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Research Article

TOXICOLOGICAL STUDY OF MEBUDIPINE AND DIBUDINE, TWO NEW CALCIUM-CHANNEL BLOCKERS, IN SACCHAROMYCES CERVISES AND ISOLATED RAT HEPATOCYTES CELLS IN-VITRO

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ABSTRACT

Mebudipine and Dibudipine are two new calcium antagosists used in laboratory animals.

Toxicological testing were evaluated in cultured mouse cells in-vitro and on growth yeast cells . It is shown that in these two system it is safe and no toxicological effect has been shown.

Therefore, it is concluded that considering effect of mebudipne on yeast cells, heptocytes cells in-vitro, and reported effect on salmonella[11], it can be said that is drug is safe and can be used in humans.

Keywords: Mebudipine, Dibudipinedihydropyridine, Calcium antagonists, Saccharomyces cerevisiae

INTRODUCTION

Calcium-channel blockers have a significant role in the treatment of several cardiovascular and non-cardiovascular disorders [1 and 2]. Currently, extensive research is being carried out on the synthesis of new compounds of this class, Synthesis of compounds with greater tissue selectivity, longer duration of action and slower rate of absorption is the main aim of the current efforts. Such improvements in the properties of new drugs will ultimately lead to fewer side effects and improved patient compliance. In our previous study [3] it was shown that mebudipine and dibudipine, two new 1,4-dihydropyridine derivatives (fig. 1) synthesized in our laboratory, were potent relaxants of vascular and ileal smooth muscles. Inhibition of calcium-induced contraction of ileal smooth muscle [3] and reduction of calcium spikes of F1 neuronal soma membrane in Helix aspersa [4] confirmed its calcium channel blocking properties. Relaxing The KCl-induced contractions of human internal mammary artery confirmed that the results obtained in animal studies [3] could be reproduced in human vascular preparations [5] It has been shown that these compounds have a longer half life [6] and more active than nifedipine [7] and shows a vascular selectivity [8]. It has been shown that these drugs, mebudipine and dibudipine are not mutgen on Aimes assay tet on Samonella T102 [9].

It has been shown that yeast cells (Saccharomyces cerevisiae) can serve as an excellent alternative model model organism to test the toxicity of various compounds and pollutants [10]. This assay method is easy and unexpensive.and has been developed to test a number of different pollutants and various compounds.

Therefore the present study was carried out to investigate its toxicity in Saccharomyces cerevisiae as a model organism and rat hepatocytes in-vitro.

MATERIAL AND METHODS

Hepatocyte incubations

Freshly isolated hepatocytes were obtained from male Sprague Dawley rats (190–210 g). Anaesthesia was induced in rats with 60mgkg 1 intraperitoneal phenobarbital sodium (Sagatal 60mgmL 1; Aventis, UK) and hepatocytes were isolated from whole livers by a two-step collagenase perfusion as described previously (Moldeus et al 1978). The viability of the cells as determined by Trypan blue exclusion was typically ‡ 85%. Portions (10 μL) of dibudipine, mebudipine and nifedipine solutions (100μM in dimethyl sulfoxide (DMSO)) were added to 10mL of hepatocyte suspension ($2.4x10^6$ cells/mL) in Krebs-Henseleit buffer pH 7.6 containing 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (12.5mM) in rotating 50mL round-bottom flasks to produce a final concentration of 100 μM. The flasks were maintained at 37 °C in a water bath in an atmosphere of O^2/CO^2 (95:5%v/v) and samples taken at specific intervals over a 2-h incubation period.

Checking cell viability

100 μl trypan blue (% 0.1) and 100 μl of cell suspension were added to a microtube. Mixed well and 10μL was put on the cell counting chamber and their viabilities were calculated as below:

Viability =(total-dead)/total*100

Yest cells toxicity testing

Haploid yeast strain used throughout this study was S, cerevisiae wild type PTCC-5052, which was obtained from biotechnological centre of the industrial and research organization (Shahriar – Iran).

Yeast cells were grown at 30 °C. Nutritional requirements appropriate for maintenance of the strains were scored on minimal media consisting of 0.67% Difco yeat nitrogen base and 2% carbon source (D-glucose), supplemented with appropriate amino acids (fulka, Switzerland) and uracil (Sigma, USA).Glucose (40% w/v) and 100 x amino acid stock solutions were filter sterilized and added after autoclaving. The pH of the prepared media was adjusted to 6.4 for chemical testing with 50 mM citate buffer. The chemical were dissolve in DMSO. All components were of analytical quality. Mebudipine and Dibudipine were synthesized in our lab as described previously [3].

RESULTS

Hepatocyte viability

The results of viability testing are shown in the figs. 2 and 3. These results showed that mebudipine and dibudipine do not show any toxicity towards rat hepatocytes at its effective concentration.

Yest cells toxicity testing

Effect of mebudipin and dibudipine is shown in Figs; 4 and 5.

DISCUSSION

It has been shown that mebudipin and dibudipineh are useful two new calcium antagonists with considerable effect on various laboratory animals [3-8]. The present work was carried out to test its toxicological effect on various cells. We tried yeast cells as a model cells.

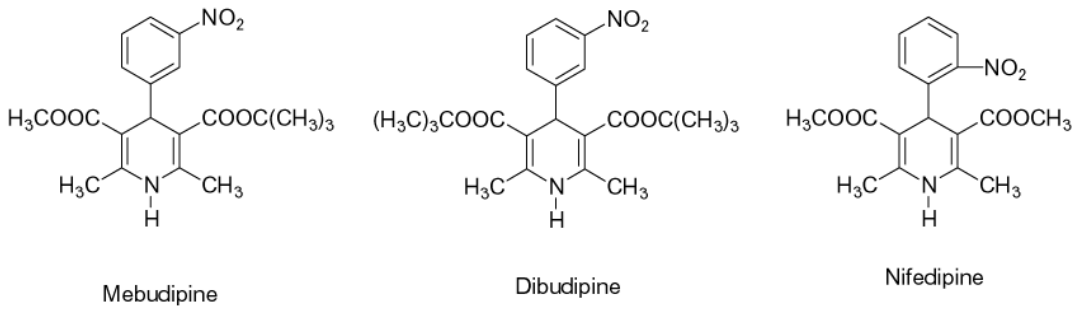

Fig. 1: Chemical structures of the 1, 4-dihydropyridine analogues mebudipine, dibudipine, and nifedipine

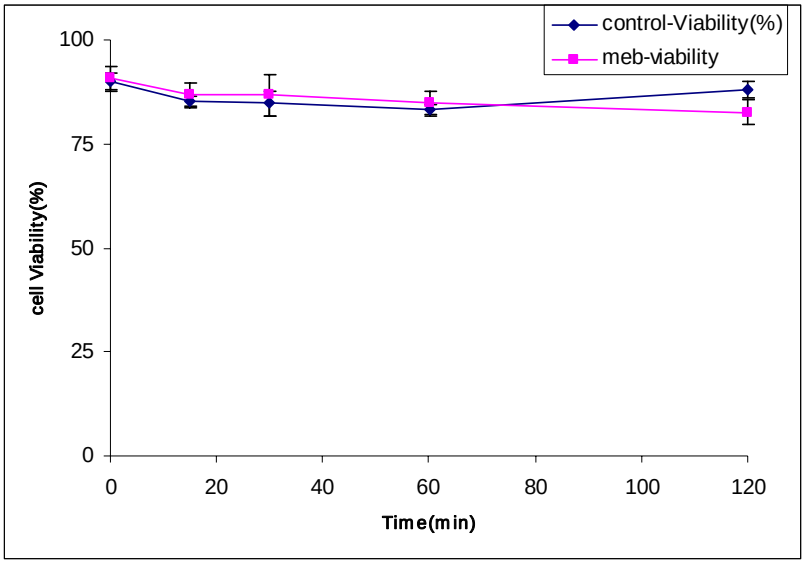

Fig. 2: Viability of isolated rat hepatocytes in the presence of mebudipine (100 µM) in the incubation media containing 2.4x10⁶ cell/ml

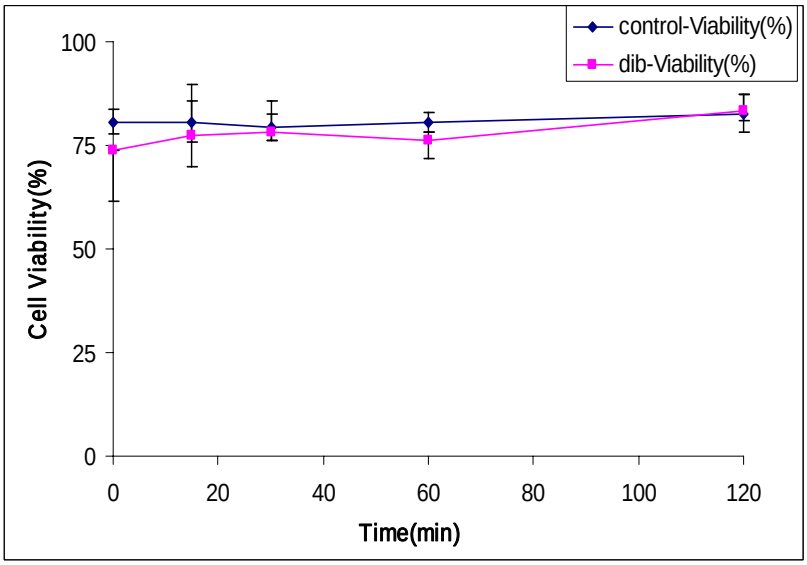

Fig. 3: Viability of isolated rat hepatocytes in the presence of dibudipine (100 µM) in the incubation media containing 2.4x10⁶ cell/ml

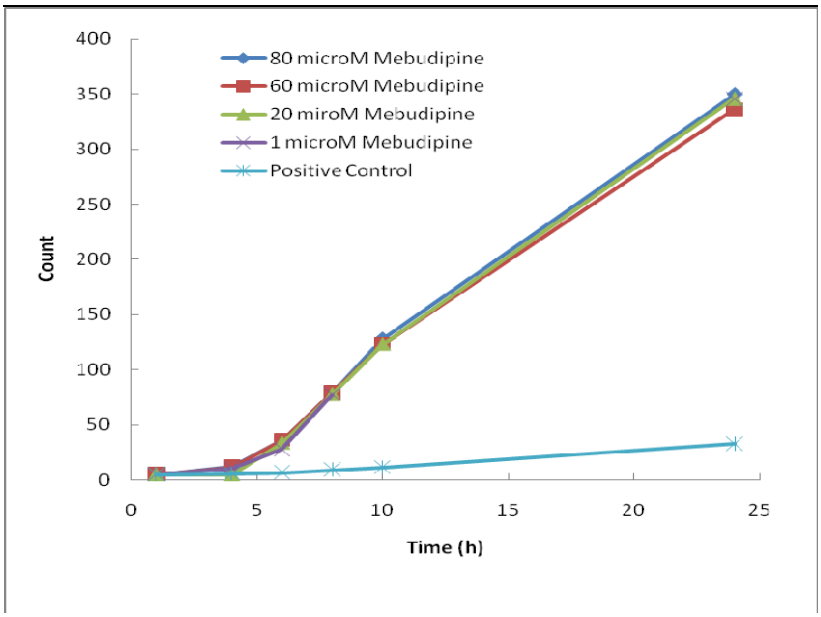

Fig. 4: Effect of of mebudipine on the viability of yeast cells. Numbers of cells shows in x access (10^5)

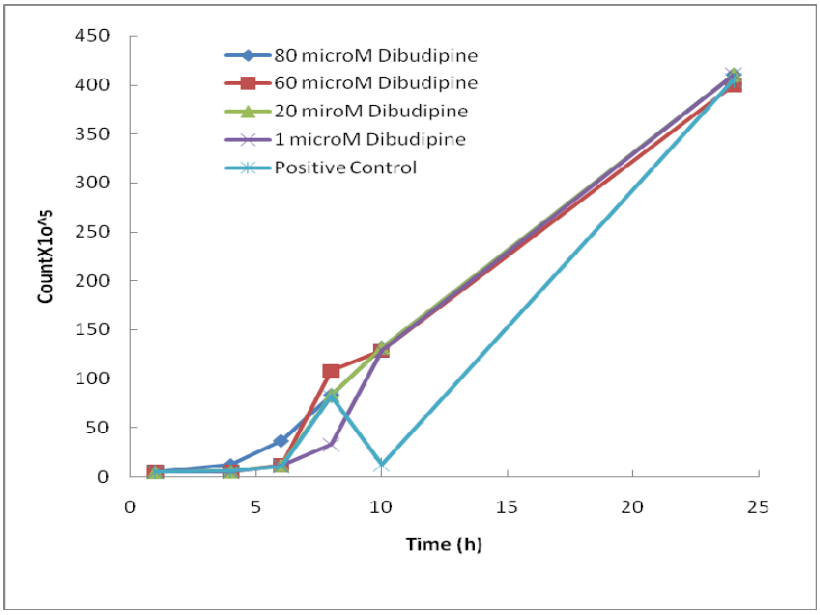

Fig. 5: Effect of of dibudipine on the viability of yeast cells. Numbers of cells shows in x access (10^5)

It has n shown that mebudipine and dibudipine did not effect the viability of hepatocyte in vitro and considering their lack of effect on mtagenicity effect on Aimes assay test on Samonella T102 [9].

It has been shown that yeast cells is a sensitive and practical model system for toxicological risk assessment has been reported by Schmitt.et al. [12].

Considering effet of mebudipne on yeast cells, heptocytes cells in-vitro, and reported effect on salmonella [11], and toxicological study in rats[13], it can be said that is drug is safe and can be used in humans.

ACKNWOLEDGEMENT

This paper is devoted to the memory of late Mrs. Mona Khoshbakhat,

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