

ALTERATIONS IN ALKALINE PHOSPHATASE ACTIVITY OF SKELETAL MUSCLES IN SALIVARIADENECTOMISED MICE

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ABSTRACT

The influence of the salivary glands, the major source of various growth factors, on skeletal muscles in male mice was studied by doing sialoadenectomy and salivariadenectomy and examining its effect on alkaline phosphatase activity in various post-operation periods. The operations were carried out in 20 days mice and they were killed on 40th, 60th, 80th and 100th days from birth. The alkaline phosphatase activity was determined from gastrocnemius, soleus and rectus abdominus muscles of control, sialoadenectomised and salivariadenectomised mice. The study showed significant increase in alkaline phosphatase activity in all three muscles in sialoadenectomised and salivariadenectomised mice as compared to control mice. Increase in alkaline phosphatase activity indicates pathological condition in muscles. Thus the results revealed that salivary gland secreted growth factors plays important role in development of skeletal muscles.

KEYWORDS: Alkaline phosphatase, Gastrocnemius, Rectus abdominus, Salivariadenectomy, Sialoadenectomy, Soleus.

INTRODUCTION

Alkaline phosphatase (AP) represents a group of isozymes that are membrane bound glycoproteins which catalyzes the hydrolysis of inorganic and organic monophosphate esters at alkaline pH in vitro (Pearse, 1968). Although this glycoprotein is widely distributed in vertebrate tissues, its physiological function is yet not fully understood. AP is ubiquitous in nature located in basal membrane of various tissues and also found in the serum (Wada *et al.*, 2001). Soluble forms of APase exist in the serum. AP are members of a rather diverse group of membrane proteins which are anchored to lipid bilayers in cell membranes by a phosphatidylinositol-glycan moiety attached to the carboxy terminus of the protein (Harris, 1989). Serum APase is a dimer, whereas the membrane bound forms of APase are probably tetramers (Safadi *et al.*, 1991). Studies reported earlier have established that APase does form an important entity in the muscle cell (Kumar and Katoch, 1994). It has been shown that advanced age and long term physical exercise cause changes in the activity of AP in rat muscle (Reznick *et al.*, 1989). In normal muscle cells of various mammals there is no demonstrable sarcoplasmic alkaline phosphates activity. In muscle associated pathologies such as neuropathies and myopathies, there have been several reports of increased AP activity in afflicted muscles (Kar and Pearson, 1972; Malhotra *et al.*, 1978; Kirkeby and Moe, 1985).

Salivary glands of the mouse especially the submandibular gland secrete number of biologically active polypeptides like epidermal growth factors (EGF), nerve growth factor (NGF), mesodermal growth factor (MGF), kallikreins, glucagon that affect the different body organs like the reproductive organs (Pillai and Walvekar 2002, 2005, 2007, 2009), gastrointestinal tract (Skinner *et al.*, 1984), as well as blood glucose level (Silverman and Dunbar, 1984). Atterdi in 1965 studied effect of a fraction of submandibular gland of mouse on tissues of mesodermal origin in vitro. He has observed almost complete loss of myosin which was indicated by loss of eosinophilia in myoblast of experimental cultures. From his experiment Atterdi had concluded that change in the muscle tissue in the culture is due to a component which is secreted by submandibular gland and having esterase and peptidase like activity, in 1967 Atterdi has again tried to characterized and purify that material. But up till now nobody has tried to find out role of submandibular gland secreted MGF in *in vivo* conditions so the purpose of this investigation is to find out the role of salivary MGF on the development of skeletal muscles. Thus to study the skeletal muscle development we have selected one of the parameter i.e. alkaline phosphatase activities.

MATERIALS AND METHODS

Swiss albino male mice (*Mus musculus*) were used for present investigation. The mice were bred and reared in departmental animal house (Registration number- CPCSEA/233). They were housed in aluminum cages in groups of three to four and supplied with Amrut rat / mouse feed (Pranav Agro Industry) and water *ad Libitum*. For these study forty eight male mice of twenty days old weighing (8 to 15gms) were used for experimentation. The thirty two male mice subjected to bilateral salivariadenectomy (removal of both submandibular and sublingual glands) and sialoadenectomy (removal of submandibular glands) at the age of 20 days and after that, they were killed on 40th, 60th, 80th and 100th day of age. Controls were sham operated. Mice from all groups were killed by decapitation and skeletal muscles viz., gastrocnemius, soleus and rectus abdominus, were dissected out. The muscles were cleaned, weighed and

homogenized in chilled distilled water. The concentration of homogenate was 1mg/ml for alkaline phosphatase estimation. The homogenates were centrifuged at 10°C at 5000 rpm for 10 minutes. The supernatants were used for estimation of alkaline phosphatase by Linhardt and Walter method (1965) by using P-nitrophenyl phosphate as substrate.

Statistical Analysis

All data is reported as mean ± SD. The significance of difference between means was assessed by one way analysis of variance for independent or correlated samples ANOVA followed by Tukey’s post hoc test.

RESULTS

Alkaline phosphatase activity in all the three muscles i.e. gastrocnemius, soleus and rectus abdominus muscle was increased significantly in salivariadenectomised and sialoadenectomised male mice as compared to control mice from all groups i.e. from 40 days to 100 days (P < 0.001) (Table no. 1, 2 and 3).

Table 1. Effect of salivary secretions on Alkaline phosphatase activity on Gastrocnemius muscle of male mice (Units AP activity/ mg protein)

Sr. No.	Groups	40 days (4)	Statistical Significance	60 days (4)	Statistical Significance	80 days (4)	Statistical Significance	100 days (4)	Statistical Significance
1.	Control	0.264± 0.00828		0.32 ± 0.0090		1.285 ± 0.0532		1.677 ± 0.04856	
2.	Sialoadenectomised	0.75± 0.09055	1:2 P<0.01	1.527 ± 0.0512	1:2 P<0.01	1.602 ± 0.0478	1:2 P<0.01	2.112 ± 0.0853	1:2 P<0.01
3.	Salivariadenectomised	1.287± 0.01707	1:3 P<0.01 2:3 P<0.01	1.692 ± 0.04113	1:3 P<0.01 2:3 P<0.01	1.907 ± 0.0427	1:3 P<0.01 2:3 P<0.01	2.32 ± 0.0645	1:3 P<0.01 2:3 P<0.01

Number in parenthesis denotes the number of animals
P<0.01- Significant

Table no.2 Effect of salivary secretions on Alkaline phosphatase activity on Soleus muscle of male mice (Units AP activity/ mg protein)

Sr. No.	Groups	40 days (4)	Statistical Significance	60 days (4)	Statistical Significance	80 days (4)	Statistical Significance	100 days (4)	Statistical Significance
1.	Control	0.305 ± 0.00420		1.29 ± 0.038		1.53 ± 0.025		1.952 ± 0.035	
2.	Sialoadenectomised	1.55 ± 0.2081	1:2 P<0.01	1.58 ± 0.89	1:2 P<0.05 1:3 P<0.01 2:3 P<0.01	1.842 ± 0.0309	1:2 P<0.01 1:3 P<0.01 2:3 P<0.01	4.122 ± 0.0932	1:2 P<0.001 1:3 P<0.001 2:3 P<0.001
3.	Salivariadenectomised	1.575 ± 0.1707		2.045 ± 0.1864		2.107 ± 0.083		4.912 ± 0.0854	

Number in parenthesis denotes the number of animals
P<0.01- Significant, P<0.05- Significant, P- NS (Non-significant)

Table no. 3 Effect of salivary secretions on Alkaline phosphatase activity on rectus abdominus muscle of male mice (Units AP activity/ mg protein)

Sr. No.	Groups	40 days (4)	Statistical Significance	60 days (4)	Statistical Significance	80 days (4)	Statistical Significance	100 days (4)	Statistical Significance
1.	Control	0.285 ± 0.0129		0.517 ± 0.0095		1.377 ± 0.015		1.837 ± 0.0126	
2.	Sialoadenectomised	0.982 ± 0.0170	1:2 P<0.01	1.27 ± 0.0182	1:2 P<0.01	1.865 ± 0.020	1:2 P<0.01	3.875 ± 0.1258	1:2 P<0.01
3.	Salivariadenectomised	1.25 ± 0.0420	1:3 P<0.01 2:3 P<0.01	1.715 ± 0.0129	1:3 P<0.01 2:3 P<0.01	1.93 ± 0.0182	1:3 P<0.01 2:3 P<0.01	4.25 ± 0.129	1:3 P<0.01 2:3 P<0.01

Number in parenthesis denotes the number of animals
P<0.01- Significant

Table 1, 2 and 3 reveals that there is remarkable increase in alkaline phosphatase activity of soleus muscle than gastrocnemius and rectus abdominus muscles. While there was much more increase in alkaline phosphatase activity in salivariadenectomised group as compared to sialoadenectomised and control group.

DISCUSSION

After 20th day of sialoadenectomy there was 3 times increase in the alkaline phosphatase (AP) activity, after 40 days there was 5 times increase while after 60 and 80 days there was 1.3 times increase in AP activity as compared to control was observed in gastrocnemius muscle. Similar type of increase in AP activity was observed in soleus and rectus abdominus muscles. While in salivariadenectomised group still more increase in AP activity was observed. This increase in AP was due to proliferation of non-contractile connective tissues in muscle (Kar and Pearson, 1972). The nonspecific increase in the activity of AP due to sialoadenectomy and salivariadenectomy causes increased transmembrane transport where AP is involved in absorption and transport across the membrane (Sandhir and Gill, 1995). An association of AP with pathological conditions has been reported for skeletal muscle of chick and rats in which striated muscles revealed increased activity in pathological conditions such as denervations (Malhotra *et al.*, 1978). It has been reported earlier that increased fiber AP positivity is correlated with an increased incidences of degenerative and regenerative changes, fibrosis and atrophy of skeletal muscles (Engel and Cumsnnimigham, 1970; Cros *et al.*, 1980). Thus, it is concluded from the present study that increased AP activity after sialoadenectomy and salivariadenectomy (due to absence of MGF) can cause pathological changes in skeletal muscles and may affect other tissues as well.

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