Evaluation Of Cell Viability Of Leukemic Cell Using MTT

In cancer research, viability tests are immensely significant. These tests are used to observe the characteristics of different kinds of cancer. In drug development, viability of hostile cells are tested against chemical substances to evaluate the potential of those substances in pharmaceutical use. Additionally, the adequate dosage for those drugs are also studied in vitro. Established in 1977 from a patient with acute myeloid lymphoma in the National Cancer Institute in the United States, human Caucasian promyelocytic leukemia (HL-60) cells are used in a variety of chemotherapeutic researches (Jackobson, 2020). The cells are characterised by the ability to self differentiate and being manipulated to differentiate continuously in vitro. Recent experiments have also given rise to specialised HL-60 cell lines.

Viability is the survival tendency of organisms. Microorganisms and single cells are studied under cytotoxic conditions to assess viability. In this study, the viability of a HL-60 cell line has been evaluated using MTT assay. The concentration of the cells have been assessed using haemocytometer. After the achievement of desired concentration, the cells have been subjected to cytarabine or cytosine arabinoside (Ara-C). The drug is used to treat several kinds of leukemia and lymphoma (Higginset al. 2018). The assessment of vitality has been done through chromatographic method. Dimethyl sulfoxide (DMSO) has been used for solubilisation of formazan crystals. The final goal of the study is to better understand the properties of HL-60 cells and overall leukemia itself. Moreover, the effects of cytarabine on leukemia have also been explored.

Method

20 ml of the provided suspension of HL-60 was taken into an eppendorf tube. 20 ml trypan blue was added to the tube. The mixture of cell suspension and trypan blue was mixed well and 20ml of it was transferred to one of the chambers of a haemocytometer by carefully touching the cover slip's edge with a pipette tip, containing the solution. The four corner squares of the haemocytometerwere observed under a microscope. The blue dots representing dead cells and the white dots representing the live cells in each chamber were counted separately. The dots on the inward left boundary and the outward bottom boundary were also taken into account.

The number of live cells and the number of dead cells were added to calculate the total number of cells. The number of viable cells in the four chambers were divided by four to calculate the average number of viable cells in each chamber. Similarly, the average number of dead cells in each chamber was counted. The average number of viable cells present in 1 ml of the suspension was counted using the volume of a single chamber and the ratio of cell suspension and trypan blue in the solution. Cell suspension containing 40000 cells was added to the wells of the MTT assay plate as marked in Figure-2. The volume of cytarabine was measured for four different concentrations with the common volume of 5ml each. Using sterile pipettes, the drug solutions were transferred to the cell containing wells on the plate. The culture was incubated overnight (24 hours) at 37° C. After incubation, 200ul MTT reagent was added to each cell containing well. The plate was covered in aluminium foil and incubated for 4-6 hours in humid air with 5% CO2 at 37° C. After 2 hours, the plate was taken out of the incubator. 100ul DMSO was added to the wells with cells. The cells were resuspended manually,

Results

Each chamber of the haemocytometer is 1 mm in length and 1 mm in width. Therefore, the surface area is 1 square mm. The height of each chamber with cover glass is 0.1 mm. Therefore, the volume of each chamber is 1 mm2 x 0.1 mm = 0.1mm3. Therefore, each chamber contained 0.1mm3 or 10-4ml trypan blue-cell suspension solution. The number of viable cells in the four corner chambers was 23, 23, 17, and 12. Thus, the average number of viable cells in each chamber is (25+23+17+12)/4 = 19.25 cell/ml.

The ratio of cell suspension and trypan blue in the solution was 1:1. Therefore, the dilution factor for cell suspension in the solution is, (1+1=)2. The average concentration of viable cells in each chamber is, (the average number of viable cells in each chamber/the volume of trypan blue-cell suspension in each chamber) or (19.25/10-4ml). Therefore the concentration of viable HL-60 cells in the pure cell suspension was, (19.25 x 2 x 104 ml) cells/ml or 385000 cells/ml or 385 cells/µl.

Therefore, the volume of cell suspension containing 40000 viable cells is, (40000/ number of viable cells in one µl cell suspension) µl or (40000/385) µl or 103.89 µl. Therefore, 103.89 µl cell suspension containing 40000 cells was added to each well.

The total stock solution of cytarabine consisted of 1000uM. The required volume of cytarabine to be added to each well is 5ml. The required 4 concentrations of cytarabine are, 0.25µM, 1µM, 5µM and 10µM. The respective amounts of diluent (DMSO) required for creating each concentration of cytarabine are calculated using the following formula. C1V1=C2V2 or V2 = C1V1/C2, where C1 = Initial concentration of cytarabine, V1 = Initial volume of the solution, C2 = Final concentration of cytarabine and V2 = Final volume of the solution. The volume of each necessary cytarabine solution of concentrations mentioned earlier is 5ml.

The volume of diluent (DMSO) to be added to each cytarabine solution was calculated by subtracting the V2 value for each solution from 5000ul. Table-1 depicts the volume of the cytarabine (stock) and diluents (DMSO) required to prepare cytarabine solutions of 0.25µM, 1uM, 5uM and 10µM respectively.

After the addition of cytarabine to the wells containing viable HL-60, and incubation for 6-8 hours, the MTT assay was performed. The recorded OD for each well containing different concentrations of cytarabine at 570nm are listed in Table-2

Discussion

Trypan blue is a low cost vital stain, and is excluded from live cells. Live cells possess intact cell membranes, which prohibit the entry of trypan blue into the cytoplasm (Lebeauet al. 2019). On the other hand, the membrane integrity of dead cells is not intact. Therefore, trypan blue can easily enter dead cells cytoplasm. When the stain is applied to a cell suspension, the alive cells do not accumulate trypan blue whereas dead cells do. When the suspension is subjected to microscopic observation, the dead cells appear deep blue, but the viable cells appear clear. On the haemocytometer, only one chamber is filled with the solution of cell suspension and stain. The solution was filled into the other chambers by capillary action. The transfer was carefully controlled so as to not overfill or underfill the chamber. Average number of viable cells present

in one chamber of the haemocytometer indicates the number of live cells present in 0.1mm3 of the provided HL-60 cell suspension. The calculation of average number of cells present in 1ml sample helped distribute more or less equal number of cells in each well in MTT assay.

Cytarabine or cytosine arabinoside (arc-c) is an antineoplastic chemotherapeutic compound used to treat different forms of leukemia through cytotoxicity (Zhang et al. 2018). Cytarabine is generally administered through intravenous or intra tracheal infusion (Islam, 2017). Intrathecal infusion is generally done to transfer the drug to cerebrospinal fluid (CSF). Due to its antimetabolite property, cytarabine seizes the multiplication of cells upon incorporation. This occurs due to termination of the cell cycle. Upon entering a cell, Ara-C is rapidly converted into ara-CTP and ara-U. The former of these two substances inhibits DNA polymerase. It renders the cell's DNA unable to replicate. A portion of Ara-C also incorporates into the DNA in the cell. The drug is cell cycle specific. The application of cytarabine involves several drawbacks. The side effects of the drug include headache, nausea and temporary decrease in blood RBC count. With precise administration, cytarabine offers promising results in cancer treatment. However, it is very important to accurately assess the quantity of the drug to be administered. Although the amount depends upon the patient's characteristics, it should be sufficient to draw significant results. Ara-C is an antimetabolite. The drug has been used in this experiment to evaluate the viability of HL-60 cells. The cell line has been administered with different concentrations of the drug and the results have been tested using the MTT assay.

Thiazolyl blue tetrazolium bromide (MTT) reduction assay is one of the most useful colorimetric assays for cytotoxicity measurement (Tobólskaet al. 2018, p.216). The method is based on the conversion of (3-[4,5- dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide or MTT into insoluble crystals of formazan. Upon entering a cell, MTT is subjected to mitochondrial enzymes. These enzymes convert MTT into formazan (Adan et al. 2016). On the other hand, dead cells do not form formazen. Therefore, the OD of dead cells and live cells subjected to MTT show different OD during colorimetry. When the formazan crystals are subjected to dimethyl sulfoxide (DMSO), the crystals dissolve. As an organosulfur dipolar aprotic solvent (Nguyen et al. 2019), DMSO dissolves both polar and non polar compounds. The formazan concentration is measurable by employing wavelengths between 540nm and 720nm. The cytotoxicity of a drug is generally evaluated based on the IC50 value. The half maximum concentration or IC50 is the potency of a substance to decrease 50% of the original viability of a cellular sample in vitro. The IC50 standard is common practice in drug evaluation.

In this experiment, the IC50 value of cytarabine has been measured. The IC50 value is greatly dependent on the characteristics of the drug being used and the sample. The results show that the change in viability of HL-60 cells does not change consistently with change in drug concentration. In the graph depicted in Figure-3, cell viability is a logarithmic function of the concentration of Ara-C. Between the drug concentration of 0.25uM and 1uM, the viability of the cells changed at a high rate. Between 1uM and 10uM, this rate is slower. Despite the increase in rational difference between two concentrations. Such phenomenon indicates that a third entity or property may be influencing the viability of the HL-60 cells. However, Chen et al.(2017) in their study on YAP inhibition effects on HL-60 cells observed no additional effects of DMSO on the viability of the leukemia cells. Kao et al. (2019) in their study also observed no effect of DMSO on HL-60 cells. Yet, the study conducted by Kao et al. was conducted in an Ara-C free medium. Many other researchers have closely studied the combined effect of the two substances (Papie? M. and Krzy?ciak, 2018). Although the researchers have not observed any additional effects of DMSO on Ara-C led apoptosis on HL-60 cells. On the contrary, DMSO has

been monitored to decrease cell proliferation in peripheral blood lymphocytes (Henrique et al. 2017). The deviation of decrease in viability can be caused by the intrinsic properties of the drug itself. However, the study itself can also be affected by various limitation. The nutrophile property of the cell line makes it vulnerable to cryopreservation related drawbacks (Al-Otaibiet al. 2019). The pre preservation and post preservation molecular states of the cells are also different. Also, minute mistakes in measurements can also cause the deviation. Besides, potential defects in the MTT assay technique must also be considered. Such problematic results have been observed in the past. In a 2016 research, the standard curve OD of the MTT assay was altered due to the presence of trisilanol phenyl and trisilanolisooctyl polyhedral oligomericsilsesquioxane particles (Almutary and Sanderson, 2016). Therefore more research is needed in the field to evaluate the cause of such deviation and inconsistency in viability of HL-60.