Sub-chronic toxicity Study in Wistar Rat to Evaluate Toxicity Profile of Ceftriaxone-Tazobactum Combination

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Citation

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Abstract

Currently, the use of -lactamase inhibitors in specific resistance mechanism of -lactamase producing organisms in combination with -lactams served as highly effective and promising therapy. It regain the susceptibility profile of bacteria to combination agent. The present study investigates toxicity profile of similar regimen, a potent and synergistic combination of third generation cephalosporins and -lactamase inhibitor, Ceftriaxone-Tazobactam by conducting repeated dose subchronic toxicity study on rat (male and female). Three dose levels were selected for the study. Outcomes of present study suggested no significant changes in physiological, biochemical as well as hematological parameters. No mortality was observed in any of the treatment groups. It was concluded that Ceftriaxone-Tazobactam combination exerts no toxicity and is safe on long-term treatmen usage.

INTRODUCTION

Resistance to third generation cephalosporins has become a major problem all over the worldwide (Arlet et al. 2006). Cephalosporins are often considered to be a 'drug of choice' and are increasingly used in empirical therapy (AitMhand et al. 2002). Due to wide spectrum of activity, good pharmacokinetics, established clinical efficacy and high tolerability, cephalosporins are among the most widely used antibiotics worldwide (Rankin et al. 2002; Pefanis et al. 1993). Ceftriaxone, a third generation cephalosporin, is unique in exhibiting an unusually long elimination half-life that allows for once-daily administration (Bijie et al. 2005; Gupta et al. 2003). These features contributes its very frequent use which resulted in loss of bacterial susceptibility and recently emerged as a group of extended spectrum b-lactamases (ESBLs) (Aldridge et al. 1994; Weill et al. 2004). Against this rising resistance, the role of b-lactamase inhibitor combinations needs to be considered.

To combat this specific resistance mechanism of -lactamase producing organisms, the use of -lactamase inhibitors in combination with -lactam antibiotics represents an effective measure against -lactamase producing Gram negative bacteria (Georgopoulos et al. 1999; Thauvin-Eliopoulos et al. 1997).

Tazobactam effectively inhibits -lactamases from a broad range of Gram-positive and Gram-negative bacteria (Sader et al. 2000) and, as a result potentiates the activities of a number of -lactam antibiotics against ESBLs bacteria (Maddux 1991; Caron et al. 1990). Although the tazobactam demonstrates little intrinsic activity against most bacteria, it alone inhibits many strains of B. fragilis (Pefanis et al. 1993).

-Lactamase inhibitors substantially enhanced the in-vitro and invivo potency against this species of ceftriaxone which as a single agent has only modest activity (Chambers and Fournier 1993; Edelstein and Edelstein 1994). Ceftriaxone-tazobactam combination would thus seem to have advantages in terms of antimicrobial activity against many -lactamase producing bacteria including B. fragilis (Mentec et al. 1992; Jacoby and Medeiros 1991).

With a high prevalence of infections due to ESBL positive bacteria (Goldstein et al. 1995), a simultaneous increase in the use of these fixed dose combinations (FDC) is being observed. Hence there is a strong urge to determine the Safety profile of combination agents which is essential to guide for empiric as well as appropriate therapy of severe infections in hospitalized patients. The present study was carried out to evaluate the Toxicity potential of ceftriaxone/ Tazobactam.

MATERIALS AND METHODS

ANIMALS

Healthy Wistar rat (male and female, 150- 175 g weight) were divided into four groups and assigned as three

treatment groups and one control group. All groups consist of 6 male and 6 female animals. Animals were provided with standard diet (pellets) supplied by Amrut feed India and water was given ad libitum. They were housed in polyurethane cages (three in each) at controlled room temperature of 29 ± 2°C and a relative humidity of 50.5%, and a constant light-dark schedule (12 hours light and 12 hour dark cycle).

EXPERIMENTAL DESIGN AND DRUG TREATMENT

Ceftriaxone-Tazobactam was administered intravenously at three dose levels i.e. 30 mg/kg, 60 mg/kg and 120 mg/kg body weight correspond to low dose, intermediate dose and high dose respectively. Control group was treated with normal saline as sham treatment. Treatment was done for continuous 28 days (once daily). The study protocol was approved by Institutional animal ethics committee of Institute for Toxicological Studies, Pune, India.

Physical parameters (body weights, food and water intake) and local injury were studied throughout the treatment. Mortality if any, in all the groups, during the course of treatment was also recorded. Autopsy was done if animal died during course of treatment. At the end of treatment hematological, biochemical (liver function tests & renal function tests) and histological parameters were studied. The organs were kept for histological studies. The organ body weight ratio of each organ was estimated and tissues were processed for H& E staining.

HEMATOLOGICAL PARAMETERS

Blood was collected by cardiac puncture. Blood samples were analyzed for routine hematological parameters. Hemogram was performed on ACT diff-2 Hematolgy Analyzer (Beckman Coulter India Ltd., Mumbai, India).

BIOCHEMICAL PARAMETERS

Biochemical parameters were evaluated in plasma and serum sample. Serum levels of alkaline phosphatase (SAP), blood urea nitrogen (BUN), Serum gluatmic oxaloacetic transaminase (SGOT), serum gluatmic pyruvic transaminase activities (SGPT), Blood Sugar and blood sugar levels were estimated. All parameters were studied by Merck semi auto analyzer using Merck kits.

HISTOLOGICAL EXAMINATION

Animals were sacrificed at the end of treatment and various organs like liver, kidney, lungs and gonads were collected for histological examinations. All the organs were immediately fixed in 10% buffered formalin.

STATISTICAL ANALYSIS

Results are shown as Mean ± SD. Significance of difference between groups was evaluated by using ANOVA. If ANOVA shows significant differences, post hoc analysis was performed with Tukey test. $P<0.05$ was considered as statistically significant.

RESULTS

The results of current study showed no alterations in physical parameters were observed throughout the dosing period. The increase in body weight, food and water intake were similar to control group in all dose levels. No significant change group mean body weight was observed in all the groups as compared to control group on 28^{th} day.

Hemogram was estimated for each animal of all doses and control. In male rat groups, no significant change was observed in hemoglobin (Hb), red blood cell counts (RBC), white blood cell counts and platelet counts in all the treated groups as compared to control group (Table 1).

{image:1}

There was no alteration observed in total leukocyte count, Hemocrit and other parameters. There was no significant change in different hematological parameters in female rat groups also as compared to respective control group (Table 2).

{image:2}

Data is presented as mean ± Standard deviation (n=6 in each group)

Liver toxicity parameters were studied. In male as well as female rat groups, no significant change in serum GPT, GOT activities, were observed in all the treated groups as compared to respective control group. No significant change in plasma sugar levels were observed in both the groups (Table 3 and 4).

{image:3}

Data is presented as mean ± Standard deviation (n=6 in each group)

{image:4}

Data is presented as mean ± Standard deviation (n=6 in each

group)

Histological examination were done for each animal and no significant and treatment related gross and histopathological changes were observed at any dose level.

DISCUSSION

Emergence of ceftriaxone, third generation antibiotic, is alarming situation spreading all over the world which have given rise to various potential combination therapy such as β-lactam antibiotic and β-lactamase inhibitors (Aldridge et al. 1994; Higgins et al. 2004). Our study demonstrates safety of a β-lactamase inhibitor Tazobactam administered with ceftriaxone.

The outcomes of this study have shown, increase in body weights and growth of treated animals of both the sex were followed similar pattern as in control groups. There were no signs of local injury at site of injection in the treated groups. Hematological parameters are the most important measure of toxicity profile. Hemogram was estimated for all treated as well as control groups and results had shown no unusual effect on blood cell count, haemoglobin, hemocrit and platelet count in treated animals as compared to control group.

It is clear that the liver and kidneys play significant roles in various metabolic processes. Therefore, emphasis was placed on the effect of FDC might have on the function of these organs. In addition, the liver plays an important role in xenobiotic function, while kidneys are sites for filtration and reabsorption. Major part of elimination of Ceftriaxone-Tazobactam takes through renal excretion (Bijie et al. 2005). Parameters of Kidney function showed, Ceftriaxone-Tazobactam have no effect on kidney function as no significant differences were observed on BUN level, Blood sugar and total protein with respect to control. Impact of this potent antibiotic combination was also evaluated in liver function. No significant changes were observed in liver parameters such as SGOT, SGPT and SAP activities in Ceftriaxone-Tazobactam treated all groups of either sex as compared to the respective control group which confirmed lacking in hepatotoxic potential of FDC .

Histopathological analysis were done and have also supported safety of Ceftriaxone-Tazobactam data infered from physiological, biochemical and heamatological parameters. There were no signs of toxicity observed in any of the organs in Ceftriaxone-Tazobactam animals at all three doses as compared to control.

The combination of ceftriaxone and Tazobactam proved to be an excellent therapeutic alternative with 94.6% of Ceftriaxone resistant strains being susceptible in vitro to this combination (Aldridge et al. 1994; Payne et al. 1994). On coadministration of Tazobactam significantly enhanced activity of ceftriaxone in animal model and has proved as synergistic combination (Brauers et al. 2005; Maddux 1991). Tazobactum improve susceptibility of range of ESBL producing bacteria towards Ceftriaxone (Prakash et al. 2005; Wust and Hardegger 1994). Tazobactum also enhanced marked activity of Ceftriaxone against resistant strains of Escherichia coli, Enterobacter cloacae and other resistance strains of bacteria as compared to ceftriaxone alone (Yoshiyama et al. 1998).

It can be concluded from findings of present study that Ceftriaxone-Tazobactam is safe even at highest dose level and offers no obvious toxicity thus can be evolve as promising regimen for treatment of various life threatening infections.

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