Inflammatory stress and idiosyncratic drugs hepatotoxicity in rabbit

H. T. Mohamed

Coll. of Vet. Med. /Unive. of Basra

Abstract

Back ground: idiosyncratic drug hepatotoxicity is none or 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 Malondialdehyd (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 3times 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 M D A, 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 M D A.

Introduction

Drug induce hepatotoxicity is an important human problem. In United Kingdom recent study found that adverse drug reaction responsible for more than 6% of hospital admission and the mortality rate is approximately 2% (1), and it is expected that the real incidence might be greater than estimated by current method (2), (3). Withdrawal of some efficacious drugs due to idiosyncratic hepatotoxicity leads to deficits in treatment, an example of this is felbamate (4), which was effective in treating severe cases of epilepsy. It's used markedly reduced because of its association with a

plastic anemia and hepatotoxicity in some patients (5). Idiosyncratic adverse drug reaction that target the liver are common cause of acute liver failure in the United States according to more than 10% of cases, the results of 5 years prospective study indicate that many dietary supplement and drugs with different pharmacological targets are associated with idiosyncratic hepatotoxicity. (6). Andrade et al 2005 (7) cited that anti-infective, and musculo skeletal and gastro intestine tract drugs are the main group of drugs, which can cause hepatotoxicity.

Material and Methods

a. *chemicals:* Thiobarbituric acid (BDH, chemical, Ltd. Pool, England.) Trichloroacetic 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 distilled water to make stock solution contain (1 mg/ml). The drug

was given to the rabbit by oral route in dose of 5 mg/kg three times daily for three days. Lipopolysaccharide of E.coli, as powder (vial contain 10 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 lower than that hepatotoxic dose of lipopolysaccharide 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

Absorbance

MDA = ----- the final result expressed in nmol/g

1.49 x 10⁻⁵

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)

L- Alanine + 2-Oxoglutarate $\leftarrow$ pyruvate + L-Glutamate.

L- Aspartate + 2-Oxoglutarate $\leftarrow$ Oxaloacetate + L-Glutamate.

The pyruvate or oxalate react with 2, 4 DNPH to form 2, 4 dinitrophenylhydrazones, which absorbed at 505 nm.

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 M D

A 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)

We used colorimetric method for determination of alkaline phosphates (ALP) as in the following scheme:

Phenyl phosphate $\rightarrow$ phenol + 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 M D A 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 L P S on level of M D A in liver homogenate of the rabbit: L P S increased the level of the M D A in the liver homogenate by 28% as compared with control. And this effect was statistically not significant ($p > 0.05$). TABLE1.

3. Effect of combination treatment (Diclofenac sod + lipopolysaccharide) on the level of M D A in liver homogenate of the rabbit: this combination lead to increase the level of M D A in the homogenate by 93% as compared with control. This effect was statistically significant ($p < 0.05$) TABLE 2.

4. Effect of combination treatment (Diclofenac sod. + Lipopoly saccharide) on level of the (ALT) in rabbit serum: This combination treatment leads to an increase in level of the (ALT) as compared with control. And this was highly significant ($p < 0.01$) and the percentage change from control was (346%) TABLE3.

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% table1 .

7 .effect combination treatment (diclofenac sod. + lipopolysaccharide) on serum level of (AST) in rabbit: this combination treatment causes an increase in level of (A S T) 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.

Group of rabbits treated with	MDA in liver tissue (nmol/gm)
Control group(N .S)	0.86 $\pm$ 0.23
Treated with diclofenac sod	1.12 $\pm$ 0.08
Treated with , lipopolysaccharide	1.10 $\pm$ 0.19
Treated with diclofenac sod. +lipopolysaccharide	1.66 $\pm$ 0.44 $\neq$

$\neq$ Significant from diclofenac sod. alone

TABLE 2: The effect of different treatment on serum ALT in liver tissue (iu/l)

Group of treated rabbit with

Control group (N.S)	62.83 $\pm$ 61.38 (iu/l)
Diclofenac sod.	72.16 $\pm$ 30.26
Lipopolysaccharide	175.66 $\pm$ 82.41
Diclofenac sod. +lipopolysaccharide	281 $\pm$ 25.25 $\neq$

 $\neq$ Significant from diclofenac sod.alone

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

Control group (N.S)	28.75 $\pm$ 4.73 (iu/l)
Diclofenac sod.	106.0 $\pm$ 32.21 $\neq$
Lipopolysaccharide	141.75 $\pm$ 80.13 $\neq$
Diclofenac sod. +lipopolysaccharide	281.0 $\pm$ 53.54 $\neq\neq$

 $\neq$ Significant from control alone. $\neq$ Significant from control alone. $\neq$ Significant from diclofenac sod., alone

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

Control group (N.S)	56.18 $\pm$ 13.1 (iu/l)
Diclofenac sod.	23.40 $\pm$ 8.97
Lipopolysaccharide	60.95 $\pm$ 25.25
Diclofenac sod.+ lipopolysaccharide	47.89 $\pm$ 18.37

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 isotony 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 of other drugs with diclofenac sodium (18), like lipopolysaccharide or Thiazolidinediones or rosiglitazone, show an increase 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, Kitleringham, Park BK "Adverse drug reaction", BMJ 1998; (316) – (7140) ; 1295 -1298
2. Bagheri H, Michel F, Lapeyre –Mester M, Lagier E, Cambus JP, Valdiguie p, Montastruc JL. "Detection and incidence of drug induced liver injuries in hospital; a prospective analysis from laboratory signals. Br. J. Clin Pharmacol; 2000; 50:479 -484.
3. Sgro C, Clinard F, Quazir K, Chanay H, Allard C, Guilleminet C, Lenoir C, Lemoine A, Hillon P : Incidence of drug-induced hepatic injuries " a French population-based study. Hepatology ,2002; 36: 451-455.
4. Pellock JM. Felbamate in epilepsy therapeutic evaluating the risk. "Drug Saf 1999;(21) 225-239.
5. Dieckhaus CM, Thompson CD, Roller SG, MacDonald TL."Mechanisms of idiosyncratic drug reaction: The case of felbamate. Chem Biol Interact :2002 (142): 99-117.
6. Chalasani N, Fontana RJ, Bonkovsky HL, Watkins PB, Davern T, Serrano J, Yang H, Rochon J ."Drug induced liver injury, network (Dilin) "causes, clinical features and outcome from a prospective study of drug-induced liver injury in United States: Gastroenterology: 2008-(135): 1924-1934
7. Andrade RJ, Lucena MI, Fernandez MC, Pelaez G, Pachkoria K, Garcia-Ruiz E, Garcia-Munoz B, Gonzalez-Grande R, Pizarro A, Duran JA, et al "Drug-induced liver injury: an analysis of 461 incidences submitted to the Spanish registry over a 10-year period." Gastro Enterology 2005; 129:512-521.
8. Weisborth SH, Flatt, Krus AI : The biology of the laboratory rabbits, Academic press 1988.
9. Bueg JA, Aust SD; Microsomal lipid peroxidation method ; Enzymol 1978 (52) : 303- 303.
10. Reitman, S, Frankel, S ;(1957) : Amer.J. Clin, Path 28:56
11. Williams BL and Wilson K (editor) :principles and techniques of practical Biochemistry.1975 1st edition, Edward Arnold, Washington.
12. Bork, C; dietary antioxidants and human cancer. Integer cancer ther.;2004(3):333-341.
13. Rhodes JM and Campbell BJ; " inflammation and colorectal cancer: IBD-associated and sporadic cancer compared, trend, MOL MED 2002; (80) 10 -16.
14. Coussens LM and Werb Z."inflammation and cancer" ;Nature 2002; 420 :860 -867.
15. Zimmerman HJ."Drug –induced liver disease. Clin .Liver Dis.(2000) 4:73-96
16. Masubuchi Y. " Metabolic and non-metabolic factors determining troglitazone hepatotoxicity : a

- review ; Drug Metapharmacokinet ;2006 oct. 21; (5):347-356.
17. Deng X, Luyendy K JP , Zou W, Lu J, Malle E, Ganey PE, Roth RA. "Neutrophil interaction with the hemostatic system contributes to liver injury in rats ,cotreated with lipopolysaccharide and ranitidine ; J .pharmacolExpther. 2007(3220):852 -861.
18. Al-Ali, H.S., Ms Thesis, for college of medicine, university of Basra, 2012.

الجهد الالتهابي والأعراض غير المتوقعة للأدوية على سمية الكبد/ في الأرانب

حسن طعمة محمد الخزاعي
كلية الطب البيطري / جامعة البصرة
الخلاصة

الأعراض السمية للكبد غير المتوقعة من بعض الأدوية والتي تحدث في فترات غير محددة يمكن ان تكون قاتلة . افترض ان الالتهاب الكبدي والمواد المؤكسدة تحدث عشوائيا عند المرضى, وبدون ظهور اعراض ونتيجتها أحداث سمية للكبد. هدف الدراسة: قياس سمية الكبد بعد أخذ دواء الايكلوفيناك الصوديوم في مصل الكبد في الأرانب وذلك قبل وبعد اجهاد بواسطة السكريات متعددة الدهون (LIPOPOLYSACCHARIDE) (LPS) وربط هذه مع مختلف قياسات الأجهاد, مثل زيادة مستوى (MDA) . الطريقة: اربعة وعشرين ارنب محلي استخدمت في هذه التجربة حيث قسمت عشوائيا الى اربعة مجاميع في كل مجموعة 6 أرانب . المجموعة الأولى: أعطيت المحلول الملحي فمويا لمدة ايام التجربة. المجموعة الثانية: أعطيت دواء الدايكلوفيناك الصوديوم (5ملغم/كغم) ثلاث مرات يوميا ولمدة ثلاثة ايام . المجموعة الثالثة: أعطيت مادة متعددة السكريات الدهنية (LPS) وبجرعة (150ميكروغرام/كغم) 24 ساعة من نهاية التجربة. المجموعة الرابعة: أعطيت دواء الدايكلوفيناك الصوديوم اضافة الى مادة متعددة السكريات الدهنية (5 ملغم/كغم, فمويا و 150 ميكروغرام/كغم عن طريق الوريد). ثم في نهاية التجربة تم قياس الملوندايديهايد (MDA) في أنسجة الكبد ونسبة انزيمات الكبد. النتائج: مادة الدايكلوفيناك سببت ارتفاع احصائي في نسبة اسبارتيت امينو ترانزفيريز (ALT) . متعدد السكريات الدهنية سبب في نسبة الأسبارتيت امينو ترانزفيريز (AST) توليفة الدايكلوفيناك مع متعدد السكريات الدهنية تسبب في زيادة احصائية ملحوظة في نسبة كلاً من المالنديالهايد ($p > 0.05$) والالانين امينو ترانزفيريز ($p > 0.01$) والأسبارتيت امينو ترانزفيريز ($p > 0.01$). أُلستنتاج: أن متعدد السكريات الدهنية يمكن ان يسبب تعزيزا لتأثير الدايكلوفيناك السمي الكبدي . التأثير يحدث بواسطة الكسدة والالتهابات كما هو واضح في زيادة نسبة المالنوندايديهايد في أنسجة الكبد وان هذه الأكسدة والالتهاب تحدث قبل التغيرات النسيجية وهذه ليس لها علاقة مع التغيريلر النسيجي .