Study The Effect of Thiadiazole and Thiazine Derivatives as Antiatherogenic and Antioxidant Agents in Induced Hyperlipidemic Mice

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Abstract
This study conducted to examine the effect of two newly synthesized compounds[3-(5-(ethylthio)-1,3,4-thiadiazol-2-yl)-2,3-dihydro-2-(3-nitrophenyl)benzo[1-3-e] thiazin-4-one (compound I) and 5(4-dimethyl amino) benzylidene amino)-1,3,4-thiadiazole-2-thiol(compound II)] as antiatherogenic and antioxidant agents, In vivo study.

The study was carried out on sixty male Wister mice aged seven to eight weeks their weight ranged(180-200 g). The mice were grouped as: group(1)(G1): control group (20 mice).Group(2)(G2):consisted of 40 mice in which the mice were daily administered cholesterol (25mg/kg/day) in coconut oil 6% and creamy cheese for 28 days. Lipid profile (total cholesterol (Tch), triglyceride (TG), high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol VLDL-c), was measured for 12 of mice chosen randomly from G2 to assure hyperlipidemia. Then group2 (G2) subdivided into two groups: group (2.A): (14 mice) which the mice were daily treated with (10^{-4})M of compound (I)via drinking water for 20 days. Group(2.B):(14 mice) which the mice were daily treated with (10^{-5})M of compound II via drinking water for 20 days. Lipid profile, catalase, total oxidative status (TOS), total antioxidant status (TAS), and atherogenic index of plasma (AIP) were determined in all studied groups. The results showed significant
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Elevation in levels of Tch, TG, LDL and VLDL, while there are significant reduction in HDL-c levels in G2 comparing to G1. After orally administration of compounds I and II from the mice, the results revealed that the levels of Tch, TG, LDL and VLDL were reduced while the levels of HDL-c was elevated. Catalase activity showed significant decrease in G2 when compared with G1. Also, a significant elevation was found in AIP, TOS and TAS in G2 when comparing to G1. Also, significant elevation was noticed in catalase activity after administration of compounds I and II, in addition to reduction in levels of AIP, TOS, and TAS. The conclusion could be drawn from this study that compounds I and II could be considered as antiatherogenic and antioxidant agents.

Key words: Lipid lowering compounds, Thiazine & Thiadiazole derivatives, In vivo study.

Introduction

Hyperlipidaemia is an increase in the lipids, and lipoproteins\(^{(1)}\). The combination of certain risk factors such as dyslipidemia can act multiplicatively or synergistically to increase the risk of atherosclerosis and heart disease\(^{(2)}\).

Oxidant generation is part of the normal metabolism of many types of cells and it is critical for cell homeostasis. To protect itself against exposure to noxious oxidants. The airway mucosa has developed an antioxidant system\(^{(3)}\). Cells infiltrating the nasal mucosa in patients with AR produce a variety of mediators, including reactive oxygen species (ROS). This leads to an imbalance between the oxidative forces and the antioxidant defense systems which is believed to favor an oxidative injury that has been causes tissue damage\(^{(4-6)}\).

Thiazines and Thiadiazole compounds have gained importance in medicinal and pharmaceutical fields due to a broad spectrum of biological activities like antioxidants, even at small concentration and thus have diverse physiology role in the body. Antioxidant act as radical scavengers and help in converting the radicals to less species and promote the repair of relative oxygen species induced damage to cell structures\(^{(7-10)}\).

The aim of the present study is to examine the effect of some derivatives of thiadiazole and thiazone which may be considered as antiatherogenic and antioxidant agents.
These compounds were prepared in previous study \(^{(11)}\).

**Subjects and Methods**

The studied groups comprised with sixty male Wister mice obtained from animal house, from the college of Medicine, Baghdad University, the mice divided into group(1): which consist of 20 mice as control,( G 2): consist of 40 mice in which the mice were daily administered cholesterol (25mg/kg/day) \(^{(12)}\) in coconut oil 6% and creamy cheese for 28 days. Lipid profile were measured for 12 mice chosen randomly from G2 to assure hyperlipidemia. After that G2 were subdivided into two groups as follows:

G2A: (14 mice) in which the mice were daily treated with \((10^{-4})\)M of compound (I) via drinking water for 20 days. G2B: (14 mice) in which the mice were daily treated with \((10^{-5})\)M of compound II via drinking water for 20 days.

The chosen concentration was based on *in vitro* and *in vivo* previous studies which given the best inhibition from the other concentrations \(^{(13,14)}\).

The blood samples were collected and blood serum was prepared by centrifuging blood at 2600 rpm for 15 min. Serum was frozen until the assay. Total cholesterol \(^{(15)}\), high density lipoprotein- cholesterol (HDL-c) \(^{(16)}\) and triglyceride (TG) \(^{(17)}\) were determined by using commercial kits (Randox, France).

Atherogenic index of plasma (AIP) calculated from the formula \(^{(18)}\):

\[
\log(TG/HDL-c)
\]

Catalase activity was measured by using the method that depends on its ability of \(H_2O_2\) decomposition to give \(H_2O\) and \(O_2\) \(^{(19)}\). Serum TOS was also determined using a automated measurement method, developed by Erel \(^{(20)}\). In this method, oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by abundant glycerol molecules in the reaction medium. The ferric ion forms a colored complex with xyleneol organ in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide and results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (\(\mu\text{mol} H_2O_2 \text{ Eq/L}\)). Serum TAS was determined using automated method \(^{(21)}\) involving the
production of hydroxyl radical, the most potent of biological radicals. The antioxidant effect of the sample against the potent free-radical reactions initiated by the hydroxyl radicals produced is measured. Student t-test was used to examine the differences of mean. The results were expressed as mean ±SD and P≤ 0.05 was considered significant.

**Result and Discussion**

Table (1) show the levels of lipid profile in G1, G2, G2A and G2B. The results showed significant elevation in levels of Tch, TG, LDL, VLDL, and AIP while there were significant reduction in HDL levels in G2 comparing to control group(G1). The results revealed that the levels of Tch, TG, LDL and VLDL were reduced while the levels of HDL-c was elevated after administration of compounds I and II in G2A and G2B respectively. Antihyperlipidemic agents which are active in cholesterol induced hyperlipidemic model function by one or more mechanisms. Therefore, higher intake of dietary cholesterol increases serum lipid profile by down regulation of LDL-c receptor synthesis thus decreasing uptake of LDL-c via these receptors(22).

Some researches demonstrated that the actions may be due to increased inhibition of intestinal absorption of cholesterol, interference with lipoprotein production, increased expression of hepatic LDL receptors and their protection etc. leading to an increased removal of LDL-c from the blood and its increased degradation and catabolism of cholesterol from the body(23).

The logarithmatically transformed ratio of plasma TG to HDL correlated closely with the LDL particle size and could sever as an indicator of the atherogenic lipoprotein phenotype (24). AIP indicates a balance between the actual concentration of plasma TG and HDL. Clinical studies have shown that AIP predicts cardiovascular risk and it is an easily available cardiovascular risk marker and a useful measure of the response to treatment (25).

Table (2) show the catalase activity, levels of TOS and TAS were significant decrease in G2 in levels of TOS and TAS when compared with G1. Also, a significant increase was found G2 compared with G1 in patient group. Catalase is the enzyme, which protects the cells from accumulating of hydrogen peroxide by dismutating it to form water.
and oxygen or by using it as an oxidant in which it works as peroxidase\(^{(26)}\).

After orally administration of compounds I and II to the mice, the results revealed that the levels of Tch, TG, LDL and VLDL were reduced while the levels of HDL-c was elevated. Catalase activity showed significant decrease in G2 when compared with G1. Also, a significant elevation was found in AIP, TOS and TAS when comparing to group 2. A, significant elevation was noticed in catalase activity after administration of compounds I and II, in addition to reduction in levels of AIP, TOS, TAS and AIP.

The main difficulties associated with the use of oxidative stress markers are the complexity and cost of the methods and equipment required. Since the measurement of individual antioxidant molecules is not feasible and since the antioxidant effects of these molecules are additive, we measured total oxidative status (TOS) and total antioxidant status(TAS)\(^{(27,28)}\). Although the complete mechanism of this elevation is unknown, it is proposed that TAS was increased as to compensate for the increase in total oxidative stress\(^{(29)}\).

The conclusion could be drawn from this study that compounds I and II could be considered as antiatherogenic and antioxidant agents.

References
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Table(1): The Levels of Lipid profile in all studied Groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>G1 n=12</th>
<th>G2 n=12</th>
<th>G2A n=12</th>
<th>G2B n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tch (mg/dl)</td>
<td></td>
<td>155±2.4</td>
<td>600±20.5</td>
<td>340±0.97</td>
<td>172±0.91</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td></td>
<td>100.3±5.1</td>
<td>325.01±15.6</td>
<td>299±9.3</td>
<td>205±0.3</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td></td>
<td>43±6.9</td>
<td>25.6±5.2</td>
<td>40.1±6.1</td>
<td>50.3±4.01</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td></td>
<td>62±3±4.3</td>
<td>310±9.3</td>
<td>240±2.7</td>
<td>81±2.4</td>
</tr>
<tr>
<td>VLDL (mg/dl)</td>
<td></td>
<td>30.1±0.19</td>
<td>65.4±0.25</td>
<td>59.8±0.43</td>
<td>41.6±0.74</td>
</tr>
<tr>
<td>AIP</td>
<td></td>
<td>0.36±0.13</td>
<td>1.10±0.56</td>
<td>0.87±0.17</td>
<td>0.60±0.40</td>
</tr>
</tbody>
</table>

P-values <0.05 was considered statistically significant

Table(2): The level of oxidant-antioxidant status in all studied Groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>G1 n=12</th>
<th>G2 n=12</th>
<th>G2A n=12</th>
<th>G2B n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (U/ mg)</td>
<td></td>
<td>0.82±0.04</td>
<td>0.09±0.001</td>
<td>0.63±0.03</td>
<td>0.54±0.01</td>
</tr>
<tr>
<td>TAS (mmol/L)</td>
<td></td>
<td>1.61±0.34</td>
<td>5.54±0.63</td>
<td>1.71±0.23</td>
<td>1.83±0.4</td>
</tr>
<tr>
<td>TOS (µmol/L)</td>
<td></td>
<td>7.29±1.5</td>
<td>12.9±3.3</td>
<td>0.49±0.03</td>
<td>0.94±0.03</td>
</tr>
</tbody>
</table>

P-values <0.05 was considered statistically significant
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دراسة تأثير مشتقات جديدة للثياديازول والثيازين كعامل مضاد لتصلب الشرايين ومضاد الاكسدة في الفئران المحتكة بارتفاع الدهون
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الخليدية:

هدف هذه الدراسة هو تقييم تأثير بعض مشتقات الثياديازول والثيازين الجديدة لتحكير كمضاد لتصلب الشرايين ومضادات الأكسدة بالإضافة إلى قياس صورة الدهون في الفئران المختبرية التي تم تحضير أفرادها من الفئران المختبرية (I) بتركيز (10^{-4}) مولاري والمركب (II) بتركيز (10^{-5}) مولاري، داخل الخليدية.

أجريت الدراسة على ستين فأرا من الذكور الذين تزودوا أعمارهم بين سبعة إلى ثمانية أسابيع (180-200 يوم) والتي تم الحصول عليها من البيوت الحيواني في كلية الطب، جامعة بغداد. تم تقسيم الفئران المختبرية إلى:

- المجموعة الأولى: مجموعات السيطرة (20 فأرا) والمجموعة الثانية: تتكون من (40 فأرا) التي كانت تعتمد يومياً بولاثيون (5mg/kg/day) 3 ميجا، زيت عصير الليمون 6 وجبات كامل الدسم لمدة (28 يوم). ثم قياس صورة الدهون في 12 فأرا من المجموعة الثانية ثم اختمار عشوائي لتشخيص ارتفاع الدهون فيها. ثم قسمت المجموعة الثانية إلى:

  - المجموعة A: (14 فأرا) حيث عولجت هذه الفئران يومياً بالمركاب الأول بتركيز (10^{-4}) مولاري عن طريق الحقن.
  - المجموعة B: (14 فأرا) حيث عولجت هذه الفئران يومياً بالمركاب الثاني بتركيز (10^{-5}) مولاري عن طريق الحقن.

ولقد أجريت هذه الدراسة على ستين ذكر من الفئران الذين تتراوح أعمارهم بين سبعة إلى ثمانية أسابيع وزنهم تراوح إلى (180-200) غرام. مستوي الدهون في الدم، الكولسترول، وتضجيم الدهون (TOS)، وثبات الدهون (TAS)، وثبات مسحوقات الأكسدة الكلي (AIP).

الشرايين من البلازم (AIP) في كل المجموعات المدرسة أظهرت النتائج ارتفاع كبير في مستويات الشرايين بالدم، من البلازم وثبات الدهون (TOS)، وثبات الدهون (TAS)، وثبات مسحوقات الأكسدة الكلي (AIP)، بعد اقتراب إعداد المركاب الأول والثاني لتأثيرات فقداً وجود ارتفاع كبير من سمعه الأكسدة الكلية (TOS).

وقد اقتراب من النتائج هذه الدراسة بأن المركاب الأول والثاني يمكن أن يقدماً كعامل مضاد لتصلب الشرايين ومضاد الأكسدة.

الكلمات المفتاحية: مركبات خفض الدهون، مشتقات الثياديازول والثيازين في الخليدية الحية.