International Journal of Anatomical Sciences, 2013, 4(2): 11-13.

Research Paper

Phenytoin Induced Up-regulation of LPL Gene in Albino Rat Testis Gene Microarray Analysis

Rajkumar R[*], Vatsala Venkatesan[‡], Sriram Thanigai[¥]

[*] *Department of Anatomy Mahatma Gandhi Postgraduate Institute of Dental Sciences, Puducherry – 605 006, India.*

[‡] *Department of Anatomy, Sri Balaji Medical College &Hospitals, Chennai – 600 044, Tamil Nadu, India.*

[¥] *Department of Orthopedics, SRM.Medical college and Research Centre, Kattankulathur 603 203, Tamil Nadu, India.*

Key Words: micro array analyses – gene expression – LPL gene – phenytoin effects

Abstract: Phenytoin is widely used against all types of partial and tonic-clonic seizures. Phenytoin may alter the release and action of different hormones which may contribute to sexual dysfunction. The present study is aimed at the effect of phenytoin induced differential regulation of LPL gene in albino rat testis. The albino rats were divided into two groups, control and test. The test group was given 150mgs/kg/body weight of phenytoin orally and equal amount of normal saline was given for the control group. After 45 days with the rat under deep anesthesia, the testis were removed from the scrotum and stored in liquid nitrogen. The stored specimens of testis of control and tests group were subjected to cDNA microarray analysis. This study showed the differential expression of gene LPL in test group when compared with the control group.

Phenytoin is an anticonvulsant used to control grandmal and psychomotor seizures. It produces chromosomal anomalies. Phenytoin is excreted in human semen in small quantities and this may possibly affect testosterone levels. Reduced plasma concentration of free testosterone has been detected in male epileptic patients receiving phenytoin. Meng *et al.* observed possible mutagenic effect on human sperm cells. According to Bauer *et al.* and Kuhn-Velter *et al.,* phenytoin acts directly on the testis to inhibit testosterone synthesis by leydig cells. Researches done by Rebuff et al. points out increased levels of testosterone increases lipolytic potential and decrease the LPL activity of adipose tissue. The lipoprotein lipase encoded by LPL genes. The LPL is secreted by adipose tissues. The testis is covered by connective tissue capsule called tunica albuginea, internal to which is a vascular layer of loose connective tissue, called the tunica vasculosa. The connective tissue extends inward from the tunica vasculosa into the testis to form interstitial connective tissue, which surrounds, binds and supports seminiferous tubules. It contains blood vessels loose connective tissue containing adipocytes which secretes LPL. The endothelium of testicular blood vessels also secretes LPL. The present study is to assess the effect of phenytoin on rat testicular LPL genes.

Correspondence to: Rajkumar R, Department of Anatomy Mahatma Gandhi Postgraduate Institute of Dental Sciences, Puducherry – 605 006, India.

Email: rrajkumar_r@yahoo.com

Accepted: 06-Sep-2013

IJAS, 2013, 4(2): 11-13.

Materials and Methods

Animal treatment and sample collection

Male adult albino rats were segregated into control and test groups, The test group were treated with phenytoin 150mgs/kg body weight/day orally for 45 days similarly control groups were given equal amount of normal saline. In life study protocols, including animal housing, dosage, sacrifice and tissue harvesting were as per IAEC guidelines. After 45 days the tissue samples from test and control were collected in Rnase free tubes and snap frozen in liquid nitrogen. Frozen tissues were stored in RNA later at-70 c until processed for RNA extraction

RNA Isolation and DNA Microarray Hybridization And Analysis

RNA was extracted from the testis preserved in RNA later using QIagen's RNeasy minikit Cat#74104 and checked for purity and concentration. The extracted mRNA labeled with Agilent's Quick-Amp labeling kit (p/n5190-0442) Hybridized with Agilent's in situ Hybridization kit5188-5242 and scanned using high throughput Agilent scanner with "Surescan" technology.

Comprehensive Data Analysis

Data analysis includes automated feature extraction using Agilent feature extraction Software, Normalisation and statistical analysis and pathway and gene ontology analysis using Agilent's Genespring GXv1o==10.0 Biological interpretation of significant gene using Genotypics Biointerpreter Tool with literature curated information.

Observations

Phenytoin induced 3.2 up regulated LPL gene expression was observed in phenytoin treated group when compared with untreated control group.

Discussion

In bulls, LPL expressed in testis, heart, kidneys, adrenal gland and the spleen (Elkattawy *et al.*). LPL encodes lipoprotein lipase. Lipoprotein lipase is a member of lipase gene family. It is water soluble enzyme that hydrolyses triglycerides in lipoprotein which are found in chylomicrons and very low density lipoproteins into two free fatty acids and one monoacylglycerol molecules, Research work done by Vijay *et al.* pointed out intratesticular level of testosterone treated with phenytoin showed considerable decline in the 2nd to 7th week of sampling time. According to Bauer *et al.* and Kuhn-Velteer *et al.,* phenytoin acts directly on the testis to inhibit testosterone synthesis by Leydigs cells. In the present study phenytoin treated test group showed 3.2 fold change of LPL gene expression which possibly reveals the reciprocal relationship between testosterone and LPL.

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