Original Article

Protective Role of Magnesium Sulphate in Dexamethasone induced Histopathological Alteration in Spermatogenic Cells of Albino Rats

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ABSTRACT

Objective: This study has been undertaken to assess the spermatoprotective role of magnesium sulphate ($MgSO_4$) on the histology of the seminiferous tubules in dexamethasone induced spermatogenic cells damage in albino rats.
Study Design: Prospective experimental study.
Place and Duration of Study: This study was conducted at the Department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi from April 2012 to May 2012.
Materials and Methods: Thirty male albino rats of 90-120 days of age and around 200-250 grams of weight were selected and divided into three groups (A, B&C). Each group comprising of ten rats. Group-A served as control, group-B was given dexamethasone (Dexa) at the dose of 4mg/kg/day intraperitoneally for 20 days. Group-C was administered $MgSO_4$ at the dose of 20mg/kg/day intramuscularly and Dexa at the same dose as given in group- B. The rats were sacrificed at the end of the experimental period and histopathological changes in the germ cells were recorded.
Results: The microscopic examination of group-B rats revealed marked changes in most of the seminiferous tubules such as, vacuolization, detachment of basement membrane, atrophy, sloughing, widening of the interstitial spaces & disorganization of the spermatogenic cells series. Group-C which was protected with magnesium sulphate, showed restoration of basement membrane and spermatogenic cell series.
Conclusion: The present study concluded that magnesium sulphate ($MgSO_4$) administration reduced the damaging effects of dexamethasone in testes.
Key Words: Dexamethasone (Dexa), Magnesium sulphate ($MgSO_4$), Testicular tissue.

INTRODUCTION

Spermatogenesis is a process that involves an array of cellular and biochemical events, collectively culminating in the formation of haploid spermatids from diploid precursor cells known as spermatogonia[1]. This highly regulated and complex process of germ cell proliferation and differentiation leads to the production and release of spermatozoa from the testes, depending upon hormonal stimulation as well as dynamic interaction between the sertoli cells and the germ cells of the seminiferous epithelium[2]. It involves four key cellular events, namely; spermatogoniogenesis, spermatocytes differentiation, spermeiogenesis and spermiation[3].

Normal spermatogenesis represents a precisely regulated balance between continuous cell proliferation and concomitant programmed cell death (PCD), the apoptosis,[4,5] this PCD is required to ensure cellular homeostasis[6]. When the testicular environment cannot support spermatogenesis, specific pathways are accelerated leading to germ cells apoptosis[7]. This abnormal apoptosis of germ cells may lead to an imbalance of cell proliferation and death, resulting in impaired spermatogenesis[8].

Glucocorticoids (GCs) are the major steroid hormones prescribed by the physicians to treat many inflammatory conditions. More than thirty steroids have been isolated from the adrenal cortex in which cortisol is the principal GCs in human and is secreted under the control of hypothalamic-pituitary-adrenal axis[9]. Dexamethasone (Dexa) a synthetic GC which is thirty times more potent than cortisol has made it an especially important drug for stimulating specific glucocorticoid activity[10]. Experimental studies have shown that excess GCs have damaging effects on spermatogenic cells i.e. reducing serum testosterone level[11], impair luteinizing hormone signal transduction and steroidogenesis in Leydig cells of adult rats[12], and also suppress the activity of hypothalamic-pituitary-gonadal (HPG) axis[13].

Minerals play a diverse role in the body. They most commonly function as essential coenzymes and cofactors for metabolic reactions and thus help support basic cellular reactions i.e., glycolysis, the citric acid cycle, lipid and amino acid metabolism, required to maintain energy production and life .They are also important in the regulation of metabolism, gene expression and may influence the development and progression of many chronic diseases[14,15]

Magnesium (Mg) is the second abundant intracellular cation after potassium (K),[16] functioning as a cofactor for more than 300 enzymes. It is essential for all energy-dependent transport system, glycolysis,

oxidative energy metabolism, biosynthetic reactions and cell membrane stabilization[17]. Many enzymes require the presence of magnesium ion for their catalytic action, especially enzymes utilizing ATP or those which use other nucleotides to synthesize DNA & RNA[18].Magnesium sulphate has effectively prevented the histopathological alteration in germ cells caused by alcohol[19], torsion[20]and radiation[21].

This study has been undertaken to evaluate the protective role of magnesium sulphate on dexamethasone induced spermatogenic cells damage in albino rats.

MATERIALS AND METHODS

This study was conducted in the department of Anatomy, Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Center (JPMC), Karachi. Thirty male albino rats 90-120 days of age, weighing around 200-250 grams, were obtained from the animal house of BMSI and were kept under observation for one week prior to the commencement of the study. They were housed in a temperature and light controlled room and fed with standard laboratory chow and water. The animals were divided into three groups (A, B&C), each comprising of ten rats. Group-A served as control, group-B received dexamethasone[22] at the dose of 4mg/kg/day intraperitonealy for twenty days. Group-C was administered dexamethasone and magnesium sulphate[23], at the dose of 20mg/kg/day intramuscularly for the above mentioned period. At the end of study period, the experimental and corresponding control animals were sacrificed under ether inhalation. Lower midline incision was given and extended up to the skin of the scrotum. The testes were dissected out carefully from each animal without damage to tunica albugina and were fixed in Bouin's fixative for 24 hours. After 24 hours each testis was cut longitudinally into two equal halves and post fixed in fresh Bouin's fluid for next 24 hours. After fixation, they were kept in 70% alcohol overnight. Dehydration was done with ascending strength of alcohol, with changes of one hour each i.e. 80%, 90%, 95%, absolute-I& absolute-II. The tissue was cleared in two changes of xylene for one hour each, and then infiltrated in two changes of paraffin in the laboratory oven at 59 degree centigrade, then paraffin blocks were made & 5micron thick sections were cut with the help of a rotatory microtome. Sections were mounted on labeled glass slides and stained with periodic acid Schiff (PAS) iron hematoxylin[24] for histological study of spermatogenic cells.

RESULTS

Group-A: Testicular sections from control animals revealed that the parenchyma of testes was formed of rounded seminiferous tubules. Most of them attained narrow lumina and lined by stratified germinal epithelium. The epithelium was formed of several types of spermatogenic cells: spermatogonia, primary spermatocytes, spermatids and Sertoli cells. Spermatogonia appeared as small rounded cells, resting on a thin basement membrane and had rounded nuclei. Primary spermatocytes were larger in size than the spermatogonia with large rounded nuclei. Early spermatids appeared as small rounded cells with paler nuclei. Sertoli cells appeared as column cells with euchromatic nuclei and prominent nucleoli in between spermatogonia. The tubules were separated by the interstitial spaces containing groups of interstitial Leydig cells with acidophilic cytoplasm (Fig-1).

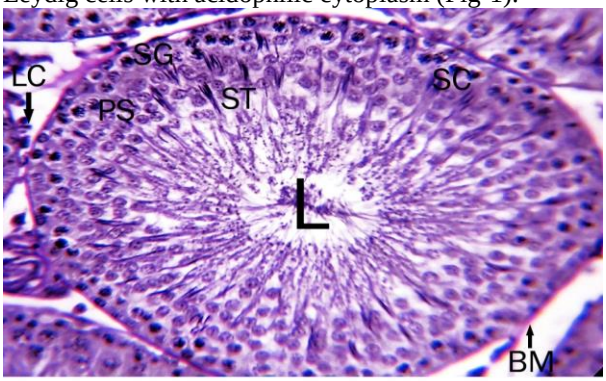

Fig-1
PAS- Iron Haematoxylin stained 5 micron thick section of seminiferous tubule of control group showing intact basement membrane (BM), spermatogonia (SG), primary spermatocytes (PS), spermatids (ST), sertoli cell (SC), lumen (L) contained spermatozoa, and Leydig cells (LC), in the interstitial space. (Photomicrograph X 40)

Group- B: Dexamethasone treated sections revealed that seminiferous tubules attained different shapes and lost the normal arrangement of germ cells. The Lumina of the affected tubules contained sloughed germ cells. The basement membrane showed irregularity or detachment and sloughing to vacuolization of seminiferous tubules, contributing to eventual atrophy. The interstitial tissues exhibited groups of Leydig cells and congested blood capillaries (Fig-2).

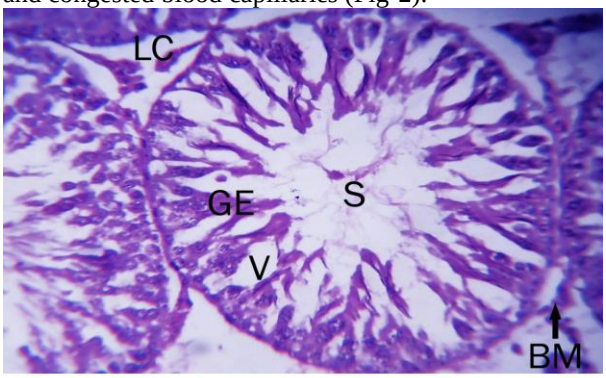

Fig -2
PAS- Iron Haematoxylin stained 5 micron thick section of seminiferous tubule after 20 days dexa treated group , showing disorganized germinal epithelium (GE), vacuolation (V), slough (S) in the lumen and detached basement membrane (BM).(Photomicrograph x40)

Group- C: The morphological examination of testes in group-C revealed seminiferous tubules with slight widening of interstitial spaces but basement membrane was intact. Lumen contained spermatozoa without slough. There was restoration of spermatogenic cell series but some vacuolation was seen (Fig 3).

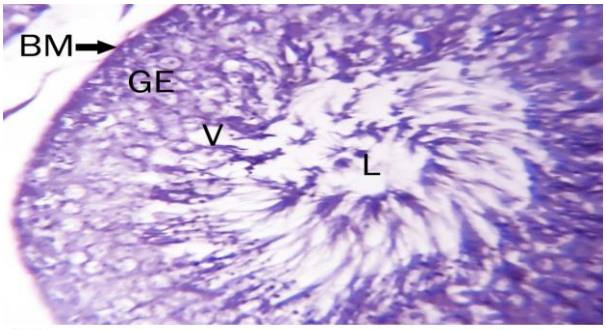

Fig-3
PAS-Iron Haematoxylin stained 5 micron thick section of seminiferous tubule, after 20 days dexa plus magnesium sulphate treated group, showing restoration of germinal epithelium (GE) and basement membrane (BM), lumen (L) contained spermatozoa and some vacuolation (V), Photomicrograph X40.

DISCUSSION

Glucocorticoids are often used therapeutically for their potent anti-inflammatory and immunosuppressive properties in the treatment of allergic, rheumatologic, neurological and autoimmune diseases. [25]

In the present study, examination of the sections of the testes treated by Dexa showed that seminiferous tubules attained different shapes and lost the normal arrangement of germ cell series, along with presence of sloughed germ cells in the lumen. This was due to retraction of the cytoplasmic processes of the sertoli cells, which extend between the different layers of germ cells, so the cells became loosely arranged and easily sloughed out. Nashwa et al (2010)[26] found disorganization of germ cells, vacoulation and sloughing in germ cell of rats, treated with dexamethasone. Orazizadeh et al (2010)[27] also demonstrated Dexamethasone induced histopathological alterations such as epithelial vacuolization, atrophy of the seminiferous tubules and reduction in spermatozoa and apoptosis of germ cells was also significantly increased. Martinez et al (2000)[28] found that intake of alcohol has been associated with marked degenerative changes in germ cells of albino rats, due to its apoptogenic effect mainly by DNA damage, oxidative stress and androgen suppression. The group-B also showed decline in the number of Leydig cells, which might be due to glucocorticoid suppression of luteinizing hormone (LH) signal transduction and steroidogenic enzyme activities. Our findings are in agreement with Sapolsky et al (2000)[11] and Hardy et al (2005)[7], who observed that elevation of glucocorticoid concentration inversely affects testosterone production by reducing the number of Leydig cells through the induction of germ cell apoptosis. The results of Yazawa (2001)[29] is in disagreement of our study who revealed that dexamethasone decreased testicular germ cells apoptosis in ischemic testes, by suppressing oxygen-derived free radicals and its anti-inflammatory effects.

In present study the simultaneous administration of magnesium sulphate with dexamethasone in group-C animals showed restorative effects on germ cells histoarchitecture. This was due to the diverse role of magnesium in metabolic reactions as a co-enzyme and regulator of hypothalamic-pituitary-adrenal axis (HPA-axis), thus it helps to support basic cellular reactions. Adivarekar et al (2005)[20] showed that treatment of torsion by detorsion alone does not prevent testicular damage however administration of $MgSO_4$ prior to detorsion, resulted improvement in semen quality, fertility and reduction in long term morbidity. Chandra et al (2012)[30] demonstrated significant increase in testosterone level along with progressive improvement in the histoarchitecture of genital organs after administration of magnesium sulphate for one and two consecutive spermatogenic cycles in albino rats.. Cinar et al (2011)[31] concluded in his study that supplementation of $MgSO_4$ increases free and total testosterone.

CONCLUSION

The present study shows cytotoxic and apoptotic effects of dexamethasone on the testes of albino rats. The study further emphasizes a definite protective role of simultaneous administration of magnesium sulphate in spermatogenic cells of albino rats.

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