Research Article

Impacts of simultaneous administration of omega-3 fatty acids with amoxicillin/clavulanic acid on albino rats' liver and bile

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ABSTRACT

Most drugs undergo some metabolism in the liver before excretion by the kidneys or bile. Thus, it is not surprising that liver injury may be provoked due to its exposure to various drugs and compounds. Drug-induced cholestatic liver injury may occur particularly under conditions of increased drug concentrations, genetic alterations in expression of enzymes or transporters. Additionally, the drug-induced cholestasis can be caused by direct toxic effects of drugs or their metabolites on different hepatic cell types or through an immune-mediated process. Amoxicillin/ clavulanic acid, an antibiotic that is therapeutically utilized for the treatment of a number of bacterial infections. Omega-3 fatty acids are unsaturated fatty acids that have roles in human physiology including α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. This study was designed to examine the impact of co-administration of omega 3 with therapeutic dose of Amoxicillin/ clavulanic acid for 14 days on rats' liver. The animals utilized in this study were allocated into 3 groups (six rats each) as negative control, amoxicillin/ clavulanic acid, amoxicillin/ clavulanic acid and omega 3. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) activities; and serum tumor necrosis factor –alpha (TNF-α), interleukin 10 level were determined. The results showed significant increase (P<0.05) in serum activities of ALT, and ALP; and in serum IL10 compared to the corresponding level in negative control rats. Moreover, a significant decrease in serum activity of ALP, TNF- α, and IL10 levels (P<0.05) were observed in group of rats treated with the combination of omega 3 and amoxicillin/ clavulanic acid compared to amoxicillin/ clavulanic acid-treated rats for 14 days. In conclusion, this study demonstrated that co-administration of omega 3 with amoxicillin/clavulanic acid for 14 days moderately alleviate the injurious effects of the intended antibiotic on rats' liver and bile.

Keywords: ALT, AST, ALP, Cholestasis, Clavulanic acid, Amoxicillin.

INTRODUCTION

The liver is the principal organ for metabolism and elimination of many drugs. The majority of oral drugs and xenobiotics are lipophilic and water-insoluble, enabling easy absorption across intestinal cell membranes.

Liver injury is a potential complication of many different drugs. This is not surprising, given the important role played by the liver in the metabolism and excretion of drugs from the body. Most drugs undergo some metabolism in the liver before excretion by the kidneys or through bile, though some drugs have little or no metabolism within the liver. Drug induced liver injury and hepatotoxicity has been reported to occur in 2–10 % of patients hospitalized for jaundice.[1,2] Furthermore, in several large retrospective DILI studies, cholestatic pattern has been found in 20–40 % of patients, hepatocellular in 48–58 % and mixed pattern in 12–20 %[3,4].

Bile is made up of the bile acids, bile pigments, and other substances dissolved in an alkaline electrolyte solution that resembles pancreatic juice. About 500 mL is secreted per day. Some of the components of the bile are reabsorbed in the intestine and then excreted again by the liver (enterohepatic circulation). In addition to its role in digestion and absorption of fats, bile (and subsequently the feces) is the major excretory route for lipid-soluble waste products.

Amoxicillin, an acid stable, semi-synthetic drug belongs to a class of Penicillins (β -lactam antibiotics). It is shown to be effective against a wide range of infections caused by wide range of Gram -positive and Gram- negative bacteria in both human and animals. Amoxicillin is a commonly used semisynthetic penicillin that is associated with a very low rate of mild hepatocellular and cholestatic liver injury when used alone.[5,6]. However, when amoxicillin is combined with the β-lactamase inhibitor, clavulanic acid or clavulanate, the estimated risk of hepatotoxicity increases from 3 to 17 per 100,000 prescriptions presumably due to the clavulanate component.[6]

Amoxicillin/clavulanate potassium is an oral antibacterial combination consisting of the semisynthetic antibiotic amoxicillin and the β-lactamase inhibitor, clavulanate potassium (the potassium salt of clavulanic acid). Clavulanic acid is produced by the fermentation of *Streptomyces clavuligerus*. It is a β-lactam structurally related to the penicillins and possesses the ability to inactivate a wide variety of β-lactamases by blocking the active sites of these enzymes. Clavulanic acid is particularly active against the clinically important plasmid-mediated β-lactamases frequently responsible

for transferred drug resistance to penicillins and cephalosporins [7] .

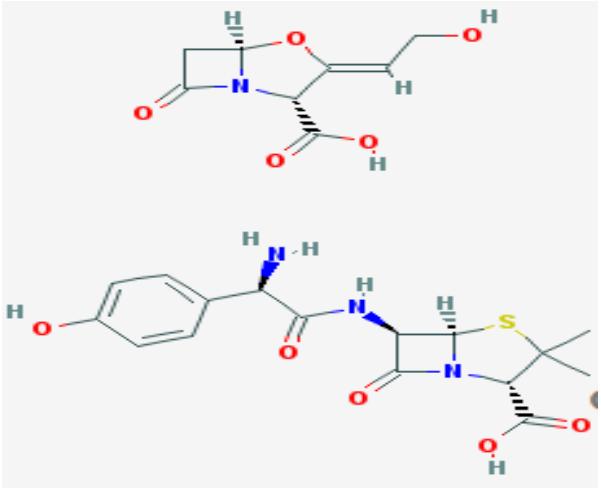

Figure 1: Chemical structure of amoxicillin/clavulanic acid

Omega-3 polyunsaturated fatty acids (PUFAs), such as Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are abundant in marine organisms. They are formed by desaturase and elongase enzymes respectively .However, most vertebrates, including humans, cannot synthesize high levels of long chain PUFAs because the essential desaturases in these species are not sufficiently efficient [8]. These fatty acids play an important role in cell membrane composition which, in turn, influences fluidity and cell surface biochemical signaling, and may serve as natural ligands for certain nuclear receptors that affect gene expression.

Animals and Methods

Animals

Eighteen female albino rats of both sexes weighing 200-300g were used in this study; they were purchased from The Animal House of the College of Sciences /University of Dhi Qar and maintained under conditions of controlled temperature and humidity and light/dark cycle in the animal house of the college of pharmacy / university of Baghdad . The animals were fed standard laboratory pellets and tap water *ad libitum* .

Experimental design

This study was approved by The Local Research Ethical Committee in College of Pharmacy, University of Baghdad. The animals utilized in this study were allocated into three groups as follows:

Group 1: six rats were orally administered distilled water and fed normal dietary pellets and considered as negative control.

Group 2: six rats received amoxicillin/clavulanic acid 185 mg/kg/day given orally twice daily for 14 days

Group 3: six rats received omega 3 100 mg /day given once daily simultaneously with amoxicillin/clavulanic acid 185 mg/kg/day given orally twice daily for 14 days.

Drugs and chemicals

No.	Chemicals	Suppliers
1	Amoxicillin 250 mg suspension	Sandoz / Austria
2	Augmentine 475 mg suspension	Mepha Pharmaceutical industries Suiss
3	Omega 3	Vitex pharmaceuticals . Australia
4	Rat ALP Elisa kit	Yh Biosearch Laboratory - China
5	Rat TNF- alfa Elisa kit	Cusabio Biotech co., ltd - China
6	Rat Alanine aminotransferase (ALT) Elisa kit	Cusabio Biotech co., ltd - China
7	RAT ASpartate Aminotransferase (AST) Elisa kit	Cusabio Biotech co., ltd - China
8	RAT INTERLEUKIN 10 (IL-10) ELISA KIT	Cusabio Biotech co., ltd - China
9	Total Antioxidant Status (TAS) Kit	Cayman Chemical Company .USA.

MATERIALS AND METHODS

Preparation of serum samples

After euthanization of rats by anesthetic ether, blood was collected from the neck and put in plain tube; the clot was dispersed with glass rod and then centrifuged at 3000 (rpm) for 15 minutes . Serum samples were utilized for determination of ALT, AST, ALP activities in addition to TNF-alpha and IL10.

Determination of Serum Alanine Aminotransferase (ALT) Activity.

The principle of this assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for ALT has been pre-coated onto a microplate. Standards and samples are pipette into the wells and any ALT present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for ALT is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ALT bound in the initial step. The color development is stopped and the intensity of the color is measured. The detection range : 3.12 mIU/ml-200 mIU/ml.

Determination of Serum Aspartate Aminotransferase (AST) Activity.

The principle of this assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for AST has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any AST present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for AST is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of AST bound in the initial step. The color development is stopped and the intensity of the color is measured. Detection range : 3.12 mIU/ml-200 mIU/ml.

Determination of Alkaline phosphatase (ALP) activity:

The principle of alkaline phosphatase (ALP) ELISA kit practices the competitive enzyme immunoassay technique utilizing a monoclonal anti-ALP antibody and an ALP-HRP conjugate. The steps of the determination of ALP are similar to those mentioned previously under ALT and AST but in this assay, the sample and buffer are incubated with ALP-HRP conjugate instead of ALT-HRP or AST-HRP conjugate. The activity of serum ALP was expressed as ng/ml.

Determination of serum Interleukin-10 (IL-10) levels :

The principle of this assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-10 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-10 bound in the initial step. The color development is stopped and the intensity of the color is measured. Detection range : 3.12 pg/ml-200 pg/ml.

Determination of serum Tumor necrosis factor alpha (TNF-α) levels:

The principle of this assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for TNF-α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF-α present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for TNF-α is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TNF-α bound in the initial step. The color development is stopped and the intensity of the color is measured. Detection range : 6.25 pg/ml-400 pg/ml.

Statistical analysis

The significance of differences between the mean values was calculated using unpaired Student's T-test. Comparisons among treated groups were made using analysis of variance (ANOVA). P-values less than 0.05 were considered significant for all data presented in this study.

RESULTS

Groups of rats orally administered therapeutic dose of amoxicillin/clavulanic acid 185 mg/kg/day given orally twice daily by gavage tube for 14 days showed significant increase in ALT activity (P<0.05) compared to negative control (a 1.35 fold increase) and significant increase in ALP activity (p<0.05) compared to negative control (a 1.34 fold increase) and non-significant difference in AST activity (p>0.05) compared to negative control as shown in table 1.

Table 1: Effects of therapeutic dose of Amoxicillin plus clavulanic acid orally administered for 14 days on the serum activities (ALT), (AST), and (ALP) in rats (N=6).

Group	ALT mIU/ml	AST mIU/ml	ALP ng/ml
Negative Control/DW	14.05±3.803	4.33±1.085	334.616±95.746
Amoxicillin/clavulanic acid-treated for 14 days 185 mg/kg/day	19.05 ±3.48*	4.35±1.492	450.733±42.302*

- Each value represents Mean ± standard deviation (SD).
- P * < 0.05 significant difference in comparison with the negative control group.
- N = number of animals.

Concerning the effects on TNF-α, rats orally administered therapeutic dose of amoxicillin/clavulanic acid 185 mg/kg/day twice daily for 14 days showed a non-significant difference (P>0.05) in serum TNF-α levels compared to negative control, while a significant increase in serum IL10 levels (p<0.05) were observed compared to negative control group as shown in table 2.

Animals orally administered therapeutic dose of amoxicillin/clavulanic acid 185 mg/kg/day with 100 mg/day ω-3 fatty acids for 14 days showed significant decrease (P< 0.05) in (ALT, and ALP) serum enzymes activities (a 0.42 and 0.75 fold decrease respectively) compared to the corresponding level in rats treated with

Table 2: Effects of therapeutic dose of Amoxicillin plus clavulanic acid orally administered for 14 days on serum levels of IL10 and TNF- α in rats (N=6).

Group	IL10 Pg/ml	TNF- α Pg/ml
Negative Control /DW	19.516±9.992	10.483±2.442
Amoxicillin plus clavulanic acid –treated for 14 days 185 mg/kg/day	50.883±11.029*	9.183±3.249

- Each value represents Mean ± standard deviation (SD).
- P^{*} < 0.05 significant difference in comparison with the negative control group.
- N = number of animals.

amoxicillin / clavulanic acid alone ; while a non-significant difference (*P*>0.05) in the level of AST was observed in serum of rats orally treated with a therapeutic dose of amoxicillin / clavulanic acid 185 mg/kg / day in combination with 100 mg / day ω-3 fatty acids compared to the group treated with amoxicillin/ clavulanic acid alone for the same period Table 3.

Table 3: Effect of co-administration of Therapeutic dose of Amoxicillin /clavulanic acid and omega 3 on the activities of serum (ALT), (AST), and (ALP) in rats compared to Amoxicillin/clavulanic acid-treated group for 14 days. (N=6).

Group	ALT mlu/ml	AST mlu/ml	ALP ng/ml
Amoxicillin plus clavulanic acid –treated 185 mg/kg/day for 14 days	19.05±3.474[A]	4.35±1.492	450.733±42.30[A]
Amoxicillin plus clavulanic acid 185 mg/kg/day and omega 3-treated 100 mg/day for 14 days	8.16±4.78[B]	5.1±0.764	338.85±35.596[B]

- Each value represents Mean ±SD.
- Values with non –identical capital letters (A, and B) are significantly different (*P*<0.05).
- N = number of animals.

Animals orally administered therapeutic dose of amoxicillin/clavulanic acid 185 mg/kg/day with 100mg/day ω-3 fatty acids for 14 days showed significant reduction (*P*<0.05) in serum levels of IL10 (a 0. 47 fold decrease) compared to the corresponding level in rats treated alone with amoxicillin / clavulanic acid, while the level of TNF- α showed non-significant difference (*p*>0.05) between groups. Table 4.

Table 4: Effect of co-administration of Therapeutic dose of Amoxicillin/ clavulanic acid and omega 3 on serum levels of (IL10, and TNF-α) in rats compared to Amoxicillin/clavulanic acid-treated group for 14 days. (N=6).

Group	IL10 Pg/ml	TNF- α Pg/ml
Amoxicillin plus clavulanic acid -treated 185 mg/kg/day for 14 days	50.883±11.029[A]	9.5±4.46[A]
Amoxicillin plus clavulanic acid – treated 185 mg/kg/day and omega 3-treated 100 mg/day for 14 days	24.416±9.491[B]	13.31±4.893[B]

- Each value represents Mean ± standard deviation (SD).
- Values with non –identical capital letters (A, and B) are significantly different (*P*<0.05).
- N = number of animals.

DISCUSSION AND CONCLUSION

The group of animals orally administered therapeutic dose of amoxicillin / clavulanic acid 185 mg/kg/day for 14 days showed significant increase in ALT activity (*p*<0.05) compared to negative control (a 1.35 fold increase) and significant increase in ALP activity (*p*<0.05) compared to negative control (a 1.34 fold increase) and non-significant difference in AST activity (*p*>0.05) compared to negative control.

Furthermore, it has been noticed that animals orally administered therapeutic dose of amoxicillin / clavulanic acid 185 mg/kg / day with 100 mg / day ω-3 fatty acids for 14 days showed significant decrease (*P*< 0.05) in (ALT, and ALP) serum enzymes activities (a 0.42 and 0.75 fold decrease respectively) compared to the corresponding level in rats treated alone with amoxicillin / clavulanic acid; while a non-significant difference (*P*>0.05) in the level of AST was observed in serum of rats orally treated with a therapeutic dose of amoxicillin / clavulanic acid 185 mg/kg / day in combination with 100 mg / day ω-3 fatty acids compared to the group treated with amoxicillin/ clavulanic acid alone for the same period.

IL-10 an immune regulatory cytokine , help regulate the immune response by inhibiting the proliferation of certain immune cells and promoting the proliferation of others; reducing the production of inflammatory cytokines; and promoting the secretion of antibodies, which bind to specific foreign molecules, thereby inactivating those molecules and marking them for destruction by other immune cells [9].

In the present study, IL10 level was increased significantly (p < 0.05) in the group received amoxicillin or

amoxicillin/clavulanic acid of 14 days (1.91 and 2.6 fold increase respectively). IL10 levels showed non-significant difference ($p > 0.05$) in the group received amoxicillin/clavulanic acid in combination with Omega-3 for 14 days as compared with negative control, which means that the noticed increase in IL-10 was resolved when Omega-3 was used.

Tumor necrosis factor (TNF) is an inflammatory cytokine produced by macrophages/ monocytes during acute inflammation and is responsible for a diverse range of signaling events within cells. TNF exerts its biological functions by interactions with two members of the TNF receptor (TNFR) super family, namely TNFR1 and TNFR2. The cytoplasmic tail of TNFR1 contains a death domain, which is essential for the induction of apoptosis.[10, 11].

In the present study TNF-α levels did not change significantly ($p > 0.05$) throughout the study which indicate that amoxicillin/clavulanic acid combination used did not initiate an inflammatory response in the acute phase (during the study), and this comes in consistence with previous studies that mentioned a considerable delay (3-4 weeks) between discontinuation of amoxicillin/clavulanic acid intake and symptoms of liver injury[12-14].

Furthermore, this study showed that TNF-α levels did not change significantly ($p > 0.05$) when Omega-3 was administered with amoxicillin/clavulanic acid combination for 14 days.

In conclusion, this study showed that amoxicillin/clavulanic acid administered for 14 days produced certain degree of cholestatic hepatitis manifested by elevated ALT and ALP and that IL-10 was elevated in a compensatory manner to regulate inflammatory mediators. Additionally the current study proved that, omega-3 removed the cholestatic hepatitis insult on hepatocyte by reducing signs of inflammation through the reduction- of IL10 level and activity of ALT, and ALP back to normal and thus, may possess a hepato-protective effects.

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