200 µl and then the counting of the cells started [9]. Briefly, the procedure was continued and carried out in the following steps:

- Day One: 100 µl of cells was added into each well (96 well plate) and incubate at 37 with 5% co₂ overnight.
- Day Two: The media was removed and diclofenac was added and incubated at 37 with 5%co₂ overnight. For control 10%FBS was added to media.
- Day Three: Diclofenac was removed from media. 20 µl of 5 mg/ml MTT was added to each well and incubated for 4 hours at 37oC. 150 µ isopropanol was added and covered with tinfoil and agitate cells on orbital shaker for 15 min. Absorbance was read at 570 nm with a reference filter of 630 nm and recorded.

2.3 Statistical Analysis

Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS 19. Differences with P<0.05 were considered significant.

3. Results

Table I shows the viability of L929 cells in response to different doses of diclofenac.

TABLE I. Viability of L929 cells in response to different doses of diclofenac

Concentration	Viability (%)
Control	100
0.001 mg/ml	91.43
0.01 mg/ml	79.41
0.1 mg/ml	70.22
1 mg/ml	51.56
10 mg/ml	28.94

Figure I shows toxicity of diclofenac in cells exposed to different doses of drug.

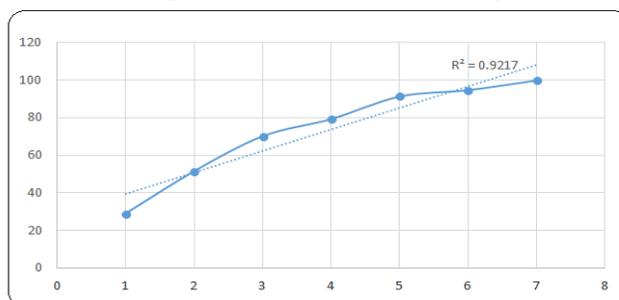


Fig.1: Toxicity in L929 cells in response to different doses of diclofenac

Our findings show that viability of L929 cell lines decreased based on dose dependent pattern; i.e., the toxicity in L929 cells increases by increasing of diclofenac concentration. There was lowest viability of L929 cells in cells exposed to 10mg/ml of diclofenac ($P < 0.001$).

4. Discussion

Our study indicated that diclofenac has anti-proliferative effects on L929 cell lines. In line with our finding there are other reports indicating that diclofenac treatment can cause a 60% decrease in tumor size. This is due to increased apoptosis of tumor cells. It has been shown that diclofenac yields a significant antiangiogenic effect, as demonstrated by a strong reduction in vascular endothelial growth factor (VEGF) tumor content, and decreased microvascular density and morphological changes in tumor blood vessels. Diclofenac treatment also remarkably increases arginase activity in tumor stroma cells, peritoneal macrophages and white blood cells [7], [8]. The anti-tumoral capacity of diclofenac is also generally attributed to its direct effect on tumor cell viability and cell cycle [10], [11].

5. Conclusion

Our findings show that diclofenac which commonly is prescribed to relieve the pain has anti-proliferative potential against L929 cells in cell culture.

6. Acknowledgment

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7. References

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