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RESEARCH ARTICLE

VITAMIN C PREVENTS STRESS INDUCED CARDIOMYOPATHY IN PRENATAL NOISE EXPOSED RODENTS

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Abstract: Potency of oral vitamin C to prevent cardiomyopathy in prenatal noise exposed newborn *Wistar* rats was studied by comparing the cardiomyocyte numbers and the extracellular matrix expressions (ECM) to controls. Twenty-four newborns (NR) of 32 pregnant mothers were divided equally into 4 groups: K1 (distilled water [DW]), K2 (150 mg/kg of BW oral vitamin C once daily [VC]), P1 (4 hours daily of white noise at 95 dB [WN]+DW), and P2 (WN+VC). VC and WN were given from D1 till birth and from D15 till birth, respectively. The hearts of NR were harvested, and processed for histology slides (2 midsagittal 4 µ cut slides/ animal) stained with hematoxyllin-eosin and Masson trichrome for the cardiomyocytes and ECM quantification at the ventricles using Image Raster 3.0 and ImageJ, respectively. Pictures from 8 visual fields/ slide were taken and analyzed in duplicate (400x magnifications under a light microscope). Data were analyzed using SPSS 17; significance level of $p<0.05$. In P1, the cardiomyocite cell numbers was significantly lowest ($p<0.001$); whilst the ECM was significantly highest than K1, K2, P2 ($p<0.001$ and $p<0.005$, respectively). Here, vitamin C could prevent the adverse effect of prenatal noise exposures in the ventricle myocardium of newborn rats.

Keywords: cardiomyopathy, hypertrophy, prenatal noise, vitamin C

INTRODUCTION

Prenatal noise exposures may alter the DNA of cardiomyocytes in the heart of the rodents. At the same time, ultrastructural of the rat cardiomyocytes showed several subcellular changes (1). Noise exposure may increase fibrosis in rat cordis. This noise effect can result in extracellular matrix changes characterized by abnormal proliferation of collagen and thus development of tissue fibrosis (2). The alterations of heart tissue may disrupt the function of this organ correlating to many pathology i.e. hypertrophy cardiomyopathy, hypertension and ischemic heart disease (3).

In newborn rats, administration of vitamin C prevented tissue injury and death. Furthermore, vitamin C can improve cardiac protection during

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myocardial repair (4). Using isolated cardiomyocytes, it was reported that vitamin C reduced the necrosis and apoptosis of the cells (5). The study on the newborn rat ventricles treated with oral vitamin C after prenatal noise exposure has yet been cleared, thus studied here.

METHODS

The experimental protocols had been approved by the Animal Care and Use Committee (ACUC) of The Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya.

Twenty-four newborns from 32 female Wistar rats divided into 4 groups equally: K1 (distilled water), K2 (vitamin C 150 mg/kg BW/day), P1 (distilled water + white noise, 95 dB, 4 hr/day), P2 (vitamin C 150 mg/kg BW/day + white noise, 95 dB, 4 hr/day). Vitamin C and distilled water was administered orally once daily at 9 am from day 1 of pregnancy until delivery (6). Noise was given to P1 and P2 group as a white noise generated from a Real-time analyzer software version 5.2.0 (Yoshimasa Electronic Inc., Japan) that connected to a loudspeaker (Sony SRS XB30, Japan) located 30 cm above the rat cages, from day 15 of pregnancy until delivery (at 10 am to 2 pm daily). The intensity of the noise was measured by a sound level meter (Krisbow, Indonesia). K1 and K2 group were kept in different chambers without noise exposures in a different room during the experiment as control groups. At birth, the newborns were sacrificed (n=6 in each group).

The hearts of the newborn rats were harvested after decapitation, fixed in 10% formaldehyde contained solution. In all series, the hearts were sectioned from the apex cordis of the ventricle. Prior to this, the whole heart was paraffinated and sliced at 4 µ midsagittaly, the midventricular fragment was selected for histology slides. Slides were stained with hematoxylin-eosin for the counting of the number of nuclei of the cardiomyocytes using Image Raster 3.0 software (Mikonos Transdata Nusantara, Indonesia). The adjacent slide stained using Masson's trichrome to calculate the fibrosis in the ventricle myocardium represented by the % area of the ECM using ImageJ software (NIH, USA) (7). All slides were analyzed under a light microscope (Olympus, Japan) twice independently at 400x of magnification (2 slides/ animal; 8 visual fields/ animal). Data were analyzed either using ANOVA and LSD post-test; or Kruskall-Wallis and Mann-Whitney post-test. Prior to these tests, data were analyzed using Saphiro-Wilks and Levene homogeneity tests. The significance level is $p<0.05$ (SPSS 17).

RESULTS

The numbers of the cardiomyocytes of all groups were presented in table 1. The % area of the ECM presented of fibrosis area from all groups was detailed in table 2.

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Table 1. The results of the number of cardiomyocyte cell heart in each group. The highest count was observed in K2, whilst the lowest in P1.

Groups	Cardiomyocyte		
	Median (Maximum-Minimum)	Kruskal Wallis	Sig. (p) of Mann Whitney post test
K1 (n=6)	89.25 (94.75 – 79.00)	p<0.001[a]	K2 p=0.004 P1 p=0.004 P2 p=0.005
K2 (n=6)	104.75 (110.56 – 100.75)		K1 p=0.004 P1 p=0.004 P2 p=0.004
P1 (n=6)	57.41 (66.75 – 56.31)[a]		K1 p=0.004* K2 p=0.004 P2 p=0.004
P2 (n=6)	90.47 (93.88 – 78.50)		K1 p=0.005 K2 p=0.004 P1 p=0.004

Data are expressed as median number ± standard of error [a] P1 vs. K1, K2, P2. *p<0.05

After normality and homogeneity tests, we found that the data of the cardiomyocte numbers were not distributed normally although homogenous. Thus we proceeded with Kruskal-Wallis and Mann-Whitney post-test. When compared to the other groups, the number of the cardiomyocytes in P1 was significantly lower (p<0.05). In the post-test, it was shown that the cardiomyocyte number in P2 was significantly higher compared to in P1 (p=0.004).

Prior to the inferential stats, the normality and homogeneity tests were conducted using Shapiro-Wilks and Levene tests. The % area of ECM was homogenous and distributed normally. Thus, the % area of ECM was analyzed using ANOVA followed by LSD post test. It was found that the fibrosis area represented by the % of ECM in P1

Tabel 2. The results of the area of fibrosis heart in each group.

Groups	Area of fibrosis		
	Rerata ± SE	ANOVA	Sig. (p) LSD post test
K1 (n=6)	9886,37 ± 557,88	p=0.001[a]	K2 p<0.0001 P1 p<0.0001 P2 p<0.0001
K2 (n=6)	14450,18 ± 719,13		K1 p<0.0001 P1 p<0.0001 P2 p<0.0001
P1 (n=6)	24275,38 ± 1058,05		K1 p<0.0001 K2 p<0.0001 P2 p=0.003
P2 (n=6)	20823,83 ± 476,12		K1 p<0.0001 K2 p<0.0001 P1 p=0.003

Data are expressed as mean ± standard error of the mean. [a] P1 vs. K1, K2, P2. The area of fibrosis in P1 was significantly highest (p=0.001).

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was significantly higher compared to the other groups ($p<0.001$) showed in table 2. In LSD post-test, it was shown that the fibrosis area in P2 was significantly lower when compared to in P1 ($p=0.003$; table 2).

The representative figures of myocardium ventricles of 4 groups were shown below (Figure 1). The cardiomyocytes counted from the nucleus of each cell, it is oval and situated centrally, coloured in darker pinkish-purple. The ECM was shown as cotton-candy like structure coloured in lighter pinkish purple.

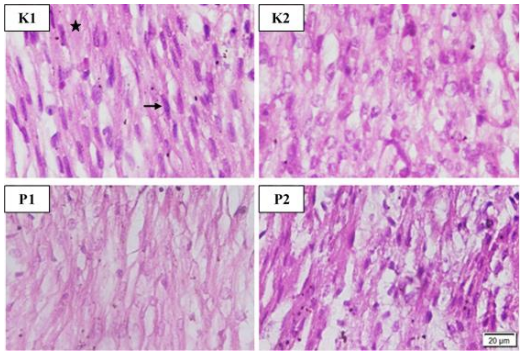

Figure 1. Cardiomyocytes cell in the newborn rat cardiovascular of K1, K2, P1 and P2 at 400x magnification with hematoxyllin-eosin staining. Scale bar 20 µ. Numbers of cardiomyocytes in P1 were decreased significantly compared to other groups ($p<0.001$). Whilst in P2, the cardiomyocytes were significantly higher compared to in P1 ($p<0.05$). Arrow showed the nucleus of the cardiomyocytes, the black star showed the ECM.

Distribution area of collagen at ECM represented the potential fibrosis shown in 5 steps of colour deconvolution using ImageJ can be seen in Figure 2. In P1, the area of fibrosis increase compared to groups K1, K2 and P2 which were statistically significant ($p = 0.001$).

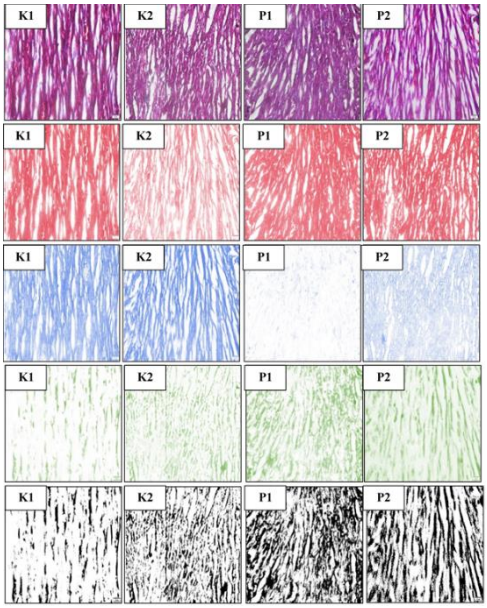

Figure 2. Area of fibrosis in the newborn rat heart of K1, K2, P1 and P2 at 400x magnification with masson trichome staining. Scale bar 20 µ. Area of fibrosis in P1 were increased significantly after a prenatal noise-stress exposures compared to other groups ($p<0.001$).

DISCUSSIONS

We observed a significant decrease in the cardiomyocyte numbers in the myocardium of P1 ventricles when compared to P2 and controls. The myocardium is a highly organized structure that contains several cell types, such as cardiomyocytes and fibroblasts. At the cellular level, cardiac growth occurs because of cellular proliferation and increased cellular volume (8). A newborn heart contains about half of the total number of myocytes compared to the heart of an adult. The volume of cardiomyocytes in humans, and also in rats, increases almost 25-folds and the number of cardiomyocytes become 3-4 times higher from birth to 2 months (9). The number of cardiomyocytes nucleus is one of the parameters that can be used

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to determine the occurrence of cardiomyocytes apoptosis. Previous study reported that noise could be a source of oxidative stress that has an adverse effect to the biological tissue including hearts. Noise can modulate the blood pressure, heart pulse and the levels of stress hormones i.e. glucocorticoids (10). In humans, fetus might response to acoustic external stimulants as early as 2 months; where noise can be transmitted via abdominal walls of the mother thus could affect the fetus directly. The approximate noise threshold was approximately 90-100 dB before producing structural damage to the hearing organs (11).

In oxidative stress, free radicals are atoms and/ molecules that have unpaired electrons in order to obtain chemical stability, immediately bound to the surrounding biology structures. Structures that are picked up by electrons will become free radicals as well so initiate a chain reaction, which eventually causes cell damages (12). The body would response to these stressors by developing general stress syndrome or general adaptation syndrome (GAS). GAS would activate the hypothalamus-hypophysis-adrenal (HPA) axis by releasing the corticotropin-releasing hormone (CRH) which then stimulates the adrenocorticotropic hormone (ACTH) secretion into the circulation. This hormone can be transported via the placentae from maternal to fetal circulation (13,14). The increase glucocorticoids may disrupt the gluconeogenesis and glycolisis by producing a relative hyperglycemia where glucose would go under pylol pathway or sorbitol-aldose reductase pathway, which can be transferred into sorbitol via aldose reduktase that transformed the nicotinamide adenine dinucleotide phophatase (NADPH) into NADP. The sorbitol would also be changed into fructose by transformation of NAD^+ into NADH. The decrease of NADH could deplete the production of glutathione (GSH) as the natural anti-oxidant thus implicating the imbalance between the oxidants and the anti-oxidants resulted in oxidative stress. This condition would then provoke the increase of reactive oxygen species (ROS) (15).

In this study we also found that there was an increase expressions of the ECM, one of potential markers of myocardium fibrosis, in P1 grup compared to in the other groups. On the other hand, the administration of oral vitamin C in P2 could prevent the over deposition of the collagen fibres in the extracellular matrix of the ventricle myocardium stained by Masson Trichrome compared to P1.

Vitamin C may act as scavengers to free radicals, including ROS, thus stopping the cell damage by producing an ascorbil that lesser reactive. Vitamin C is considered as the principal water soluble antioxidant in plasma and after uptake into cells, is able to exert many protective anti-apoptotic and anti-inflammatory actions. Vitamin C is a potent endogenous antioxidant crucial for maintaining antioxidant status and scavenging free radicals (16). These may be the cause of the cardiomyocytes lesser degree loss seen in P2 compared to in P1 in the current study.

Furthermore, vitamin C plays an important role for balancing the synthesis and deposition of collagen

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protein, which is the most abundant protein in the body and has a strong impact on the composition, structure and biomechanics of extracellular matrix tissue (16). The ECM is a complex mix of fibrillar polysaccharides and proteins synthesized and secreted by the cells in the extracellular space (17). The ECM homeostasis requires a balance between the degradation and synthesis of the collagen and, changes in this interaction may result in an abnormal collagen network in the heart (18,19). In the heart, fibroblasts are important cells in the remodeling that occurs in response to pathology changes such as, hypertension, myocardial infarction and heart failure (19). The clinical consequences of its presence would be inherent to the deposition of collagen in the cardiomyocytes and around the cardiac vessels leading to myocardial stiffness, left ventricular dysfunction, modifications in coronary flow reserve and ventricular arrhythmias (20).

CONCLUSIONS

In this study, vitamin C may prevent stress induced damage on the heart of prenatal noise exposed rodents.

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