Research Article

The Effect of Daunorubicin on the Normal and Cancer Myeloid Stem Cells Cultures at 48 hours

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ABSTRACT

Daunorubicin is an anthracycline antitumor antibiotic agent. This study was aimed to evaluate the cytotoxic effect of daunorubicin on the normal and cancer myeloid stem cell in the primary cell cultures at 48 hours. Cancer cells were isolated from 12 patients with chronic myeloid leukemia (6males and 6 females, newly diagnosed) and normal myeloid stem cells were isolated from 12 persons (6males and 6 females). The ages of both normal and leukemic volunteers were 50 - 72 years who attended to the National Center of Hematology/ Al – Mustansiyria University, during the period from September 2016 to December 2018. Daunorubicin were prepared in the different concentrations "300,150, 75, 37.5, 18.75µg/ml", incubation at 37°C for 48 hours. The viable cells were measured by MTT (Methyl thiazolyl tetrazolium) assay. This study showed that the cytotoxic effect or inhibition growth rate of daunorubicin depended on the dose or concentration for normal and cancer myeloid cells ($P<0.01$). In the high concentration of drug "300µg/ml" the inhibition rates were 42.3% and 77.2% for normal and cancer cells respectively while in the low concentration of drug "18.75 µg/ml", the inhibition rates were 12.77% and 30.6% for normal and cancer cells respectively and there were significant differences for inhibition rates between normal and cancer cells. This study was concluded that the daunorubicin was higher cytotoxic effect on the cancer cells than normal myeloid stem cells in the primary cell cultures at 48 hours.

Keywords: Normal and cancer myeloid stem cells, daunorubicin, MTT assay.

INTRODUCTION

Daunorubicin "daunomycin" is an anthracycline antitumor antibiotic agent that isolated from *Streptomyces peucetius* [1-2]. It interacts with DNA by blocking topoisomerase II enzyme, preventing the DNA double helix from being resealed and stopping the process of replication result in the cell cannot split into new cancer cells [3- 4] This drug used for treatment acute lymphocytic leukemia, acute myeloid leukemia and Kaposi's sarcoma [5].

Pluripotent hematopoietic stem cells (HSCs) are the stem cells from which all types of blood cells are derived. HSCs gives both of myeloid and lymphoid lineages by haematopoiesis process. Myeloid stem cells give neutrophils, eosinophils, basophils, monocytes, macrophages, erythrocytes, dendritic cells and megakaryocytes while lymphoid stem cells give T cells, B cells, and natural killer cells [6-7]. Chronic myeloid leukemia (CML) is a malignant clonal, myeloproliferative disorder of the pluripotent hematopoietic stem cells [8- 9]. It is characterized by abnormal chromosome (Philadelphia chromosome) due to translocation of *ABL1* on chromosome 9 to the region of the *BCR* gene on chromosome 22 [10-11]. CML is about 15% of adult leukemias and its affected males, females and rare in children [12]. The important clinical features of CML are splenomegaly, ganulocytosis and shift to the left in the differential WBCs count [13-14].

MATERIALS AND METHODS

Volunteers

Present study was carried out in the laboratories of the National Center of Hematology/ Al – Mustansiyria University in Baghdad governorate, during the period from September 2016 to December 2018. Cancer myeloid stem cells were isolated from 12 patients (6malesand 6 females) with CML (newly diagnosed, chronic phase) and normal myeloid stem cells were isolated from 12 persons (6males and 6 females) after taking the approvals. The ages of both normal and leukemic volunteers were 50 to 72 years. All volunteers were carrying Iraqi nationality.

Myeloid Cells Cultures

Ficoll- opaque was used to isolate human myeloid cells from bone marrow that taken from the posterior iliac crest by using aspiration needle under local anesthesia "10 ml xylocaine", cells were placed into 25 cm falcon [15-16], then added 10 ml of RPMI – 1640 (20 % fetal calf serum), RPMI – 1640 was prepared by dissolving 16.35g powder medium with Hepes buffer and L– glutamine. Sodium bicarbonate (2 g), ampicillin(1 ml), streptomycin (0.5 ml) and 200 ml of fetal calf serum (20 % FCS) were added to one liter of RPMI – 1640, then incubated cell cultures at 37° C [17]. The same viable cells count for both normal and leukemic myeloid cells "4× 10^6" also the same procedures used for both cells.

Daunorubicin Preparation

Daunorubicin (Pfizer. Actavis, Italy S.P.D,) was prepared in different concentrations "300, 150, 75, 37.5, 18.75µg/ml".

200µl of daunorubicin from each concentration was added to the cell cultures "200 µl of cell suspension in the each well of micro titration plate of 96 wells flat bottom". Five replicates were used for each concentration of drug. Cells were incubated at 37°C for 48hours (hrs) for normal and cancer myloid cells [18-19].

Solution "2 mg /ml" of MTT "Methyl thiazolyltetrazolium" was added to the cells culture [20-21]. ELISA reader was used to measure viable cells at 550 nm. Calculation of inhibition growth rate was (A – B) / A X 100. A : is optical density of non treated wells (control). B: is optical density of treated wells [22].

Statistical Analysis

In this study data was represented as means, ranges, percentages, standard errors and LSD (P≤0.01) to compare the inhibition rates according to concentrations for 48hrs [23].

Ethical Approval

In the present study, oral and written agreeing were taken from each volunteer.

RESULTS AND DISCUSSION

The results showed that the percentage of inhibition growth rates of normal and cancer myeloid cells at 48hrs depended on the concentration of daunorubicin (P<0.01). In the high concentration "300µg/ml" the inhibition rates were 42.3% and 77.2% for normal and cancer cells respectively while in the low concentration "18.75 µg/ml", the inhibition rates were 12.77% and 30.6% for normal and cancer cells respectively. There were significant and non significant differences (P<0.01) between inhibition rates of the normal cells as well as in the cancer cells according to the drug concentration as in tables 1, 2 and figures 1,2 for the normal and cancer cells respectively. In comparison between the normal and cancer cells showed there were significant differences for the inhibition rates as in table 3 and figure 3.

The present study showed that the inhibition growth rate "cytotoxic effect" of daunorubicin depend on the concentration, Thus the inhibition growth rates were increased on the normal and cancer myeloid stem cells when increased concentration of drug. Also this study recorded that the cytotoxic effect of daunorubicin on the cancer myeloid cells was higher than on the normal myeloid stem cells in the primary cell cultures at 48 hrs.

The major modalities treatment of human cancers are drugs chemotherapy, surgery, radiotherapy and immunotherapy [24]. In addition to systemic toxicity of anti-cancer and drugs resistance[25], also the major problems with the anti-cancer drugs therapy that have sever adverse effects [26] and lack the specificity of these drugs to certain target cancer cells, and may extend the effect to other cells or tissues and not distinguish between normal and cancer cells [27].

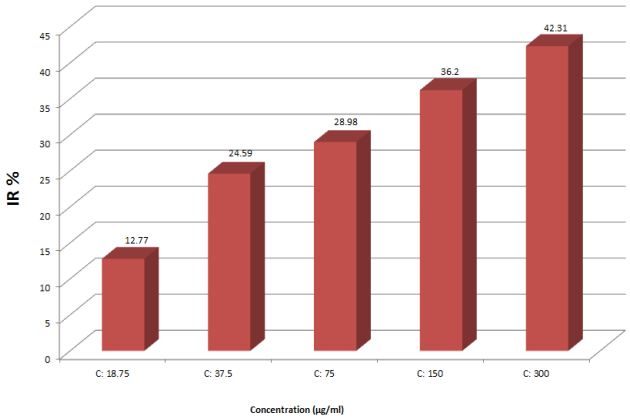

Figure 1: Inhibition rates of daunorubicin on the normal myeloid stem cells at 48 hrs.

Table 1: Inhibition rate of daunorubicin on the normal myeloid stem cells at 48 hrs.

Concentration (µg/ml)	Mean ± SEM of IR %
18.75	12.77 ± 0.70 c
37.5	24.59 ± 1.31 b
75	28.98 ± 1.66 b
150	36.21 ± 2.08 a
300	42.31 ± 2.15 a
LSD value	6.946 **
P-value	0.0001
*** (P<0.01).*	

SEM= standard error of mean. IR= inhibition rate %. Same letters= non significant differences. Different letters = differed significantly. **=significant differences.

Table 2: Inhibition rate of daunorubicin on the cancer myeloid stem cells at 48 hrs.

Concentration (µg/ml)	Mean ± SEM of IR %
18.75	30.67 ± 1.72 d
37.5	40.43 ± 2.15 c
75	45.92 ± 2.46 c
150	61.11 ± 3.51 b
300	77.26 ± 3.64 a
LSD value	7.933 **
P-value	0.0001
** (P<0.01).	

SEM= standard error of mean. IR= inhibition rate %. Same letters= non significant differences. Different letters = differed significantly. **=significant differences.

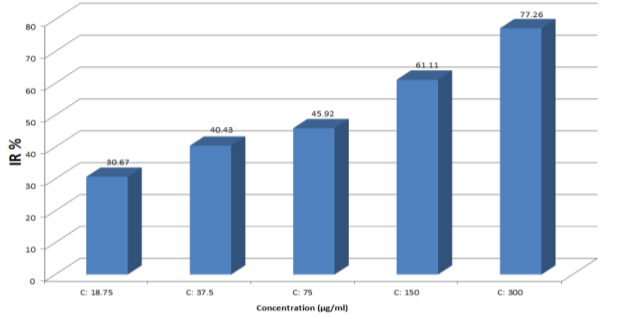

Figure 2: Inhibition rate of daunorubicin on the cancer myeloid stem cells at 48 hrs

Table 3: Comparison of inhibition rates for daunorubicin on the normal and cancer myeloid stem cells at 48 hrs.

Concentration of drug (µg/ml)	Mean ± SEM of IR %		
	Cancer myeloid stem cells	Normal myeloid stem cells	Comparison between inhibition rates for normal and cancer cells
18.75	30.67 ± 1.72 d	12.77 ± 0.70 c	**
37.5	40.43 ± 2.15 c	24.59 ± 1.31 b	**
75	45.92 ± 2.46 c	28.98 ± 1.66 b	**
150	61.11 ± 3.51 b	36.21 ± 2.08 a	**
300	77.26 ± 3.64 a	42.31 ± 2.15 a	**
LSD value	7.933 **	6.946 **	---
P-value	0.0001	0.0001	0.0001
** (P<0.01).			

SEM= standard error of mean. IR= inhibition rate %. Same letters= non significant differences. Different letters = differed significantly.**=significant differences.

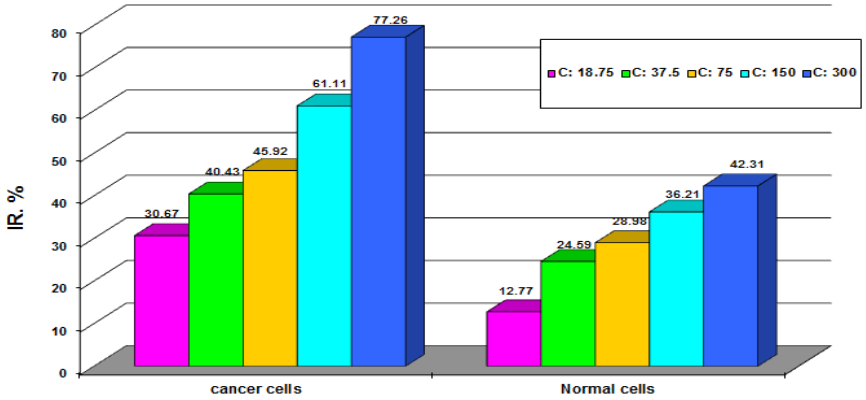

Figure 3: Inhibition rates of daunorubicin on the normal and cancer myloid stem cells at 48 hrs

For these reasons, many studies to establish the specific tumor therapy and distinguish between normal and cancer cells result in strategies targeting oncogenic mutations or tumor suppress pathways deficiency in cancers [28].

In this study, anthracycline as daunorubicin used to study cytotoxic effect on the normal and cancer myeloid stem cells that isolated from patients with chronic myeloid leukemia, moreover daunorubicin was used for acute myeloid leukemia induction therapy for decades [29], thus daunorubicin was used to evaluate the cytotoxic effect on the normal and myeloid disorders. Combination of daunorubicin and other drugs or extracts of herbs may be used to obtain less cytotoxic effect on the normal myeloid cells, in contrast high cytotoxic effect on the cancer myeloid stem cells [30, 31].

Present study used MTT assay to determine the cytotoxic effect of daunorubicin on the normal and cancer cells because this method is widely use in researches and rapid spectrophotometric for screening cytotoxicity of anti-cancer drugs in the cell cultures [32]. Also the viable cells can measure by MTT assay though measuring the mitochondrial reductase enzyme activity of viable cells that could reduce MTT to formazan which appears in purple color [33].

CONCLUSION

The present study showed that the cytotoxic effect of daunorubicin depened on the concentration of drug on both normal and cancer myeloid stem cells in the primary cell cultures, also daunorubicin was higher cytotoxic effect on the cancer cells than normal myeloid stem cells in the primary cell cultures at 48 hours.

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