Cytotoxic Effects of Digoxin on Mesenchymal Stem Cells: An in Vitro Study

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ABSTRACT

Introduction: Cardiac glycosides such as digoxin or digitoxin are the natural products that are traditionally used to increase cardiac contractile force in patients with heart failure and cardiac arrhythmias. It has been shown that digoxin can directly inhibit the cell proliferation and lead to cell apoptosis. Present study was conducted to analyze the effect of digoxin in the cohorts of mesenchymal stem cells(MSCs) -based therapy.

Methods: MSCs were cultured and treated with different concentrations (0.1, 0.5, 1, 5, 7, 10, 15, 20, 30 and 40 µM) of digoxin for 6, 12, 24 and 48 hours. MTT assay was performed to study cell viability and proliferation.

Results: After 24 and 48 hours, cell viability was significantly decreased in 20, 30 and 40 µM concentrations ($P<0.05$), but significant decline in cell viability was observed only in 40 µM after 12 hours ($P<0.05$). After 6 hours, cell viability of experimental groups had no significant differences toward control group ($P>0.05$).

Conclusion: It is suggested that digoxin may lead to decline in cell survival ability and increase in cell apoptosis in a dose-time dependent pattern. It is recommended to consider application of glycosoids in concurrence with stem cell therapy.

1. Introduction

Bone marrow mesenchymal stem cells (MSCs) are defined as a class of the multipotent adult stromal stem cells that differentiate into a variety of tissues including cartilage, bone, fat as well as myocytes like cardiomyocytes in vitro and in vivo [1]. This property makes them as a promising source for cell-based therapy. Recently, several studies have demonstrated that cell transplantation therapy with bone marrow mesenchymal stem cells (MSCs) is safe and has great potential in regenerating infarcted myocardium and restoring the impaired heart function [2, 3]. Wen et al. have observed that administration of MSCs by intravenous, intraventricular or intra myocardial injection can improve myocardial function before and

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after and also duration of survival after cardiopulmonary resuscitation in Myocardial infarction rats [4, 6]. Myocardial infarction or heart failure is one of the major causes of cardiovascular mortality [1, 7]. Stem cell based therapy has generated significant interest and to date (today) preclinical research has shown its therapeutic potential. As yet, clinical studies have reported that this therapeutic modality may lead to an overall improvement of cardiac function [1, 8, 9]. In spite of more benefits of stem cell therapy, its application in clinics encountered to several problems such as apoptosis and low survival due to toxic environments resulted from free radicals and some drugs interaction.

Cardiac glycosides such as digoxin or digitoxin are the natural products that traditionally used to increase cardiac contractile force in patients with heart failure and cardiac arrhythmias [10, 11].

Digoxin expressed selective toxicity against tumor cells from patients [10, 12]. Moreover, it has shown that digoxin can directly inhibit the proliferation of the androgen dependent prostate cancer cell line LNCaP and the androgen independent prostate cancer cell lines DU145 and PC3, too. Indeed, its efficacy is done by elevation in intracellular Ca^{2+} and by apoptosis [13].

It is well known that digitalis has the ability to suppress the activity of Na^{+}/K^{+}ATPase and results in increased intracellular Ca^{2+}. Deregulation of these ions lead to activation of a number of intracellular pathways such as a change in cellular structure of gene expression. An increase of intracellular Ca^{2+} may also activate various hydrolyticenzymes, including proteases, nucleases and lipases,which have been implicated as effectors of Ca^{2+} elicited toxicity [10, 14]. Also, the anti-tumor effects of cardiac glycosides in cancer patients are due to the increase in cytosolic Ca^{2+} caused by cardiac glycoside [15].

Recently, stem cell therapies are recommended for cardiac patients. One study have shown that stem cell treatment by coronary artery injections of stem cells derived from their own bone marrow resulted in small but statistically significant improvements in left ventricular ejection fraction and end-diastolic volume are not seen with placebo after an MI [16]. Interaction between glycosoids and stem cells viability, survival and apoptosis has not been studied completely. Therefore, the present study follows cytotoxic effects of digoxin as a glycosoids on mesenchymal stem cells.

2. Materials & Methods

Mesenchymal stem cells isolation, culture and treatment

Mesenchymal stem cells (MSCs) were obtained from the femoral and tibial bones of 6 weeks old wistar albino male rat by flushing the rat femurs and tibias with Dulbecco's Modified Eagle's medium (DMEM) (Gibco, Invitrogen, Germany). Then, MSCs were suspended in DMEM medium supplemented with 10% FBS (Gibco, Invitrogen, Germany) and incubated at 37°C in a humidified chamber with 5% CO^{2}. The culture media was completely replaced every 3 days and non adherent cells were discarded. MSC were

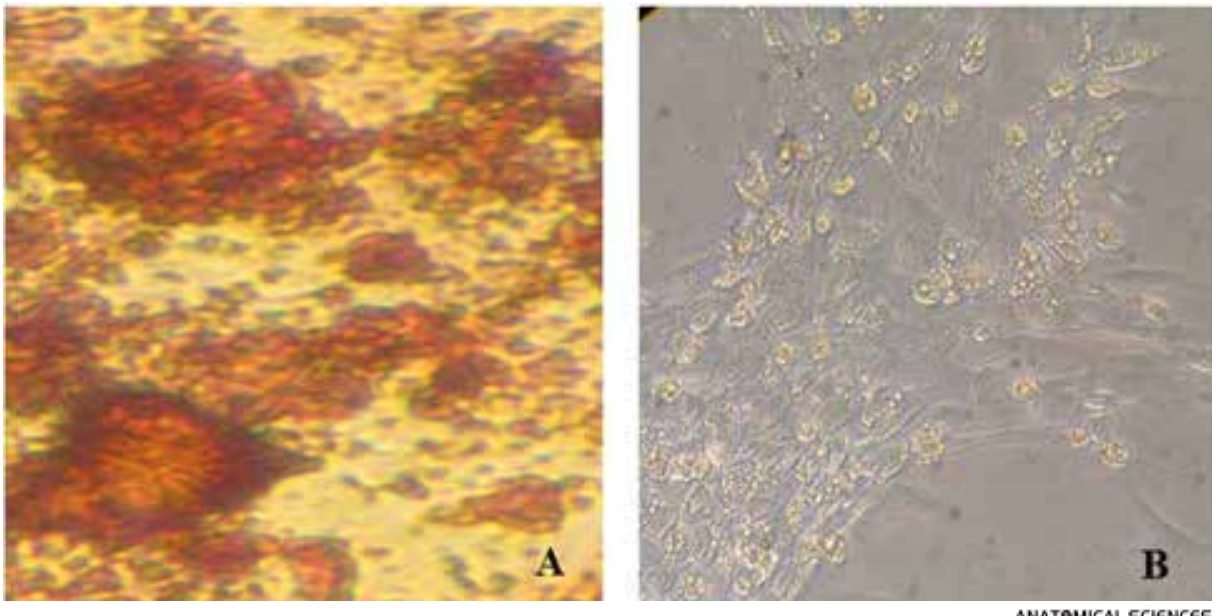

Figure 1. MSCs were cultured with differentiation media. Osteogenesis occurred after 21 days (A) and adipogenesis after 15 days (B).

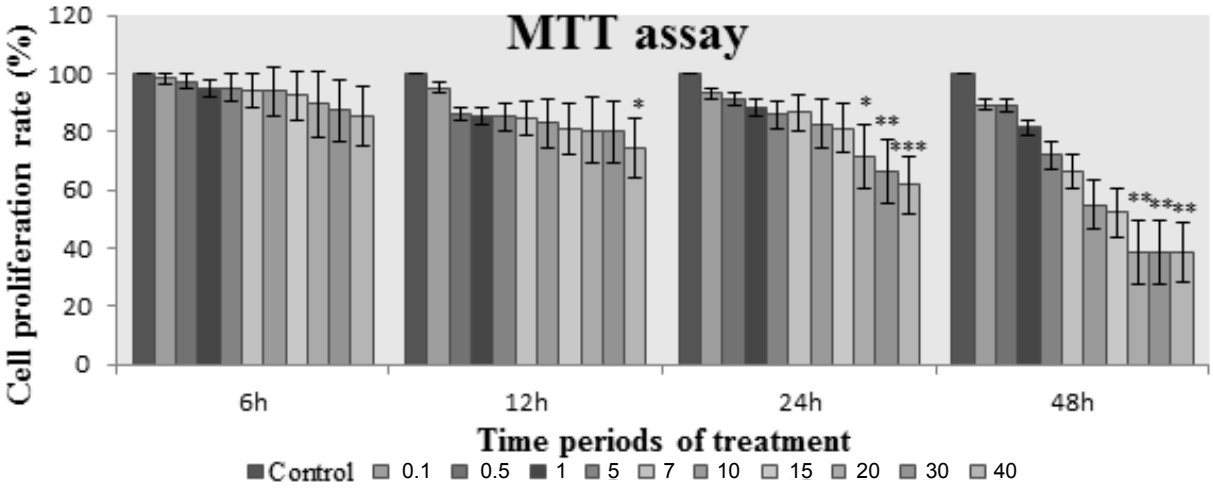

Figure 2. The diagram shows proliferation rate of MSCs after 6, 12, 24 and 48 h in different concentration of digoxin.

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recognized by their ability to proliferate in culture with an attached well-spread morphology. Once cells were more than 80% confluent, adherent cells were detached and replated 1:3 by flask (passage 1). The multi-potency of MSC was confirmed by induction of osteogenic and adipogenic differentiation using specific differentiation media (Figure 1). Passage 4 of MSCs was incubated with digoxin at different concentrations (0.1, 0.5, 1, 5, 7, 10, 15, 20, 30 and 40 µM) for 6, 12, 24 and 48 hours.

MTT Assay

Cell proliferation evaluation was confirmed by MTT assay. MSCs were cultured in 96 well plates and treated with digoxin. After 6, 12, 24 and 48h, 100µl of the culture medium was removed and 15 µlMTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (5mg/ml) was added. 3 hours later the wells were substituted with 200 µl of DMSO. The wells were pipetted and read with spectrophotometer (ELISA reader, TECAN/ sunrise, Magellan program, Austria) at optical density of λ570. % of viable cells was measured by:

$$\frac{\text{OD of experimental groups}}{\text{OD of control}} \times 100$$

Statistical analysis

Results were presented as mean±SD in triplicate experiment. Differences were determined using ANOVA with the Tukey–Kramer multiple comparisons test at significant difference of 0.05.

3. Results

Digoxin decreased cell proliferation

The evaluations of cell proliferation rate by MTT assay showed that digoxin decreased the MSCs proliferation rate in a dose and time dependent manner. Indeed, after treatment with different concentrations of digoxin in time period of 6 hours, the cell viability and proliferation of experimental groups had no significant differences toward control group ($P>0.05$), but after 12 hours of treatment, cell viability was declined in the digoxin concentration of 40µM, significantly ($P<0.05$). Furthermore, after 24 hours of cell treatment, cell viability in concentrations of 20 µM, 30 µM and 40 µMhad significant decrease compared with control group ($P<0.05$), but the other experimental groups showed no significant differences toward control group ($P>0.05$). The same results were observed in 48 hours of treatment, too (Figure 2).

4. Discussion

Recently, there has been interest in cell transplantation therapy with bone marrow mesenchymal stem cells to treatment of heart failure. The therapeutic efficacy of mesenchymal stem cells is dependent on their survival and differentiation ability in the target tissue [17]. On the other hand, many patients with cardiac disease consume cardiac glycosides such as digitalin and digoxin. A variety of reports suggested that cardiac glycosides may have anticancer properties in breast [14, 15, 18, 19] and prostate [20] cancers. Probably, reduction in transplanted MSCs viability in heart failure is due to apoptotic effects of cardiac glycosides. Hence, present paper evaluated the effects of

different concentrations of digoxin on mesenchymal stem cells.

Our results showed that digoxin decreases the cell viability in a dose-time dependent manner. Several studies have confirmed the apoptotic and cytotoxicity effects of digoxin [21, 22]. The cardiac glycosides, like digoxin, act by inhibiting the activity of the Na^+/K^+-ATPase. Inhibiting Na^+/K^+- ATPase leads to higher levels of intracellular Ca^{2+}. However, the decrease in intracellular K^+ and increase in intracellular Na^+ and Ca^{2+} following inhibition of the Na^+/K^+-ATPase are early key steps in apoptosis [23-25]. Moreover, inhibition of IL-8 production and the TNF-α/NF-κB pathway; inhibition of DNA to poisomerase II and activation of the Src kinase pathway occurs [14]. However, finding suitable concentrations of digoxin with lower apoptotic effects is necessary. Lopez-Lazaro et al. suggested that Digitoxin inhibits the growth of cancer cell lines at concentrations commonly found in cardiac patients [21]. We examined the effect of different concentrations of digoxin on mesenchymal stem cells. According to MTT assay, obtained results showed that 0.1, 0.5, 1, 5 and 7 μM of digoxin in time periods of 6, 12, 24 and 48 hours have safe effects on mesenchymal stem cells.

Finally, it is recommended to consider application of stem cell therapy in concurrence of drug in patients with heart diseases.

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