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Propranolol induced chromosomal aberrations in Chinese hamster ovary cell line

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ABSTRACT

Propranolol (PL), a non-selective beta-blocker, is a cardiovascular drug widely used to treat hypertension. The present study was concerned with assessing the cytogenetic effects of this drug on Chinese hamster ovary (CHO) cell line. MTT assay was then carried out to determine the cytotoxicity index (IC₅₀) of the drug. The IC₅₀ value of PL was 0.43±0.02 mM. To investigate the clastogenic effects of the drug, chromatid and chromosome breaks and polyploidy in metaphases were analyzed. CHO cells were exposed to different concentrations of the drug (0.1, 0.2, 0.3, 0.4 mM) for 24 hours. Considering that PL has liver metabolism, experiments were carried out in the presence and absence of the metabolic activation system (S9 mix). Mitomycin-C and sodium arsenite were used as positive controls. It was observed that in cells treated with different PL concentrations as 0.1, 0.2 and 0.3 mM, the frequency of chromatid and chromosome breaks as well as polyploidy increased when compared with untreated CHO cells. The addition of S9 mix significantly decreased the chromatid breaks, chromosome breaks and polyploidy compared to the treatment of PL alone. It is concluded that, PL causes chromatid and chromosome aberrations in CHO cell line and the metabolic activation system (S9 mix), playing an important role in drug cytotoxicity reduction.

Key words: Propranolol; Chromatid breakage; Chromosome breakage

INTRODUCTION

Propranolol (PL), a non-selective beta-blocker, is known as a cardiovascular drug. A number of studies have demonstrated the anti-proliferative, anti-migratory and cytotoxic properties of PL against lung adenocarcinoma [1, 2], colon carcinoma [3], breast carcinoma [4], nasopharyngeal carcinoma [5], ovarian cancer [6], pancreatic cancer [7-10]

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and gastric cancer, leukemia, human corneal epithelial and retinal pigment epithelial cell lines, skin keratinocytes, fibroblasts, and hemangioma-derived endothelial [11-14].

The induction of a significant increase in the frequency of micronuclei in erythrocytes of Swiss albino mice was observed at higher dose levels of PL. However, PL in germ cells of mice, failed to induce significant chromosomal aberrations at any dose tested [15]. Anti-inflammatory and anti-tumoral effects of PL have also been reported [16-18].

Infantile haemangiomas are the most common benign tumors of infancy. PL is thought to exert its effects on growing haemangiomas by three different molecular mechanisms: vasoconstriction, inhibition of angiogenesis and induction of apoptosis [19]. It is suggested that PL may be able to increase the efficacy of chemotherapy by potentiating the anti-proliferative and/or anti-angiogenic effects of chemotherapeutic drugs [19]. Taken together, it is very likely that PL has interaction with DNA and chromosomes. We thus decided to investigate the effect of PL on the chromosomes of cultured Chinese hamster ovary (CHO) cell line.

MATERIALS AND METHODS

Cell culture: In the present study, experiments were carried out using 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 with the addition of penicillin (100 U/ml) and streptomycin (100 mg/ml). For the present experiment we used propranolol hydrochloride (1-isopropylamin -3-(1-naphthoxy)-2-propanol) in liquid form (Inderal, 1mg/ml; Tolidaro Corporation, Iran)

Growth inhibition assay: The CHO cells were seeded into 96-well plates at 2×10^4 cells/well in 100 μ l complete medium. After clinging to plates, the cells were treated with various concentrations of PL (0.025, 0.05, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 mM) for 24h. The final volume in each well was 200 μ l. Subsequently, the cell viability was measured by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay [20]. The metabolic conversion of MTT into formazan crystals by living cells, which determines mitochondrial activity, was measured by an ELISA reader. After obtaining the absorption at 570 nm, the percentage inhibition of cell growth was calculated. Cell inhibition was expressed as the percentage of untreated control cells. The IC₅₀ value is defined as the cytotoxicity index that reduces the cell number to 50% compared with untreated-control CHO cells.

Chromosome and chromatid aberrations: Mitomycin-C (0.06 μ g/ml) and trivalent sodium arsenite (NaAsO₂; 1 μ gM) were used as positive controls [21-25]. The CHO cells were seeded at the density of 1.8×10^6 cells/petridish in a 10 ml volume. After 48h, the cells were treated with different doses of PL (0.1, 0.2, 0.3, 0.4 mM) in RPMI-1640 for 24h. Chromosomes were conventionally stained with Giemsa. The metaphases were analyzed for the number and type of chromosome aberrations, classified as chromatid breaks, chromosome breaks (such as dicentric chromosome, ring chromosome, etc) and

polyploidy. Some examples of the aberrations were shown in Figure 1. To determine the mitotic index (expressed as percentage of cells in mitosis), the number of mitosis were counted in 1000 cells per treatment. In each treatment, 100 mitotic cells were counted to determine the chromosome aberrations. All experiments were performed in triplicate.

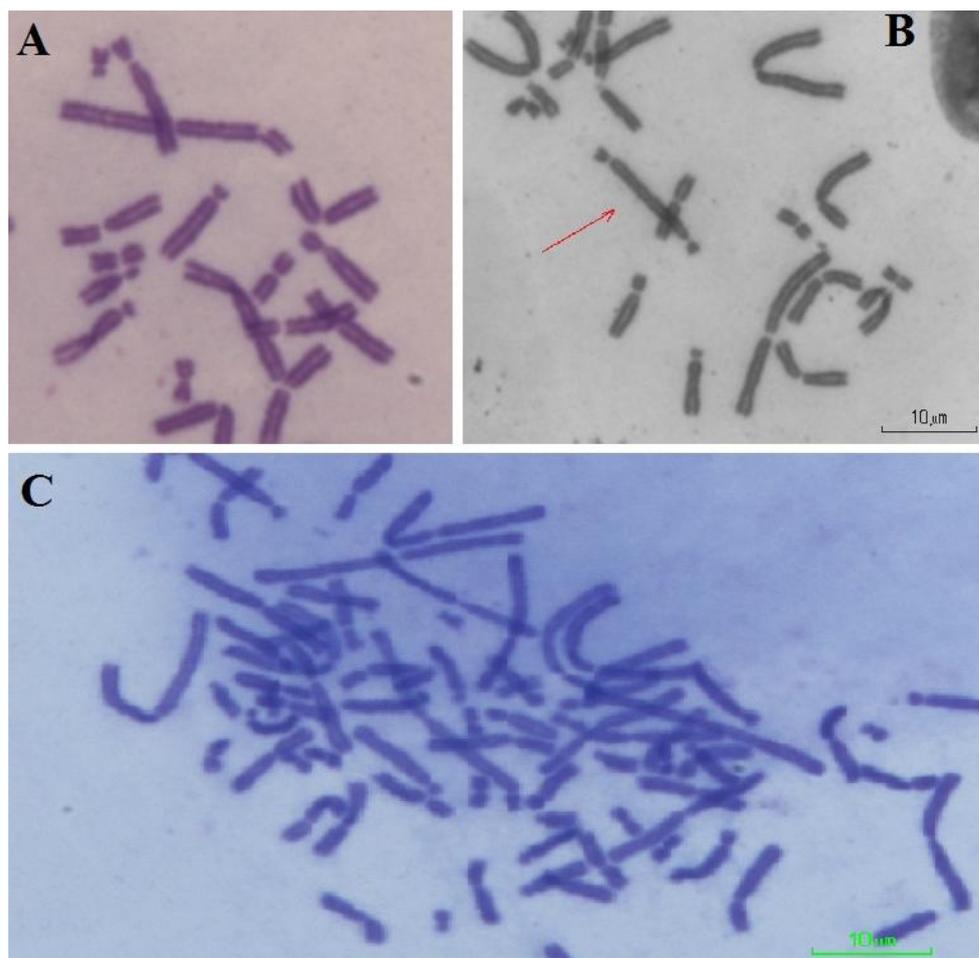


Figure 1. Chromosome aberrations in CHO cells treated with propranolol. Chromatid break, dicentric chromosome and a polyploidy metaphase were shown in A, B and C sections, respectively.

Metabolic activation system: Liver S9 fraction was obtained from Wistar male rats induced with Phenobarbital. The livers from three rats were removed, pooled and S9 fraction (10000 x g) supernatant was prepared following the standard procedure. Immediately before use, an S9 mix (S9 fraction with cofactors) was prepared: 1ml of S9 mix contained: 0.3ml phosphate solutions, 0.2ml KCl, 0.2ml MgCl₂, 0.1ml S9 fraction, 0.1ml glucose-6-phosphate (Fluka) and 0.1ml NADP (Sigma). The S9 mix was added to 50 μl of all the cultures.

Statistical analysis: The level of the chromatid and chromosome breaks and also polyploidy, cells with aberrations and mitotic index are presented as means \pm standard deviations (SD). Comparisons of the mean values of the studied indices were done using one way analysis of variance. Duncan test was used 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 as statistically significant. All P-values were two-tailed.

RESULTS

The effect of PL on the proliferation of CHO cells were analyzed using an MTT assay. Induction of inhibition increased as a function of PL concentration ($P < 0.001$). The IC_{50} for PL was estimated to be 0.43 ± 0.02 mM. With the elongation of the incubation time (48h), the sensitivity of cells was increased (Fig. 2).

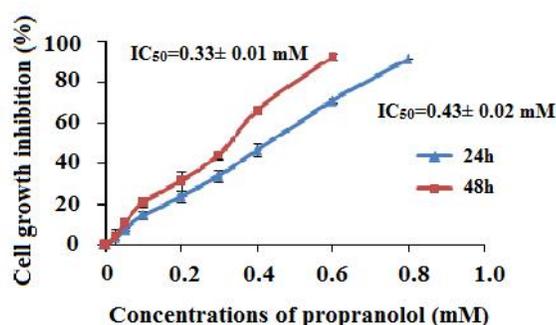


Figure 2. Effect of propranolol on Chinese hamster ovary (CHO) cell line for 24 and 48 hours. The results are presented as percentage of inhibition of cell growth obtained by MTT assay. Data are mean \pm SD of triplicate cultures.

The induced chromosomal aberrations in treated CHO cells are shown in Table 1. Significant differences were found between positive- and negative-controls for the frequencies of chromatid breaks, chromosome breaks and polyploidy ($P < 0.01$).

Treatment with PL significantly induced chromatid breaks, chromosomal breaks and polyploidy (Table 1). All of the studied aberrations and frequencies of aberrant cells significantly increased as a function of propranolol concentration. By increasing the drug concentration, the number of cells with aberrations significantly increased ($r = 0.999$, $df = 2$, $P = 0.001$). There was significant negative correlation between concentration of PL and mitosis index ($r = -0.972$, $df = 2$, $P = 0.028$).

Considering that PL is metabolized enzymatically [26, 27], further experiments were performed to investigate the effect of PL treatment in the presence of the S9 mix on the chromosomal integrity of CHO cells. As shown in Table 1, the addition of the S9 mix significantly decreased quantities of the chromatid breaks, chromosome breaks and polyploidy compared to the treatment of PL alone. The percentage of aberrant cells

decreased compared to the experiment cells were treated with only PL. It should be mentioned that in this situation, the induced damages to the chromosomes are higher than the control level (Table 1).

Table 1. Induction of chromatid and chromosome aberrations in CHO cells by propranolol.

Treatment	Aberrations per 100 metaphases			Cells with aberrations (%)	Mitotic index (%)
	Chromatid breaks	Chromosome breaks	Polyploidy		
Propranolol (mM)					
Without S9 Mix					
0	5.33±1.96 ^a	3.67±0.51 ^a	6.83±2.78 ^a	7.0±1.26 ^a	7.87±0.38 ^e
0.1	10.67±1.53 ^b	6.67±0.57 ^{ab}	14.33±2.08 ^b	17.67±1.53 ^b	4.81±0.17 ^c
0.2	14.67±1.53 ^c	11.67±1.53 ^c	21.67±1.15 ^c	27.0±1.73 ^c	3.55±0.18 ^b
0.3	21.0±4.36 ^d	15.33±4.04 ^d	20.33±2.08 ^c	36.33±2.08 ^d	2.28±0.27 ^a
0.4					
With S9 Mix					
0	3.67±1.53 ^a	4.0±1.0 ^a	6.33±2.08 ^a	6.67±1.53 ^a	7.53±0.35 ^e
0.2	9.0±1.73 ^b	7.33±2.08 ^{ab}	12.0±2.64 ^b	16.67±4.93 ^b	6.16±0.15 ^d
0.3	11.0±1.0 ^b	8.0±1.0 ^b	11.67±4.50 ^b	18.67±0.57 ^b	4.93±0.15 ^c
Positive controls					
MMC (0.06 µg/ml)	32.0±13.0	26.67±10.6	23.33±5.13	46.33±8.96	4.01±0.53
Sodium arsenite (1 µM)	30.33±3.78	16.33±5.5	16.0±5.19	42.33±5.13	3.53±0.75

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

As mentioned in Table 1, at the concentration of 0.4 mM of PL, the number of metaphase cells was very low. The aberrations were therefore, not studied.

Using an MTT assay of eight clinical beta-blockers (including propranolol, alpernolol, adenolol, labetalol, metoprolol, pindolol, timolol, and bisoprolol), the in vitro cytotoxicity on human corneal epithelial and retinal pigment epithelial cell lines, skin keratinocytes and fibroblasts were compared. Relatively small differences in cytotoxicity were observed between different cell lines for the same drug. The obtained results indicate that the mechanism of cytotoxicity is not cell-specific [11, 12, 15, 28, 29].

In a study performed recently by Aruna et al., the mutagenic effect of PL was confirmed and it was observed that at the elevated doses, this drug can increase mouse erythrocytes micronucleus [15]. This study suggests that PL causes chromosomal aberrations in different concentrations. Moreover, a correlation between drug concentration and the quantity of chromosomal abnormalities was observed.

In our study, the S9 mix reduced the effects of PL on CHO chromosomes (Table 1). As reported already, PL is metabolized rapidly by the S9 mix [30]. The drug is converted to its metabolites by cytochrome *CYP2D6*, *CYP1A2* and *CYP2C19* [31] which are present in the S9 fraction. By studying Table 1 it can be concluded that the use of the S9 mix reduced the rate of anomalies and increased the mitotic index. These results suggest that PL metabolites are less toxic than the original drug. The role of the S9 mix in reducing toxicity has been reported previously [26]. The antioxidant properties of catalase (as existing in the

S9 mix) are in strong agreement with our findings because our results demonstrated that the S9 mix can reduce the toxicity of PL.

We know that the S9 fraction from rodent liver mixed with cofactors NADP and glucose-6-phosphate (S9 mix) form an activation system, enabling the biotransformation of the drugs. The mixtures have many enzymes including the antioxidant enzyme catalase which *scavenge* the *active* free radicals, especially hydroxyl radicals [26]. In conclusion, PL can cause chromatid and chromosome aberrations in CHO cell lines, and the metabolic activation system (S9 mix) plays an important role in the drug cytotoxicity reduction.

PL has been assigned to pregnancy category C by the FDA. Some studies have revealed adverse effects of PL on embryos [32, 33]. However, further experiments are necessary to clarify the significance of the present findings.

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