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ORIGINAL RESEARCH

Superiority of Ceftriaxon to Cefazolin in a Rat Model of Obstructive Jaundice: An Experimental Study

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ABSTRACT

Objective: The objective of this study was to evaluate the serum and bile concentrations of cefazolin and ceftriaxone at the third and sixth hours in an experimental obstructive jaundice model and to identify the rate of excretion of these antibiotics into the bile. *Material and methods:* Thirty-two Wistar albino rats were used in this study. The bile and serum levels of cefazolin were measured at the third hour in the A1 group and at the sixth hour in the A2 group, with cefazolin administered as 5 mg/rat; while the bile and serum levels of ceftriaxone were studied at the third hour in the B1 group and at the sixth hour in the B2 group, with ceftriaxone administered as 5 mg/rat. *Results:* After 3 hr of cefazolin administration, the serum concentration in the A1 group reached a mean of 1.8 μg/ml, while the bile concentration was 90% of the serum concentration, with a mean of 1.6 μg/ml; whereas in the B1 group, the third-hour serum concentration of ceftriaxone was 18.6 μg/ml, while the bile concentration was found to be as high as 330% of this level, i.e., 56 μg/ml. The serum value of cefazolin decreased to 1.4 μg/ml in the A2 group and ceftriaxone decreased to 3.7 μg/ml in the B2 group at the sixth hour. *Conclusions:* Although the excretory level of cefazolin and ceftriaxone into the bile reaches therapeutic doses, the duration for which these levels are above those required for bactericidal activity is short. Ceftriaxone is better concentrated in the serum and bile than cefazolin.

Keywords: jaundice; cephalosporins; HPLC; excretion; MIC

INTRODUCTION

Biliary stasis may result in cholangitis, which can cause significant morbidity and mortality that occurs secondary to bacterial colonization through translocation [1, 2]. The obstruction leads to impairment in the biliary excretion of hepatocytes or cholangiocytes [3, 4]. The resultant increase in the biliary pressure may impair the biliary excretion of antibiotics [2]. For this reason, antibiotic selection in cases of obstructive jaundice and resultant cholangitis is of particular importance, and attention should be paid to the selected antibiotic to be an effective agent diffusing or secreting well into the bile. There are several studies in the literature using various methods to determine efficient antibacterial drugs and the biliary concentrations of antibiotics used in the treatment of biliary infections [5–9].

In a guide for intra-abdominal infections published by the Surgical Infection Society and the Infectious Diseases Society of America in 2009 [10], the treatment regime and some rules to be applied for the initial empirical treatment of biliary infections in adults were emphasized. Accordingly, cefazolin, sefuroksim, and ceftriaxone, each was reported to be useful in community-acquired mild-to-moderate infections in adults with suspected infection. It was also emphasized that both acute cholecystitis and acute cholangitis should receive antimicrobial therapy.

The antimicrobial effect is directly associated with the drug concentration reaching via the serum to the

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infection area [7, 11]. The effectiveness of beta-lactams depends on the minimum inhibitory concentration (MIC) and the duration for which the serum concentration of the used antibiotic is greater than its MIC value (time > MIC) [11, 12].

The objective of this study was to evaluate the serum and bile concentrations of cefazolin and ceftriaxone, which play an important role in the initial empirical treatment of hepatobiliary infections, at the third and sixth hours in an experimental obstructive jaundice model and to identify the rate of excretion of these drugs into the bile.

MATERIAL AND METHODS

This experimental protocol was submitted to and approved by the Istanbul Kartal Kosuyolu Yuksek Ihtisas Training and Research Hospital and the Experimental Medicine Research Center, Yeditepe University (YUDETAM) Ethical Committee. Thirty-two Wistar albino rats of both genders, weighted between 250 and 300 g, were used in the study. The rats were supplied by YUDETAM. The rats were anesthetized with a combination of 10 mg/kg xylazine HCl (Rompun® 2%; Bayer HealthCare AG, Leverkusen, Germany) and 100 mg/kg ketamine HCl (Ketasol® 10%; Richter Pharma AG, Weis, Austria) through the i.m. route following a fasting for 12 hr. The surgical procedures were performed under sterile conditions. A povidone-iodine 7.5% liquid soap (Polyod®) and physiological serum combination was used for cleaning the abdominal region. After hair removal, the abdomen was cleaned with 1% antiseptic povidone-iodine solution and a 3-cm midline laparotomy was done. The bile duct was ligated and obstructive jaundice was induced within one week (Figures 1 and 2). During this period, all rats were housed in single cages in a room with controlled temperature (22 ± 2°C), humidity (50 ± 5%), and a 12-hr cycle of light and dark. They were fed laboratory pellet chows and water was given ad libitum. The evaluation of the general condition, food intake, and postoperative movements of rats showed that there was no need of additional analgesic administration. After seven days, the animals were anesthesied again, following by cervical dislocation and immediate re-laparotomy. The rats were divided into four groups. The bile and serum levels of cefazolin were measured with HPLC (high-performance liquid chromatography) at the third hour in rats from the A1 group and at the sixth hour in rats from the A2 group, with cefazoline administered as 5 mg/rat (Cefamezin, i.m. 1 g, Eczacıbası Ilaç ve Tic A.S., Istanbul, Turkey); while the bile and serum levels of ceftriaxone (Cefaday, i.v.–i.m. 1 g, Biofarma Ilaç San ve Tic A.S., Istanbul, Turkey) were studied at the third hour in rats from the B1 group and at the sixth hour in rats from the B2 group, with ceftriaxone administered as 5 mg/rat.

TABLE 1 Serum and bile concentrations of cefazoline

	3 hr (A1)	6 hr (A2)	Test statistic and p
Bile concentration (μg/ml)	1.6 ± 0.2	0.0 ± 0.0	$Z = -2.524$ $p = 0.012$
Serum concentration (μg/ml)	1.8 ± 0.2	1.4 ± 0.3	$Z = -2.100$ $p = 0.036$

The values are expressed in mean ± standard deviation.

TABLE 2 Serum and bile concentrations of ceftriaxone

	3 hr (B1)	6 hr (B2)	Test statistic and p
Bile concentration (μg/ml)	56.0 ± 2.4	0.0 ± 0.0	$Z = -2.521$ $p = 0.012$
Serum concentration (μg/ml)	18.6 ± 3.1	3.7 ± 0.5	$Z = -2.521$ $p = 0.012$

The values are expressed in mean ± standard deviation.

Statistics

The data obtained were coded and recorded on a computer using SPSS for Windows 16.0.0. The changes in the serum and bile levels of antibiotics at the third and sixth hours in the groups that received cefazolin and ceftriaxone were separately evaluated using the Wilcoxon matched-pairs test. p values < .05 were considered statistically significant in the test.

RESULTS

After 3 hr of cefazolin administration, the serum concentration in the A1 group reached a mean of 1.8 μg/ml, while the bile concentration was found as 90% of the serum concentration, i.e., 1.6 μg/ml; whereas in the B1 group, the third-hour serum concentration of ceftriaxone was 18.6 μg/ml, while the bile concentration was found to be as high as 330% of this level, i.e., 56 μg/ml. The serum value of cefazolin dropped to 1.4 μg/ml in the A2 group and to 3.7 μg/ml in the B2 group at the sixth hour. No amount in the bile could be detected with HPCL for the two drugs at the sixth hour. A significant difference was found between the serum concentrations ($p = 0.036$) and the bile concentrations ($p = 0.012$) of cefazolin at both the third and the sixth hour. A significant decrease was observed between the serum concentrations ($p = 0.012$) and the bile concentrations ($p = 0.012$) of ceftriaxone at both the third and the sixth hour (Tables 1 and 2).

DISCUSSION

In the studies investigating the effectiveness of antibiotics or some biological agents used in the treatment of

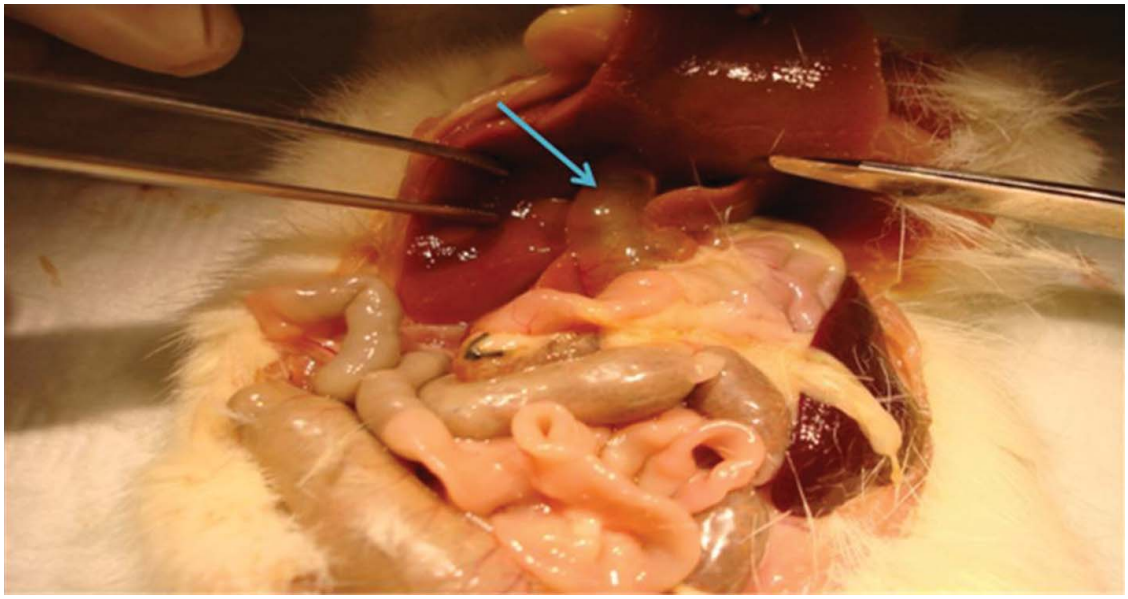

FIGURE 1 Ligated bile duct.

obstructive jaundice, either the inhibitory effect of the drug on direct bacterial translocation [13–15] or the excretion of the drug into the bile [5, 7–9] was studied. In the infections due to obstructive jaundice, the clinical picture presents a serious spectrum, from local biliary infections to sepsis, including organ failure [16]. Although the diagnosis of acute cholangitis is established with Charcot's triad, recommendations were made at the International Tokyo Consensus [1] regarding the early diagnosis and treatment. This disease was classified into mild, moderate, and severe, and early diagnosis and early bile drainage were recommended in all cases of the mild form. Bile drainage is essential, accompanied by surgical or other invasive methods, in

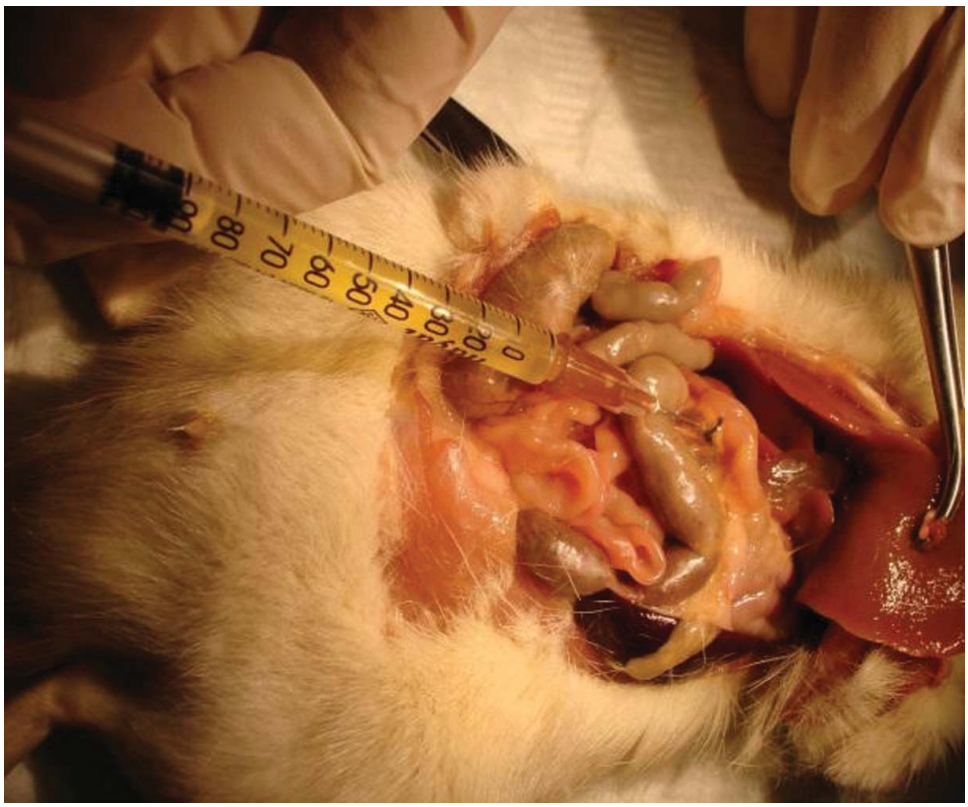

FIGURE 2 A rat with the bile duct ligated, obstructive jaundice induced, and the bile aspirated.

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treating obstructive biliary infections. In addition, supportive and antibiotic therapies are also necessary. The effectiveness of antibiotics increases with the drainage [1, 5, 8]. In obstructive jaundice, adjustment of the dose of antibiotics and application of a careful supportive treatment become necessary due to renal impairment (in the excretion of antibiotics) [2, 5]. The serum and infection area concentrations of the drugs must exceed a sufficient level over the MIC values of the active microorganism in order for the antibiotic therapy to be effective [7, 17]. Furthermore, taking specimens for culture antibiogram to identify the biliary infection factor is very difficult. Therefore, the initial treatment is generally empirical. Although the biliary infections are usually polymicrobial, about 70% of the aerobes most frequently isolated are *Escherichia coli* (*E. coli*) and bacteroides [2, 7]. In this study, we chose to investigate the excretion of cefazolin and ceftriaxone into the bile because these two drugs play an important role in the prophylaxis and empirical treatment of bile duct infections [10, 18]. Given the most frequently isolated microorganisms in the bile, especially Gram (−) bacteria, in the case of the obstructive jaundice, antibiotics used in this study are the drugs that have been proven effective.

Cefazolin is a drug that belongs to the first-generation cephalosporins, used in abdominal surgery for prophylaxis purposes. Its effect on Gram (+) bacteria is in the foreground, although it is also reported to be effective on Gram (−) bacteria, while the effectiveness of ceftriaxone against Gram (−) bacteria is more prominent. Therefore, ceftriaxone is known to be more effective than cefazolin in the treatment of hepatobiliary infections [10, 18].

Although the basic elimination route of cephalosporins is known to be the kidneys [19, 20], in a study by Wright and Line [9] investigating the biliary excretion of some cephalosporins, elimination of some cephalosporins in the rats was reported to be from the bile. They found in their study that the excretion of cephalosporins into the bile is directly proportional to the molecular weight of these cephalosporins. In that study, they stated that 60%–80% of cefoperazone, which is one of the highest-molecular-weight cephalosporins, was excreted into the bile. When these results are compared with our study, our values of 90% for cefazolin and 330% for ceftriaxone at the third hour conflict with that study. Nevertheless, that study is important to demonstrate that the elimination of cephalosporins is not only from the kidneys in the rats.

Rippin et al. [21] showed in their study that the transport system in rat hepatocytes involves transport proteins (polypeptides) found in the basolateral and canalicular plasma membranes. They underlined that excretion into the bile occurred either through diffusion or by active transport through these proteins, and these mechanisms may be affected to varying degrees in the case of cholestasis.

In their study investigating the effect of cefoperazone and ceftazidime on biliary excretion, Leung et al. [5] found that both drugs were concentrated in the bile during active and passive excretion, but obstructive jaundice affected both mechanisms, and active excretion was not improved even after the obstruction was resolved. They concluded that these drugs should be used in 20- to 40-fold doses in order to reach the MIC value following obstruction, and that the drugs may be effective against pathogens only after resolution of the obstruction. In our study, because ceftriaxone reached a bile concentration over 300% of the serum level at the end of the third hour, we can say that this drug was excreted into the bile through active transport. On the other hand, serum concentration of cefazolin reached a mean value of 90%, suggesting that passive excretion was prominent. Again, the concentration mentioned above was observed in the bile for both drugs, suggesting that obstructive jaundice did not impair the excretion of these two drugs.

Kohner et al. [22] conducted an in vitro study to measure the MIC values of some drugs for *E. coli* and *Klebsiella*, which are among the bacteria that can develop resistance when exposed to extended-spectrum beta-lactamase (ESBL) and plasmid-mediated AmpC betalactamase (pAmpC). In this study with 264 isolates used, the MIC value was found to be 1–2 μg/ml for cefazolin and $\geq$64 μg/ml for ESBL or pAmpC gene resistance. The MIC value was found to be $\geq$0.03–0.06 μg/ml for ceftriaxone and $\geq$16–32 μg/ml for ESBL or pAmpC gene resistance. Considering the mean values of both antibiotics in our study, we can say that the MIC value was reached both in the serum and in the bile at the third hour, while only in the serum at the sixth hour. The effectiveness of cephalosporins is directly proportional not only to the increasing MIC value but also to the duration (time > MIC) [5, 6]. In a study by Andes and Craig [12] evaluating treatment of infections caused by organisms that produce ESBL, along with pharmacokinetic and pharmacodynamic aspects, the importance of the time > MIC value for cephalosporins was emphasized. In that study, they stated that the time > MIC value of the drug must be over 40%–50% (for the bacteria in the given environment) for the beta-lactam antibiotics to achieve maximal bacteriological eradication in the infection area. Considering that the most frequently isolated bacterium in the bile is *E. coli*, when we look at the concentrations of the two antibiotics in our study, we can conclude that the time > MIC value of 40%–50% was obtained for the serum. However, we need more evidence to evaluate this for the bile. The bile concentration in our study reached 90% of the serum concentration at the end of the third hour, whereas a significant decrease was observed at the end of the sixth hour compared with the third hour, although this value was over the expected value, i.e., inconsistent with the 2-hr half-life of cefazolin. This shows us that obstructive jaundice

might affect the elimination of cefazolin from the kidneys and the bile. The absence of cefazolin in the bile at the sixth hour may suggest that the transport proteins involved in the diffusion of cefazolin might have become saturated. On the other hand, the bile concentration of ceftriaxone reached 330% of the serum concentration at the sixth hour. This shows that ceftriaxone can be excreted into the bile at a high rate and in concentrated form. This is possible due to its property to bind to transport proteins at a high rate and be excreted into the bile by active transport. However, its absence in the bile and its significantly decreased levels in the serum at the third hour conflict with the 8-hr half-life of ceftriaxone. Again, following up obstructive jaundice for one week, ceftriaxone was concentrated in the bile at the third hour and was not detected at the sixth hour, suggesting that its excretion was not impaired but increased. Accordingly, ceftriaxone should be administered at more frequent intervals in cases of obstructive jaundice.

In conclusion, the excretory level of cefazolin and ceftriaxone into the bile reaches therapeutic doses, although the duration for which these levels are above the level required for bactericidal activity, which represents their main effectiveness, is short. Ceftriaxone is better concentrated in the serum and bile than cefazolin. Better treatment outcomes can be achieved by administering ceftriaxone at more frequent intervals. How the serum and bile levels will be affected in case these drugs are administered at more frequent intervals can be assessed in further studies.

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