Influence of Swimming on Occurrence of Apoptosis by Experimental Diabetic Myopathy in Rats

M. Hashemi, A.R. Azizi Saraji, M. Bayat and M. Entezari

1Department of Genetics, Islamic Azad University, Tehran Medical Branch, Tehran, Iran
2Department of Veterinary Laboratory of Sciences, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Branch, Tehran, Iran
3Department of Medical and Veterinary Mycology, Faculty of Specialized Veterinary Sciences, Islamic Azad University, Science and Research Branch, Tehran, Iran

Abstract: By increasing insulin sensitivity and removing glucose as well as decreasing body fat, swimming accounts as a very robust non-drug tools for preventing and treating diabetes mellitus, generally type1 and type2. Diabetic myopathy is the main factor damaging muscle cells in diabetic patients. This study aimed to determine swimming role on apoptosis differences by experimental diabetic myopathy. 56 male Wistar rat, 12 weeks age old, weighting 250-300g were selected for this study. Rats were divided into two groups of 28; then they were experimentally affected to diabetes by streptosotocin (50 mg for a kg weight) using intra-peritoneum injection. Treatment group remained in the same conditions (feeding and maintaining) for 12 weeks; forcing to swim 5 days a week and an hour a day orderly. During this period, control group had no physical activities and exercise, but in the same maintaining and feeding conditions. After 12 weeks, both groups were sampled from gastrokenimous muscle tissue and were fixed in formalin10%, sectioned using regular methods for pathological sectioning and provided 5-6 micron thick sections, then stained by hematoxilin-eosin and Tanel specific technique. Tissue pathology in the control group indicates apoptosis and necrosis in the gastrokenimous muscle tissue. Such differences in treatments were insignificant and both groups were significantly different. Results indicate that swimming can reduce pathological differences and may relatively improve damages of muscle tissue in diabetic myopathies.

Key words: Apoptosis • Diabetic Myopathy • Swimming

INTRODUCTION

Diabetes mellitus is the most prevalence metabolic disease diagnosing by hyperglycemia arising from absolute or relative lack of insulin [1]. More than 150 people in the world and nearly 3 million people in Iran affected by this disease and it is expected increasing to 221 million people in 2010. Based upon anticipation of world health organization (WHO), this figure in adults will be increased to 300 million people in 2025. While decreasing the blood glucose in such patients may not be enough using standard methods and chemical medicines to prevent its effects such as muscle, arterovascular disorders, eye diseases, neuropathy and kidney failure and it assumes that to treat this disease, a hidden epidemic one, it is necessary seeking for other methods. There are three important lesions in diabetic myopathy: (1) cell injury, (2) atrophy, (3) myocyte [2]. The most important muscle lesion include atrophic alterations and cell degeneration such as decreased width and length of a muscle fiber and forming vacuoles in muscle cell’s sarcoplasm. Generally, it assumed that two processes play role in muscle tissue lesions. First, metabolic failure occurs in all patients and may be due to final advanced glycosylated end products responsible for atrophic alterations and cell degeneration; second, increased oxidative stress which is the most important factor of cell death. In diabetic patients, muscle cells may affected by

Corresponding Author: M. Hashemi, Department of Genetics, Islamic Azad University, Tehran Medical Branch, Tehran, Iran

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degenerative alterations due to over-aggregation of glycogen. In rigorous hyperglycemia, osmolality of muscle cells may occasionally be increased, so it may leave cells with degenerative alterations. As mentioned above, it indicates the importance of diabetes in occurring muscle lesions; anyway, one of the preventive and treatment procedures for this is regular physical activity a day in patients; for this reason, exercise is considered as the main treatment program in diabetes treatment [1]. This study aimed to examine preventive role of swimming on apoptosis in experimental diabetic myopathy to make clear whether regular swimming may reduce apoptosis, cell death, in diabetic patients.

MATERIALS AND METHODS

A total number of 56 male Wistar rats was selected, 12 weeks age old, weighting 250-300g. They divided into two groups of 28. Both groups remained in the same conditions (feeding and maintaining).

To make diabetes, streptosotocin, 50mg for a kg of body weight was interaperitonioally, injected. 48h after injection, to ensure making diabetes in rats, there was bleed from their tail and its blood glucose was read using glucometer (Boehringer Mannheim Indianapolis IN). Blood with glucose concentration more than 300mg/dl is considered as diabetic index. They forced to swim 5 days a week and an hour a day orderly. At the end of 12th week, both groups killed using cervical dislocation then sampled gastrokenimus muscle, were fixed in formalin10%, sectioned using regular methods for pathological sectioning and provided 5-6 micron thick sections, then stained by hematoxilin-eosin and Tanel specific technique [1, 3, 4]. A number of necrotic cells and apoptotic ones were counted accidentally. Besides, there was made photomicrographs with 5.1Mpix resolution.

Results were analyzed using SPSS, Ver13 and statistical t-Test with p<0.05 significance. This study was done by intervening experimental method with its analyzing variables (1) exercise as independent variable (2) apoptosis variations in muscle tissue as a dependent variable.

Running Tanel Technique to Diagnose Apoptotic Cells: For this reason, there was used Tanel kit (in situ cell death detection kit, POD; Roche Company, Germany).

- Sections were deparaphinized, hybridized, then added to proteinase K, incubated for 30 min in 37°C and washed with buffer phosphate.
- Sections then added by Tanel reagent, 50 Ml, for 60 min in 37°C and washed by buffer phosphate.
- In this stage, after incubation, added to convertor solution (50MI) for 30min in 37°C, washed by buffer phosphate; then added to di amino benzidin tetrachloride and incubated for 20min in 25°C.
- Sections then washed by buffer phosphate and stained by Tuloiden blue [3].

RESULTS

Histological Studies in Treatment and Control: Atrophic and necrotic variations were observed in different parts of rats’ muscle tissue. Pathologically such tissue variations were due to reducing the muscle cell size accompanying with sarcoplasm vacuolation. Necrotic cells indicate increased eosinophil with a part of it related to basophile loss arising from RNA in cytoplastm and a part related to increased eosin link to denaturized intra cytoplasm proteins. Cell may have an even and glass appearance mainly relates to losing the glycogen particles. Above mentioned changes in treatment group was ignorable (Fig 1 and 4). Sever density in chromatin of muscle cells and its fragmentation was a sign to diagnose the type of cell death pattern by Tanel staining as indicated in Figs 5 and 6 for positive Tanel cells in tissue sections of gastrokenimus tissue. There was less apoptotic cells proportion in the muscle tissue of treatment than control group.

Fig. 1: Magnified image of gastroknimus tissue of treatment group (above) and control group (below), to atrophic variation in the muscle tissue of controls comparing with treatments.
Fig. 2: Magnified image of gastroknimous tissue of controls in where degenerative variations and picnosis of muscle nucleous (flash 1 and 2) accompanying with zinker necrosis (flash 3) and dissection of mono nucleous cells in inta tissue space (flash1). Specifically, pay attention to denaturized sarcoplasmic proteins of muscle cells (hematoxilin- eosin staining, magnification ×100).

Fig. 3: Magnified image of gastroknimous tissue of controls with dissection of mono nuclear cells and collagen precipitation initializing fibrosis formation (flash 2) atrophy and necrosis of muscle tissue (flash 1) (hematoxilin- eosin staining, magnification ×100).

Fig. 4: Magnified image of gastroknimous tissue of treatments with necrosis of muscle cells (flash 2) accompanying with inter edema (flash 1) (hematoxilin- eosin staining, magnification ×100).

Fig. 5: Magnified image of gastroknimous tissue of controls with apoptotic cells reacting positively with tanel (flashes) (Tanel staining, magnification ×100).

Fig. 6: Magnified image of gastroknimous muscle tissue of treatments comparing with controls; there are seen fewnumbers of apoptotic cells with positive tanel reaction (flash) (Tanel staining, magnification ×100).

Diagram 1: Cell death mean variations of muscle tissue of treatment and controls (n=28). Data indicated as Mean ± SEM, P<0.001, **comparing with treatments

Statistical analysis indicated that mean difference of cell death pattern and apoptosis was significant between treatment and control groups (Table 1). Results of this statistical analysis, mean ± SEM, indicated in Tables 1 and 2.
DISCUSSION

Diabetic myopathy has been studied by scientists as a very important lesion and scientists through the world always tried to reduce muscle tissue lesions arising from diabetes. There has been used various medicines for this reason, but they could not reduce effects of diabetes in the muscle tissue; until recently there was disposed reduce damages induced by diabetes. Exercise will increase gene expression of IGF-II or Insulin like Growth Factor in tissue; as this factor has protective role in muscle cells and increases insulin sensitivity in muscle tissues, thus positive role of exercise on reducing diabetes effect and accompanying with results of current study is verified. It must be mentioned that IGF-II is an insulin agonist and in carcino of muscle tissue may supply glucose for cancerous cells using more glucose.

Various mechanisms are involved in apoptosis induction following diabetes in myocytes of muscle tissue in which one can mention the role of oxidative stress in the muscle tissue of diabetic rats. Free toxic oxygen factors arising from oxidative stresses as well as inactivating kinase enzyme ERK1/2 and activating other kinase enzymes, C-JUN/C and JUN/AP-1, can indicate occurring apoptosis following the oxidative stresses. It is obvious that remaining pathway for cell death is due to caspase 3 and poly adenosine di phosphate polymerase (PARP) [3, 5, 6]. How over aggregation of glycogen in muscle cells can induce cell death of apoptosis type? Robert et al. [2005] answered to this question accompanying with results of this study; they stated that inhibition role of enzyme GSK-3β in launching apoptosis by TNF. α pathway loss due to over aggregation of glycogen in muscle cells and make apoptosis by phosphorylation of P65 and gene expression of NF.KB of muscle cell [7-10]. Thus there are three main factors inducing the apoptosis: (1) increased glycozylated hemoglobin (2) oxidative stresses (3) over aggregation of glycogens in cells. Influencing the expression swimming generally reduces diabetes effects in the muscle tissue, as stated by Ronald et al. [2005] [8, 11]. Laaksonen et al.

[2000] stated that reducing the oxidative stress account as the main factors in reducing damages induced by diabetes. This is clear in current study comparing treatment and control groups [12, 13, 14] Cotter et al. [1989] [15]. indicated that diabetes has some effects in muscle tissue. Atalay et al. [2002] [3] stated that reducing oxidative stress, exercise is of most important factors to reduce damages induced by diabetes. Exercise will increase gene expression of IGF-II or Insulin like Growth Factor in tissue; as this factor has protective role in muscle cells and increases insulin sensitivity in muscle tissues, thus positive role of exercise on reducing diabetes effect and accompanying with results of current study is verified. It must be mentioned that IGF-II is an insulin agonist and in carcino of muscle tissue may supply glucose for cancerous cells using more glucose.

So it can be deduced that this factor has always no positive role, thus it is better for diabetic patients to continuously exercise in the initial stages, but if patient suffers neoclassical changes, it may not has its positive effect [2]. for the first time Ehrich et al. [1883] indicated glycogen aggregation in the cell nucleolus involving in tuberculosis, septicemia, hepatitis, autoimmune diseases besides diabetes such that its over aggregation may irretrievably damage cells. Current study also indicates damage induced by over aggregation of glycogen forming fibrosed tissue following more precipitation of extra cellular matrix [15, 16].

Making hypoglycemia, exercise reduces glucose access by muscle cells, thus it will reduce glycogen storing disease in muscle cells [17, 18], by which one can find the positive role of exercise on the side effects of diabetes with the same results in current study [19]. Glycozylated hemoglobin (HbA1c) will be reduced by exercise, such that occurring non-enzymatic glycozilation, glucose with no enzyme interference and chemically links to the amine groups of proteins, by which make glycozilation products; they may be suffered to rearrangement and produce early and stable glycozilation products called Amadori- Type. Enzymatic glycozilation

Table 1: mean for apoptosis variations in the gastrokenimus muscle tissue of treatments; data indicated as mean±SEM

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Error deviation</th>
<th>Standard deviation</th>
<th>Error deviation ± mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis</td>
<td>0.2683</td>
<td>1.420</td>
<td>2.357±0.2683</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0.2708</td>
<td>1.433</td>
<td>2.143±0.2708</td>
</tr>
</tbody>
</table>

Table 2: mean for apoptosis variations in the gastrokenimus muscle tissue of controls; data indicated as mean ± SEM

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>Error deviation</th>
<th>Standard deviation</th>
<th>Error deviation ± mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis</td>
<td>0.4012</td>
<td>2.123</td>
<td>11.714±0.4012</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0.2654</td>
<td>1.404</td>
<td>8.250±0.2654</td>
</tr>
</tbody>
</table>
rate is directly dependent on blood glucose value. While they must be degraded, newly produced glycolysis products change chemically and rearrange slowly on collagen and other long-life proteins in the connective tissues and vessels’ wall and finally produce irreversible and advanced end products resulted from glycolysis called AGE (Advance glycosylation end products). AGE can be made on proteins, fats and nucleic acids; they make cross connections between polypeptids of collagen and trap plasma or connective proteins. In large vessels, for example, trapping the low density lipoproteins results in decreased exiting from the vessel wall and increased precipitation of cholesterol in antima, it will consequently accelerate atrogenesis. In capillaries, plasma proteins like albumin may link to the glycolysated basal membrane and this can increase its thickness which is the characteristics of diabetes micro angiopathy. Proteins cross linked with AGE are resistant to proteolytic digestion. So, cross linking not only results in reduced proteins removing, but also may disrupt reactions between collagen anf other parts of connective material (laminin, proteoglycans) and causes structural and functional drawbacks in the basal membrane and connective tissue. AGE can link to the receptors on most cells (endothelium, monocytes, macrophases, lymphocytes and mesanshimal cells) (Figs 2, 4, 5). Such connections may result in various biological activities such as monocytes migration, releasing cytokins and growth factors from macrophages, increasing the endothelial penetrability, increasing the coagulation of endothelial surfaces and macrophages, increasing the proliferation of extra cell matrix by fibroblasts and smooth muscle cells. With regard to above mentioned and results from current study, reduced cell damage may be due to formation of AGE following the swimming [1]. Stephen et al. [2006] stated the role of diabetes on the structure of muscle tissue. Their results indicated that vessel damages like microangiopathy may be due to: (1)increased oxidative stress, (2) decreased high density lipoproteins, (3)increased protein glycolysisation, (4) hyperglycemica, (5) increased fat peroxidation as well as production of 8-iso-PgF2α. Besides vessel changes, it can be indicated increased connective matrix in the connective tissue of muscle cells, as indicated in Doustar et al. [2007], Zinman et al. [2003], Chiascerva et al. [2000] and Delissio et al. [1991], all mentioned the role of regular exercise on reducing the effects of diabetes corresponding with results of current study.

One of the most important growth factors play role in diabetes called TGF-β. Hyperglycemia accounts as the most important inducer factors in releasing TGF-β from differentiated miofibroblasts of muscle cells. Rigorous increased blood glucose invokes proliferation of miofibroblasts as well as increased collagens of types 1 and 4 and TGF-β [20, 21]. Increasing insulin sensitivity and hypoglycemia induction, exercise can prevent proliferation of miofibroblast cells, by which inhibits producing and releasing TGF-β and collagens of type 1 and 4 [1, 5]. It is obvious that controlling blood glucose, exercise can inhibit production and release of TGF-β and reduces fibrosis of muscle tissue in diabetic patients. In this study, there was also indicated significant difference in fibrosis of muscle tissue between treatments and controls conforming above mentioned findings [12, 14, 22, 23]. Based on Amie et al. [2006], in diabetics, more Ca²⁺ in cytosol and across cell membrane and mitochondria as well as activating kaplin factor and overexpression of Bax protein, may induce cell death, while regular physical activities can balance Ca²⁺ in the sarcoplasm of muscle cell and inhibits effects of diabetes on cell damage [1]. Consequently, by different mechanisms, swimming may play positive role in improving the diabetes myopathy. Thus, it can be deduced that swimming can be a treatment and preventative approach of diabetes effects in the muscle tissue of patients, but further researches need to study more effective reasons for exercise role in diabetes myopathy.

REFERENCES