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Medicine

MEMBRANE STABILIZING ACTIVITY OF ARUMUGA CHENDOORAM ON MEMBRANE BOUND ENZYMES IN EXPERIMENTAL RATS

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ABSTRACT Membrane fluidity plays a major role to maintained the mitochondrial activity and its evidences through the functional loss of membrane bound enzyme ATPases such as Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase Supplementation of Arumuga chendooram to methimazole treated rats restored the decreased activity of Na^+/K^+, Ca^{2+} and Mg^{2+} ATPases in thyroid of methimazole treated rats were observed in the present study. The results of the present study indicate that the protective role of Arumuga chendooram in methimazole treated induced oxidative stress in rats may be related to counteraction of free radicals. These findings suggest that the membrane stabilizing activity of Arumuga chendooram may indeed play a pivotal role in attenuating free radical damage on thyroid membrane.

KEYWORDS : ATPase, Membrane bound enzymes, Arumuga chendooram, Methimazole

INTRODUCTION

ATPases are membrane bound enzymes and are mostly present on the basolateral membrane. They help in maintaining the ionic gradients between aqueous intra and extracellular phases. Na^+/K^+ ATPase activity pumps Na^+ ions out of the cell. The cytosol of animal cells contains a concentration of potassium ions twenty times higher than that in the extra cellular fluid. Conversely, the extra cellular membrane contains a concentration gradient of sodium ions 10 times greater than that within the cells. These concentration gradients are established by the active transport called the Na^+/K^+ ATPase. As a result, the intracellular concentration of Na^+/H^+ exchange is established across the brush border membrane which splits up the ATP for energy purpose. The activity of Na^+/K^+ ATPase can be regulated by hormones, G-proteins, and secondary messengers (Veena et al., 2012).

Membrane fluidity plays a major role in elucidating the processes in aging and its evidences through the functional loss of membrane bound enzyme ATPases. The plasma membranes are of fundamental importance to cell structure and function. It surrounds the cell, and other membranes form a continuous intracellular surface (endoplasmic reticulum) and the structural basis of intracellular organelles such as mitochondria. Membrane function is vital to many cellular processes including the role of membrane enzymes and receptors in cell growth and signaling. A number of factors are thought to modulate membrane function including dietary components. The dominant lipids in animal cell membranes are phospholipids based on glycerol, such as phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol each with variable fatty acid side chains. The phospholipid bilayer forms the basic structure of all membranes and the presence of a wide range of different proteins confers on membranes a great diversity of function. Cholesterol is found in large amounts in plasma membranes (often equimolar with the phospholipid) whereas endoplasmic reticulum, mitochondrial, and nuclear membranes have a low cholesterol content (Zachowski, 1993). Mg^{2+} ATPases is poised to regulate the flow of potential energy from the mitochondria and from the cytoplasm. The decreased activities of Na^+/K^+ ATPase and Mg^{2+} ATPase in liver cancer bearing animals may be due to increase in the production of free radicals that leads to cell injury. The inhibiting function of ion dependent ATPases leads to disturbances in ion homeostasis. Disturbances in ion homeostasis results in impaired signal transduction, altered cellular metabolism, changes in cell membrane permeability and integrity and an elevation in membrane fluidity and disturbances of vital function. Thus the activities of all three ATPases in liver tissue have been found to be inhibited in carcinoma bearing animals (Daisy glory and Devaki, 2012). Keeping this in view, the present study was to monitoring the effect of Arumuga chendooram on membrane ATPase activity in experimental hypothyroid rats.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 180-190g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals

Nitroblue tetrazolium (NBT), ethylenediaminetetra acetic acid (EDTA), Trichloro acetic acid (TCA), Thiobarbituric acid (TBA), 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (reduced), glutathione (oxidized) and Nicotinamide adenine dinucleotide phosphate ($NADP^+$/NADPH) were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Preparation of Arumuga chendooram

The Siddha medicine Arumuga chendooram was prepared at its different stages of preparation in departmental laboratory with the help of a traditional siddha medical practioners as per the IMCOPS method.

In the first stage of the preparation of Arumuga chendooram. Five parts of purified mercury (Suththi seitha rasam), nine parts of purified sulphur (Suththi seitha kanthakam), seven parts of purified lode stone (Suththi seitha kantham), twelve parts of purified iron filing (Sutht.hi seitha ayapodi), four parts of rock salt (Induppu) and eight parts of desiccated borax (Poriththa venkaram) were ground with sufficient quantity of aloe juice (Kumari charu for five days continuously. This was then made into small cakes and dried. It was then sealed in discs and burnt for 24 hours. If the colour of the chendooram does not appear as dark purple the grinding and burning are usually repeated equal to pH and then attractive particle interations predominate which may influence the drug delivery.

Experimental design

Body weights of the animals were recorded and they were divided into **4** groups of 6 animals each as follows. First group was normal rats fed with standard diet and served as a control which received saline. Second group was negative control administered Methimazole (40mg/kg) induced experimental hypothyroidism for 40 consecutive days Third group was treatment group treated with Methimazole (40mg/kg) along with Arumuga chendooram (10mg/kg) for 40 days. Fourth group was positive control treated with Methimazole (40mg/kg) along with standard throxine sodium (20µg/kg) for 40 days.

Collection of samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with or without EDTA as anticoagulant. Blood, plasma and serum were separated for the estimation of various biochemical parameters. The thyroid tissues were dissected out, washed in ice-cold saline, and weighed. A known weight of them was used for homogenate preparation and used for various biochemical analyses.

Biochemical Estimation

The activity of Na^+/K^+ATPase and Ca^{2+}ATPase were assayed according to the method of Bonting (1970). The activity of Mg^{2+} ATPase was assayed by the method of Ohniski *et al.* (1982).

RESULTS AND DISCUSSION

Membrane fluidity plays a major role to maintained the mitochondrial activity and its evidences through the functional loss of membrane bound enzyme ATPases such as Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase Supplementation of Arumuga chendooram to methimazole treated rats restored the decreased activity of Na^+/K^+, Ca^{2+} and Mg^{2+} ATPases in thyroid of methimazole treated rats were observed in the present study. The results of the present study indicate that the protective role of Arumuga chendooram in methimazole treated induced oxidative stress in rats may be related to counteraction of free radicals. These findings suggest that the membrane stabilizing activity of Arumuga chendooram may indeed play a pivotal role in attenuating free radical damage on thyroid membrane (Table 1).

Table 1: Effect of *Arumuga chendooram* on ATPase activity in experimental rats

Parameters	Group I	Group II	Group III	Group IV
Na^+K^+ATPase (moles of Pi/min/mg)	8.62 0.62[a]	6.11 0.53[b]	7.85 0.50[a]	8.35 0..55[a]
Ca^{2+} ATPase (moles of Pi/min/mg)	12.65 0.84[a]	10.55 0.74[b]	11.95 0.75[a]	12.44 0.80[a]
Mg^{2+}ATPase (moles of Pi/min/mg)	19.11 1.54[a]	12.46 1.02[b]	18.78 1.11[a]	18.92 1.19[a]

Values were expressed as mean ± SD for six rats in each group.
[a] Significantly different from Group II (P < 0.05)
[b] Significantly different from Group I, III and IV (P < 0.05)

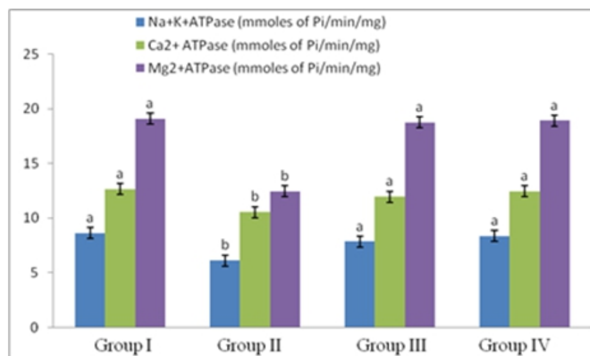

Figure 4.18: Effect of *Arumuga chendooram* on ATPase activity in experimental rats

Values were expressed as mean ± SD for six rats in each group.
[a] Significantly different from Group II (P < 0.05)
[b] Significantly different from Group I, III and IV (P < 0.05)

Nuclear mechanisms of thyroid hormone action have been extensively described but an increasing number of nongenomic effects of the hormone at the cellular level have been recognized in the past 10 years (Cheng *et al*. 2010). Actions of thyroid hormone are by definition independent on nuclear receptors for the hormone and have been described at the plasma membrane, various organelles, the cytoskeleton, and in cytoplasm. The actions include alterations in the transport of solutes like $Ca^{++,}$ Na^+ and glucose, changes in activities of several kinases, including protein kinase C, cAMP-dependent protein kinase and mitogen-activated protein kinase. Iodothyronines also can regulate nongenomically through a protein kinase C activation of neutral lipids, phospholipids and phosphatidylinositol 4, 5-bisphosphate (Axelband *et al*., 2011).

Membrane fluidity plays a major role in elucidating the processes in aging and its evidences through the functional loss of membrane bound enzyme ATPases. The plasma membranes are of fundamental importance to cell structure and function. It surrounds the cell, and other membranes form a continuous intracellular surface (endoplasmic reticulum) and the structural basis of intracellular organelles such as mitochondria.

Membrane function is vital to many cellular processes including the role of membrane enzymes and receptors in cell growth and signaling. A number of factors are thought to modulate membrane function including dietary components. The dominant lipids in animal cell membranes are phospholipids based on glycerol, such as phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositol each with variable fatty acid side chains. The phospholipid bilayer forms the basic structure of all membranes and the presence of a wide range of different proteins confers on membranes a great diversity of function. Cholesterol is found in large amounts in plasma membranes (often equimolar with the phospholipid) whereas endoplasmic reticulum, mitochondrial, and nuclear membranes have a low cholesterol content (Zachowski, 1993).

ATPases help in maintaining the ionic gradients between aqueous intra and extracellular phases. Na+/K+ ATPase activity pumps Na+ ions out of the cell. The cytosol of animal cells contains a concentration of potassium ions twenty times higher than that in the extra cellular fluid. Conversely, the extra cellular membrane contains a concentration gradient of sodium ions 10 times greater than that within the cells. These concentration gradients are established by the active transport called the Na+/K+ ATPase. As a result, the intracellular concentration of Na+/H+ exchange is established across the brush border membrane which splits up the ATP for energy purpose. The activity of Na+/K+ ATPase can be regulated by hormones, G-proteins, and secondary messengers (Veena *et al*., 2012).

Mg^{2+} ATPases is poised to regulate the flow of potential energy from the mitochondria and from the cytoplasm. The decreased activities of Na^+/K^+ ATPase and Mg2+ ATPase in methimazole trreated animals may be due to increase in the production of free radicals that leads to cell injury. The inhibiting function of ion dependent ATPases leads to disturbances in ion homeostasis. Disturbances in ion homeostasis results in impaired signal transduction, altered cellular metabolism, changes in cell membrane permeability and integrity and an elevation in membrane fluidity and disturbances of vital function. Thus the activities of all three ATPases in thyroid tissue have been found to be inhibited in methimazole treated animals (Kim *et al*., 2001). The action of membrane-bound enzymes depends on the cellular membrane integrity; an altered integrity of membrane following derangement of membrane lipids due to lipid peroxidation may affect the functioning of membrane ATPases, subsequently leading to ionic imbalances in the cells. This is evident from the results of the present investigation, which revealed increased lipid peroxidation.

The reductions in the activities of ATPase also suggest an alteration in the ionic transport and clearly indicate the toxic effects of methimazole. From the present study it is evident that methimazole decreased the activities of ATPase. Thus methimazole seems to have differential influence over membrane bound enzymes in the thyroid gland of rat. Supplementation of Arumuga chendooram -treated group showed a significant increase in the activities of Na+/K+ATPase, Ca2+ATPase and Mg2+ATPase. Present finding is similar to the Vanisthasree *et al*., (2011) and LeGrow *et al*. (1999) studies.

The results of the present study demonstrated that the effect of Arumuga chendooram on membrane bound enzymes in normal and experimental rats were investigated. The protective role of methimazole induced hypothyroid in rats may be related to counteraction of free radicals. The active constituents of Arumuga chendooram could be reported in this study for its *in vivo* antioxidant properties and these properties indirectly helps to maintain the levels of membrane bound enzymes such as Na^+/K^+ATPase, Ca^{2+}ATPase and Mg^{2+}ATPase on methimazole induced hypothyroid rats. These findings suggested that the membrane stabilizing activity of Arumuga chendooram may indeed play a pivotal role in attenuating free radical damage on cellular structural integrity.

REFERENCES

1. Veena MSP, Nithya G, Dhivya S, Sakthisekaran D. Biochemical alterations due to

membrane damage in benzo (a)pyrene induced experimental lung cancer. International Journal Of Pharmacy&Technology 2012. 4(2) p4325-4336.
2. Daisy Glory M and Devaki T. Chrysin attenuates the instability of xenobiotic metabolizing and mitochondrial enzymes during Diethyl nitrosamine induced liver carcinoma. Journal of Pharmacy Research 2011,4(6),1839-1842
3. Zachowski, A. (1993). Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. Biochem. J. 294, 1-14.
4. Vanisthasree K., A. Gopala Reddy, B. Kalakumar, C. Haritha, and B. (2011) Anilkumar, Hepatotoxicity Studies in the Progeny of Pregnant Dams Treated With Methimazole, Monocrotophos and Lead Acetate. Toxicol Int. Jan-Jun; 18(1): 67–69.
5. Kim HO, Tae-Hoon Lee, Young Sun Hwang, Mi Ae Bang, Kang Hwa Kim, Jae Mi Suh, Hyo Kyun Chung, Dae-Yeul Yu, Kyung-Kwang Lee, O-Yu Kwon, Heung Kyu Ro, And Minho Shong. (2001) Methimazole As an Antioxidant and Immunomodulator in Thyroid Cells: Mechanisms Involving Interferon- Signaling and H2O2 Scavenging Mol Pharmacol 60:972–980.
6. LeGrow A B, Fielding D C and Pressley T A. (1999) Stimulation of Na,K-ATPase by hypothyroidism in the thyroid gland. Journal of Endocrinology 160, 453–460.
7. Cheng, S. Y., Leonard, J. L. & Davis, P. J. Molecular aspects of thyroid hormone actions. Endocr. Rev. 31, 139–170 (2010).
8. Axelband, F.; Dias, J.; Ferrão, F.M. & Einicker-Lamas, M. (2011). Nongenomic signaling pathways triggered by thyroid hormones and their metabolite 3-iodothyronamine on the cardiovascular system. Journal of Cellular Physiology, Vol. 226, No.1, (January, 2011), pp. 21-28