PREGABALIN EFFECTS ON CELLULAR AND HUMORAL COMPONENTS OF BLOOD OF MICE (*Mus musculus*)

Wissam Sajid Hashim Al-Uboody

Department of Physiology and medical Physics, College of Medicine, Al-Muthanna University. Al-Muthanna, Iraq.

(Received 17 October2017 ,Accepted 18 December 2017)

Keywords: Pregabalin, Blood, Electrolytes.

Corresponding author E.mail: dr.wissam2013@gmail.com

ABSTRACT

The oral use of pregabalin was evaluated in this study to reveal its effects on some hematological and biochemical parameters of laboratory mice. The animals of this study were divided into three groups (twelve mice each). The control group was fed on a standard ration. The first treated group (Pregabalin 1) were dosed orally with one ml of distilled water containing Pregabalin (20 mg/ml/mice/ day). The second treated group (Pregabalin 2) were dosed orally with one ml of distilled water containing pregabalin (40mg/ml/mice/ day). The experiment continued for two successive months. The results showed that the use of pregabalin caused a significant declination in R.B.C., HGB, HCT, W.B.C., M.C.V., MCH, and MCHC of both treated groups compared them with control group and the declination was more significant in (pregabalin 2) group compared with (pregabalin 1) group. The RDW-CV, RDW-SD, and PLT increased significantly in both treated groups as compared with those of the control group. However, PCT, PDW and MPV were not affected by the use of pregabalin except the MPV of (Pregabalin 2) group was significantly less than that of (Pregabalin 1) but without a significant difference compared with that of control. The Fe^{++}, and GLU decreased significantly in both treated groups compared with the control and they declined more significantly in (Pregabalin 2) compared with (Pregabalin 1). Both the TP and Ca^{++} did not affected by the use of pregabalin compared with the control group ($P \leq 0.05$).

INTRODUCTION

Pregabalin is well known as an anti-seizure drug which consists of a GABA molecule that binds to a lipophilic isobutane (1). Pregabalin binds with a protein in the membranes of the cortex of the brain which has a sequence of amino acids resembling that of the subunit α2δ-1 of calcium (2). Pregabalin is absorbed after being used orally and it suffers no metabolism and secreted unchanged in urine as it is not bound to plasma proteins (1). The oral LD50 of pregabalin is more than 5000 mg/kg of mice (3). Many studies and reports have been made about this drug but approximately all of them focus on the role of pregabalin as an anti-epilepsy drug and the relation of which with the pain and little is known about its side effects on the other functions of the body systems such as the blood and the proteins, therefore; this study is focusing on finding out the side effects of pregabalin on the blood and other biochemical aspects. Of the studies which comprised pregabalin are; the effect of pregabalin as antinociceptive against hypersensitivity of rectum in rats after septicemic shock (4), pregabalin effects on brain neurotransmitters (5), the role of pregabalin as protector for neurons after spinal injury of rat (6), its effects on libido (7), the pregabalin effects on the changes in behavior of rats after being induced by ketamine (8), the effects of pregabalin on different hormones like reproductive hormones and the weights of rats (9), the effect of pregabalin against neuronal hyperexcitability of the posterior thalamus of neuropathic rats (10), the role of pregabalin as protector in spinal ischemia of rats (11), its effect against the hypophyseal axis and histological structure of testis of rat (12), its effects against neuralgia in rats (13) and pregabalin effect against neuropathic pain of rats (14). Thus far, the neurological role of pregabalin was well documented. To date, no information are available on the effects of pregabalin. This study aimed to spot a light on these effects of pregabalin on blood and biochemical markers.

MATERIALS AND METHODS

• Experimental Design

1- Control group: Twelve male mice were maintained on a standard ration for two months.

2- First treatment group (Pregabalin 1): Twelve male mice were dosed orally (20mg/ml/mouse) of pregabalin via oral gavage daily for two months.

3- Second treatment group (Pregabalin 2): Twelve male mice were dosed orally (40mg/ml/mouse) of pregabalin via oral gavage daily for two months. Pregabalin was dissolved in distilled water and dosed to the mice in both treated groups.

** Oral LD50 of pregabalin for mice = 5000 mg/kg (3).

• Preparation of specimens

At the end of the experimental period, mice were anaesthetized by the use of chloroform and blood samples were withdraw from myocardium directly. Part of blood samples were put in Ependorf gel tubes to separate and obtain sera and other part were put into anticoagulant tubes containing EDTA.

• Parameters of the study

PKL (POKLER ITALIA) and its special included kits was the device that used to perform the serological tests of the study and its special included kits while the hematological tests were done by the use of Sysmex Automated Hematology Analyzer device.

• Statistical analysis

One way ANOVA test was used to find out the least significant difference (LSD) among groups used IBM SPSS version 20.

RESULTS AND DISCUSSION

It is very obvious from this study that pregabalin affects the blood cellular and electrolyte components. The current study revealed that the oral use of pregabalin causes a significant decrease in red blood cells count (RBC), hemoglobin concentration (HB), packed cell volume (HCT), and leukocytes count (WBC) of the treated groups compared them with those of the control group. There was a significant decrease of these parameters when higher dose of pregabalin was used (table 1). For the red blood cells indices, there was a significant decrease in the mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) of both treated groups, while the red cell

Distribution widths (both RDW-CV and RDW-SD) were not affected in both treated groups as compared with control group (table 2). Considering the platelets (thrombocytes), the results showed a significant increase in platelets count (PLT) and

there was more significant increase in PLT when the dose of pregabalin increased in pregabalin2 group as compared with pregabalin1 and control groups. The mean platelets volume (MPV) was not affected significantly in pregabalin1 group but it was significantly decreased in pregabalin2 group, while the platelets distribution width (PDW) and the plateletcrit (PCT) were not affected significantly in both treated groups compared them with control group (table 3).

For the biochemical parameters, the oral use of pregabalin causes a significant decrease in serum glucose (GLU) and iron (Fe^{++}) and the decrement was more significant when the dose of pregabalin increased in pregabalin2 group compared with that of control and pregabalin1 groups, while the total protein (TP) and calcium (CA) were not affected significantly in both treated groups as compared with control group at ($P \leq 0.05$).

The declined number of R.B.C, HB, HCT, MCV, MCH, and MCHC beside the declined Fe^{++} which were caused by the oral use of pregabalin can be diagnosed as a microcytic hypochromic anemia and the subsequent thrombocytosis (increased PLT count) is due to the anemia and Fe^{++} deficiency (15). The mechanism by which pregabalin causes these malicious effects might be explained as the follow: anti-epilepsy medicines were reported to affect the hypothalamus-pituitary axis and the subsequent glands like thyroid gland, adrenal gland and gonads, and they might cause a disturbances in hormones like thyroid hormones, cortisols and others (16). The effect of thyroid hormones like thyroxin is well known on all cells and functions of the body where it induces all forms of carbohydrates metabolism like glucose intestinal absorption, gluconeogenesis, enhanced glycolysis, intestinal mineral and vitamins absorption, cellular growth, DNA replication and others. In addition, the effect of adrenal corticosteroid hormones especially glucocorticoids is also well understood and represented by increased blood glucose levels and others (17). So, when pregabalin affects these hormones, the resulted decreased blood GLU, blood cells and anemia are expected and explained.

Table (1). Pregabalin effects on cellular components of blood.

GROUPS / Parameters	R.B.C $\times 10^{12}$ /L	HGB g /dL	HCT %	W.B.C $\times 10^{9}$ /L
Control	a 9.33 ± 0.51	a 13.17 ± 0.40	a 42.5 ± 1.22	a 3.5 ± 0.54
Pregabalin 1	b 8.17 ± 0.40	b 10.5 ± 0.54	b 36.76 ± 0.63	b 2.17 ± 0.40
Pregabalin 2	c 7.33 ± 0.51	c 10 ± 0.6	c 33 ± 1.41	b 1.67 ± 0.51
LSD	0.833	0.50	3.766	1.333

Table (2). Pregabalin effects on red blood corpuscles indices.

GROUPS / Parameters	M.C.V fL	MCH pg	MCHC g/dL	RDW-CV %	RDW-SD fL
Control	a 47.50 ± 1.22	a 14.91 ± 0.76	a 31.17 ± 1.16	b 14.67 ± 0.51	b 24 ± 1.26
Pregabalin 1	b 45.67 ± 1.03	b 13 ± 0.55	b 27.83 ± 1.16	a 18 ± 0.63	a 26 ± 0.89
Pregabalin 2	c 43.33 ± 0.81	b 12.96 ± 0.55	b 28.5 ± 1.87	a 17.33 ± 0.51	a 26 ± 1.54
LSD	1.83	1.91	2.66	2.66	2

Table (3). Pregabalin effects on thrombocytes and their indices.

GROUPS / Parameters	PLT $\times 10^9$ /L	MPV fL	PDW fL	PCT %
Control	a 503.50 ± 16.53	a 6 ± 0.1	a 14.5 ± 0.54	a 0.35 ± 0.05
Pregabalin 1	b 568.83 ± 40.20	a 6.33 ± 0.81	a 14 ± 1.26	a 0.367 ± 0.1
Pregabalin 2	c 676.17 ± 52.15	b 5.33 ± 0.51	a 13.83 ± 0.40	a 0.383 ± 0.04
LSD	65.33	1.00	-------	-----

Table (4). Pregabalin humoral components of blood.

GROUPS / Parameters	GLU mg /dl	CA mg /dl	TP g /dL	Fe^{++} mg /dL
Control	a 106.83 ± 5.34	a 9.83 ± 0.4	a 5.67 ± 0.51	a 403.5 ± 22.52
Pregabalin 1	b 78.67 ± 5.12	a 10 ± 0.3	a 6 ± 0.6	b 276.67 ± 20.65
Pregabalin 2	c 59.83 ± 6.70	a 9.75 ± 0.5	a 6.17 ± 0.75	c 204.17 ± 7.73
LSD	18.83	------	-------	72.5

تأثير البريكابالين على مكونات الدم الخلوية والخلطية للفئران المختبرية

وسام ساجد هاشم العبودي

فرع الفسلجة الطبية والفيزياء , كلية الطب , جامعة المثنى ، المثنى، العراق .

الخلاصة

في هذه الدراسة تم تقييم تاثير الاستخدام عن طريق الفم للبريكابالين لمعرفة تاثيراته على بعض المعايير الدمية والكيموحيوية للفئران المختبرية. تم توزيع حيوانات التجربة عشوائيا الى ثلاث مجاميع مكونة من اثنتا عشر فارا لكل مجموعة. مجموعة السيطرة تم تغذيتها على عليقة قياسية. مجموعة المعاملة الاولى (بريكابالين ١) تم تجريعها فمويا بمل واحد من الماء المقطر المحتوي على بريكابالين (٢٠ ملغم/مل/فار/باليوم). مجموعة المعاملة الثانية (بريكابالين ٢) تم تجريعها فمويا بمل واحد من الماء المقطر المحتوي على بريكابالين (٤٠ ملغم/مل/فار/باليوم). استمرت التجربة لمدة شهرين متتابعين. اظهرت نتائج الدراسة ان استخدام البريكابالين سبب انخفاض معنوي في عدد كرات الدم الحمراء، الهيموغلوبين، حجم الخلايا المرصوصة، خلايا الدم البيضاء، معدل حجم الكريات الحمراء، معدل الهيموغلوبين في الكريات، ومعدل تركيز الهيموغلوبين في الكريات لكلا مجموعتي المعاملة مقارنة بمجموعة السيطرة وكان الانخفاض اكثر معنوية لمجموعة المعاملة (بريكابالين ٢) مقارنة بمجموعة المعاملة (بريكابالين ١). معدل توزيع الكريات الحمراء بنوعيه والصفيحات الدمية قد ازدادت بصورة معنوية في كلا مجموعتي المعاملة مقارنة بمجموعة السيطرة بينما حجم الصفيحات المرصوصة، معدل توزيع الصفيحات، ومعدل حجم الصفيحات لم تتاثر باستخدام البريكابالين عدا معدل حجم الصفيحات لمجموعة المعاملة (بريكابالين ٢) والذي كان اقل معنويا مما هو عليه في مجموعة المعاملة (بريكابالين ١) وبدون فرق معنوي مقارنة مع مجموعة السيطرة. بالنسبة للحديد والكلوكوز فلقد انخفضت مستوياتهما بصورة معنوية لكلا مجموعتي المعاملة مقارنة مع مجموعة السيطرة وكان الانخفاض اكثر معنوية بمجموعة (بريكابالين ٢) مقارنة مع مجموعة (بريكابالين ١). كلا معدلات البروتين الكلي والكالسيوم لم تتاثر باستخدان البريكابالين مقارنة مع مجموعة السيطرة عند مستوى احتمال ($P\leq0.05$).

REFERENCES

1- Brunton, L. L.; Chabner, B. A.; and Knollmann, B. C. (2011). Goodman & Gilman's, The Pharmacological Basis of Therapeutics. Twelfth edition, USA. Pp: 571.

2- Gee, N.S.; Brown, J.P.; Dissanayake, V.U. (1996). The novel anticonvulsant drug, gabapentin (Neurontin) binds to the 2 subunit of a calcium channel. *J Biol Chem*, *271*:5768–5776.

3- Pfizer pharmaceuticals group (2012). Material safety data sheet. Pp: 5.

4- Eutamene, H.; Coelho, A. M.; Theodorou, V.; Toulouse, M.; Chovet, M.; Doherty, A.; Fioramonti, J.; and Bueno, L. (2000). Antinociceptive effect of pregabalin in septic shock-induced rectal hypersensitivity in rats. The journal of pharmacology and experimental therapeutics. Vol. 295, No. 1.

5- Errante, L. D. and Petroff, A. C. (2003). Acute effects of gabapentin and pregabalin on rat forebrain cellular GABA, glutamate, and glutamine concentrations. Seizure; **12:** 300–306.

6- Ha, K. Y.; Carragee, E.; Cheng, I.; Kwon, E. S. and Kim, H. Y. (2011). Pregabalin as a neuroprotector after spinal cord injury in rats. Korean Med Sci. 26: 404-411.

7- Bucur, M. and Jeczmien, P. (2011). Pregabalin and libido. The open neuropsychopharmacology journal. 4, 8-9.

8- Nunes, E. A.; Canever, L.; De Oliviera, L.; De Luca, D. R.; Quevedo, J.; Zugno, A.; Peregrino, A.; Crippa, S. A. J.; Dursum, M. S.; Baker, B. G.; and Hallak, C. E. J. (2012). Effects of pregabalin on behavioral alterations induced by ketamine in rats. Rev Bras Psiquiatr. 34:329-333.

9- Tawfeeq, N. E.; Yanzeel, H. J.; and Fakhrildin, B. M. (2015). Effects of pregabalin drug on level of reproductive hormones, sexual Activity, number and weight of offspring in female albino rats. International journal of science and research. Vol. 6, No. 8: 814-819.

10- Patel, R.; and Dickenson, H. A. (2016). Neuronal hyperexcitability in the ventral posterior thalamus of neuropathic rats, modality selective effects of pregabalin. J. neurophysiol. 116: 159–170.

11- Kazanci, B.; Ozdogan, S.; Kahveci, R.; Gokce, C. E.; Yigitkanli, K.; Gokce, A.; and Erdogan, B. (2016). Neuroprotective effects of pregabalin against spinal cord ischemia-reperfusion injury in rats. JTN.17959-16.1.

12- Bostanian, S.; Shariati, M.; and Zamanpoor, M. (2016). Effect of pregabalin on pituitary – gonad axis and testis histological changes in adult rat. J. Appl. Environ. Biol. Sci., 6(3)165-171.

13- **Chen, W. R.; Liu, H.; An, X. J.; Qian, Y. X.; Jiang, D. Y.; Cope, K. D.; Williams, P. J.; Zhang, R.; and Sun, N. L.** (2017). Cognitive effects of electro-acupuncture and pregabalin in a trigeminal neuralgia rat model induced by cobra venom. Journal of Pain Research: 10 1887–1897.

14- **Meymandi, S. M.; Sepehri, G.; Abdolsalamadi, M.; Shaabani, M.; Heravi, G.; Yazdanpanah, O.; and Aghtaei, M. M.** (2017). The effects of co-administration of pregabalin and vitamin E on neuropathic pain induced by partial sciatic nerve ligation in male rats. Inflammopharmacology: Vol.25, No. 2, pp 237–246.

15- **Stockham, S. L. and Scott, M. A.** (2008). Fundamentals of veterinary clinical pathology. 2nd edition. Blackwell publishing, USA.

16- **Wagner, B. S.; Bauer, J.; Flugel, D.; Brennemann, W.; Klingmuller, D.; and Elger, C. E.** (1998). Serum sex hormones are altered in patients with chronic temporal lobe epilepsy receiving anticonvulsant medication. Epilepsia 39:1164–1173.

17- **Guyton, A. C.; Hall, J. E.** (2006). Textbook of medical physiology. 11th edition. Elsevier Inc. USA.