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Inhibition of Inflammation and Oxidative Stress by Phospholipase A_2 Inhibitor in DOCA-salt Hypertensive Rats.

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Abstract: Background: *Hypertension is a chronic pathophysiological stress in the cardiovascular system, and it is a significant cause of generalised cardiovascular remodelling. Generally compounds that have the ability to decrease free radicals and inflammatory agents in the both can provide good remedies for these chronic clinical conditions associated with cardiovascular remodelling.* ***Objectives:*** *The current study was aimed to evaluate the inhibition of inflammation and oxidative stress by phospholipase A_2 inhibitor in DOCA-salt hypertensive rats.****Materials ad Methods:*** *14 Wistar rats were taken in this experiment, and the rats aged from eight to ten weeks old. Rats were fed and drank normal food and water for 4 days prior to the date for surgery. The surgery was performed to remove the left kidney from these selected rats, and after surgery the rats were divided into two experimental groups of 5-7 rats each. Group one was given 1% sodium Chloride in drinking water and a subcutaneous injection of deoxycorticosterone acetate (25 mg in 0.4 mL of dimethylformamide) every fourth day. In addition to this, group one was daily given phospholipase inhibitor (PLAi) in a form of dose of 5mg/kg/day by oral gavage as treatment, and the rats were given PLAi for 32 days. Group two which called UNX have been given normal food and normal water without any further treatment and no DOCA injection so as to act as a control fot the experiment. Moreover, the body weight measurements were taken on a daily basis, that is for water initial, water final, food initial and food final. All experiment took 32 days which is 4 days before surgery and 28 days after surgery, and the results for all parameters were recorded. Whole data sets were represented as mean ±standard error of the mean. Comparisons of findings between groups were made via statistical analysis of data sets using Student t- test to determine differences between treatment groups. $P<0.05$ was considered statistically significant.* ***Results:*** *First the water intake of the treated DOCA-rats is highly elevated. However, those rats exposed to Phospholipase inhibitor indicated a lowered values of increased water intake. The food intake by the DOCA-salt rats showed a decrease and there was a much more effect especially in the group one rats. The food intake by the DOCA-salt rats showed a decrease and there was a much more effect especially in the group one rats. The graph of force of contraction (mN) versus Noradrenaline concentration (log M) in DOCA-salt rats shows that there is a marked decreased contractile coefficient of the vessel, whereas the DOCA+PLAi rats have marked increased contractile coefficient, with increased concentration of noradrenaline. In DOCA-salt rats the graph shown that the force of relaxation (mN) versus acetylcholine concentration (log M) is dramatically declined while in DOCA+PLAi group the relaxation abilities are still finding. The group with both the DOCA and PLAi indicates a retention of both contractile and relaxation abilities as compared to the group with only DOCA. This is also seen in the graph of force of relaxation (mN) versus concentration of sodium nitroprusside (log M), where by there is a similar trend of decreased relaxation abilities of the blood vessels in the rats exposed to only DOCA-salt whereas in DOCA+PLAi rats the relaxation abilities are improved. The relaxation abilities response in blood vessels to sodium nitroprusside indicated more than relaxation abilities response to acetylchline.* ***Conclusion:*** *The DOCA-salt Wistar rat model is a typical example of induced cardiovascular remodelling which occurs in the human beings with chronic hypertension and heart disorders. The anti-inflammatory substances in the body usually decrease the DOCA-salt cardiovascular remodelling especially those related to fibrosis. The research can be utilised as opportunities to develop new noble therapeutic agents for chronic cardiovascular disease, and this is a major task in clinical medicine management of aged populations in our society.*

Keywords: Inflammation, Oxidative Stress, Phospholipase A_2 Inhibitor, DOCA-salt Hypertensive, Rats.

Introduction

Hypertension is a chronic pathophysiological stress in the cardiovascular system, and it is a significant cause of generalised cardiovascular remodelling. Chronic high blood pressure without control generates excessive deposition of collagen and other extracellular matrix proteins in myocardium, and ultimately resulting to hypertrophy, fibrosis, stiffness and electrical conduction changes in the heart. In addition, peripheral blood vessels are affected by chronic hypertension which leads to smooth muscle and endothelial dysfunction (Seifi et al., 2007). Prolonged and uncontrolled hypertension leads to activation of the inflammatory reaction and oxidation that generate cardiovascular remodelling in humans. An inflammatory response is a normal defence mechanism by the body immune system cells, expressed whenever they are exposed to infection and injury, but it usually take short period. However, this inflammation process becomes pathological when this mechanism continues for a prolonged period of time. This is because an increased complement factor in the cell lead to activation of inflammatory cells such as macrophages, monocytes, T cells, neutrophils, and mast cells in the damaged tissue, as well as the release of inflammatory cytokines. Therefore the infiltration of immunoinflammatory cells enhances the development of cardiovascular remodelling.

Oxidative stress which refers to the damage to the cardiovascular cells, induced by reactive oxygen containing free radicals, such as peroxynitrite (ONOO-), superoxide (O2-) and hydroxyl (OH-) which normally exist in form of unpaired electrons may occur. The body cells create oxidative free radicals by enzymes such as cyclooxygenases, myeloperoxidases, lipoxygenases, xanthine oxidase, uncoupled nitric oxide synthase, cytochrome P450, monooxygenase, peroxidases, haeme oxygenases, NADPH oxidases and the enzymes of the mitochondrial electron transport chain (Iyer, Chan & Brown 2010). Superoxide has significant roles which is cell signalling pathways and activate immune cells in the function of normal cells. Superoxide and other oxidative free radicals have the ability to momentarily react with nitric oxide (NO) to produce oxynitrite or generates hydrogen peroxide to create hydroxyl radicals. The free radicals are eliminated by enzymes which includes glutathione peroxidases, superoxide dismutases, thioredoxin reductase and catalase as well as by molecule antioxidants including polyphenol, ascorbic acid and glutathione, which removes these free radical intermediates and inhibit other oxidation reaction (Takimoto and Kass, 2007). These enzymes and molecule antioxidants maintain physiological concentration of superoxide. Therefore, cellular damage is caused by rise in superoxide and oxidative free radicals concentration, and this is produced by decreased removal from the body or increased production in the body. High concentrations of activated tissue inflammatory cells which produces oxygen containing free radicals usually perpetuate oxidative stress.

Inflammation and oxidative stress are two and closely related physiological processes that generates a chronic pathophysiological body stress, as seen especially in the cardiovascular remodelling. In experimental research study deoxycorticosterone acetate (DOCA-salt acts as mineralocorticoid family such as Aldosteron which cause reabsorption of sodium, secretion of potassium, and increase water retention. Therefore, blood volume will be increase which lead to increasing blood pressure. All these mechanisms' happen in kidney (Karatas et al. 2008) and sodium chloride were administered to DOCA-salt hypertensive rats, with single kidneys (uninephrectomised) to offer reliable animal models of oxidative and inflammatory damage in the cardiovascular system.).

Phospholipase inhibitor (PLAi) is an anti-inflammatory agent which limits the degree of cardiovascular damage caused by inflammatory agents in the body. Chronic hypertension usually results to excessive deposition of collagen fibres (also referred as fibrosis) in the endothelium and ventricular wall. This causes ventricular hypertrophy and endothelial dysfunction, and eventually remodelling due to a rigid myocardium. The DOCA-salt rat experiment indicates that cardiac fibrosis is enhanced by inflammatory processes which arising through involvement of inflammatory cells such as macrophages into left ventricles. This role of inflammatory elements is initiated by a cascade of body reactions that generates arachidonic acid by cell membranes through phospholipid hydrosis sn-2 ester bond. This PLAi prevents the pro-inflammatory enzymes from initiating collagen deposition in the ventricular walls and the endothelium which is a significant component of cardiovascular remodelling of chronic hypertension patients (Levick et al., 2006).

Generally compounds that have the ability to decrease free radicals and inflammatory agents in the both can provide good remedies for these chronic clinical conditions associated with cardiovascular remodelling. These compounds will thus abolish the excessive deposition of collagen and other extracellular matrix proteins in myocardium, and ultimately preventing hypertrophy, fibrosis, stiffness and electrical conduction changes in the heart.

Objectives

The current study was aimed to evaluate the inhibition of inflammation and oxidative stress by phospholipase A_2 inhibitor in DOCA-salt hypertensive rats.

Materials and Methods

14 Wistar rats with weights of about 300-350g were taken in this experiment, and the rats aged from eight to ten weeks old. Rats were fed and drank normal food and water for 4 days prior to the date for surgery. The surgery was performed to remove the left kidney from these selected rats (to obtain uninephrectomised rats), and after surgery the rats were divided into two experimental groups of 5-7 rats each. Group one was given 1% sodium Chloride in drinking water and a subcutaneous injection of deoxycorticosterone acetate (25 mg in 0.4 mL of dimethylformamide) every fourth day. In addition to this, group one was daily given phospholipase inhibitor (PLAi) in a form of dose of 5mg/kg/day by oral gavage as treatment, and the rats were given PLAi for 32 days. Group two which called UNX have been given normal food and normal water without any further treatment and no DOCA injection so as to act as a control fot the experiment. A control group of DOCA without further treatment was previously prepared by Hemant Poudyal (unpublished data). Moreover, the body weight measurements were taken on a daily basis, that is for water initial, water final, food initial and food final. All experiment took 32 days which is 4 days before surgery and 28 days after surgery, and the results for all parameters were recorded.

In the experiment the rats were anaesthestised by the administration of Zoletil (which is at 15 mg/kg of tiletamine and 15 mg/kg of zolazepam) via intraperitoneal injection, and the systolic blood pressure was taken, using an MLT844 Pizo-Electric Pulse Transducer (AD Instruments, Sydney, Australia) and inflatable tail-cuff connected to an MLT844 Physiological Pressure Transducer (AD Instruments) and Power Lab data acquisition unit (AD Instruments). Pentobarbitone sodium (100 mg/kg intraperitoneally) was given to rats for euthanization. The buffer was prepared for the Langendroff and organ bath chambers as shown in the constituents below.

Preparation of the buffer for Organ Bath Chambers

Compound	5L	Final Concentration in Tyrode's

NaCl	320g	136.9mM
KCl	16.1g	5.4mM
MgCl2	8.6g	1.05mM
NaH2PO4	2.6g	0.42mM
NaHCO2	75.9g	22.6mM
Mix them in 3500ml of distilled water then add 1500ml of distilled water		
CaCl2	10.6g	1.8mM

Preparation of the buffer for Langendroff buffer

Compound	**5L**	**CONCENTRATION(Mm)**
NaCl	34.8g	**119.10**
KCl	1.77g	**4.75**
MgSO4.7H2O	1.47g	**1.19**
KH2PO4	0.81g	**1.19**
NaHCO3	10.50g	**25.00**
Glucose	9.91g	**11.00**
Mix them in 3500ml of distilled then add 1500ml of distilled water		
CaCl2.2H2O	**1.59g**	**2.16**

The rats were left for about one to two minutes before starting to take organs and blood from them, Heparin (0.1um) was given to the rats through the femoral artery. After that the blood was taken from the abdominal artery, was put into a centrifuge at 5000 rpm for 5 minutes to take plasma. The heart was the first organ to be taken from the rats for langendorff. Thoracic aorta was removed from the rats, and it cleared from the fats then it cut into four rings (4 mm in length). The weights for rats' organs which are heart and kidney were taken for each rat.

The four rings of thoracic aorta were suspended in an organ bath chamber under a resting tension of ten mN. In addition, the organ bath chamber has been filled with about 20mL of Tyrode's solution, and the the valve on the aeration line was opened and adjust the flow of the oxygen/carbon dioxide mixture through the aeration frit to create a plume of small bubbles. Then the organ bath was washed until the tension of vessel stable in 10mN. After that, noradrenaline 40ul (2x10-6) which is prestart contraction (70%) was added to the number two, three and four of organ bath, and it lift until the curve arrived to highest contraction and stable. Then noradrenaline was added to organ bath chamber number one; further, sodium nitroprusside was added to organ bath chamber number two, and acetylcholine was added to organ bath chamber three and four. All drug doses were added by accumulative concentration as following table.

Table 1 Cumulative concentrations

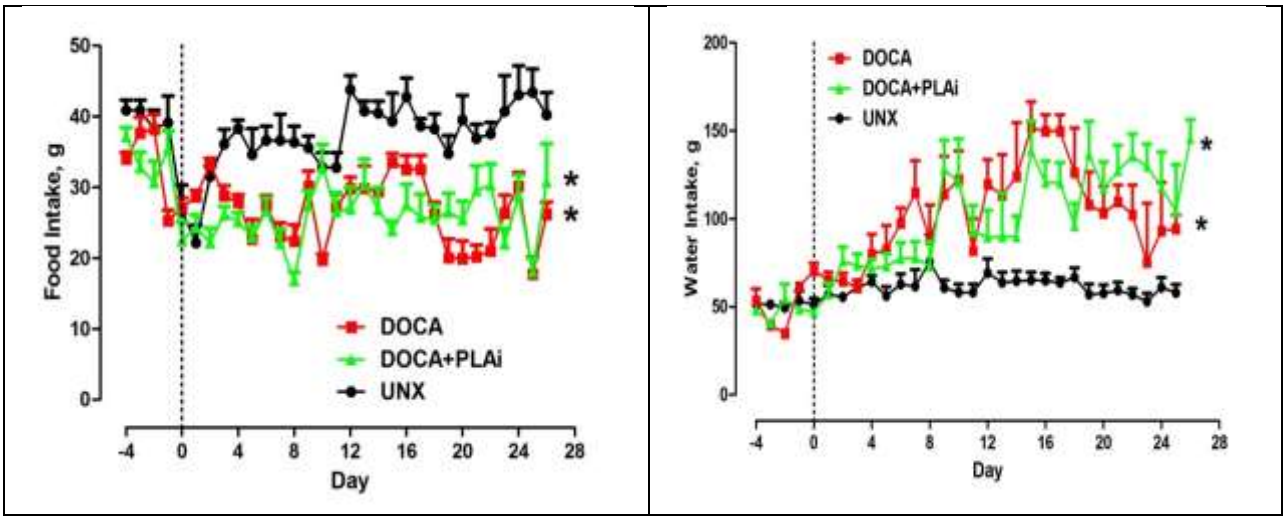
Bath concentrations (M)	To 20 ml bath add Volume of (µl)
1×10^{-9}	20
3×10^{-9}	40
1×10^{-8}	14
3×10^{-8}	40
1×10^{-7}	14
3×10^{-7}	40
1×10^{-6}	14
3×10^{-6}	40
1×10^{-5}	14
3×10^{-5}	40
1×10^{-4}	14
3×10^{-4}	40

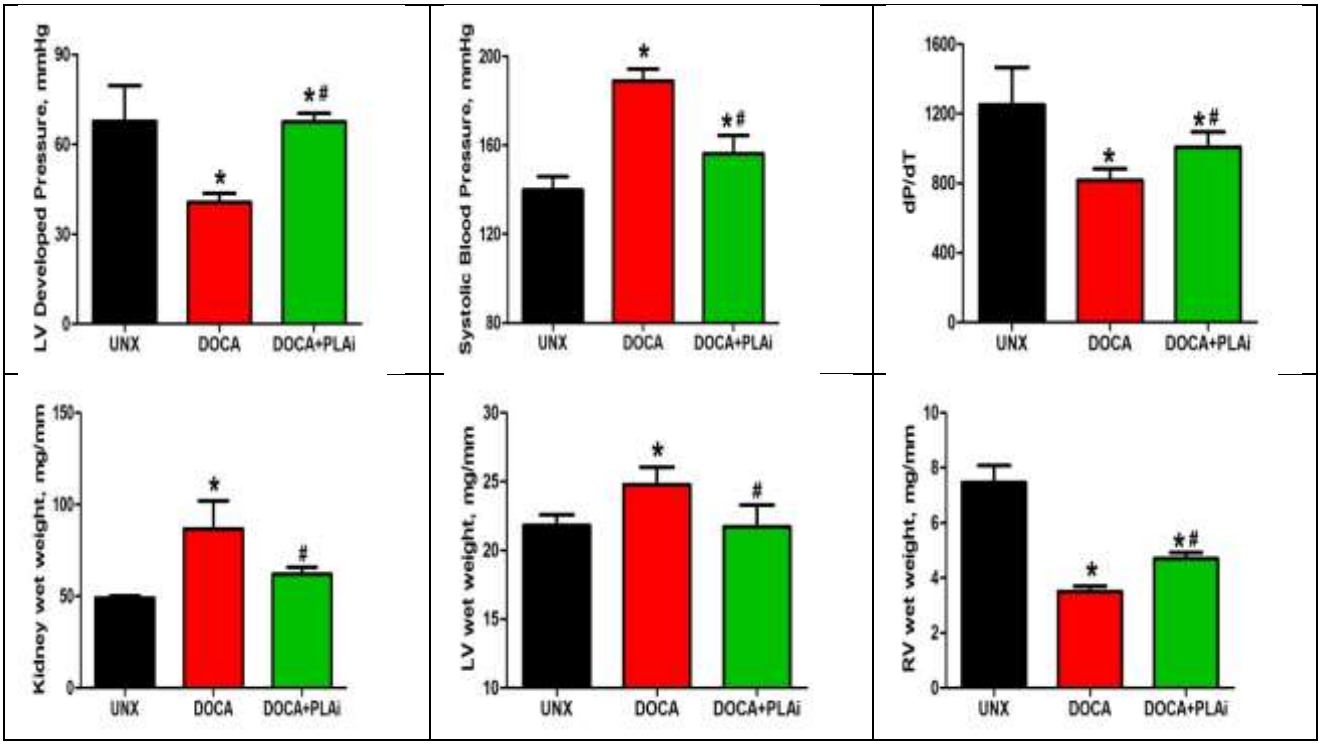
Statistical Analysis

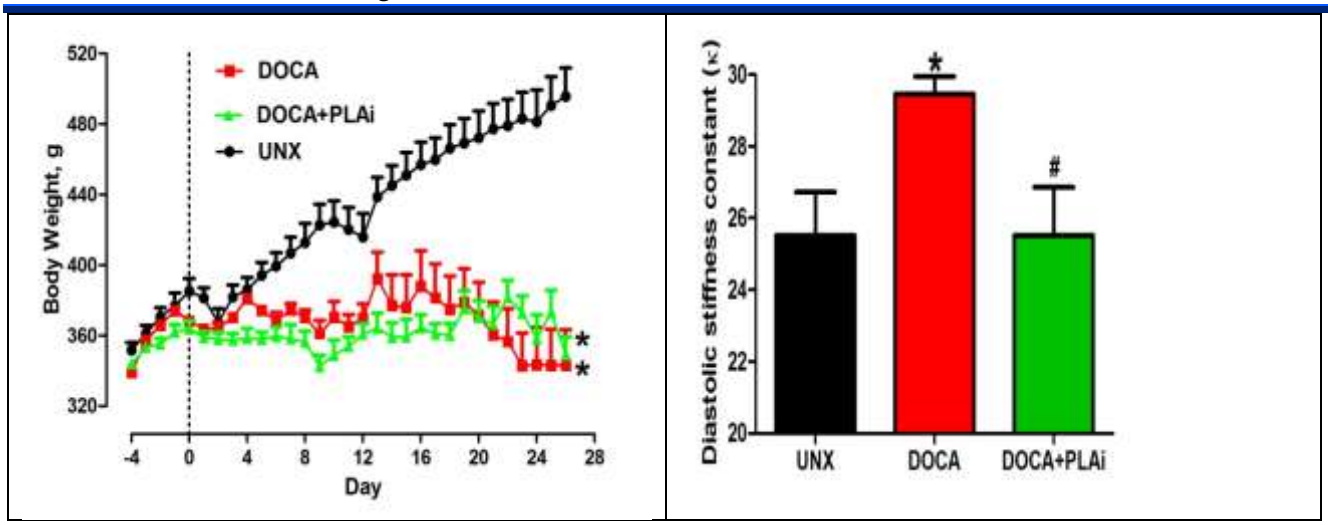
Whole data sets were represented as mean ±standard error of the mean. Comparisons of findings between groups were made via statistical analysis of data sets using Student –*t*- test to determine differences between treatment groups. $P<0.05$ was considered statistically significant (Iyer, Chan & Brown 2010).

Results

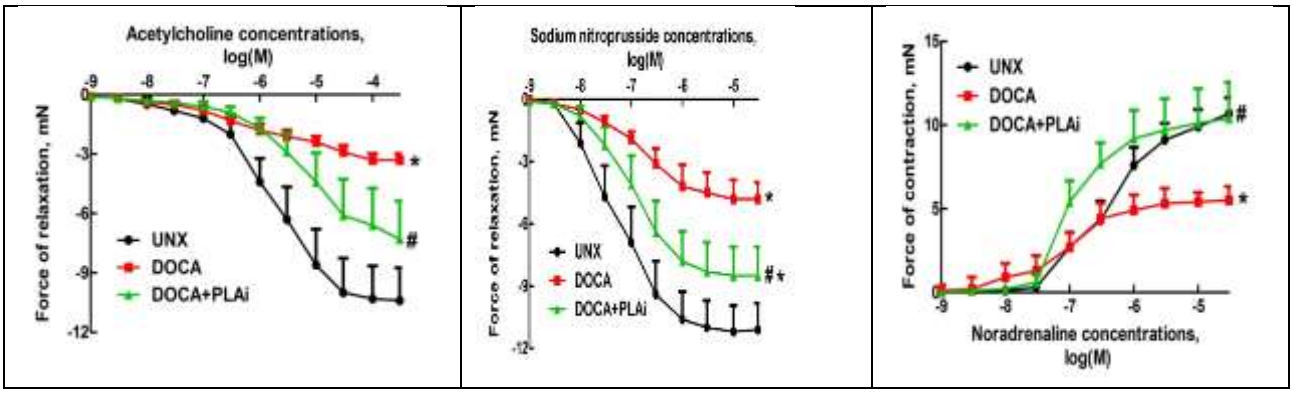
The results obtained from this experiment is shown as from the values of food and water intake, weights of the kidney, whole body, left and right ventricles, systolic blood pressure and diastolic stiffness constants for the entire group of rats. First the water intake of the treated DOCA-rats is highly elevated, and this indicated the corresponding levels of cardiovascular remodelling. However those rats exposed to Phospholipase inhibitor (PLAi) indicated a lowered values of increased water intake, and this is explained by abolition of the inflammatory distress such as those due to fibrosis in the cardiovascular system. The UNX rats showed a relatively constant value of water and food intake. The food intake by the DOCA-salt rats showed a decrease and there was a much more effect especially in the group one rats.







The body weight of the treated wistar rats does not show any increase as recorded by the control experiment animals, and this indicated the cardiac failure effects. The kidney weight for DOCA-salt rats and DOCA+PLAi is increased as a direct mechanism of compensatory effect to renal overload and this effect is much pronounced in DOCA-salt rats. Also the systolic blood pressure is observed to undergo elevation and this is accompanied by an increased diastolic stiffness constant. Unlike the decreased wet weight of the right ventricle, the left ventricle is recorded to have increased its wet weight due to compensatory hypertrophy to ensure effecient cardiovascular function regardless of increased peripheral vascular blood pressure. The left ventricle develope pressure is markedly reduced in DOCA-salt rats and slightly decreased in DOCA+PLAi rats.



From the organ bath results, the experiment indicates that a graph obtained indicates that in the DOCA-salt rats there is a cardiovascular remodelling due to development of chronic hypertension and endothelia dysfunction. The graph of force of contraction (mN) versus Noradrenaline concentration (log M) in DOCA-salt rats shows that there is a marked decreased contractile coefficient of the vessel due to fibrosis in smooth muscle whereas the DOCA+PLAi rats have marked increased contractile coefficient, with increased concentration of noradrenaline. In DOCA-salt rats the graph shown that the force of relaxation (mN) versus acetylcholine concentration (log M) is dramatically declined because fibrosis in endothelial layer of vessels while in DOCA+PLAi group the relaxation abilities are still finding. The group with both the DOCA and PLAi indicates a retention of both contractile and relaxation abilities as compared to the group with only DOCA. This is also seen in the graph of force of relaxation (mN) versus concentration of sodium nitroprusside (log M), where by there is a similar trend of decreased relaxation abilities of the blood vessels in the rats exposed to only DOCA-salt whereas in DOCA+PLAi rats the relaxation abilities are improved. The relaxation abilities response in blood vessels to sodium nitroprusside indicated more than relaxation abilities response to acetylchline. Due to sodium nitroprussid is endothelial independent, while acetylchline is endothelial dependent.

Discussion

The DOCA-salt rats show changes that resembles what is demonstrated in the chronic cardiovascular remodelling in humans. This includes hypertrophy, fibrosis, electrical conduction abnormalities, hypertension, as well as vascular hypertrophy and

dysfunction. Cardiac hypertrophies is also a characteristic sign in the cardivascular remodelling as indicated by the increase in weight per mm which signify both left and right hypertrophy or thickening. This is a concentric hypertrophy which is facilitated by cardiac fibrsosis and scar tissue formation which develops in both ventricular walls and this is followed by the increased expression of the collagen I and III mRNA resulting to excessive interstitial and perivascular deposition. This increased scar tissue formation is accompanied by a severe inflammatory distress indicated by the excessive extravasation of white blood cells into the ventricular tissue. Electrical remodelling is elevated as shown by the raised action potential, in duratioqns of a fifth, a half and nine tenth fractions, in repolarisation. This cardiovascular remodellingcan also be displayed by functional changes and they includes lowered values of left ventricular pressure or the E/A flow ratio, cardiac output,relaxation and contractile values of the cardiac muscles (+dp/dt, -dp/dt) and elevated values of diastolic stiffness in the DOCA-salt rats.

This is what happens to chronic cardivascular and hypertension in most human patients, and generally vascular hypertrophy is severe in small or large arteries in a these experimental rats, with extensive thickening of the media. Lowered sensitivities to stimulants of acetylcholine and sodium nitroprusside is an indication of endothelial and smooth muscle dysfunction, caused by myocardial fibrosis. This has a reduced effect in the second group which is exposed to PLAi as this inhibits the effects of pro-inflammatory agents in the body that causes fibrosis. Generally there is a markedly depression of reniangiotensin system in plasma, and elevated plasma aldosterone results to increased reabsorption of water and sodium ions from epithelial cells in distal renal tubules raising the blood pressure levels. Genetically the aldosterone may bind to mineralcorticoid receptor which is a member of the ligand-dependent transcription factors, and this leads to inheritance of these chronic disorders to offsprings. Increased concentrations of aldosterone activate oxidative stress by upregulated NADPH oxidase in this rats, and this can be mimicked by human hypertensive patients (Takimoto and Kass, 2007). Aldosterone usually induce superoxide and other oxidative free radicals secretion through mineralocorticoid receptor-mediated activation of NADPH oxidase as well as Rac1 in the vascular endothelium, and this causes vascular damage. NADPH oxidase amplifiess the free radical damage especially by myocardial fibrosis and this can lead to heart failure induced by NO synthase uncoupling and xanthine oxidase activities.

Therefore inhibition of production of free radicals such as superoxide generally improves the cardiovascular structure and subsequent functioning of DOCA-salt rats. This can be achieved by small molecules that can inhibit NAPDH oxidase as seen in the second group of experimental rats, which are exposed to PLAi. The superoxide production by NADPH oxidase is elevated by angiotensin II and endothelin through activation of selective receptors, therefore receptor antagonism will facilitate prevention or reverse the oxidative damage of the cardiovascular system. This can be implicated by ventricular hypertrophy attenuation and prvention of monocyte or macrophage accumulation in the left ventricles which is mostly affected through fibrosis (Brown et al, 1999). There is diminished in ventricular stiffness, action potential duration prolongation, and improved peripheral vascular function. Through rapid removal of superoxide there is a general improvement of the cardiovascular system effeciency in human patients with chronic hypertension or heart disorders as indicated by these DOCA-salt rats model experiment.

Conclusion

The DOCA-salt Wistar rat model is a typical example of induced cardiovascular remodelling which occurs in the human beings with chronic hypertension and heart disorders. Leucocytes and other inflammatory cells increases effects and activity of pro-oxidant enzymes such as myeloperoxidase and NADPH oxidases facilitating in generation of even more reactive oxygen free radicals. Generally any interventions directed at decreasing the body concentrations of oxygen-containing free radicals for instance superoxides, by either decreasing their production in the body or facillitating their removal from the body may reduce or eliminates this cardiovascular remodelling of heart and peripheral vasculature as shown by this experiment. Also the anti-inflammatory substances in the body usually decrease the DOCA-salt cardiovascular remodelling especially those related to fibrosis. From this reasearch study, we can give a conclusion that there is a combination effort by free radicals' oxidative damage as well as inflammatory reactions that enhances the development of cardivascular remodelling, through natural and synthetic compounds with anti-oxidative and anti-inflammatory effects and how they affects a cardivascular remodelling. This can be very significant in the fact that, the research can be utilised as opportunities to develop new noble therapeutic agents for chronic cardiovascular disease, and this is a major task in clinical medicine management of aged populations in our society.

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