ELSEVIER

Pathophysiology 21 (2014) 293–300

ISP PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Comparative study of the effect of verapamil and vitamin D on iron overload-induced oxidative stress and cardiac structural changes in adult male rats

Eman S.H. Abd Allah [a,*], Marwa A. Ahmed [a], Asmaa Fathi Abdel Mola [b]

[a] *Medical Physiology Department, Faculty of Medicine, Assiut University, Assiut, Egypt*
[b] *Histology Department, Faculty of Medicine, Assiut University, Assiut, Egypt*

Received 11 February 2014; received in revised form 24 May 2014; accepted 11 June 2014

Abstract

The present study was designed to compare the effect of verapamil and vitamin D on the expression of the voltage-dependent LTCC alpha 1c subunit (Cav1.2) and thereby on iron overload-induced cardiac dysfunction in adult male rat. Forty rats were randomly divided into four groups. Control group received the vehicle, iron overload group received ferrous sulfate intraperitoneally (IP) for 4 weeks, iron overload + verapamil received ferrous sulfate and verapamil IP concurrently for 4 weeks and iron overload + vitamin D group received ferrous sulfate IP and vitamin D3 orally concurrently for 4 weeks. Serum ferritin, total antioxidant capacity (TAC), total peroxide (TP) and cardiac iron and calcium were determined. Oxidative stress index (OSI) was calculated. Histopathological studies using H&E, Masson trichrome and Prussian blue stains and immunohistochemical studies using Cav1.2 antibody were also carried out. Administration of ferrous sulfate induced a significant increase in serum ferritin, OSI, cardiac iron and calcium contents. Moreover, cardiomyocytes were degenerated and the expression of Cav1.2 protein was increased in iron overload group as compared to control. Verapamil decreased ferrous sulfate-induced increase in serum ferritin, OSI and cardiac iron deposition. In addition, verapamil improved myocardial degeneration and decreased the expression of Cav1.2 protein. In contrast, vitamin D produced insignificant changes in ferrous sulfate-induced increase in cardiac iron content, myocardial degeneration and the expression of Cav1.2 protein. These results indicate that verapamil has a protective effect against iron overload-induced cardiac dysfunction, oxidative stress and structural changes, while vitamin D has an insignificant effect on these parameters.

Keywords: Iron overload; Adult rat; Verapamil; Vitamin D; L-type Ca^{2+} channel

1. Introduction

Beta-thalassemia major (BTM) is the most common genetically determined chronic haemolytic anemia (85.1%) in Egypt [1] and is most common among people of African or Asian ancestry. BTM patients need frequent blood transfusions which in turn lead to iron overload. Iron overload in these patients leads to iron deposition in many tissues, particularly in the heart resulting in cardiomyopathy, heart failure and eventually death. It is the commonest cause of death among patients receiving chronic blood transfusion [2]. In iron overload, transferrin is saturated and non transferrin-bound iron (NTBI) is released into the circulation and enters the cardiomyocytes through L-type calcium channels (LTCC) which is toxic to the heart [3]. Excess iron is catalyzed by the rapid Fenton reaction producing hydroxyl radicals from hydrogen peroxide and superoxide [4], which induces lipid peroxidation and cellular damage [5].

LTCC have been suggested as a possible pathway for cardiac iron uptake [6]. LTCC mediate long lasting Ca^{2+} currents [7]. They consist of five subunits with α1 subunit is the ion-conducting pore [8]. Four different LTCC α1 subunits have been cloned with Cav1.2 (α1c) and Cav1.3 (α1D) being the

* *Corresponding author at*: Physiology Department, Faculty of Medicine, Assiut University, Assiut, Egypt. Tel.: +20 882316384/1123392260; fax: +20 88 2332278.
E-mail address: eman_sayyed2@yahoo.com (E.S.H. Abd Allah).

http://dx.doi.org/10.1016/j.pathophys.2014.06.002
0928-4680/

principle isoforms expressed in the heart. Cav1.2 is the major isoform in the working myocardium, while Cav1.3 is the major isoform in the sinoatrial node (SAN) [9]. LTCC is primarily used for Ca^{2+} transport; however, several researches suggested that LTCC can be used for other divalent cations such as Fe^{2+} [3].

Calcitriol (1,25(OH)2-vitamin D3), which is the active form of vitamin D was found to play an important role for the growth, proliferation and normal structure of the cardiomyocytes [10]. It exerts its action through cytosolic and membrane bound receptors, which are present in most cells including cardiomyocytes [10]. Vitamin D deficiency has been linked to numerous cardiovascular complications including high risk of coronary heart disease, myocardial infarction, hypertension [11] and heart failure [12]. Moreover, vitamin D deficiency is highly prevalent among β-thalassemia patients [13] and this may contribute to the growth impairment [1] and cardiovascular disease observed in these patients.

It has been shown that verapamil reduced cardiac iron uptake [14,15] and thereby it may be considered the drug of choice for the prevention of iron overload cardiomyopathy. Accumulating data suggested that vitamin D supplementation may be beneficial for the prevention of cardiovascular diseases. However, it is not known if vitamin D could be cardio-protective in iron overload-induced cardiac dysfunction. Therefore, vitamin D supplementation may represent an alternative treatment for preventing iron overload induced cardiomyopathy, especially in those cases where verapamil has been contraindicated.

Thus, the present study was designed to compare the ameliorative effect of verapamil and vitamin D on iron overload-induced cardiac dysfunction, oxidative stress, structural changes and expression of Cav1.2 protein in adult male rat.

2. Materials and methods

2.1. Animals

Adult male Wister Albino rats 6 weeks of age were used for this study. Animals were housed in clean capacious cages (up to 4 per cage) in animal house of Faculty of Medicine, Assiut University. They maintained on a natural 12:12-h light–dark cycle in an aerated room, temperature (25 ± 5 °C), food (standard rat pellets) and water available *ad libitum*. The experimental protocol was approved by the Institutional Animal Research Committee of the Faculty of Medicine, Assiut University, Egypt.

2.2. Experimental design

Forty rats were randomly divided into four groups, 10 animals each. Control group animals received the vehicle either sodium chloride 0.9% intraperitoneally (IP) or oil orally for 4 weeks. Animals of iron overload group were treated with 3 mg/kg/day ferrous sulfate dissolved in sodium chloride 0.9% IP for 4 weeks [16]. Iron overload + verapamil group received concomitant treatment with 3 mg/kg/day ferrous sulfate IP and 8 mg/kg/day verapamil dissolved in 5 ml sodium chloride 0.9% IP [17] for 4 weeks. Animals of iron overload + vitamin D group were treated with 3 mg/kg/day ferrous sulfate IP and 1 mg/kg/day cholecalciferol (vitamin D3) dissolved in 6 ml of coconut oil orally [18] for 4 weeks.

2.3. ECG recording

Surface ECG was recorded in rats anesthetized with an intraperitoneal injection of etamine and xylazine (125 and 12.5 mg/kg, respectively). Three surface probes were inserted into the subcutaneous space following a lead II configuration. Lead II was achieved by placement of the negative electrode near the right shoulder and the positive electrode to the left of the xyphoid space, analogous to the human right arm – negative electrode and left leg (LL) – positive electrode configuration. The following measurements were made: P–R interval, R–R interval (to monitor heart rate) and the duration of the QRS complex. PR interval was measured as the distance between the beginning of a P wave and the beginning of R wave. Data were averaged from three measurements from well-defined P waves.

2.4. Biochemical examinations

Blood samples were obtained from each rat via retro-orbital vein. Then, all animals were decapitated under anesthesia with urethane. Blood samples were initially centrifuged at 3000 round per min (rpm) for 15 min. The clear, non hemolysed supernatant sera were removed and kept at −20 °C until use for analysis. Serum ferritin was estimated using commercial available ELISA kit. Serum total antioxidant capacity (TAC) was measured colorimetrically using the commercial available kit (Bio-Diagnostics, Giza, Egypt) according to the method of Koracevic et al. [19]. The determination of the antioxidative capacity (TAC) depends on the reaction of antioxidants in rats' serum with a defined amount of exogenously provided hydrogen peroxide (H_2O_2). The antioxidants in the serum eliminate a certain specific amount of H_2O_2. The residual H_2O_2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3,5-dichloro-2-hydroxy benzensulphonate to a colored product. Serum total peroxide (TP) was measured as described by Harma et al. [20]. Briefly, TP is determined colorimetrically by an enzymatic reaction which involves the oxidation of xylenol orange into colored product. Oxidative stress index, an indicator for oxidative stress was calculated as the percentage ratio of TP to TAC in mM/L [20].

For determination of cardiac iron and calcium, part of the ventricle was quickly removed from each animal and homogenized. The homogenate was then dissolved in equal aliquots of analytical-grade 70% nitric acid (Sigma Aldrich) and 30%