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# DEFINING MINIMAL EFFECTIVE CONCENTRATIONS OF DNA-DAMAGING AGENTS VIA MICROSCOPIC OBSERVATION

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# DECLARATION

I hereby declare that the thesis is my original work and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis. This thesis has also not been submitted for any degree in any university previously.

Arsen Orazbek

28 April 2023

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## SUMMARY

DNA-damaging agents (DDAs) are efficient chemotherapeutic drugs in combating malignant cells. The different types of these agents may, in certain concentrations, induce cell arrest and consequent cell death. Therefore, concentrations can be differentiated as cytostatic and cytotoxic. The identification of minimal effective concertation is important for clinical and research applications. The current traditional methods of defining effective drug concentrations are performed via $IC_{50}$ concentrations (e.g., MTT assay) that represent the concentration of drug required to achieve "50% of its maximal efficacy".

However, this term in measuring cell viability for cancer studies can be vague. Sometimes $IC_{50}$ values can represent a 50% decrease in cell number, thus assessing cell death. On the other hand, they also can represent a 50% decrease in cell division, thus, assessing the proliferation rate. Thus, the $IC_{50}$ values can fail to represent the detailed characterization of concentration-dependent drug effect.

Therefore, the novelty of our research is the detailed identification of minimal effective DDA concentrations by microscopic analysis. In this research, our goal was to identify effective DDA concentrations that a) significantly decrease proliferation rate and b) significantly increase cell death (or cytotoxic concentration). The results demonstrate that the effect of drugs was drug dependent, and Doxorubicin was more toxic compared to Camptothecin and Mitomycin-C. In conclusion, our research was able to identify novel data with defined cytostatic and cytotoxic concentrations.

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## ABBREVIATIONS

| | |
|---|---|
| DDAs | DNA-damaging agents |
| DOX | Doxorubicin |
| CPT | Camptothecin |
| MMC | Mitomycin-C |

# 1 INTRODUCTION

## 1.1 Overview of different DNA-damaging agents

Globally, cancer is the leading reason of death, thus, its treatment is under intensive research (World Health Organization: WHO, 2022). Chemotherapy is one of the main treatments for these malignancies. Different types of chemotherapeutic drugs inhibit uncontrollable cell division (Amjad et al., 2023). Among them, the drugs that damage DNA are considered one of the efficient types of chemotherapeutic agents (Woods & Turchi, 2013).

Interest in cancer treatment using DNA-damaging agents (DDAs) rose in the 1960-70s that are used in the treatment of hematological and solid cancers. Since then, numerous types of DNA-damaging agents have been developed and used in cancer treatment (Cheung-Ong et al., 2013). They directly (e.g., DNA intercalation and alkylation) or indirectly (e.g.. topoisomerase I and II inhibitors) damage DNA that triggers cell cycle arrest and consequently may cause cell death or escape from this arrest in rapidly dividing cells (Weber, 2015; Rixe & Fojo, 2007).

DDAs can be classified by their mechanisms of action, which include direct base modification, DNA intercalation, interstrand and intrastrand DNA crosslinking, interference with DNA replication, and interference with topoisomerases (Weber, 2015). The DNA crosslinks result in the formation of stalled replication forks; while inhibition of topoisomerases I and II results in single or double-strand breaks, respectively. Among numerous DDAs, for this research, three different DNA-damaging drugs were selected: Mitomycin-C (MMC), Doxorubicin (DOX), and Camptothecin (CPT). Their chemistry and mechanisms of action are described in Table 1.

**Table 1.** The structures, nature, and mechanisms of action of four DNA-damaging agents

| | Nature and chemistry | Mechanism of action |
|---|---|---|
| MMC | Antitumor quinone agent isolated from *S. caespitosus* (Green, 1977) | An alkylating agent which can crosslink DNA bases (Rahman et al., 2019) |
| DOX | Anthracycline isolated from *Streptomyces peucetius var. caesius* (Thorn et al., 2011) | DNA intercalation, inhibition of topoisomerase II (Morelli et al., 2022), and generation of free radicals (Taymaz-Nikerel et al., 2018) |
| CPT | Pentacyclic alkaloid isolated from the wood of *Camptotheca acuminata* (Li et al., 2017) | Inhibition of topoisomerase I (Li et al., 2017) |

The abovementioned stalled replication fork, ssDNA or dsDNA breaks may trigger a DNA damage response that activates cell cycle checkpoint and DNA repair through ATM/ATR and CHK1/CHK2 pathways and consequent p53 phosphorylation. If DNA repair fails and cell cycle arrest takes a prolonged time, cell apoptosis can be initiated (Garrett & Collins, 2011). Since the cell cycle in cancer cells is more rapid, DNA damage checkpoints in cancer cells are more likely to initiate apoptosis compared to normal cells. However, larger doses of DNA-damaging agents (DDAs) can affect the normal rapidly proliferating cells like bone marrow, skin, and gastrointestinal tract (Hijiya et al., 2009). Therefore, the identification of minimal effective concentration of different DDAs is crucial to minimize these negative consequences. This is especially important in a combinational treatment of DDAs with other chemotherapeutic agents such as microtubule-targeting ones.

The effective concentrations can be classified as cytotoxic and cytostatic. The concentration that leads to significant cell death (hence, a significant decrease in cell number) is considered cytotoxic. Compared to that, the concentration that only leads to a significant decrease in proliferation with no significant cell death is considered cytostatic. The concentration-dependent effect of DDAs can be divided into cytotoxic and cytostatic effects based on drug concentration (Rixe & Fojo, 2007).

## 1.2 Traditional measurement of effective chemotherapeutic concentrations

Defining the effective concentration range for chemotherapeutic drugs is important in understanding their pharmacological characteristics. These concentrations are usually determined either by the half-maximal inhibitory concentration, which is called $IC_{50}$, either by the half-maximal response concentration, which is called $EC_{50}$ (or $ED_{50}$), or by half-maximal inhibition of cell proliferation, which is called $GI_{50}$. However, the last two methods ($EC_{50}$ and $GI_{50}$) were rarely used in the literature, while $IC_{50}$ is a commonly used method as a drug response metric for cancer studies (Brooks et al., 2019).

One of the most popular assays that allow determining $IC_{50}$ is the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay which is a calorimetric test that can measure NAD(P)H-dependent oxidoreductase activity (Cheng & Prusoff, 1973). Also, there are MTT assay analogs like WST-8 and MTS tests that do not require solubilizing chromogenic products as in MTT assay (Ishiyama et al., 1997). However, there is controversy surrounding $IC_{50}$ measurements for chemotherapeutic agents despite its usage for more than 30 years.

## 1.3 Literature review

According to He et al. (2016), the $IC_{50}$ values are inconsistent with each other between different laboratories and even in some cases within the laboratory. For example, 24h Doxorubicin vs. A549 cells offers $IC_{50}$ values that range from 86nM to 1μM concentration range (Punia et al., 2017; Mishra et al., 2022). These differences probably resulted from the differences between manufacturers and formulae of MTT or analogous assay (He et al., 2016). Moreover, since the $IC_{50}$ value is estimated from cells at the end of the experiment, changes in initial seeding density, duration of the experiment, and growth conditions can significantly influence the $IC_{50}$ values; thus, making biological repeats inconsistent (He et al., 2016; Hafner et al., 2016).

Additionally, the $IC_{50}$ metric was initially developed to assess the inhibition of certain biological activities like enzyme inhibition or fluorescence reduction (Brooks et al., 2019). Therefore, the “50% inhibition” from $IC_{50}$ is a relatively vague metric for cancer studies and cannot demonstrate the clear cytostatic and cytotoxic effect of the

chemotherapeutic drug. In rare cases, some articles tried to differentiate $IC_{50}$ into "antiproliferative" and "cytotoxic" (Pessina et al., 2001), but the majority of $IC_{50}$ values are not characterized.

To identify the minimal effective drug concentrations, we proposed to determine them by microscopic observation and cell counting as a direct cell viability test. The cell fates under DDA treatment can be easily determined under a microscope. We expected to have more accurate minimal effective concentrations (i.e., cytostatic and cytotoxic). This thesis research is a part of a larger project on the sequential treatment of cancer cells with microtubule-targeting agents followed by DDAs. Based on the results, the effect of DNA-damaging agents on different cancer cell lines was found to be drug-dependent. Specifically, the treatment of Doxorubicin had a cytotoxic effect against all selected cell lines, while Mitomycin-C and Camptothecin mainly halted cell proliferation.

# 2 MATERIALS AND METHODS

## 2.1 Reagents and culturing media

**Table 2**. List of DNA-damaging agents

| Name | Abbreviation | Catalog Number | Source |
|---|---|---|---|
| Mitomycin-C from *Streptomyces caespitosus* ≥98% (HPLC), γ-irradiated | MMC | M7949-2MG | Sigma-Aldrich |
| Camptothecin, 98% | CPT | 276721000 | Fisher Scientific |
| Doxorubicin hydrochloride, 10 mg/ml in distilled water | DOX | AAJ67364XF | Fisher Scientific |

**Table 3.** List of reagents and cell culture media

| Name | Usage | Catalog Number | Source |
|---|---|---|---|
| DMEM (Dulbecco's Modified Eagle Medium), high glucose | Cell culture media component | 11965092 | Gibco™ |
| DMEM/F-12 | Cell culture media component | 11320033 | Gibco™ |
| PBS, pH 7.4 | For cell passaging | 10010023 | Gibco™ |
| Image-iT™ TMRM Reagent (mitochondrial membrane potential indicator) 100uL | Viable cell visualization | I34361 | Invitrogen™ |
| Penicillin-Streptomycin (10,000 U/mL) | | 15140122 | Gibco™ |
| Fetal Bovine Serum (FBS) | Cell culture media component | F2442-500ML | Sigma-Aldrich |
| Trypsin-EDTA (0.05%), phenol red | For cell passaging | 25300054 | Gibco™ |
| CO2 Independent Medium | Cell culture media component | 18045088 | Gibco™ |
| L-Glutamine (White Crystals or Crystalline Powder) | Cell culture media component | MFCD00008044 | Fisher BioReagents |

## 2.2 Cancer cell lines

**Table 4.** List of cell lines

| Name | Origin | Tissue | Company |
|---|---|---|---|
| HeLa (cervical cancer line) | *Homo sapiens* | Cervix | Sigma-Aldrich |
| HaCaT (immortalized nontumorigenic human epidermal cell line) | *Homo sapiens* | Keratinocyte | Sigma-Aldrich |
| A549 (lung carcinoma epithelial cell line) | *Homo sapiens* | Alveolar basal epithelia | Sigma-Aldrich |
| PC-3 (prostate cancer cell line) | *Homo sapiens* | Prostate | Sigma-Aldrich |

Apart from PC-3, all cells were cultured in DMEM (10% FBS and 4-8 mM L-Glutamine), while PC-3 will be cultured in F12 medium (10% FBS and 4-8 mM L-Glutamine). Cells were cultured in a humidified atmosphere in an incubator under 5% CO2 at 37°C. The passage number of each cell line was <30 which is optimal for our research.

## 2.3 Microscopic analysis of DDAs

The cancer cells were seeded into a 48-well plate for a day (for PC-3, two days are needed) with seeding densities of $2\times10^4$ cells/cm$^2$ for HeLa, $3 \times 10^4$ cells/cm$^2$ for HaCaT, $6\times10^4$ cells/cm$^2$ for A549, and $2\times10^4$ cells/cm$^2$ for PC-3 cells. Then, we added 10nM-10μM (physiologically relevant doses) DNA-damaging drug concentrations. The negative control was the untreated cells. The 100μl of 50nM TMRM was added into each well for visualization of alive cells under the microscope. The samples were monitored by High throughput time-lapse imaging (Zeiss Cell Observer) microscope with 20X magnification and 0.5 numerical aperture objective for 48 hours. The time-lapse images were analyzed in ImageJ Fiji and Zen Blue 3.7 software by selecting random 50 cells at the 1$^{st}$-hour frame, and we followed the cell fates of these cells throughout 48h. The presence of fluorescent signal from TMRM stain was interpreted as viable cells, while the absence or significant decrease of TMRM fluorescence was interpreted as cell death (Figure 1). The data was processed in Excel and GraphPad Prism 8 programs. For statistical significance, the experiments were repeated 3 times.

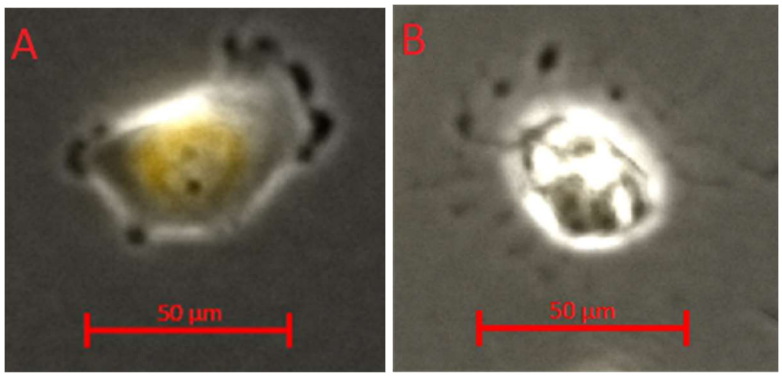


**Figure 1.** The images of a) alive alive A549 cell with fluorescence (represented yellow) from TMRM stain and a non-fluorescent dead A549 cell after treatment with 3µM DOX.

## 2.4 Data analysis

To define effective concentrations, a T-test with Welch correction was used to measure statistical significance ($p<0.05$) between 1) the number of cells after 48h of the control group and of the treatment group, as well as 2) the initial number of cells (50 cells) and the number of cells after 48h treatment. The correction was used in the cases of the different variances in each time group. The statistical analysis was performed by Prism GraphPad 8 software.

# 3 AIMS OF THE THESIS PROJECT

- To define and compare minimal cytostatic and cytotoxic doses of DNA-damaging agents (i.e., Doxorubicin, Camptothecin, Mitomycin-C) on different cancer cell lines (i.e., HaCaT, HeLa, A549, and PC-3)

# 4 RESULTS

Overall, we were able to observe the dose-dependent response where 48h DDA treatment resulted in one of the following: 1) no significant difference from negative control, 2) a significant decrease in cell proliferation (cytostatic concentration), or 3) a significant decrease in cell number (cytotoxic concentration). The data was consistent within three biological replicates. All three DDAs resulted in a cytostatic effect against all cell lines and only DOX treatment at high concentrations resulted in a cytotoxic effect. On the other hand, MMC and CPT treatment had a cytotoxic concentration in the HaCaT cell line.

Based on the results of 48h MMC treatment, the cytostatic concentrations were 1μM for A549 cells and 300nM for other cancer cell lines (Figure 2). The resulting number of cells after 48h of treatment with mentioned MMC concentrations were only 75±2, 68±7, 67±23.5, and 63±19 for A549, HeLa, PC-3, and HaCaT cells, respectively. However, the significant cell death resulting from MMC was observed only in the HaCaT cell line (10 μM) where cell number decreased to 42± 11 (Figure 2D).

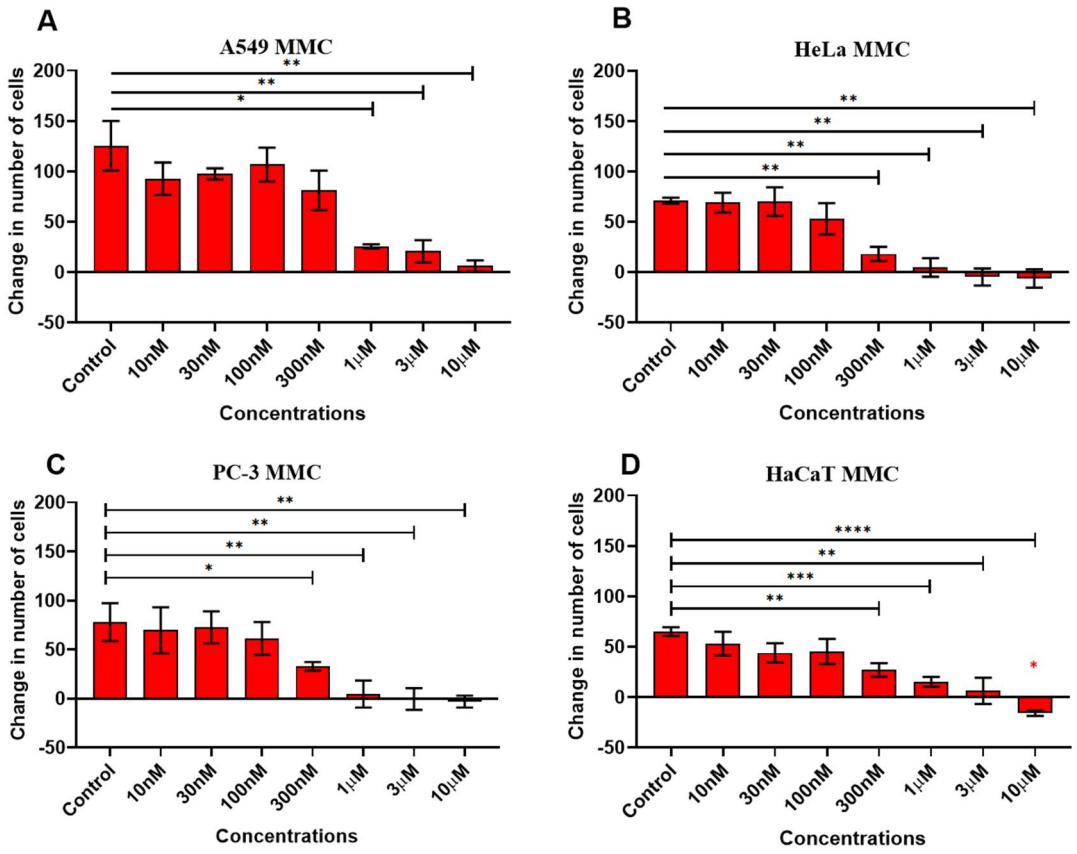


**Figure 2**. The change in the number of TMRM-positive cells untreated (control) and treated with 30nM-10uM Mitomycin-C concentrations for 48h in a) A549, b) HeLa, c) PC-3, and d) HaCaT cell lines. The cells were counted manually by following 50 cell fates throughout 48h observation. As the statistical significance test, T-test with Welch correction was performed ($p>0.05$ is ns, $p<0.05$ is *, and $p<0.01$ is **; bars represent standard deviation). The black stars represent significant decrease in cell number compared to control number after 48h, and red stars represent significant decrease in cell number compared to initial cell number (50 cells). The number of cells after treatment with DDA (48h) was compared to 1) the number of cells in control after 48h and 2) the number of cells initially selected at time 0h.

The results of CPT treatment are nearly similar to the results of MMC treatment. The minimal cytostatic CPT concentrations after 48h treatment were 300nM for HeLa cells and 1μM for other cancer cell lines (Figure 3). The resulting number of cells after 48h of treatment with mentioned CPT concentrations were 49±7, 57±3, 53±3, and 48±7 for A549, HeLa, PC-3, and HaCaT cells, respectively. However, the cytotoxic MMC concentration was observed only in the HaCaT cell line (3μM) where cell number decreased to 32±3 (Figure 3D).

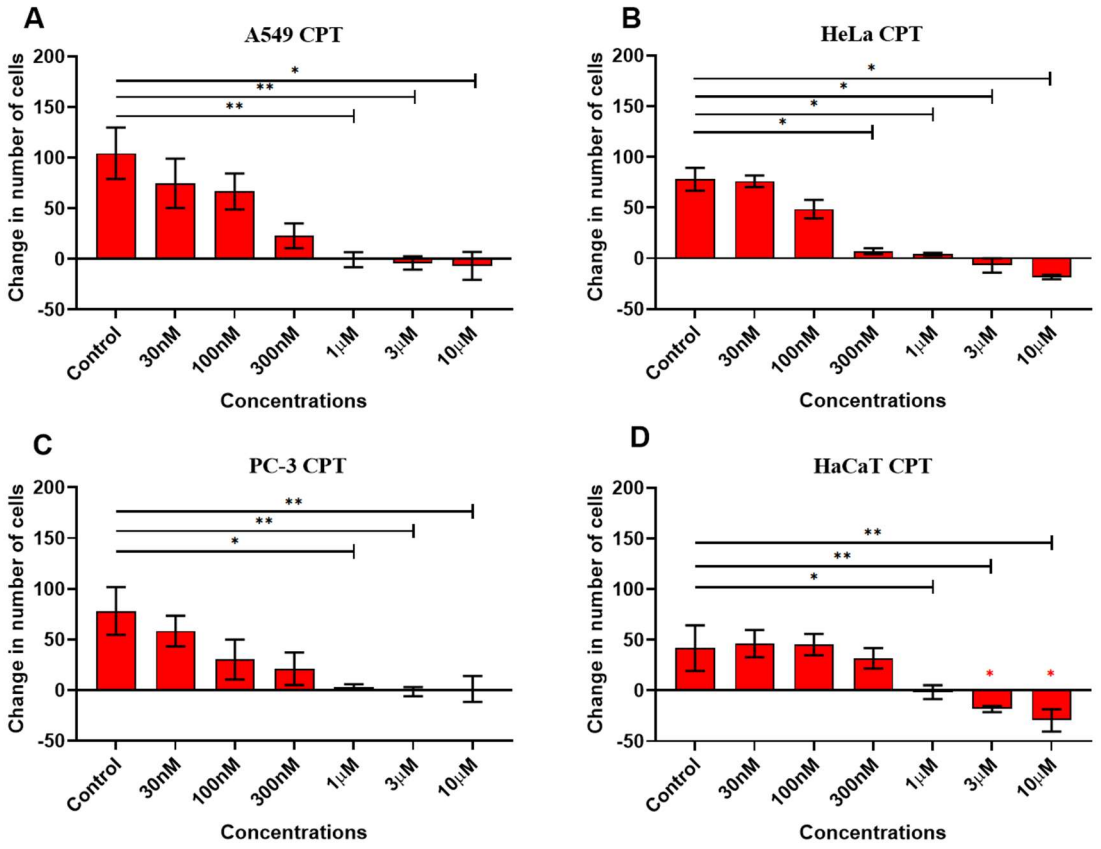


**Figure 3.** The change in the number of TMRM-positive cells untreated (control) and treated with 30nM-10uM Doxorubicin concentrations for 48h in a) A549, b) HeLa, c) PC-3, and d) HaCaT cell lines. The cells were counted manually by following 50 cell fates throughout 48h observation. As the statistical significance test, T-test with Welch correction was performed ($p>0.05$ is ns, $p<0.05$ is *, and $p<0.01$ is **; bars represent standard deviation). The black stars represent significant decrease in cell number compared to control number after 48h, and red stars represent significant decrease in cell number compared to initial cell number (50 cells). The number of cells after treatment with DDA (48h) was compared to 1) the number of cells in control after 48h and 2) the number of cells initially selected at time 0h.

On the other hand, 48h DOX treatment required significantly less concentration to significantly decrease cell proliferation. The minimal cytostatic DOX concentrations were 10nM for A549 and HeLa cells, 30nM for HaCaT cells, and 300nM for PC-3 (Figure 4). The resulting number of cells after 48h of treatment with mentioned DOX concentrations were 91±10, 87±12, 51±2, and 65±11 for A549, HeLa, PC-3, and HaCaT cells, respectively.

Moreover, 48h DOX treatment resulted in significant cytotoxicity in all cell lines. The minimal cytotoxic concentrations of DOX were 1μM for HeLa cells, 3μM for A549 and HaCaT cells, and 10μM for PC-3 cells. The microscopic image of 48h 3μM DOX treated A549 cells is illustrated in Figure 5 as an example. The number of cells after 48h

treatment with minimal cytotoxic concentrations was 30±1, 12±1, 26±8, and 6±1 for A549, HeLa, PC-3, and HaCaT cells, respectively.

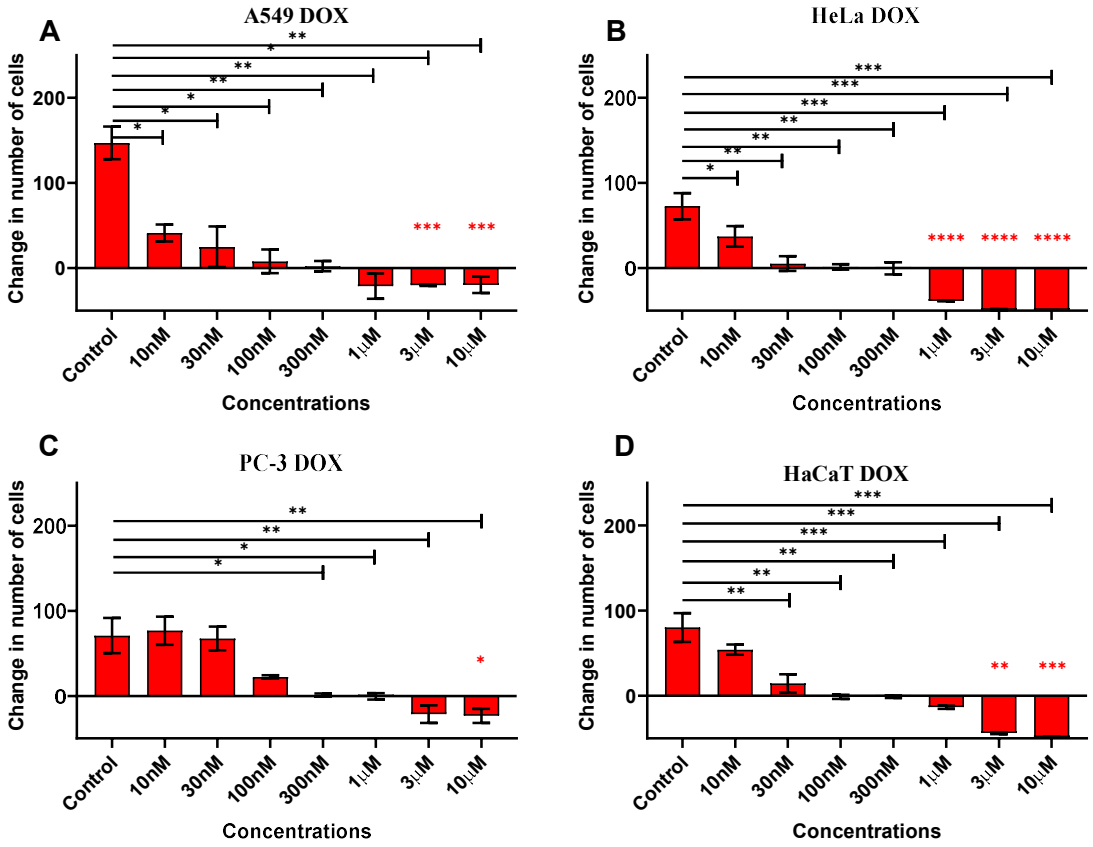


**Figure 4.** The change in the number of TMRM-positive cells untreated (control) and treated with 30nM-10uM Camptothecin concentrations for 48h in a) A549, b) HeLa, c) PC-3, and d) HaCaT cell lines. The cells were counted manually by following 50 cell fates throughout 48h observation. As the statistical significance test, T-test with Welch correction was performed ($p>0.05$ is ns, $p<0.05$ is *, and $p<0.01$ is **; bars represent standard deviation). The black stars represent significant decrease in cell number compared to control number after 48h, and red stars represent significant decrease in cell number compared to initial cell number (50 cells). The number of cells after treatment with DDA (48h) was compared to 1) the number of cells in control after 48h and 2) the number of cells initially selected at time 0h.

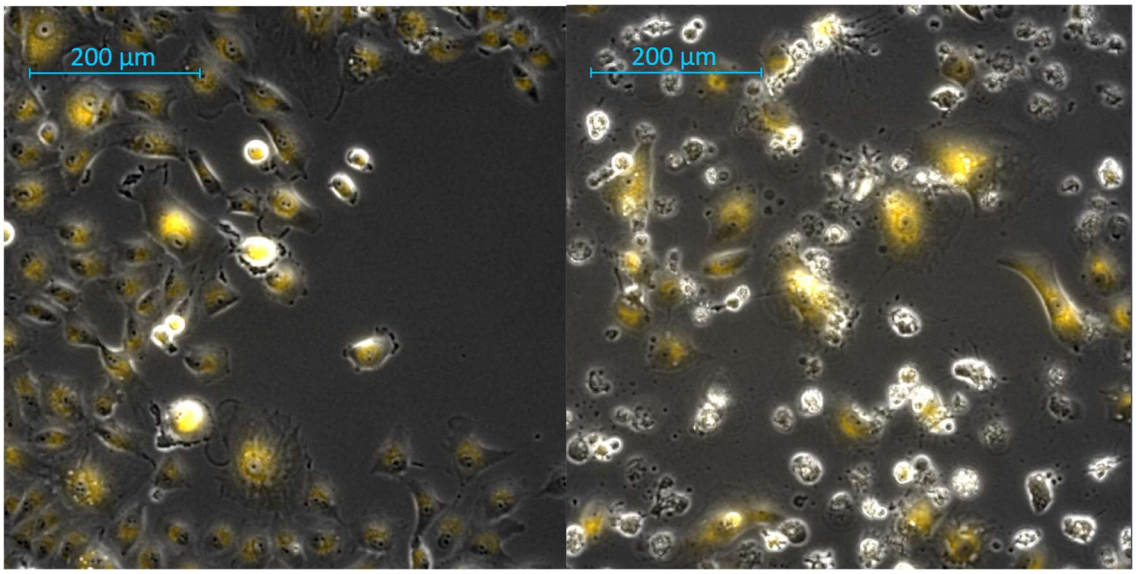


**Figure 5.** Microscopic images of A549 cells treated with 3 µM DOX. The left image represents treatment at 1h, and the right image represents treatment at 48h. Cells were stained with TMRM where the fluorescent signal (yellow) represents viable cells.

The minimal cytostatic and cytotoxic concentration results are summarized in Table 5. Not all drugs demonstrated the presence of cytotoxic concentration in the selected concentration range. The highest cytostatic and cytotoxic concentrations in all cell lines were observed in PC-3 cells, hence PC-3 cells can be considered more resistant to the mentioned DDAs compared to other cell lines. On the other hand, all three drugs were toxic in the given concentration range against the HaCaT cell line. Additionally, the cytotoxic to cytostatic concentration ratio was highest in DOX vs. A549 cells where its minimal cytotoxic concentration is 300-fold higher than its minimal cytostatic one.

**Table 5**. The overall cytostatic and cytotoxic DDA concentrations (in µM). The values in brackets represent the cytotoxic to cytostatic concentration ratios.

| **Cell line** | **MMC** | | **DOX** | | **CPT** | |
|---|---|---|---|---|---|---|
| | Cytostatic | Cytotoxic | Cytostatic | Cytotoxic | Cytostatic | Cytotoxic |
| **PC-3** | 1 | N/A | 0.3 | 10 | 1 | N/A |
| **A549** | 0.3 | N/A | 0.01 | 3 | 1 | N/A |
| **HeLa** | 0.3 | N/A | 0.01 | 1 | 0.3 | N/A |
| **HaCaT** | 0.3 | 30 | 0.03 | 3 | 1 | 3 |

# 5 DISCUSSION

It was aimed to define and compare effective drug concentrations (i.e., cytostatic and cytotoxic) of Mitomycin-C, Doxorubicin, and Camptothecin in different cell lines by directly observing cell fates under the microscope. The biological repeats were similar to each other with minor standard deviations; thus, microscopic results are repeatable within the laboratory. Moreover, the $IC_{50}$ concentrations for the CPT and MMC against most selected cell lines were not available. Since $IC_{50}$ values do not specify clear drug effect, our results were characterized as cytostatic and cytotoxic, hence concentrations can be selected for specific purposes.

The only relevant research was a study by Pessina et al., 2001. They used a similar drug and cell line (CPT vs. HaCaT cells) and was able to separately define $IC_{50}$ values as antiproliferative (half the number of cells arrested) and cytotoxic (half the number of cells dead). However, the authors did not explain the rationale and methods to define cytotoxic and antiproliferative $IC_{50}$ values. The $IC_{50}$ cytotoxic concentration (3.02µM) was very similar to our observed cytotoxic concentration (3µM). In contrast, cytostatic concentration was significantly different: they observed 0.0051µM which is 200 times lower than our results. Since the criteria for the characterization of antiproliferative $IC_{50}$ concentration are unknown, it can be possible that the characterization of antiproliferation was different in their research.

Based on the results of different DDA treatments in cancer cell lines, the effect of drugs was mainly depended on the drug type, among which DOX was the most cytotoxic compared to CPT and MMC. The DOX treatment was toxic to all cell lines at the selected concentration range, while CPT and MMC were only toxic to the HaCaT cell line. The toxicity of DOX can be explained by its broad spectrum of activity where it blocks the topoisomerase II and intercalates DNA (Morelli et al., 2022; Taymaz-Nikerel et al., 2018).

Cell lines also behaved differently in response to the same drug. At high concentrations, all three drugs resulted in cytotoxicity in the HaCaT cell line. According to Pessina et al. (2001), HaCaT cells overexpress topoisomerase I (60-fold higher expression compared to normal cells), thus, the topoisomerase inhibitors (CPT and DOX) are very effective in killing them. However, it is still unclear why MMC had similar effective cytotoxicity as other topoisomerase inhibitors. On the other hand, PC-3

cells had the highest cytostatic and cytotoxic concentrations compared to other cell lines. This is most probably due to the lack of p53 proteins, enhancer of apoptosis in response to DNA damage stress (Lin et al., 2018). Therefore, apoptosis was significantly reduced by the absence of pro-apoptotic proteins.

In conclusion, our study was able to define minimal cytostatic and cytotoxic concentrations using direct microscopic observation instead of using traditional MTT assay or its analogs to determine $IC_{50}$ value. Although our method is laborious, it achieves specific and consistent results of drug effect. Moreover, these data can be beneficial in research of cancer cells. As a future direction, cytotoxic concentrations of DDAs could be used in the sequential treatment with microtubule targeting agents. In the scope of the future research, we plan to analyze the survival of multinucleated cells resulting from microtubule-stabilizing agents, such as Paclitaxel and Epothilone B.

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# 7 APPENDICES

**Table 6.** The average numbers with standard deviations (SD) of TMRM-positive cells untreated (control) and treated with 10nM-10uM MMC concentrations for 48h in A549, HeLa, PC-3, and HaCaT cells.

| MMC | A549 | | HeLa | | PC-3 | | HaCaT | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Control | 175,3 | 24,6 | 121,0 | 2,8 | 89,0 | 55,1 | 82,5 | 46,0 |
| 10nM | 142,7 | 16,0 | 119,0 | 9,9 | 84,8 | 49,2 | 76,5 | 37,5 |
| 30nM | 147,3 | 5,5 | 120,0 | 14,1 | 86,3 | 51,4 | 71,9 | 31,0 |
| 100nM | 156,7 | 16,9 | 103,0 | 15,6 | 80,8 | 43,5 | 72,6 | 31,9 |
| 300nM | 131,0 | 19,7 | 68,0 | 7,1 | 66,6 | 23,5 | 63,5 | 19,0 |
| 1µM | 75,3 | 2,1 | 54,5 | 9,2 | 52,4 | 3,4 | 57,6 | 10,7 |
| 3µM | 70,7 | 11,0 | 45,0 | 8,5 | 49,8 | 0,3 | 53,1 | 4,4 |
| 10µM | 56,0 | 5,6 | 43,5 | 9,2 | 48,4 | 2,2 | 41,9 | 11,4 |

**Table 7.** The average numbers with standard deviations (SD) of TMRM-positive cells untreated (control) and treated with 10nM-10uM DOX concentrations for 48h in A549, HeLa, PC-3, and HaCaT cells.

| DOX | A549 | | HeLa | | PC-3 | | HaCaT | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Control | 194,7 | 43,0 | 122,7 | 15,3 | 120,8 | 20,6 | 130,3 | 16,8 |
| 10nM | 91,3 | 9,8 | 87,2 | 12,0 | 126,7 | 16,5 | 104,3 | 6,0 |
| 30nM | 75,0 | 23,9 | 55,3 | 8,5 | 117,5 | 13,9 | 64,7 | 10,8 |
| 100nM | 58,0 | 14,0 | 51,3 | 3,2 | 72,5 | 1,8 | 48,8 | 2,3 |
| 300nM | 52,3 | 6,1 | 49,7 | 7,1 | 51,0 | 2,0 | 49,2 | 1,5 |
| 1µM | 29,0 | 14,9 | 11,7 | 0,6 | 49,7 | 3,8 | 36,7 | 1,9 |
| 3µM | 30,0 | 1,0 | 1,0 | 1,0 | 28,7 | 10,3 | 6,3 | 1,3 |
| 10µM | 30,3 | 9,6 | 1,0 | 0,0 | 26,5 | 8,3 | 1,7 | 0,8 |

**Table 8.** The average numbers with standard deviations (SD) of TMRM-positive cells untreated (control) and treated with 30nM-10uM CPT concentrations for 48h in A549, HeLa, PC-3, and HaCaT cells.

| CPT | A549 | | HeLa | | PC-3 | | HaCaT | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Control | 97,3 | 10,4 | 162,5 | 9,2 | 128,1 | 23,6 | 91,8 | 22,5 |
| 30nM | 76,7 | 19,9 | 154,5 | 19,1 | 108,3 | 15,0 | 96,2 | 13,4 |
| 100nM | 71,7 | 18,7 | 98,5 | 9,2 | 80,2 | 19,7 | 95,3 | 10,6 |
| 300nM | 72,8 | 12,3 | 57,0 | 2,8 | 71,1 | 16,1 | 81,7 | 10,1 |
| 1μM | 49,3 | 7,5 | 54,0 | 1,4 | 53,2 | 2,7 | 48,4 | 6,8 |
| 3μM | 46,0 | 6,6 | 43,0 | 7,1 | 48,5 | 4,5 | 31,7 | 2,9 |
| 10μM | 43,2 | 13,8 | 31,5 | 2,1 | 51,1 | 12,8 | 20,4 | 11,0 |