The Influence of some Anti-pyretic and Anti-inflammatory Drugs on the Activity of Lactate Dehydrogenase ; Kinetic Study.

Dr. Perry H. Saifullah (Ph.D. Biochemistry)
Chemistry Department, College of Science for Women, University of Baghdad.

Abstract

Lactate dehydrogenase (LDH) is an enzyme EC (1. 1.1.27) that functions in anaerobic glucose metabolism and glucose synthesis [(1).] LDH is present in a wide variety of organisms including plants and animals [(2)]. LDH has known effectors like lactate , pyruvate, NAD^+, NADH, pH, buffers composition, ionic strength[(4)], fructose 1,6 bisphosphate, divalent cations such as Mn^{2+} or Co^{2+} [(5)]. Proline and trimethylamine oxid (2μ) also affect the activity of LDH[(6)]. Drugs like antipyretic and anti-inflammatory were reported to affect lactate dehyydrogenase activity [(7,8)].

In this study we study the effect of some antipyretic drugs (aspegic, paracetamol, diclofenac) and anti-inflammatory drugs(hydrocortisone) on the activity of LDH and the result reveled that aspegic and paracetamol increased the activity of LDH for 2-folds,diclofenac for 1-fold and hydrocortisone for 1.8-fold, and only the actiavation in the presence of paracetamol was concentration dependant.

The measurement of kinetic parameter reveled that LDH do not obeys Mechelis-Menten equation. Aspegic and paracetamol increased the km values at approximant low conc. of pyruvate and decreases the km values for approximant 10- fold at high conc. of pyruvate , while diclofenac increases the km values at all conc. of pyruvate .On the other hand hydrocortisone at as a moderate activator for LDH. This difference in the effect of these drugs on the activity of LDH could be due to the difference in the polarity of the drugs.

Key word: lactate dehydrogenase, LDH, aspegic, paracetamol, diclofenac, hydrocortisone ,inhibitor ,activator .

Introduction

Lactate dehydrogenase (LDH) is an enzyme EC (1. 1.1.27) that functions in anaerobic glucose metabolism and glucose synthesis [(1)]. LDH is present in a wide variety of organisms including plants and animals [(2).] Human beings have two identical isozymic polypeptide chains with molecule mass of 30-35 kD for this enzyme: the H isozyme, highly expressed in heart and M isozyme found in skeletal muscle. The functional enzyme is tetrameric, and many different combinations of the two subunits are possible, H_4, H_3M, H_2M_2, HM_3, and M_4.[(1)] .

Lactate dehydrogenas from some bacteria are allosteric enzymes with sigmoidal kinetics for pyruvate (homotropic activation), unlike non-allosteric vertebrate LDHs[(3)]. LDH catalyses the conversion of pyruvare and lactate with concomitant interconversion of NADH and NAD^+.At high concentration of lactate the enzyme exhibits feedback inhibition and the rate of conversion to pyruvate is decreased[(1)].

LDH has known effectors like lactate , pyruvate, NAD^+, NADH, pH, buffers composition, ionic strength[(4)], fructose 1,6 bisphosphate, divalent cations such as Mn^{2+} or Co^{2+}[(5)]. Proline and trimethylamine oxid (2µ) also affect the activity of LDH[(6)].

Drugs like antipyretic and anti-inflammatory were reported to affect lactate dehyydrogenase activity [(7,8)].It was reported that diclofenac increased blood lactate dehydrogenase [(9)], and acetaminophen caused a slight increase in cellular release of LDH[(10)].

The best of our knowledge , however, there are no report of a case in:

1- THE effect of different concentrations of analgesic and anti-inflammatory drugs on the activity of LDH in sera of healthy pre-menopausal women.

2- Discussion these effects in the term of extrapolation of enzymatic kinetic data.

Material and Methods

Material:

LDH kit were provided by Biocon Diagnostik (Germany)

Drugs: drugs were provided from different sources

Aspegic (injectable 0.5IM .IV/ 5 ml water) from Laboratories Synthelablol / Synth labo Group (France)

Hayamol (Paracetamol) (injection 375 mg/ 5ml water) from IBn Hayyan Pharmaceuticals. R.Faysal & Co. (Syria)

Olfen-100 injection(Diclofenac sodium 100/2 ml water) from Merckel-GmbH-Blaubeuren-Weiler (Germany).

Hydrocortison injection (100mg/ 2ml water) from Hemoform ,(Yugoslavia).

Subjects:

Thirty healthy pre-menopausal female subjects with age ranging (20-40 year) were included in this study. They were not complaining from any illness or using any drugs.

Blood Sampling:

Blood sample (5 ml.s.) were collected from each subject by vein puncture, centrifuged at 3000 rpm(1500xg) for 5 min after allowing the blood to clot at room temperature. The sera were liquated and frozen at -20C^0 until the assay day, although all the samples used in this study almost collected freshly. (Note: serum enzyme LDH is stable for 6 weeks at -20C^0).

Methods

LDH Assay:

LDH activity in the direction of keto acid reduction by monitoring the oxidation of NADH at 340nm at 30 C^0. The standard reaction mixture 0.2M NADH, 1.6mM pyruvate in 80mM Trise buffer pH7.5, the final volume of the reaction mixture was 1.040 ml.s.[(11)].

Calculation:

Absorption decrease were calculated per minutes (ΔA/min) and multiplied with the corresponding factor[(11)]:

340 ΔA/min x 4127 = Activity of Lactate Dehydrogenase IU/L

Influence of the Drugs on the Enzyme Activity:

The experiments were performed at fixed concentration of pyruvate and NADH 0.0246mM; .0.2 mM respectively, and a rang of drugs concentrations:

Aspegic (3.15 , 6.36, 9.38 , 12 , 15mM)

Paracetamol (4.78 , 9,52 , 14 , 19 , 23 mM)

Dichlofenac (4.23 , 8.41 , 13 , 17 , 21 mM)

Hydrcortisol (3.30 , 5.58 , 9.82 , 13, 16 mM)

The final volume of the reaction mixture in each case (with drug) was 1.040 ml.s. and the initial absorbance was read immediately at 340nm and again after exactly 1 ,2, 3 min

Calculation:

Calculations equation were done as mentioned in LDH assay.

Determination of Kinetic Parameters (*Km* and *V max)*of LDH

- **In the Absence of Drugs**

The Km and V max of LDH for pyruvate in the sera of pre-menopausal healthy females wrer determined in the presence of increasing pyruvate concentrations (0.018 , 0 .0246 , 0.025 , 0.026 , 0.0265 , 0.0271 mM) .(Note: 0.0246 mM was the concentration present in the assay to determine maximal activity) and increasing amount of NADH (0.067 , 0.068 , 0.070 , 0.073 , and 0.075 mM). the initial rate of the reaction were measured by monitoring the decreasing in A340 nm associated with NADH oxidation.

- **In the Presence of Fixed Concentration of each Drugs**

[Apegic 12mM , Paracetamol 23mM , Diclofenac 17mM , Hydrocortisol 13mM.]

The Km and V max of LDH for pyruvate in the sera of pre-menopausal healthy females were determined with increasing pyruvate concentrations (0.018 , 0 .0246 , 0.025 , 0.026 , 0.0265 , 0.0271 mM) .(Note: 0.0246 mM was the concentration present in the assay to determine maximal activity) and increasing amount of NADH (0.067 , 0.068 , 0.070 , 0.073 , and 0.075 mM), and a fixed concentration of each drug (Aspegic 12mM , Paracetamol 23mM , Diclofenac 17mM , Hydrocortisol 9.82mM).

The initial rate of the reaction was measured by the following the decreasing in A340 nm associated with NADH oxidation.

Result and Discussion

LDH Assay :

Lactate dehydrogenase activity for 30 healthy pre-menopausal females were measured at fixed concentration of pyruvate and NADH and the mean value of LDH activity was (46.60 ±13.70 (SD)).This result s a good agreement with that of other authors who have reported that normal values for total LDH activity can be up to approximately 200 IU/L but is usually found with 45-90 IU/L[12].

Influence of Drugs on LDH activity:

The effect of four types of analgesic and anti-inflammatory drugs with different concentrations on the activity of LDH in the sera of healthy females was examined and the results are shown in (Fig.1-4).

As shown in (Fig 1) , aspegic (12mM) increases the activity of LDH (49.52 IU/L) for 2-fold, and decreases the activity (8.254 IU/L) for approximately 3-folds at concentration (9.38mM) as compared to control (23.7 IU/L) . The result of increment is in agree with [13] who reported that after the subject injection with acetyl salicylate, the lactate dehydrogenase activity in both urine and cells was higher than normal[13]. The author didn't find references that reported the decrement in LDH activity in the presence of aspegic. It's clear from Fig.1 the effect of the drugs on enzyme activity is concentration independent.

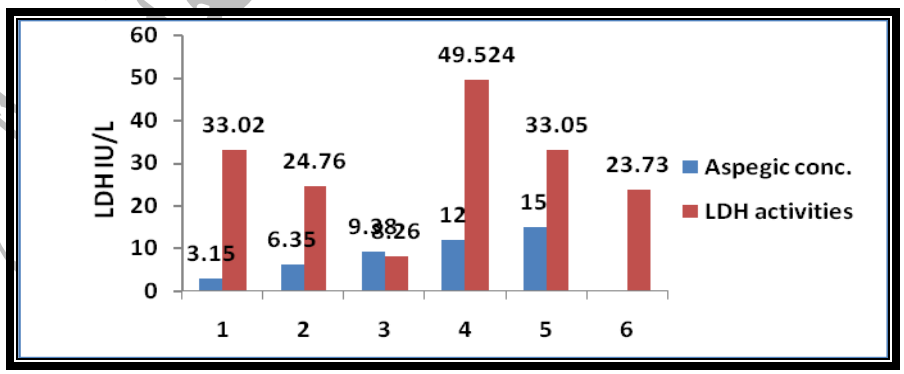


Fig1. Shows the effect of different conc. of Aspegic on LDH activity

In the presence of Paracetamol as shown in (Fig. 2) the activity of the sera LDH shows maximum increment (49.52 IU/L) for 2-fold at (23mM) of Paracetamol, and

lowest decrement in the activity (10.32 IU/L) for 1.8 fold at (4.78 mM) as compared to control (23.73 IU/L). The increment results are in agreement with Peter *et al* [14] who reported that the increment in the sera LDH activity could reach 833IU/L for 8 mg of Paracetamol daily. Milam and others also reported a slight increase in the cellular release of lactate dehydrogense at (3 mM) Acetaminophen[15] , but an induction in LDH –leakage was reported by other in low concentrations of Paracetamol (1mM) [16,17] .the data in (fig .2) shows that the effect of paracetamol en LDH activity is concentration dependent.

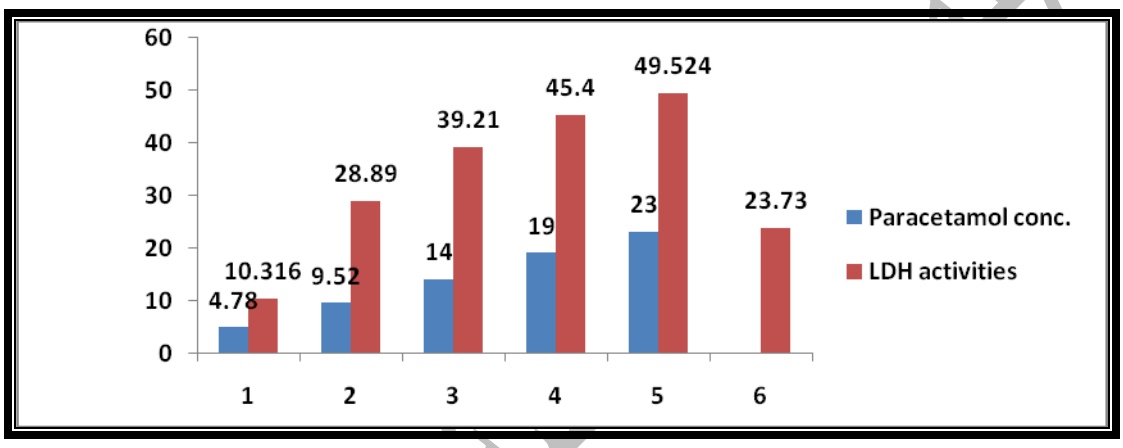


Fig -2 Shows the effect of different conc. Of Paracetamol on LDH activity

Diclofenac also shows different effect on LDH activity within the range of conc. (4.23- 21m M) as shown in (Fig.3) . Maximum increment in the activity of LDH activity (30.95 IU/L) for 1.3 fold was shown at conc.(17 mM) and a maximum decrement (6.21 IU/L) about 4-fold at conc.(4.23 mM) of diclofenac as compared to control. These data revealed that diclofenac tend to be an inhibitor to LDH at low concentrations and a modulate activator in high concentrations. This results are in agreement with a Japanese Physician who reported an increment in blood lactate dehydrogenase for a Diclofenac dosage :250 mg/d [9]. Rossoni et al.[18] reported that the increment of LDH activity in the precense of diclofenac sodium could be concentration dependant, however the activity is reduced in low concentration.[18].

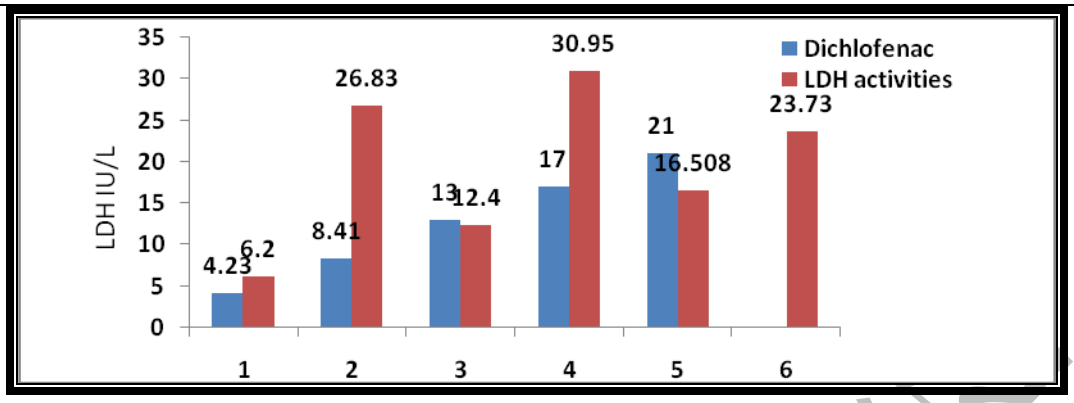


Fig -3. Shows the effect of different conc. Of Diclofenac on LDH activity

Finally, Fig.4 shows the effect of different concentrations of Hydrocortison on LDH activity . As seen there are a slight increment in LDH activity (43 IU/L) for 1.8 fold at conc. (9.82 mM) of hydrocorticol and a decrement in the activity (16.51 IU/L) for 1.4 fold at conc,(16 mM) of the drug as compared to control (23.73IU/L). This results are in agreement with Kusen 's who reported that the addition of hydrocortisone to the medium does not affect significantly the LDH activity [19]. While Lawrence et al. reported that Cortisol injection and handling stress increased LDH activities [20].

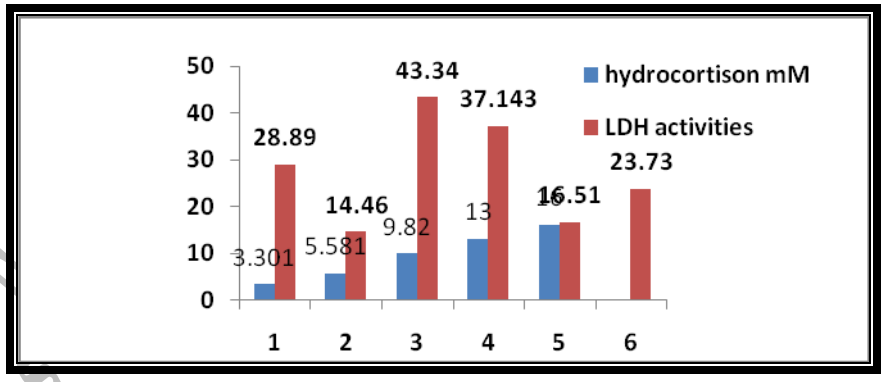
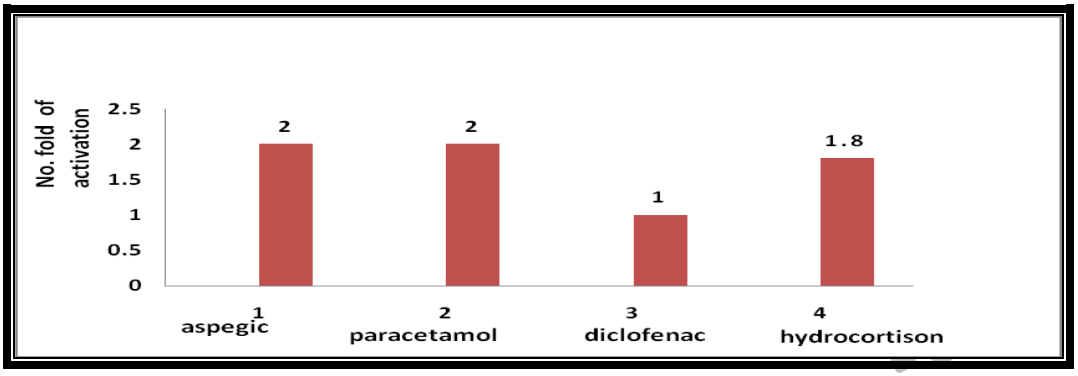


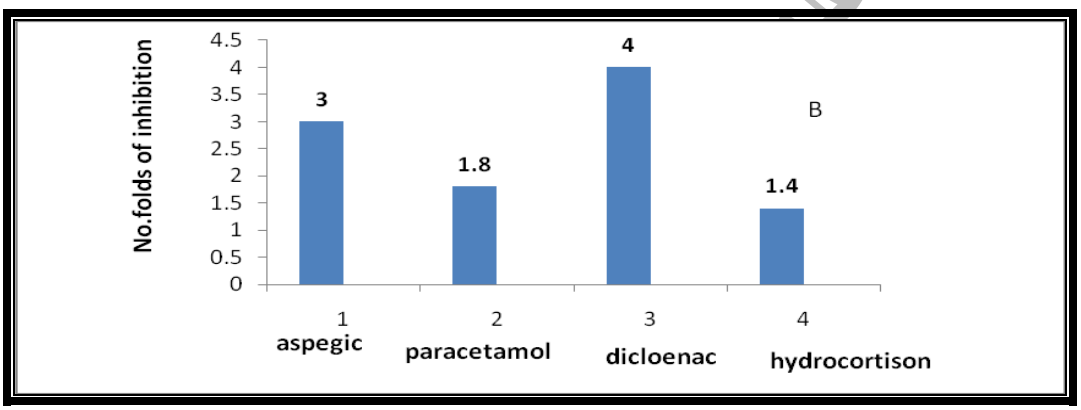
Fig -4 Shows the effect of different conc. Of Hydrocortison on LDH activity

As a comparison between drug effect on the activation or the inhibition of LDH (Fig.5- A,B) shows that Aspegic and paracetamol activate LDH in 2-fold (49.52), while diclofenac has the lowest ability to activate LDH (1.3 fold).Diclofenac seems to have the

higher ability to inhibite LDH activity (4-fold) , while hydrocortisone has the lower ability to inhibit LDH activity (1.4 fold).



A



B

Figure (5) A: shows the no. of folds of activation for the four drugs
B: shows the no. of folds of inhibition for the four drugs

Effect of pyruvate concentration on LDH activity

(Determination of Km and Vmax of LDH activity : in the abence and presence of drugs)

As mentioned previously lactate dehydrogenase obeys Mechelis-Menten kinetics (eq-1), initially a plot of reaction velocity (v) as a function of substrate conc (pyruvate)[1] [S] (fig-6).

$$v = \frac{Vmax\,[S]}{Km + [S]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

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As shown in (Fig-6)LDH activity is activated at (0.025mM) of the pyruvate and inhibited by higher conc.of the substrate. (Figure -7 A-D) shows the LDH activity in the presence of different conc. of pyruvate and a fixed conc. of each drug .

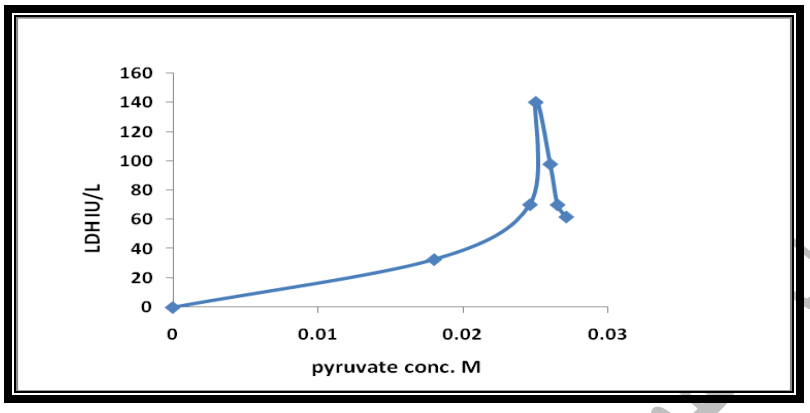
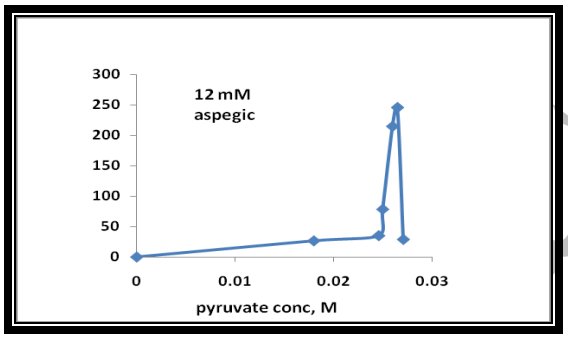
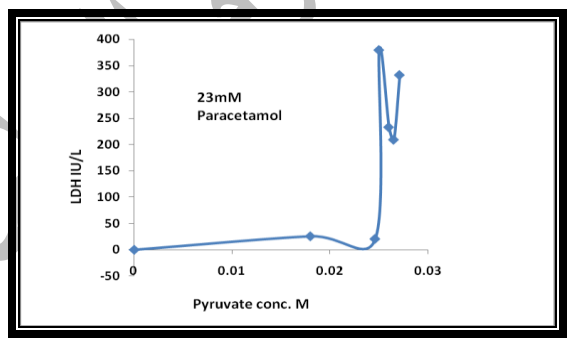


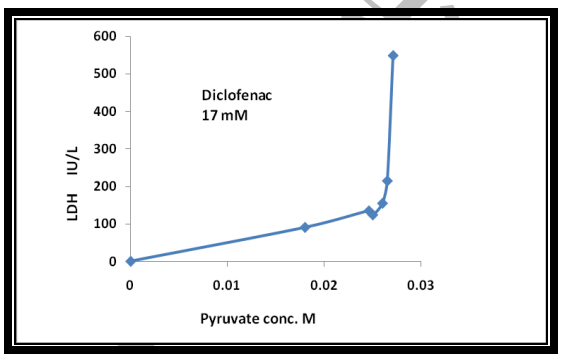
Figure (6) LDH activity in the presence of different conc. of pyruvate (high conc.)



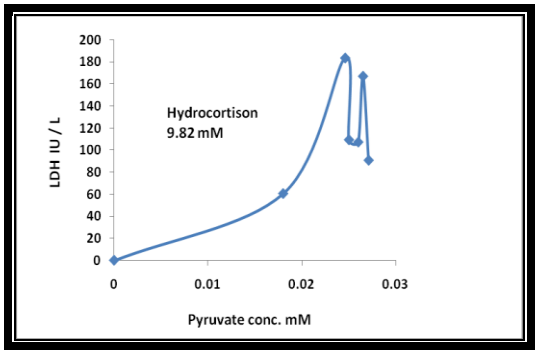
A



B



C



D

Figure (7) LDH activity in the presence of different conc. of pyruvate (high conc.) and in the presence of fixed conc. of : A: Aspegic(12mM); B:Paracetamol(23mM); C: Diclofenac (17mM); D: Hydrocortison(9.82mM).

Table -1 shows the Km and Vmax values in the absence of drugs and in the presence of a fixed conc. of each (the conc. that gave maximum activity) with increasing conc. of pyruvate and NADH , the activities of LDH were measured as the amount of pyruvate converted to lactate at 340nm. As shown in table (1) LDH do not obeys Mechelis-Menten kinetics because it has different Km at each[S][21].

Table (1) Kinetic parameter of LDH in the absence and presence of the four drugs Aspegic ; Paracetamol ; Diclofenac ; Hydrocortisone

[S]pyruvate mM	**0.018**	**0.0246**	**0.025**	**0.026**	**0.0265**	**0.0271**	**V_{max} IU/L**
Km (mM) Control	0.059	0.0114		0.0112	0.027	0.0342	140.318 0.025mM pyruvate
Km (mM) Aspegic(12mM)	0.148	0.148	0.053	0.0037		0.024	245.56 0.0265mM pyruvate
Km (mM) Paracetamol (23mM)	0.146	0.43		0.0164	0.022	0.0039	379.684 0.025mM pyruvate
Km (mM) Diclofenac (17mM)	0.091	0.076	0.086	0.066	0.0422		548.9 0.0271mM pyruvate
Km (mM) Hydrocortisone (13mM)	0.037		0.015	0.018	0.0026	0.028	183.65 0.0246mM pyruvate
f_{ES} =v/Vmax Control	0.2333	0.5008	1.00	0.700	0.500	0.4412	

The results for LDH activity in the absence of drugs are in agreement with Zew et al [22, 23] who reported that rabbit muscular LDH have Km=209µM at pH 7.15 , $28C^0$, and Km=162 µM at pH6.8 , $28C^0$.Stambaugh et al[24] also reported a Km value for rabbit muscular LDH equals 350 µM at pH7.4 , $25C^0$. The variation in the derived kinetic in the derived kinetic parameter is believed to be mainly to differences in experimental condition between the studies [22,23]. DeArriaga et al [25] reported that the $[S]_{0.5}$ values for pyruvate increased from 1.5mM at pH 7.2 up to 12mM at pH 7.7[24]. As shown in the table (1) the pyruvate has inhibitory effect at higher concentration .This result is in agreement with that who reported that LDH activity inhibited by increasing amount of pyruvate with a rang of pH and fixed conc. of NADH ,but the inhibition percentage increased with increasing NADH concentration, the coenzyme could act as an uncompetitive inhibitor[25] .

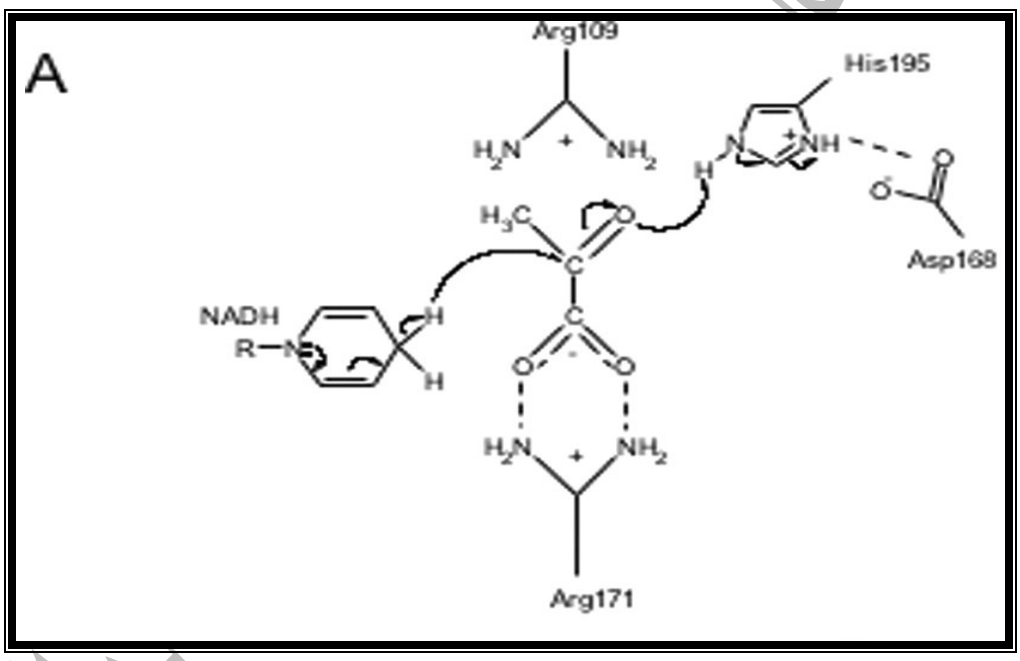
The fraction of sites filled (f_{ES}) of LDH in the absence and presences of fixed concentration of each drug are shown in (table -1). As shown in the table 0.5 percent of active site of LDH are occupied at pyruvate conc (**0.0246**mM) reaching 1.0 at (**0.025mM) ,** and decreases in higher conc.This result is with agreement with Lushchak *et al*** how reported that for some reasoned species(fish) LDH do not inhibited by pyruvate in high conc.

As shown in table (1) diclofenac shows the highest inhibitory effect on ldh activity and that is shown from the high values of km. paracetamol and aspegic shows the same effect on ldh activity giving highest activity effect at (0.026m pyruvte , km 0.0037m) and (0.027m pyruvate ,Km=0.0039m)for aspegic and paracetamol respectively. On the other hand hydrocortisone shows an activation effect on LDH activity giving it highest effect at (0.026mmpyruvate, km=0.0026mm)

As shown from(table -1) the apparent Km values for aspegic and paracetamol obtained in the presence of aspegic and paracetamol were increased for 2-10 fold approximately at low conc. of pyruvate, but the Km values at the higher conc. of pyruvate (0.0271 mM) decreased for about 10-folds approximately for the same drugs, while the km values for diclofenac were increased for (2-7 folds) at all conc. of pyruvate.km values for hydrocortisone on the other hand shows a decrement in the its values at all pyruvate conc. This result is in agreement with other studies that reported that the presence of 30-

mM fructose 1,6 bisphosphate appear to increases the Km value for 10-folds ,and the reaction shown to be competitivly inhibited in the presence of the effectors[25] and that the increasing concentration of pyruvate could overcome the inhibitory effect of the drugs[25]

Lactate dehydrogenase is equipped with an imidazole-acid pair , His^{296}—Glu^{264} and His^{195}—Asp^{168} respectively,as an acid –base ctatalyst that transfers H^+ between the substrate carboxyl and the solvent[26]. On the other hand , the substrate – binding site of LDH contains only Arg residue, Arg^{235}. the three- dimensional structure implies that the role of Arg^{235}may be supplemented by the main chain amide group Gly78 and Val 79 on a loop structure in the active site , which act as bi - dentate hydrogen bond donors to the substrate carboxyl group[26].Fig-8



(Fig. 8) Reaction scheme of reduction of pyruvate by L- lactate dehydrogenase

Several major types of interactions affect the binding of drugs to the active site of LDH [27].

Ionic bonds between the (- ve) charge of carboxylate groups in aspegic and Diclofenac and the (+ ve) charge for the guanidine group in the Arg.

Hydrogen- bonds between (-OH) grougs in the drugs and the side chain oh His and Asp. In the active site of LDH.

Hydrophobic interactions which involves the non polar groups in both drugs and the active site.

If we take in consideration the structure of the four drugs (fig-9)[28] we can conclude that all these drugs are pyruvate analogs because they all have the (_$COCH_3$) group specially Aspegic and Paracetamol , this group could bind to the active site of the enzyme and inhibited it in different folds .The polarity of these drugs could play a role on the activity of LDH. Aspegic and Paracetamol have the same polarity so they have the same effect on LDH activity (approximantly the same km values). Diclofenac because of the low polarity of it , this could effect negatively on the binding of pyruvate to the active site of LDH, while the high polarity of hydrocortisone could play a positive role in the binding.

Finally the author did not fin references that support these results.

Fig.9 The Structures of Aspegic, Paracetamol, Diclofenac Hydrocortisn and Pyruvate)[28]

Conclusions:

Our results revealed

1-The activation of LDH is not conc. dependant for each of Diclofenac, hydrocortisone, and aspegic and it is for paracetamol.

2- All four drugs have different effect on the activity of LDH.

3- All the drugs used in this research were have competitive inhibition effect on enzyme accept for the hydrocortisone which have activation effect on the enzyme.

4- The polarity of the drugs could play a major role in there effect on the LDH activity

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دراسة حركية عن تاثير بعض الادوية الخافضة للحرارة والمضادة للالتهابات على فعالية انزيم اللاكتيت ديهايدروجنيز

د.بري حبيب سيف الله (دكتوراه كيمياء حياتية)

قسم الكيمياء ، كلية العلوم للبنات، جامعة بغداد

الخلاصة:

انزيم اللاكتيت ديهايدروجنيز(HDL) (١,١,١,٢٧) يعمل في مسارات تحلل الكلوكوز اللاهوائية وفي مسارات تخليق الكلوكوز[١] وهو يوجد في العديد من الكائنات الحية النباتية منها والحيوانية[٢] . هناك العديد من المركبات التي تعمل كمؤثرات على فعالية انريم مثل الاكتيت ، البروفيت،DAN، H DAN،Hp، محتوى البفر ، القوى الايونية،
الفركتوز١،٦ ثنائي فوسفيت، الايونات الموجبة الثنائية مثل ايوني المنغنيز والكوبلت الثنائيي التكافؤ، كم يعتبر البرولين والتراي مثل امين اوكسايد وبحجم(μ من المؤثرات على فعالية الانزيم. كما لوحظ ان الادوية الخافضة للحرارة والادوية المضادة للالتهاب تؤثر ايضا على فعالية الانزيم

في هذا البحث قمنا بدراسة تاثير بعض الادوية الخافضة للحرارة(الاسبيجك، الراسيتامول، و الديكلوفين) والادوية اللمضادة للالتهاب(الهايدروكورتيزون) على فعالية الانزيم وقد لوحظ ان فعالية الانزيم تزداد بمقدار مرتين عند استخدام الباراسيتامول والاسبيجك، وبمقدار مرة واحدة عند اضاف الدكلوفين ، وبمقدار ١,٨ مرة عند اضافة الهيدروكورتيزون.ولوحظ ان التحفيز باضافةة البراسيتامول يكون معتمد على تركيز الراسيتامول المضاف.

القياسات الحركية اظهرت ان انزيم اللاكتيت ديهايدروجنيز لايطيع معادلة ميكاليس عند استخدام تراكيز عالية من البايروفيت، كم لوحظ ان اضافة الاسبيجك والبراسيتامول يعمل على زيادة قيمة معامل ميكالس في التراكيز لاواطئة نسبيا للبايروفيت الا انه يزداد بقدار ١٠ مرات عند زيادة تركيز البروفيت، اما الدكلوفين فيعمل على زيادة قيمة الثابت في مختلف تراكيز البايروفيت. من ناحية اخرى فان الهايدروكورتيزونيعمل كمنشط معتدل للانزيم. هذا الاختلاف بالتاثير قد يعزى الى اختلاف قطبية الادوية المستخدمة.