Antioxidant Status and Hepatic Lipid Peroxidation in Chloramphenicol-Treated Rats

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E. Olatunde Farombi. Antioxidant Status and Hepatic Lipid Peroxidation in Chloramphenicol-Treated Rats. Tohoku J. Exp. Med., 2001, 194 (2), 91-98 —— The present study reports the enzymatic and non-enzymatic antioxidant status and hepatic microsomal lipid peroxidation in chloramphenicol treated rats. Chloramphenicol at a dose of 28 mg/kg body weight orally administered to rats increased the activity of cytosolic superoxide dismutase by 63% while the activities of glutathione peroxidase and catalase were decreased by 57% and 44%, respectively. In vitro, chloramphenicol altered the activities of these enzymes though not as pronounced as the effect of the drug on the enzymes in vivo. The levels of serum vitamins A, C and β-carotene were significantly decreased following chloramphenicol treatment. Microsomal lipid peroxidation was markedly and significantly increased by chloramphenicol treatment. The drug elicited 69% and 71% increases in the levels of malondialdehyde and lipid hydroperoxide respectively. Glutathione level and glutathione S-transferase activity were decreased by 42% and 58%, respectively, compared to untreated controls. Overall, the results of the present investigation indicate alteration of enzymatic and non-enzymatic antioxidant status and induction of lipid peroxidation by chloramphenicol. The clinical implications in the detoxification of toxic metabolites of lipid peroxidation caused by chloramphenicol warrant co-administration with antioxidant vitamins in chloramphenicol treatment regimen. —— antioxidant status; lipid peroxidation; chloramphenicol; non-enzymic antioxidants; enzymic antioxidants

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The formation of free radicals during the metabolism of xenobiotics is an important mechanism of action through which some toxic agents may cause cellular damage and cytotoxic effects. Organic free radicals also are formed via reduction and also by oxidation reactions of several compounds (Younes 1999). Free radical reactions have been suggested to be involved in the toxic effects of several antibiotics (Halliwell and Gutteridge 1989).

The antibiotic, chloramphenicol, active against a wide range of bacteria, is used in the

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treatment of purulent meningitis and typhoid fever (Trevett et al. 1992; Kumar and Verma 1993). Therapeutic use of this drug is severely restricted in advanced countries due to the potential occurrence of reversible hematopoietic depression and irreversible aplastic anemia (Yunis 1978). However, it is heavily relied upon and widely used for the treatment of childhood meningitis in many parts of the developing countries where the cost of the third generation antibiotics like cephalosporins is prohibitively expensive.

Chloramphenicol is known to block and inactivate several key enzymes including hepatic microsomal P450 enzymes (Miranda et al. 1998; Kraner et al. 1994). We have also demonstrated that chloramphenicol can inhibit hepatic esterase and amidase activities (Farombi et al. 2000a). Derache et al. (1982) reported that chloramphenicol decreases the activity of microsomal reductases, the production of malondialdehyde and conjugated dienes in microsomes suggesting that chloramphenicol could act like an antioxidant derivative. However, recent studies have revealed that the drug does not only interfere with enzyme system but also possesses the ability to produce free radicals capable of damaging molecular structures of cells. Thus, the generation of reactive oxygen species in chloramphenicol treated cells (Karbowska et al. 1999) and in rat liver microsomes (Teo et al. 1986) treated with amino chloramphenicol in vitro have been reported. It has also been hypothesized that free radicals are involved in chloramphenicol-induced formation of megamitochondria (Matsuhashi et al. 1996; Karbowska et al. 1999).

In living cells, the biological effects of free radicals are controlled by nonenzymic antioxidants such as glutathione, tocopherols, ascorbic acid, carotenoids (Akkus et al. 1996), enzymic antioxidants like superoxide dismutase, glutathione peroxidase and catalase are involved in the detoxification of superoxide anion, hydrogen peroxide and lipid hydroperoxides (Guemouri et al. 1991).

Lowering of antioxidant defense status might result in increased peroxidation of cell membrane lipids resulting in elevation of lipid peroxides, which can damage cells and tissues. There is paucity of information on the effect of chloramphenicol on the antioxidant defense status. The present investigation therefore reports the effect of chloramphenicol on the non-enzymatic, enzymatic antioxidant activities and lipid peroxidation of rats.

**MATERIALS AND METHODS**

**Chemicals**

Chloramphenicol sodium succinate (100 mg), a product of Shangai, China was obtained from the Jaja Clinic University of Ibadan, Nigeria. Glutathione, 1, 2 dichloro-4-nitrobenzene (CDNB) thiobarbituric acid, β-carotene, epinephrine, p-nitroaniline, glycyl glycine and hydrogen peroxide were purchased from Sigma Chem., Co. (London, UK). All other chemicals were of analytical grade and were obtained from British drug houses (Poole, UK).

**Animals and treatment**

Male albino rats (Wistar strain) weighing between 180-200 g were used for the study. They were bred and housed in the Primate Colony Department of Biochemistry, University of Ibadan, Nigeria. They were kept in wire meshed cages and fed with commercial rat chow (Ladokun Feeds Nigeria Limited) and liberally supplied with water.

Twenty rats were divided into two groups of ten animals each. The first group was used as controls and received physiological saline. The second group of rats was treated with chloramphenicol (28.6 mg/kg body weight) administered orally for ten consecutive days. The animals were sacrificed 24 hours after the last treatment.
Preparation of serum

The rats were sacrificed by cervical dislocation and dissected. The liver was removed and rinsed in ice-cold 1.15% KCl and weighed. Blood was collected from rats by heart puncture technique into centrifuge tubes. Serum was prepared by centrifugation for 10 minutes at 3000×g in an MSC (Essex, UK) bench centrifuge. The clear supernatant was used for the estimation of serum enzymes.

Preparation of microsomal and cytosolic fractions

The liver excised from rat, blotted of bloodstains, rinsed in 1.15% KCl was homogenised in a Tris-sucrose buffer (10 mM Tris, 1.25 M sucrose, 1 mM EDTA, pH 7.4).

The homogenates were centrifuged at 10 000×g for 20 minutes to obtain the supernatant fraction. The supernatants were subjected to ultracentrifugation at 100 000×g for 90 minutes. Microsomal and cytosolic fractions were obtained after the centrifugation.

Determination of antioxidant enzyme activities

The procedure of Misra and Fridovich (1972) as described by Magwere et al. (1997) was used for the determination of superoxide dismutase activity by measuring the inhibition of autoxidation of epinephrine at pH 10.2 at 30°C.

Activity of catalase was determined according to the procedure of Claiborne (1989) by following the absorbance of hydrogen peroxide at 240 nm, pH 7.0 and 25°C.

Glutathione-peroxidase activity was determined according to Gunzler and Flohe (1989) by measuring the oxidation of NADPH at 340 nm, pH 7.0 and 37°C.

Effects of chloramphenicol on antioxidant enzymes in vitro

Cytosolic fractions obtained after ultracentrifugation of the 10 000×g super-

natant fractions of physiological saline-treated rats were used for the assay. Chloramphenicol, at a final concentration of 0, 10, 25, 50, 75 and 100 μM were added to each of the assay media for superoxide dismutase, catalase and glutathione peroxidase. The assays were carried out as described above.

Assay of non enzymatic antioxidants

Serum vitamin C was determined chemically according to the procedure described by Erel et al. (1997) using dinitrophenylhydrazine (DNPH).

Serum β-carotene and vitamin A were assayed according to the method of Suzuki and Katoh (1990) as described by Kokcman and Naziroglu (1999).

Assessment of lipid peroxidation

Lipid peroxidation was assessed in the rat liver microsomes by measuring the thiobarbituric acid reactive substances (TBARS) at 532 nm using a Jenway 6105 UV/Visible spectrophotometer (Essex, UK) according to the procedure described by Farombi et al. (2000 b). Malondialdehyde (MDA) was quantitated by using Σ = 1.56×10³ M⁻¹ cm⁻¹ (Buege and Aust 1978). Lipid hydroperoxides were also determined in microsomes using the thiocyanate method according to Cavallini et al. (1983).

Determination of glutathione and glutathione S-transferase activity

Glutathione was determined in the 10 000×g supernatant fraction of liver homogenate according to Jollow et al. (1974). Microsomal glutathione S-transferase activity was determined by the method described by Gibson and Skett (1994) using 1, 2-dichloro-4-nitrobenzene as substrate.

Protein determination

Protein content of all fractions was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard.
Statistics

The data were analysed by a two-tailed Student's t-test. p-Values less than 0.01 were considered statistically significant between controls and treated rats.

RESULTS

Table 1 shows the results of chloramphenicol (28.6 mg/kg body weight) treatment on cytosolic superoxide dismutase, glutathione peroxidase and catalase activities. Chloramphenicol increased superoxide dismutase level by 63% while it elicited 57% and 44% decreases in the activities of glutathione peroxidase and catalase, respectively. In vitro, chloramphenicol affected the activities of the enzymes, though not as pronounced as the effect of chloramphenicol on the enzymes in vivo (Fig. 1).

Table 1 also represents the effect of chloramphenicol on nonenzymic antioxidants. Serum vitamins A, C and β-carotene levels were markedly decreased following chloramphenicol treatment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated</th>
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<tbody>
<tr>
<td>Superoxide dismutase (Units/mg protein)</td>
<td>40.3 ± 2.5</td>
<td>65.5 ± 2.2*</td>
</tr>
<tr>
<td>Catalase (Units/mg protein)</td>
<td>36.1 ± 2.8</td>
<td>20.3 ± 1.7*</td>
</tr>
<tr>
<td>Glutathione peroxidase (Units/mg protein)</td>
<td>2.8 ± 0.2</td>
<td>1.2 ± 0.1*</td>
</tr>
<tr>
<td>Vitamin A (µmol/liter)</td>
<td>2.1 ± 0.3</td>
<td>1.3 ± 0.4*</td>
</tr>
<tr>
<td>Vitamin C (µmol/liter)</td>
<td>63.1 ± 8.8</td>
<td>44.2 ± 6.2*</td>
</tr>
<tr>
<td>β-Carotene (µg/100 ml)</td>
<td>13.2 ± 1.1</td>
<td>8.6 ± 1.4*</td>
</tr>
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*Significantly different from control p < 0.001.

1 unit of superoxide dismutase (SOD) is the amount that inhibits autoxidation of epinephrine by 50% at pH 10.2, 30°C.

1 unit of GSH peroxidase (GSH-Px) represents 1 µmol NADPH oxidised/min at 7.0 and 37°C.

1 unit of catalase (CAT) decomposes 1 µmol H₂O₂/min at pH 7.0 and 25°C.

The values are the mean ± s.d. for 10 rats in each group.

Fig. 1. Effect of chloramphenicol on the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) in vitro. Each point represents the mean ± s.d. for 10 rats and each measured in triplicates.

--- SOD; --- CAT; --- GSH Px.
Table 2. Effects of chloramphenicol (28.6 mg/kg) treatment on malondialdehyde, lipid hydroperoxide, glutathione and glutathione S-transferase activity of rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Treated</th>
</tr>
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<tr>
<td>Malondialdehyde (nmol/mg protein)</td>
<td>366 ± 23.2</td>
<td>620 ± 23.3*</td>
</tr>
<tr>
<td>Lipid hydroperoxide (nmol/mg protein)</td>
<td>586 ± 34.5</td>
<td>1001 ± 33.2*</td>
</tr>
<tr>
<td>Glutathione (nmol/mg protein)</td>
<td>5.5 ± 0.2</td>
<td>3.2 ± 0.5*</td>
</tr>
<tr>
<td>Glutathione S-transferase (nmol/min/mg protein)</td>
<td>2.6 ± 0.1</td>
<td>1.1 ± 0.2*</td>
</tr>
</tbody>
</table>

The values are the mean ± s.d. for 10 rats in each group.

*Significantly different from control *p* < 0.001.

The effects of chloramphenicol on malondialdehyde, lipid hydroperoxide, glutathione and glutathione S-transferase are presented in Table 2. Malondialdehyde level was increased by 69% and lipid hydroperoxide by 71% compared to untreated controls. Glutathione level and glutathione S-transferase activity were decreased by 42% and 58%, respectively (*p* < 0.001) compared to control.

**DISCUSSION**

It is known that aromatic nitro compounds such as nitrofurantoin, metronidazole and chloramphenicol undergo enzymatic one electron reduction to corresponding nitro radicals, and these radicals react with molecular oxygen resulting in the formation of superoxide (Trush et al. 1982; Clark et al. 1985). The formation of free radicals in microsomes by chloramphenicol, has also been reported (Teo et al. 1986). Moreover, the drug is known to undergo biotransformation by hepatic glutathione S-transferases to aldehyde derivatives, which in turn may generate free radicals due to oxidation by xanthine and aldehyde oxidases (Holt et al. 1995, 1997). Hence, metabolism of chloramphenicol appears to play a pivotal role in its toxicity and reactive oxygen species have been ascribed to be the proximal toxicant (Holt et al. 1997).

Our data indicate that chloramphenicol treatment affects both the enzymatic and non-enzymatic antioxidant profiles and causes membrane lipid peroxidation. The increase in superoxide dismutase level following chloramphenicol administration confirms our earlier study on the effect of amodiaquine, an antimalarial on this enzyme (Farombi 2000). This could be due to the response of liver to an increased production of reactive oxygen species particularly superoxide anion following exposure to chloramphenicol or its metabolites. The generation of superoxide anion radicals during oxidative metabolism of amino chloramphenicol by rat liver microsomes had been reported (Teo et al. 1986). Also, superoxide was produced during spontaneous autooxidation of hydroxyl amino chloramphenicol metabolites as well as during chemical reduction of nitroso chloramphenicol by NADPH (Teo et al. 1986).

The results further indicate that chloramphenicol decreased glutathione peroxidase and catalase activities. It is generally accepted that hydrogen peroxide can be detoxified by catalase, which removes it when present at high concentration and glutathione peroxidase, which destroys it when present at a steady state (Casado et al. 1995). Therefore, the reduction in the levels of these enzymes by the drug may render the liver more susceptible to hydrogen peroxide and hydroxyl radical-induced oxidative stress.

Chloramphenicol treatment reduced the levels of serum vitamin A, β-carotene and ascorbic acid. Carotenoids are known to protect cells and tissues against damage by preferentially reacting with radicals and being consumed in the process, thus acting as sacrificial radical-trapping antioxidants (Farombi and Britton 1999a, b; Kokcam and Naziroglu 1999). β-Carotene is a precursor of vitamin A and there is ample evidence that they are effective quenchers of reactive oxygen species (Burton
and Ingold 1984; McDowell 1989). In addition to vitamin A and β-carotene, ascorbic acid is known to represent the first line of antioxidant defense (Frei et al. 1988, 1989) and this vitamin is likely to be most susceptible to free radical oxidation. Nishikimi (1975) had reported its oxidation by superoxide anion radical.

A notable negative side effect of chloramphenicol mostly involves blood dyscrasiasis such as irreversible non-dose dependent aplastic anemia (deVries et al. 1994). The apparent decreases in serum vitamins observed in this study following chloramphenicol pretreatment suggest treatment regimens could be devised to involve co-administration with antioxidant vitamins. This may possibly abate or attenuate clinical manifestations of chloramphenicol toxicity.

The remarkable elevations of malondialdehyde and lipid hydroperoxides in rat liver microsomes after treatment with chloramphenicol indicate toxicity. Lipid peroxides in terms of lipid soluble fluorophores both in mitochondria and microsomes of the liver of chloramphenicol-treated animals had been previously reported (Matsuhashi et al. 1996).

The level of reduced glutathione is a measure of the cellular redox status (Chance et al. 1979). Hence alteration in glutathione concentration may affect the overall redox status of the cell. Our results show a decrease in glutathione by chloramphenicol with attendant decrease in glutathione S-transferase. Although chloramphenicol is known to be metabolized by glutathione S-transferase to its aldehyde derivatives (Holt et al. 1995) the ability of aldehydes to generate free radicals capable of inactivating enzyme proteins is well recognized (Holt et al. 1997). Aniya and Naito (1993) had reported that severe oxidative stress might result in decrease in glutathione S-transferase with concomitant depletion of glutathione. Similar inactivation of microsomal drug metabolizing enzymes and other hepatic enzymes by chloramphenicol has been reported (Kraner et al. 1994; Farombi et al. 2000b).

Taken together, the present observation suggests that the profile of enzymatic and non-enzymatic antioxidants is altered by chloramphenicol treatment. This may have clinical implications in the detoxification of toxic metabolites of lipid peroxidation caused by chloramphenicol-induced oxidative stress particularly under prolonged chloramphenicol treatment of health threatening diseases such as purulent meningitis and typhoid fever.

References


Matsushashi, T., Liu, X., Nishizawa, Y., Usukura, J.,


