Inflammatory stress and idiosyncratic drugs hepatotoxicity in rabbit
H. T. Mohamed
Coll. of Vet. Med. /Unive. of Basra

Abstract
Background: Idiosyncratic drug hepatotoxicity is non-time related, unpredictable, occurs infrequently and can be fatal. It was proposed that inflammatory or oxidative stress occurs randomly in patients even after asymptomatic incidence can precipitate drug hepatotoxicity.

Aim: To measure hepatotoxicity of diclofenac in rabbit serum following the incidence of inflammatory stress by lipopolysaccharide (LPS) and correlate this to various stress parameters as Malondialdehyed (MDA).

Method: 24 rabbits were divided into four groups (6 each) according to type of treatment.
Group 1: control (received normal saline).
Group 2 received diclofenac sod. (5mg/kg, orally 3 times daily for three days).
Group 3 received lipopolysaccharide (150µg/kg, i.v, 24 hours before killing.
Group 4 received diclofenac sod. + Lipopolysaccharide (5mg/kg orally + 150µg/kg, i.v 24 hours before killing).
Then for each animal were measure, liver MDA, liver enzymes.

Conclusion: LPS potentiated the hepatotoxic effect of diclofenac sod. The effect is mediated by oxidative and inflammatory reactions as demonstrated by increase in liver tissue MDA.

Material and Methods
a. Chemicals: Thio barbituric acid (BDH ,chemical ,Ltd. Pool, England.) Trichloro acetic acid (T C A) from Thomas Baker, Ltd, India. Lipopolysaccharide (LPS) from Sigma- Aldrich, France.
b. Drugs: Diclofenac sodium: (100 mg/tab) (triveni chemicals; India); powdered and dissolved in 100 ml distal water to made stock solution contain (1 mg/ml). The drug was given to the rabbit by oral rout in dose of 5 mg/kg three times daily for three days. Lippolysaccharide of E.coli, as powder (vial contain 1o mg) which equivalent to (10.000 µg), dissolved in (100 ml) of 0.09% normal saline to make a stock solution has concentration equal to (100µg/kg). The dose used low than that hepatotoxic dose of lipopolysacchride of (150 µg/kg)
c. Animals: the experiment was carried out on 24 local bred sexually mature male rabbits. The range of body weight was (1 - 1.5 kg), the animal were housed in special room for acclimatization. Rabbits were maintained on free access to food and drinking water. Groups of animals were divided randomly into four groups as following:
Group (1) received normal saline (0.9%)
Group (2) received diclofenac sod. (5 mg/kg orally, three times daily for three days).
Group (3) received lipopolysaccharide (150µg/kg, i.v, 24 hours before killing).
Group (4) received diclofenac sod. + Lipopolysaccharide (5mg/kg, orally + 150µg/kg, i.v 24 hours before killing).
Prior to the day of experiment each rabbit was kept for more than 10 hours in restraint cage.
On the day of experiment the groups of rabbits were allocated randomly to receive either active treatment or physiological saline via pediatric stomach tube advanced through wooden clinical tongue depressor with hole in the center to control jaw movement and prevent the animal from chewing the tube (8).
After 3 days of treatment the rabbits were killed by sharp blow on the back of the head, the abdomen was opened and liver removed.
The concentrations of the drug used in this study represented the double therapeutic dose of the drug.

Preparation of liver homogenate and MDA measurement:
A. the liver was removed and cut into small pieces then washed with cold phosphate buffer saline (PBS) and squeezed between filter paper to remove excess phosphate buffer saline.
B. five grams of the liver tissue was homogenized by electric homogenizer in (100 ml) of cold phosphate buffer saline until large particles disappeared.
C. centrifuge at 2000 rpm for 10 minutes and the supernatant was taken
D. (0.1 ml) of this solution add to (1.5 ml) of trichloroacetic acid (TCA).
E. after 10 minutes incubation at 37 °C, centrifuge at 3000 rpm for 15 minutes.
F. all the supernatant was removed and treat with (1.5ml) of clear solution of 0.067% thiobarbituric acid (TBA)
H. incubated in water bath for 30 minutes at 100C.
I. absorbance was measured at 532 nm UV wave length.
MDA concentration was calculated using the following equation (9)
\[
\text{MDA} = \frac{\text{Absorbance}}{1.49 \times 10^{-5}}
\]
the final result expressed in nmol/g
Measurement of serum liver enzymes : (ALT, AST): colorimetric method, hazy, to white and umber and adapted for determination of the activity in serum by Reitman (1957).(10)
\[
\text{L- Alanine} + 2-\text{Oxoglutarate} \rightarrow \text{pyruvate} + \text{L-Glutamate}.
\]
\[
\text{L- Aspartate} + 2-\text{Oxoglutarate} \rightarrow \text{Oxaloacetate} + \text{L-Glutamate}.
\]
The pyruvate or oxalate react with 2, 4 DNPH to form 2, 4 dinitrophenylhydrazones, which absorbed at 505 nm.
We used colorimetric method for determination of alkaline phosphates (ALP) as in the following scheme:
\[
\text{Phenyl phosphate} \rightarrow \text{phenol} + \text{phosphate}
\]
Free phenol liberated by hydrolysis of substrate reacts then with 3 – amino antipyrine in the presence of alkaline from potassium ferric cyanide to form red colored complex which absorbance measured at 510 nm is directly proportional to the (ALP) activity in the serum.
Statistical Analysis

Analysis was made by using SPSS package version 15. Data were analyzed by one way ANOVA. Paired t-test was used to compare between different concentration and control. The differences are considered to be significant when p<0.05. The correlation between various parameters was evaluated by parsons' correlation analysis.

Results

1. The effect of diclofenac sodium on level of MDA in the liver homogenate of the rabbit:
   Diclofenac sodium increased the level of MDA in the liver homogenate at a dose of (5 mg/kg) by 39% as compared with control. And this effect was statistically not significant (p>0.05) table 1.

2. Effect of LPS on level of MDA in liver homogenate of the rabbit: LPS increased the level of MDA in the liver homogenate by 28% as compared with control. And this effect was statistically not significant (p>0.05).

3. Effect of combination treatment (Diclofenac sod + lipopolysaccharide) on the level of MDA in liver homogenate of the rabbit: this combination lead to increase the level of MDA in the homogenate by 93% as compared with control. This effect was statistically significant (p<0.05).

4. Effect of combination treatment (Diclofenac sod + Lipopolysaccharide) on level of the (ALT) in rabbit serum: This combination treatment leads to an increase in level of (ALT) in the rabbit serum as compared with control. And consider as highly significant (p<0.01) and percentage change from control was 256%

5. Effect of Diclofenac sodium on level of (ALP) in the serum of the rabbit:
   Administration of diclofenac sodium to the rabbit causes decrease in the level of (ALP) as compared with control, but this elevation was statistically not significant (p>0.05) and the percentage change from control(-55.4%) table 1.

6. Effect of the diclofenac sodium on serum level of (AST) in rabbit:
   Administration of diclofenac sodium to the rabbit show an increase in (AST) level as compared with control ,and this elevation was statistically significant (p<0.01) and percentage change from control was 256%. 

7. Effect combination treatment (diclofenac sod. + lipopolysaccharide) on serum level of (AST) in rabbit: this combination treatment causes an increase in level of (AST) in the rabbit serum as compared with control. And consider as highly significant (p<0.01) and percentage change from control was (884%) TABLE4.

8. Effect of lipopolysaccharide on ALP in serum rabbit: when we LPS to the rabbit there was an increase in level of ALP as compared with control. But this elevation was statistically not significant (p>0.05) and percentage change from control (8.5%) TABLE4.

9. The effect of combination treatment (diclofenac sod. +lipopolysaccharide) on ALP level in serum rabbit: when we give this combination to the rabbit, lead to decrease in level of ALP as compared with control. And this was statistically not significant (p>0.05) and the percentage change from control was(-14.7%) TABLE4.

Table 1: the effect of different treatment on MDA of liver tissue.

<table>
<thead>
<tr>
<th>Group of rabbits treated with</th>
<th>MDA in liver tissue (nmol/gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (N.S)</td>
<td>0.86 ± 0.23</td>
</tr>
<tr>
<td>Treated with diclofenac sod.</td>
<td>1.12 ± 0.08</td>
</tr>
<tr>
<td>Treated with lipopolysaccharide</td>
<td>1.10 ± 0.19</td>
</tr>
<tr>
<td>Treated with diclofenac sod. + lipopolysaccharide</td>
<td>1.66 ± 0.44 ≠</td>
</tr>
</tbody>
</table>

≠ Significant from diclofenac sod. alone
TABLE 2: The effect of different treatment on serum ALT in liver tissue (iu/l)

<table>
<thead>
<tr>
<th>Group of treated rabbit with</th>
<th>Control group (N.S)</th>
<th>Diclofenac sod.</th>
<th>Lipopolysaccharide</th>
<th>Diclofenac sod. +lipopolysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>62.83 ± 61.38</td>
<td>72.16 ± 30.26</td>
<td>175.66 ± 82.41</td>
<td>281 ±25.25 ≠</td>
</tr>
</tbody>
</table>

≠Significant from diclofenac sod. alone

Table 3: the effect of different treatment on serum (AST) in liver tissue.

<table>
<thead>
<tr>
<th>Group of treated rabbit with</th>
<th>Control group (N.S)</th>
<th>Diclofenac sod.</th>
<th>Lipopolysaccharide</th>
<th>Diclofenac sod. +lipopolysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28.75± 4.73</td>
<td>106.0±32.21</td>
<td>141.75 ± 80.13</td>
<td>281.0 ±53.54 ≠≠</td>
</tr>
</tbody>
</table>

≠Significant from control alone.
≠Significant from control alone.
≠significant from diclofenac sod., alone

Table 4: the effect of different treatment on ( ALP) in serum of the rabbit.

<table>
<thead>
<tr>
<th>Group of treated rabbit with</th>
<th>Control group (N.S)</th>
<th>Diclofenac sod.</th>
<th>Lipopolysaccharide</th>
<th>Diclofenac sod. +lipopolysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>56.18 ±13.1</td>
<td>23.40 ±8.97</td>
<td>60.95 ±25.25</td>
<td>47.89 ±18.37</td>
</tr>
</tbody>
</table>

Discussion

We used rabbit in the present study as a model for detecting idiosyncratic drug hepatotoxicity by using the interaction of drugs with LPS. The previous studies were conducted on rodents using either mice or rat. The rabbit is easier to handle and can easily be given by intravenous injection in the marginal ear vein. The large size of the liver also helps better handling, necked eye examination and histopathological examination. Homogenization is a disorganization stage where tissue is converted to so call homogenate (11). The aim of this process is to disrupt tissue and break open the boundaries (cell membrane) of the cell to release the cellular contents. The use of liquid rather than solid (sand or glass ) for homogenization is based on the fact that force needed one for animal cell disruption is mild. This is in contrast to bacterial or plant cells which need greater force because of presence of cell wall in these organisms that makes cell disruption more difficult. Choosing the medium in which to suspend the tissue during homogenization is also essential. Phosphate buffer saline (PBS) was selected in this study to provide the required pH and isotonisty as that for human body (11). Oxidative stress has been implicated list of human disease such as colonic cancer and inflammatory bowel disease (12), (13). Oxidative stress is also one characteristic finding of inflammation. Tissue undergoing chronic inflammation is at an increased risk of undergoing malignant changes. Many cancers arise from sites of infection and chronic irritation as well as inflammation and oxidative stress may be an important contributor (14). Diclofenac sodium when given alone produce statistically significant increase in AST levels, it also produced histopathological changes in 3/6. Diclofenac sodium is widely used non-steroidal anti-inflammatory drug that can rarely cause severe liver injury, due to immune-allergic idiosyncratic reaction with involvement of
leukotriene (IL-17 and IL-1B) (15). It was also found that there is an important role of mitochondrial permeability transition in the pathogenesis of hepatocyte injury induced by diclofenac and possible contribution to human idiosyncratic hepatotoxicity (16). This hepatotoxic effect was shown to be precipitated by LPS in laboratory animals (17). This is agreement with our results. The administration other drugs with diclofenac sodium (18), like lipopolysaccharide or Thiazolidinediones or rosiglitazone, show an increases in AST and no changes in MDA or liver enzymes. It also produced mild histopathological changes in 1/3 of the experimental animals.

References

1. Pirmohamed M, Brekenridge AM, Kitteringham, Park BK "Adverse drug reaction". BMJ 1998; (316) – (7140); 1295 -1298
16. Masubuchi Y. “ Metabolic and non-metabolic factors determining troglitazone hepatotoxicity : a
الجهد الالتهابي والأعراض غير المتوقعة للإدوية على سمية الكبد في الأرانب

حسن طمعة محمد الخزاعي
كلية الطب البيطري / جامعة البصرة

الخلاصة
الأعراض السمية للكبد غير المتوقعة من بعض الأدوية والتي تحدث في فترات غير محددة يمكن أن تكون قاتلة. افترض أن الالتهاب الكبدíي والمواد المؤكسدة تحدث عشوائيا عند المرضى. وبدون ظهور أعراض ونتيجة أحداث سمية للكبد.

هدف الدراسة: قياس سمية الكبد بعد إدخال الإلايكوليفينك الصوديوم في معالجة الفئران وذلك قبل وبعد إعطاء ليفولوبوليسكاريد (LPS) بواسطة السكريات متعددة الدهون (LIPOLYPSACCHARIDE) مثل زيادة مستوى (MDA).

الطريقة: أربع وعشرين آرنباً محلي استخدمت في هذه التجربة حيث قسمت عشوائياً إلى أربعة مجموعات في كل مجموعة 6 آرناب.

المجموعة الأولى: أعطيت المحلول المليجي فوميا لمدة يوم التجريبة.

المجموعة الثانية: أعطيت دواء الإلايكوليفينك الصوديوم (5مل/كم²) ثلاث مرات يوميا ولعدة ثلاثة أيام.

المجموعة الثالثة: أعطيت مادة متعددة السكريات الدهنية (LPS) وجرعة (150ميكروغرام/كم²) ساعتين من نهاية التجربة.

المجموعة الرابعة: أعطيت دواء الإلايكوليفينك الصوديوم إضافة إلى مادة متعدد السكريات الدهنية (5مل/كم²) فوميا و 150 ميكروغرام/كم² عن طريق الوريد.

النتائج:

مدة الإلايكوليفينك سببت ارتفاع أحيائي في نسبة طسعريت أمينوترازيفيز (ALT) معدل السكريات الدهنية سببت في نسبة الأسبارتيت أمينوترازيفيز (AST) توليفة الإلايكوليفينك مع معدل السكريات الدهنية سببت في زيادة أحيائيًة متحركة في نسبة سكال في المانديباللام (p<0.05)

(الالاتين أمينوترازيفيز (0.01<p) الأسبارتيت أمينوترازيفيز (0.01<p)

الاستنتاج: أن معدل السكريات الدهنية يمكن أن يسبب تعزيزاً تأثير الإلايكوليفينك السمي الكبد. التأثير يحدث بواسطة الكبد والالتهابات كما هو واضح في زيادة نسبة المانديباللام في النسبة الكبد. يس تأثير هذه الاكتشاف والالتهاب تحدث قبل التغيرات النسيجية وسبيت لسياقة التغيير النسيجي.