Effect of toprimate on blood glucose level and lipid profile in normal and diabetic rabbits

Mohammed Abdul Muttalib Abdul Bari Msc . pharmacology Baghdad collage of pharmacy

Prof. Dr. Faruk H. Al-Jawad M. B.CH. B , Ph. D. pharmacology and therapeutics.

AL-Yarmouk university –Dept. Of pharmacology

Abstract

This study was performed on 72 normal and diabetic rabbits allocated to groups to evaluate the effect of anti-epileptic drug topiramate . Both Hyperglycemia and dyslipidemia, are concern with the disorders of chronic metabolic syndromes related to dysfunctional endocrine system , clinically referred to as diabetes mellitus (DM) . DM is defined and categorized on the basis of intrinsic and extrinsic causative factors. Physicians have used the antiepileptic topiramate to treat neuropathic pain in diabetic patient & bipolar disorder , This study was performed to evaluate the effects of anti-epileptic drugs (topiramate) when used in a dose dependent manner on induction of Cytchrom-P450 enzyme with diabetic disease on lipid profile in normal and diabetic rabbits . the obtained results in this study showed that topiramate in a dose of 50 mg/kg has no significant effect on blood cholesterol , LDL and HDL levels with the exception of triglyceride that show a significant changes in compare to both negative and positive control group and also in the same animal group between day 5 to day 20.

Keyword : topiramate , lipid profile , epilepsy , diabetic rabbits

تاثير التوبريميت على مستوى السكر بالدم والصورة الجانبيه للدهون في الارانب الاعتيادية والمصابة بالسكري

محمد عبد المطلب (ماجستير فارماكولوجي) ، الاستاذ د. فاروق حسن الجواد – فرع الفارماكولوجي / جامعه اليرموك

الخلاصه

لقد استعمل عقار التوبريميت المضاد للصرع لمعالجة الالم العصبي لمرضى داء السكري وكذلك اختلال ثنائي القطبي العصبي. انجزت الدراسه على اثنان وسبعون ارنبا اعتياديا او مصابا بالسكري بطريقه الاثاره وزعوا على مجموعات وذلك لغرض تقييم تاثير التوبريميت على احداث انزيمات سايتوكروم ٤٥٠ عندما استعمل العقار بجرع مختلفه وارتباط ذلك بمرض السكري والصورة الجانبيه للدهون في الارانب الاعتيادية والمصابه بالسكري. اظهرت النتائج ان جرعه ٥٠ ملغ/كغم من التوبريميت ليس لها من تاثير معتد على مستوى الكوليسترول بالدم ومستوى الكولسترول الواطئ والعالي الكثافه بالدم في حين حصلت متغيرات في الكلسيريدات الثلاثية بالدم في مقارنه مع مجموعتي الضبط السالبه والموجبه واخرى في نفس المجموعه من الارانب مابين اليوم الخامس واليوم العشرين.

الكلمه المفتاح: التوبريميت ، الصورة الجانبية للدهون ، الصرع ، الارانب المصابه بالسكري

Introduction

Both Hyperglycemia and dyslipidemia, are part of the other disorders of chronic metabolic syndromes related with a dysfunctional endocrine system clinically referred to as diabetes mellitus (DM). DM is heterogeneous group of disorder categorized on the basis of intrinsic and extrinsic causative factors (1), Although the etiology of DM is multifaceted, the occurrence of the disease worldwide is often linked to genetic/physiologic factors, inactive lifestyle, and obesity, poor dietary habits such as high ingestion of sugars and saturated fats in addition to low intake of polyunsaturated fatty acids (PUFAs) have been implicated to be major contributory factors toward the progression of the disease. (2) (3)

Forms of dyslipidemia in DM (Atherosclerosis-induced coronary heart disease) are major causes of increasing rate of fatalities among patients with DM. (4)

Topiramate is considered to be an antiepileptic drug (AED) with a wide range of anticonvulsant activity. It was licensed in the UK since 1995. It is found to have multiple mechanisms of action. It can inhibit L-type calcium channels of the high voltage activated currents so that controlling neuronal depolarization with subsequent anticonvulsant activity (5).

Also topiramate enhances GABA mediated chloride flux by increasing the opening and burst frequency of GABA-A receptor channels (6). Beside topiramate action on GABA-A receptors, it was found that it can selectively inhibit pre/postsynaptic GABA-B receptors in the interneurons, an action that eventually results in elevation of GABA release (7)

In children, topiramate is indicated for the treatment of Lennox-Gastaut syndrome, a disorder that causes seizures and developmental delay. It is also approved by Food and Drug Administration (FDA) for most frequently prescribed for the prevention of migraines. Physician have used topiramate to treat neutopathic pain in diabetic mellitus patient , bipolar disorder, although the available evidence does not support its use in any phase of bipolar disorder treatment. (8)(9)

AIM OF THE STUDY

This study was performed to evaluate the effects of anti-epileptic drugs (topiramate) –cytp-450 enzyme induction in a dose dependent manner – on lipid profile in normal and diabetic rabbits .

Materials and methods

Animals

Seventy two Local domestic healthy rabbits of both sexes ; purchased from center of technical institution (AL-Nahrien university) they housed one per cage which is provided with wire-mesh floor. They were kept in well controlled hygienic environment

drugs that used in the experiment- Toprimate tablet 50 mg tab.

Animals design.

Animals were allocated into two main groups , group A and B . According to induction of diabetes . Each group has been given same dose of toprimate orally (Topamax®) 50mg/kg , group A-received drugs but without any induction of diabetes) and the group B- received drugs with induction of diabetes.

Induction of Diabetes:

The rabbits were injected with alloxan monohydrate dissolved in sterile saline (0.9% NaCl) at a single dose of 150 mg/kg intraperitoneally. The baseline fasting blood glucose was determined before intraperitoneal administration of alloxan. After 6 h alloxan administration, 5% glucose solution was infused orally in feeding bottle for a day to overcome the early hypoglycemic phase as a result of acute massive pancreatic release of insulin. Hyperglycemia was confirmed by elevated serum glucose level, determined at 3rd day post-induction. Rabbits that became hyperglycemic (fasting blood glucose level around 200-250 mg/dl) and stable were include in the study .(10)

The rabbits were shifted to placement restraints (wooden holder) and the drugs administered orally by using a catheter .

Extraction of serum from blood.

After 12 hours fasting the blood of the rabbits was collected in plane tube. Then blood samples centrifuged at 3000 rpm for 10 min . after centrifugation and isolation of cellular fraction; the obtained plasma fraction was stored frozen at degree -4 C˚ until analysis performed.

Measurement of Serum Lipid Profile:

Serum total cholesterol was estimated according to (11) where a readymade kit is used for this purpose,

Serum triglyceride levels were determined according to the method of Fossati and Prencipe (12)

Serum HDL-C levels were estimated according to the method of Burstein (13) through which LDL-C and VLDL-C was determined calorimetrically by measurement of light absorbance at 505 nm, using a readymade kit for this purpose.

Statistical analysis:

- The results were expressed as mean ±SD.
- Student t-test for paired and unpaired samples and ANOVA test was used to examine the degree of significance, P-value less than 0.05 considered significant.

Results

Effect of topiramate on blood cholesterol level in normal and diabetic rabbits

days	Normal rabbits	normal rabbits trated with topiramate	Diabetic rabbits	diabetic rabbits treated with topiramate
5	67.66±4.5	68.00 ±3.6	183.00 ± 3.6	183.66±3.51
20	68.66±4.0	72.33± 2.08	200.00±5.0	196.00±1.0
total	67.91±3.5	69.33 ± 3.5	188.33±7.9	189.08±5.6
p*.value		NS		NS
p.value**		NS		P<0.01
p *a*		2.06%		0.3%
p *a a*	1.47%	6.3%	9.2%	6.7%

Table 1 means of cholesterol level from day 5 to day 20 of the study for negative , positive control group and drugs treated groups p* represent p.value in comparing the results between treated group with the control . P** represent p.value in comparing the results from day 5 to day 20 on the same group. P ª Represent percentage of change between total mean of each treated group with control group p ª ª Represent percentage of change between day 5 to day 20 of same group

The mean of negative control group arrange between 67.66 **±** 4.5 mg/dl on day 5 to 68.6 ± 4 mg/dl On day 20 of the study .

Group treated with topiramate showed a non-significant difference in compare to negative control group . Total mean value of cholesterol increased by 2.06 % in topiramate treated group . Topiramate treated group started by 68 ±3.6 mg/dl on day 5 to 72.3 ± 2.08 mg/dl on day 20 and cholesterol mean value increased by 6.3 %

The mean of positive control group arranged between 183 **±** 3.6 mg/dl on day 5 to 200 ± 5 mg/dl On day 20 of the study . Group treated with topiramate showed a non - significant difference in compare to positive control group(0.3% increase) .
Topiramate treated group started by183.66 ±3.51 mg/dl on day 5 to 196 ± 1 mg/dl on day 20 and cholesterol mean value increase by 6.7 % (p<0.01) .

Effect of topiramate on blood triglyceride level in normal and diabetic rabbits

days	**Normal rabbits**	**Normal rabbits treated with topiramat**	**Diabetic rabbit**	**Diabetic rabbit treated with topiramat .**
5.00	**85.0±5**	**84.00±4**	**133.66±4.04**	**136.66±4.7**
20.00	**86.00±3**	**92.33±1.5**	**153.66±3.2**	**175.33±3.2**
total	**85.25±2.9**	**88.15±3.8**	**144.33±8.4**	**156.50±15.25**
P*.value		**P<0.05**		**P<0.001**
P.value**		**P<0.05**		**P<0.01**
p ^{a}		**3.4%**		**8%**
p $^{a\,a}$	**1.17%**	**9.91%**	**8%**	**14.5%**

Table 2 means of triglyceride level from day 5 to day 20 of the study for negative , positive control group and drugs treated groups p* represent p.value in comparing the results between treated group with the control . P** represent p.value in comparing the results on from day 5 to result of day 20 on the same group. P ^{a} Represent percentage of change between total mean of each treated group with control group p $^{a\,a}$ Represent percentage of change between day 5 to day 20 of same group

The mean of negative control group arrange between 85 **±** 5 mg/dl on day 5 to 86 ± 3 mg/dl On day 20 of the study . group treated with topiramate show a significant difference in compare to negative control group (p<0.05) Total mean value of triglyceride increased by 3.4 %. Topiramate treated group started by84 ± 4 mg/dl on day 5 to 92.3 ± 1.5 mg/dl on day 20 with and mean value increased by 9.91 % (p<0.05) .

The mean of positive control group arranged between 133.6 ± 4.04 mg/dl on day 5 to 153.6 ± 3.2 mg/dl On day 20 of the study . topiramate treated group showed a very high significant differences in compare to positive control group (8% increase in compare to positive control), Topiramate treated group started by 136.66 ± 4.72 mg/dl on day 5 to 175.3 ± 3.2 mg/dl on day 20 with and triglyceride mean value increased by 14.5 % (p<0.01)

Effect of topiramate on blood LDL level in normal and diabetic rabbits .

days	Normal rabbits	Normal rabbits treated with topiramate	Diabetic rabbits	Diabetic rabbits treated with topiramate
5.00	46.00± 3	44.0±2	100.33± 4.5	95.0± 5
20.00	45.00± 1	47.0± 5	120.0± 5	113.90± 0.85
total	45.5± 2.7	45.75± 3.3	111.41± 8.7	104.37± 7.9
P*.value		NS		P<0.001
p**.value		NS		P<0.05
P ^{a}.*value*		0.4%		- 6.3%
P $^{a\,a}$.*value*	-2%	6.38%	19.6%	19.8 %

Table3 means of LDL level from day 5 to day 20 of the study for negative , positive control group and drugs treated groups , p* represent p.value in comparing the results between treated group with the control . P** represent p.value in comparing the results on from day 5 to day 20 on the same group. P ^{a} Represent percentage of change between total mean of each treated group with control group p $^{a\,a}$ Represent percentage of change between day 5 to day 20 of same group

The mean of negative control group arrange between 46 ± 3 mg/dl on day 5 to 45 ± 1 mg/dl On day 20 of the study .

Groups that treated with topiramate showed un significant change in compare to negative control group , 0.4 % , Topiramate treated group started by 44 ± 2 mg/dl on day 5 to 47 ± 5 mg/dl on day 20 with and LDL mean value increased by 6.38 % with no significant difference in compare between day 5 and day 20 .

The mean of positive control group arrange between 100.3 ± 4.5 mg/dl on day 5 to 120 ± 5 mg/dl On day 20 of the study . group treated with topiramate showed a very high significant difference in compare to control group ($p<0.001$) with 6.3 % decrease in compare to control group .

Topiramate treated group started by 95 ± 5 mg/dl on day 5 to 113.9 ± 0.85 mg/dl on day 20 with and LDL mean value increase by 19.8 % with a high significant difference in compare between day 5 and day 20 .

Effect of, topiramate on blood HDL level in normal and diabetic rabbits .

days	Normal rabbits	Normal rabbits treated with topiramate	Diabetic rabbits	Diabetic rabbits treated with topiramate
5.00	**32.6± 1.52**	**33.00± 2**	**27.3 ± 4.04**	**26.00± 0.5**
20.00	**33.66± 0.57**	**34.0± 1**	**20 ± 5**	**24.66± 0.4**
total	**33.41± 0.99**	**33.0± 1.87**	**24.33± 4.0**	**25.4± 3.05**
p*. value		**NS**		**NS**
p. value**		**NS**		**P<0.01**
P ^{a}.*value*		**-1.1%**		**4.5 %**
P $^{a\,a}$.*value*	**3%**	**3%**	**-26.7%**	**-5.3%**

Table4 means of HDL level from day 5 to day 20 of the study for negative , positive control group and drugs treated groups , p* represent p.value in comparing the results between treated group with the control . P** represent p.value in comparing the results on from day 5 to result of day 20 on the same group. P ^{a} Represent percentage of change between total mean of each treated group with control group p $^{a\,a}$ Represent percentage of change between day 5 to day 20 of same group

The mean of negative control group arrange between 32.6 ± 1.5 mg/dl on day 5 to 33.6 ± 0.5 mg/dl On day 20 of the study .

Groups that treated with topiramate showed no significant change in compare to negative control group -1.1 % decrease respectively in compare to negative control group .Topiramate treated group started by33± 2 mg/dl on day 5 to 34 ± 1 mg/dl on day 20 with and HDL mean value increase by 3 % with no significant difference in compare between day 5 and day 20 .
The mean of positive control group arranged between 27.3 ± 4.04 mg/dl on day 5 to 20 ± 5 mg/dl On day 20 of the study .

Group treated with topiramate showed non-significant change in compare to control group with 4.5% changes .
Topiramate treated group started by 26 ± 0.5 mg/dl on day 5 to 24 ± 0.404 mg/dl on day 20 with and HDL mean value decreased by 5.3 % with a high significant change in compare between day 5 and day 20 ($p<0.01$).

Discussion

The anti-epileptics drugs had been shown to have multiple molecular targets , in addition to their effects on central nervous system they take another effects on hormones and enzymes that play crucial roles in the synthesis or regulation of lipids concentration . Animal data showed that a particular enzyme, CYP51A1, catalyzes the conversion of lanosterol into cholesterol intermediates .When these intermediates build up through inhibition of the enzyme, they in turn inhibit the rate-limiting step of cholesterol synthesis, 3-hydroxy-3- methylglutaryl-coenzyme A reductase (HMG-CoA reductase), and slow the synthesis of cholesterol. It follows that induction of CYP51A1 should therefore increase cholesterol production through metabolism of these intermediates and reduced feedback inhibition.(14) increase of the activity of the cholesterol synthesis rate limiting (enzyme HMG-CoA reductase)

may also lead to increase in LDL-C levels (15) on the other hand Cytp450 enzymes play a role in the synthesize of apolipoprotein A (the main HDL lipoprotein particle) leading to increase the concentration of HDL-C level in the blood (15) (16)

There is a strong evidence that drugs assumed to have an inhibitory or stimulatory effect on cyp-450 enzymes had an effects on lipid profile , like ketoconazole which is the potent CYP450 inhibitor has been shown to reduce cholesterol production in studied animals , and this effect is documented clinically by the finding that patients taking valproate, (CYP450- inhibiting properties) have lower cholesterol levels than controls (17).

These findings suggest that serum lipids parallel to the activity of the CYP-450 enzyme system, so that treatment with a CYP450 inducing agent increases lipids, and upon withdrawal of the drug lipids return to the patients baseline. (18)

Topiramate found to induce at least some CYP450 enzymes at high doses (19), but there is no evidence of any CYP450 induction when it is used at low to moderate doses(20).

Lipid levels in TPM-treated neurological patients have been examined in few previous investigations (21). These studies found no change in TC after TPM treatment; one found a small decline in HDL-C only (22).

These data, along with those of previous studies, propose that TPM at low doses may not affect the activity of the CYP450 system, at least with regard to the enzymes responsible for cholesterol synthesis. This is clinically applicable , since TPM has been found to induce the metabolism of oral contraceptive hormones at high doses, but not at low doses (19 , 20)

It is likely that the CYP450-inducing effects of TPM are both enzyme- and concentration-specific, happening only for certain enzymes, and even then only at certain serum levels . (23)

This is compatible to the results obtained in the present study

Conclusion

Topiramate in a dose of 50 mg/kg has no significant effect on blood cholesterol , LDL and HDL levels with the exception of triglyceride level that show a significant differences in compare to both negative and positive control group and also in the same group between day 5 to day 20.

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