Assay and biochemical effect of long term high dose and abuse administration of ceftriaxone In experimental animals

Mansour Hamed Mohamed
National Organization for Drug Control and Research NODCAR

ABSTRACT

Ceftriaxone is a drug proved to have efficacy on community acquired infection including uncomplicated gonorrhea, acute pyelonephritis and various infections in children. Recent studies have demonstrated that ceftriaxone induces reversible precipitates in the gallbladder. This complication is referred to as "biliary pseudolithiasis", and it has symptoms similar to the liver dysfunction usually occurs in children receiving high doses of ceftriaxone. The patient's jaundice subsides, and the liver function test results usually improve, and return to baseline levels after the end duration of treatment. The present study was designed to describe the effect of high dose and abuse treatment of experimental rats with ceftriaxone (500 and 1000 mg/kg B.W.) every 12 hours for one week on liver function tests.. After 7 days of therapy, (ALT, AST), total, direct, and indirect bilirubin levels were evaluated in the experimental rats and the concentration of the drug was determined by high pressure chromatography. The results of the study showed significant elevation in all measured parameters by the end of one week ceftriaxone therapy. Therefore, it could be concluded that the choice of a more safe and potent antibiotics require selective investigation concerning the group of antibiotic, the dosage and the duration as well as the type of disease[(1)].

INTRODUCTION

Antibiotics are substances, produced in substrates during the growth of microorganism, which in low concentration destroy or inhibit the growth of other species of microorganism .Therefore, the antibiotics are generally considered as antimicrobial and in other conditions are used as anti-infection drug . Third-generation cephalosporins is commonly used and proved to have antimicrobial activity against many gram-positive and gram-negative organisms. Generally, ceftriaxone is a safe antibiotic[(1)]; however, symptomatic biliary sludge has been reported in rare instances, most of which have involved children. It is uncommon for ceftriaxone to cause increases in laboratory indices, such as bilirubin levels and (AST,ALT), used similarly to cefataxime for the treatment of susceptible[(2)] infections. They include Chancroid, endocarditis. shigellosis, gonorrhea, lyme diseases, Meningitis .Pneumonia , septicaemia , syphilis, and typhoid fever . It is also used for surgical infection prophylaxis

[3].The present study deals with the HPLC assay to evaluate the efficacy of the drug ceftriaxone on the long term high dose and abuse administration effect on experimental animal rats. The dosage as well as duration of the antibiotic administration were also considered.

MATERIALS & METHODS

1-Test drug

Ceftriaxone, SANDOZ 500 mg and 1 gm as (ceftriaxone sodium cephalosporins antibiotics)

2-Dosage and Administration

20 to 50 mg/kg once daily, the maximum dose should not exceed 50 mg/kg once daily (equivalent to 500 and 1000mg/vial) were administrated two time every 12 hrs in a dose of 20 and 50 mg/kg body weight for a one week, These doses were equivalent to human therapeutic dose of the test drug

3-Experimental Design

40 Male albino rats from the animal house of National Organization for Drug Control and Research (NODCAR) weighting 150-250 grams were used in thestudy. Control animals were included simultaneously with experimental groups .Rats were divided equally into four groups ten in each .All rates were fed on the normal basal diet[4]and treated with the equivalent therapeutic dose of antibiotic drug ceftriaxone.

Group (1) rats fed on the normal basal diet for a period of one week and not treated with ceftriaxone used as a control.

Group (2) rats fed on the normal basal diet and treated with ceftriaxone 500 mg/kg b.w for a period of one week.

Group (3) rats fed on the normal basal diet and treated with ceftriaxone 1000 mg/kg b.w, for a period of one week

Group (4) rats fed also on the normal basal diet and treated twice daily with ulternative doses of ceftriaxone one time with a dose (500) mg/kg b.w. and the other with (1000) mg/kg b.w for a period of one week .

4-Chemicals

All chemicals were of pure analytical grade (HPLC 100 %), Purchased from Biosystems , Randox and Biomerieux

HPLC assay of ceftriaxone according to[5].

Mobile phase

Buffer PH **7** **13.6**gm dibasic potassium sulfate + **4** grams monobasic potassium phosphates complete with distilled water to **1** liter , adjust PH till **7** with H3PO4 or KOH Buffer PH **5** 25.8 gm of sodium citrate in **500** ml distilled water, adjust PH till **5** with H3PO4 or KOH **3.2** gm heptansulphonic acid in **400** ml Acetonitril + **44** ml buffer PH **7** + **4** ml buffer PH **5** and complete with distilled water to **1** liter

***HPLC Conditions*:**

Column C18 phenomenx (4.6 mm x 125 mm x 5u) or equivalent
Flow rate = 2 ml/min
Wave length 270 nm
Chart speed 0.5 cm/min

Retention time 1.458 min to separate the test drug antibiotic ceftriaxone and the percentage of the drug concentration in each vial was determined

5- Blood samples were withdrawn from retinobulbar venous plexus by

means of fine capillary glass tube. Sera were separated and kept at –2 degree centigrade until the time of determination of the following parameters:

a- Total, direct, and indirect bilirubin serum levels[6].
b- AST(SGOT) and ALT(SGPT) aminotransferase enzyme activities[7].

Statistical analysis:

The obtained data were statistically analyzed by NOVA using[8] for different groups, and $p<0.05$ was considered significant.

RESULTS

The data shown by high pressure liquid chromatography (HPLC) revealed that the mean peak area of the ceftriaxone standard= 524405, the mean peak area in either case of the ceftriaxone tests are = 54355 (500 mg/kg b.w) and 540353 (1000 mg/kg b.w)

The mean values of the peak area of the, test, standard and concentration of ceftriaxone in either case (500, 1000) mg/kg b.w respectively by high pressure liquid chromatography (HPLC) conditions.

Table 1: Retention time,peak area and concentration of ceftriaxone in the different groups of the study.

	Retention time by min	Area by mV	500 mg/v2	1000 mg/v
Test	1.555		54300 54571	53450 54657
Standard	1.555	52788 52093		
Mean		524405	544355	540535
Concentration of ceftriaxone %			103.8% 519.5 mg/v	103.75% 1037.5mg/v

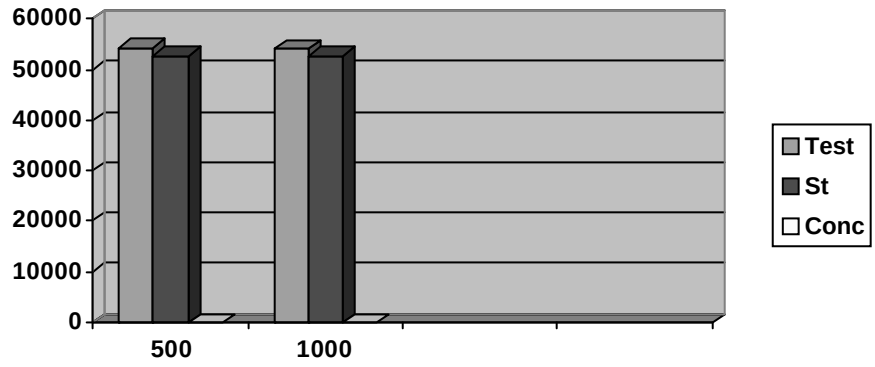

Fig 1: Mean peak area of ceftriaxone test and standard

The concentration of ceftriaxone in the two cases can be determined by dividing the mean peak area of the test divided on the mean peak area of the standard x100 as 544355 / 524405 **x**100 =103.8 % , (103.8 x 500) = 519.02 mg/kg b.w and 103.75 % which equivalent 1037.5 mg/kg b.w respectively and system suitability was adjust.

Elevations concomitant with antibiotic ceftriaxone administration were detected in serum total and conjugated bilirubin , serum ALT (SGPT) and AST (SGOT). However, aggravated hyperbilirubinaemial effect were noticed in long term high dose and abuse administration in groups 3 and 4 as shown in table 2.

Table 2: Serum Total, direct and indirect bilirubin (mg/100) (Mean values ±SE) of experimental male albino rats treated with long term high dose and abuse of ceftriaxone (500 and 1000 /mg/b.w) compared to normal duration and control.

Biochemical Parameters (mg/100)	Group (1) Control	Group (2) Normal duration	Group(3) long term high dose	Group(4) Abuse
Totalbiliru T. Bilirubin	0.35±0.5	0.40**±0.15	0.70**±0.03	0.82**±0.02
Direct bilir D. Bilirubin	0.15±0.002	0.20*±0.002	0.25 **±0.002	0.28**± 0.002
Indirect bi I. Bilirubin	0.20±0.002	0.20±0.002	0.35**±0.007	0.47**±0.007

P> 0.05 Non Significant
***P< 0.01 Highly Significant*

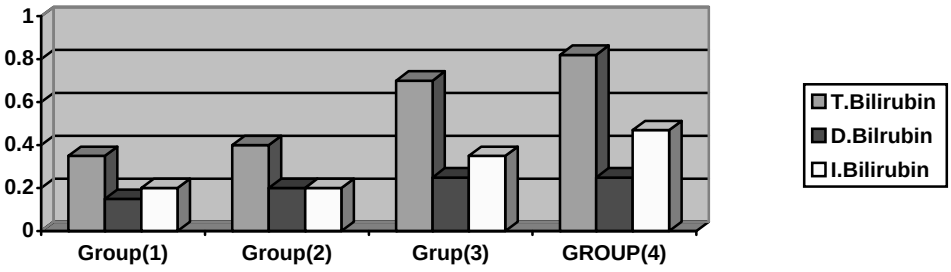

Fig 2: Mean values of serum total, direct and indirect bilirubin in the four different groups

The transient elevation in liver enzyme (AST and ALT) values reported during the normal duration of the test drug antibiotic administration in group (2) was replicated nearly twice time or more in long term high dose and abuse of ceftriaxone. Mainly significantly elevated values were recorded in ALT (SGPT) and AST (SGOT) enzymes in groups (3 and 4). Meanwhile, AST was extended in the elevation from group (3) to group (4) and maintained elevated after the administration of the ceftriaxone due to the extend of the half life elimination of AST immunoglobulin complex as shown in the table 3.

Table 3: Serum ALT (SGPT) and AST (SGOT) u/L (Mean values ±SE)of experimental male albino rats treated with long term high dose and abuse of ceftriaxone compared to control and normal duration of the test drug

Biochemical Parameters (U/L)	Group (1) Control	Group (2) Normal duration	Group (3) long term high dose	Group(4) Abuse
ALT (SGPT)	6. 5±0.5	13.8**±0.7	20.8**±1.2	24. 5** ±1.4
AST (SGOT)	9.8±0.5	11. 5*±0.7	14.8 **±0.9	18.7±1.2
AST/ALT ratio	1.5	0.83**	0.71**	0.76**

P> 0.05 Non Significant
***P< 0.01 High Significant*

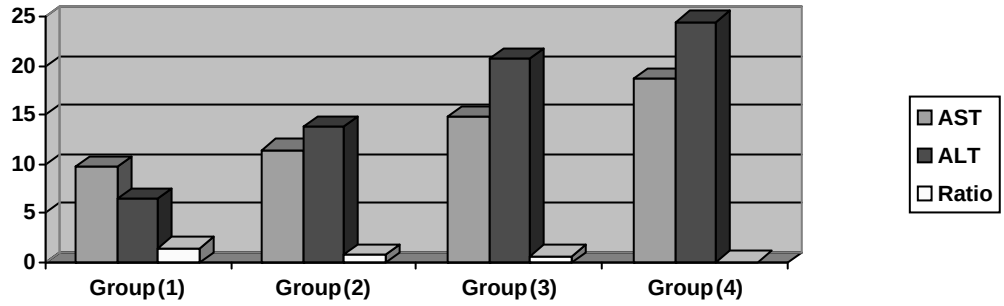

Fig 3: Mean values of serum ALT, AST u/L and AST/ALT ratio in the four different groups

DISCUSSION

In the present study, the biological effect of long term and abuse administration of ceftriaxone were studied in experimental animals.

The results of this study showed that the administration of cefatriaxone 500 and 1000 (mg/kg.b.w) did not cause any marked elevations in the serum direct bilirubin this means that no displacement for the conjugated bilirubin has occurred in normal rats injected with ceftriaxone[9,10].

Similar finding were demonstrated [11], who failed to demonstrate any measurable displacement of bilirubin from albumin in newborn infants given the antibiotic. Therefore, the recorded increase in serum total bilirubin as in groups (3 and 4) could be referred to increase in unconjugated form[12].

It may be pointed out that unconjugated hyperbilirubinaemia[9] can result from toxin induced liver dysfunction such as that caused by chloroform, arsphenamines, carbon tetrachloride, acetaminophen, hepatitis virus and cirrhosis etc. Although most of this acquired disorders could be due to pranchymal cell damage, there is frequently a component of obstruction of biliary tree within the liver that may results in the presence of some conjugated hyperbilirubinaemia[13]. The undue effect of ceftriaxone seemed to be belonging to symptoms similar to the last disorder, since, it has been indicated in groups (3 and 4).

The results shown in group (3 and 4) revealed that the two cefatriaxone doses (500 and 1000) mg/kg.b.w caused elevation in the serum total bilirubin level at the end of experimental period. Such an effect could be due to residual property appeared after the excretion of the antibiotic from tissues to the bile. these explanation seemed consistence with previous reports stated that there is extensive excretion of ceftriaxone in bile[12].

The recorded elevations seemed consistent with previous studies which reported that ceftriaxone competition with bilirubin for albumin binding[14] therefore, it may increase serum bilirubin level[15].

Elevations in serum total bilirubin that was not coupled with similar elevations of serum direct bilirubin started from initial administration of ceftriaxone and maintained with the same significance of magnitude (in case of long term high dose and abuse administration) until the end of the experimental period (one week) of rats, could be referred to the competition between the excess serum total bilirubin and ceftriaxone with the high affinity sites of albumin where excess bilirubin can be bound only loosely to the low affinity[16] site.

It may be also pointed out that a number of compounds such as antibiotics and other drugs compete with bilirubin for the high affinity binding site on albumin[17].

As it has been previously recorded that the treatment of experimental rats with ceftriaxone in normal duration to the end of experimental period (one week) not cause any effect on serum AST, ALT and T.bilirubin .Therefore , any significant fluctuations in these enzymes would be consequences of the long term high dose and abuse administration of ceftriaxone only.

The data shown in (group 3, and 4) proved that this was the case where, almost always, identical significance of magnitude for the recorded elevations under long term high dose and abuse conditions were indicated after administration of the ceftriaxone. Meanwhile, similar duration of effects were also recorded.

The recorded elevations in serum level of AST and ALT seemed consistent with the general properties of the groups to which the ceftriaxone were recorded[18].

According to these authors the abnormality in liver enzymes during ceftriaxone were probably with no clinical significance, other authors also reported that cephalosporins liver enzymes elevations have been transient , returning to normal after withdrawal of treatment at the end of experimental period (one week) as in group (2) .

The data shown in group (3 and 4) revealed that the elevations recorded in AST and ALT level after the administration of the two dosages (500,1000 mg/kg/b.w) of cefatriaxone to normal rats were persistent until the end of the experimental period. This finding could be due to the so called macro AST. In this conditions the specific enzyme protein is bound to an immunoglobulin (usually to **IgG**) and the prolonged half-life elimination of the enzyme immunoglobulin complex is thus extended[19].

It may be pointed out that the recorded elevations in the serum in AST and ALT seemed to be characteristic for the ceftriaxone antibiotic, these results were consistent with those reported by [20] for the third–generation cephalosporin.

The AST/ALT ratio that was performed according to[21]was significantly varied from the corresponding controls under normal duration to the long term high dose and abuse conditions. Similar findings were indicated after investigating the relationship between therapy with cephalosporins antibiotic (ceftriaxone) and alteration in serum level enzymes and bilirubin in human subjects)

Conclusion

The prementioned results strongly suggest that antibiotic test cefatriaxone as well as the long term high dose treatment and abuse administration displayed variable side effects on serum total bilirubin pattern as well as liver enzymes under normal conditions. Meanwhile, in case of long term high dose and abuse administration of ceftriaxone aggravated the recorded side effect showing symptoms similar to liver dysfunction in many case of hyperbilirubinaemia and jaundiced conditions. However, calcium ceftriaxone salts was a major component of bile stone associated with biliary sludge and pesudolithiasis[22,23].

This study confirms the possibility of precocious biliary lithiasis under ceftriaxone therapy in childhood and their spontaneous dissolution after discontinuation of the drug. Therefor, caution in the treatment of neonates , ill children aging less or equal 10 years , (hypoprothrombinemial children) [24] and some jaundiced adult by high dose and abuse of ceftriaxone . Therefor ,it may also convenient to

reduce ceftriaxone dosage during t treatment to prevent the habitation and synthesized conditions of pesudolithiasis , biliary sludge [25] and intractable hiccups [26].Clinicians need to be aware of the association of ceftriaxone with biliary pseudolithiasis , and jaundiced patients, were monitor accordingly

It may pointed out that biliary pesudolithiasis were also occur in children receiving high dose of ceftriaxone. The antibacterial and pharmacokinetic benefits of ceftriaxone outweigh the problem of reversible biliary pesudolithiasis with this drug.

REFERENCES

1. **Reynolds, J., Parsons, A., and Sweetmans,S (2005)** MARTINDALE. The Extra pharmacopoeia, 34 edition th London Chicago P 183- 186.
2. **Brogden RN Ward A. (1988)** Ceftriaxone: a reappraisal of its antibacterial activity and pharmacokinetic properties, and an update on its properties, use with particular reference to once daily administration. Drugs ; 35: 604- 45
3. **Perry TR, and Schentag JJ. (2001)** Clinical use of ceftriaxone: a Pharmacokinetic Pharmacodynamic prospective on the impact of minimum inhibitory concentration and serum portion binding. Clinical pharmacokinetic 40: 685 – 94
4. **Ahamed , A.S (1976)** Breeding and housing of experimental animals NAMERU-3, Abbassia , 3rd Edit ., 43-73.overview .J. Infect .137; 560 –573
5. **USP NF (2006)** ASIAN EDIATION, The official Compendia of Standards
6. **Jendrassik, L., Grof, P., (1938)** Biochem. Z.81, 297
7. **Reitman, S., and Frankel , S (1957)** A micromethod for the determination of aminotrnsferase AM.J. Clin. Patho., 28; 56- 63
8. **PC-STAT (1985)** : Statistical programs .Coded by Mohn Rao, Kathleen Blane and Marc Zonnenberg , Univ. of Georgia
9. **Isselbacher, KJ. (1994)**: Bilirubin metabolism and hyperbilirubinaemia. In: Harrisons, principles of internal medicine, 13 th ed. Isselbacher, K J., (editors) McGraw -Hill
10. **Fink S. (1987)**: Ceftriaxone effect on bilirubin- albumin binding pediatrics , 80 : 873-5
11. **Broderson, R. and Ebbesen, F. (1983):** Bilirubin - displacing effect of cephalosporins , in vitro
12. **Smithivas T Hyams, PJ., and Rehal, Jr (1971):** Ceftriaxone and ampicillin in human bile.J.Infect Dis .124 (Suppl 24) 102 :106
13. **Wolkoff, AW., Chowhurhy, JR and Arias (1983):** Hereditary jaundice and disorder of bilirubin. In: The metabolism Basis Inherited Disease 5 th ed. Stanury JB et al ., McGraw Hill , p 55 –70
14. **Robertson, A. Fink, S. and Karp W. (1988):** Effect of cephalosporins on bilirubin - albumin binding. J Pediatric 112:291-294. and in newborn infants. J. pharm Sci. 72: 248 – 253

15. **Marti, MC. Farquet, C and Fabre, J. (1981):** Pharmacokinetics and biliary excretion of cephalosporin in patients with bile duct drainage. Infection q (Suppl 1) : 534 – 36
16. **Takase, Z. Shirafuki, U. and Uchida M. (1980):** Fundamental and Clinical studies of cephalosporin (T -1551) in the filed of antibiotics and gynecology. Chemother, 28 (Suppl 6) : 825 – 826
17. **Marry, K., Granner, K., Mayes, P., et al. (1998):** Harper's Biochemistry 32 th ed ,Norwalk, Connecticut ,SanMatea , California P 442 – 262
18. **Olin, B. Hebel, S. Connell, S. et al., (2004):** Drug facts and comparison. Facts and Comparison P. (1586)
19. **Moss D., and Rosalki, S (2002):** Enzyme tests in diagnosis, Oxford university press Inc., Newyork. London Sydney. Auckland. P34
20. **Mor, F. Leibovici , L,Cohen et al. (2004):** Prospective evaluation of liver function tests in patients treated with cephalosporins united state Feb 32 (3): 235: 37
21. **DeRitis, F. and Cacciatara, L. (1983):** Differential diagnosis of Liver diseases. In: Csmos G, Thaler H eds. Clinical Hepatolgy. Berlin : springer - Verlag 16- 28
22. **Park HZ (1991):** Ceftriaxone associated gallbladder sludge :identification of calcium ceftriaxone salts as a major component of gallbladder precipitate, gastroentrology; 100 : 1665 – 70
23. **Kucers, A and Bennett, N. (1975):** The use of Antibiotics, 2 nd ed. William Heinemann Medical Books Ltd., London, England.
24. **Scimeca PG (1996):** Homolysis after treatment with cefatriaxone J pediatr ; 128- 163
25. **Schaad UB (1988):** Reversible cefatriaxone associated biliary pesudolithiasis in children. Lancet ii ; 1411- 13
26. **Bonioli E (1995):** Pesudolithiasis and intractable hiccups in a body receiving ceftriaxone. N Engl. J Med.; 331: 332.

دراسة حيوية لتأثير السيفاترياكسون كمضاد حيوي بجرعات مفرطة وعالية وطويلة على حيوانات تجارب بعد فصلة كروماتوجرافيا

منصور حامد محمد غيط

رئيس شعبة المضادات الحيوية الهيئة القومية للرقابة والبحوث الدوائية (نود كار)

تم إجراء التجربة على ٤٠ فأرا من النوع الألبينو قسمت الى أربع مجا ميع كل مجموعة تحتوى على ١٠ فئران: المجموعة الأولى تتغذى على الوجبة الأساسية وتستخدم كمجموعة ضابطة والمجموعة الثانية تتغذى على نفس الوجبة السابقة وتخضع للحقن العضلي للمضاد الحيوي المذكور ٥٠٠ مجم والمجموعة الثالثة تتغذى على نفس الوجبة وتخضع للحقن العضلي بجرعات عالية التركيز ١٠٠٠مجم و المجموعة الرابعة تتغذى على نفس الوجبة المذكورة وتخضع للاستخدام المفرط للمضاد الحيوي ٥٠٠مجم و ١٠٠٠ مجم هذا وقد استغرقت التجربة أسبوعا تم بعدة سحب عينات الدم وبعد فصل المصل تم تقدير الآتي :-١- مستوى الصفراء الكلية ٢- الصفراء المرتبطة وحساب الصفراء الحرة .

وكذا الأنزيمات الناقلة للأمين كالألنين والأسبرتات هذا وقد سبق ذلك فصل المضاد الحيوي على أجهزة التحليل الدقيق تحت الضغط العالي (الكروماتوجرافى) بعد حقن المادة القياسية للمستحضر تحت نفس الظروف ونفس الوقت لتحديد تركيز المضاد الحيوي قبل البدء في استعماله للأخذ في عين الاعتبار كفاءته لعلاج الأمراض المنوط باستخدامه لها وقد أظهرت النتائج ما يلي :-

١- تركيز الدواء يصل إلي ١٠٣% قبل البدء فى استخدامه كعقار وذلك بعد فصلة على أجهزة التحليل الدقيق مما يدل على كفاءته المنتظرة عند الاستخدام .

٢-احدث الحقن زيادة مؤقتة للأنزيمات الناقلة للأمين حيث تزول هذه الزيادة بزوال المؤثر أي تعود إلى معدلاتها لطبيعية بعد انتهاء الحقن كما في المجموعة الثانية وأخرى أظهرت ارتفاعات ممتدة ومقاومة للعودة إلى المستوى الطبيعي لها (نظرا لطول فترة نصف العمر للمركب المكون من المستحضر و إنزيم امينوجلوبيولن اى جى جى)

٣-أحدث الاستخدام الطويل والمفرط لجرعات عالية من المستحضر إلى زيادة في مستوى الصفراء الكلية والصفراء المرتبطة وكذا احدث خللا في إنزيمات الكبد الناقلة للأمين كما فى المجموعة الثالثة والرابعة

٤-وبذلك قد خلق الحقن بالمضاد الحيوي أعراضا مشابهة للأعراض الوبائية للكبد وقد تكون هذه الأعراض مؤشرا حقيقيا لحدوث حصوات مرارية او انسداد مؤقتا في متفرعات الحويصلة المرارية نتيجة استخدام الجرعات المفرطة وعالية التركيز للمستحضر على المدى الطويل .

٥- وتدل النتائج السابقة أن الحقن بالمضاد الحيوي المذكور أدى إلى تعميق الآثار السلبية السابقة كما يستحسن اخذ الحيطة و الحذر في العلاج بالمضاد الحيوي المذكور للأطفال حديثي الولادة الذين يعانون من صفراء فسيولوجية والآخرين المرض باعتلال فى الكبد و الأقل من عشر سنوات وكذا البالغين الذين يعانون من أمراض للكبد كالنزف وغيرة من الأمراض المرارية المختلفة .