ORIGINAL ARTICLE

GROSS HEPATIC CHANGES IN DEVELOPING ALBINO RATS EXPOSED TO VALPROIC ACID

Muhammad Khan, Sania Tanweer Khattak*, Maqbool Elahi**

Department of Anatomy, *Obstetrics and Gynaecology, Saidu Medical College, Swat,
****Department of Anatomy, Khyber Girls' Medical College, Peshawar, Pakistan**

Background: Valproid Acid (VPA) is a broad spectrum antiepileptic drug. Its use during pregnancy has been associated with congenital anomalies and hepatotoxicity. This study was designed to assess the effects of VPA on the gross structure of liver in developing albino rats exposed to the drug during various trimesters of pregnancy. **Methods:** In this experimental study 40 pregnant rats were divided into 4 equal groups A, B, C and D. Group A received VPA in a dose of 500 mg/Kg/day intraperitonealy (I/P) on days 3, 4 and 5 of gestation. Group B received the drug in a dose of 500 mg/Kg/day I/P on days 8, 9 and 10 of gestation. Group C received VPA in a dose of 500 mg/Kg/day I/P on days 16, 17 and 18 of gestation. Group D received no treatment and was kept as a control group. On day 21, the rats were euthanised by cervical dislocation. The liver of the foetuses were dissected out for the assessment of their gross structure. **Results:** Foetal liver of the experimental groups showed significant decrease in weight as well as relative tissue weight index (RTWI) as compared to the control group, although the gross appearance of the foetal liver was normal in all the groups. **Conclusion:** The use of VPA during various trimesters of pregnancy produces hepatotoxicity in the developing rats. So, the use of this drug during pregnancy should be carefully decided.

Keywords: Valproic acid, Developing albino rats, Hepatotoxicity

INTRODUCTION

Epilepsy being an important problem from a medical, social and legal point of view[1] is the third most common serious neurologic disorder following Stroke and Alzheimer's disease[2]. It affects approximately 0.5–1% of pregnant women.[3] In most women seizures are well controlled during pregnancy but if fit frequency changes, it is usually for the worse.[4] It is generally agreed that the avoidance of generalised seizures during pregnancy is paramount and the avoidance of all seizure types is desirable for psychosocial and socioeconomic reasons as well as for the physical well-being of the mother and fetus.[5]

VPA is a branched carboxylic acid[6] and is an established broad spectrum antiepileptic drug[7]. It is the only drug capable of controlling all types of seizures associated with the idiopathic generalised epilepsies.[8] Valproic acid plasma protein binding is from 80–94%.[9] The multiple physiological changes that occur during the course of pregnancy can influence the drug disposition. VPA plasma protein binding is significantly decreased in pregnant women with increase in free fraction.[5] VPA undergoes extensive placental transfer in animals as well as humans.[10] It crosses the placenta and may reach pharmacologically toxic concentration.[11] VPA is a potential hepatotoxic drug[12] and its use during pregnancy has been associated with congenital malformations and hepatotoxicity[13].

MATERIAL AND METHODS

In this experimental study 40 female and 14 male albino rats of sprague dawly strain, weighing 250–300 gm were used. After conception pregnant rats were divided in to four groups A, B, C and D, each containing 10 animals. The rats in group A received sodium valproate, i.e, Epilim (Sanofi-Synthelabo) in a dose of 500 mg/Kg/day I/P on days 3, 4 and 5 of gestation. Group B had the same dose I/P on days 8, 9 and 10 of gestation. Group C received the similar dose of 500 mg/Kg/day I/P on days 16, 17 and 18 of gestation. The rats in group D received no drug and were kept as control group. On 21st day of gestation, the rats were euthanised by cervical dislocation. Hysterotomy was done and the foetuses were harvested. The livers of the foetuses were dissected out for the assessment of their gross structure.

RESULTS

In group D (Control), the external surface of the foetal liver was smooth and shiny and its colour was reddish brown. The gross appearance of the foetal liver in group A, B and C was found to be similar to that of foetal liver in group D and therefore no abnormality was noted. The mean weight of foetal liver for the control group D was found to be 0.45±0.00 gm, 0.40±0.09 gm for group A, 0.24±0.00 gm for group B and 0.28±0.00 gm for group C (Table-1). The mean weights of the foetal liver in experimental groups A, B and C were significantly reduced as compared with the control group D (p<0.01). The reduction in the weight of the foetal liver in group A versus B, A versus C and C versus B was also statistically significant (p<0.01) (Table-2).

The mean relative tissue weight index for group D (control) foetuses was calculated to be

8.21±0.00, 6.07±0.07 for group A, 6.54±0.15 for group B and 7.50±0.05 for group C foetuses (Table-1). The mean relative tissue weight indices of the groups A, B and C showed statistically significant reduction when compared with the control group D (p<0.01). The reduction in RTWI of the group C versus A, C versus B and B versus A was also statistically significant (p<0.01) (Table-3).

Table-1: Effects of VPA on weight of liver and relative tissue weight index (RTWI) of the rat foetuses

Parameters	Groups			
	A (n=28)	B (n=37)	C (n=42)	D (n=54)
Liver weight (gm)	0.40±0.09	0.24±0.00	0.28±0.00	0.45±0.00
RTWI	6.07±0.07	6.54±0.15	7.50±0.05	8.21±0.00

All values are expressed as Mean±SD, n= Number of foetuses

Table-2: Effects of VPA on the weights of the foetal liver

Source of variation	Sum of square (SS)	Degree of freedom (DF)	Mean square (MS)	Variation ratio (F)
A vs B	0.05	1	0.05	52.32**
A vs C	0.00	1	0.00	5.08*
A vs D	0.45	1	0.45	492.17**
B vs C	0.03	1	0.03	31.33**
B vs D	0.98	1	0.98	1069.17**
C vs D	0.71	1	0.71	771.95**
Between groups	1.24	3	0.41	451.33**
Within groups	0.14	157	0.00	
Total	1.39	160		

VPA=Valproic Acid, D=Control Group, A, B, C=Experimental Groups, **p<0.01,*p<0.05, Based on one way ANOVA

Table-3: Effect of VPA on relative tissue weight indices (RTWI)

Source of variation	Sum of square (SS)	Degree of freedom (DF)	Mean square (MS)	Variation ratio (F)
A versus B	3.54	1	3.54	12.88**
A versus C	34.65	1	34.65	126.23**
A versus D	84.37	1	84.37	307.35**
B versus C	18.33	1	18.33	66.75**
B versus D	61.10	1	61.10	222.55**
C versus D	11.67	1	11.67	42.52**
Between Group	109.95	3	36.65	133.51**
With in Groups	43.10	157	0.27	
Total	153.05	160		

VPA=Valproic Acid, D=Control Group, A, B, C=Experimental Groups, **p<0.01, Based on one way ANOVA

DISCUSSION

There are reports of VPA induced hepatotoxicity in adult liver.[14,15] However very little data is available on the effects of VPA on the foetal liver. The present study was designed to assess the effects of VPA on the gross structure of the foetal liver in albino rats exposed to the drug during various trimesters of pregnancy. VPA is an anticonvulsant agent used in the management of various forms of epilepsy including absence, myoclonic and tonic clonic seizures.[16]

The mechanism by which VPA induces liver injury remains unknown. It is hypothesised to involve the generation of toxic metabolites and/or reactive oxygen species.[17] The reaction of toxic metabolites with glutathione in mitochondria produces a localised depletion of glutathione that would result in oxidation stress.[18] Oxidative stress precedes the onset of steatosis and necrosis in liver.[19]

The current study showed that VPA induces liver injury in all the three experimental groups. Though gross appearance of the foetal liver was normal in all the groups, foetal liver of the experimental groups showed significant decrease in weight as well as RTWI as compared to their control. The reason may be the loss of hepatic parenchyma due to necrosis and also due to incomplete formation of the trabeculae during hepatic development.

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Address for Correspondence:

Dr. Muhammad Khan, Department of Anatomy, Saidu Medical College, Swat, Pakistan. **Cell:** +92-342-9622205
Email: drkhansmc@hotmail.co.uk