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Protective role of the novel hybrid 3,5-dipalmitoyl-nifedipine in a cardiomyoblast culture subjected to simulated ischemia/reperfusion

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ABSTRACT

This work investigated the acute effects of the calcium channel blocker nifedipine and its new fatty hybrid derived from palmitic acid, 3,5-dipalmitoyl-nifedipine, compared to endocannabinoid anandamide during the process of inducing ischemia and reperfusion in cardiomyoblast H9c2 heart cells. The cardiomyoblasts were treated in 24 or 96-well plates (according to the test being performed) and maintaining the treatment until the end of hypoxia induction. The molecules were tested at concentrations of 10 and 100 μM, cells were treated 24 h after assembling the experimental plates and immediately before the I/R. Cell viability, apoptosis and necrosis, and generation of reactive oxygen species were evaluated. Nifedipine and 3,5-dipalmitoyl-nifedipine were used to assess radical scavenging potential and metal chelation. All tested molecules managed to reduce the levels of reactive oxygen species compared to the starvation + vehicle group. In in vitro assays, 3,5-dipalmitoyl-nifedipine showed more antioxidant activity than nifedipine. These results indicate the ability of this molecule to act as a powerful ROS scavenger. Cell viability was highest when cells were induced to I/R by both concentrations of anandamide and the higher concentration of DPN. These treatments also reduced cell death. Therefore, it was demonstrated that the process of hybridization of nifedipine with two palmitic acid chains assigns a greater cardioprotective effect to this molecule, thereby reducing the damage caused by hypoxia and reoxygenation in cardiomyoblast cultures.

1. Introduction

Cardiovascular disease is a global public health problem. Damage after myocardial ischemia and reperfusion (I/R) is the leading cause of morbidity and mortality globally [1,2]. Hypertensive patients are most affected by I/R [3], stressing the importance of studies investigating drugs that attenuate blood pressure while concurrently preventing or mitigating I/R myocardial damage.

Evidence indicates that several interrelated factors, such as the decrease in cellular ATP levels, the production of reactive oxygen species (ROS), the accumulation of hydrogen ions and the generation of reactive nitrogen species, contribute to the damage caused by I/R [4]. Oxidative stress contributes to the cascade of events leading to cell death: increased ROS production may modify the expression of various inflammatory mediators during cardiac injury [5]. During reoxygenation, the membrane integrity is compromised by the oxidation of the phospholipids which leads to uncontrolled ion permeability [6]. The ROS can still compromise the function of cardiac proteins, such as ion channels, calcium pumps and contractile proteins involved in the excitation mechanism of heart contraction [7]. Thus, the restoration of blood flow and the return of the oxygen supply generate ROS that trigger cell death through apoptosis and necrosis [6].

Substances that have anti-hypertensive and antioxidant action can bring benefits for the treatment of hypertensive patients prone to ischemia and reperfusion injury. According to Benzie and Tomlinson [8], antihypertensive drugs such as captopril, fosinopril,

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enalapril, perindopril, quinapril, and ramipril can act as iron ion scavengers, using the ferric reduction antioxidant power test (FRAP), although these substances are not as powerful as the classic antioxidant ascorbic acid. An antioxidant substance can protect biomolecules from damage mediated by both in vivo and in vitro free radicals by preventing or slowing oxidation of macromolecules. It does this by acting against the toxicity of metals, connecting these metals and avoiding reactive oxygen species generation. It also works through the chelation of those metals maintaining the redox state of the molecule [8].

Another commonly used antihypertensive drug is nifedipine (NIF) [9]. It is a member of the dihydropyridine family, which is characterized by blocking the calcium channel, and is also a potent vasodilator that is widely used as an antihypertensive [9]. However, one of the side effects of nifedipine is tachycardia, a risk factor for ischemia diseases such as acute myocardial infarction [10,11]. The treatment of cardiovascular diseases with hybrid molecules may involve more than one pharmacological action in a single drug, thereby providing an efficient alternative to potentiate already known effects or assign new effects to molecules [10].

NIF is also effective in inhibiting the activation of tumor necrosis factor kappa beta (NFkB), thereby contributing to decreased inflammation, and increased endothelial function in coronary circulation [12,13]. NIF has been shown to promote re-endothelialization after vascular injury, and is considered to protect against atherosclerosis by inhibiting endothelial cell apoptosis and suppressing vascular inflammation, effects attributed to the antioxidant properties of the drug [13,14].

Previous studies have shown the relationship between cannabinoid receptors and oxidative stress, confirming the antioxidant action of the ligands of these receptors in human pancreatic tumor cell lines [15–17]. However, the antioxidant effect of endocannabinoids is not only connected to their receptors. Some studies have shown that endocannabinoids play an important modulatory role in the function of the cardiovascular system in various pathological conditions, such as hypertension, myocardial infarction and heart failure [16,17].

To assist in mitigating the damage caused by I/R, we report in this study a convenient synthesis of novel hybrid molecule, 3,5-dipalmitoyl-nifedipine (DPN), via a one-pot Hantzsch multicomponent reaction using sulfamic acid as an inexpensive and nontoxic catalyst (Fig. 1). In the process of joining molecules, the fatty amides show an essential biological activity, whose importance is likely to facilitate the permeability of the cells of these new molecules [18]. Since the myocardial ischemia process is a global health problem, the authors aimed to study the acute effects of the calcium channel blocker nifedipine and its hybrid fatty acid during hypoxia/reoxygenation in a H9c2 cardiac cell line (cardiomyoblast) and compare these effects with the effect of anandamide, as well as in vitro testing the antioxidant potential of nifedipine and its hybrid fatty acid.

2. Methodology

2.1. Apparatus and chemistry

Sulfamic acid (98%) was supplied by Aldrich Chemical Co., and the methanol was supplied by Merck. The other reagents were purchased from Aldrich Chemical Co. and used without further purification. All organic solvents used for the synthesis were of analytical grade. The palmitic β-keto esters were synthesized through transesterification of methyl acetoacetate with the respective alcohol derived from palmitic (C16:0) acid. Anandamide (AEA) was synthesized by reacting arachidonic acid with ethanol amine, triethylamine, and a catalytic amount of dimethylaminopyridine (DMAP), and dicyclohexylcarbodiimide (DCC). Nifedipine was synthesized using the same experimental protocol described for 3,5-dipalmitoyl-nifedipine (DPN) in the item 2.2. The spectroscopy data used for anandamide (AEA) and nifedipine (NIF) are according to the literature. The reactions were monitored using thin-layer chromatography (TLC) performed with plates containing silica gel (Merck 60GF245), and the spots were visualized using iodine. Column chromatography was performed using Silica Gel 60 A (ACROS Organics, 0.035–0.070 mesh). Yields refer to chromatographically and spectroscopically homogeneous materials. The NMR spectra were recorded using a Varian VNMRS 300 spectrometer (^{1}H at 300 MHz and ^{13}C at 75.5 MHz) and deuterochloroform ($CDCl_3$) as the solvent. The chemical shift data are reported in units of δ (ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard.

2.1.1. Experimental procedure for synthesizing 3,5-dipalmitoyl-nifedipine (DPN)

Palmitic β-keto ester (2 mmol), 2-nitro-benzaldehyde (1 mmol), ammonium acetate (3 mmol), methanol (5 mL) and

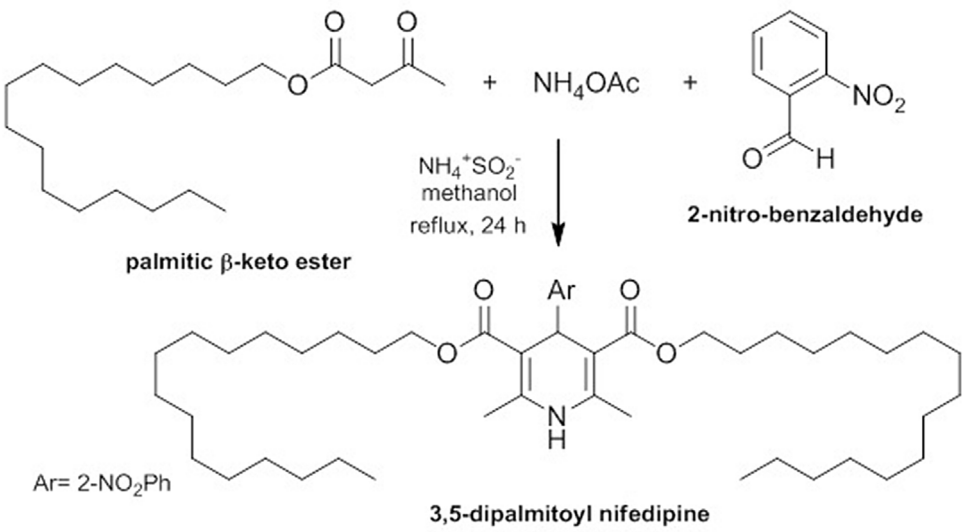

Fig. 1. Synthesis of the new hybrid 3,5-dipalmitoyl-nifedipine (DPN) through a Hantzsch multicomponent reaction using sulfamic acid.