

## Importance of Cell line Selections from Different Tissues in Cellular Survival Assays

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### Abstract

**Introduction:** Cell Culture is a simple and accurate way to evaluate the effect of various factors on different cell lines. One of the most important factors in cell culture is selection of the right cell line for different experiments which might dramatically affect our final conclusions. The purpose of this study was to examine the importance of cell line selection in cellular survival assays.

**Methods and Results:** To reach this purpose, we used four different cell lines, A2780, A549, HT29, and MIA paca-2, and three widely used pharmaceutical chemicals. MTT assay was performed in order to evaluate the effect of cisplatin, dexamethasone, and progesterone on cell survival after 24 hours exposure. *In-vitro* MTT assay showed that dexamethasone had proliferation effects on A549, no significant effect on A2780 and MIA paca-2 cell lines, and also cytotoxic effects in high concentrations of HT29 cells. Higher concentrations of progesterone had cytotoxic effects on all four cell lines, furthermore low concentrations of progesterone has proliferative effects on A2780. On the other hand, it did not show any significant effect on A549, HT29, and MIA paca-2 cells in lower concentrations. Cisplatin had cytotoxic effect on all four cell lines with different EC<sub>50</sub>s.

**Conclusions:** The results of this study revealed that choosing the proper cell line is important for gaining the reliable results in cell culture experiments.

**Key words:** Cisplatin, Dexamethasone, Progesterone, A2780, A549, HT29, MIA paca-2

## 1. Introduction

No Cell culture experiments are increasingly used in biological researches as a simple and accurate model set up for living adventures. They provide a simple system to examine hypothesis and get an initial idea on consequence events of biological interventions. Nowadays, cell culture is very much applied not only in the biological investigations, but also in biochemical, anatomical, pharmacology and toxicology, ecosystem and astronomical, physical, and even social researches. The protocols, however, are

more general and focus on cell events rather than on area of application. Many important factors need to be considered in the application of cell culture experiments for an accurate and precise result and conclusion, including type of cell, culture media, experimental assay, duration of experiment, and other interfering factors. A review of thousands of published articles on this very important method presents a weakness in the precise selection of aforementioned factors. Here, and to present the importance of cell line selection for a reliable result and conclusion, we have set up a simple routine assay

using different cell lines to explore variations in a very simple concept of cell survival.

Four different epithelial cell lines with distinct characteristics are selected for this purpose; MIA paca-2 is a human pancreas carcinoma cell line with epithelial morphology [1]. HT-29 is a human colorectal adenocarcinoma cell line with epithelial morphology [2]. A2780 is a human ovarian carcinoma cell line with epithelial morphology [3], and A549 is a human lung adenocarcinoma cell line with epithelial morphology [4]. All of these cell lines grow as monolayer, adherent to the culture flask.

**Table 1: Cell lines characterizations**

Cell line	Tissue	Cell type
MIA paca-2	Pancreas	Epithelial
HT29	Colon	Epithelial
A2780	Ovary	Epithelial
A549	Lung	Epithelial

To present cellular outcome following a simple assay of exposure to different variables, three widely used pharmaceutical chemicals were used. Cisplatin is a cytotoxic agent. When cisplatin enters the cell, in the cytoplasm, chloride atoms on the cisplatin are replaced with water molecules, as a result, cisplatin becomes activated. This product is an electrophile that can react with different nucleophiles, for instance, atoms such as nitrogen on nucleic acids. Cisplatin binds to the N7 reactive center on purines and causes deoxyribonucleic acid (DNA) damage in cancer cells. This process can block cell division and causes cell death [5].

Dexamethasone is a glucocorticoid (GC). In biological and clinical significance, there is a phenomenon which is called GC induced apoptosis. In biology, GCs have been considered as regulators of immune responses and in clinic, they have been used in lymphoid malignancies therapy [6]. Such a phenomena may cause cell death in some cells such as bone, hippocampus, eosinophils, fibroblasts, and certain cancer cells. On the other hand GCs have some proliferative effects on erythroblasts, neutrophils, ovary, and liver [6]. Gundisch *et al.* worked on 17

different cell lines with different origins, such as epithelial, mesenchymal, and neuroectodermal cells [7]. They found out that proliferation induced by dexamethasone, is mediated by the GC receptor. In their study dexamethasone increased tumor cell proliferation in nine out of seventeen cell lines from solid tumors [7]. In another study, the effect of dexamethasone was evaluated on glioma cells. Based on this study, dexamethasone had cytotoxic effect in high concentration, on the other hand it did not show any significant effect in low concentration [8].

Progesterone is a hormone which is synthesized in female and male and it is natural and neurosteroidal [9]. Progesterone has vital role in female reproductive system. Progesterone participates in cells' proliferation during menstrual cycle. In the uterus, it has both proliferative and cytotoxic effects [10]. In addition, progesterone has cytotoxic and apoptotic effects in breast, endometrial, ovarian, colon, and salivary gland tumors *in-vitro* and *in-vivo* [4, 5]. Clinical data suggest that progesterone has some chemo-preventive effects on some cancers [11]. Heijmans *et al.* studied the chemo-preventive effect of progesterone in colorectal cancer and they found that progesterone did not have any effect on proliferation of colorectal cancer cells [11]. In their further investigation they evaluated the presence of progesterone receptor (PR) and they found that PR is not expressed in not only neoplastic but also in normal intestinal tissue. As a result, the effect of progesterone on different cells is based on the presence of receptors [11].

To further investigate the importance of tissue and cell line selection in evaluating drugs survival curve, we have set up the following experiments in this research.

## 2. Materials and Methods

### 2.1. Materials

Human colon cancer HT29 cell line (IBRC C10097), human lung adenocarcinoma A549 cell line (IBRC C10681) were provided by the Iranian Biological Resource Center. Human ovary carcinoma A2780 cell line (C461), human pancreas cancer MIA paca-2 cell line (CRL-1420) were obtained from American Tissue

Cell Culture. The tetrazolium dye MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide) and dimethyl sulfoxide (DMSO) were both purchased from Merck®. Dexamethasone, cisplatin, and progesterone were purchased from Caspian, Mylan®, and Aburaihan pharmaceutical companies, respectively.

## 2.2. Methods

### 2.2.1. Cell culture

Cell lines were grown in RPMI 1640 medium (GIBCO®) supplemented with 10% fetal bovine serum (FBS) (GIBCO®) and 1% penicillin/ streptomycin (GIBCO®). Before any experiment, the cells were passaged three times. They were kept in humid incubator (5% CO<sub>2</sub>) at 37°C during the experiment.

### 2.2.2. Cell proliferation assay

MTT assay was performed in order to evaluate the effects of cisplatin, dexamethasone, and progesterone on the cell survival. Briefly, the cells were seeded in 96 well plates at density of 3000 cells per well and incubated at 37°C for 24 hours. Then, the cells were exposed to different concentrations of cisplatin (0-1 mg/mL), dexamethasone (0-4 mg/mL), and progesterone (0-50 mg/mL). After 24 hours, 20 µL MTT was added to each well and incubated for 4 hours. Then, the supernatant was discarded and 200 µL of dimethyl sulfoxide (DMSO) was added to each well. The plates were shaken for 30 minutes and the absorbance was measured at 570 nm using ELISA reader. The Graph Pad Prism® software was used to create graphs and also one-way ANOVA analysis was used to determine whether there are any statistically significant differences between different concentrations.

## 3. Results

S Dexamethasone showed variable patterns on survival curve of these cell lines. The results of the present study revealed that the survival rate of HT29 cells decreased in

higher concentrations of dexamethasone in comparison with the control group ( $P < 0.0001$ ). On the other hand, there was not any significant change in the range of lower concentrations. Furthermore, A549 cell line's survival rate increased in the highest concentration of dexamethasone ( $P = 0.0048$ ), while there was not any significant change in other concentrations. The survival rate of A2780 cells and MIA paca-2 cells were not affected following exposure to different concentrations, neither low, nor high. Based on the previous reports, these controversial effects are because of the presence or absence of GR [7].

These results showed that the survival rate of A2780 cells decreased at higher concentrations of progesterone, and increased at lower concentrations ( $p < 0.0001$ ). The survival rate of HT-29 cells were not affected following exposure to lower progesterone concentrations, even though the higher concentrations of progesterone decreased the cells' survival rate ( $p < 0.0001$ ). Lower concentrations of progesterone did not have any effect on A549 and MIA paca-2 cells' survival, while the higher concentrations decreased the survival rate. Based on the previous reports, the proliferative effect of progesterone is only in the cell lines which have progesterone receptors [11]. One hypothesis might come to the conclusion that unlike other cell lines (A549, MIA paca-2, and HT29), A2780 cells may contain PR. Yu et al. evaluated the effect of two different concentrations of progesterone on ovarian cell line. After 24 hours the higher concentration ( $\cong 31$  mg/mL) induced cell death, while the lower concentration ( $\cong 3$  mg/mL) had proliferative effect [12]. In another study, they examined the effect of progesterone on cervical cell lines. They found that progesterone had cytotoxic effect and induced apoptosis in cervical cells [13]. So, we can conclude that progesterone effect on proliferation of A280 cells is positively related to the drug concentration.

Cisplatin had similar sigmoidal pattern in A549, A2780, HT29, and MIA paca-2 cell lines, however, with different EC<sub>50</sub>s.

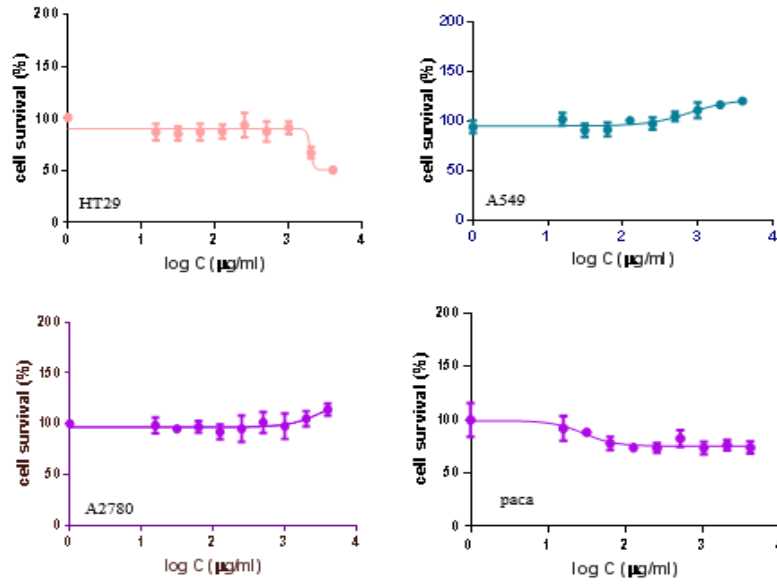


Figure1. Cell survival curves after 24h exposure to dexamethasone

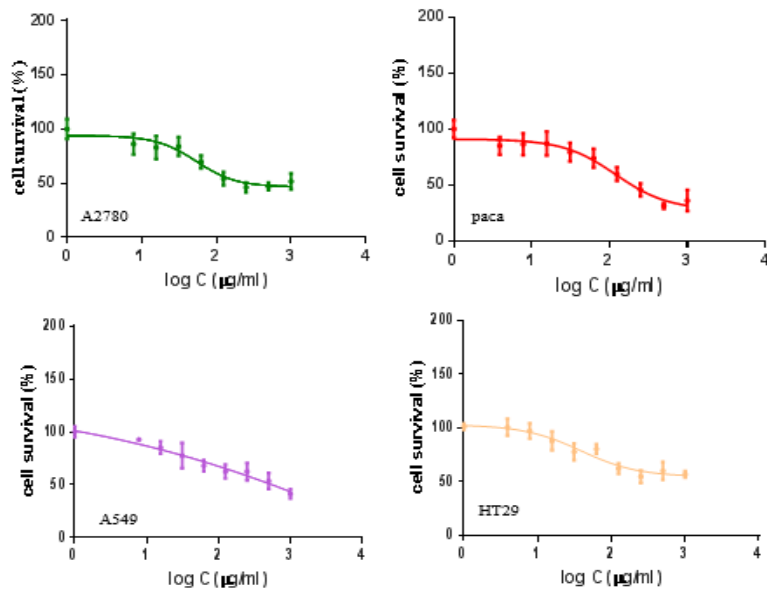


Figure2. Cell survival curves after 24h exposure to cisplatin

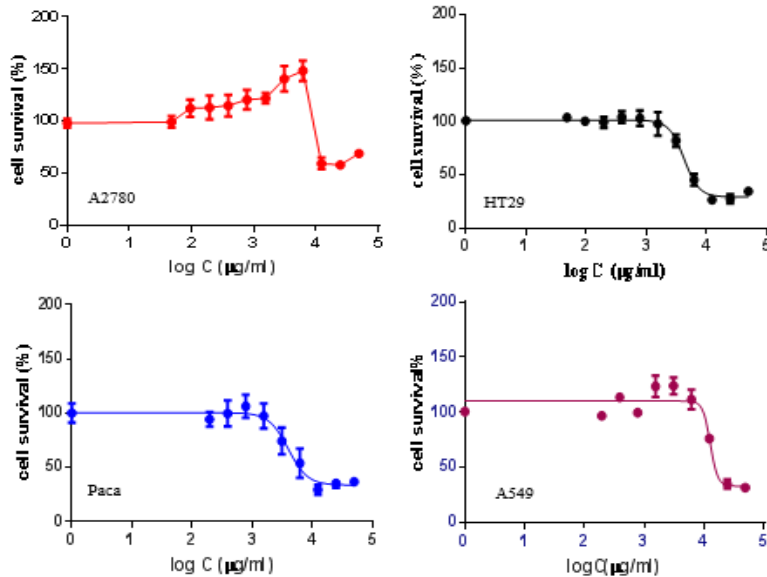


Figure3. Cell survival curves after 24h exposure to progesterone

Table 2: Cell lines' EC50

Cell lines	Dexamethasone EC50s ± SD (mg/mL)	Progesterone EC50s ± SD (mg/mL)	Cisplatin EC50s ± SD (mg/mL)
A2780	0.0033±1.151	8.77±0.0001	0.054±0.07
A549	0.79±0.18	12.73±0.39	0.003±0.14
MIA paca-2	0.028±0.11	3.95±0.04	0.12±0.09
HT29	1.96± 13.52	0.0042±0.001	0.038±0.11

**4. Discussion and Conclusion**

T Cisplatin exhibited cytotoxic effects in all four cell lines. The most sensitive cell line is MIA paca-2 (EC<sub>50</sub> = 0.12±0.09 mg/mL) and the least sensitive one is A549 (EC<sub>50</sub> = 0.003e±0.14 mg/mL).

Although progesterone showed proliferative effects on A2780 cells at concentrations below 12.5 mg/mL, there was no significant difference in proliferation of HT29, MIA paca-2, and A549. On the other hand, higher concentrations of progesterone showed strong cytotoxic effects on all these four cell lines (*p* < 0.0001).

Dexamethasone had proliferative effects on A549 and HT29 cell lines in high concentrations, but show neither proliferative nor cytotoxic effects on A2780 and MIA paca-2 cell lines.

These diverse patterns might reflect the existence of various receptors and signaling pathways in each of these four cell lines. These results revealed the importance of selecting the cell line in study of drugs with various mechanisms. Researches have to consider very well documented and investigated base for the selection of cell line in cell culture experiments to acquire reliable results and conclusions.

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