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RESEARCH ARTICLE

ASCORBIC ACID (VIT-C) INDUCED BIOCHEMICAL ALTERNATION OF COLLAGEN CHARACTERISTICS IN THE HEART TISSUES OF SWISS MICE

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ABSTRACT

Collagen is a fibrous structural protein that provide strength to the different tissue of the body. Vitamin C plays an important role in collagen synthesis and stabilization by hydroxylation of the proline residue. As a person ages the quality of the collagen degrades due to the intra molecular and inter molecular cross-linking. In our present study when Swiss a mice were treated with vitamin C a insignificant increase in collagen concentration was found in the heart tissue. Heart contains less quantities of collagen protein when compared with other organs. Earlier workers suggest that the newly synthesized collagen may helpful for enhancing the function of efficiency of the heart. Since heart also response to the vitamin C for collagen synthesis, a required amount of vitamin C intake may help to/forage related heart problems. The values for salt soluble, acid soluble and total collagen content of control and experimental condition are 8.212, 5.852, 23.163, 37.229 and 10.619, 11.142, 31.070, 52.832 respectively. Similarly the values of % of salt solubility, % of acid acid solubility of control and experimental values are 21.798, 15.214 and 20.289, 23.444 respectively. The values of salt soluble / salt insoluble and acid soluble / acid insoluble are 0.290, 0.181 and 0.256, 0.335 respectively. Values are not significant at 0.05 confident levels.

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INTRODUCTION

Vitamin C or Ascorbic acid is a water soluble organic compound which is well known for its anti oxidant property. It is white crystalline in appearance and stable in dry form. Vitamin C has many functions it can function as a coenzyme or as a cofactor in the body. It appears to be necessary for the normal function of cellular units and sub-cellular structures. In metabolism, vitamin C functions to accept and donate hydrogen. Production of collagen, a protein substance in fibrous tissue, depends on ascorbic acid. Vitamin 'C' is essential in collagen synthesis, wound healing blood vessel maintenance and immunity. It is act as an antioxidant in the body and is used as a preservative and it is easily destroyed by oxygen. It is act as a co-enzyme in the hydroxylation of lysine and proline in the synthesis of collagen and elastin.

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It is also needed to make and repair collagen, move fat into cells, where it can be converted into energy (Beray, 2005). Vitamin C act as an electron donor for eight different enzymes. Three enzymes participates in collagen hydroxylation. Hydroxylation allows the collagen molecules to assumes its triple helix structure and thus vitamin C is essential to the development and maintenance of scar tissue, blood vessel and cartilage (Chvapil *et al*., 1973). Hydroxyproline and hydroxylysine are essential for the collagen cross-linking and the strength of the fiber. So that vitamin C is necessary for maintenance of normal connective tissue and wound healing process (Bailey and Robins, 1973). There are four amino acids found in the collagen like Glycine, Proline, Hydroxylysine, Hydroxyproline. Collagen fibrils are semi crystalline aggregates of collagen molecules. Collagen fibers are bundles of fibrils and arranged in different combination and concentration in various tissues to provide varying tissue properties (Chen and Rostlethwait, 1961). The distribution of collagen depends on the mechanical strength required of each tissue.

Table 1. In vivo effects of Ascorbic acid on collagen (dose 50 µg/kg body wt.) characteristics of Heart muscle of Swiss mice. Values for soluble, insoluble and total are mg/g tissue wet-weight (mean ± SEM), Numbers in parentheses indicate sample size, NS, Not significant at 0.05 confidence level

Experimental condition	Salt soluble	Acid soluble	Insoluble	Total	% of salt solubility	% of acid solubility	Salt soluble/ Salt insoluble	Acid soluble/ Acid insoluble
CONTROL	8.212 ± 1.885 (8)	5.852 ± 1.882 (8)	23.163 ± 4.004 (8)	37.229 ± 7.018 (8)	21.798 ± 2.863 (8)	15.214 ± 2.263 (8)	0.290 ± 0.046 (8)	0.181 ± 0.030 (8)
(P)	NS	NS	NS	NS	NS	NS	NS	NS
EXPERIMENT	10.619 ± 1.261 (8)	11.142 ± 1.606 (8)	31.070 ± 6.272 (8)	52.831 ± 2.297 (8)	20.289 ± 1.251 (8)	23.444 ± 4.232 (8)	0.256 ± 0.019 (8)	0.335 ± 0.075 (8)

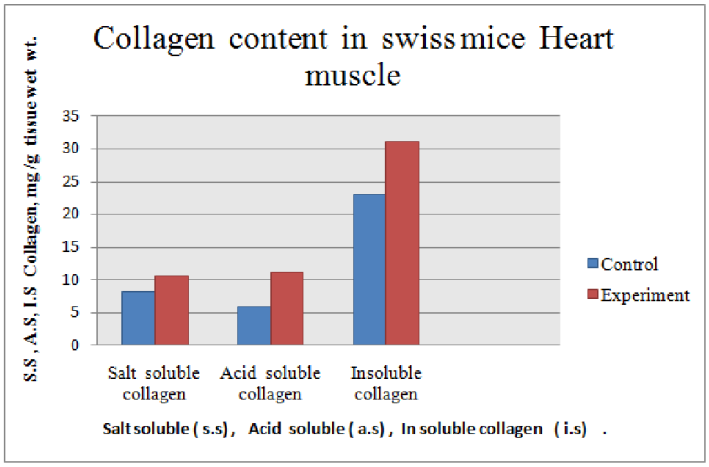

Fig. 1. Effect of ascorbic acid (50 µg/g) on salt-soluble (s.s), acid soluble (a.c), insoluble collagen (i.s) collagen of Heart Muscles of Swiss mice. Values are mg/g tissue wet-wt, columns represents the mean values and vertical bars SEM

It is known that the highest amounts of collagen are expected in those tissues which have primarily a mechanical function (Harkness *et al*., 1954). There are many way to promote the synthesis of new healthy collagen that provides the heart with a reserve of vitamin C, as a necessary co-factor in collagen synthesis. Vitamin C is proved to increase the production of collagen. It is shown that extended exposure of mammalian connective tissue cells to vitamin C stimulates an eight fold increase in the synthesis of collagen (Silva *et al*., 2000).

Aim and Objective

The main aim of the present study was to evaluate the anti-oxidant effect of vitamin C on Heart collagen of Swiss mice. Studies combining physical measurements and morphological observations indicates that the collagen mainly function to maintain the form and to limit deformation of tissues.

Choice of Parameters

The biochemical parameters chosen were the changes in the characteristics of the structural protein, collagen. In vertebrates, collagen constitute about 30-40% of total body protein. It is distributed in almost all tissues of the body. Salt-soluble collagen refers to newly synthesized collagen. Neutral saline solubilises collagen not covalently linked into fibers by labile aldimine type bonds (Everitt and Steele, 1970).

Studies on the effect of chemicals, on the collagen characteristics are considered important in view of their involvement in the manipulation of cross-links. A chemical agent found to prevent cross link formation might beuseful since cross-linked collagen is known to interfere with a variety of physiological processes (Kohn and Hamlin, 1978). While the total collagen content reflects a balance between its synthesis and degradation, the changes in solubility and soluble/insoluble collagen ratio are indirect indicators of alterations in the degree of cross-linkages of collagen molecules (Walford *et al*., 1969; Hall, 1976).

MATERIALS AND METHODS

Swiss albino mice (*Mus musculus*) procured from live animal supply farm Mr. Ghosh Enterprises, Kolkata, India, were supplied as the test animal in the present study. They were reared in the animal house of the department in perfect hygienic conditions.

The animal house was maintained at 23 ± 2^0C and 12 hours light and dark cycle. The mice cages and beds of papers and wood scarps were cleaned regularly. Balanced diet of prepared baked cake and tap water were provided to the animal. From the reared stocked healthy male mice of 10 to 12 weeks old and 15 to 20 gm body weight each were selected for experimental use.

Experimental Protocol

Thirty Swiss mice were utilized to derive the data for the present study and were divided into fifteen sets. Each sets have two mice, one of them injected intraperitonially with distilled water at the rate of 1 ml/kg body weight which served as the vehicle control and another sets of mice were injected intraperitonially with (vitamin – C) 10 mg/kg body weight. All the fifteen sets of mice were caged separately and the named its sex, body weight and dose allotted is printed on cover. After one month the first, second, thirds etc. sets of Swiss mice were sacrificed respectively to derive the data for estimation of collagen on the heart.

Tissue Processing

The Heart muscle for both control and experimental of Swiss mice were processed for the extraction and estimation of collagen fractions. Heart muscles were clean properly removing the adherent materials like fat, other muscles etc. and cut into small pieces and soak in filter paper. Then the cut of dried muscle were weighted (25 mg) in electronic balance and were taken for extraction of different collagen fraction.

The salt-soluble collagen was extracted in 0.14N NaCl solutions for 24hr at 8°C. The salt-soluble and salt-insoluble fractions were separated by centrifugation (REMI) at 1500 rpm in an IEC centrifuge for 10 minutes. Acid soluble collagen fraction was extracted from the salt-insoluble fractions in 0.45N acetic acid for 24hr at 8°C and the acid-soluble and insoluble fractions were separated by centrifugation at 1500 rpm in the above centrifuge for the same period of time. All the soluble fractions like as salt-soluble, acid-soluble and insoluble were hydrolyzed in hydrochloric acid (HCl) final concentration 6N, in sealed tubes at 110°C for 16 hrs., following hydrolysis, the hydroxyproline contents of neutralized samples were estimated by the method of Newmann and Logon (1950) as modified by Leach (1960). Values of hydroxyproline were converted to collagen contents by multiplying with the factor 7.46 as suggested by Jackson and Cleary (1967). The percentages of salt and acid solubility were calculated from the total collagen content of each tissue. Then the amount of collagen in Heart tissue of experimental and control mice were calculated. To evaluate the statistical significance of the data student's t-test was used.

RESULTS

Salt-soluble (s.s), Acid soluble (a.s), Insoluble (i.s) Collagen

After treatment with ascorbic acid (vitamin – C) salt soluble collagen content of heart muscle of Swiss mice was found to be not significant (P, NS) statistically when compared to their corresponding control. Acid-soluble collagen content of heart-region of Swiss mice was found to be increase insignificantly (P, NS) following treatment of 10 mg ascorbic acid/kg body weight. After treatment with ascorbic acid (Vitamin C) insoluble collagen content of heart muscle of Swiss mice was found to be not significant (P, NS) statistically. When compared to their corresponding control (Table – I, Fig. – 1).

Total Collagen content

The total collagen content of heart muscle of Swiss mice did not show any appreciable change (P, NS) when induced in ascorbic acid (vitamin – C) as compare to their controls (Table – I, Fig. – 2).

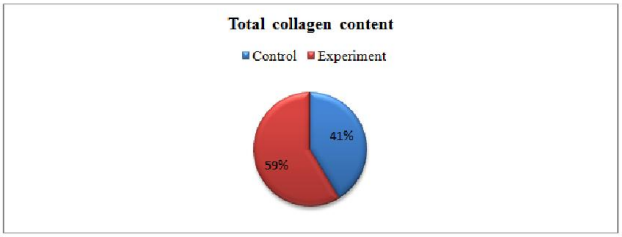

Fig. 2. Effect of ascorbic acid (50 µg/g) on Total collagen of Heart Muscles of Swiss mice Values are mg/g tissue wet-wt, columns represents the mean values and pie bars SEM

Salt soluble and Acid soluble %

After treatment with ascorbic acid (vitamin – C) salt solubility % (Salt soluble collagen as % of total collagen) of heart muscle of Swiss mice was found to be not significant statistically (P, NS) when compared to their corresponding control. Acid soluble % content of heart regions of Swiss mice was found to be increased insignificantly (P, NS) following treatment of 10 mg ascorbic acid/kg body weight (Table – I, Fig. – 3).

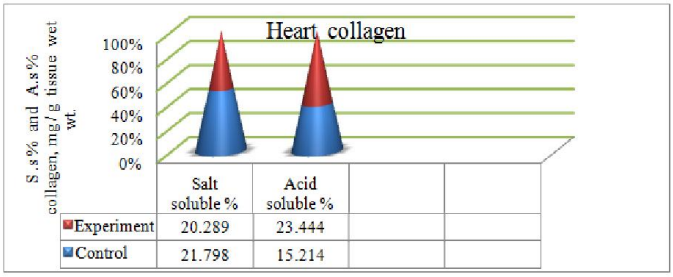

Fig. 3. Effect of salt solubility % and acid soluble % collagen of Heart Muscles of Swiss mice Values are mg/g tissue wet-wt, columns represents the mean values and vertical bars SEM

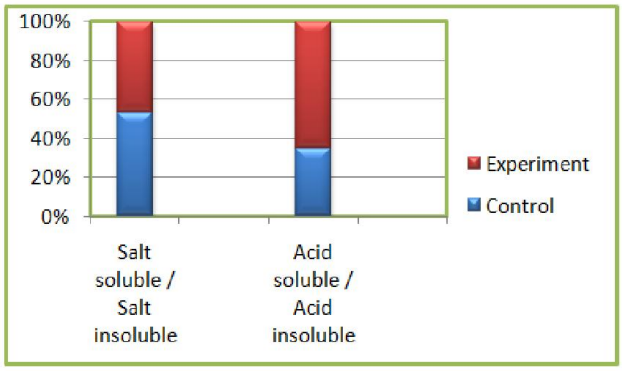

Fig. 4. Effect of salt soluble/salt insoluble and acid soluble / acid insoluble collagen of Heart Muscles of Swiss mice Values are mg/g tissue wet-wt, columns represents the mean values and vertical bars SEM

Salt soluble / Salt insoluble and Acid soluble / Acid insoluble

After treatment with ascorbic acid (vitamin – C) salt soluble/salt insoluble collagen content of heart muscle of Swiss mice was found to be not significant (P, NS) statistically when compared to their corresponding control. Acid soluble/Acid insoluble collagen content of heart regions of Swiss mice was found to be increased insignificantly (P, NS) following treatment of 10 mg ascorbic acid / kg body weight (Table – I, Fig. – 4).

DISCUSSION

The heart is a myogenic muscular organ found in all animals with a circulatory system, that is responsible for pumping blood throughout the blood vessels by repeated, rhythmic contractions. The vertebrate heart is composed of cardiac muscle, which is an involuntary striated muscle tissue found only in this organ and connective tissue. Collagen fibrils and the fibrous matrices they form are stabilized by covalent cross-links. Normal cross-link formation is essential to the development of functional tissue and organs. Our understanding of the role covalent collagen cross-linking plays in heart function or in the development of various cardiac pathologies is still in its early stages. Several observations, however, make collagen cross-linking an intriguing focus for investigations involving heart extracellular matrix (ECM). For instance, alterations in cross-link concentrations are associated with lethal pathologies as well as physiological adaptations, reported by McCormick *et al*. (1994).

In the adult heart, perimysium may exist structurally as a weave of collagen fibers surrounding bundles of myocytes, as tendon-like collagen strands interconnecting the perimysial weave or as coiled perimysial fibers running in the direction of the muscle fibers (Caulfield and Borg, 1979). Collagen status make individual myocytes-to-myocytes and/or myocytes-to-capillary connections. They help maintain constant myocytes length during diastole and capillary and coronary blood during systole (Medugorac, 1982). Collagen molecules undergo extensive post-translational modifications. Intracellular, selected proline and lysine residues are enzymatically hydroxylated and some hydroxylysine are then glycosylated (Nimni and Harkness, 1988).

The cross-link pathway which apparently predominates in mammalian heart collagen is the one based on hydroxyl lysine (Gunja-Smith *et al*., 1996). The mature cross linking residues on the hydroxyl lysine pathway are trivalent, 3-hydroxypyrldinium (HP) residues and lysyl pyridinium, with the latter present in negligible amounts in most tissues except bone. The progression of cross-links from divalent to trivalent forms during maturation is significant because multivalent cross-links have the potential to markedly increase the strength of the myocardial interstitium by linking together adjacent fibrils as well as individual collagen molecules (Reiser *et al*., l992). An experimental study conducted by Last *et al*. (1990) relatively little is known of the mechanisms which regulate cross-link ing in muscle, including heart. Levels of lysine hydroxylation influence cross-linking patterns in tissues, including proportions of HP to its ketoamine precursor and the ratio of lysine aldehyde to hydroxylysine aldehyde cross-links. There is variability in levels of lysine hydroxylation among collagen types and among different tissues; however, levels or degree of variability in myocardial collagen lysine hydroxylation have not been reported . The observation that cross-linking residues on adjacent molecules or fibrils must be precisely aligned for cross linking to proceed, suggests that spatial relationships between types I and III collagen may be a controlling factor in cross-link formation (Last *et al*., 1990). A mechanism by which spatial relationships among collagen molecules may be regulated involves the binding of decorin to fibrillar collagen. Interactions between the core protein of decorin and collagen govern the rate and extent of collagen fibrillogenesis (Zimmerman, 1997). The result of the present study showed that the salt-soluble collagen in heart muscles increased non-significantly. This fraction represents the newly synthesized collagen not co-valently linked into fibers by labile aldimine type bonds. A decrease in acetic acid extractability indicate an increased number of cross linkages.

As acid-soluble collagen increased non-significantly in heart muscle. It clearly shows an anti ageing effect was reported by Davison *et al*. (1972). Brog *et al*. (1981) reported that, insoluble-collagen from both heart muscle increase non-significantly and taken as a very good indicator of increased number of cross-linkages. Moreover, there are indications that both tissues protein form disulphide bonds with other proteins leading to the formation of insoluble protein complexes. The total collagen content of a tissue reflects a balance between its synthesis and degradation. Total collagen from both heart muscles increased non-significantly indicates an enhanced synthesis and its incorporation into fibers, a fact supported by higher level of soluble and insoluble collagens (Borg and Caulfield, 1981). An increase in the number of cross linkages of collagen leads to alterations in the physiological activities of the cells, eventually leading to the ageing of the whole organism. The molecular stability of collagen decreases while its solubility increases.

The result of the present study shows that there is an increase non-significantly of acid solubility % of collagen and decreases non-significantly of salt-solubility % of collagen in heart muscles. The result of salt soluble/salt insoluble is decrease non-significantly and acid soluble/salt insoluble is increased non-significantly in heart muscle.

Almost all parameters showed non-significantly increases in collagen content following ascorbic acid treatment to Swiss mice. As high dose of ascorbic acid acts as an antioxidant and accelerate the antioxidant effect (Kohn and Hamlin, 1978; Walford *et al*., 1969; Hall, 1976). From the work Nusgen *et al*. (2002) it has been proved that with the administration of vitamin C there is an increased in collagen a corresponding increase in birefringence, which is similar as our report (Borg and Caulfield, 1981). Patiyal (2006) suggested that collagen is a non-contractile protein which play an important role in regulating myocardial contractility and hence it is instrumental in controlling many hermodynomic functions (Patiyal and Kotch, 2006).

Conclusion

Vitamin C or ascorbic acid has vital and dynamic role on heart muscle of Swiss mice . Its releasing effect in the present study has been evaluating the quantitative change in heart muscle collagen content of Swiss mice. The quantity of salt-soluble, acid-soluble, insoluble, total collagen, % of salt solubility & % of acid solubility in vitamin C or ascorbic acid treated mice increased non-significantly in heart muscle of Swiss mice as compared to the values of control indicating its anitageing effect as a potential antioxidant.

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