STUDY CYTOTOXIC EFFECT OF CISPLATIN DERIVATIVES OR DRUGS ON HUMAN CANCEROUS CELL LINES

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Abstract

Cell culture studies are very important and desirable, as they provide a system to study mechanism, direct access ,and evaluation of tissues. The technique of tissue culture is a valuable tool to study problems of clinical relevance, especially those related to diseases, screening, and studies of cell toxicity mechanisms. In the present study, heterocyclic aromatic cisplatin derivatives were used. Four cell lines viz. HepG2, ZR-75, HL-60, and Daudi were cultures in Dulbecco's Modified Eagle's medium (DMEM) medium. The cells were harvested after 24hr incubation. Various concentrations (10-100µl) of these eight cisplatin derivatives were used to determine their effect on cell proliferation. MTT- cell cytotoxicity assay was used to determine the effect of these cisplatin derivatives on various cell lines, the cytotoxic effect of eight cisplatin derivatives viz. 1100.001, 1100.002, 1100.003, 1100.004, 1100.006, 1100.008, 1100.009 and 1100.013 on cancerous cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Those selected cell lines are ZR-75, HL-60, Daudi, and HepG2. Various concentration of each compound was evaluated for this study.

Key words: cell lines, MTT assay, DMEM

1. Introduction

Cell culture studies are very important and desirable, as they provide a system to study mechanism, direct access ,and evaluation of tissues. The technique of tissue culture is a valuable tool to study problems of clinical relevance, especially those related to diseases, screening, and studies of cell toxicity mechanisms (Allen et al., 2005). The cellular mechanism can be studied in the cell which may suggest new potential drug targets. In the case of pathological-derived tissue, it has an interesting application in the evaluation of therapeutic agents that potentially may treat the dysfunction. In many studies, cell culture is used for screening of test drugs (Allen et al., 2005; Donato et al., 2008).Harvey (2001) state that in the 16th century, the cell culture phenomenon was discovered. They observed that a piece of myocardium kept in the palm of his hand covered in his own saliva could remain contractile for extended periods. In this context, Rensberger (1998), late in 19th century, showed that embryonic chick cells can be successfully maintained on saline solution In vitro. Harrison (1910) showed that amphibian spinal cord can be cultured in a lymph clot and axons are produced in vitro at the end of the single nerve cells. However, they found that the very soon the formed cultures cells eventually underwent senescence and death. Effect of test molecules / cisplatin derivatives on cell proliferation can be assessed by cell-based assays which show direct cytotoxic effects that eventually lead to cell death. Cell-based assays also are routinely used for measuring receptor binding and a variety of signal transduction events that may involve the expression of genetic reporters, trafficking of cellular components, or monitoring organelle function (Romijn et al., 1988). It is important to know how many viable cells are remaining at the end of the experiment, regardless of the type of cell-based assay being used. There are many assay

methods that can be used to estimate the number of viable cells. Those methods are tetrazolium reduction, resazurin reduction, protease markers, and ATP detection. Methods for flow cytometry and high content imaging can be also done.Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are usually done in the dark since the MTT reagent is sensitive to light.Measurement of cell proliferation have been done with radioactive tritiated thymidine incorporation assay but now a day's MTT assay was used and it was first widely accepted method that replaced the previous method. However, there are some limitations associated with using the MTT assay. Then also, this method is reliable, reproducible and fast. Many studies have reported the use of MTT assay for cell viability test (Slater et al., 1963; Romijn et al., 1988; Berridge and Tan, 1993; Berridge et al., 1996). The MTT test gives a high degree of precision and is easy to do, it is suitable for the purpose of (large-scale) chemosensitivity testing (Romijn et al., 1988).The cisplatin derivatives used in this study contain heterocyclic aromatic molecules and those cisplatin derivatives are cisplatin derivatives. Aromatic rings (also known as aromatic cisplatin derivatives or arenes) are hydrocarbons that contain benzene, or some other related ring structure. Benzene, C6H6, is often drawn as a ring of six carbon atoms, with alternating double bonds and single bonds:In the present study four cell lines were selected. The detailed information about the cell line was given below.HepG2 cell line Derivation: HepG2 was derived from a liver hepatocellular carcinoma of a 15-year-old Caucasian male. ZR-75 cell line Derivation: ZR-75 cell line was derived from a malignant ascitic effusion in a 63 year old female Caucasian with infiltrating ductal carcinoma. Both the wild type and variant oestrogen receptors, progesterone receptor and other steroid hormones are expressed in this cell line. HL-60 cell line is a promyelocytic cell line derived by S.J. Collins, et al. Peripheral blood leukocytes were obtained by leukopheresis from a 36-year-old Caucasian female with acute promyelocytic leukemia. Daudi cell line was derived from a 16-year-old Black male with Burkitt's lymphoma by E. Klein and G. Klein in May, 1967.

2. Material and methods

2.1 Cell culture

The culture medium was removed from the culture flask. The fresh medium was prepared using 25 ml of DMEM, 2.5 ml of Serum, 1 ml of penicillin antibiotics, 0.5ml of Nestytin. The fresh medium was poured on the cultured cells in aseptic conditions. The cultured flasks were incubated at 37°C for 24 hr in a CO_2 incubator**.**

2.4 Harvesting of cells

After 24 hr incubation of all cell lines, the culture flasks were observed under the inverted light microscope (Labomed, model TCM 400) for cell growth. The cell line was then scraped and used for the MTT-based cytotoxicity assay.

2.3 Test (cisplatin derivatives) sample collection

Eight test cisplatin derivatives were collected from Wadia College. These compound names are depicted in Table 1. All these cisplatin derivatives are cisplatin derivatives**.**

Table 1: List of cisplatin derivatives selected for the study.

Cisplatin derivatives number	Cisplatin derivatives name
1	**1100.001**
2	**1100.002**
3	**1100.003**
4	**1100.004**
5	**1100.006**
6	**1100.008**
7	**1100.009**
8	**1100.013**

2.4 Preparation of sample stocks

Each drug was dissolved in dimethylsulfoxide (DMSO). One mg per ml stock was prepared. The stock was prepared freshly and used for the MTT-based cytotoxicity assay.

2.5 MTT-based cytotoxicity assay

In the 96-well plates, 10µl cell culture suspension was added. To this 10-100µl test cisplatin derivatives were added. In the control reaction/well 100 µl DMSO was added. The plate was allowed to incubate at 37°C for 28hr in a CO2 incubator. After incubation 10µl MTT dye was added to each well. The plate was wrapped carefully with aluminum foil and incubated at 37°C for 4hr in a CO2 incubator.

After incubation, a solution containing 100µl DMSO and 20µl of glycine buffer was added to each well. The supernatant was taken in separate 96 well plate and read the absorbance at 595nm in ELASA plate reader (Bio-Rad, i-Mark).

2.6 Statistical analysis

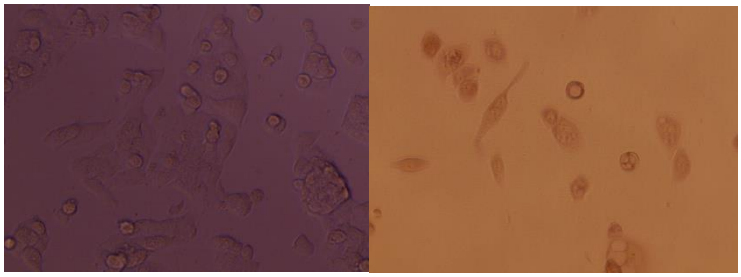
All the assays were performed in triplicate. Results were presented as Mean ± Standard Error (SE).

3. Results

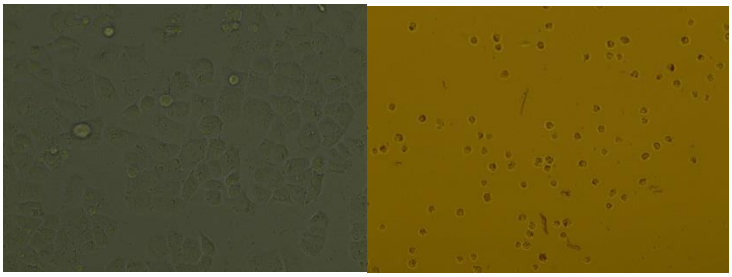
In this study, we have evaluated the cytotoxicity of selected eight cisplatin derivatives against four cancerous cell lines by MTT-based cytotoxicity assay. The 8-cisplatin derivatives are namely 1100.001, 1100.002, 1100.003, 1100.004, 1100.006, 1100.008, 1100.009 and 1100.013. The four-cell line viz. ZR-75, HL-60, Daudi and hepG2 were used in the present study.

3.1 Cell culture

The cells are subcultures in the DMSO medium and incubated at 37°C for 24hr in CO_2 incubator. Figure 1 showed the culture flasks after cells inoculation. The cultures were observed under inverted microscope for cell growth and characteristics. Figure 1 depicts the cell growth under inverted microscope.



(A) (B)



(C) (D)

Figure 1: Microscopic images of the (A) HepG2 cell line, (B) ZR-75 cell line, (C) HL-60 cell line and (D) the Daudi cell line.

All Cultures were incubated at 37°C for 24 hr in CO2 incubator

3.2 Harvesting of cells

The cells were harvested successfully and used for further study.

3.3 Preparation of sample stocks

Each time fresh cisplatin derivatives stock were prepared and used in the MTT assays

3.4 MTT-based cytotoxicity assay

The eight-cisplatin derivatives were evaluated for their cytotoxic potential against four different cell lines. The percentage survival was showed in the Tables 1-4.

4.4.1. Effect of cisplatin derivatives in the HepG2 cell line

Figure 5.8A-H depicts effect of cisplatin derivatives on percentage of survival of HepG2 cell line at various concentrations. Table 5.1 shows effect of cisplatin derivatives on the percentage of survival of HepG2 cell line. Varied patterns of the survival were observed at various concentrations.

Cisplatin derivatives 1100.002, 1100.004, 1100.006, 1100.008 and 1100.013 showed maximum survival percentage at a concentration 80µl. while Cisplatin derivatives 1100.001 and 1100.003

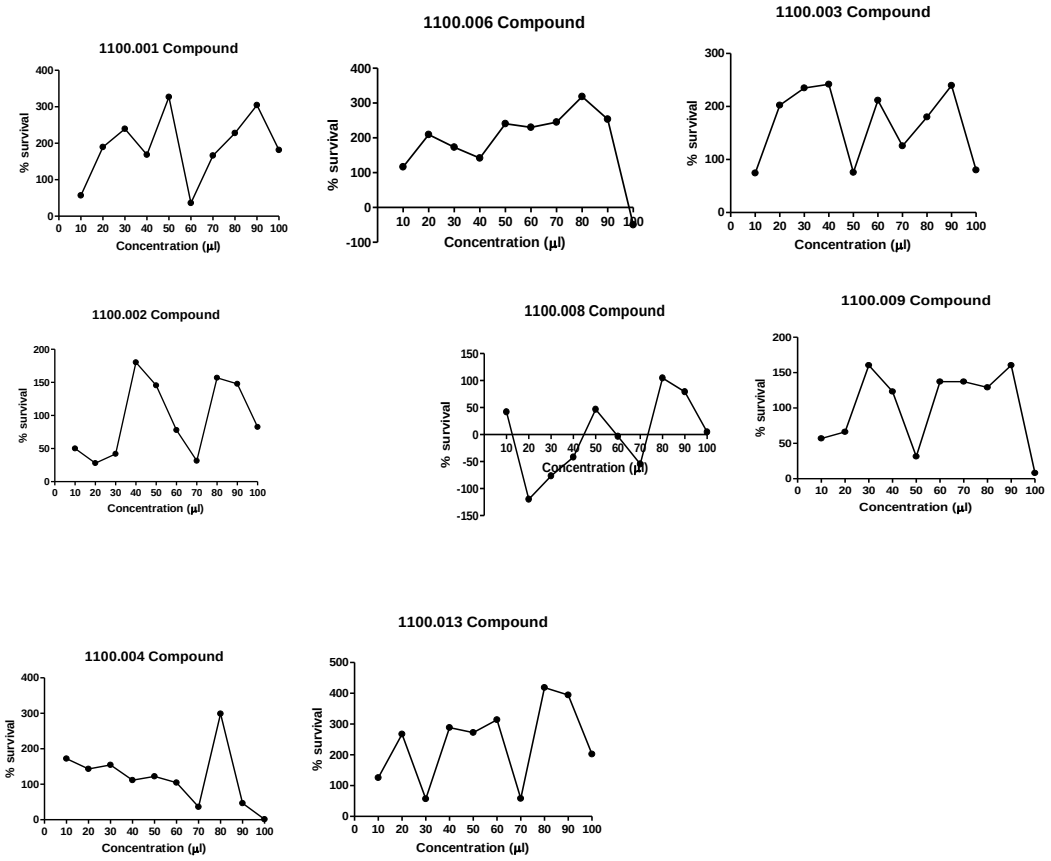
showed maximum survival percentage at a concentration 50µl and 90µl respectively. Compound 1100.009 showed maximum survival percentage at two concentrations i.e. 30µl and 90µl.

Cisplatin derivatives 1100.001, 1100.002, 1100.003, 1100.008, and 1100.013 showed minimum survival percentage at a concentration 60, 20, 10, 20 and 70µl respectively. Compounds 1100.004, 1100.006 and 1100.009 were observed minimum survival percentage at a concentration 100µl.

Table 5.1: Percentage survival of HepG2 cell line after treatment of selected cisplatin derivatives

Concentration	1100.001	1100.002	1100.003	1100.004	1100.006	1100.008	1100.009	1100.013
10 µl	56.98	50.00	74.42	172.09	116.28	41.86	56.98	125.58
20 µl	189.53	27.91	202.33	143.02	209.30	-119.77	66.28	267.44
30 µl	239.53	41.86	234.88	154.65	173.26	-76.74	**160.47**	56.98
40 µl	168.60	180.23	241.86	111.63	141.86	-41.86	123.26	288.37
50 µl	**326.74**	145.35	75.58	122.09	240.70	46.51	31.40	272.09
60 µl	36.05	77.91	211.63	104.65	230.23	-3.49	137.21	313.95
70 µl	166.28	31.40	125.58	36.05	245.35	-54.65	137.21	58.14
80 µl	227.91	**156.98**	180.23	**298.84**	**318.60**	**104.65**	129.07	**418.60**
90 µl	304.65	147.67	**239.53**	46.51	253.49	79.07	**160.47**	394.19
100 µl	181.40	82.56	80.23	1.16	-50.00	4.65	8.14	202.33

re 5.8: Effect of the selected cisplatin derivatives on HepG2 cell line[$]



5.4.2. Effect of cisplatin derivatives in the ZR-75 cell line

Figure 5.9A-H depicts the effect of cisplatin derivatives on percentage of survival of ZR-75 cell line at various concentrations. Table 5.2 shows effect of cisplatin derivatives on the percentage of survival of ZR-75 cell line. Varied patterns of the survival were observed at various concentrations.

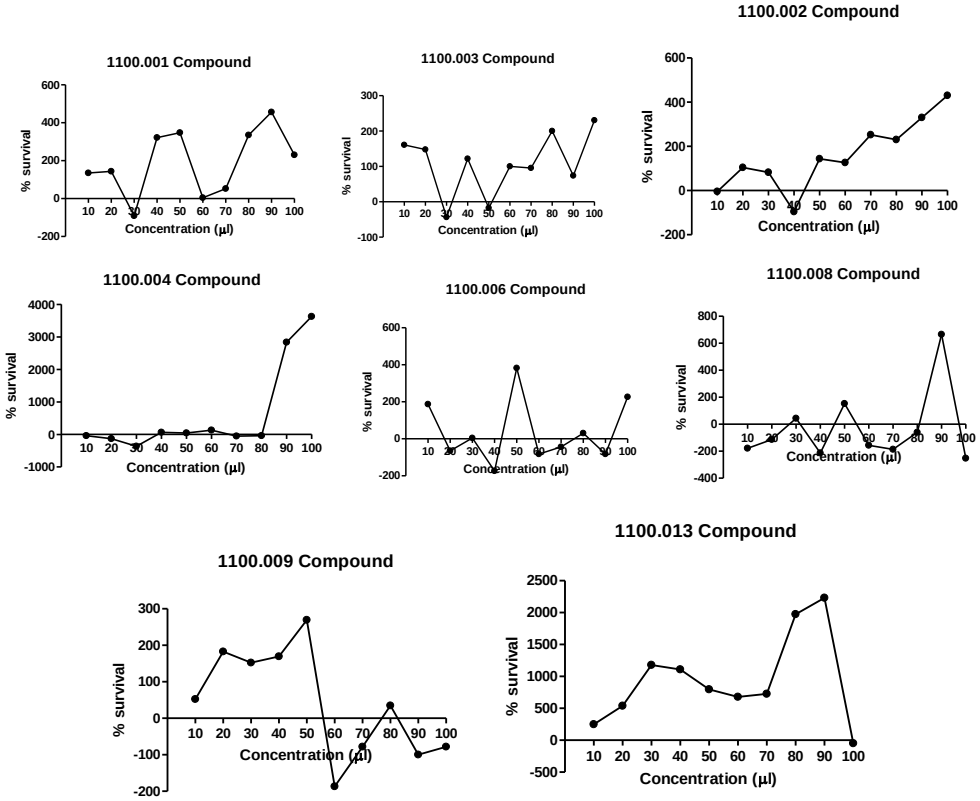
Cisplatin derivatives 1100.001, 1100.008 and 1100.0013 showed maximum survival percentage at concentration 90µl. Cisplatin derivatives 1100.002, 1100.003 and 1100.004 showed maximum survival percentage at concentration 100µl. Cisplatin derivatives 1100.006 and 1100.009 showed maximum survival percentage at 50µl.

Cisplatin derivatives 1100.001, 1100.003 and 1100.004 showed minimum survival percentage at 30µl. Cisplatin derivatives 1100.002 and 1100.006 showed minimum survival percentage at concentration 40µl. Cisplatin derivatives 1100.008 and 1100.013 showed minimum survival percentage at a concentration 100µl while compound 1100.009 showed minimum survival percentage at a concentration 60µl.

Figure 5.2: Percentage survival of ZR-75 cell line after treatment of selected cisplatin derivatives

Concentration	1100.001	1100.002	1100.003	1100.004	1100.006	1100.008	1100.009	1100.013
10 µl	134.78	-4.35	160.87	-39.13	186.96	-178.26	52.17	247.83
20 µl	143.48	104.35	147.83	-130.43	-65.22	-113.04	182.61	539.13
30 µl	-91.30	82.61	-43.48	-365.22	4.35	43.48	152.17	1178.26
40 µl	321.74	-95.65	121.74	65.22	-173.91	-213.04	169.57	1108.70
50 µl	347.83	143.48	-17.39	43.48	**382.61**	152.17	**269.57**	795.65
60 µl	4.35	126.09	100.00	130.43	-82.61	-156.52	-186.96	678.26
70 µl	52.17	252.17	95.65	-52.17	-43.48	-186.96	-78.26	726.09
80 µl	334.78	230.43	200.00	-39.13	30.43	-60.87	34.78	1973.91
90 µl	**456.52**	330.43	73.91	2834.78	-82.61	**665.22**	-100.00	**2230.43**
100 µl	230.43	**430.43**	**230.43**	**3626.09**	226.09	-252.17	-78.26	-47.83

Figu 5.9: Effect of the selected cisplatin derivatives on ZR-75 cell line[$]



5.4.3. Effect of cisplatin derivatives in the HL-60 cell line

Figure 5.10A-H depicts the effect of cisplatin derivatives on percentage of survival of HL-60 cell line at various concentrations. Table 5.3 shows effect of cisplatin derivatives on the percentage of survival of HL-60 cell line. Varied patterns of the survival were observed at various concentrations.

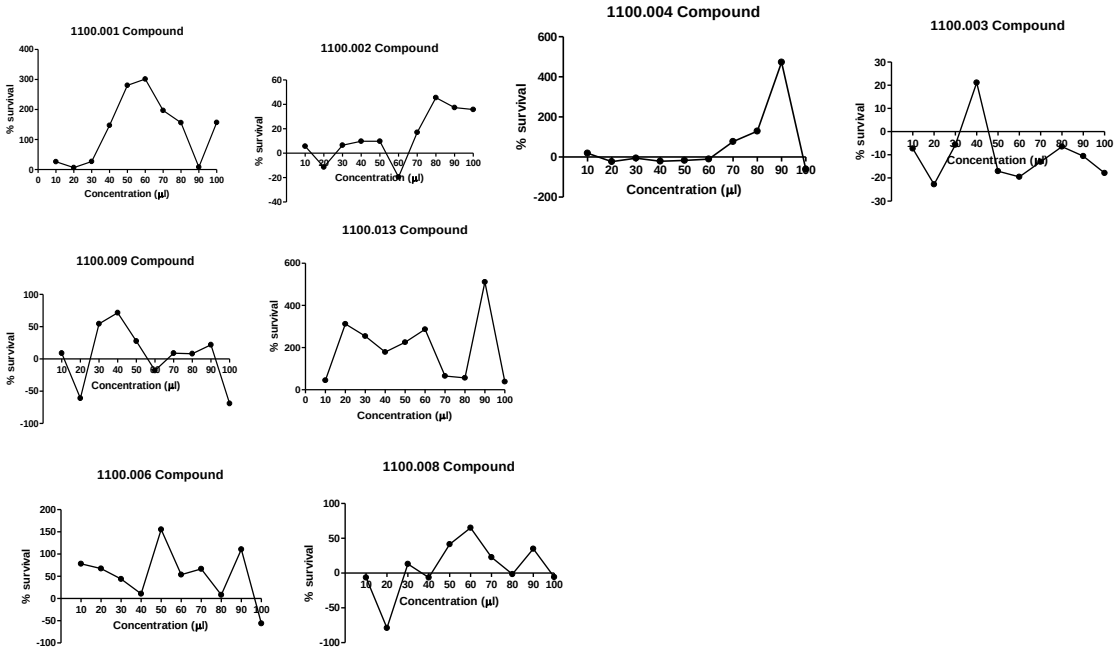
Cisplatin derivatives 1100.001 and 1100.008 showed maximum survival percentage at a concentration 60µl. Cisplatin derivatives 1100.003 and 1100.009 showed maximum survival percentage at a concentration 40µl. Cisplatin derivatives 1100.004 and 1100.013 showed maximum survival percentage at 90µl. Cisplatin derivatives 1100.002 and 1100.006 showed maximum survival percentage at 80µl and 50µl respectively.

Cisplatin derivatives 1100.001, 1100.003 and 1100.008 had minimum survival percentage at a concentration 20µl. Cisplatin derivatives 1100.004, 1100.006, 1100.009 and 1100.013 showed minimum survival percentage at a concentration 100µl while compound 1100.002 showed minimum survival percentage at a concentration 60µl.

Figure 5.3: Percentage survival of HL-60 cell line after treatment of selected cisplatin derivatives

Concentration	1100.001	1100.002	1100.003	1100.004	1100.006	1100.008	1100.009	1100.013
10 µl	26.02	5.69	-7.32	18.70	78.05	-6.50	8.94	44.72
20 µl	6.50	-11.38	-22.76	-22.76	67.48	-78.86	-60.98	312.20
30 µl	26.83	6.50	-5.69	-5.69	43.90	13.01	54.47	254.47
40 µl	147.15	9.76	**21.14**	-21.14	10.57	-6.50	**71.54**	178.86
50 µl	280.49	9.76	-17.07	-17.07	**155.28**	41.46	27.64	225.20
60 µl	**301.63**	-19.51	-19.51	-10.57	53.66	**65.04**	-17.89	286.99
70 µl	196.75	17.07	-13.01	77.24	66.67	22.76	8.94	65.04
80 µl	156.10	**45.53**	-6.50	129.27	8.13	-1.63	8.13	56.10
90 µl	8.13	37.40	-10.57	**473.17**	110.57	34.96	21.95	**511.38**
100 µl	156.91	35.77	-17.89	-61.79	-56.10	-5.69	-69.11	38.21

re 5.10: Effect of the selected cisplatin derivatives on HL-60 cell line[$]



$The results are represented as means of three replicated.

% survival = (ODsample / ODcontrol)*100

5.4.4. Effect of cisplatin derivatives in the Daudi cell line

Figure 5.11A-H depicts the effect of cisplatin derivatives on percentage of survival of Doudi cell line at various concentrations. Table 5.4 shows effect of cisplatin derivatives on the percentage of survival of Doudi cell line. Varied patterns of the survival were observed at various concentrations.

Cisplatin derivatives 1100.001 and 1100.002 had maximum survival percentage at a concentration 100µl. Cisplatin derivatives 1100.004, 1100.006, 1100.008 and 1100.009 had maximum survival

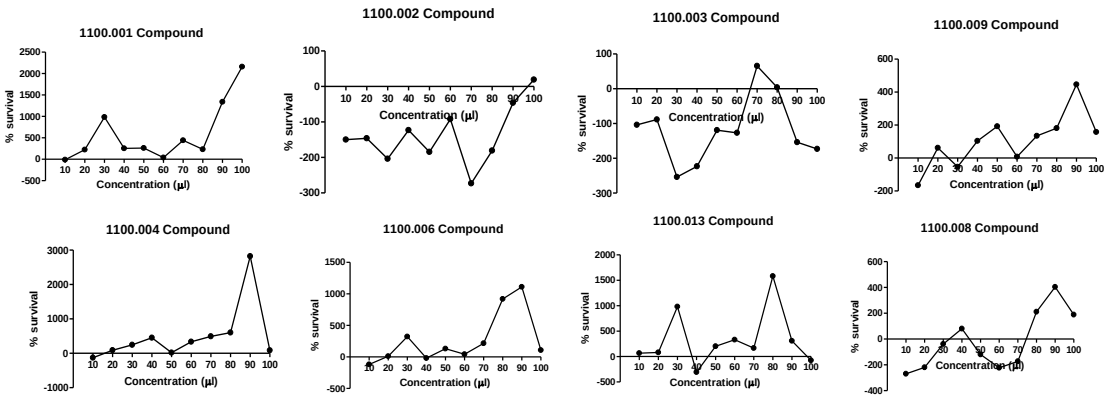
percentage at a concentration 90µl. Cisplatin derivatives 1100.003 and 1100.013 showed maximum survival percentage at 70µl and 70µl respectively.

Cisplatin derivatives 1100.001, 1100.004, 1100.006, 1100.008 and 1100.009 had minimum survival percentage at a concentration 10µl. Cisplatin derivatives 1100.002, 1100.003 and 1100.013 showed minimum survival percentage at a concentration 70µl, 30µl and 40µl respectively.

Table 5.4: Percentage survival of Daudi cell line after treatment of selected cisplatin derivatives

Concentration	1100.001	1100.002	1100.003	1100.004	1100.006	1100.008	1100.009	1100.013
10 µl	-11.54	-150.00	-103.85	-126.92	-119.23	-269.23	-165.38	65.38
20 µl	223.08	-146.15	-88.46	88.46	11.54	-219.23	61.54	80.77
30 µl	984.62	-203.85	-253.85	246.15	323.08	-38.46	-53.85	980.77
40 µl	253.85	-123.08	-223.08	450.00	-19.23	80.77	103.85	-307.69
50 µl	261.54	-184.62	-119.23	19.23	130.77	-119.23	192.31	200.00
60 µl	38.46	-92.31	-126.92	334.62	42.31	-223.08	7.69	330.77
70 µl	442.31	-273.08	**65.38**	492.31	215.38	-173.08	134.62	165.38
80 µl	230.77	-180.77	3.85	600.00	919.23	211.54	180.77	**1580.77**
90 µl	1338.46	-46.15	-153.85	**2823.08**	**1111.54**	**403.85**	**446.15**	307.69
100 µl	**2157.69**	**19.23**	-173.08	84.62	107.69	188.46	157.69	-76.92

Figure 5.11: Effect of the selected cisplatin derivatives on Daudi cell line[$]



In the present study, all cisplatin derivatives showed varied rate of cell survival in all four selected cell lines.

Discussion

In the present study, we studied eight heterocyclic aromatic cisplatin derivatives against four cancer cell line (HepG2, ZR-75, HL-60 and Daudi). The percent viability is assessed by MTT cytotoxicity assay.

Donato et al. (2008) proposed different cell line models for drug metabolism screening. Human liver-derived cell lines would be ideal models for this purpose given their availability, unlimited life span, stable phenotype, and the fact that they are easy to handle. Threes are many limitations of traditional method such as in vitro phenotypic instability of hepatocytes, the irregular availability of fresh human liver for cell harvesting purposes, and the high batch-to-batch functional variability of hepatocyte preparations obtained from different human liver donors, seriously complicate their use in routine testing.

Romijn et al (1988) studied the MTT assay for the quantitation of growth modulating effects on prostate cancer lines on cultured. Dose-dependent reduction of MTT converting activity was observed, reflecting the impaired survival of the drug-treated cells. Good correlations of the results obtained with the MTT-test, as compared with a thymidine incorporation assay or with direct DNA measurements, were observed. Mode of action of the drugs was be measured by the serial measurements. This can be used to discriminate between cytostatic and cytotoxic drug effects (Romijn et al., 1988). Ho et al. (2012) studied cytotoxicity effect of tamoxifen on breast cancer cell line (MCF-7) and reported to have its cytotoxic effect. Results are in correlation with the cultures' response assessed by (lactate dehydrogenase) LDH release assay. LDH release assay was cannot used for detecting a wide range of cytotoxicity due to high basal background reading. Hence, the author used MTT assay for a better indicator to apoptosis event in comparison to the LDH release assay.

Price and McMillan (1990) studied three human tumor cell lines of widely differing radio sensitivity were used to examine the characteristics of the 3-4, 5-dimethyI (thiazol-2-yl)- 3, 5-diphery tetradium bromide (MTT) assay and to select suitable conditions for its use in assessing the response of cells to ionizing radiation. The relationship between absorbance and cell number was not linear over the wide range of cell numbers that were used. Our results are in accordance with this report. In the present study, all cisplatin derivatives showed varied patterns of percent survival. Price and McMillan (1990) used calibration curve of absorbance against cell number for each cell line. When cells were in exponential growth, accurate surviving fractions were calculated. Using this modification to its interpretation, the MTT assay was able to provide a reproducible measure of survival, which compared well with clonogenic cell survival measurements.

Carmichael et al. (1987) reported significant degrees of cell killing and low number of viable tumor cells using the dye exclusion assay. These results agree with previously published data, claiming good correlation between dye exclusion assays and clonogenic assays (Weisenthal et al., 1983; Bosanquet et al., 1985). This particular dye exclusion assay has the advantage over other short-term assays, in that tumor cells can be distinguished from non-tumorous cells by differential staining techniques, which is beneficial in the testing of primary tumor samples. The dye exclusion assay used in this study, is highly labor intensive, particularly at the time of reading. The MTT assay, in contrast, offers an excellent opportunity for the rapid testing of large numbers of drugs with good reproducibility, particularly in adherent cell lines. However, the assay is less optimal for the testing of floating cell lines where high degree of standard deviations was observed. The increased variability in absorbance with floating cultures may be caused by variation in residual volume following aspiration of medium, prior to solubilization of the MTT formazan. This problem could be resolved by the production of a tetrazolium salt, which on reduction forms a water-soluble formazan dye, allowing microtiter plates to be read directly.

Therefore, in the present study, all cisplatin derivatives showed aberrant cell toxicity for selected four cell lines.

Conclusion

The selected cisplatin derivatives namely, 1100.001, 1100.002, 1100.003, 1100.004, 1100.006, 1100.008, 1100.009 and 1100.013 did not showed any cytostatic and cytotoxic effect on all four-cell lines (HepG2, ZR-75, HL-60 and Daudi).

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