Effect of Ascorbic Acid on Lipid Peroxidation Induced by Ceftazidime

Devbhuti P1*, Devbhuti D2, Saha A3, Sengupta C4

1Gupta College of Technological Sciences, Asansol, West Bengal, India
2Sree Ramkrishna Shilpa Vidyapith, Suri, Birbhum, West Bengal, India
3Department of Chemical Technology, University of Calcutta, Kolkata, India
4Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India

Abstract
Lipid peroxidation is the oxidative deterioration of polyunsaturated lipids which is a free radical related process and responsible for the development of many diseases and disorders like diabetes mellitus, hypertension, cancer etc. End products of lipid peroxidation are malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), etc. which are the ultimate mediator of toxicity. Antioxidants have the capability to inhibit lipid peroxidation. Keeping in mind this fact, the present in vitro study was carried out to evaluate lipid peroxidation induction potential of ceftazidime, a cephalosporin antibiotic and its suppression with ascorbic acid considering some laboratory markers of lipid peroxidation like MDA, 4-HNE and reduced glutathione (GSH). Goat liver was used as the lipid source. After treatment of the liver homogenate with drug and/or antioxidant the levels of 4-HNE, MDA and GSH were estimated in different samples at different hours of incubation. The results showed that the drug ceftazidime could significantly induce lipid peroxidation and the antioxidant ascorbic acid has the capability to inhibit ceftazidime-induced lipid peroxidation.

Keywords: Lipid peroxidation, drug toxicity, ceftazidime, ascorbic acid

Introduction
Oxidative deterioration of polyunsaturated lipid is known as lipid peroxidation which is found to be involved in the pathogenesis of several diseases and ailments. Oxidative stress may cause lipid peroxidation which is a free radical related process. Reactive oxygen species and other prooxidants are involved in the formation of aldehydic end products including malondialdehyde (MDA), 4-hydroxy-2-nonenals (4-HNE), which are found to be the ultimate mediators of toxicity. To minimize the deleterious effects of lipid peroxidation, antioxidants are used as possible suppressor of lipid peroxidation series of 2-substituted benzimidazole analogs were synthesized and antimicrobial activity were evaluated.

Materials and Methods

Materials
Liver collected from goat (capra capra) was used as the lipid source. The goat liver was selected because of its easy availability and close similarity to the human liver in its lipid profile. Goat liver was collected in a sterile vessel containing phosphate buffer (pH 7.4) solution. The buffer solution was drained completely and the liver was immediately grinded to make a tissue homogenate (1 g/ml) using freshly prepared phosphate buffer (pH 7.4). The homogenate was divided into four equal parts as C (control), D (only drug treated), DA (drug plus antioxidant treated) and A (only antioxidant treated). Drug and antioxidant were added at a concentration of 1.3332 mg/g and 0.1666 mg/g of liver tissue respectively. After drug and/or antioxidant treatment, the different portions of liver homogenate were shaken for 1 hour and incubated below 20°C for up to 24 h.

Estimation of 4-hydroxy-2-nonenal (4-HNE) level
The estimation was done at 2 and 24 hours of incubation and it was repeated in 5 animal sets. In each case three samples of 2.0 ml of tissue homogenate were treated with 2.0 ml of 10% TCA solution. The mixture was then centrifuged at 3000 rpm for 30 minutes. Then 2.0 ml of the filtrate was treated with DNPH (100 mg/100 ml of 0.5 M HCl) and kept for 1 hour at room temperature. The samples were then extracted with hexane and the extract was evaporated at 400°C. After cooling, 2.0 ml of methanol was added to each sample and the absorbance was measured at 350 nm against methanol as blank. The concentration of 4-HNE was calculated from a standard curve.

*Address for Correspondence:
E-mail: p_devbhuti@rediffmail.com
Tel. 9433515149
Fax: +91341-2314604
Estimation of malondialdehyde (MDA) level

The malondialdehyde content was measured using thioarobitic acid (TBA) method.\textsuperscript{16} The estimation was done at 2 and 24 hours of incubation and repeated in five animal sets. In each case three samples of 2.5 ml of incubation mixture was treated with 2.5 ml of 10\% trichloroacetic acid (TCA) and centrifuged at 3000 rpm for 30 minutes to precipitate protein. Then 2.5 ml of the filtrate was treated with 0.002 (M) TBA solution (5.0 ml) and the volume was made up to 10.0 ml with distilled water. The mixture was heated for 30 minutes in boiling water bath. Then the tubes were cooled to room temperature and the absorbance was measured at 530 nm against a TBA blank (prepared from 5.0 ml of TBA solution and 5.0 ml of distilled water) using WPA light wave diode array spectrophotometer (version 2.3). The concentration of MDA was calculated from a standard curve.

Estimation of reduced glutathione (GSH) level

Reduced glutathione was measured by its reaction with 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) to give a colored compound which absorbs at 412 nm (Ellman's method).\textsuperscript{17} The estimation was done at 2 and 5 hours of incubation and repeated in five animal sets. In each case three samples of 1.0 ml of tissue homogenate were treated with 1.0 ml of 5\% TCA in 1 mM EDTA and centrifuged at 2000 g for 10 minutes. Then 1.0 ml of the filtrate was mixed with 5.0 ml of 0.1 M phosphate buffer (pH 8.0) and 0.4 ml of DTNB (0.01\% in phosphate buffer) was added to it. The absorbance of the solutions was measured at 412 nm against a blank (prepared from 6.0 ml of phosphate buffer and 0.4 ml of DTNB) using the above-mentioned spectrophotometer. The concentration of GSH was calculated from a standard curve. The percent changes in 4-HNE, MDA and GSH level of different samples at different hours of incubation were calculated with respect to the control of the corresponding hours of incubation. The calculation of percent changes in 4-HNE, MDA and GSH content with corresponding t-values, average changes in five animal sets with corresponding standard errors were done directly from raw spectrophotometric data.

Results and Discussion

The results of the studies on ceftazidime-induced lipid peroxidation and its suppression with ascorbic acid have been illustrated in Fig. 1, 2, and 3. Interpretation of the results is supported by Student's t-test. Incubation of the liver homogenates with ceftazidime caused an increase in 4-HNE and MDA content with respect to control of different hours (Fig. 1-2). This observation suggests the lipid peroxidation induction potential of the drug ceftazidime. It was further observed that...

\begin{figure}[h!]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Effect of ascorbic acid on ceftazidime induced 4-HNE content increase. Key: D, DA, and A indicate ceftazidime-treated, ceftazidime and ascorbic acid treated and only ascorbic acid treated samples, respectively.}
\end{figure}

\begin{figure}[h!]
\centering
\includegraphics[width=\textwidth]{fig2.png}
\caption{Effect of Ascorbic acid on ceftazidime induced MDA content increase. Key: D, DA, and A indicate ceftazidime-treated, ceftazidime and ascorbic acid treated and only acid treated samples, respectively.}
\end{figure}

\begin{figure}[h!]
\centering
\includegraphics[width=\textwidth]{fig3.png}
\caption{Effect of ascorbic acid on ceftazidime induced GSH content decrease. Key: D, DA, and A indicate ceftazidime-treated, ceftazidime and ascorbic acid treated and only a scorbic acid treated samples, respectively.}
\end{figure}
enhancement of 4-HNE and MDA content due to ceftazidime was significantly suppressed when the liver homogenate was incubated with both the drug and antioxidant. This indicates that ascorbic acid could reduce ceftazidime induced lipid peroxidation due to its free radical scavenging property. When the liver homogenate was treated with only ascorbic acid, the 4-HNE and MDA content was further decreased with respect to control due to antioxidant property of ascorbic acid. Reduced glutathione plays a very important role in the defense mechanism for tissues against the free radical attack.

Conclusions
The present results suggest lipid peroxidation induction capacity of ceftazidime, which may be related to its toxic potential. The results further suggest antiperoxidative effect of ascorbic acid that prevents ceftazidime induced lipid peroxidation. Free radical scavengers have the capability to reduce iatrogenicity of drugs by way of inhibiting drug induced lipid peroxidation. From the present study, it is clear that ascorbic acid has the potential to reduce ceftazidime induced toxicities. However, any definite conclusion can only be drawn after a detail study using more parameters and an in vivo system.

References