Studying the Effect of Some Thiadiazole Derivatives on the Activity of LDH Enzyme

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Abstract

This work involved studying the effect of some thiadiazole derivatives on the activity of lactate dehydrogenase enzyme (LDH) in the serum of normal control and thalassemia patients. The results showed that all the compounds used had inhibitory effect on LDH enzyme activity, the inhibition percent was between (7.87%-55.22%) depending on compound structure and its concentration. Studying kinetic properties showed that these compounds behave as non-competitive inhibitors.

Keywords: Thiadiazoles; Lactate dehydrogenase; Thalassemia.

Introduction

Thiadiazole derivatives, are used therapeutically in various diseases due to the properties such as antifungal [1], antiviral [2], antibacterial [3], anticonvulsant [4], antimicrobial and anti-inflammatory [5]. It is also stated that thiadiazole derivatives show anti-thyroid activity [6]. Thalassemia is genetic disorder of the blood, which is inherited and, passed from parent to child. In these conditions production of the protein portions (globin) of the hemoglobin molecule is decreased and the globin protein itself is abnormal of this abnormality red cells produced are smaller, as result and more pale than normal, more fragile and prone to early destruction [7]. Thalassemia is a genetically determined defect in hemoglobin synthesis. There is an inability to manufacture sufficient of globin chains [8]. The defect may affect α, β and δ chain or may affect some combination of the α, β and δ chain in the same patient, but never α and β chain together, unmatched globin's crepitate and damage
red blood cell RBC membranes causing their destruction while still in the marrow [9,10].

**There are Different forms of thalassemia**

There are four genes coding for alpha chain production. These genes are located on chromosome 16. There are at least five forms of alpha thalassemia pending up on the number and location of the abnormal genes alpha thalassemia occurs when one or more of the four alpha chain genes fail to function. Alpha chain protein production, for practical purposes, is evenly divided among the four genes. With alpha thalassemia, the "failed" genes are almost invariably lost: from the cell due to a genetic accident [11]. The genes controlling beta chain production are located on chromosome 11. If both genes fail, then the patient is said to have beta thalassemia major. If only one gene fails, then the patient has beta thalassemia minor [12].

Treatment of thalassemia depends on the type and severity of the disease. People who are carriers (thalassemia trait) usually have no symptom and need no treatment. Those with moderate or severe forms of thalassemia may be treated by [13]. Blood transfusion, Iron chelating agents, Surgery, Bone Marrow or stem cell transplants & Folic acid. The natural history of thalassemia has shown substantial change during these years. This applies for each aspect of the pathology (for example, endocrinological, hepatological and psychological) and also for the pathology that has presented and still presents the main cause of death: myocardial dysfunction [4].

Cardiac complications are a main feature of the clinical spectrum of β-thalassemia.

They are the leading cause of death. The prominent finding in this condition is left ventricle dysfunction, which is attributed mainly to iron overload and leads gradually to cardiac failure and cardiogenic death. Cardiac involvement may be different in the latter because these patients live longer and generally have low hemoglobin levels and lower iron loads [14]. Lactate dehydrogenase is an enzyme found in almost all body tissues. It plays an important role in cellular respiration, the process by which glucose from food is converted into usable energy for our cells. Lactate dehydrogenase (LDH) is an intracellular enzyme, which catalyzes the readily reversible reaction involving the oxidation of lactate to pyruvate with nicotine amide
adenine dinucleotide (NAD+) serving as coenzyme, an essential step in producing cellular energy [15] as shown in the following equation [16].

Although LDH is abundant in tissue cells, blood levels of the enzyme are normally low. However, when tissues are damaged by injury or disease, they release more LDH into the bloodstream. Even though an LDH test is useful in diagnosing tissue damage, other tests are usually necessary to pinpoint the location of the damage. One such test is called the LDH isoenzymes test. LDH isoenzymes are five kinds of the LDH enzyme that are found in specific concentrations in different organs and tissues. By measuring the blood levels of these isoenzymes, doctors can get a better idea of the type, location, and severity of the cellular damage. The LDH test is generally used to screen for tissue damage. This damage may be acute (as in the case of a traumatic injury) or chronic (due to a long-term condition such as liver disease or certain types of anemia). It also may be used to monitor progressive conditions, such as muscular dystrophy and HIV [17]. LDH level can be elevated in many conditions such as stroke, heart attack, various kinds of anemia, low blood pressure, liver disease (for example, hepatitis), muscle injury, muscular dystrophy, pancreatitis [18].

**Materials and Methods:**

**Subjects:** The Samples were conducted in AL-Karama Hospital. They have been classified into two groups; control group which included healthy male individual with no previous disease which may interfere with the parameters analyzed in this study and β-thalassemia major group which included (19) male patients. The samples were collected during six months

**Blood sampling:** Blood samples (5 ml) were collected into plain tube for serum separation, centrifuged at 3500 rpm for 10 min. Serum samples were used for determination of LDH enzyme in the present and absence of inhibitors.
Effect of thiazolium compounds on LDH in vitro

Principle

Lactate dehydrogenase (LDH) catalyzes the reduction of pyruvate to lactate in the presence of reduced nicotinamide adenine dinucleotide (NADH) at pH 7.5. The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD⁺ proportional to the activity of LDH present in the sample.

\[ \text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{L-Lactate} + \text{NAD}^+ \]

This test has been formulated according the standardized method described by SFBC1. Colorimetric determination of LDH was performed as follows [19, 20]

Reagent composition

R1: LDH substrate. TRIS buffer 100 mmol/L pH 7.5, pyruvate 2.75 mmol/L, sodium chloride 222 mmol/L.

R2: LDH coenzyme NADH 1.55 mmol/L.

Reagent preparation

Working reagent: 4ml of R1 + 1ml of R2, stable for 2 months at 2-8 °C and should be protected from light.

Procedure

1) Pre-incubate working reagent samples and controls to reaction temperature 30/37 °C.
2) Set the photometer to 0 absorbance with distilled water.
3) Pipette into a cuvette:

| Reaction temperature | Working reagent | Sample or control |
|----------------------|-----------------|-------------------|
| 30/37 °C             | 1.0 mL          | 20 L              |

Mix gently by inversion. Insert cuvette into the cell holder and start stopwatch.
4) Incubate for 30 seconds and record initial absorbance reading.
5) Repeat the absorbance reading exactly after 1, 2 and 3 minutes.
6) Calculate the difference between absorbance.
7) Calculate the mean of the results to obtain the average change in absorbance per minute (ΔA/min).

**Calculations:**

\[
U/L = \Delta A/\text{min} \times 8095
\]

Samples with ΔA/min exceeding 0.150 at 340nm should be diluted 1:10 with saline and assayed again. Multiply the results by 10. If results are to be expressed as SI units apply:

\[
U/L \times 0.01667 = \mu k \text{at/L}.
\]

The compounds used in this research were derived from thiadiazole and they were supplied by Dr. Emad AL-Saraj, as shown below:

[5-(-4-Ethoxy phenyl)-[1,3,4]thiadiazol-2-yl-[3-(5-nitro-furan-2-yl) allylidene]-amine

**Compound M1**

(4-nitro methoxy -but-2-enyldene) -[5- (4- propoxy- Phenyl) -[1,3,4]thiadiazol-2-yl]-amine

**Compound M2**
[5- (4- Hexyl oxy- Phenyl)-[1,3,4] thiadizol -2-yl]-[3-(5-nitro-furan -2-yl)-allyldene]-
amine
Compound M3

4- Hexyl- benzoic acid-4{[5-(4-hydroxy- Phenyl)- [1,3,4]thidiazol- 2-ylimino]-
methyl]-Phenyl ester
Compound M4

A stock solution (1×10^{-2} M) of the compounds was prepared and the following concentration of (1×10^{-4}, 1×10^{-6}) M were prepared by diluting with dimethyl sulfoxide (DMSO). The enzyme LDH activity was measured in human serum to determine the action of the compounds by using the same method of enzyme activity determination with adding 0.05 µL of the prepared compounds to the test tube. The inhibition percentage was calculated by comparing the activity with and without inhibitor under the same conditions, according to the equation:

\[ \% Inhibition = 100 - \left( \frac{X}{Y} \right) \times 100 \]

X= The activity in the presence of inhibitor.
Y= The activity in the absence of inhibitor

Two concentration of each inhibitor (1×10^{-4}, 1×10^{-6} M) were used with different substrate concentrations (1.50, 1.75, 2.00, 2.25, 2.50, 2.75 mmole/L) to study the type of inhibition. These different concentrations were prepared from stock solution of (mmole/L). The enzyme activity was determined with and without the inhibitor using the Line Weaver-Burg method by plotting 1/V vs. 1/[S], and the values of Ki, Vmax
and type of inhibition were estimated. The overall predictive values for the results in all studied groups were performed according to program of SPSS version 22, and the results were expressed as mean±S.D. T-test was used to compare the significance of the difference in the mean values of any two groups, (P ≤ 0.05) was considered statistically significant.

Results and Discussion

The biochemical tests revealed that the compounds had inhibitory effect on LDH enzyme activity. The normal value of LDH enzyme was (103.36±13.38 U/I) in control subjects while it was (252.17±19.21 U/I) in thalassemia patients, with P ≤ 0.05. The effects of the four derivatives were studied by preparing two concentrations (1×10⁻⁴, 1×10⁻⁶) M and it were found that all these compounds act as inhibitors. Tables (1, 2, 3 and 4) show that the enzyme activity decreased with increased concentrations of inhibitor.

Also, inhibition percent (Inh. %) for these compounds on LDH are shown in Fig. 1, 2, 3 and 4, in which the notice that the Inh. % is increased with increased concentrations of inhibitor.

So four compounds derived from thiadiazole were examined regarding their effect on the activity of LDH enzymes. The LDH enzyme considered as important enzymes in determining cardiac function. The difference in the inhibitory effect for the compounds used could be attributed that either the LDH enzyme consist of number of isoenzymes that it has which differ in their reaction with the inhibitors or to the difference in the activity of these compound on the active site of the enzyme, and the enzyme could have a change in their a stereo structure during the progress of thalassemia which can lead to higher conjugation between the inhibitors and the enzyme and that will give higher inhibition. Type of inhibition, Vmax, Kmax were estimated by measuring the enzyme activity in absence and presence of inhibitor at different concentrations of substrate under the same conditions using Line weaver-Burk equation and plots, as shown in Fig. 5, 6, 7 and 8.
These results suggest that all compounds act as non-competitive inhibitors, in non-competitive inhibition Km changes and Vmax remains constant.

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Table 1: LDH Enzyme Activity in Control and Patients in the Presence of Compound M1

| Inhibitor Compound M2 | Conc. | 10^{-4}M | 10^{-6}M |
|----------------------|-------|----------|----------|
| LDH activity U/I     | (Control) Mean ± S.D. | 61.06±11.84 | 82.49±10.95 |
| LDH activity U/I     | (Patient) Mean ± S.D. | 144.03±27.16 | 202.34±35.06 |
| \( P \) value        |       | 0.09     | 0.02     |

Table 2: LDH Enzyme Activity in Control and Patients in the Presence of Compound M2

| Inhibitor Compound M2 | Conc. | 10^{-4}M | 10^{-6}M |
|----------------------|-------|----------|----------|
| LDH activity U/I     | (Control) Mean ± S.D. | 63.47±14.76 | 78.56±9.55 |
| LDH activity U/I     | (Patient) Mean ± S.D. | 144.13±22.87 | 220.13±36.98 |
| \( P \) value        |       | 0.05     | 0.02     |

Table 3: LDH Enzyme Activity in Control and Patients in the Presence of Compound M3

| Inhibitor Compound M2 | Conc. | 10^{-4}M | 10^{-6}M |
|----------------------|-------|----------|----------|
| LDH activity U/I     | (Control) Mean ± S.D. | 41.58±2.45 | 74.68±12.64 |
| LDH activity U/I     | (Patient) Mean ± S.D. | 150.53±10.17 | 190.61±37.58 |
| \( P \) value        |       | 0.02     | 0.03     |

Table 4: LDH Enzyme Activity in Control and Patients in the Presence of Compound M4

| Inhibitor Compound M2 | Conc. | 10^{-4}M | 10^{-6}M |
|----------------------|-------|----------|----------|
| LDH activity U/I     | (Control) Mean ± S.D. | 64.95±8.46 | 85.64±3.95 |
| LDH activity U/I     | (Patient) Mean ± S.D. | 160.88±8.46 | 210.68±18.84 |
| \( P \) value        |       | 0.09     | 0.01     |

Fig. 1: Comparison between inhibition percentage of compound M1 on LDH in control and patient

Fig. 2: Comparison between inhibition percentage of compound M2 on LDH in control and patient
Fig. 3: Comparison between inhibition percentage of compound M3 on LDH in control and patient.

Fig. 4: Comparison between inhibition percentage of compound M4 on LDH in control and patient.

Fig. 5: Type of inhibition of compound M1 on LDH in control and patient groups.

Fig. 6: Type of inhibition of compound M2 on LDH in control and patient groups.
Fig. 7: Type of inhibition of compound M3 on LDH in control and patient groups

Fig. 8: Type of inhibition of compound M4 on LDH in control and patient groups