Evaluation of dihydropyridine calcium antagonist effects on the stress bioindicator organism *Saccharomyces cerevisiae*

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ABSTRACT

Nifedipine is the subject of several studies since their introduction into clinical medicine in 1975. Some of them found it as a protective solution against stress increase, but others works demonstrate that can be a stress source. That's why in this present paper we want to put in evidence the effect which has this molecule on bioindicator stress: the yeast *Saccharomyces cerevisiae*. These effects have been studied by measuring the cell proliferation, respiratory activity and the levels of some biomarkers (CAT, MDA). The results obtained show a significant inhibition on cell proliferation of yeast and respiratory metabolism. We noted also a significant/high significant increase in all studied biomarkers following treatment with a dose-response manner.

Keywords: Biomarkers, Catalase, Cell growth, Lipid peroxidation, Oxidative stress, Respiratory metabolism, *Saccharomyces cerevisiae*.

INTRODUCTION

Nifedipine is a calcium channel blocker of the dihydropyridine type which is mainly used for the treatment of cardiovascular diseases such as hypertension, angina pectoris and coronary artery spasm [1,2,3,4] Thereby nifedipine has the potential to attenuate the development of cardiac hypertrophy and Left Ventricular dysfunction by acting directly on the signaling mechanisms in cardiac myocytes [5].

Nifedipine can be used as another therapeutic option due to its inhibitory effects of stress and ROS increases [6,7], as in the case of the protective effect against renal tubular toxicity caused by gentamicin [8]. Furthermore, nifedipine has been shown to protect the B-cells against an endoplasmatic reticulum stress and apoptosis, due to the high concentration of glucose, by the inhibitions of Ca²⁺ release [9]. Another study showed that nifedipine treatment ameliorated endothelium injury in patients with Systemic sclerosis and attenuated oxidative stress [10]. Also nifedipine GITS and atenodol are effective in preventing the development of wall-motion abnormalities or overall left ventricular dysfunction in response to mental stress, though the two therapies display different mechanisms of action [11].

All these papers spotlight the protective role played by the dihydropyridine calcium channel blocker against stress (hypersomotic stress, oxidative stress, mental stress…) and its inhibitory effects on superoxide production. [12] Paradoxically others studies found that molecule induced a strong and protracted stress response [13, 14] and caused a sperm antifertility [15].

In this work, we are interested to bring out the effect of this dihydropyridine -stress prevention or stress cause- on an alternative model to animal testing, a stress bioindicator organism, *Saccharomyces cerevisiae*. 
MATERIALS AND METHODS

Biological material
The biological material used is fungus unicellular eukaryotes: the yeast *Saccharomyces cerevisiae*, an optimal eukaryotic model system to study toxic effects and mammalian biological responses upon exposure to exogenous and endogenous perturbations [16,17]. The choice of this material is due not only for its organization highly similar to higher eukaryotic cells at both macromolecules that organelles levels [18,19], but especially for the presence of L-type calcium channels sensitive to dihydropyridine drugs [20,21,22].

Cultivation and treatment:
*Saccharomyces cerevisiae* was isolated in a culture medium favoring respiration (0.25 g / L glucose, 10 g yeast extract / L, 25 mL of glycerol and 940 mL of distilled water) [23] and treated during 4 hours with nifedipine.

Chemical material
The chemical material is a dihydropyridine calcium antagonist: nifedipine (C_{17}H_{18}N_{2}O_{6}) which selectively inhibits the transmembrane calcium by blocking the L-type calcium channels. [21,24] Nifedipine was dissolved in acetone and further diluted in distilled water with 1 % final concentration of acetone.

Fig. 1: Chemical structure of nifedipine (1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-dicarboxylic acid dimethyl ester)  

Four concentrations of this xenobiotic were chosen: 0.01mM, 0.05mM, 0.5mM and 1mM. All precautions have been taken to avoid denaturation/photodegradation of the molecule.

Parameters Studies:
Kinetics of Growth: The growth kinetics of yeast *Saccharomyces cerevisiae* is done by measuring the optical density (OD) at wave length $\lambda = 660$nm. [23]

Calculating the Percentage Response: The percentage of response which assesses response of yeast in presence of xenobiotic, according to the equation:

$$PR = \left[ \frac{(NC - BN)}{NC} \right] \times 100$$

The positive values of response percentage indicate an inhibition of growth, while negative values indicate a stimulation of growth.

Assay of Catalase Activity (CAT): The method used for determination of catalase activity (CAT) in yeast is the method of Regoli and Principato.[26]

Measurement of Respiratory Activity: The respiratory activity of yeast is measured by the method of Djebar and Djebar. [27]

Measurement of malondialdehyde: The proportionnig of malondialdehyde is carried out according to method of Draper and Hadley, [28] by using the colorimetric method, based on the reaction of thiobarbituric acid with MDA.

Statistical Analysis: Statistical evaluations were performed by Minitab version 15. The analysis of variance with two controlled factors is used to estimate the differences reported for the different studied parameters [29]. The data are represented by the mean more or less the standard deviation (m ± s). Differences were considered significant when *p < 0.05; very significant when **p < 0.01; and very high significant when ***p < 0.001.
RESULTS

Kinetics of Growth:
We found a significantly inhibitory/disruptive effect dose-dependent of the calcium antagonist on cell proliferation of *Saccharomyces cerevisiae*; it is respectively 40%, 52%, 64% and 60% in the treated concentrations of 0.01mM, 0.05mM, 0.5mM and 1mM nifedipine.

![Fig. 2: Effect of Nifedipine on Saccharomyces cerevisiae cell growth](image)

Response percentage:
The percentage of response is a parameter for evaluating the effect of the calcium antagonist at different concentrations confirms the results obtained concerning the kinetics of growth of the studied microorganism.

Figure (3) shows that the response percentage of *Saccharomyces cerevisiae* is dose-dependent and proportional to increasing concentrations of the Nif, it is respectively 56%, 68%, 72% and 68% for the concentration of 0.01mM, 0.05mM, 0.5mM and 1mM Nifedipine.

![Fig. 3: Evolution of the response percentage of Saccharomyces cerevisiae in presence of different concentrations of nifedipine](image)

Respiratory activity:
The results in figure 4 reveal disturbances recorded at the respiratory metabolism and shown an inhibition of respiratory activity for the yeast treated with different doses of Nifedipine. This inhibition is significantly for the concentration of 0.01mM (p = 0.047) and 1mM (p = 0.046) (with respectively an oxygen consumption of the order of 186nmoles/min/ml, 124nmoles/min/ml and an inhibition of about 58% and 39%) by contribution to control.
Fig. 4: Evolution of respiratory activity of *Saccharomyces cerevisiae* depending on the different concentrations of nifedipine

**Catalase Activity assays:**

The measurements of catalase enzyme activity showed an increase in the Yeast treated with different concentrations of Nifedipine.

Indeed, after 4 hours Nifedipine treatment, the level of catalase increased from 5.06 nmol/min/mg prot in controls (4.76 to acetone controls) to 19.26 nmol/min/mg prot in cells treated by 1mM concentration of Nifedipine.

**Measurement of malondialdehyde level:**

The results obtained show a significant (0.002 <p > 0.042) elevated in the Malondialdehyde levels on yeast treated with different concentrations of Nif.

Calcium antagonist causes significant oxidative stress in yeast with increased malondialdehyde level.
Fig. 6: Evolution of MDA level as a function of different Nifédipine concentrations in Saccharomyces cerevisiae

DISCUSSION

In this present study we have showed that nifedipine increased lipoperoxidation as well as antioxidant enzyme activities, with a decrease of a cell overgrowth and respiratory activity. These results point to indicate an occurrence of oxidative stress on Saccharomyces cerevisiae.

This toxicity is initially pronounced by the inhibition of yeast cells proliferation with a concentration-dependently manner. A similar finding has been reported in cultured cardiac nonmyocytes of neonatal rats \[30\], SHR and WKY fibroblasts \[31\] and in vascular smooth muscle cells (VSMCs) \[32\].

This antiproliferative action of dihydropyridines calcium channel blocker was due to different mechanisms, as an alteration of the cell cycle and a reduce of DNA synthesis caused by G\(_0\)/G\(_1\) arrest \[33\] (Nifedipine affected the transition of cells from G\(_0\)/G\(_1\) to S phase). \[31\]

The suppress role playing by our molecule can also be mediated by the activation of p21 (Waf1/Cip1) gene via the action of the glucocorticoid receptor GR and the transcription factors C/EBP-\(\alpha\). \[32\] or/and via the activation of LKB1-AMPK pathway (as upstream, a kinase LKB1 is required for nifedipine induced a phosphorylation of AMP-activated protein kinase (AMPK) in a dose-and time-dependent manner, and inhibited VSMCs proliferation). \[33\]

Concerning the catalase, an essential enzyme in the detoxification mechanisms, catalyses the conversion of H\(_2\)O\(_2\) to molecular water and oxygen \[34\], the increase in the catalase activity observed in our work is an indicator of cellular lesions and can be explained by the activation of an anti-oxidant mechanism to prevent the accumulation of ROS. These significant increases (\(p < 0.05\)) in the catalase enzyme activities of drug-treated yeast with nifedipine compared with controls is due to disrupt of the fine balance between the production and scavenging of ROS by calcium antagonists \[14\].

In the other hand, the treatment of Saccharomyces cerevisiae by the different concentrations of the calcium antagonist increase the Malondialdehyde (MDA) levels, an organic compound formed during the lipid peroxidation of cell membrane caused by ROS and free radical. This marker of oxidative stress can affected and damaged the macromolecules as proteins, lipoproteins and DNA.

The augmentation of MDA levels noted in present paper was at the same wavelength with other works on the nifedipine antifertility effect on sperm \[14\] was caused by an inhibition of acrosomal reaction which modify the sperm and by the loss in motility and lipid damage, the factors responsible for causing aging and finally infertility. \[35\]

Lastly, the measure of cellular respiratory, used as a mitochondrial system dysfunctionary and a tool of a level toxicity evaluation \[36\], show an inhibition of respiratory activity on saccharomyces cerevisiae treated with Nifedipine. Thus this dihydropyridine had an effect on mitochondrial metabolism by reducing the oxidative ATP synthesis rate \[37\]. This suggests that nifedipine has a direct effect on mitochondrial function. It is possible that this
effect is brought about by nifedipine influence on intracellular calcium. More investigations will be required to put in evidence the mechanisms of this effect.

Moreover, Nif can caused a macrovascular steatosis, considerate like a consequence of a mitochondrial dysfunctionnement, bring about an accumulation of triglycerides on cell, by activating transcription factor involved in hepatic lipogenesis such as PXR. [37,38]

To conclude this work, it seems obviously that the dihydropyridine calcium channel blocker exercise a toxic effect on *Saccharomyces cerevisiae* and through that all the biochemical parameters measured. By the way our results highlight an important oxidative stress translated by the inhibition of yeast growth and respiration, in addition to a lipoperoxydation (MDA), along with the outburst of detoxification system by the stimulation of catalase and peroxidise, and finally showed that the yeast *Saccharomyces cerevisiae* can be used as an exemplary model drug to animal testing.

REFERENCES


