BIOCHEMICAL EFFECTS OF LONG DURATION OF SEDATION ON NEUROTRANSMITTERS, PROTEIN FRACTIONS AND IMMUNOGLOBULINS

Mohamed K. Mahfouz[1], El-Far, A. H.[2], Hussein A. Abdel Maksoud[1]

[1]Department of Biochemistry, Fac. of Vet. Med., Moshtohor, Banha Univ., Egypt
[2]Department of Biochemistry, Fac. of Vet. Med., Damanhour Univ., Egypt
e-mail: aboufares90@yahoo.com

Abstract

The present study was planned to know the biochemical effect of the long period of sedation on neurotransmitters, protein fractions and immunoglobulins. For this aim thirty adult male albino rats were allotted into two groups, control group of 10 rats were injected with an equivalent volume of physiological saline and the remained 20 rats were utilized as experimental group, injected with a long period of sedation (midazolam)-12 weeks. After that period, some neurotransmitters (adrenaline, dopamine and histamine) and serum components (serotonin, cholinesterase, protein electrophoresis, IgG, IgM, IgE and IgA) were investigated. Our study revealed that, injection of midazolam for 12 weeks has no obvious changes in the investigated neurotransmitters, serum electrophoretic pattern and immunoglobulins except a significant increase in Cholinesterase enzyme activities at 3rd and 4th weeks of experiment.

Key words: Midazolam, Neurotransmitters, Protein Fractions, Immunoglobulins

INTRODUCTION

The brain contains separate neuronal systems that utilize three different catecholamines (dopamine, norepinephrine, and epinephrine) each system is anatomically distinct and serves separate, but similar, functional roles within their fields of innervations [1].

Neurotransmitters include chemical categories of amino acids (aspartete, glutamate and glycine) and its derivatives (GABA) amines and neuropeptides. Other substances that may participate in central synaptic transmission include purines (such as adenosine and ATP), nitric oxide, and arachidonic acid derivatives [2].

Drugs acting on the CNS have five major functional classes as stimulation, tranquilization, sedation, hypnoses, narcotsis, and antidepressants where sedatives mainly relieve anxiety and produce relaxation without causing sleep [3]. While, drugs that primarily induced sleep are called hypnotics [4]. The CNS depressant, sedatives and hypnotics generally divided into two categories: barbiturates and nonbarbiturates [5].

Midazolam is one of the CNS depressants belongs to a class of medications called benzodiazepines and it is used before medical procedures or before anesthesia for surgery to cause drowsiness, relieve anxiety, and prevent any memory of event [6]. Midazolam induced side-effects which might be serious, such as agitation, restlessness, uncontrollable shaking of a part of the body, stiffening and jerking of the arms and legs, and aggression. This paradoxical excitement occurs in <1% of all patients receiving midazolam [7]. Pharmacologically, midazolam is used as an agent for the procedural sedation and analgesia (PSA). It takes up to 45 min for the patients to recover fully and be discharged, and adverse events such as respiratory depression are a known occurrence [8].

The aim of this experiment is to study the effect of midazolam on neurotransmitters (adrenaline, dopamine, histamine, serotonin and cholinesterase), electrophoretic pattern of serum protein and immunoglobulin (IgG, IgM, IgE and IgA) for a period of 3 months (12 weeks) of injection.

MATERIAL AND METHOD

Experimental animals

For the experimental investigation of these study 30 adult male albino rats of average body weight 150 -160 g were used. Rats were housed in separate metal cages for each group. Fresh and clean drinking water and food were supplied.

Diet

Composition of the experimental diet (g/kg diet) was according to the formula of [9]. It included fat (5%), carbohydrates (65%), proteins (20.3%), fiber (5%), salt mixture (3.7%) and vitamin mixture (1%).

Experimental design

Thirty male adult white albino rats were used in the present study and allotted as following: control group (10 rats) was to be injected with an equivalent volume of physiological saline and midazolam group (20 rats) was to be injected with the midazolam for 12 weeks.

Sedative dose for intravenous midazolam in human is 0.1 mg/Kg= 7 mg/Kg according to [10]. The dose was modified to rats according to [11]. Each ampoule of midazolam of 5 mg was dissolved 1 mL of physiological saline. For every 100 g B.W. of rats give 0.02 mL midazolam intravenously.

Biochemical analysis

Blood samples were to be withdrawn through 1, 2, 3, 4, 6, 8, 10 and 12 weeks from the median canthus by a hematocrit micro-capillary tube at seven minutes post injection [3] and poured into plastic two tubes (One tube contains EDTA and another without EDTA for each rat). All tubes are centrifuged and the clear plasma samples were used to the hormones level assay by using of enzyme immunoassay (EIA) kit where adrenaline and dopamine according to the method of [12]**,** histamine according to the method of [13]**.** Serum serotonin according to the method of [14]**,** acetylcholinesterase activity was determined colorimetrically according to [15]**,** Electrophoretic pattern of serum protein was determined according to [16], Quantificatation of IgG, IgA and IgM was determined according to the method of [17] and quantitative determination of IgE concentration in human serum by a microplate enzyme immunoassay was determined according to [18]**.**

Statistical analysis was done by [19].

RESULTS AND DISCUSSIONS

The present data illustrated in Table (1) revealed a non significant experimental changes in plasma (adrenaline, dopamine and histamine) and in serum serotonin in midazolam injected group. While serum cholinesterase activity was significantly increased ($P<0.05$) in midazolam injected group at 3^{rd} and 4^{th} week of experiment. The obtained data in Table (1) revealed a non significant decrease in the level of plasma adrenaline as compared to the control group. This data was in contrary with the results of [20] who recorded a decrease in plasma adrenaline through sedation which related to midazolam used for induction of anesthesia results in a transient depression of baroreflex function and a sustained decrease of sympathetic tone. Whereas our results were came in accordance with the results of [21] in cat and in human by [22] who found that; medetomidine, midazolam, and their combination did not significantly change the plasma epinephrine concentration

The data illustrated in Tables (1) revealed no significant increases in plasma dopamine as compared to the control group. The results agreed with [23] who performed his study in human as a ten volunteers administered loading doses of 0.07 mg/kg of midazolam followed by 0.7 mg/kg of ketamine and related his study to the development of a method for detoxification that decreases the stimulation of the sympathetic nervous system will prevent changes of catecholamine levels. Direct transition from heroin to oral naltrexone after deep sedation with midazolam in conjunction with naloxone, droperidol, ondansetron and clonidine treatment for 24 hours has no effect on the plasma level of epinephrine and dopamine [24].

The obtained data in Tables (1) recorded a non significant decrease in plasma histamine as compared to the control group. These results were disagreed with the study of [25] who proved that, histamine levels

decreased significantly after 5 min from midazolam injection. Also, our results similar to [26] and [27] who stated that midazolam is not a histamine releaser.

The obtained data in Tables (1) stated a non significant increase in the level of plasma cholinesterase as compared to the control group but at 3rd and 4th week exhibit a significant increase in cholinesterase activity. This result agreed with that of [28] who stated that midazolam did not inhibit the pseudocholinesterase (CHE) activity.

The obtained data in Tables (1) revealed no significant changes in the level of serum serotonin as compared to the control group. This results was came in accordance with the results of [29] who studied the influence of long-term immobilization stress on regional blood-brain barrier permeability, cerebral blood flow and serotonin level in conscious normotensive and sedated rats not induce any changes in serotonin level.

The data illustrated in Table (2) and shown in Figure (1) stated no significant alteration between control and midazolam groups in serum total protein, albumin, total globulin, α-globulin, β-globulin and γ-globulin. This result is confirmed by the study of [30].

The obtained data in (Tables 3) revealed no significant changes in the level of serum IgA as compared to the control group. IgA is the major immunoglobulin in seromucous secretion. It is increased in liver disease and increased or decreased in sinopulmonary infection and not affected in neural diseases [31].

The obtained data in (Tables 3) stated a non significant change in the level of serum IgE as compared to the control group. IgE increased in parasitic and allergic diseases as homocytotropic reaction [31]. Also, this data was confirmed by [26] who found that midazolam is not a histamine releaser. So since the level of histamine is decreased with midazolam then this should lead to a normal IgE which increases with high level of histamine.

The obtained data in (Tables 3) revealed no significant changes in the level of serum IgG as compared to the control group. [31] stated that IgG is increased in liver disease and chronic infection and reduced in B cell depression. Since the liver enzymes are normal and no infection occurred and midazolam had a dose-dependent inhibitory effect on mast cell chemotaxis and exocytosis as stated by [32]**,** thus there were no changes in IgG or IgM and since the dose used for midazolam was a small dose the results revealed no significant changes as compared to the control group.

The obtained data in (Tables 3) revealed no significant changes in the level of serum IgM as compared to the control group. IgM is increased in infection and reduced in B cell depression [3]. Since there is no recent infection the results revealed no significant changes as compared to the control group.

Table 1: Mean values of plasma adrenaline, histamine, dopamine and serum cholinesterase, serotonin in control and midazolam injected rats

	Control	Midazolam							
		1st week	2nd week	3rd week	4th week	6th week	8th week	10th week	12th week
Adrenaline (pg/ml)	111.48 ±3.75	106.49 ±3.15	105.48 ±5.31	108.4 ±5.81	109.09 ±4.15	109.81 ±5.99	108.70 ±7.84	109.12 ±1.98	111.38 ±2.88
Dopamine (pg/ml)	119.30 ±4.34	139.61 ±1.35	148.59 ±2.25	149.95 ±1.08	153.39 ±0.81	149.39 ±0.81	151.84 ±0.72	139.29 ±1.35	131.49 ±2.79
Histamine (ng/ml)	0.44 ±0.01	0.44 ±0.05	0.43 ±0.06	0.44 ±0.08	0.42 ±0.05	0.41 ±0.18	0.40 ±0.08	0.40 ±0.01	0.42 ±0.03
Cholinesterase (U/L)	5.78 ±0.12	5.78 ±0.18	9.75 ±0.54	11.66 ±0.27*	10.86 ±0.09*	6.70 ±0.18	6.82 ±0.45	8.90 ±0.09	7.70 ±0.18
Serotonin (ng/ml)	77.63 ±1.50	76.95 ±1.17	76.94 ± 1.18	76.96 ±1.17	76.96 ±1.17	76.86 ±1.17	76.76 ±1.17	76.76 ±1.17	76.86 ±1.17

* Indicate significant difference from control at (P<0.05).

Table 2: Mean values of serum total protein, albumin, total globulin, α-globulin, β-globulin and γ-globulin in control and midazolam injected rats

	Control	Midazolam							
		1st week	2nd week	3rd week	4th week	6th week	8th week	10th week	12th week
Total protein (g/dl)	6.91 ±0.71	7.05 ±0.72	7.19 ±0.74	7.32 ±0.75	7.46 ±0.77	7.53 ±0.77	7.60 ±0.78	7.67 ±0.79	7.74 ±0.80
Albumin (g/dl)	3.98 ±0.37	4.06 ±0.38	4.14 ±0.28	4.22 ±0.39	4.30 ±0.40	4.34 ±0.40	4.38 ±0.41	4.42 ±0.39	4.46 ±0.46
Total globulin (g/dl)	2.91 ±0.35	2.97 ±0.26	3.03 ±0.36	3.08 ±0.37	3.14 ±0.38	3.17 ±0.38	3.20 ±0.39	3.23 ±0.28	3.26 ±0.19
α-globulin (g/dl)	0.98 ±0.18	1.00 ±0.17	1.02 ±0.13	1.04 ±0.19	1.06 ±0.16	1.07 ±0.21	1.08 ±0.28	1.09 ±0.20	1.10 ±0.26
β-globulin (g/dl)	0.67 ±0.13	0.68 ±0.18	0.70 ±0.15	0.71 ±0.19	0.72 ±0.14	0.73 ±0.14	0.74 ±0.24	0.74 ±0.19	0.75 ±0.15
γ-globulin (g/dl)	1.26 ±0.21	1.29 ±0.31	1.31 ±0.22	1.34 ±0.28	1.36 ±0.23	1.37 ±0.23	1.39 ±0.33	1.40 ±0.28	1.41 ±0.29

* Indicate significant difference from control at (P<0.05).

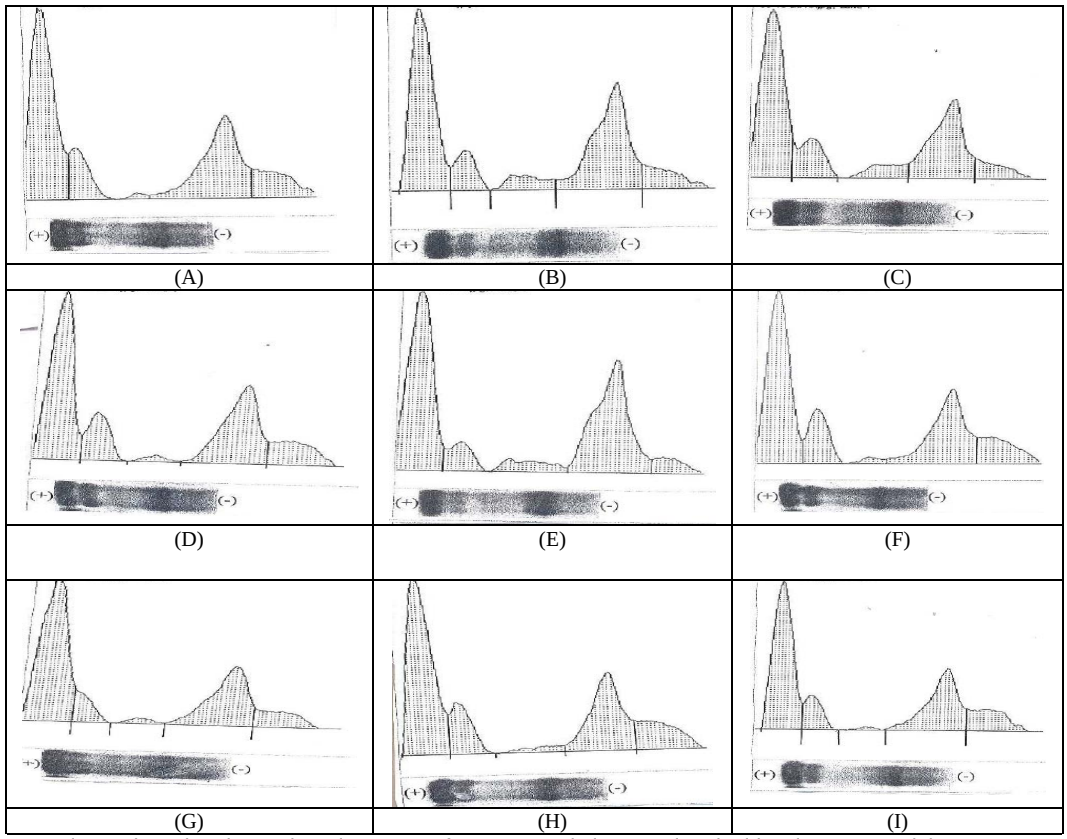

Fig. 1: show the electrophoretic pattern of serum protein in control and midazolam group: (A) Control, (B) midazolam group at 1st week, (C) midazolam group at 2nd week, (D) midazolam group at 3rd week, (E) midazolam group at 4th week, (F) midazolam group at 6th week, (G) midazolam group at 8th week, (H) midazolam group at 10th week, (I) midazolam group at 12th week.

Table 3: Mean values of Serum IgA, IgE, IgG and IgM in control and midazolam injected rats 4 weeks experiment

	Control	Midazolam							
		1st week	2nd week	3rd week	4th week	6th week	8th week	10th week	12th week
IgA (mg/dl)	33.08 ±1.29	29.35 ±0.54	30.11 ±1.08	29.84 ±1.17	28.68 ±1.35	30.74 ±0.99	30.69 ±0.79	30.01 ±0.81	29.23 ±1.44
IgE (IU/ml)	2.38 ±0.10	2.10 ±0.09	2.13 ±0.18	2.06 ±0.18	2.17 ±0.08	2.08 ±0.18	2.15 ±0.18	2.21 ±0.27	2.19 ±1.44
IgG (mg/dl)	535.37 ±5.73	493.69 ±5.85	488.97 ±4.77	507.62 ±4.47	485.28 ±4.95	488.41 ±4.05	509.86 ±5.15	511.03 ±5.13	525.23 ±4.41
IgM (mg/dl)	55.80 ±1.30	50.78 ±1.35	49.54 ±1.08	50.14 ±0.99	49.41 ±0.88	51.23 ±0.99	50.31 ±1.08	50.70 ±1.98	49.48 ±1.88

* Indicate significant difference from control at (P<0.05).

CONCLUSIONS

The conclusion from the present study, midazolam injected for 12 consecutive weeks has no significant changes in neurotransmitters (except actylecholinestrase at 3rd and 4th week were significantly increased), protein electrophoretic fractions and immunoglobulins.

REFERENCES

[1] Lewis, D. A.: Dopamine systems in the primate brain. In, Handbook of Chemical Neuroanatomy. Vol. 13. (Bloom, F. E., Bjorklund, A. and Hökfelt, T. eds.) Elsevier, Amsterdoun, 1997, pp. 263-375.

[2] Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Merighi, S.; Varani, K.; Borea, P.A. and Spalluto, G.: A(3) adenosine receptor ligands: history and perspectives. Med. Res. Rev., 2000, 20: 103-128.

[3] Gindi, R., R.: Biochemical Studies of Some Sedatives in rats, PhD thesis, Faculty of Veterinary Medicine (Moshtohor), Benha University, 2007.

[4] Moffett, D. F.; Moffett, S. B. and Schauf, C. L.: Human Physiology Foundations and Frontiers. Second edition, 1993, 249, 250.

[5] Ebadi, M.: Sedatives, Hypnotics and Alcohol. From: Core Concepts in Pharmacology. First edition. Lippincott-Raven, Philadelphia, New York, 1997, pp. 83.

[6] Weinbroum A. A.; Szold O.; Ogorek D. and Flaishon R.: The midazolam-induced paradox phenomenon is reversible by flumazenil. Epidemiology, patient characteristics and review of the literature. Eur J Anaesthesiol., 2001, 18:789–797.

[7] Luciano S. C.; Ana S. S.; Pilar E. R. and Manuel S. P.: Paradoxical reaction to midazolam reversed with flumazenil. J Emerg Trauma Shock; 2010, 3(3): 307.

[8] Rahman N. H. and Hashim A.: Is it safe to use propofol in the emergency department? A randomized controlled trial to compare propofol and midazolam, Int. J. Emerg. Med.; 2010, 3(2): 105–113.

[9] Kim, J., H., Hahm, D., H., Yang, D., C., Kim, J., H., Lee, H., J., Shim, I.: Effect of crude saponin of Korean red ginseng on high-fat diet-induced obesity in the rat. J Pharmacol Sci., 2005, 97:124-31.

[10] Richard, A.; Griffiths, M. and Scully, C.: Wide variation in patient's response to midazolam sedation for outpatient oral surgery. Oral Surg. Oral Med. Oral Pathol., 1993, 76(4): 408- 411.

[11] Paget, G., E. and Barnes, J., M.: Evaluation of Drug Activities, vol. I. Academic Press, 1964.

[12] Kopf, D.; Goretzki, P. E.; and Lehnert, H.: Clinical management of malignant adrenal tumors. J. Cancer Res. Clin. Oncol., 2001, 127: 143-155.

[13] Hermann, K.; Frank, G. and Ring, J.: Contamination of heparin by histamine: measurement and characterization by high-performance liquid chromatography and radioimmunoassay. Allergy, 1994, 49(7): 569–572.

[14] Attanasio, A.: Diurnal rhythms of N. acetylserotonin and serotonin. J. Pineal res., 1986, 3: 251-256.

[15] Deb Blawen, D. H.; Poppe, W.A. and Trischler, W.: Deterioration of cholinesterase activity. J. Clin. Chem. Biochem., 1983, 21:381-386.

[16] Alper, C., A.: "Plasma proteins" Measurments as a diagnostic aid. Neig. J. Med., 1974, (29), pp. 287 – 291.

[17] Whicher, J. T.; Warren, C. and Chambers, R. E.: Immunochemical assays for immunoglobulins. Ann. Clin. Biochem., 1984, 21: 78-91.

[18] Plebani, M.; Bernardi, D.; Basso, D.; Faggian, D. and Borghesan, F.: "Measurement of specific immunoglobulin E; intermethod comparison and standardization", Clin. Chem., 1998, 44, 9.

[19] SAS: Statistical Analysis System. Users Guide Statistics, SAS Institute Cary, North Carolina, 1996.
[20] Marty, J.; Gauzit, R.; Lefevre, P.; Couderc, E.; Farinotti, R.; Henzel, C. and Desmonts, J. M.: Effects of diazepam and midazolam on baroreflex control of heart rate and on sympathetic activity in humans. Anesth. Analg., 1986, 65(2): 113- 119.
[21] Kanda T. and Hikasa Y.: Effects of medetomidine and midazolam alone or in combination on the metabolic and neurohormonal responses in healthy cats. Can J Vet Res.; 2008, 72(4): 332–339.
[22] Kong K. L.; Willatts S. M; Prys-Roberts C.; Harvey J. T. and Gorman S.: Plasma catecholamine concentration during sedation in ventilated patients requiring intensive therapy. Intensive Care Med., 1990, 16:171–174.
[23] Morse, Z.; Sano, K. and Kanri, T.: Effects of a midazolam-ketamine admixture in human volunteers. Anesth. Prog., 2004, 51(3): 76- 79.
[24] Macedo, T. R.; Relvas, J.; Fontes Ribeiro, C. A.; Pacheco, F.; Morgadinho M. T.; Pinto, C. M.; Games, P. C.; Ventura, M.; Henriques, V.; Nunes, S. V.; Ruis, G. R.; Ramalheira., C.; Boto, I. and Vale, L. L.:Plasma catecholamines during an ultrarapid heroin detoxification Ann. N. Y. Acad. Sci., 2000, 914: 303- 310.
[25] Facon, A.; Divry, M. M.; Dessaint, J. P. and Scherpereel, P.: Evaluation of the histamine-liberating property of midazolam, Cah. Anesthesiol., 1988, 36(2): 111-113.
[26] Behne, M.; Janshon, G.; Lischke, V. and Asskali, F.: The plasma histamine level during anesthesia induction using midazolam. Anaesthesist., 1989, 38(7): 348-352.
[27] Kimura, K.; Adachi, M.; Kubo, K. and Ikemoto, Y.: Incidence of histamine release after the administration of midazolam-ketamine in allergic patients. Fukuoka Acta Medica, 1999, 90(12): 448-456.
[28] Rump, A. F.; Schierholz, J.; Biederbick, W.; Theisohn, M.; Diefenbach, C.; Abel M.; Borner, U; Buzello, W. and Klaus, W.: Pseudocholinesterase-activity reduction during cardio-pulmonary bypass: the role of dilutional processes and pharmacological agents. Gen. Pharmacol., 1999, 32(1): 65- 69.
[29] Sharma, H. S. and Dey, P. K.: Influence of long-term immobilization stress on regional blood-brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. J. Neurol. Sci., 1986, 72(1): 61-76.
[30] Walter-Sack, I., de Vries J. X., Rudi J. Conradi, R. , Kohlmeier, M. , Kohl, B. , and Weber E.: Lack of accumulation of midazolam in plasma and lipoproteinfractions during intravenous lipid infusions in patients on artificial respiration Eur J Clin Pharmaco, 1992, 42:71- 75.
[31] Hayes, A. W.: Principles and Methods of Toxicology third edition, 1994, p.1069.
[32] Fujimoto, T.; Nishiyama, T. and Hanaoka, K.: Inhibitory effects of intravenous anesthetics on mast cell function. Anesth. Analg., 2005, 101(4): 1054-1059.