Lipid peroxidation, has been broadly defined by A.L. Tappel as the oxidative deterioration of polyunsaturated lipids. It leads to the generation of peroxides and lipid hydroperoxides that can decompose to yield a wide range of cytotoxic products most of which are aldehydes, as exemplified by 4-hydroxy-nonenal (4-HNE) and malondialdehyde (MDA) (1). Different types of aldehydes which are generated endogenously during the process of lipid peroxidation are causally involved in some of the pathophysiological effects associated with oxidative stress in cells and tissues.

Lipid peroxidation on its own is amplifier for the initial free radicals, and the reactive aldehydes generated in this process may well act as “second toxic messengers” of the complex chain reactions which are initiated if polyunsaturated fatty acids of the membrane bilayer are converted to lipid hydroperoxides (3-5). MDA is cytotoxic and chemically reactive, but 4-HNE is more aggressive than MDA and leads to cell damage at nanomolar concentrations (6). Oxidative free radicals are known to cause peroxidation of membrane polyunsaturated fatty acids. 4-HNE may contribute to the cytotoxic effects of oxidative stress (2, 7, 8). 4-HNE prevents NO production in cells by inhibiting nuclear factor-κB-dependent transcriptional activation of inducible NO synthase (9). Nitric oxide, a reactive radical, controls various vital physiological functions in the body. Estimation of nitrite and nitrate, the stable end products of nitric oxide oxidation, is a common indirect method used to monitor nitric oxide (NO) levels in various body fluids and tissues (10). Endogenous NO production is highly correlated with nitrite/nitrate levels in serum, plasma and urine. Hence estimation of nitrite/nitrate is a relative measurement of NO production.

Antioxidants are substances that, when present at low concentrations compared with those of an oxidizable substrate, significantly prevent or delay a pro-oxidant initiated oxidation of the substrate (11). Use of suitable antioxidants, as adjuvants with peroxidative drugs, appears to become a promising

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EVALUATION OF ASCORBIC ACID, PROBUCOL, AND α-TOCOPHEROL AS SUPPRESSORS OF DEXAMETHASONE INDUCED LIPID PEROXIDATION

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Abstract: Lipid peroxidation is a highly destructive process, which induces a plethora of alterations in structure and function of cellular membranes. It is involved in number of diseases and poisoning by several toxins. Lipid peroxidation occurs in response to oxidative stress, and a great diversity of aldehydes are formed when lipid hydroperoxides break down in biological systems. Among the aldehydic endproducts, most intensively studied is 4-hydroxy-nonenal (4-HNE), the major toxic mediator of consequences of lipid peroxidation. The present study deals with the lipid peroxidation induction potential of dexamethasone (DEX), a commonly used anti-inflammatory drug, and the scavenging capacity of some conventional antioxidants like ascorbic acid (AA), probucol (PR) and α-tocopherol (TOC) on drug induced lipid peroxidation. The drug (dexamethasone) induced lipid peroxidation studies have been performed using goat liver homogenate. Liver being the major site for detoxification is the primary target for environmental or occupational toxic exposure in biological system. The results of the present study indicate that DEX increased the 4-HNE content vis-à-vis decreased the nitric oxide (NO) level of tissue. This suggests that DEX caused significant extent of lipid peroxidation, which may be related to the toxic potential of the drugs, and the above antioxidants have potential protective effects against DEX induced lipid peroxidation to the significant extent.

Keywords: dexamethasone, ascorbic acid, α-tocopherol, probucol, 4-hydroxy-nonenal, nitric oxide

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approach for reducing iatrogenic disorders. A major contribution of antioxidants is to prevent degenerative diseases associated with aging, cancer, cardiovascular disease, immune-system decline, brain dysfunction, and cataracts. Ascorbic acid (AA), probucol (PR), \( \alpha \)-tocopherol (TOC) are well known antioxidants (12-15), which protect cells and tissues from oxidative damage induced by free radicals (16).

A part of our ongoing effort to explore drug induced lipid peroxidation and inhibitory actions of different antioxidants on drug induced lipid peroxidation (17-22), our present study has been made to explore the lipid peroxidation induction potential of steroidal anti-inflammatory drug dexamethasone (DEX) on liver homogenates using 4-HNE and NO as markers of lipid peroxidation and suppressive actions of different antioxidants like ascorbic acid, probucol and \( \alpha \)-tocopherol on dexamethasone induced lipid peroxidation. As a whole, the aim of the study was to explore dexamethasone induced lipid peroxidation and search some potential antioxidants that might have possible prospect and promise for reducing toxicity due to dexamethasone induced lipid peroxidation. Corticosteroids are the most effective therapeutic agents for the treatment of inflammatory diseases. Functionally, they act partly by inducing anti-inflammatory genes such as secretory leukocyte proteinase inhibitor, lipocortin-1 and interleukin-1 receptor antagonist, but mainly by repression of inflammatory genes, such as cytokins, adhesion molecules, inflammatory enzymes and receptors (23). They act by binding to a cytosolic glucocorticoid receptor (GR), which upon binding is activated and rapidly translocated to the nucleus. Within the nucleus, the GR either induces gene transcription by binding to specific deoxyribonucleic acid elements in the promoter/enhancer regions of responsive genes or reduces gene transcription by transrepression. The GR reduces gene transcription by interaction with pro-inflammatory transcription factors such as activation protein-1 and nuclear factor-kappa B. These effects of the GR on gene expression involve changes in the chromatin structure localized to the promoter sites of responsive genes. Many of the detrimental side effects of corticosteroids are believed to be due to gene induction (23, 24). Dexamethasone has various toxic effects such as gastrointestinal upset, nausea, visual disturbances, abdominal pain etc. (25). Thus, evaluation of antioxidants as suppressors of the dexamethasone induced lipid peroxidation provides a scope to select free radical scavengers, which on co-administration in vivo, may reduce toxic effects of the drug used for therapeutic purpose, in case of reduced endogenous antioxidants defence.

**MATERIALS AND METHODS**

**Chemicals**

Dexamethasone (DEX) was gifted by Cadila Pharmaceuticals Ltd., Ahmedabad, Gujrat. Trichloroacetic acid was purchased from Ranbaxy, S.A.S. Nagar; 2,4-dinitrophenylhydrazine (DNPH) from SD Fine Chem. Ltd., Mumbai. The standard sample of 4-HNE was purchased from ICN Bio-medicinals Inc., Aurora, Ohio. All other reagents were of analytical grade.

Liver collected from goat (Capra capra) was used as the lipid source. Goat liver was selected because of its availability and close similarity of its lipid constituents to that of human liver (26).

**Preparation of tissue homogenate**

Goat liver, perfused with normal saline through hepatic portal vein, was harvested and its lobes were briefly dried between filter papers to remove excess of blood and thin-cut with a heavy-duty blade. These small pieces were then transferred to a homogenizer to prepare homogenate (1 g, w/v) in cold condition temp. 25°C. The work has been done as per the guidelines of the Institutional Animal Ethics Committee.

**Incubation of tissue homogenate with drug and/or antioxidant**

For each antioxidant (AA/PR/TOC), the tissue homogenate was divided into four parts of 50 ml each. The first portion was kept as the control (CL) which was not treated with drug or antioxidant, while the second portion was treated with the drug along with antioxidant (AA/PR/TOC). The fourth portion was treated with only antioxidant (AA/PR/TOC). Ascorbic acid (AA), probucol (PR) and \( \alpha \)-tocopherol (TOC) were used at effective concentrations of 0.12 mg/g, 0.1 mg/g and 0.1 mg/g, respectively. After treatment with drug and/or antioxidant, liver homogenates were stirred for 1 h below 20°C and then incubated at 15°C up to 24 h along with the control sample.

**Estimation of lipid peroxidation end products measured as 4-HNE from tissue homogenate**

The extent of lipid peroxidation was estimated in terms of 4-HNE content using 2,4-dinitrophenylhydrazine (6, 27). The estimation was done at 4 and
24 h of incubation during addition of drug and/or antioxidant and for the control portion of the liver sample. In each case three samples of 2.5 ml of incubation mixture were transferred to tubes containing 2.5 ml of 10% trichloroacetic acid. Then the tubes were centrifuged at 3000 rpm for 30 min to precipitate the protein part. The TCA soluble fraction was fully separated and then the supernatant was treated with DNPH solution (5 mM) and kept aside for 1 h at room temperature i.e. 25°C. Then the samples were extracted with hexane and after extraction, the hexane layers were collected into graduated stopper tube and evaporated in a water bath at a temperature not exceeded 40°C. Then the residue was cooled to room temperature, 2 ml of methanol was added to it and shaken well. Then the absorbance was measured at 350 nm in a Beckman DU-64 UV/VIS spectrophotometer. The values were determined from the standard curve obtained by using standard 4-hydroxynonenal.

Estimation of nitric oxide (NO) level from tissue homogenates

Nitric oxide was measured as the amount of nitrite (the stable metabolite of NO) present in tissue homogenates. Nitrite was measured colorimetrically by the Griess reaction method (28, 29). The Griess reagent was prepared by gentle mixing 100 µl of 1.0% sulphanilamide (prepared in 3M HCl) and 100 µl of 0.1% N-naphthylethylenediamine.

The estimation was done at 4 and 24 h of incubation during addition of drug and/or antioxidant and for the control portion of the liver sample. In each case three samples of 3 ml of incubation mixture were transferred to tubes containing 3 ml of 10% trichloroacetic acid. Then the tubes were centrifuged at 3000 rpm for 30 min to precipitate the protein part. The TCA soluble fraction was fully separated and then 5 ml of supernatant was treated with 0.5 ml of Griess regent and kept aside for 15 min at room temperature i.e. 25°C. After 15 min, the absorbance was measured at 540 nm against a blank solution containing the same concentrations of ingredients but no biological sample. The amount of nitric oxide level was calculated from standard curve using sodium nitrite as the standard.

The per cent changes in 4-HNE content and NO level of tissues of different samples at different hours of incubation were calculated with respect to the control of the corresponding hours of incubation and the change in 4-HNE/NO level was considered as indicator of the extent of lipid peroxidation. The calculations of per cent changes in 4-HNE/NO content with corresponding ‘t’ values, average changes in five animal sets with corresponding standard errors and analysis of variance (ANOVA) (30, 31) were done directly from raw spectrophotometric data. When more than two treatments are to be used in an experiment, ANOVA may be used for comparing the means of two or more treatment groups. If the F test is significant and more than two treatments are included in the experiment, it may not be obvious immediately which treatments are different. Some or all of the treatments may be different. Various multiple-comparison procedures have been proposed to solve this problem. The general procedure is to test the ranked means from lowest to highest and two means included in the same parenthesis that are not statistically significantly different from each other. Two means not included in the same parenthesis are statistically significantly different at p<0.05. The procedure is carried out by calculating a 5% allowance, which is defined as the critical difference between means (31). This allows one to reject the null hypothesis (µi=µj) and accept the alternative hypothesis (µi≠µj) for any two sample means X i and X j at p=0.05.

In case of the least significant difference (LSD) procedure 5% allowance is given by the

\[ t \cdot \sqrt{\frac{\text{s}^2}{n_i} + \frac{\text{s}^2}{n_j}} \]

where t is the value of t from two tail t table at p=0.05 which depends upon the df (degree of freedom), \( \text{s}^2 \) is the pooled variance from the analysis of variance and n i, n j are the numbers of observations from which the means \( \bar{X}_i \) and \( \bar{X}_j \) were determined, respectively.

RESULTS

4-Hydroxy-nonenal (4-HNE) content in liver homogenate

The results of the studies on dexamethasone induced lipid peroxidation, measured as change in 4-HNE content, and its inhibition with ascorbic acid, probucol and α-tocopherol are listed in Tables 1, 2 and 3. Interpretation of the results is supported by Student’s t-test and also by a statistical multiple comparison analysis using the significant different procedure (30, 31) (Tables 1, 2 and 3).

The per cent changes in 4-HNE content with respect to control of the corresponding hours of incubation for different samples and their averages are shown along with statistical analysis in Tables 1, 2 and 3. From the results, it is evident that the treatment of liver homogenates with DEX, significantly increases the 4-HNE content with respect to control of the corresponding hours. It may be inferred that this drug could significantly induce the lipid peroxi-
idation process. It was further found from the study that when the liver homogenates were incubated with drug (DEX) in combination with antioxidants (AA/PR/TOC) then an increase in 4-HNE content due to DEX was significantly arrested in comparison to drug-treated liver homogenates. It happened due to the free radical scavenging capacity of these antioxidants. When the liver homogenates were treated only with antioxidant AA/PR/TOC, the 4-HNE content decreased in comparison to the control but in case of the ascorbic acid some increase in 4-HNE content was observed.

### Table 1. Effects of ascorbic acid (AA) on dexamethasone (DEX) induced lipid peroxidation: percentage change in 4-HNE and NO content

<table>
<thead>
<tr>
<th>Hrs of incubation</th>
<th>Animal set</th>
<th>Percentage changes in 4-HNE content with respect to control</th>
<th>Analysis of variance and multiple comparison</th>
<th>Time of incubation</th>
<th>Animal set</th>
<th>Percentage changes in NO content with respect to control</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>DAA</td>
<td>AA</td>
<td></td>
<td>D</td>
<td>DAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 hr</td>
<td>A1</td>
<td>105.55'</td>
<td>35.19'</td>
<td>-11.61'</td>
<td>F1=8.71'</td>
<td>[df=(2, 8)]</td>
<td>F2=1.41</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>234.96'</td>
<td>216.21'</td>
<td>-6.92'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>389.47'</td>
<td>340.16'</td>
<td>-3.26'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A4</td>
<td>174.95'</td>
<td>149.68'</td>
<td>-9.96'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A5</td>
<td>328.42'</td>
<td>319.69'</td>
<td>-7.66'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Av. (SD)</td>
<td>246.67 212.18</td>
<td>-7.88 114.31</td>
<td>125.68 3.18)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hr</td>
<td>A1</td>
<td>321.26'</td>
<td>267.27'</td>
<td>24.71'</td>
<td>F1=19.11'</td>
<td>[df=(2, 8)]</td>
<td>F2=0.69</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>276.76'</td>
<td>247.66'</td>
<td>05.51'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>244.67'</td>
<td>224.03'</td>
<td>05.20'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A4</td>
<td>361.21'</td>
<td>300.99'</td>
<td>-04.84'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A5</td>
<td>294.99'</td>
<td>284.47'</td>
<td>-00.31'</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Av. (SD)</td>
<td>299.77 264.88</td>
<td>06.05 (44.22)</td>
<td>(30.24)</td>
<td>(11.27)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D, DAA, and AA indicate only DEX-treated, DEX & AA-treated and only AA treated samples, respectively.

* Per cent changes with respect to controls of corresponding hours are shown.

Significance of ‘t’ values of the per cent changes of 4-HNE/NO content (df = 4)
a: >99%; b: 98-99%; c: 95-98%; d: 90-95%; e: 80-90%; f: 60-80%; g: <60%.

F₁ and F₂ correspond to variance ratios between samples and between animals, respectively.

F values are significant at p<0.05.

Av. = Average of five animal sets; S.E. = Standard Error (df=4); df=degree(s) of freedom.

* Error mean square, # Critical difference according to least significant difference procedure (Ref. 30 and 31).

** Two means not included within the same parenthesis are statistically significantly different at p<0.05.
Nitric oxide (NO) level in liver homogenate

The tissue levels of NO were also measured in the present study. NO is metabolized to its stable products nitrite and nitrate. Nitrite levels were measured as an index for NO generated in the tissues (29). The per cent changes in the nitric oxide level (NO) with respect to the control of different hours of incubation for various samples collected from different animals and their averages are shown along with statistical analysis in Tables 1, 2, 3. The tables also show the effects of AA< PR and TOC as free radical scavengers. Interpretation of the results is

Table 2. Effects of probucol (PR) on dexamethasone (DEX) induced lipid peroxidation: percentage change in 4-HNE and NO content

<table>
<thead>
<tr>
<th>Hrs of incubation</th>
<th>Animal set</th>
<th>Percentage changes in 4-HNE content with respect to control</th>
<th>Analysis of variance and multiple comparison</th>
<th>Time of incubation</th>
<th>Animal set</th>
<th>Percentage changes in NO content with respect to control</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 hr</td>
<td></td>
<td>D DPR PR</td>
<td>F1 = 23.59&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>A1 PR</td>
<td>F1 = 13.33&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[df = (2, 8)]</td>
<td></td>
<td></td>
<td></td>
<td>[df = (2, 8)]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>F2 = 2.93</td>
<td></td>
<td></td>
<td></td>
<td>F2 = 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>[df = (4, 8)]</td>
<td></td>
<td></td>
<td></td>
<td>[df = (4, 8)]</td>
</tr>
<tr>
<td></td>
<td>A1 PR</td>
<td>101.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-0.79&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td>A1 PR</td>
<td>-19.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>06.04&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>260.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A2 PR</td>
<td>260.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-9.81&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>A2 PR</td>
<td>-27.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.94&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>275.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-4.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A3 PR</td>
<td>402.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-4.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>A3 PR</td>
<td>-16.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>09.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-5.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A4 PR</td>
<td>306.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-4.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>A4 PR</td>
<td>-27.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.62&lt;sup&gt;a&lt;/sup&gt;</td>
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<td></td>
<td></td>
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<td>-4.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>A5 PR</td>
<td>269.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-5.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>A5 PR</td>
<td>-24.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>08.47&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>108.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(3.28)</td>
<td></td>
<td></td>
<td></td>
<td>(5.31)</td>
</tr>
</tbody>
</table>

D, DPR, and PR indicate only DEX-treated, DEX & PR-treated and only PR treated samples, respectively.

<sup>1</sup> Per cent changes with respect to controls of corresponding hours are shown.

Significance of ‘<’ values of the per cent changes of 4-HNE/NO content (df = 4)

a: >99%; b: 98-99%; c: 95-98%; d: 90-95%; e: 80-90%; f: 60-80%; g: <60%.

F<sub>1</sub> and F<sub>2</sub> correspond to variance ratios between samples and between animals, respectively.

V<sub>1</sub> F values are significant at p <0.01; V<sub>2</sub> F values are significant at p<0.05.

Av. = Average of five animal sets; S.E. = Standard Error (df=4); df=degree(s) of freedom.

* Error mean square; # Critical difference according to least significant difference procedure (Ref. 30 and 31).

** Two means not included within the same parenthesis are statistically significantly different at p<0.05.
supported by Student’s t-test and also by the statistical multiple comparison analysis using the least significant different procedure (30, 31) (Tables 1, 2 and 3).

Incubation of the liver homogenate with dexamethasone significantly decreased the NO level with respect to the corresponding controls. Again, when the liver homogenates were treated only with the antioxidant (AA/PR/TOC), the NO level was increased in comparison to the control and drug-treated samples of corresponding incubation periods except in some cases with ascorbic acid. This increase in NO level may be due to the free radical scavenging properties of these antioxidants.

When the liver homogenates were treated with the drug (DEX) along with antioxidants (AA, PR and TOC), the decrease of NO level was less than that of drug treated samples of the same incubation periods.

Table 3. Effects of α-tocopherol (TOC) on dexamethasone (DEX) induced lipid peroxidation: percentage change in 4-HNE and NO content

<table>
<thead>
<tr>
<th>Hrs of incubation</th>
<th>Animal set</th>
<th>Percentage changes in 4-HNE content with respect to control</th>
<th>Analysis of variance and multiple comparison</th>
<th>Time of incubation</th>
<th>Animal set</th>
<th>Percentage changes in NO content with respect to control</th>
<th>Analysis of variance and multiple comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>DTOC</td>
<td>TOC</td>
<td></td>
<td>D</td>
<td>DTOC</td>
<td>TOC</td>
</tr>
<tr>
<td></td>
<td>4 hr</td>
<td></td>
<td></td>
<td></td>
<td>A2</td>
<td>-16.92</td>
<td>05.21</td>
</tr>
<tr>
<td></td>
<td>A1</td>
<td>-21.91</td>
<td>-04.38</td>
<td>12.50</td>
<td>Pooled variance (c) = 46205.99</td>
<td>Critical difference (p=0.05) LSD=313.49</td>
<td>Ranked means**: (D, DTOC) (TOC)</td>
</tr>
<tr>
<td></td>
<td>A2</td>
<td>-18.40</td>
<td>-06.32</td>
<td>07.57</td>
<td>Pooled variance (c) = 17007.00</td>
<td>Critical difference (p=0.05) LSD=190.19</td>
<td>Ranked means**: (D, DTOC) (TOC)</td>
</tr>
<tr>
<td></td>
<td>A3</td>
<td>-21.01</td>
<td>-03.04</td>
<td>17.97</td>
<td>Critical difference (p=0.05) LSD=70.37</td>
<td>Ranked means**: (D, DTOC) (TOC)</td>
<td>F1=23.28</td>
</tr>
<tr>
<td></td>
<td>A4</td>
<td>-17.36</td>
<td>-00.52</td>
<td>15.78</td>
<td>Critical difference (p=0.05) LSD=70.37</td>
<td>Ranked means**: (D, DTOC) (TOC)</td>
<td>F1=23.28</td>
</tr>
<tr>
<td></td>
<td>A5</td>
<td>-15.90</td>
<td>-07.82</td>
<td>22.22</td>
<td>Critical difference (p=0.05) LSD=70.37</td>
<td>Ranked means**: (D, DTOC) (TOC)</td>
<td>F1=23.28</td>
</tr>
<tr>
<td></td>
<td>Av. (SD)</td>
<td>290.91</td>
<td>255.12</td>
<td>-00.17</td>
<td>Av. (SD)</td>
<td>(158.01)</td>
<td>(150.08)</td>
</tr>
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<td>-02.12</td>
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<td>A5</td>
<td>-15.90</td>
<td>-07.82</td>
</tr>
</tbody>
</table>

D, DTOC, and TOC indicate only DEX-treated, DEX & TOC-treated and only PR treated samples, respectively.

* Per cent changes with respect to controls of corresponding hours are shown.

Significance of ‘t’ values of the per cent changes of 4-HNE/NO content (df = 4)

ψ Per cent changes with respect to controls of corresponding hours are shown.

a: >99%; b: 98-99%; c: 95-98%; d: 90-95%; e: 80-90%; f: 60-80%; g: <60%.

F1 and F2 correspond to variance ratios between samples and between animals, respectively.

V1 F values are significant at p<0.01; V2 F values are significant at p<0.05.

Av. = Average of five animal sets; S.E. = Standard Error (df=4); df=degrees of freedom.

F1 and F2 correspond to variance ratios between samples and between animals, respectively.

* Error mean square, # Critical difference according to least significant difference procedure (Ref. 30 and 31).

** Two means not included within the same parenthesis are statistically significantly different at p<0.05.
period, i.e. increased the NO level in comparison to only drug treated samples with respect to control of the corresponding hours.

DISCUSSION

The results of the present study suggest that dexamethasone treatment can result in increased generation of lipid peroxidation end product 4-HNE content in liver homogenates. Supplementation of antioxidants resulted in decreasing the formation of the lipid peroxidation products with respect to the control values of the corresponding hours. The increase in the generation of 4-HNE content indicates the involvement of free radical mediated lipid peroxidation due to dexamethasone. Again, when the liver homogenates were treated with drug (DEX) and antioxidant (AA/PR/TOC), the 4-HNE content of the tissue was reduced in comparison to drug-treated liver homogenates, and when liver homogenates were treated only with antioxidant (AA/PR/TOC), the 4-HNE content of the tissue was reduced significantly in comparison to the control of the corresponding hours. This implies that the antioxidants could reduce the extent of dexamethasone induced oxidative stress. This is due to their protective effects against free radicals that may have been generated within the system due to the presence of the drug. It is worthy of mentioning that when the liver homogenates were treated only with ascorbic acid, then some increase in 4-HNE content with respect to control of the corresponding hours was observed. In the case of ascorbic acid it is due to its double role (antioxidant and prooxidant) with respect to free radical reactions (32). The ability of ascorbic acid to release transition metals from a protein complex or to maintain transition metals, such as iron and copper, in a reduced state is the likely mechanism involved (33). Iron released from ferritin molecules by superoxide radicals is capable of initiating the Haber-Weiss reaction and generating oxygen radical species (34).

When the liver homogenates were treated with drug (DEX), the NO levels were decreased significantly with respect to control of the corresponding hours. Dexamethasone is known to be an inhibitor of nitric oxide synthase (35, 36). The inhibitory effect on NOS could result in the decreased levels of NO noted in the dexamethasone treated tissue homogenates.

High 4-HNE/NO ratio indicates an increase in pro-oxidant status in the tissues. NO reacts with superoxide to form peroxynitrite (37, 38). NO when produced in excess is capable of quenching superoxide anion (39), but if NO levels are low, then increased amounts of peroxynitrite radical are formed which can cause an increase in the generation of products (37).

From the present study we found that supplementation with antioxidant (AA/PR/TOC) resulted in the elevation of NO levels in the liver homogenates with respect to control of the corresponding hour. Further, when the liver homogenates were treated with DEX along with AA/PR/TOC, the NO level increased with respect to drug treated samples of the corresponding hours. This indicates that supplementation with antioxidants reduced the per-oxidative damage induced by steroidal drug dexamethasone.

CONCLUSION

Based on the results of the present study, increased levels of 4-HNE content and decreased levels of NO content in drug treated liver homogenates with respect to control of the corresponding hours suggested that the steroidal drug dexamethasone has lipid peroxidation inducing capacity. The present study also indicates the potential of different antioxidants, e.g., ascorbic acid, probucol and α-tocopherol, for suppression of lipid peroxidation induced by dexamethasone. These observations imply that the antioxidants merit further extensive studies to explore their possible potential in reducing drug-induced toxicity that may be mediated through free radical mediated processes. The concept of antioxidant co-therapy may also be exploited during future formulation design with an aim of reducing drug-induced toxicity. Moreover, lipid peroxidation induction capacity of a drug may be tested at the individual level to determine the extent of risk from a drug in case of a particular individual in view of variable in vivo antioxidant defence, and accordingly, the decision about safe use of a drug and necessary co-administration of antioxidants may be taken. However, further extensive study is required to advance such hypothesis.

Acknowledgements

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REFERENCES


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