Centrifaxone-induced Lipid Peroxidation and Its Inhibition with Various Antioxidants

Comparative studies on possible in vitro suppressive actions of three antioxidants, ascorbic acid (AA), cysteine hydrochloride (CH) and hydroquinone (HQ) on ceftriaxone sodium (CTS)-induced lipid peroxidation were performed using goat whole blood as the lipid source to explore possible potential of the antioxidants to reduce the drug-induced toxicities. AA was found to significantly suppress CTS-induced lipid peroxidation while CH and HQ failed to do so. Lipid peroxidation being a toxicity mediating process, AA might have potential to reduce the toxic effects of CTS on co-administration in vivo.

Lipid peroxidation is a molecular mechanism of cell injury bringing about several changes in structures and functions of biological membranes with potential injurious consequences. It is a free radical chain reaction promoting generation of peroxyl radicals and lipid hydroperoxides (LH). LH can decompose to yield a wide variety of cytotoxic products including malondialdehyde (MDA) which interacts with DNA and other cell materials leading to mutagenesis and carcinogenesis.

Lipid peroxidation involves highly reactive oxygen species formed constantly in the human body and are removed by enzymatic (e.g., superoxide dismutase, catalase, glutathione peroxidase etc. which are mostly intracellular) and non-enzymatic (e.g., α-tocopherol, ascorbic acid, caeruloplasmin, albumin, bilirubin etc. which are mostly extracellular) antioxidant defence systems.

Oxidative damage is increased by the processes like ion release, lipoygenase activation, phagocyte stimulation, disruption of mitochondrial electron transport chain and/or decrease in antioxidant defences. When antioxidant defence of the body is inadequate, oxidative stress can damage a variety of biomolecules, inactivate receptors and membrane-bound enzymes. Reactive oxygen species seem to play a role in the mediation of some pathological states, e.g., joint injury in rheumatoid patients, formation of atherosclerotic plaques, some pulmonary and hepatic diseases, neurological disorders etc. Recently, doxorubicin-induced cardiotoxicity has been reported to be mediated through free radical reaction sequence.

In the present study, drug-induced lipid peroxidation and its suppression with selected antioxidants have been focussed in an attempt to correlate toxic potential of the drug to its lipid peroxidation induction capacity and to find out a suitable antioxidant which on co-administration in vivo with the drug might reduce toxic action and increase therapeutic index of the drug. Ceftriaxone sodium (CTS) a third generation cephalosporin, has been chosen as the representative drug and in vitro inhibition of CTS-induced lipid peroxidation by three antioxidants, viz, ascorbic acid, cysteine hydrochloride and hydroquinone has been comparatively studied.

MATERIALS AND METHODS

For each antioxidant, separate sets of experiments were performed. The experimental method involved following steps:
Collection and preservation of blood—Goat whole blood was collected in a sterile vessel containing anticoagulant solution (sodium citrate in saline) and filtered to remove foreign materials like dust and hair and then preserved at 15° for further work.

Treatment and incubation of blood with drug and/or antioxidant—The blood collected and preserved as above was divided into six portions of 50 ml each. The first portion was kept as the control (CL) while the second portion was treated with the drug (CTS) at a concentration of 80 mg% (equivalent to 4 g human dose). The third and fourth portions were treated with both drug and antioxidant at two dose levels of antioxidant. The equivalent human dose levels of antioxidants were 250 mg and 500 mg for both AA (CTSA250, CTSA500) and CH (CTSC250, CTSC500) while 50 mg and 100 mg for HQ (CTSH50, CTSH100). The fifth and sixth portions were treated with only antioxidant at two different doses (AA: A250, A500; CH: C250, C500; HQ: H50, H100). The solutions of the drug and the antioxidants were prepared using normal saline. Calculated volumes of saline were added to different samples to keep the volume of additives same in all cases. After treatment with drug and/or antioxidant, blood samples were stirred for 1 h below 20° and then incubated at 15° for 32 h along with the control sample.

Estimation of lipid peroxidation breakdown products measured as MDA—The extent of lipid peroxidation was estimated in terms of MDA content using thiobarbituric acid (TBA) method as described by Tarladgis et al.13 with some modifications. The estimation was done at 2.5, 5, 8, 24 and 32 h of incubation for all samples and additionally at 0 h (during addition of drug and/or antioxidant) for the control sample.

In each case three samples of 2.5 ml blood were withdrawn and each aliquot was treated with 2.5 ml 10% solution of trichloroacetic acid and centrifuged at 3000 rpm for 30 minutes to precipitate the protein part. The supernatant was filtered and mixed with 5 ml 0.002 molar TBA solution and volume was made up to 10 ml with distilled water. The mixture was boiled for half an hour and then cooled to room temperature. Absorbance of the resulting solution was measured at 530 nm against a TBA blank (prepared from 5 ml TBA solution and 5 ml distilled water).

RESULTS AND DISCUSSION

Effects of the antioxidants (AA, CH, HQ) on CTS-induced lipid peroxidation have been presented in figures 1, 2 and 3. The control blood samples (CL) did not show any significant change in MDA content in different hours of incubation.

CTS produced time-dependent increase in MDA content with respect to control (0 h). It is evident that in vitro incubation of blood with CTS induced lipid peroxidation.

Fig. 1: Effects of AA on CTS-induced lipid peroxidation: Percent changes in MDA content with respect to control (0 h) (Averages of five animal sets with corresponding standard errors have been shown)

Fig. 2: Effects of CH of CTS-induced lipid peroxidation: Percent changes in MDA content with respect to control (0 h) (Averages of five animal sets with corresponding standard errors have been shown)
Table 1 - Effects of Ascorbic Acid on CTS induced lipid Peroxidation:
ANOVA and Multiple Comparison Analysis of data

<table>
<thead>
<tr>
<th>Incubation Time (h)</th>
<th>F ratio Between sample*</th>
<th>F ratio Between animals**</th>
<th>Critical difference at 5% level with ranked sample means***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LSD*</td>
</tr>
<tr>
<td>2.5</td>
<td>31.480 (a)</td>
<td>7.904 (a)</td>
<td>3.117 (AE)(EFD)(FDC)(B)</td>
</tr>
<tr>
<td>5</td>
<td>52.066 (a)</td>
<td>5.297 (a)</td>
<td>3.327 (A)(EF)(CD)(B)</td>
</tr>
<tr>
<td>8</td>
<td>72.693 (a)</td>
<td>2.790 (d)</td>
<td>3.153 (A)(F)(DC)(B)</td>
</tr>
<tr>
<td>24</td>
<td>31.894 (a)</td>
<td>0.242 (e)</td>
<td>6.889 (A)(EF)(DC)(B)</td>
</tr>
<tr>
<td>32</td>
<td>78.895 (a)</td>
<td>0.610 (e)</td>
<td>5.349 (A)(EF)(DC)(B)</td>
</tr>
</tbody>
</table>

Significance levels of F values: a>99%, b>97.5%, c>95%, d>90%, e<90%
Keys for sample means: A=CL, B=CTS, C=CTSA250, D=CTSC500, E=A250, F=A500
*d.f. = (5,20); **d.f. = (4,20); # Ref. 16;
## Two means not included in same parentheses are statistically significantly different at P<0.05;
+ Least significant difference procedure; ++ Studentized range procedure

Table 2 - Effects of Cysteine Hydrochloride on CTS induced lipid Peroxidation:
ANOVA and Multiple Comparison Analysis of Data

<table>
<thead>
<tr>
<th>Incubation Time (h)</th>
<th>F ratio Between sample*</th>
<th>F ratio Between animals**</th>
<th>Critical difference at 5% level with ranked sample means***</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>LSD*</td>
</tr>
<tr>
<td>2.5</td>
<td>16.323 (a)</td>
<td>8.973 (a)</td>
<td>7.249 (AFE)(CBD)</td>
</tr>
<tr>
<td>5</td>
<td>16.720 (a)</td>
<td>5.619 (a)</td>
<td>7.706 (AFE)(DCB)</td>
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<tr>
<td>8</td>
<td>30.284 (a)</td>
<td>7.700 (a)</td>
<td>8.350 (EFA)(CD)(DB)</td>
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<tr>
<td>24</td>
<td>28.456 (a)</td>
<td>3.269 (c)</td>
<td>12.216 (AFE)(CDB)</td>
</tr>
<tr>
<td>32</td>
<td>55.494 (a)</td>
<td>7.786 (a)</td>
<td>10.151 (FAE)(CDB)</td>
</tr>
</tbody>
</table>

Significance levels of F values: a>99%, b>97.5%, c>95%, d>90%, e<90%
Keys for sample means: A=CL, B=CTS, C=CTSC250, D=CTSC500, E=C250, F=C500
*d.f. = (5,20); **d.f. = (4,20) # Ref. 16;
## Two means not included in same parentheses are statistically significantly different at P<0.05;
+ Least significant difference procedure; ++ Studentized range procedure
Lipid peroxidation, being a destructive process, causes denaturation of biological membranes by changing fluidity and permeability, inactivation of membrane bound enzymes and receptors, and loss of -SH group. Thus lipid peroxidation induction capacity of the drug may be related to its toxic potential.

In case of blood sample treated with both drug and antioxidant, different effects were observed depending on the antioxidant used. Increase in MDA content was significantly decreased with respect to drug-treated blood in case of AA (Fig. 1), but not in the cases of CH (Fig. 2) and HQ (Fig. 3). The results imply that AA significantly suppressed CTS-induced lipid peroxidation where as CH and HQ failed to do so.

It was also observed that AA and HQ per se produced slight but significant increase in MDA content with respect to control (0 h) when used without drug but CH did not produce any such change in MDA content. The increase in

![Graph showing the effect of HQ on CTS-induced lipid peroxidation](image)

**Fig. 3: Effect of HQ on CTS-induced lipid peroxidation:**
Percent changes in MDA content with respect to control (0 h) (Averages of five animal sets with corresponding standard errors have been shown)

<table>
<thead>
<tr>
<th>Table 3 - Effects of Hydroquinone of CTS induced lipid Peroxidation: ANOVA and Multiple Comparison Analysis of Data</th>
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<tr>
<td><strong>Hrs of Incubation</strong></td>
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<tr>
<td></td>
</tr>
<tr>
<td>2.5 hr</td>
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<tr>
<td>5 hr</td>
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<tr>
<td>8 hr</td>
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<tr>
<td>24 hr</td>
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<tr>
<td>32 hr</td>
</tr>
</tbody>
</table>

Significance levels of F values: a>99%, b>97.5%, c>95%, d>90%, e<90%
Keys for sample means: A = CL, B=CTS, C=CTSH50, D=CTSH100, E=H50, F=H100
*d.f. = (5,20); ** d.f. = (4,20); # Ref. 16;
## Two means not included in same parentheses are statistically significantly different at P<0.05;
* Least significant difference procedure; ++ Studentized range procedure
MDA levels in the blood samples treated with only AA (A250, A500) may be explained on the basis of the fact that AA reduces Fe$^{3+}$ to Fe$^{2+}$ which promotes generation of hydroxyl radicals and other highly reactive species accelerating lipid peroxidation\cite{14,15}. The fact that HQ treated samples (H50, H100) showed higher MDA content than the control suggests that HQ per se might have peroxide generation potential. Interpretation of the results from the figures is supported by statistical multiple comparison analysis by least significant difference procedure and studentized range procedure\cite{16} (Tables 1, 2 and 3).

In summary, among the antioxidants used, only AA could effectively suppress CTS-induced lipid peroxidation though it is reported that it itself may generate peroxides in presence of metal ions, especially iron. Thus it may be presumed that AA might have potential in reducing toxic effects of CTS on co-administration in vivo if blood level of iron is carefully monitored. However, these in vitro results give only predictive idea about the nature of actions of the antioxidants on lipid peroxidation and are not to be treated as conclusive. Variations may be found between in vitro and in vivo results and this necessitates further in vivo studies to confirm the predictions.

ACKNOWLEDGEMENTS

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REFERENCES

2. Esterbauer, H., Zollner, H. and Scharf, R.J., Biochemistry, 1988, 1, 311.