Journal University of Kerbala , Vol. 15 No.1 Scientific . 2017

The effect of digoxin on the enzymatic activity of glutamate oxaloacetate transaminase.

تأثير الدجوكسين على الفعالية الانزيمية للانزيم الناقل لمجموعة الامين (الكلوتاميت – الاوكزالواسيتات).

Ali Mohammed Abbed
Department of chemistry, College of Science, Al-Mustansiriya University
Mob: 07702982679

Abstract

The investigation of glutamate oxaloacetate transaminase (GOT) activity to search the effect of digoxin drug on the enzyme activity of GOT, which this work carried out on sera (22) sample healthy individual in vitro. The result demonstrate that increased activity and non significance variation ($P< 0.07$), when comparison of enzymatic activity with drug with that of enzymatic activity without drug for the same each sample .The activation percentage equal to (251%). Also experimented the volume effect from stock (0.5mg/2ml), which the volume was give maximum activation equal to (40 µℓ).

الخلاصة:-

دراسة كيموحيوية للتحري عن الفعالية الانزيمية للانزيم الناقل لمجموعة الامين من اجل بحث تاثير المادة الدوائية (الدجوكسين) في هذا الانزيم. اذ اجري هذا العمل على مصول (22) شخصا اصحاء خارج الجسم. واوضحت النتائج وجود زيادة غير معنوية في الفعالية الانزيمية عند احتمالية (0.07) بوجود الدواء، وذلك من خلال المقارنة بين الفعالية الانزيمية مع وجود الدواء و الفعالية الانزيمية مع عدم وجود الدواء و كانت نسبة التنشيط 251%. وكذلك تم تجريب تاثير الحجم في الفعالية الانزيمية حيث اعطى اعلى فعالية انزيمية عند الحجم 40 مايكروليتر من التركيز الاصلي (0.5 ملغم/2مل).

Introduction

Aspartate aminotransferase (EC 2.6.1.1., GOT), which belonged to the transport enzymes group. AST formerly is called glutamate oxaloacetate transaminase (GOT). This enzyme is widely distributed among plant, animal and microorganism which found in these livings mainly in the heart cells and in less level in liver, red blood cells, muscle tissue and other organs such as pancreas and kidney[1,2,3], and exist as two isoenzyme form, the mitochondrial form (M-GOT) and the cytosol form (S-GOT). It is from porcine heart has a molecular weight in the range (91000-94000)[4]**.** GOT level in healthy serum are low, but the level are significantly elevated in number of clinical cases such as a cute and chronic hepatitis, obstruction jaundice, carcinoma of liver, myocardial infraction and muscular dystrophy, therefore the determination of serum level GOT has great clinical and diagnostic significance[5]. GOT in fact underlie role very important in nitrogen metabolism. Such as some of compound containing carbonyl group substituted amino acids, dicarboxylic acids and pyridoxal phosphate analogues react with the enzyme[6,7], where GOT activity required pyridoxal-5-phosphate the active form of vitamin B_6 as a coenzyme for this enzymatic activity[8]**.** This enzyme can be used in diagnosis the many heart and liver disease or damaged AST is released in to the blood stream lead to elevation in enzyme level. The normal concentration is equal (5-40) U/L[9,10,11]**.**

Digoxin is large distributed used medicine to treat heart failure and atrial fibrillation[12,13].

Digoxin is extracted from plants of the genus Digitalis[14]. This drug belonged to the group of drugs (cardiotonic steroids), the steroid nucleus of this drug with a 5- or 6-member lactone ring at C17[15]**.** This drug has a narrow therapeutic range, with toxic effects at (Digoxin ≥ 2ng/ml) of plasma[16]**.** The cardiotonic action of digoxin is increase the force of cardiac contraction also all Na^+pump[17].

The digoxin anti - hypertensionogenic effect was confirmed in salt - sensitive model of hypertension[18].

Kinetic evidence of digoxin is uptake transporter in kidney[19]. While other searcher proved active sodium-dependant digoxin uptake in a human embryonic kidney cell line[20]**.** This drug can be able to interact with other drugs such as carvedilol, diltiazem and lead to changes in the digoxin plasma than the clinical threshold value of 1.2 [21]**,** and the digoxin at 10 nM concentrations, reversibly constrict rat mesenteric small arteries with myogenic tone[22]**.**

Experimental

Materials and methods

Drug

Digoxin as pharmaceutical ampoule (0.5mg/2ml), which manufactured in Athens Greece by ANFARM HELLAS S. A.

Biochemical study

Collection samples

The blood samples were collected (22) sample from healthy male individual. Five milliliters of venous blood samples and allowed to clot for 30 min at room temperature., centrifuged at 3000xg for 5 minute, and then sera were collected and stored at -15°C until to use. The serum was utilized for the estimation of biochemical parameters SGOT activities with and without digoxin drug.

Assay of GOT activity

Colorimetric kinetic determination of SGOT assays were performed and the method applied according to the recommendations of the expert panel of the IFCC (International Federation of Clinical Chemistry), which was depend on the following reactions by using BioSystem kit[23,24].

$$\text{Aspartate} + \text{2-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate} + \text{Glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{Malate} + NAD^+$$

A 100µℓ of serum sample was mixed with 1000µℓ of buffer (Tris pH = 7.9). The mixture was incubated at 37°C for 5 minutes, then was added 250 µℓ of substrate (2-Oxoglutarate) and the absorbance was read at 365 nm through time of reaction 1 minute and 3minute. Then the enzyme activity was calculated, by applying the following equation:

Enzymatic Activity (U/L) = (ΔA⁄3min) × 3235

The influence of drug on the enzymatic activity was studied by applying the same procedure but differ in addition of certain amount (50 µℓ) of digoxin in the cuvette after read of absorbance through time of reaction 1 minute, and after addition complete the time reaction and read at 3 minute.

The activation percentage was calculated by comparing the activity with and without the digoxin drug under the same conditions, according to the equation:-

$$\%\text{Activation} = \frac{\text{Activity in the presence of activator}}{\text{Activation without presence of activator}} \times 100 - 100$$

Result

Serum GOT level in vitro was determined colorimatically by using BioSystemkit, which the table showed comparison between the S- GOT activity in the natural state without drug and the S-GOT activity with drug which is found in the cuvette of enzymatic reaction at fixed drug volume (50 µℓ) of (0.5mg/2ml) as stock solution. The result in this table refer to the S-GOT activity in the healthy individual non significance variation ($p< 0.07$) when compared between the activity with drug with that of activity without of digoxin for the same individual, and this effect indicated to modulation of enzymatic activity by digoxin. Which the results reveal to increase the activity with drug, that is mean this drug has been activation effect on this enzyme as well as modulator. Where the activation percentage value with drug increased in limit 251% in comparison with that of enzymatic activity without drug, which are illustrated in the following table.

Table (1): GOT enzymatic activity in the sera of healthy individual with and without drug.

Groups	N (Numbers)	Age/year	Range of Activity (U/L)	Mean of Activity (U/L)	SD	% activation	P-Value
With drug	22	20-32	20.8-61.4	41.5	13.05	251	P< 0.07
Without drug	22	20-32	5.4-17.3	11.8	3.7		

When experiment the volume of drug effect on the enzymatic activity the result indicated that there are many volume (20, 40, 60, 80, 100, 150, 200, and 300) µℓ from stock (0.5mg/2ml) as an ampoule manifest difference effect on the enzymatic activity. Which the volume of drug equal to 40 µℓ lead to increased the activity where is reach a maximum, while the volume over 40µℓ refer to drop effect in activity until 300µℓ less activity. This result demonstrated in the following figure.

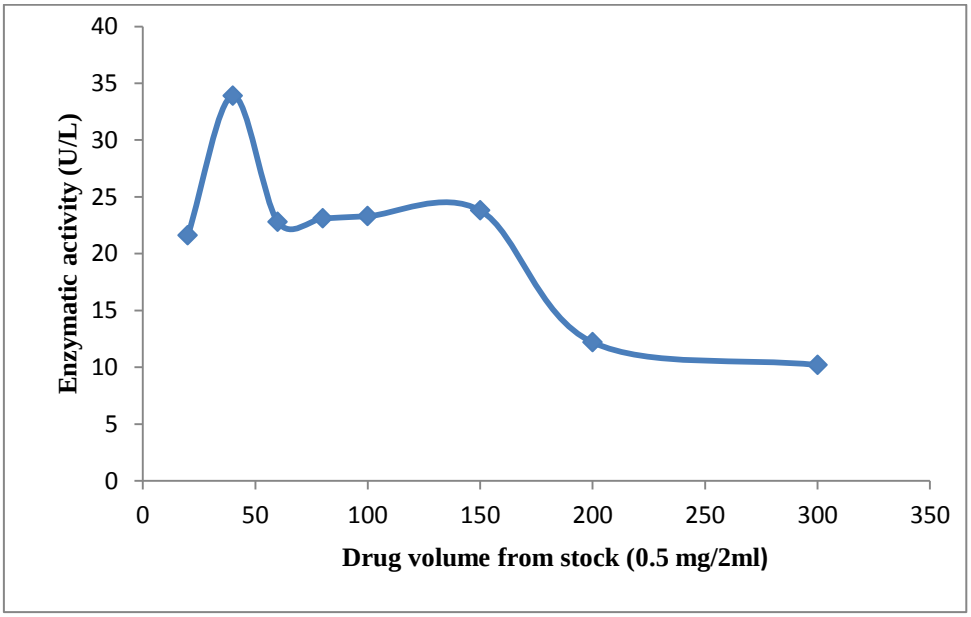

Figure (1): Effect of drug scaling from the stock (0.5mg/2ml) on the S-GOT enzymatic activity.

The activation was non-competitive in that velocity will be increased by increasing the volume activator; this found in small range volume was used (20-40) µℓ of drug. And the activation is becoming competitive that observed drop in velocity at range (50-300) µℓ[(25)].

Discussion

Researchers were referred by their studies that the change of GOT enzymatic activity, this change lead to many causes such as heart and liver diseases, drug, metals and toxins. These effectives lead to increased or decreased of S-GOT concentration level in serum, or GOT metabolism occurs[26,27,28]. While the observed result in this work indicate that the increasing the GOT enzymatic activity by adding the digoxin drug., which related to biochemical effects in GOT molecule and binding site of enzyme. The amino acid side chain that make up the active site are molded into the precise position that enable the enzyme to perform it catalytic function[29]. Enzyme are static structures not rigid and flexible structure, instead they have complex internal dynamic motion that is movements of the enzymes structures such as individual amino acids residues[30,31]. Some of drugs possible bind to enzyme with some residue amino acid of the enzyme and induced the enzyme to bind with substrate and finaly increased in catalytic function. description of the size of an active site and the number of properties of sub-sites, such as details of the binding interaction, as well as proteolytic enzymes are targets for some drugs[32].

Pyridoxal phosphate act as a coenzyme in all transamination reactions[33]. The aldehyde group of pyridoxal phosphate form a shiff base with ε-amino group of aspecific lysine group of the amino transminase enzyme[34]. Pyridoxalphosphate used by amino transferases which it is act upon dosamine and perosamine sugar. In these reaction pyridoxal phosphate react with glutamate, which transfer its α-amino group to pyridoxal phosphate to making pyroxamine phosphate, then pyroxamine transfer this nitrogen to the sugar and formation amino sugar[35]. The fact of our results for increasing of enzymatic activityin assay of S-GOT by using the commercial kit procedure with adding the digoxin, which that the digoxin structure contains a suitable chemical structure as well as glucose bind with basic ring structure of digoxin to form aglycoside drug[36]. Therefore the digoxin act as an intermediate compound via its sugar to accept amino group and finaly accelerate of enzymatic transamination of amino group from the α-amino group of substrate glutamate to sugar group of the digoxin that is regard as an activator of enzyme.

Conclusion

The result of the current study, it can be concluded by consideration is that the assumption of the observed mean value enzyme velocity with activation by activator as well as digoxin. Therefore in order to investigate the action of the activator in any case it is desirable to have more information that can be obtained from measurement of velocity with varying amount of added the activator. We can be concluded that the activation of enzyme by this drug increased in the enzymatic activity *invivo* when the administration of drug for patient with heart failure disease which the enzyme found in heart muscle.

References

1. Sampson E.J., Whitner V.S., Burtis C.A., McKneaily S.S., Fast D.M., Bayse D.D. An interlaboratory evaluationof the IFCCmethod for aspartate aminotransferase with use of purified enzyme materials. Clin. Chem. (1980). 26., p: 1156-1164.
2. Jacobs D.S. Laboratory Test Handbook. 4th ed. Cleveland, OH: Lexi-Comp Inc.; 1996.
3. Purcell G.V., Behenna D.B., Walsh P.R. Alanin aminotransferase and aspartate aminotransferase measurements with two automated analyzers, SMAC and the ABA-100, compared. Clin. Chem. (1979). 25., p: 780-782.
4- Banks B. E. C. & Vernon C. A. Transamination. Part I. The isolation of the apoenzyme of glutamic-aspartic transaminase from pig heart muscle. J. Chem. Soc. (Lond). (1961). P: 1698-1705.
5- Screiber G. Eckstein M. Oeser A & Holzer H. The concentration of aspartate aminotransferase from brewers yeast. Biochem. Z. (1964). 340., p: 13-20.
6- E. Braunstein. "The enzymes". Vol IX (Ed3rd), Academic press Inc., New York, N. Y., 1973; P: 379.
7- V. L. Florentiev, V. I. Ivanov and M. Y. Karpeisky, "Method in Enzymology" Vol XVIII A, Academic Press Inc., New York, N. Y., 1970, p: 567.
8- Percudani R. I., Peracchi A. A genomic overview of pyridoxal-phosphate- dependent enzymes. EMBO Rep. (2003). 4.,9., p: 850-4.
9- Hafkenscheid J. C. M., Dijt C. C. M. Determination of serum aminotransferases: activation by pyridoxal-5-phosphate in relation to substrate concentration. Clin. Chem. (1979). 25., p: 55-59.
10- Sampson E. J., Whitner V. S., Burtis C. A., McKneaily S. S., Fast D. M., Bayse D. D., An interlaboratory evaluation of the IFCC method for aspartate aminotransferase with use of purified enzyme material. Clin. Chem. (1980). 26., p: 1156-1164.
11- Jacobs D. S. Laboratory Test Handbook. 4th ed. OH: Lexi-Comp Inc.; 1996.
12- The Digitalis Investigation Group. The effect of digoxin on mortality and morbidity in patients with heart failure. N Engl J Med. (1997). 336., p: 525–533.
13-Wyse A. L., DiMarco J. P, Domanski M. J., Rosenberg Y., Schron E. B., Kellen J. C., Greene H. L., Mickel M.C., Dalquist J. E. Atrial Fibrillation Follow-up Investigation of Rhythm Management (AFFIRM) Investigators a comparison of rate control and rhythm control in patients with atrial fibrillation. N Engl J Med. (2002). 347., p: 1825–1833.
14- Manunta P[1], Hamilton J, Rogowski A.C., Hamilton B.P., Hamlyn J.M. Chronic hypertension induced by ouabain but not digoxin in the rat: antihypertensive effect of digoxin and digitoxin. Hypertens Res. (2000). 23., Suppl., p: S77–85.
15- Hollman A. Digoxin comes from Digitalis lanata. British Medical Journal. (1996). 312., 7035., p: 912.
16- Müller F., Fromm M.F. Transporter-mediated drug-drug interactions. Pharmacogenomics. (2011). 12., p: 1017–1037.
17- Blanco G, Mercer RW. Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity in function. Am J Physiol Renal Physiol. (1998). 275., p: F633–F650.
18- Huang B.S., Kudlac M, Kumarathasan R, Leenen F.H. Digoxin prevents ouabain and high salt intake-induced hypertension in rats with sinoaortic denervation. Hypertension. (1999). 34., p: 733–738.
19- Acharya P. O., Connor M.P., Polli J.W., Ayrton A, Ellens H, Bentz J. Kinetic identification of membrane transporters that assist P-glycoprotein-mediated transport of digoxin and loperamide through a confluent monolayer of MDCKII-hMDR1 cells. Drug Metab Dispos. (2008). 36., p: 452–460.
20- Taub K, Sane R.S., Watson C.A., Chen L, Ellens H, Hirakawa B, Reyner E.L., Jani M, Lee CA. Digoxin is not a substrate for organic anion-transporting polypeptide transporters OATP1A2, OATP1B1, OATP1B3, and OATP2B1 but is a substrate for a sodium-dependent transporter expressed in HEK293 cells. Drug Metab Dispos. (2011). 39., p: 2093–210

21- Harma E, etal. Application of Receiver Operating Characteristic Analysis to Refine the Prediction of Potential Digoxin Drug Interactions. Drug MetabDispos. (2013). 41., 7., p: 1367-1374.
22- Hong S, Eiji K, John M. H and Mordecai P. B. Ouabain-digoxin antagonism in rat arteries and neurons. J Physiol. (2014). 592., Pt5., p: 941-969.
23- Gella F. J *et al* . A simple procedure for routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. Clin Chim Acta. (1985). 153., p: 241-247.
24- Tietz Textbook of clinical chemistry and molecular diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
25- Malacon Dixon & Edwin C. W. Enzymes. 3rd edition C Longman Group.; 1979, p: 384.
26- Sawao M; Toyokazu N; Noriko Katayama and Hiroto N. New Aspartate Aminotransferase Inhibitor (Gostatin) produced by streptomyces sumanensis nov. sp., NK-23. Agric. Biol. Chem. (1981). 45., 4., p: 1039-1041.
27- Savita K; Zachary B and Cameron R. Inducible Glutamate Oxaloacetate Transaminase as a therapeutic target against Ischemic stroke. Antioxid Redox Signal. (2015). 22.,2., p: 175-186.
28- Salma A R; Amer H A; Shaemaa H A and Abdulrahman K A. The effect of Gold and Silver nanoparticles on transaminase enzymes activities. Int. J. Chem. Res. (2011). 1., 4., p: 1-11.
29- Vasella A, Davies GJ, Bohm M. Glycosidase mechanisms. Current Opinion in Chemical Biology. (2002). 6., 5., p: 619-29.
30- Fields P. A. Protein function at thermal extremes: balancing stability and flexibility. Comp. Biochem. Physiol. A Mol. Integr. Physiol. (2001). 129., p: 417-431.
31- Ramanathan A, Savol A, Burger V, Chennubhotla CS and agarwal PK. Protein conformational populations and functionally relevant substates. Acc. Chem. Res. (2014). 47.,1., p: 149-56.
32- Schechter I. Mapping of the active site of protease in the 1960_s and rational design of inhibitors/drugs in the 1990_s. Current Protein and peptide Science. (2005). 6., p: 501-512.
33- David D, Poulson R and Avramovic O. Vitamin B6: pyridoxal phosphate coenzyme and cofactors. Wiley Interscience, New York. 1986; Volume 1, Part B.
34- Toney, M. D. Reaction specifity in pyridoxal enzymes. Archives of biochemistry and biophysics. (2005). 433., p: 279-287.
35- Samuel G and Reeves P. Biosynthesis of O-antigens: genes and pathways involved in nucleotide sugar precursor synthesis and O-antigen assembly. Carbohydrate research. (2003). 338., p: 2503-2519.
36- Hollman A. Digoxin comes from Digitalis Lanata. British Medical Journal. (1996). 312., 7035., p: 912.