International Journal of Anatomical Sciences, 2013, 4(1): 09-11

Research Paper

Phenytoin Induced Up Regulation of DEFB1 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 DEFB1 gene in albino rat testis .The Albino rat were divided into two groups, control and test, The test group were given 150mgs/kg of phenytoin orally and equal amount of normal saline was given for 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 for cDNA microarray analysis. This study showed the differential expression of gene DEFB1 compared to the control group.

Phenytoin is effective against all types of partial and tonic clonic seizures. Its toxic effects are associated with chronic medication. Tania et al reported depression of cellular or humoral immunity or both, in a significant number of phenytoin treated patients. They found that phenytoin therapy was associated with failure of antibody response to Salmonella typhi antigen. Phenytoin is a major cause of antiepileptic drug hypersensitivity syndrome (AHS), which is rare but potentially fatal complication. Ito *et al.*, reported agranulo-cytosis following phenytoin induced hypersensitivity syndrome. Kim *et al.,* reported a case of 40 year old man who had drug rash with Eosinophilia and systemic symptoms (DRESS) syndrome associated with anticonvulsant drugs which are rare but potentially life threatening disease that occurs in response to arene oxide producing anticonvulsant drugs like phenytoin and carbamazepine. Human Beta-defsin DEFB1 is the most important antimicrobial peptide. It is coded by DEFB1 gene. It is the only innate immunity gene that shows long-term balanced selection and heterozygote advantage. It is capable of differentially regulate gene expression upon inflammatory or microbial stimuli

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

Email: rrajkumar_r@yahoo.com

Accepted :27-FEB-2013

IJAS, 2013, 4(1): 09-11

Materials and Methods

Animal treatment and sample collection

Male adult albino rats were segregated into control and test groups. The test groups were treated with phenytoin 150 mg/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 collected in Rnase free tubes and snap frozen in liquid nitrogen. Frozen tissues were stored in RNA later at -70^{o} 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 Bio interpreter Tool with literature curated information.

Observations

Phenytoin induced 2.41 folds up regulated DEFB1 gene expression was observed in phenytoin treated group when compared with untreated control group.

Discussion

Suresh yenugu *et al*., study of Systemiatic suties to identify and characterize novel antimicrobial proteins and peptides revealed that the majority of defensins are expressed predominantly in the male reproductive tract. Ito *et al*., reported agranulocytosis following phenytoin induced hypersensitivity syndrome. Subbannan Gujral reported necrotising lymphadenitis associated with the phenytoin induced hypersensitivity syndrome. Kim *et al.,* reported drug hypersensitivity to previously tolerated phenytoin carbamazepine induced DRESS. All these reports confirm that phenytoin can cause hypersensitivity, necrotizing lymph-adenits, drug rash with eosinophilia and systemic symptoms (DRESS) syndrome. Prado-montes de oca E found that DEFB1 is capable of upregulation upon inflammatory or microbial stimuli. The present study also confirms the 2.41 folds up regulated expression of DEFB1 which is due to hypersensitivity induced by phenytoin toxicity.

References

Sorrell TC, Forbes IJ, Rishbieth B (1971) Depression of immunological function in patients treated with phenytoin sodium (sodium diphenylhydan-toin) *The Lancet*, 298: 1233-1235.

Mendis-Handagama SMLC, Ariyaratne HBS, Teunissen-van-Manen KR, Haupet RL (1998) Differentiation of adult leydig cells in the neonatal rat testis is arrested by hypothyroidism. *Biology of Reproduction*, 59: 351-357.

Yuan G, Z-Al-Shali K, Hege RA (2007) Hyper-triglyceridemia: its etiology, effects and treat-ment, *CMAJ* 176:.doi:10.1503/cmaj.060963

Saltiel AR (2000) Another hormone –Sensitive triglyceride lipase in fat cells? *PNAS*, 97: 535-537.

Moody DE (2001) Genomics techniques:An overview of methods for the study of gene expression. *J Anim Sci*, 79:E128-E135.

Hamadeh HK, Bushel PR, Supriya J, Bennet DO, Leping Li, Raymond SJ, Barrett C, Paules RS,

Blanchard K, Afshari C (2002) Prediction of Compound Signature Using High Density Gene Expression. *Profiling Toxicol Sci*, 67: 232-240.

Bjorntorp P (1997) Hormonal Control of regional fat distribution. *Human Reproduction*, 12: Supplement 1.