**EFFECT OF TOBRACEF IN CARBAPENEM RESISTANT PNEUMONIA INFECTION**

A. Ahmad, V.K. Dwivedi*, and M. Chaudhary

*Preclinical Division, Venus Medicine Research Centre, Hill Top Industrial Estate, Bhatoli Kalan Baddi, H.P.-173205 India

**ABSTRACT**

To determine effect of Tobracef and imipenem drug on antioxidant enzyme activity and lipid peroxidation level and some biochemical parameters in carbapenem resistant pneumonia infection rat model. Total 40 rats were selected and divided into 4 groups of 10 rats each. Group I was control group; group II was infected via A. baumannii bacterial strain. Group III and IV were infected plus treated group with tobracef and imipenem drugs.

Our results showed that a significant (p <0.001) decrease in enzymes activities (Superoxide dismutase, Catalase, Glutathione reductase and ascorbic acid) along with increased lipid peroxidation level as well as cytokine parameters (tumor necrosis factor-a ,interlukin-6, interlukin-10) and biochemical parameters in plasma of infected group as compared to control group. These activities were increased along with decreased in lipid peroxidation level and biochemical parameters in both treated group as compared to infected group after nine days treatment with tobracef and imipenem drugs. When tobracef treated group was compared with imipenem treated group, all above parameters were improved in the plasma of tobracef treated group. These findings concluded that tobracef has better efficacy than imipenem in carbapenem resistant pneumonia infection.

**Key words:** Fixed dose combination, tobracef, imipenem, lipid peroxidation, antioxidant enzymes

**Introduction**

Pneumonia infection is severe infection that causes high morbidity and mortality rate worldwide. It is caused by Acinetobacter baumannii microorganism which has a rapidly progressive clinical course that is often complicated by multilobular involvement and causes lung abscesses. Acinetobacter baumannii is a significant problem in critically ill patients. It is widespread, can colonise patients quickly and causes virulent infections. It is a well recognized pathogen that causes nosocomial infection in intensive care units in Europe and Asia. Nosocomial pneumonia (NP) is currently the most common and leading causes of death. The incidence of acquiring nosocomial pneumonia infection ranges from 17.8% to 44.8% and is influenced by the duration of hospital and Intensive care unit stay. Bacterial lung infection commonly occur in infants with underlying lung injury, it is unclear whether exposure to hyperoxia can directly increase the risk for invasive pulmonary infection. Bacterial adherence leading to colonization, is the first step in the process of invasive pulmonary infection with phagocytosis by alveolar macrophages critically important in preventing invasive pulmonary infection. Free radicals are generated during pneumonia infection. Alveolar macrophages are essential components of lung innate immunity. Pneumonia infection can cause oxidation and inactivation of a variety of macro molecule in the lung, including protein, lipid and DNA.

Tobracef is a novel fixed dose combination of ceftazidime and tobramycin. Ceftazidime is third generation class of cephalosporin antibiotic. It has...
broad spectrum activity against Gram-positive and Gram-negative bacteria. Tobramycin is an aminoglycoside used to treat various types of bacterial infections, particularly Gram-negative infections. Several studies have been reported that cephalosporin has potential property of free radical scavenger against hypochlorous acid driven oxidative injury\textsuperscript{10}.

Imipenem is subgroup class of carbapenems antibiotics which has broad spectrum of antibacterial activity. It has a broad spectrum of activity against aerobic and anaerobic Gram positive as well as Gram negative bacteria. In the present study, the authors have been tried to determine the role of tobracfe on some biochemical parameters and antioxidant enzyme activity along with free radical mediated damage in imipenem resistant pneumonia infection rat model.

Materials & Methods

All biochemicals used in the study were procured from Sigma, St. Louis, MO, USA. Other chemicals were of analytical grade. Biochemical kits were procured from Bayer Diagnostics India Ltd., Baroda, Gujrat, India for estimation of Creatinine, SGOT, SGPT, urea, uric acid and total bilirubin etc. Interlukin-6, Interlukin-β and TNF-α were estimated by using In-vitrogen kits. Camarillo, CA 93012 USA. Culture medium Acinetobacter baumannii was obtained from IMTECH (Institute of Microbial Technology), Chandigarh, India. Antibiotics such as ceftazidime plus tobramycin and imipenem were obtained from Venus Remedies, India. The ratio of fixed dose combination of ceftazidime + tobramycin was 8.3: 1 respectively.

Bacterial Inoculum

Bacterial strains were grown in septic culture in nutrient broth at 37\textdegree{} C for 18 hours and maintained on nutrient agar slant. Organisms were harvested by centrifuged at 2348 x g for 15 minutes, washed 3 times and suspended in phosphate-buffered saline (0.2 M, pH 7.0) to the desired concentration.

Animals

Animals were quarantined for a period of three weeks to ensure stabilization before use. Forty wistar rats (all males, weighing 120 to 140 g) were used in the experiment. The rats were fed standard pelleted diet and water \textit{ad libitum}. The test room was air conditioned with temperature 23 ± 2\textdegree{}C, humidity 65 ± 5\%, and with artificial fluorescent light (10 and 14 hours of light and dark, respectively). The study was approved by the institutional animal ethical committee.

Pneumonia Model

Respiratory tract infection model in rats were prepared by selecting carbapenem resistant bacterial strain Acinetobacter baumannii (MTCC No. 1425) for induction of pneumonia infection in rat model, bacterial dose ranges from 10\textsuperscript{2} to 10\textsuperscript{7} colony-forming units [CFU]/mL and this dose was determined prior to studying the course of pneumonia. Overnight MH broth culture was used to prepare inoculum approximately 6 log\textsubscript{10} CFU/mL. Immediately prior to infection the culture was diluted 1:10 in molten nutrient agar maintained at 40\textdegree{}C to give a final bacterial inoculum of approximately 7 log\textsubscript{10} CFU/mL in MH broth.

For intranasal instillation of the bacterial inoculum, the method of Held et al.,\textsuperscript{11} was employed. The rats were anesthetized under light ether and infection was created by administration of 75 µl (10\textsuperscript{7}) bacterial inoculum in to nasal opening while holding the rat up right for nine days. The pneumonia infection was developed within nine days.
Groups and Treatment
Total forty rats were randomly selected and divided into 4 groups. Each group contains 10 rats as given below:
Group I: Control, normal saline treated group
Group II: Infected group (7 log_{10} CFU/mL A. baumannii bacterial stain)
Group III: Infected + imipenem treated group (25.0 mg/Kg/ body weight)
Group IV: Infected + tobracef treated group (18.66 mg/Kg/ bodyweight)

After pneumonia infection, tobracef and imipenem drugs were given intravenously to group III and IV for nine days and blood samples were collected in sodium citrate (3.8%) containing vials by retro orbital vain and samples were centrifuged at 0-4 °C at 6000 rpm for 15 minute and plasma was separated out to determine enzyme activity along with malonaldehyde level and biochemical parameters in plasma of all groups.

Antioxidant enzymes
Superoxide dismutase (SOD) activity was determined by the Method of Misra and Fridovich\(^\text{12}\). Catalase activity was measured by the method of Luck\(^\text{13}\). Glutathione reductase (GR) activity was measured by Carlberg & Mannervik\(^\text{14}\) with minor modification. The ascorbic acid was determined by the method of Roe et al.\(^\text{15}\).

Lipid peroxidation level
Lipid peroxidation level was measured in term of malonaldehyde (MDA) formed. It was determined by thio barbutric reaction. The levels were assayed according to method of Ohkawa et al.\(^\text{16}\).

Biochemical parameters
Serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT) alkaline phosphatase, total bilirubin, creatinine uric acids, erythrocyte sedimentation rate (ESR), white blood cell count (WBC) and urea were determined by using commercially available diagnostic kits (Bayer Diagnostics India Ltd., Baroda, Gujrat, India).

TNF α, Interlukin-6 and Interlukin-β determination:
Cytokine parameters (TNF α, Interlukin-6 and interlukin-β) were assayed by ELISA Reader, (Merck, Serial No- 21041098, MIOS -junior) according to manufacturer's instruction.

Statistical analysis
The results are expressed in mean ± SD. Statistical evaluation of the data was performed by one way- ANOVA followed by student Newman-Keuls using INSTAT 3.0 software package. The statistical difference was analyzed between control vs infected group as well as infected vs drug treated group. p<0.05 was considered statistically significant.

Results
In the present study, no mortality was seen.

Clinical Symptoms
In the present study, there was significantly decreased body weight along with increased body temperature in infected group as compared with control group. After administration of imipenem and tobracef drugs for nine days treatment, body weight and temperature were significantly improved in tobracef treated group when compared with infected group. When imipenem treated group was compared with tobracef treated group, body weight and temperature was significantly improved in tobracef treated group in comparison to imepnem treated group after nine days of treatment. The data was represented in Fig.1 a, b.

Enzymatic and non enzymatic antioxidant enzymes activities (Superoxide dismutase, Catalase
Effect of Tobracef and Imipenum drug on body weight and temperature on carbapenem resistant pneumonia infection

Figure1. Wistar rats infected with A. baumanni represents decreased body weight (a) and increased body temperature (b) on days 0-9 after intranasal infection. After treatment with tobracef and imipenum drugs for 9 days, bodyweight and temperature was improved. When tobracef treated group was compared with imipenum treated group, the body weight and temperature was highly improved in tobracef treated group and comes back to control level.

Glutathione reductase and Ascorbic acid level) were significantly decreased (p <0.001, 55.8%; p <0.001, 61.9%; p <0.001, 28.2% and p <0.001, 40.2%) in infected group as compared with control group. These enzyme activities were significantly increased (p <0.01; 25.2%, p>0.05, 13.5%; p<0.001, 6.3% and p>0.05 13.8%) in imipenem treated group as well as (p <0.001; 94.2%; p <0.001, 118.7% ; p>0.05, 17.6% and p <0.01, 38.4%) in tobracef treated group when compared with infected group after nine days treatment. But in case of imipenum treated group vs tobracef treated group, all enzymes
activities were found to be significantly (p <0.001, p <0.01, p <0.05, ) elevated in tobracef treated group after ten days treatment (Tab.1).

Table 1. Effect of Tobracef and imipenem drug on antioxidant enzyme levels and lipid peroxidation level in pneumonia induced rat model

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Control group (N=10)</th>
<th>Infected group (N=10)</th>
<th>Imipenem treated group (N=10)</th>
<th>Tobracef treated group (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SOD (nmolemin/ml)</td>
<td>0.197 ± 0.023</td>
<td>0.087 ± 0.007***</td>
<td>0.109 ± 0.013**</td>
<td>0.169 ± 0.013***</td>
</tr>
<tr>
<td>2</td>
<td>Catalase (nmole/min/ml)</td>
<td>0.349 ± 0.023</td>
<td>0.133 ± 0.008***</td>
<td>0.151 ± 0.022**</td>
<td>0.291 ± 0.025***</td>
</tr>
<tr>
<td>3</td>
<td>GR (nmole/min/ml)</td>
<td>1.19 ± 0.099</td>
<td>0.854 ± 0.048***</td>
<td>0.908 ± 0.032***</td>
<td>1.005 ± 0.054***</td>
</tr>
<tr>
<td>4</td>
<td>Ascorbic acid (mg/ml)</td>
<td>8.45 ± 1.5</td>
<td>5.05 ± 1.21***</td>
<td>5.75 ± 0.98**</td>
<td>6.99 ± 1.03**</td>
</tr>
<tr>
<td>5</td>
<td>MDA (mmole/ml)</td>
<td>3.35 ± 0.62</td>
<td>5.90 ± 0.54***</td>
<td>4.55 ± 0.46***</td>
<td>3.39 ± 0.25***</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SD. N= numbers of animals. Where a= p<0.001; highly significant, b= p<0.01 significant, C= p>0.05 in significant. Statistical analysis was performed between control vs infected group as well as infected vs both infected plus treated group. Where SOD; Superoxide dismutase, GR; Glutathione reductase

MDA level was significantly higher (p <0.001, 76.1%) in infected group when compared with control group. The MDA level was lowered (p <0.001, 22.8%; p <0.001 42.5%) in imipenem as well as tobracief treated group after treatment of nine days. When imipenem treated group was compared with tobracief treated group, the level was gradually decreased (p <0.001, 25.4%) in tobracief treated group and comes near back to control level (table.1). ESR and WBC were significantly (p <0.001, 475%; 249%) increased in infected group in comparison to control group. After treatment with imipenem, and tobracief drugs for nine days, ESR and WBC levels were significantly decreased (p <0.001, 13.0 %; 90%) in imipenem treated group and (p <0.001, 65 %; 65.8%) tobracief treated group. When imipenem treated group was compared with tobracief treated group, the levels of ESR and WBC were significantly lowered (p <0.001, 60.0 %; 62.4%) in tobracief treated group after nine days treatment (Fig.2, 3).
A significant increased Tumor necrosis factor; TNF-α, Interlukine-6 and Interlukin- β were observed (57.3%, 66.4% and 101% ) in infected group when compared with control group. These levels were significantly decreased about 8.5%, 1.05% and 0.57% in imipenem treated group as well as about 29.0%, 38.1% and 48.2% in tobracef treated group after nine days treatment as compared with infected group. These levels were significantly lowered (22.4%, 37.5% and 48.5%) in tobracef treated group after nine days treatment when compared with imipenem treated group (Fig 4, 5 and 6).
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Fig 5. Values are expressed in Mean ± SD. Where ***p<0.001; highly significant, **p<0.01 significant, *p<0.05 less significant ns p>0.05 in significant. Statistical analysis was performed between control vs infected group as well as infected vs both infected plus treated group.

Fig 6. Values are expressed in Mean ± SD. Where ***p<0.001; highly significant, **p<0.01 significant, *p<0.05 less significant ns p>0.05 in significant. Statistical analysis was performed between control vs infected group as well as infected vs both infected plus treated group.

The levels of SGOT, SGPT, urea, creatinine, uric acid, alkaline phosphatase and total bilirubin were found to be significantly increased (79.0%; 70.2%, 46.1%, 104%, 84.2%, 76.25% and 64.1%) in infected group as compared with control group. These biochemical parameters were significantly lowered in imipenem treated group as well as in tobracing treated group after ten days of treatment as compared to infected group. When tobracing treated group was compared with imipenem treated group, these biochemical parameters were significantly lowered in tobracing treated group after nine days treatment (Tab. 2).

Total protein level was significantly lowered (56.8%) in infected group in comparision with control group. The protein level was increased about 11.02% in imipenem treated group as well as 116% in the tobracing treated group when compared with infected group after nine days treatment. When tobracing treated group was compared with imipenem...
treated group, protein level was increased and reached almost to control level. (Tab.2).

**Table. 2.** Effect of Tobracef and imipenem drug on biochemical parameters in pneumonia Induced rat model

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Control group (N=10)</th>
<th>Infected group (N=10)</th>
<th>Imipenem treated group (N=10)</th>
<th>Tobracef treated group (N=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Urea (mg/dL)</td>
<td>26.19 ± 2.61</td>
<td>40.37 ± 2.75***</td>
<td>37.63 ± 1.87*</td>
<td>27.13 ± 2.50***</td>
</tr>
<tr>
<td>2</td>
<td>Uric acid (mg/dL)</td>
<td>1.65 ± 0.07</td>
<td>3.04 ± 0.06***</td>
<td>2.86 ± 0.15 **</td>
<td>1.93 ± 0.16 ***</td>
</tr>
<tr>
<td>3</td>
<td>Creatinine (mg/dL)</td>
<td>0.25 ± 0.01</td>
<td>0.51 ± 0.02***</td>
<td>0.49 ± 0.03*</td>
<td>0.26 ± 0.02***</td>
</tr>
<tr>
<td>4</td>
<td>Total bilirubin (mg/dL)</td>
<td>0.39 ± 0.04</td>
<td>0.64 ± 0.07***</td>
<td>0.58 ± 0.06*</td>
<td>0.48 ± 0.03***</td>
</tr>
<tr>
<td>5</td>
<td>Total protein (mg/dL)</td>
<td>8.63 ± 0.74</td>
<td>3.72 ± 0.39***</td>
<td>4.13 ± 0.64*</td>
<td>8.03 ± 0.46***</td>
</tr>
<tr>
<td>6</td>
<td>ALP (IU/L)</td>
<td>122.8 ± 5.58</td>
<td>216.5 ± 3.86***</td>
<td>210.6 ± 7.56</td>
<td>134.33 ± 3.94***</td>
</tr>
<tr>
<td>7</td>
<td>SGOT (IU/L)</td>
<td>47.67 ± 4.53</td>
<td>85.33 ± 4.46***</td>
<td>81.17 ± 3.53</td>
<td>48.67 ± 4.35***</td>
</tr>
<tr>
<td>8</td>
<td>SGPT (IU/L)</td>
<td>39.83 ± 4.26</td>
<td>67.83 ± 3.39***</td>
<td>63.17 ± 2.41</td>
<td>42.67 ± 1.97***</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SD. N= numbers of animals. Where ***= p<0.001; highly significant, **= p<0.01 significant, *= p<0.05 significant and  ns = p>0.05 insignificant. Statistical analysis was performed between control vs infected group as well as infected vs both infected plus treated group. Where ALP; Alkaline phosphatase, SGOT; Serum glutamyl oxaloacetic transaminase, SGPT; serum glutamyl pyruvic transaminase

**Discussion**

Pneumonia is one of the most common and severe form of infections treated by health care practitioners. Alveolar macrophages are essential components for lung innate immunity. Alveolar macrophages phagocyte and kill pathogens by the production of free radicals. Alveolar macrophages (AMs) are main cellular component of defense system that maintains the integrity of the lower respiratory tract. Macrophages modulate a variety of complex host functions, including immunoregulatory, phagocytic and secretory processes. Among the numerous secretory products of AMs are the reactive oxygen metabolites.

The imbalance between oxidants and antioxidants is referred as oxidative stress. Oxidative stress has been associated with various type of respiratory disorders. Increased oxidative stress involved in the pathogenesis of both airways and parenchymal lung diseases. Asthma, chronic obstructive pulmonary disease and bronchiectasis have been associated with inflammation that causes increased levels of oxidative stress. During bacterial pneumonia, rapid and massive influx of activated phagocytes into the distal airways has been observed. These phagocytic cells release excess free radicals when they encounter bacteria, as part of host defense against infection. ROS are also produced by bacteria during aerobic respiration. Enhanced production of ROS may induce peroxidative lipid damage. This damage may be scavenged off by antioxidant enzymes. Superoxide dismutase (SOD), Catalase and glutathione reductase (GR) are free radical scavenging enzymes that inhibits the generation of free radicals.

In the present study, all antioxidant enzymes (SOD, Catalase and GR) activities were significantly decreased along with increased malonaldehyde levels (MDA) in pneumonia infected group as compared with control group. Similar results are reported by other researchers.

Katsoulis et al. has been reported that, occurrence
of oxidant/antioxidant imbalance through decreased total serum antioxidant status (TAS) in pneumonia patients. Cemek et al. have been reported that enzymatic and non enzymatic antioxidant activities were decreased along with increased oxidative stress in children with acute pneumonia. WBC and ESR were higher in infected group when compared to control group. WBC and ESR level were increased due to presence of bacterial infection that causes inflammatory response during pneumonia. WBC and ESR are non specific inflammatory parameters that reflect the severity of acute phase reaction. Korppi et al. has reported that the level of ESR and WBC were found higher in pneumonia in children. During pneumonia infection, there are some alteration in hepatic and renal enzymes. Our study, SGOT and SGPT levels were significantly increased in infected group as compared with control group. Kang et al. has been reported that, increased levels of hepatic enzymes were frequently observed during mycoplasma pneumonia infection in children. The elevated SGOT and SGPT levels may be associated with possible hepatocellular dysfunction induced by severe inflammation and sepsis. Hepatic enzymes were frequently observed during M. pneumoniae in children. Nikolic et al. reported that the higher plasma activities of SGOT and SGPT and higher malonaldehyde level production in damage liver. The levels of renal enzymes (total bilirubin, alkaline phosphatase, urea, uric acid and creatinine) were significantly increased in infected group as compared to control group. It has been reported that the renal enzymes induced by vasoactive mediators triggered by bacterial components. Wang et al. reported that, bilirubin and creatinine levels were significantly increased in pneumonia infected immunocompetent mice.

The induction of proinflammatory cytokines may play an important role in the pathogenesis of pneumonia infection. It is known that cytokines are important mediators in both lung defense and inflammation, in response to bacterial infection. Cytokines (TNF-α, IL-1β and IL-6) are important mediators in host defence against bacterial and viral infections. In pneumonia the initiation, maintenance, and resolution of inflammation is dependent upon the complex network of pro-inflammatory and anti-inflammatory cytokines. In this study, we found that cytokines were significantly increased in infected group as compared with control group. These levels were increased due to bacterial infection. Various researchers have been reported that the cytokines is necessary for host defense against the invasive pathogen.

Pneumonia infection is cured by the empirical therapy of antibiotics. A combination of antibiotics provides a broader spectrum of coverage than any single antibiotic alone. Combination therapy should be synergistic & provides an anti-bacterial spectrum greater than the sum of individual activities. Several studies reported that combination therapy have a better efficacy than monotherapy in nosocomial pneumonia infection. After administration of a fixed dose combination of ceftazidime plus tobramycin (tobracef) and imipenem alone for a week treatment, antioxidant enzymes activities were improved along with significant decreased MDA level as well as lowered the hepatic and renal enzymes levels in tobracef treated group in comparision to imipenem treated group. Similarly the cytokines levels were improved in tobracef treated group in comparision to imipenem alone treatment.

**Conclusion**

Our findings concluded that tobracef has better efficacy than imipenem therapy in carbapenem resistant pneumonia infection. It
improved antioxidant enzyme activities by reducing lipid peroxidation level during bacterial infection.

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