

Effects of Dose Amount for Antiepileptic Drugs on Lipid Profile of Male Rats (Rattus Norvegicus).

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Abstract:

The present study aimed to estimate the changes in lipid profile depend on dosage amount of two AEDs ,Phenytoin (PHT) and Carbamazepine (CBZ) , and make a comparison between them in rats. A 30 healthy male Wistar rats (150-200 g) (subdivide to five groups) first group was control , the second and third groups received (50 mg/kg and 100 mg/kg) of Phenytoin respectively, fourth and fifth groups received (25 mg/kg and 50 mg/kg) of Carbamazepine respectively. Serum total cholesterol (TC), triglycerides (TGs) and High-density lipoprotein (HDL) levels were measured after 20 days period of treating, Low-density lipoprotein (LDL) and Very low-density lipoprotein (VLDL) was calculated also. Our data revealed that, the administration of PHT (50 mg/kg) and CBZ (25 mg/kg) didn't affect serum lipid profile significantly compared to control group in male rats except the levels of VLDL in CBZ (25 mg/kg) group which increased significantly (p-value <0.05) compared to control group, on the other hand we found that the administration of double dose (PHT 100 mg/kg and CBZ 50 mg/kg) in group3 and group5 respectively caused a significant increase in TC, TGs, LDL and VLVL in both groups compare to control group. According to our results we conclude that the doubling the dose of CBZ and PHT cause an increase in cholesterol, TGs, LDL and VLDL and switching from CBZ and PHT to another drugs or decrease the dosage could contribute to avoid effects on the lipid metabolism profile of patients with chronic epilepsy.

Keywords: AEDs, CBZ ,PHT, lipid profile, Cholesterol, TGs, HDL, LDL, VLDL.

تأثير كمية الجرعة للأدوية المضادة للصرع في مستوى الدهون لذكور الجرذان المختبرية.

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الخلاصة:

تهدف الدراسة الحالية إلى تقدير التغيرات في مستويات الكوليسترول الكلي والبروتينات الدهنية اعتماداً على كمية الجرعة لاثنتين من أدوية الصرع، الفينيتوين (PHT) و الكاربامازيبين (CBZ)، وإجراء مقارنة بينهما. (30) من ذكور الجرذان المختبرية (150-200غم) (قسمت إلى خمس مجموعات) وكانت المجموعة الأولى مجموعة السيطرة، جرعت المجموعتين الثانية والثالثة بـ (50 ملغ / كغ و 100 ملغ / كغ) من الفينيتوين على التوالي، المجموعة الرابعة والخامسة تم تجريعها بـ (25 ملغ / كغ و 50 ملغ / كغ) من كاربامازيبين على التوالي. و بعد فترة 20 يوماً من التجريع اليومي تم قياس الكوليسترول الكلي (TC)، والكليسيريدات الثلاثية (TGs) والبروتينات الدهنية عالية الكثافة (HDL)، أما البروتينات الدهنية منخفضة الكثافة (LDL) و

البروتينات الدهنية ذات الكثافة الواطئة جدا (VLDL) تم حسابها من خلال معادلة خاصة. أظهرت نتائج الدراسة أن الجرعة (50 ملغ \ كغم) فينتوين و (25 ملغ \ كغم) كاربامازيبين لم يؤثر على مستوى الدهون في الدم بشكل ملحوظ مقارنة مع مجموعة السيطرة في ذكور الجرذان باستثناء مستويات VLDL في المجموعة التي عوملت بـ (25 ملغ \ كغم) كاربامازيبين ارتفعت معنويًا عند مستوى معنوية ($p\text{-value} < 0.05$) مقارنة بمجموعة السيطرة، من ناحية أخرى وجدنا أن إعطاء جرعة مضاعفة من العلاجين تسبب في زيادة في مستويات TC، TGs، LDL و VLDL في كلا المجموعتين مقارنة بمجموعة عند مستوى معنوية ($p\text{-value} < 0.05$). وفقًا لنتائج الدراسة الحالية، نستنتج أن مضاعفة جرعة الفينيتوين و الكاربامازيبين سبب زيادة في الكوليسترول، TGs، LDL و VLDL، وإن التحول من هذين العلاجين إلى أدوية أخرى أو تقليل الجرعة يمكن أن يساعد في تقليل آثارهما الجانبية.

Introduction:

Epilepsy is defined as a chronic neurological disorder characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness (Sridharan, 2002). AEDs are used to treat seizures disorders and epilepsy. Phenytoin (PHT) has been widely and effectively used in the treatment of epilepsy and arrhythmias (Tamura *et al.*, 2000). Epilepsy needs long-term and in some cases lifelong treatment. Thus, prolonged antiepileptic treatment could have some undesirable effects, and lot of reports have already shown that AEDs affects lipoprotein and cholesterol levels in serum (Franzoni *et al.*, 1992; Isojarvi *et al.*, 1993). While, high concentration levels of serum lipoproteins and lipids are associated with an high probability risk of coronary disease (Castelli *et al.*, 1977). There has been an availability of medications to treat epilepsy for over 100 years. For example, the anticonvulsant ability of phenytoin was discovered in 1938, and since then, it has become an established antiepileptic drug therapy. In Europe phenytoin and valproic acid was approved for use during the 1960s and in the USA in 1978 (McNamara, 1996). Both drugs are classified as first-generation AEDs (Perucca *et al.*, 2007). Carbamazepine in 1953 was introduced as a AED and became one of the first antiepileptic drugs developed by pharmaceutical company (Geigy). CBZ is considered one of the first drugs in treatment of secondarily generalized and partial seizures (Alberto *et al.*, 2009). Long term AEDs therapy have a significant effects on TC, HDL, LDL and TGs (Rai *et al.*, 2010). Hyperlipidemia considered as one of the major risk factors for atherosclerosis, the first sign of which

can be detected during childhood, AEDs may affect the serum lipid profile of children in such a way that maybe facilitate the development of atherosclerosis (Garcia & Moodie, 2004). Total cholesterol and low-density lipoprotein have been identified as risk factors for atherosclerosis along with changes in anatomy of great vessels and its function (Poli *et al.*, 1988). Although many new AEDs have been introduced over the past 15 years, the consensus first choice for focal seizures has traditionally been (CBZ) (Karciski *et al.*, 2005).

This work aimed to assess the effect of dose amount on serum lipid profile (TC, TGs, HDL, VLDL and LDL) of two AEDs (PHT and CBZ) and investigate the difference in effects between PHT and CBZ.

Materials and methods:

A (150-200 g) twelve week old healthy male Wistar rats (*Rattus Norvegicus*) were used in this study. All animals were housed under controlled conditions of 23 ± 2 °C temperature and 10-14 h of light-dark cycles respectively, it housed in cages throughout the study and had free access to animal chow and water *ad libitum*, 30 rats were randomly segregated to 5 groups (6 animals each) (Group1: Control, Group2: PHT (50 mg/kg), Group3: PHT (100 mg/kg), Group4: CBZ (25 mg/kg), Group5: CBZ (50 mg/kg)), body weights were recorded weekly to calculate corresponding dose. PHT and CBZ was obtained from local pharmacies. Dose and way of giving was based on earlier studies. Drugs powder dissolved in DMSO (0.2 ml for each animal) and administered orally daily, control group received only DMSO. After last exposure to the drugs (period of 20 day) animals were sacrificed. Serum total cholesterol (TC) was measured spectrally using a test reagent kit according to the method of

Richmond (Richmond, 1973). Serum Triglycerides were measured spectrally using a test reagent kit according to the method of (Fossati & Prencipe, 1982). Serum HDL was measured colorimetric ally using a test reagent kit according to the method of (Lopes-virella *et al.*, 1977). Serum LDL was calculated according to the formula: LDL-cholesterol (mg/dl) =Total Cholesterol (Triglycerides/5+ HDL-c), Serum VLDL was calculated according to the formula: [VLDL (mg/dl) = Triglycerides/5] (Friedwald *et al.*, 1972). We used the Student's t-test and One-Way Analysis of Variance (ANOVA) in order to detect statistically significant differences between the groups. All tests were considered to be significant at a p-value <0.05.

Results:

The results of table (1) illustrated non-significant changes (p-value <0.05) in serum levels of TC, TGs, VLDL and LDL in PHT (50 mg/kg) group compared with control group and the other groups. Phenytoin (100 mg/kg) cause a significant increase (p <0.05) in serum total cholesterol levels, serum TGs levels, serum VLDL levels and serum LDL levels, while there was non-significant difference in HDL compared with control group, also the TGs and VLDL levels increased significantly in group (3) compared with groups (2,4 and 5). Serum TC, TGs, HDL and LDL concentrations didn't change significantly in CBZ (25 mg/kg) group compared with control group, only VLDL level of CBZ (25 mg/kg) group increased significantly compared with controls. Levels of serum TC, TGs, ,VLDL and LDL showed a significant increases by administration of CBZ (50 mg/kg) as compare to control group, also LDL level increased significantly compared to groups (2,3 and 4). The results of the different variables in the studied groups and controls are shown in the table and the figure below:

Table (1):Effect of phenytoin (PHT) and Carbamazepine (CBZ) on lipid profile of male rats.

Groups	TC Mean ± S.D.	TGs Mean ± S.D.	HDL Mean ± S.D.	VLDL Mean ± S.D.	LDL Mean ± S.D.
Group1	93.29 ^b ±31.12	31.37 ^{cd} ±5.12	39.79 ^{ab} ±10.98	6.27 ^d ±1.02	47.23 ^c ±10.97
Group2	94.66 ^b ±12.91	34.18 ^c ±4.66	39.18 ^{ab} ±10.54	6.84 ^{cd} ±0.93	48.64 ^{bc} ±4.87
Group3	125.35 ^a ±20.27	54.98 ^a ±9.14	46.59 ^a ±3.89	10.99 ^a ±2.22	67.76 ^b ±6.72
Group4	102.4 ^b ±2.26	28.23 ^d ±11.02	31.01 ^b ±8.77	7.63 ^c ±2.20	63.74 ^{bc} ±16.15
Group5	130.65 ^a ±54.67	47.72 ^b ±4.91	30.81 ^b ±12.69	9.54 ^b ±0.98	90.29 ^a ±6.45
LSD	22.83	5.68	8.4	1.13	16.39

-The different letters refers to a significant difference at p-value <0.05.
-The same letters refers to non-significant difference at p-value <0.05.

Discussion:

Results showed that administration of phenytoin (100 mg/kg) significantly increase in serum total cholesterol, TGs, , VLDL and LDL in comparison with control. These results in agreement with previous studies (Itemobong *et al.*, 2007) in rats, and data are in line with the results of (Luoma *et al.*, 1979) in healthy volunteers and epileptic patients treated with phenytoin. In many studies phenytoin increased total cholesterol and/or LDL concentrations were found, and elevated HDL is also frequently reported (Dewan *et al.*, 2008). Palkonen *et al.*, (1975) also reported an increase in TC and TGs in epileptics on long term treatment with Phenytoin. Our findings were contradictory with Sudhir *et al.*, (2016) who found that PHT and CBZ did not reveal any significant difference on lipid profile. Belcastro *et al.*, (2010) found that PHT is the potent inducer of the cytochrome P450 system, which exerts strong effects on serum lipid profile. It follows that this enzyme inducing drug may substantially increase the risk of atherosclerosis.

The enzyme-inducing AEDs phenytoin (PHT), carbamazepine (CBZ), increase the activity of the hepatic cytochrome P450 system, which is involved in synthesis of serum cholesterol. Animal data show that a particular enzyme, CYP51A1, catalyzes the conversion of lanosterol into cholesterol intermediates (Gibbons,

2002). Current study found that the administration of CBZ (50 mg/kg) caused a significant increases in serum TC, TGs, VLDL and LDL in comparison with control these results in agreement with (Bramswig *et al.*, 2002) Who found that levels of serum TC, TGs and LDL were significantly increased in patients treated with CBZ.

Also our results is consistent with Bramswig *et al.*, (2003) who reported that during treatment with carbamazepine a significant increases in total cholesterol, LDL, and triglycerides, but not in HDL were observed. Many investigations have suggested that treatment with CBZ is associated with increases in TC and various lipid fractions, including LDL, HDL and serum triglycerides (Sonmez *et al.*, 2006). Our results could be explained on the basis of hepatic enzyme inducing effect of antiepileptic drugs including CBZ which is metabolized mainly in the hepatic P-450 system. These enzymes also catalyze the biotransformation of cholesterol and other lipids. Thus, chronic use of CBZ may compete with lipids in the utilization of these enzymes, leading to decreased biotransformation of these lipids and causing increase of their levels in the blood (Mahmoudian *et al.*, 2005). Also Hosny *et al.*, (2013) found that lipid abnormalities encountered in patients with epilepsy during CBZ therapy cannot be explained by hepatic enzyme induction as the only or the main cause but subclinical hypothyroidism caused by CBZ, appear to have an important role in the pathogenesis of elevated serum lipids in these patients. Changes in serum lipids caused by antiepileptic treatment have often been discussed controversially. The risk of atherosclerosis has been the main point of discussion (Zeitlhofer *et al.*, 1993). High serum cholesterol, LDL, and TGs levels are considered risk factors for development of atherosclerosis and coronary heart disease, whereas HDL is acknowledged protective against these diseases (Austin, 1991). In our study the serum levels of HDL did not affected in all groups compared with control group and it in agreement with study of Sozuer *et al.*, (1997), who found that CBZ-treated children with epilepsy failed to demonstrate any change in HDL during treatment. In contrast of current study Svalheim *et al.*, (2010) showed that treatment with PHT is significantly associated with

increased blood levels of HDL. On the other hand Yilmaz *et al.*, (2001) found that CBZ medication is associated with an increase serum HDL concentrations.

Conclusions:

According to our results we conclude that the doubling the dose of CBZ and PHT cause an increase in cholesterol, TGs, LDL and VLDL and switching from CBZ and PHT to another drugs could generate a positive effect on the lipid metabolism profile of patients with chronic epilepsy.

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