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EVALUATION OF CHROMOSOME ABERRATIONS INDUCED BY DIGOXIN IN CHINESE HAMSTER OVARY CELLS

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ABSTRACT

Digoxin is a cardiac glycoside that has been reported to inhibit growth of multiple tumor cell types in vitro. The present study was assessing the cytogenetic effects of this drug on Chinese Hamster Ovary (CHO) cells. All experiments were performed in triplicate. The IC_{50} was 22.5 ± 0.8 µg/ml. To investigate the clastogenic effects of drug, chromosomal aberration in metaphase cells were analyzed. Chromatid breaks and polyploidy were the main types of aberration. Mitomycin-C and sodium arsenite were used as positive controls. CHO cells were exposed to different concentration of drug (5, 10, 15, 20 µg/ml) in 24 hours. All of the study aberrations and frequency of aberrant cells significantly increased as a function of digoxin concentration (for chromatid breaks: $r = 0.881$, $df = 13$, $P < 0.001$; for polyploidy: $r = 0.777$, $df = 13$, $P = 0.001$; for cells with aberrations: $r = 0.926$, $df = 13$, $P < 0.001$). The mitotic index negatively correlated with the concentration of digoxin ($r = -0.978$, $df = 13$, $P < 0.001$). All concentrations that cause chromosomal aberrations are in the cytotoxic range of the drug. The peak serum digoxin concentration (5 - 20 ng/ml) was very lower than concentrations we used in the present experiments. Further studies on valuation of chromatid breaks, micronuclei, and sister chromatid exchange in lymphocytes of patients who received digoxin, were recommended.

Keywords: Digoxin, chromatid breakage, mitotic index, polyploidy

INTRODUCTION

Digoxin is a purified cardiac glycoside extracted from the foxglove plant, *Digitalis lanata*. Digoxin is widely used in the treatment of various heart conditions, namely artial fibrillation, artial flutter and sometimes heart failure that cannot be controlled by other medication (Chao et al., 1992). Digoxin preparations are commonly marketed under the trade names, such as Lanoxin.

Several epidemiological studies indicated that breast cancer cells obtained from women on digitalis therapy (for congestive heart failure) were characterized by a series of more benign features compared with cancer cells from control patients (Stenkvist 1999; Stenkvist et al., 1979, 1980, 1982; Goldin & Safa 1984). The growth inhibitory effects of cardiac glycosides such as digoxin in the several cancer cell lines have been reported (Haux 1999; Johansson et al., 2001; Lindholm et al., 2002; López-Lázaro et al., 2005; Svensson et al., 2005; Prassas and Diamandis 2008). Taken together, it is

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very probable that digoxin has effect(s) on DNA and chromosomes.

Considering that there is no published data about effect of digoxin on induction of chromosomal aberrations, the present study was carried out. The aim of the present study was to assess the effect of digoxin on chromatid and chromosomal aberrations on Chinese hamster ovary (CHO) cells.

MATERIAL AND METHODS

Cell culture and chromosome study

In the present study, experiments were carried out using the Chinese hamster ovary (CHO) cell line. The cells were maintained in RPMI-1640 medium (from GIBCO) supplemented with 10% inactivated fetal calf serum (from GIBCO), 2mM L-glutamine and with the addition of penicillin (100 U/ml) and stereptomycin (100 mg/ml). For the present experiment we used Lanoxin (a trade name of digoxin, a liquid form, 0.25 mg/ml; KERN Company).

Cells were seeded into 96-well plates at 2×10^4 cells/well in 100 µl complete medium. After the cells clung to plates, the cells were treated with various concentration of digoxin (2.5, 5, 10, 15, 20, 25, 30, 35 µg/ml) for 24 h. Final volume in each well was 200 µl. Subsequently, the cell viability was measured by MTT dye reduction assay (Mosmann, 1983; Liu et al., 1997). The formazan dye was measured by ELISA reader. After obtaining the absorption, percentage inhibition of cell growth was calculated. The IC_{50} is defined as the cytotoxicity index that reduces the cell number to 50 % compared with untreated-control CHO cells.

Mitomycin-C (0.06 µg/ml) and sodium arsenite (1 µgM) were used as positive controls (Mahata et al., 2004; Celikler et al., 2008; Samuel et al., 2011; Sulaiman, 2012; Sedigh-Ardakani et al., 2013). The CHO cells were seeded at the density of 1.8×10^6 cells/petridish in the volume of 10 ml. After 48 h, the cells were treated with different doses of digoxin (5, 10, 15 and 20 µg/ml) in RPMI-1640 for 24 h. Chromosomes were conventionally stained with Giemsa.

The metaphases were analyzed for the numbers and types of chromosome aberrations. Chromosome aberrations were classified to chromatid breaks and polyploidy. To determine the mitotic index (percentage of cells in mitosis), 1000 cells in each slide were observed. In each slide, 100 mitotic cells were counted to determine the chromosome aberrations.

All experiments (for determining either cytotoxiciy or chromosomal breakage) were performed in triplicate.

Statistical analysis

The level of the chromatid and chromosome breaks and also polyploidy, cells with aberrations and mitotic index are presented as mean $\pm$ standard deviation (SD). Comparisons of the mean values of the studied indices were done using one way analysis of variance. We used Duncan test as a Post hoc test.

Statistical analysis was performed using SPSS statistical software package (version 11.5) for windows (SPSS Inc., Chicago, IL, USA). A probability of $P < 0.05$ was considered statistically significant. All P-values were two-tailed.

RESULT AND DISCUSSION

Effect of digoxin on proliferation of CHO cells was investigated. After 24 h treatment of CHO cells with 2.5, 5, 10, 15, 20, 25, 30, 35 µg/ml of digoxin, the percent of cell inhibition were 4.5, 9.4, 19.4, 28.3, 39.9, 60.8, 83, and 97, respectively (Figure 1). Percent of growth inhibition rate increased as a function of digoxin concentration ($r = -0.980$, $df = 25$, $P < 0.001$). The IC_{50} for digoxin was estimated 22.5 $\pm$ 0.81 µg/ml.

The results of chromosome aberration test were presented in Table 1. There were significant difference between positive controls and negative control for frequencies of chromatid breaks and polyploidy (For all comparisons P-values were less than 0.01). Digoxin at final concentration of 25 µg/ml results very low metaphases, therefore, the analysis did not perform. All of the study

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aberrations and frequency of aberrant cells significantly increased as a function of digoxin concentration (for chromatid breaks: r = 0.881, df = 13, P < 0.001; for polyploidy: r = 0.777, df = 13, P = 0.001; for cells with aberrations: r = 0.926, df = 13, P < 0.001). However, the mitotic index negatively correlated with the concentration of digoxin (r = -0.978, df = 13, P < 0.001).

Based on more than a thousand published scientific articles, it is well established that using *in vitro* system, cardiac glycosides showed anti-cancer properties (Prassas and Diamandis, 2008). Although the molecular mechanisms under lying the increased susceptibility to cancer cells to cardiac glycosides are not fully elucidated, some mechanisms such as alterations in homeostasis of K^+, Na^+, and Ca^{2+}, inhibition of tumor necroses factor and nuclear factor κB, increased production of reactive oxygen species, inhibition of toposiomerase II, etc, might be involved (McConkey et al., 2000; Prassas and Diamandis, 2008). Considering that digoxin increased the frequency of chromatid and chromosome breaks, it is suggested that digoxin may interact with

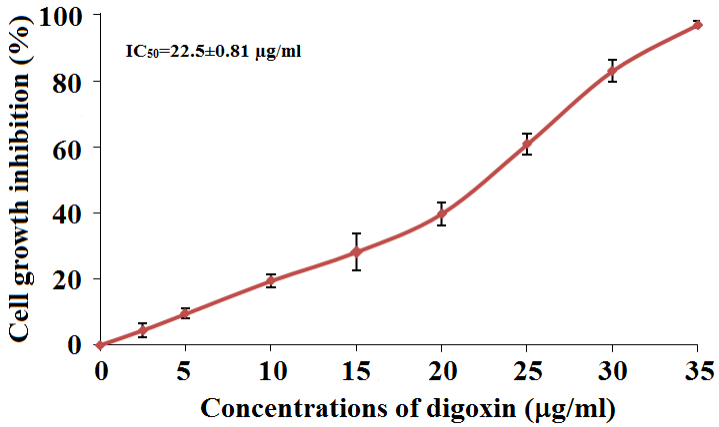

Figure 1: Effect of digoxin concentration on CHO cell line for 24 hours: The results are presented as percent of inhibition of cell growth obtained by MTT assay. Data are mean ± SD of triplicate cultures.

Table 1: Induction of chromatid breaks, polyploidy and mitotic index in CHO cells by digoxin

Treatment	Aberrations per 100 metaphases		Cells with aberrations (%)	Mitotic index (%)
	Chromatid breaks	Polyploidy		
Digoxin (µg/ml)				
0	4.3 ± 1.53[a]	5.6 ± 3.21[a]	5.30 ± 0.58[a]	7.8 ± 0.10[a]
5	4.6 ± 0.58[a]	5.0 ± 1.00[a]	7.67 ± 1.53[ab]	6.1 ± 0.20[b]
10	6.0 ± 1.00[a]	5.6 ± 1.16[a]	11.0 ± 1.00[b]	5.4 ± 0.15[c]
15	12.3 ± 4.16[b]	10.6 ± 1.53[b]	25.6 ± 0.58[c]	4.5 ± 0.25[d]
20	16.0 ± 1.00[b]	11.6 ± 1.53[b]	28.0 ± 5.57[c]	3.6 ± 0.26[e]
25	Very low metaphases			
Statistical analysis				
F (df = 4, 10)	18.59	30.06	47.63	187.28
P-value	< 0.001	0.003	< 0.001	< 0.001
Positive controls				
MMC (0.06 µg/ml)	35.0 ± 8.02	20.6 ± 2.88	51.6 ± 3.21	3.7 ± 0.10
Sodium arsenite (1 µM)	25.3 ± 8.02	15.0 ± 4.35	38.6 ± 1.52	3.3 ± 0.20

Note: The results are average of three independent experiments. Same alphabets mean no statistically significant difference between groups (P > 0.05).

DNA and subsequently inhibits cell division. The peak serum digoxin concentration (5-20 ng/ml) was much lower than concentrations we used in the present experiments (Marcinkowska-Królewicz and Feldmann, 1998; Tayrouz et al., 2003; Kothare et al., 2005). Based on the results of the present study, all concentrations that cause chromosomal aberrations are in the cytotoxic range of digoxin.

Tissue specific response to DNA and chromosomal damages due to drugs were reported. Therefore, it is possible that digoxin shows different patterns level of toxicity depended to cell type. On the other hand patients are exposed for longer periods compared to our cell culture system. Taken together, it is necessary to evaluate the chromatid breaks, micronuclei, and sister chromatid exchange in lymphocytes of patients who received digoxin.

Finally it should be mentioned that digoxin must be used cautiously in generally and particularly in children and pregnant women. Further experiments are necessary to clarify the significance of the present findings.

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DISCLOSURE STATEMENT

No competing financial interests exist.

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