Subchronic toxicity study of fixed dose combination of ceftriaxone-vancomycin in swiss albino mice.

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Abstract:
Antibiotics are highly successful in controlling bacterial infection but they have many toxic effects, sometimes very fatal. Aim of the present study was to evaluate subchronic toxicity of fixed dose combination of ceftriaxone-vancomycin in mice. Male and female mice were divided into four groups depending on dose of ceftraixone-vancomycin (0, 10, 100 and 500 mg/kg i.m.). Hematological, biochemical parameters were studied along with histological examination.
No significant alteration in hematological and biochemical parameters (SGOT, SGPT, BUN, sugar) were observed in all the group as compared to respective controls, indicating non toxic nature of fixed dose combination of ceftriaxone-vancomycin, which was further confirmed histologically as no structural alteration were observed in liver, kidney, stomach and gonads. In conclusion, sub chronic dose of fixed dose combination of ceftriaxone-vancomycin does not have any toxic effect, especially on liver and kidneys.

**Key Words:** Ceftriaxone-vancomycin, GOT, GPT.

**Introduction**

Antibiotics are the powerful tool to fight against bacterial infections. Although, scientific community has avidly pursued the discovery of new antibiotics that are more effective and have lesser side effects, these efforts have met with limited success.

Vancomycin became available for clinical use more than 50 years ago but was soon discarded in favor of other antibiotics that were deemed to be more efficacious and less toxic. The advent of pseudo membranous enterocolitis, coupled with the spread of methicillin-resistant *Staphylococcus aureus*, led to a resurgence in the use of vancomycin (1). The drug continues to have activity against a wide variety of gram-positive pathogens (2). Ceftriaxone exhibits the widest antibacterial spectrum of third generation cephalosporins and this is reflected in clinical responses (3).

Antibiotics within a structural class will generally have similar patterns of effectiveness, toxicity and allergic potential. Several antibiotics can cause severe hepatic injury, nephrotoxicity, gastrointestinal toxicity and hemolytic anemia. Vancomycin can induce renal toxicity (4). Ceftriaxone is reported to cause hemolytic anemia, renal damage (5) and lipid peroxidation
Keeping this in view, we planned to study subchronic toxicity of fixed dose combination (FDC) of ceftriaxone-vancomycin in mice.

**Materials and Methods**

Forty eight healthy swiss albino mice (weighing 25-30g) were divided into four groups (12 animal each) depending upon the dose of ceftriaxone-vancomycin. Each group was further subdivided into two groups depending on sex of mice having 6 animals each. Mice were housed in polypropylene cages under hygienic conditions and acclimatized to the laboratory conditions for a period of seven days prior to initiation of dosing. Animals were kept in 12h light and dark cycle and received food and water *ad libitum*. Animals were given freshly prepared intramuscular injection of ceftriaxone-vancomycin for 28 days.

The mixture of FDC of ceftriaxone-vancomycin was dissolved in distilled water and was injected intramuscularly at following dose levels.

- **Group I**: 0 mg/kg
- **Group II**: 10 mg/kg
- **Group III**: 100 mg/kg
- **Group IV**: 500 mg/kg

Control group was injected distilled water only. All the animals were observed for clinical signs and were monitored for mortality. The body weight were monitored weekly throughout the study.

Overnight fasted animals were sacrificed on 29th day, blood and tissues samples were collected. The Institutional animal ethics committee of Jadavpur University, Kolkata had approved the study protocol.
**Hematological and Biochemical parameters**

Hemogram was performed on Sysmax K1000 Cell counter.

**Biochemical Parameters**

Serum glutamic pyruvic transaminase (GPT), glutamic oxaloacetic acid (GOT), blood urea nitrogen (BUN), total proteins and plasma sugar levels were performed on Robonik ASP-300 biochemistry analyzer using diagnostic kits.

**Histopathological examination**

Liver, kidney, stomach, lungs and gonads were removed from the sacrificed animals and were preserved in 10% buffered formalin for histological examination.

**Statistical analysis**

The results are expressed as mean ± S.D. Dunnett’s test was used for the statistical evaluation of data using Sigma-stat software and p < 0.01 accepted as significant.

**Results**

**Physical parameters**

No behavioral and clinical signs of intoxication were observed throughout the dosing period. No significant change in group mean body weight was observed in all the treated groups as compared to respective control groups on 28th day (Table 1). Mortality rate was zero in all the groups.
Table. (1) Effect of sub chronic dosing of ceftriaxone-vancomycin FDC on group mean body weight (g).

<table>
<thead>
<tr>
<th>Groups / Days</th>
<th>Male mice</th>
<th>Female mice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0  7 14 21 28</td>
<td>0  7 14 21 28</td>
</tr>
<tr>
<td>Group I</td>
<td>24.22±1.13 24.52±1.07 24.77±1.11 25.10±1.09 25.10±1.14</td>
<td>24.28±1.42 24.55±1.42 24.82±1.38 25.08±1.39 25.33±1.40</td>
</tr>
<tr>
<td>Group II</td>
<td>24.03±0.49 24.30±0.50 24.57±0.48 24.70±0.48 24.97±0.48</td>
<td>24.07±1.22 24.25±1.21 24.42±1.23 24.57±1.20 24.75±1.21</td>
</tr>
<tr>
<td>Group II I</td>
<td>24.02±0.47 24.20±0.40 24.32±0.42 24.28±0.53 24.27±0.59</td>
<td>25.90±1.24 25.82±1.26 25.63±1.29 25.53±1.25 25.40±1.22</td>
</tr>
<tr>
<td>Group IV</td>
<td>23.50±0.64 23.57±0.59 23.55±0.59 23.42±0.58 23.28±0.56</td>
<td>25.78±2.95 26.00±2.97 26.05±2.84 26.00±2.72 25.83±2.71</td>
</tr>
</tbody>
</table>

**Hemogram**

In male mice groups, no significant change was observed in RBC, total leukocyte, retics and platelet counts in all the treated groups as compared to control group. Significant increase in Hb level was observed in group II (10mg/kg) animals (Fig. 1).
Fig. (1) Effect of sub chronic dose of ceftriaxone-vancomycin FDC on hemogram in male mice.

In female mice groups no significant change in different hematological parameters was observed as compared to control group (Fig. 2).

Fig.(2) Effect of sub chronic dose of ceftriaxone-vancomycin FDC on hemogram in female mice.

**Biochemical parameters**

No significant change in serum GPT, GOT activities, total protein and sugar levels were observed in all the treated groups as compared to respective control group. Blood urea
nitrogen was not elevated in any of the treated group as compared to respective control group (Fig 3, Fig 4).

Histological examination

Histological examination of heart, lungs, liver, stomach and gonads were found to be normal in high dose groups of either sex.
Discussion

All the animals in treated as well as control groups showed no clinical signs of intoxication and alteration in behavior. Increase in mean group body weight in all the treated groups was comparable with the increase in mean group body weight of respective control group animals. These physical parameters suggested that no toxic effect of sub-chronic dosing FDC of ceftriaxone-vancomycin in mice of either sex.

There are reports indicating that antibiotics may cause hemolytic anemia and jaundice (5, 7). In contrast, sub-chronic dosing of FDC of ceftriaxone-vancomycin to mice did not cause anemia as clearly indicated by no significant change in hemoglobin levels. Moreover, no significant change in TLC, RBC, Retics and platelets count was observed in all the treated groups as compared to respective control group clearly indicating sub chronic dose of FDC did not induce hematological toxicity.

With the wide-spread use of antimicrobial agents, however, hepatic injury is not an infrequent occurrence. Several antibiotics cause hepatotoxicity (8). Penicillins remains a widely used class of antimicrobials with a well defined record of hepatotoxicity. The combination of clavulanate with amoxicillin may be associated with the greatest risk for liver injury from any antimicrobial agent (9). Vancomycin induces increase in liver enzyme activities suggesting its hepatotoxic effect (10). No significant variation in serum total protein levels, serum GOT and GPT activities was observed in all the treated groups as compared to respective control groups indicating ceftriaxone-vancomycin FDC is not hepato-toxic to swiss albino mice. Histological examination of liver was normal in high dose groups (500mg/kg) further confirming non toxic nature of FDC ceftriaxone-vancomycin.
Some of the antibiotics are also known to induce renal dysfunction. Concurrent administration of vancomycin and aminoglycoside induced slight and transient toxic tubular effects in septic patients with normal renal functioning (11). Ceftriaxone and ampicillin sulbactam induced oliguria, immune hemolytic anemia and acute renal failure (7). Nephrotoxicity of cephalosporin antibiotics in rabbit kidney cell lines has also been reported (12). Vancomycin was reported to elevate serum urea levels in rats along with decrease in body weight leading to renal dysfunction (13).

Plasma levels of blood urea nitrogen and creatinine were reported to be increased significantly in vancomycin-treated rats by an AH-SOD-inhibitable mechanism. Histological examination revealed that vancomycin also elicited a marked destruction of glomeruli and necrosis of proximal tubule by an AH-SOD inhibitable mechanism. These results suggest that oxidative stress underlies the pathogenesis of vancomycin-induced nephrotoxicity (4). Our especially formulated FDC of ceftriaxone-vancomycin had no toxic effect on renal function as no significant increase in serum BUN levels was observed in all the treated groups as compared to respective control groups, which was further confirmed by normal histological examination of kidneys from high dose group. Ceftriaxone has also been reported to have protective effect on antibiotic induced nephrotoxicity (14, 15). Comerski et al (16) reported testicular toxicity of different cephalosporin analogues. Histological examination of heart, lungs, liver, kidney, stomach and gonads was found to normal in the high dose groups of either sex, thus confirming non toxic nature of fixed dose combination of ceftriaxone-vancomycin in swiss albino mice.

In conclusion, the especially formulated FDC of ceftriaxone-vancomycin is neither hepatotoxic nor nephrotoxic to swiss albino mice.
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References


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