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Effect of acetazolamide on morphometry of Wistar rat foetus central nervous system

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ABSTRACT

Present study elucidates the relation between dose-related defects and morphometry of the central nervous system in Wistar rat foetus. Brain measurements of the dorsal surface of 16-20 days old pregnant Wistar rats foetuses were measured by using Vernier callipers. Adult pregnant rats were randomly divided into two groups. Control group received 0.25ml of normal saline and treatment group received 80 &160 mg/kg/b. Wt. of acetazolamide on 8th, 9th and 10th day of pregnancy respectively. Pregnancy was terminated on 16th, 18th, 20th day. All foetuses brain parameters were measured. Results show that high dose group rats were observed a significant reduction in all parameters when compared to control. Hence we can conclude that high doses may be neurotoxic to developing a nervous system of Wistar rats.

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INTRODUCTION

Acetazolamide is a carbonic anhydrase inhibitor, which is used to treat congestive heart failure, glaucoma, seizures, raised intraocular pressure, high altitude sickness and intracranial hypertension in humans (Bergstom WH, 1952; Oles KS, 1989; Reiss WG, 1996; Supuran CT, 2015). However, many studies found that unilateral forelimb abnormalities particularly right side in experimental animals with the administration of acetazolamide (Layton, 1965; Wilson JG, 1968; Tellone CI, 1980; Scott WJ, 1981; Biddle FG, 1988; Beck SL, 1991).

The central nervous system malformations occur when teratogen is administrated before or during the closure of the neural groove (Ezurumlu RA, 1982). Acetazolamide administration to pregnant mothers showed rare effects like sacrococcygeal teratoma, metabolic acidosis, hypocalcemia, and hypomagnesemia in newborns. On crossing placental barrier renal tubular acidosis and low birth weight was found. A case study on maternal acetazolamide reported ectrodactyly and syndactyly at birth and oligodontia was noticed at 12yrs age. Pre-clinical studies on animals evidenced forelimb abnormalities, low fetal body weight with high doses indicating the drug as teratogenic. So if possible all drugs should be restricted in the first trimester due to drug-induced fetal teratogenicity.

A study done to know the relation between dose and response of acetazolamide on cerebral blood flow (CBF) and cerebral blood flow velocity (CBFV) determined that dose according to body weight and age should be considered, as the severity of side effects occur at a higher dose. As large doses of acetazolamide cross the blood-brain barrier and can dilate brain arterioles. Though there are reports of developmental toxicity of acetazolamide in laboratory animals, no morphometric study on

the teratogenic effect of acetazolamide on the spinal cord, cerebellum, and cerebrum of the foetuses reported (Grossmann WM, 2000; Ozawa H, 2001; Al-Saleem AI, 2016; Sethi HS, 2016; Arslan EK, 2016).

The present study focused on alterations in the measurements of foetal weight, crown rump length, tail length, brain weight, medial-anteroposterior diameter, lateral anteroposterior diameter, transverse diameter in the cerebrum, of foetuses following administration of acetazolamide to pregnant Wistar rats.

MATERIAL AND METHODS

Drug: Acetazolamide pre-weighed 250mg available from commercial sources.

Animals: Nine adult Wistar female rats weighing about 170–250 gm were obtained from animal house Jeeva labs, Hyderabad. Three female rats were mated with one male overnight. The female rats were examined for sperms in the vaginal smear the next morning, and positive sperm ones were considered as 0.5 days pregnant. The pregnant rats were divided into two groups: (control and treatment group). The rats were kept under hygienic conditions, fed ad libitum and all had free access to water. This study is carried out in strict accordance with the recommendations detailed in the Guide for the Care and Use of Laboratory Animals and protocols are approved by the animal ethical committee.

Intervention

Group-1 (control group): The rats were given 0.25 ml of normal saline orally on the 8th, 9th, and 10th days of the pregnancy. As per dosage, it is divided into two treatment Groups 60mg group-2 and 160 mg group-3 respectively. The rats received 80 and 160 mg/kg/b. Wt. of acetazolamide orally from 8th, 9thand 10th days of the pregnancy. The eighth-day rats which received acetazolamide 8o and 160 mg were terminated on the 16th day by ether inhalation. Similarly, 9th and 10th day rats were terminated on 18th and 20th day. Transverse abdominal incision removed foetuses and placentas.

All the foetuses were weighed and a ruler measured the crown-rump lengths. The foetuses were sacrificed with ether and examined for gross external malformation. A total of 81 foetuses (34 from the control group and 47 from the treatment group 80mg/kg/b. wt. -25litters, 160mg/kg/b. wt. -22 litters) were obtained.

Methodology

Immediately after death, the heads were dissected through incisions along the dorsal aspect and the dorsal cranial bones of the skull were removed to expose the brain, and immersed in 10% neutral buffered formal saline for 24 hours. After removal from the skull, the brain was blotted dry with a filter paper and weighed. The following brain measurements of the dorsal surface of 16- 20 days old Wistar rat foetuses were taken with the aid of Griffin Vernier callipers: cerebral lateral anteroposterior diameter (LAPD); cerebral medial anteroposterior diameter (MAPD); cerebral transverse diameter (TD).

Statistical analysis

Performed using one-way analysis of variance (ANOVA) and experimental data are presented as mean+/- standard deviation (SD). For all the statistical analysis SPSS software was used.

P-Value < 0.05 were taken to be statistically significant.

RESULTS

A total of 81 foetuses which include 34 from the control group and 47 from the treatment group (80mg/kg/b. wt. -25litters, 160mg/kg/b. wt. -22 litters) were obtained which were used for the morphometric study. The relation between dose-related defects and morphometry of the central nervous system on 8th, 9th, 10th day are given in Tables 1, 2, 3.

The Wistar rats were administered acetazolamide on the 8th day of pregnancy and divided into two groups based on dosage as 80 mg and 160mg groups and terminated on the 16th day of pregnancy. These groups were compared with the control group. The foetal weight (1.37±0.03; 1.20±0.02), crown-rump length (1.86±0.02; 1.79±0.04), tail length (0.76±0.03; 0.66±0.07) and brain weight (0.17±0.02; 0.19±0.03) of 80 mg and 160 mg groups were found respectively. These values were statistically significant with the control group foetal weight (2.45±0.07), crown-rump length (2.880.0±6), tail length (1.300.0±6) and brain weight (1.340.0±6).

The Wistar rats were administered acetazolamide on the 9th day of pregnancy and divided into two groups based on dosage as 80 mg and 160 mg groups and terminated on the 18th day of pregnancy. These groups were compared with the control group. The foetal weight (1.4±0.04; 1.20±0.04), crown-rump length (1.87±0.05; 1.83±0.01), tail length (0.78±0.09; 0.70±0.03) and brain weight (0.21±0.06; 0.23±0.01) of 80 mg and 160 mg groups were found respectively. These values were statistically significant with the control group foetal weight (2.4±0.11), crown-

Table 1: Effect of acetazolamide on the 8th day of pregnancy in Wistar rats on foetal weight, crown-rump length, tail length and cerebral parameters

Parameters	Control (Mean±SD)	Treatment (80mg/kg/b. wt.)(Mean±SD)	Treatment (160mg/kg/b. wt.) (Mean±SD)	F-value	P-value
Foetal-weight(gms)	2.45±0.07	1.37±0.03	1.20±0.02	1575.76	<.0001
Crown-rump Length cms)	2.88±0.06	1.86±0.02	1.79±0.04	1356.14	<.0001
Tail-length (cms)	1.30±0.06	0.76±0.03	0.66±0.07	338.23	<.0001
Brain –weight (gms)	1.34±0.06	0.17±0.02	0.19±0.03	1917.41	<.0001
Medial Anterior – Posterior diameter (cms)	0.57±0.05	0.51±0.07	0.42±0.02	18.85	<.0001
Lateral Anterior – posterior diameter (cms)	0.43±0.02	0.37±0.04	0.32±0.01	35.66	<.0001
Transverse diameter (cms)	0.65±0.04	0.59±0.08	0.47±0.04	21.98	<.0001

Table 2: Effect of acetazolamide on the 9th day of pregnancy in Wistar rats on foetal weight, crown-rump length, tail length and cerebral parameters

Parameters	Control (Mean±SD)	Treatment (80mg/kg/b. wt.)(Mean±SD)	Treatment (160mg/kg/b. wt.) (Mean±SD)	F-value	P-value
Foetal-weight(gms)	2.4 ± 0.11	1.4 ± 0.04	1.2 ± 0.04	658.3	<.0001
Crown-rump Length cms)	2.93±0.05	1.87±0.05	1.83 ± 0.01	1528.75	<.0001
Tail-length (cms)	1.29 ± 0.03	0.78 ± 0.09	0.70 ± 0.03	249.53	<.0001
Brain –weight (gms)	1.42 ± 0.05	0.21 ± 0.06	0.23 ± 0.01	1567.63	<.0001
Medial Anterior – Posterior diameter (cms)	0.57 ± 0.03	0.45±0.01	0.44±0.07	81.15	<.0001
Lateral Anterior – posterior diameter (cms)	0.43±0.03	0.41±0.04	0.33±0.01	74.66	<.0001
Transverse diameter (cms)	0.66±0.02	0.63±0.07	0.57±0.03	46.98	<.0001

rump length (2.93±0.05), tail length (1.29±0.03) and brain weight (1.42±0.05).

The Wistar rats were administered acetazolamide on the 10th day of pregnancy and divided into two groups based on dosage as 80 mg and 160mg groups and terminated on the 20th day of pregnancy. These groups were compared with the control group. The foetal weight (1.3±0.08; 1.20±0.04), crown-rump length (1.82±0.05; 1.82±0.01), tail length (0.74±0.03; 0.71±0.03) and brain weight (0.26±0.07; 0.23±0.01) of 80 mg and 160 mg groups were found respectively. These values were statistically significant with the control group foetal weight (2.4±0.04), crown-rump length (2.47±0.04), tail length (1.36±0.06) and brain weight (1.46±0.09).

The Wistar rats were administered acetazolamide on the 8th day of pregnancy and divided into two groups based on dosage as 80 mg and 160mg groups and terminated on the 16th day of pregnancy. These groups were compared with the control group. The cerebrum, medial anteroposterior diameter (0.51±0.07; 0.42±0.02), lateral anteroposterior diameter (0.37±0.04; 0.32±0.01) and transverse diameter (0.59+/-0.08; 0.47±0.04) of 80mg and 160mg groups were found respectively. These values were statistically significant with the control group medial anteroposterior diameter (0.57±0.05), lateral anteroposterior diameter (0.43±0.02) and transverse diameter (0.65±0.04).

The Wistar rats were administered acetazolamide on the 9th day of pregnancy and divided into two groups based on dosage as 80 mg and 160mg groups and terminated on the 18th day of pregnancy. The cerebrum, medial anteroposterior diameter (0.45±0.01; 0.44±0.07), lateral anteroposterior diameter (0.41±0.05; 0.35±0.02)

Table 3: Effect of acetazolamide on the 10th day of pregnancy in Wistar rats on foetal weight, crown-rump length, tail length and cerebral parameters

Parameters	Control (Mean±SD)	Treatment (80mg/kg/b. wt.)(Mean±SD)	Treatment (160mg/kg/b. wt.)(Mean±SD)	F-value	P-value
Foetal-weight(gms)	2.4±0.04	1.3±0.08	1.2±0.04	1442.6	<.0001
Crown-rump Length cms)	2.47±0.04	1.82±0.05	1.82±0.01	781.94	<.0001
Tail-length (cms)	1.36±0.06	0.74±0.03	0.71±0.03	544.04	<.0001
Brain –weight (gms)	1.46±0.09	0.26±0.07	0.23±0.01	816.98	<.0001
Medial Anterior – Posterior diameter (cms)	0.63±0.06	0.45±0.01	0.44±0.07	54.86	<.0001
Lateral Anterior – posterior diameter (cms)	0.54±0.05	0.35±0.03	0.34±0.01	77.17	<.0001
Transverse diameter (cms)	0.68±0.04	0.68±0.06	0.54±0.01	26.66	<.0001

and transverse diameter (0.66±0.07; 0.57±0.04) of 80mg and 160mg groups were found respectively. These values were statistically significant with the control group medial anteroposterior diameter (0.57±0.03), lateral anteroposterior diameter (0.44±0.06) and transverse diameter (0.66±0.042).

The Wistar rats were administered acetazolamide on the 10th day of pregnancy and divided into two groups based on dosage as 80 mg and 160mg groups and terminated on the 20th day of pregnancy. The cerebrum, medial anteroposterior diameter (0.45±0.01; 0.44±0.07), lateral anteroposterior diameter (0.35±0.03; 0.34±0.02) and transverse diameter (0.68±0.06; 0.54±0.01) of 80mg and 160mg groups were found respectively. These values were statistically significant with the control group medial anteroposterior diameter (0.63±0.06), lateral anteroposterior diameter (0.54±0.05) and transverse diameter (0.68±0.04).

DISCUSSION

To assess foetal growth and growth retardation body weight, crown-rump length, tail length has been commonly used as indices in experimental animals (Singh S, 1972; Lassen NA, 1987). According to many studies, it is found that there is a relation between malformations of the nervous system and various brain measurements. In cerebrum, lateral and medial, anteroposterior and transverse diameter were measured and found that these measurements are lower than the control group. It is in line with the line with studies related to developmental toxicity. These may be due to toxic effect exhibited on developing brain during neurulation (Crag BG, 1972; Singh S, 1978; Gutsaeva DR, 2004; Mesembe OE, 2004; Huang KC, 2006; Eluwa MA, 2009).

Earlier studies have observed acetazolamide has bi-directional influences on central nervous system oxygen toxicity. At low doses it would act as an anticonvulsant, under high doses oxidation of brain tissue increases due to increase in blood flow which causes central nervous system oxygen toxicity (Huang JL, 2010), it seems that large doses of acetazolamide cross the blood-brain barrier and dilate the brain arterioles. This might be the reason for the toxic effect on the brain (Grossmann WM, 2000; Ozawa H, 2001; Sethi HS, 2016; Arslan EK, 2016).

The results of this study revealed lower foetal body weight, crown-rump and tail lengths, medial anteroposterior diameter, lateral anteroposterior diameter, transverse diameter. These may indicate intrauterine growth retardation. This confirms that central nervous system malformations occur when teratogen is administered during the neurogenic period leading to developmental anomalies (Xu JH, 1996 Irigi AO, 1999).

The present study may prove that retarded intrauterine growth and fetal weight reduction with drug administration, is in line with previous research (Calvano C, 2000; Xu D, 2006). The foetal resorption was observed in 160mg/kg group and it may be due to the toxicity of acetazolamide and this finding is in line with previous research (Zhao L, 2008).

Morphometric methods may help to identify the difference between normal and severity of a disease. Magnetic resonance and imaging are widely used to find neuroanatomical, developmental and neurological disorders. In schizophrenia, autism, Alzheimer, dyslexia and Turner's syndrome shape of the brain are analyzed to correlate. In the present study brain weight,

cerebrum and cerebellar parameters were measured and compared with control to know the relation between day and dosage. The Present study is in line with previous research (Chandra Sekhar Rao T, 2015).

CONCLUSION

High doses of acetazolamide may cause severe retardation in the development of the nervous system in Wistar rats foetuses as it has teratogenic property. Hence its use requires further evaluation.

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