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INTRPERITONEAL INJECTION OF GABA $_B$ RECEPTOR ANTAGONIST (CGP 35348) FOLLOWING NEONATAL BRAIN DAMAGE AFFECTS THE BLOOD CHEMISTRY IN ALBINO MICE

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ABSTRACT

Aim of this study was to demonstrate the effect of GABA $_B$ receptor antagonists (CGP 35348) on hematological and serum biochemical profile of albino mouse injected for 12 days following neonatal hypoxic ischemic insult. Blood samples from 35 albino mice [CGP35348 treated (N = 19) and saline treated (N= 16)] were collected from direct cardiac puncture and various hematological [blood glucose, packed cell volume (PCV), total WBC count, total RBC count] and selected serum biochemical parameters (cholesterol, AST, ALT, HDL, LDL total protein, triglycerides) were determined. Glucose ($P < 0.001$), TRBC ($P < 0.001$), MCV ($P < 0.001$), TWBC ($P = 0.02$) and PCV ($P = 0.005$) concentrations were significantly lower in CGP35348 treated as compared to saline treated albino mice. Gender based data analysis revealed that CGP 35348 had more drastic effects in blood chemistry of female than male albino mice as glucose ($P = 0.002$) and TRBC ($P = 0.007$) were the only significantly different parameters when compared between $GABA_B$ receptor antagonist and saline treated male albino mice while glucose ($P = 0.01$), TRBC ($P = 0.01$), TWBC ($P = 0.01$), PCV ($P = 0.01$) and MCV ($P = 0.047$) concentrations were significantly lower in CGP 35348 treated female albino mice than their respective control group indicating gender specific effect of hypoxic ischemic brain damage and CGP 35348 in albino mice.

Key words. $GABA_B$ receptor antagonist, Hypoxia ischemia, Albino mouse, Hematology, Serum biochemistry.

INTRODUCTION

Hematological profile provides information about the severity of the disease and the responses to the treatment, metabolic state of an animal and it also helps in establishing a prognosis (Satue *et al*., 2009). Serology is actually clinical plasma analysis which is routinely used for the investigation of various therapeutic strategies used in multifactorial and polygenic human diseases and also the involvement of various organs (Aigner *et al*., 2012). The term Hypoxic- ischemic encephalopathy (HIE) is widely used for an event in which patients suffer pure hypoxic event without the involvement of cardiac vascular collapse and is a common cause of neonatal brain injury (David and Geer, 2006). In neonates Hypoxic- ischemic (HI) injury either results from birth asphyxia, i-e, premature separation of placenta, compression of umbilical cord, excessive contraction of uterus and excessive anesthesia to the mother, which depresses oxygenation of blood (Guyton and Hall, 2000) One to three per 1000 live full-term births are affected by Perinatal hypoxic-ischemic encephalopathy (HIE) (Graham *et al*., 2008). 15-20 % of affected new born are die in postnatal period while 25 % of the remaining who survive suffer from severe and long lasting neuropysiological consecution such as cerebral palsy and epilepsy, mental abnormalities, cognitive problems, increased hyperactivity and visual perceptive dysfunction (Chilai and Yang, 2011).

The term GABA refers to γ –amino butyric acid (H_2N-CH_2-CH_2-CH_2-COOH) which is the major inhibitory neurotransmitter and plays a vital role in regulating the activity of neuronal cells (Bettler *et al*., 2004). GABA brings about its function through $GABA_A$ which is ionotropic and $GABA_B$ metabotropic receptors (Sieghart, 1995).

$GABA_B$ receptors are widely used in the treatment of neurologic and psychiatric disorders including absence seizures, gamma- hydroxybutyrate toxicity and more recently used for the treatment of autoimmune limbic encephalitis (Eduardo and Benarroch, 2012). Activation of $GABA_B$ receptors produces anesthetic effects in animals with neuropathy and chronic inflammation (Pin and Prezeaul, 2000). CGP 35348 is among the most extensively studied, commercially available $GABA_B$ receptors antagonists. $GABA_B$ antagonists have antidepressant activity (Cryan and kaupman, 2005), cognition improvement (Froestl *et al*., 2004) and beneficial effects in rat models of absence epilepsy (Manning *et al*., 2003). Behavioral work on the effect of blockage of $GABA_B$ receptor antagonist CGP 35348 has produced results ranging from memory facilitation to impairment (Bianchi and Panerai, 1993; Carletti *et al*., 1993; Mondadori *et al*., 1993; Brucato *et al*., 1996; Getova *et al*., 1996) but little information is

available regarding the effect of CGP 35348 supplementation on blood chemistry following hypoxic ischemic encephalopathy. Present study was conducted to investigate the effect of HI stress followed by $GABA_B$ receptors antagonist injection and its effects on the hematology and serum biochemical profile of albino mice.

MATERIALS AND METHODS

Subjects: Adult albino mice [N = 37, Male = 16, Female = 19] were used during these experiments. Breeding pairs of albino mouse were purchased from veterinary Research Institute, Ghazi road Lahore, Pakistan. Mice were maintained in cages filled with wood chips at the core Animal facility, at Bio Park of Bahauddin Zakariya University, Multan. In breeding colony, albino mice were housed in individual cages, standard mouse diet and water was available at libitum. 22±1°C room temperature was maintained, room was lighted at an intensity of about 200 x 1 Watt 2m from 8 a.m. to 7 p.m. Albino mouse was housed in individual cages. All the experimental protocol was approved by the ethical committee of Institute of Pure and Applied Biology at Bahauddin Zakariya University Multan, Pakistan.

Murine Model of Hypoxia Ischemia Encephalopathy: On postnatal day 10, isoflurane inhalation (3%) was used to anesthetize the mouse pups. A right lateral incision was made in neck region and the right common carotid artery was ligated by using polypropylenedalcon USP 6 suture. During the surgery temperature was maintained at 36 °C by keeping pups on a hot plate. The surgical procedure was completed within 10 min. Pups were returned to their dams for I hour and then placed in a hypoxic chamber for 25 min with constant supply of 8% Oxygen balanced with Nitrogen. The hypoxic chamber was kept on hot plate to maintain the ambient temperature inside the chamber at 36 °C. Pups were returned to their mothers for recovery after hypoxic exposure.

Experimental Design: On 18-20th day of life, mice were separate from their parents and fed on normal mouse diet until 13th week of life when they started receiving intra peritoneal injections of the $GABA_B$ receptor antagonist, CGP 35348, (1mg / kg body weight / ml of solvent) [N = 19] for 12 days. Separate control groups were maintained in parallel that also underwent the hypoxic ischemic insult on postnatal day 10 and after 13 weeks of life, they received intrapertoneal saline injection for 12 days [N = 16].

Blood and serum collection: Following the intra peritoneal injections for 12 days, mice were anesthetized with 3% Isoflurane and blood was sampled either from retro-orbital sinus or through direct cardiac puncture. Blood was divided into two parts; one for the study of hematological parameters and second for serum biochemical profiling.

Hematological and serum biochemical profiling: Hematological parameters (blood glucose level, mean corpuscular volume, packed cell volume, total red and white blood cell count) and serum biochemical parameters [Cholesterol, Aspartate transaminase (AST), Alanine transaminase (ALT), High density lipoprotein (HDL), Low density lipoprotein (LDL), Total protein and triglycerides] were determined in treated and untreated male albino mouse blood samples by using Hitachi 902 automatic analyzer (Japan).

Statistical analysis: All the data is expressed as Mean ± Standard deviation. Statistical package Minitab (version 16, Pennsylvania) was used for the analysis of results. 2 sample t- tests was applied to compare various parameters of hematology and serum biochemical profile of albino mouse between CGP35348 treated and their respective untreated controls following hypoxia ischemia encephalopathy.

RESULTS AND DISCUSSION

Analysis of the results revealed that glucose ($P < 0.001$), TRBC ($P < 0.001$), MCV ($P < 0.001$), TWBC ($P = 0.02$), PCV ($P = 0.005$) concentrations were significantly lower in CGP 35348 treated than untreated albino mice following brain damage at postnatal day 10 (Table 1). Gender based data analysis revealed that CGP 35348 had more drastic effects in blood chemistry of female than male albino mice as glucose ($P = 0.002$) and TRBC ($P = 0.007$) were the only significantly different parameters when compared between $GABA_B$ receptor antagonist and saline treated male albino mice (Table 2) while glucose ($P = 0.01$), TRBC ($P = 0.01$), TWBC ($P = 0.01$), PCV ($P = 0.01$) and MCV ($P = 0.047$) concentrations were significantly lower in CGP 35348 treated female albino mice than their respective control group indicating gender specific effect of hypoxic ischemic brain damage and CGP 35348 in albino mice (Table 3).

Hypoxia is a general term denoting lack of oxygen in the air being breathed and ischemia is a blood supply shortage to an organ results in the damage to tissues because of oxygen deficiency and nutrients (Johansen *et al*., 2006). Blood is the special type of connective tissue that performs multiple functions including transportation of various gases, nourishment from digestion site and hormones from glands to their final destination. It also transports immunity providing substances to tissues and waste to kidney (Robert *et al*., 2006). Hence, hematological studies are useful in determining the health status of animals (Amat *et al*., 2006) as various diseases affects the blood production and also its components such as blood cells, proteins,

glucose, hemoglobin, mechanism of coagulation, etc (Aaron *et al*., 2003)

Our results indicated white blood cells count (WBC) was significantly different (P = 0.02) when compared between CGP35348 and saline treated (control) albino mice (Table 1). This parameter also varied significantly (P = 0.01) when compared between CGP35348 and saline treated female albino mice (Table 3) indicating a severe effect of hypoxic ischemic insult and CGP35348 supplementation on this parameter confirming the findings of Khadim *et al*. (2013) who had also reported a decrease in number of white blood cells in CGP 55845 treated female albino mice as compared to saline treated controls following hypoxic ischemic insult.

The PCV value indicates oxygen carrying capacity of the blood which measures the degree of stress on animal health (Larson *et al*., 1985). Data analysis revealed a significant elevation in the level of PCV and RBC count in saline treated mice understudy following HI insult and these findings are in agreement with Ambali *et al*. (2010) who had reported a rise in these parameters following a stress. While in CGP 35348 treated albino mice, we have observed a decrease in PCV and RBC followinh postnatal brain damage and these results are in agreement with Khadim *et al*. (2013) who had reported a similar decreasing pattern of PCV and RBC count in both male and female albino mice treated with $GABA_B$ receptor antagonist CGP 55845.

The decreased MCV levels may indicate a decrease in size of erythrocytes due to stressful conditions (Rao and Vidyunmala, 2009). This parameter showed significant decrease in CGP 35348 treated as compared to the saline treated (P > 0.001) albino mice (Table 1) and a similar trend (P = 0.0 47) was observed in CGP 35348 treated female albino mice when their MCV values were compared with saline treated control females following hypoxic ischemic encephalopathy at post natal day 10 (Table 3). These results are again in accordance with those reported by Khadim *et al*. (2013) who had observed decreased MCV concentrations in male albino mice injected with CGP 55845 following brain damage.

Total Red blood cell count (TRBC) was significantly different when compared between CGP35348 (P > 0.001) (Table 3.1) and saline treated albino mice as well as between treated and untreated male (P = 0.007) and female (P = 0.01) albino mice (Table 2-3).

Table 1. Comparison of various hematological and serum biochemical parameters between $GABA_B$ receptor antagonist (CGP35348) and saline treated (control) albino mice following hypoxic ischemic insult. Data is expressed as Mean ± Standard deviation. P- Value indicates the results of 2-sample t-test.

Parameters		Saline treated male control group (N = 7)		CGP35348 treated male group (N = 9)		p-value
		Mean ± SD	Range	Mean ± SD	Range	
Hematological profile	TWBC (x 10^3 μL^{-1})	8153 ± 3599	0.01 - 0.003	4679 ± 3164	0.012 - 0.001	0.02 *
	TRBC (x 10^3 μL^{-1})	678500 ± 1531276	9.2 - 4.67	141011 ± 623194	26.28 - 3.69	P < 0.001 ***
	PCV (%)	38.99 ± 7.02	47.7 - 29.7	27.63 ± 6.43	42.2 - 13.8	0.005 **
	Glucose (mg/dl)	210.2 ± 45.2	265 – 147	132.8 ± 35.6	223 - 79	P < 0.001 ***
	MCV (fp)	58.81 ± 9.75	75.3 – 0	25.6 ± 17.0	79.13 - 10.51	P < 0.001 ***
	Total protein (g/dl)	4.900 ± 0.79	5.8- 4.3	4.650 ± 0.35	5 - 4.3	0.66
	Triglycerides (mg/dl)	136.8 ± 95.6	307 – 86	109.5 ± 42.9	165 - 66	0.59
	Cholesterol (mg/dl)	100.7 ± 48.5	208.7 - 41.5	113.3 ± 23.0	155.1 - 84.2	0.52
	HDL (mg/dl)	23.83 ± 4.4	27.8 - 17.8	34.85 ± 9.5	48.5 - 28	0.1
	LDL (mg/dl)	113.9 ± 54.1	208.6 - 72.1	59.6 ± 33.3	93.4 ± 22.4	0.11
	ASAT(µ/l)	145 ± 103	295 – 69	783 ± 454	1104 - 462	0.3
	ALAT(µ/l)	41.6 ± 19.3	68 – 15	56.5 ± 22.9	99 - 35	023

P .> 0.05 = Non significant, P < 0.05 = Least significant (*), P < 0.01 = Significant (**), P < 0.001 = highly significant (***)

Glucose is the primary source of energy for the body cells. Blood glucose level outside the normal range may be an indicator of medical condition or illness (Walker *et al*., 2006). In present study, the glucose level was significantly lower in CGP35348 albino mice when compared with saline treated (control) albino mice (P = 0.00) (Table 1). Similar results were observed upon comparison of this parameter between CGP35348 and saline treated male albino mice (P = 0.002) (Table 2) and female albino mice (P = 0.01) (Table 3) following

neonatal brain damage. These results are in accordance with those reported by Khadim *et al*. (2013) who had observed decreased glucose concentrations in male albino mice injected with CGP 55845 following brain damage. Our results are contradictory to Nourian *et al*. (1996) who had injected CGP35348 both intra peritonealy (IP) and intra cerebroventricularly (ICV) to male albino mice and had reported that $GABA_B$ receptor influences blood glucose level by regulating endocrine pancreatic secretion and itself has inhibitory effect on glucagon release but its antagonist CGP35348 blocks the inhibitory effect of GABA and results in increment in blood glucose level in circulation. The contradiction in results is probably due to the application of brain damage during our study which was missing in Nourian *et al*. (1996).

In conclusion, we have observed a gender specific effect of hypoxic ischemic brain damage and CGP 35348 in albino mice with more pronounced changes observed in the blood chemistry of female albino mice. The present work can be used as a baseline reference data which will be helpful in monitoring the health status of the subjects suffering from hypoxic ischemic insult.

Table .2. Comparison of various hematological and serum biochemical parameters between $GABA_B$ receptor antagonist (CGP 35348) and saline treated (control) male albino mice following hypoxic ischemic insult. Data is expressed as Mean ± Standard deviation. P- Value indicates the results of 2-sample t-test.

Parameters		Saline treated male control group (N = 7)		CGP35348 treated male group (N = 9)		p-value
		Mean ± SD	Range	Mean ± SD	Range	
Hematological profile	TWBC (x 10^3 μL^{-1})	7360 ± 4968	0.02 - 0.003	4203 ± 3703	0.012 - 0.001	0.27 ns
	TRBC (x 10^3 μL^{-1})	59200 ± 69914	6.84 - 5.22	15518 ± 730065	26.28 - 3.69	0.007 **
	PCV (%)	37.65 ± 6.2	42-33. 30	29.54 ± 7.7	42.2 - 22.4	0.28
	Glucose (mg/dl)	217.7 ± 45.1	265 – 147	123.2 ± 42.1	223 - 79	0.002 **
	MCV(fp)	69.53 ± 8.11	75.27 - 63.79	16.01 ± 5.73	22.66 - 10.51	0.07
Serum biochemical profile	Triglycerides (mg/dl)	89.00 ± 2.83	91 – 91	92.5 ± 37.5	119 - 66	0.92
	Cholesterol (mg/dl)	154.9 ± 76.0	208 – 101	127.1 ± 27.9	55.1 - 99.4	0.71
	ASAT (µ/l)	107.5 ± 26.2	126 – 89	783 ± 454	1104 - 462	0.28
	ALAT (µ/l)	48.7 ± 15.5	60 – 31	50.7 ± 11.0	62 - 40	0.87

P .> 0.05 = Non significant, P < 0.01 = Significant (**)

Table .3. Comparison of various hematological and serum biochemical parameters between $GABA_B$ receptor antagonist (CGP35348) and saline treated (control) female albino mice following hypoxic ischemic insult Data is expressed as Mean ± Standard deviation. P- Value indicates the results of 2-sample t-test

Parameters		Saline treated female control group (N = 9)		CGP35348 treated female group (N = 10)		p-value
		Mean ± SD	Range	Mean ± SD	Range	
Hematological profile	TWBC (x 10^3 μL^{-1})	8945 ± 1702	0.01 - 0.007	5154 ± 2687	0.008 - 0.001	0.01**
	TRBC (x 10^3 μL^{-1})	736166 ± 171164	9.2 - 4.67	128411 ± 521932	18.98 - 6.08	0.01**
	PCV (%)	39.52 ± 7.95	47.7 - 29.7	26.44 ± 5.70	31.2 - 13.8	0.01**
	Glucose (mg/dl)	203.9 ± 47.8	263 – 155	141.5 ± 28.0	170 - 97	0.01**
	MCV (fp)	57.9 ± 26.3	101.1 - 34.94	23.9 ± 14.9	51.32 - 7.91	0.047*
Serum biochemical profile	Total protein (g/dl)	4.900 ± 0.8	5.8 - 4.3	4.950 ± 0.07	5 - 4.9	0.9
	Triglycerides (mg/dl)	148 ± 106	307 – 86	92.5 ± 37.5	165 - 88	0.9
	Cholesterol (mg/dl)	82.6 ± 23.7	110 - 72.1	106.4 ± 19.0	130.7 - 84.2	0.1
	HDL(mg/dl)	38.2 ± 32.5	27.8 - 17.8	28.40 ± 0.56	28.8 - 28	0.5
	LDL (mg/dl)	56.3 ± 12.6	66.5 – 42.2	40.80 ± 3.11	43 – 38.6	0.2
	ALAT (µ/l)	47.2 ± 30.8	68 – 15	62.3 ± 33.0	99 - 35	0.55

P .> 0.05 = Non significant, P < 0.05 = Least significant (*), P < 0.01 = Significant (**), P < 0.001 = highly significant (***)

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