Chapter

Cytotoxicity Study

Bhavin R. Chavda and Bhavesh N. Socha

Abstract

The nature of being poisonous to cells is cytotoxicity. A number of cell fates can result in the treatment of cells with a cytotoxic compound. In this study, the nauplii were exposed to different concentrations of compounds for 24 hours. Experiments were concluded with control and different concentration of the compound. The whole set was triplicate to get an accurate result. The LC50 was determined from the best fit line, of a graph of % mortality vs. concentration of nauplii. The LC50 value was obtained from the antilogarithm of log [complex] at 50% mortality (LC50), all data were collected from three independent experiments.

Keywords: cytotoxicity study, Cytotoxicity, Ag-SMX

1. Introduction

The nature of being poisonous to cells is cytotoxicity [1–3]. In evaluating the possible toxicity of a research material, whether plant extracts or biologically active substances derived from plants, cytotoxicity tests are a helpful tool for initial step. For the effective production of a pharmaceutical or cosmetic preparation, minimal to no toxicity is necessary and cellular toxicity studies play a crucial role in this respect. While contemplating the interaction between acute toxicity and cytotoxicity, the principle of basal cytotoxicity, where deleterious effects are noted on structures and functions common to all human cells, is important [4–6]. To classify substances with promising biological activity and marginal cytotoxicity, the selectivity index is a significant test. In order to determine the cytotoxicity of African medicinal plants, various bioassays and a variety of different cell lines have been used. In addition, the extraction of solvents differing in polarity has been used to remove various parts of plants, adding to the wide range of African plants cytotoxicity findings [7–10].

A number of cell fates can result in the treatment of cells with a cytotoxic compound. The cells can undergo necrosis in which as a result of cell lysis, they lose membrane integrity and die quickly. The cells can stop growing and dividing aggressively (a decline in cell viability), or the cells can trigger a regulated cell death genetic programme (apoptosis).

Usually, cells undergoing necrosis show accelerated swelling, lose the cohesion of the membrane, shut down metabolism, and release their contents into the atmosphere. Cells undergoing rapid in vitro necrosis do not have enough time or resources to activate the apoptotic machinery and do not produce apoptotic markers [11–13]. Apoptosis is distinguished by well-defined cytological and molecular events, including cell refractive index transition, cytoplasmic shrinkage, nuclear condensation, and regular fragment cleavage of DNA. Cells undergoing apoptosis in culture ultimately undergo secondary necrosis. The metabolism will be shut down, membrane integrity and lysis will be destroyed.

In compound libraries, cytotoxicity assays are commonly used by the pharmaceutical industry to test for cytotoxicity. If they are interested in creating a therapeutic that targets, for example, rapidly dividing cancer cells, researchers can either look for cytotoxic compounds; or they can scan 'hits' from initial high-throughput drug screens for unintended cytotoxic effects before investing in their development as a pharmaceutical.

2. Examples

Examples of toxic agents are an immune cell or some types of venom, e.g. from the puff adder (*Bitis arietans*) or brown recluse spider (*Loxosceles reclusa*).

3. Application

The prediction of cytotoxicity of chemical compounds based on prior measurements, i.e. in-silico research, is a highly important topic. Several QSAR and virtual screening methods have been suggested for this purpose. In the "Toxicology in the 21st century" project, an objective comparison of these approaches has been carried out.

Chemotherapy as a cancer treatment also relies on the ability of cytotoxic agents to kill or destroy reproductive cells, preferably targeting the rapid division of cancer cells.

Antibody-dependent cell-mediated cytotoxicity (ADCC) explains the ability of certain lymphocytes to kill cells, and involves an antibody to recognise the target cell. On the other hand, cytotoxicity mediated by lymphocytes does not need to be mediated by antibodies; neither does complement-dependent cytotoxicity (CDC) mediated by the complement system.

4. Measurement

One of the most common approaches to test cell viability and cytotoxic effects is to determine cell membrane integrity. Compounds which have cytotoxic effects frequently undermine the integrity of cell membranes. Vital dyes such as trypan blue or propidium iodide are typically removed from the inside of healthy cells; however, they easily cross the membrane and stain components which is intracellular.

Alternatively, by controlling the movement of molecules that are usually sequestered within cells to the outside, membrane integrity can be measured. Using LDH research, one enzyme, lactate dehydrogenase (LDH), is normally tested. LDH reduces NAD to NADH, which, by contact with a particular probe, induces a colour transition.

Protease biomarkers have been established that enable researchers inside the same cell population to quantify relative numbers of live and dead cells. Live cell protease is involved only in cells with a stable cell membrane and, if the cell is infected and the protease is exposed to the external environment, it loses activity. After cells have lost their membrane integrity, the dead-cell protease can not cross the cell membrane, and can only be tested in culture media.

It is also possible to track cytotoxicity using 3-(4, 5-Dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) or 2,3-bis(2-methoxy-4-nitro-5-sulphophenyl)-2H-tetrazolium-5-carboxanilide (XTT) developed by a water-soluble substance or MTS assay. Using a colorimetric reaction, this assay tests the decreasing potential of the cell. The MTS reagent would be reduced to a coloured formazan substance by viable cells. Using the fluorescent dye, resazurin, a

related redox-based assay was also produced. In addition to using dyes to display cells' redox potential to track their viability, researchers have developed assays that use the substance of ATP as a viability marker. These ATP-based assays provide bioluminescent assays in which ATP is the luciferase reaction's limiting reagent.

Cytotoxicity can also be measured by the sulforhodamine B (SRB) assay, WST assay and clonogenic assay. Suitable assays can be combined and performed sequentially on the same cells in order to reduce assay-specific false positive or false negative results. A possible combination is LDH-XTT-NR (Neutral red assay)-SRB which is also available in a kit format.

A label-free approach to follow the cytotoxic response of adherent animal cells in real-time is based on electric impedance measurements when the cells are grown on gold-film electrodes. This technology is referred to as electric cell-substrate impedance sensing (ECIS). Label-free real-time techniques provide the kinetics of the cytotoxic response rather than just a snapshot like many colorimetric end-point assays.

1. A highly important topic is the prediction of cytotoxicity of chemical compounds based on previous measurements, i.e. in-silico testing [5]. For this purpose many QSAR and virtual screening methods have been suggested. An independent comparison of these methods has been done within the "Toxicology in the 21st century" project.

2. Chemotherapy as a treatment of cancer often relies on the ability of cytotoxic agents to kill or damage cells which are reproducing; this preferentially targets rapidly dividing cancer cells.

3. Antibody-dependent cell-mediated cytotoxicity (ADCC) describes the cell-killing ability of certain lymphocytes, which requires the target cell being marked by an antibody. Lymphocyte-mediated cytotoxicity, on the other hand, does not have to be mediated by antibodies; nor does complement-dependent cytotoxicity (CDC), which is mediated by the complement system.

5. Experimental details

In this study, the nauplii were exposed to different concentrations of compounds for 24 hours. Experiments were concluded with control and different concentration of the compound. The whole set was triplicate to get an accurate result. The LC50 was determined from the best fit line, of a graph of % mortality vs. concentration of nauplii. The LC50 value was obtained from the antilogarithm of log [complex] at 50% mortality (LC50), all data were collected from three independent experiments (**Figure 1**).

6. Materials and physical measurements

Sulfamethoxazole (SMX) sulfa derivatives and d-block transition metal Ag salts are used from Sigma Aldrich and Alfa Aesar respectively, all other reagents are of the highest grade, commercially available and used without further purification. The cellular level in vivo cytotoxic analysis of the free SMX molecule and its Ag -SMX complex were carried out using eukaryotic *S. pombe* (*Schizosaccharomyces pombe*) cell via trypan blue assay.

Figure 1.
125 ml live baby brine shrimp coral fish [14].

7. Synthesis of the complex

The silver complex of SMX was synthesized by sulfamethoxazole (SMX) ($C_{10}H_{10}N_3O_3S$, Sigma Aldrich) (0.5 gm, 2 mmol) dissolving in 15 mL methanolic solution (2 mmol, pH 8–9), the metal solution of silver nitrate hydrate ($AgNO_3$, Alfa Aesar) (0.37 gm, 2 mmol) dissolved in deionized water and added drop wise in SMX solution under reflux at 25°C temperature. After stirring for 3 hours, the resultant white colored precipitate of silver compound filtered and washed with mixture of methanol and deionized water (10 ml) respectively, dried in a desiccator. The anhydrous yield of the complex was around 70%. The CHN analysis results for $Ag[C_{10}H_{10}N_3O_3S_1]_2$ are: Anal. Cal. (%): C, 33.35; H, 2.78; N, 11.66. Found (%): C, 33.39; H, 2.94; N, 11.81. pure synthesized compound dissolved in β-picoline solvent and kept for recrystallization at 30°C constant temperature. After almost three months, colorless transparent diamond shaped single crystals were possible to grow (**Figure 2**).

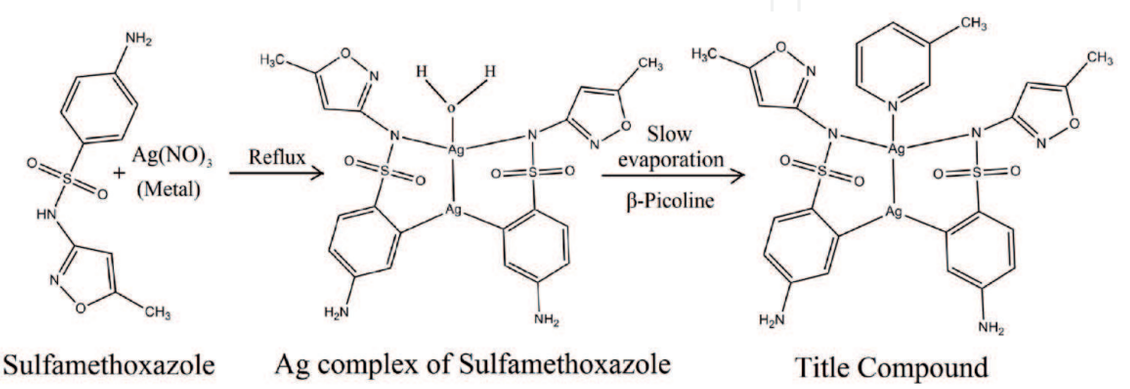

Figure 2.
Steps of reaction proposed.

8. Result and discussion

Brine shrimp lethality assay test protocol was carried out with control and different concentration of the compound. The whole set was triplicate to get an accurate result. The LC50 was determined from the best fit line, of a graph of % mortality vs. concentration of nauplii. The LC50 value was obtained from the antilogarithm of log [complex] at 50% mortality (LC50), all data were collected from three independent experiments. The LC50 (50% lethal concentration) value of the complex had been obtained from the plot of % of the brine shrimp nauplii killed against the complex concentrations and the best-fit line was found from the data through regression analysis. The significant LC50 value of the free Sulfamithoxazole (SMX) drug and its Ag-SMX complex are 12.88 μM and 6.30 μM respectively, predicting higher toxicity of SMX molecule than that of Ag-SMX complex and also, Ag-SMX complex displays less toxic behaviour compared to reported data of Cd sulfapyridine (8.32 μM) and Zn-sulfapyridine (6.31 μM) complexes [12].

The cellular level in vivo cytotoxic analysis of the free SMX molecule and its Ag -SMX complex were carried out using eukaryotic *S. pombe* (*Schizosaccharomyces pombe*) cell via trypan blue assay. **Figure 3** is the plot of % viability of the cells treated with a series of various concentrations 2, 4, 6, 8 and 10 μg/mL of free SMX molecule and its Ag-SMX complex along with control after 17 hours of treatment.

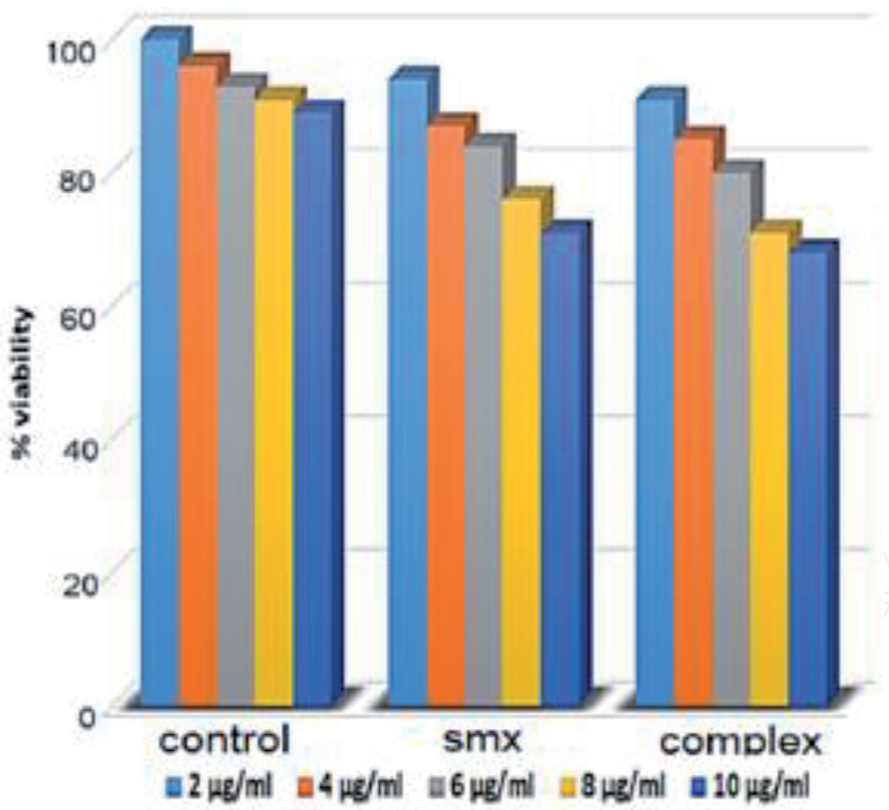

Figure 3.
The plot of % viability of the cells treated with a series of concentrations 2, 4, 6, 8 and 10 μg/mL of SMX molecule and its silver complex respectively along with control after 17 hours of treatment.

9. Conclusion

The significant LC50 value of the free Sulfamithoxazole (SMX) drug and its Ag-SMX complex are 12.88 μM and 6.30 μM respectively, predicting higher toxicity

of SMX molecule than that of Ag-SMX complex and also, Ag-SMX complex displays less toxic behaviour compared to reported data of Cd sulfapyridine (8.32 μM) and Zn-sulfapyridine (6.31 μM) complexes.

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References

[1] Lewinski, Nastassja, Vicki Colvin, and Rebekah Drezek. "Cytotoxicity of nanoparticles." small 4.1 (2008): 26-49.

[2] Korzeniewski, Carol, and Denis M. Callewaert. "An enzyme-release assay for natural cytotoxicity." Journal of immunological methods 64.3 (1983): 313-320.

[3] Russell, John H., and Timothy J. Ley. "Lymphocyte-mediated cytotoxicity." Annual review of immunology 20.1 (2002): 323-370.

[4] Banati, Richard B., et al. "Cytotoxicity of microglia." Glia 7.1 (1993): 111-118.

[5] Amos, D. B., et al. "A simple micro cytotoxicity test." Transplantation 7.3 (1969): 220-223.

[6] Chavda, Bhavin R., et al. "Coordination behavior of dinuclear silver complex of sulfamethoxazole with solvent molecule having static rotational disorder: Spectroscopic characterization, crystal structure, Hirshfeld surface and antimicrobial activity." Journal of Molecular Structure (2020): 129777.

[7] Chaudhary, Kaushik P., et al. "Coordination behavior of succinylsulfathiazole–Crystal structure of [Cu (SST).(Pyridine) 3. H2O] n, DNA interaction and cytotoxic studies." Journal of Molecular Structure 1225: 129262.

[8] Chaudhary, Kaushik P., et al. "Divalent zinc complex of succinylsulfathiazole: Synthesis, spectral, molecular structure, DNA interaction profile and Hirshfeld surface analysis." Inorganic Chemistry Communications 122 (2020): 108318.

[9] Socha, Bhavesh N., et al. "Gas-phase DFT studies, quantum chemical calculation and 3D energy framework analysis of novel copper complex of sulfadimethoxine in the presence of secondary ligand 3-methyl pyridine." Inorganic and Nano-Metal Chemistry (2020): 1-9.

[10] Dubey, Rahul P., et al. "Quantum chemical calculations, molecular docking, Hirshfeld surface analysis, biological activity, and characterizations (IR and NMR) of cadmium complex of triple sulfa drugs constituent sulfamethazine." Inorganic and Nano-Metal Chemistry (2020): 1-10.

[11] Pandya, Sachin B., et al. "Combined experimental and computational studies on molecular structure of nickel complex of 4-amino-N-(1, 3-thiazol-2-yl) benzenesulfonamide with coordinated pyridine." Inorganic and Nano-Metal Chemistry (2020): 1-11.

[12] Socha, Bhavesh N., et al. "Role of metal Cu (II) and Ag (I) on molecular structure of 4-amino-N-(2, 6-dimethoxy-pyrimidin-4-yl) benzenesulfonamide in presence of 3-methyl pyridine: Synthesis, spectral, crystallographic and DNA interaction studies." Polyhedron 188 (2020): 114696.

[13] Chavda, Bhavin R., et al. "A quantitative analysis of weak inter-molecular interactions & quantum chemical calculations (DFT) of novel chalcone derivatives." AIP Conference Proceedings. Vol. 1728. No. 1. AIP Publishing LLC, 2016.

[14] https://www.algaebarn.com/blog/live-foods/brine-shrimp/a-beginners-guide-to-brine-shrimp/