Saudi Pharmaceutical Journal (2015) 23, 483–486

King Saud University

Saudi Pharmaceutical Journal

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ORIGINAL ARTICLE

Antiproliferative effects of aspirin and diclofenac against the growth of cancer and fibroblast cells: In vitro comparative study

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Received 24 October 2014; accepted 1 January 2015
Available online 10 January 2015

KEYWORDS

Cancer cells;
In vitro;
Anticancer;
Diclofenac;
Aspirin

Abstract Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the growth of several cancer cell lines. The aim of this study is to compare the cytotoxic effect of aspirin with diclofenac on the growth of HeLa cell, mammary cell carcinoma, rhabdomyosarcoma and fibroblast cell lines in the culture media. The cells are cultured in RPMI-1640 culture media supplemented with 5% fetal calf serum and antibiotics. Aspirin (5 mg/well) and diclofenac (0.625 mg/well) significantly inhibit the growth of HeLa, rhabdomyosarcoma and fibroblast cells. The cytotoxic effect of aspirin against rhabdomyosarcoma is significantly ($p < 0.001$) higher than that of diclofenac with a potency approximated 2.6. It concludes that aspirin and diclofenac inhibit the growth of fibroblast and cancer cell by inhibiting the up-regulation of cyclooxygenases enzymes in cancer cells. Aspirin is more effective than diclofenac against the growth of rhabdomyosarcoma cell line.

1. Introduction

Regular uses of non-steroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of cancers of colon, breast, lung and ovary (Cuzick et al., 2009; Rothwell et al., 2012; Wernli et al., 2008).

Experimental studies demonstrated that diclofenac inhibits the growth of tumor xenografts (Mayorek et al., 2010; Moody et al., 2010) as well as the proliferation of cancer cell in a concentration dependent manner (Smirnova et al., 2012). Diclofenac inhibits the growth of melanoma cell by increasing the cellular level of reactive oxygen species due to inhibition

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jsps.2015.01.002

the activity of superoxide dismutase enzyme in mitochondria (Albano et al., 2013).

Aspirin induced apoptosis and inhibits the proliferation of HeLa cells in a concentration dependent manner by a mechanism related to the inhibition of the proto-oncogene (ErbB2) downstream cell survival signaling pathways (Xiang et al., 2010).

Aspirin interferes with the pathways of the proliferation rhabdomyosarcoma cells by inhibiting the induction of the matrix metalloproteinase enzyme activity (Ito et al., 2004). Recently, Siddesha et al. (2014) demonstrated that aspirin inhibits the migration of mouse cardiac fibroblast by attenuating the level of interleukin-18, an interleukin involved in different pathways that ultimately lead to reduce the migration of fibroblast and reducing fibrosis. In one study, aspirin reduced the breast cell line KM22 adhesion to human umbilical vein endothelial cell while diclofenac failed to demonstrate that effect (Bischofs et al., 2012).

The rational for doing this study is that aspirin or diclofenac exerts a specific inhibitory action against the proliferation of cells that links to its selectivity against cyclooxygenases enzymes. This study aimed to compare the inhibitory effect aspirin and diclofenac (non-selective cyclooxygenases enzyme inhibitors) on the growth of three different cancer cell line and fibroblast.

2. Materials and methods

This study was conducted in the Department of Pharmacology in the College of Medicine incorporation with National Center of Cancer Research at Al-Mustansiriya University, Baghdad, Iraq during 2014. The study was approved by the Institutional Scientific Committee at the College of Medicine.

2.1. Cell Culture treatment and viability

HeLa, human rhabdomyosarcoma, mammary (AMN3) adenocarcinoma and primary rat embryo fibroblast cell lines (provided from The National Center of Cancer Research in Baghdad, Iraq) were maintained in RPMI-1640 media with 5% fetal calf serum. All cultures were maintained in medium supplemented with 0.5% ampicillin and 0.5% streptomycin in 5% CO_2 at 37 °C. Several trials of cell reactivation are done in order to obtain a monolayer cells in a specific falcon (volume 25 ml) which can be adjusted under the microscope to look for the presence of monolayer cells. Once the growth of monolayer cells has been formed, the old growth media discarded, washed with phosphate buffer saline (PBS) buffer once, add 0.5–1 ml Trypsin-versin solution, then add 10 ml sterile growth media (supplemented with 5% fetal calf serum. Then the dispersed cells transferred into a sterile microplate. A total volume of 200 µl cells suspension is transferred into the wells of the microplate, incubated at 37 °C for 24 h to achieve a monolayer cells growth. The cells were plated at 1×10^5 cells per well in 200 µl of complete culture medium containing aspirin (5 mg) or diclofenac (0.625 mg) and incubated at 37 °C for 24 h. The concentration of aspirin that used in this study amounted 8 times of that of diclofenac in attempt to mimic the maximum tolerated therapeutic doses of these drugs.

The sterilization of the herbal tea achieved by mechanical filtration (using 0.2 µm millipore filter device) prior to the addition. On the next day (i.e. after 24 h), the viability of cells was determined by the MTT assay. A 30 µl of prepared tetrazolium dye (3-[4,5-dimethylth-iazol-2-yl]-2,5-diphenyl tetrazoliumbromide (5 mg/ml in phosphate buffer solution) was added to each well in a dark room (to avoid oxidation of the dye), and incubated for 2 h at 37.5 °C in the incubator. Then after, whole wells contents are discarded, 100 µl dimethyl sulfoxide added, shake the microtitre plate for 15 min by using horizontal shaker before recording the absorbance of each well at 540 nm by ELISA reader.

2.2. Statistical analysis

The results are expressed as number, percent and whenever possible as mean ± SD. The data are analyzed using Student's *t* test (unpaired, two tailed) and the difference between percentages test taking the probability of $\leqslant 0.05$ is the lowest limit of significance.

3. Results

Aspirin significantly ($p < 0.001$) inhibits the growth of fibroblast cell (53.7% ± 6.3%) compared with un-treated cells (100 ± 23%) (Fig. 1). A significant ($p < 0.001$) inhibitory effect of aspirin against the growth of HeLa cell line is observed (17.0 ± 3.3%) compared with untreated cells (100 ± 18.5%) (Fig. 1). A non- significant ($p > 0.05$) cytotoxic effects of aspirin is observed (103.1 ± 28.7%) against rhabdomyosarcoma cell line compared with un-treated cell line (100.0 ± 18.5%) (Fig. 1). The growth of rhabdomyosarcoma cell line is significantly ($p < 0.001$) inhibited with aspirin (26.1 ± 10.6%) compared with un-treated cell line (100 ± 26.1%) (Fig. 1). As with aspirin, diclofenac has no significant effect against the growth of AMN3 mammary cell line compared with un-treated cell line (118.5 ± 25.9% versus 100 ± 18.5%, respectively). The significant effects of diclofenac against fibroblast, HeLa and rhabdomyosarcoma cell lines are inferior to that observed with aspirin (Fig. 2). The percents of fibroblast, HeLa and rhabdomyosarcoma cell lines growth decreased into 74.9 ± 10.8%, 21.2 ± 5.5% and 67.0 ± 25.3% respectively. The cytotoxic effect of aspirin against rhabdomyosarcoma is significantly ($p < 0.001$) higher

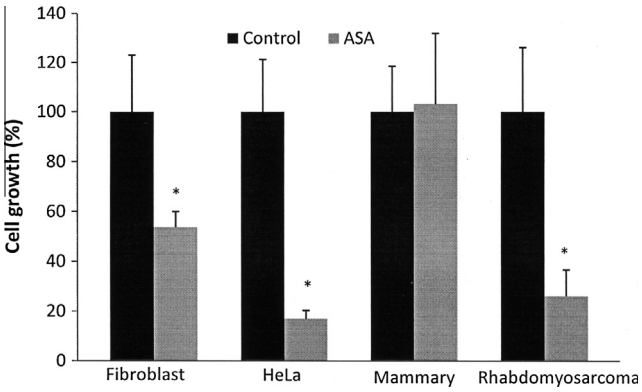

Figure 1 Effect of aspirin on the growth of different cell lines. The results are expressed as mean ± SD of each treatment ($n = 8$), $^*p < 0.001$.