



Research Paper

Evaluation of days-dependent chloramphenicol dosage on rat liver microsomal lipid peroxidation and catalase activity

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ABSTRACT

The cellular functions associated with oxidative metabolism can be altered by drugs and chemical agents, thereby stimulating ROS production. This research study is focused on the *in vivo* study of the inhibitory effect of chloramphenicol on hepatic microsomal enzyme system and the effect on lipid peroxidation using Thiobarbituric Acid (TBA) reactive as index of peroxidation damage. The rats were randomly divided into three (3) groups with group 1 serving as the control receiving saline water, while group 2 received chloramphenicol at a dose of 28.6 mg/kg body weight/day for 5 days and group 3 for 7 days. Liver protein content, lipid hydroperoxides and catalase activity were all determined. TBARS concentration significantly increased statistically with time of exposure. After 5 days of treatment, the level of TBARS increased by 103.56% with exogenous oxidant and 141.54% without oxidant, as compared to the respective control. On day 7, TBARS level in the liver was approximately 90.75% higher than in the control group with oxidant and 117.03% higher than the control without oxidant. The antibiotic elicited significant increase in rat liver lipid peroxidation compared to control. There were decreases in microsomal protein content of the liver in the drug-treated rats when compared with the control rats. It also showed that chloramphenicol treatment affects cytosolic catalase activities. It decreases catalase levels by 15.75% in 5 days dosage treatment and 17.81% in 7 days dosage treatment, respectively. There were significant difference ($P < 0.05$) in the treated and control groups irrespective of the days of dosage. The catalase level in hepatocyte in 5 and 7 days group was significant at $p < 0.01$. In conclusion, it was evident that treatment of rats for 5 and 7 days with the therapeutic doses of chloramphenicol altered the antioxidant system and the intensity of effects was concentration/dosage days dependent, which resulted in membrane lipid peroxidation, protein damage and inhibition of microsomal catalase due to increased generation of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS).

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INTRODUCTION

Several chemical agents can alter the cellular functions associated with the oxidative metabolism, thereby stimulating ROS production (Paez et al., 2008). Free radical reactions have been suggested to be involved in the toxic effects of several antibiotics (Vazifeh et al., 2002; Okuyan et

al., 2005). Several side effects and diseases have been reported with the use and administration of different antibiotics (Paez et al., 2008). Generally, the cytotoxic effects of therapeutic drugs include diverse metabolic changes that affect the host cell's normal functioning, with

oxidative stress being one of the alterations provoked (Paez et al., 2008). Similarly, some antibiotics seem to affect the oxidative state of cellular components, for example, the action of chloramphenicol on cytochrome P450. This is related to enzymatic oxidation through an increase in ROS, while the co-administration of antioxidant vitamins may attenuate its toxic action (Farombi et al., 2002).

Chloramphenicol is derived from the bacterium *Streptomyces venezuelae*; it is a broad spectrum antibiotic that was first synthesized on a large scale and a bacteriostatic drug that hinders bacterial growth by binding to the 50S subunit of the 70S ribosome of the bacterial and preventing peptide bond formation by inhibiting the peptidyl transferase activity of the bacteria ribosome, thus, inhibiting protein synthesis (Adesanoye et al., 2014). Chloramphenicol is effective against a wide variety of micro-organisms. It is widely used in many parts of the world, especially in the developing countries for the treatment of life-threatening infections such as typhoid fever and meningitis (Turton et al., 1999). It exerts mainly a bacteriostatic effect on a wide range of gram-positive and gram-negative organisms and is active against Rickettsia and Chlamydia (*Psittacosis lymphogranuloma* organisms), and Mycoplasma. It is also indicated in severe salmonella infections and regarded as an alternative agent for pneumococcal, meningococcal and the ampicillin-resistant *Haemophilus influenzae* infection (Holt et al., 1993).

Chloramphenicol administration is associated with quite a number of adverse effects. Idiosyncratic aplastic anaemia can occur in predisposed patients after chloramphenicol is administered, irrespective of the dosage. This is thought to be due to the production of a nitro-reduction derivative of chloramphenicol by the gut flora. This derivative can induce DNA damage in replicating haematopoietic stem cells, resulting in marrow hypocellularity and progressive pancytopenia (Paez et al., 2008). The most common presentation, however, is a reversible, dose-dependent bone marrow suppression, which usually occurs when serum chloramphenicol levels exceed 25 mg/L for prolonged periods of time. This condition is associated with the inhibition of mitochondrial protein synthesis and characterized by mild marrow hypocellularity, anaemia, neutropenia and thrombocytopenia (Walker et al., 1998).

As non-idiosyncratic aplastic anaemia and 'grey baby' syndrome are dose-dependent complications of chloramphenicol usage, sufficient absorption of chloramphenicol must occur through the nasolacrimal duct in order to increase the serum concentrations (Lam et al., 2002). These side effects limit the successful use of certain drugs. Both *in vitro* and *in vivo* results indicate that the toxicity of diverse chemotherapeutic drugs involves an increased production of ROS and oxidative stress with lipid peroxidation and protein oxidation (Lu and Cederbaum, 2006). This research if focused on the *in vivo* study of the inhibitory effect of chloramphenicol on hepatic microsomal enzyme system and the effect on lipid peroxidation.

MATERIALS AND METHODS

The male Wistar rats with body weight 120 to 150 g, were kept in metal cages at room temperature with 12 h light: dark cycling. They had free access to drinking water and standard granulated diet. The rats were purchased from the the Central Animal House of the Faculty of Basic Medical Sciences, University of Ibadan, Oyo state, Nigeria. The handling and treatment of rats were in conformation to the guidelines of the National Institute of Health (NIH publication 85-23, 1985) for laboratory animal care and use. Acclimatization of rats was done two (2) weeks before commencement of drug administration and treatment. Chloramphenicol used for this study was obtained from Smithkline Beecham. All other reagents were of analytical grade.

Experimental design

The rats were randomly divided into three (3) groups. The first group served as the control and received physiological saline, while the second group received chloramphenicol at a dose of 28.6 mg/kg body weight/day for 5 days and was sacrificed on the 6th day after an overnight fasting and the third group received chloramphenicol also at a dose of 28.6 mg/kg body weight/day for 7 days and was sacrificed on the 8th day after overnight fasting (to avoid excess of glycogen in the liver which can stick to the homogenate). Rats were maintained on a normal diet throughout the experimental period. The study was approved by the Ethics Committee of the University College Hospital, University of Ibadan, Oyo state, Nigeria.

Collection and preparation of tissues

After 5 and 7 days of the experiment, rats were sacrificed by cervical dislocation. The livers were quickly removed, rinsed in ice cold 1.15% KCl solution and weighed. The liver samples were chopped and homogenized in 4× liver weight volume of homogenizing buffer using Potter-Elvehjem homogenizer with a loose fitting pestle. The homogenates were centrifuged at 10,000 g, 4°C for 15 min in a Sorvall RC-5B centrifuge. The post nuclear supernatant was further centrifuged at 10,500 g, 4°C for 55 min in a Beckman L-8-80M ultracentrifuge and the resultant pellet weighed and resuspended in the incubation buffer – 130 mM KCl/ 20 mM Tris, pH 7.2 to a volume of 1 ml/g weight. The preparation was stored at -80°C until required.

Biochemical assays

Protein content of the microsomal fractions of the livers was determined by the Biuret method (Gornall et al., 1949)

Table 1: Effect of Chloramphenicol treatments for a period of 5 and 7 days at therapeutic doses of 28.6 mg/kg body weight/day on body weight of rats (mean \pm standard deviation).

Treatment	Average loss in body weight (g)	% Weight loss
Control (5 days)	5.00 \pm 0.55 ^a	4.17 \pm 2.36 ^a
Chloramphenicol (5 days)	15.15 \pm 6.75 ^{b*}	12.63 \pm 1.95 ^{b*}
Control (7 days)	7.07 \pm 1.36 ^a	5.89 \pm 4.12 ^a
Chloramphenicol (7 days)	25.69 \pm 3.79 ^{c*}	21.41 \pm 0.98 ^{c*}

Values in the same column with different letters are significantly different (P<0.05), *denotes significance across dosage days (P<0.05).

Table 2: Effect of Chloramphenicol treatments on lipid peroxidation (Liver TBARS level).

Treatment	With oxidant (μ mol MDA/mg protein)	Without oxidant (μ mol MDA/mg Protein)	% Change with and without oxidant
Control (5 days)	22.50 \pm 0.71 ^a	13.00 \pm 1.41 ^a	73.07
Chloramphenicol (5 days)	45.80 \pm 0.28 ^{b*}	31.40 \pm 0.85 ^{b*}	45.86
% change	103.56%	141.54%	
Control (7 days)	28.65 \pm 4.03 ^a	20.55 \pm 7.57 ^a	39.42
Chloramphenicol (7 days)	54.65 \pm 10.39 ^{b**}	44.60 \pm 14.71 ^{b**}	22.53
% change	90.75%	117.03%	

Values in the same column with different letters are significantly different (P<0.05), */**denotes significance across dosage days (P<0.05).

using bovine albumin as standard. Lipid peroxidation was assessed as an index of oxidative stress by measuring the formation of Thiobarbituric Acid Reactive Substances (TBARS) according to the method described by Rice-Evans et al. (2000). Lipid hydroperoxides were also determined in microsomes using the thiocyanate method as described by Cavallini et al. (1983). Activity of catalase was determined according to the procedure of Claiborne (1989) following the absorbance of hydrogen peroxide at 240 nm, pH 7.0 and 25°C.

Statistical analysis

Results were expressed as means \pm S.D. Data were analyzed by one-way ANOVA and T-test. Values were considered statistically significant when p< 0.05.

RESULTS

The results present the toxicological effects of single administration of chloramphenicol to rats for a period of 5 and 7 days at therapeutic doses of 28.6 mg/kg body weight/day. Table 1 shows the changes in the body weights of the animals treated with the drugs (5 and 7 days) when compared with the control animals. The highest weight loss was observed in rats administered with chloramphenicol for 7 days (21.41 \pm 0.98%) followed by the group administered for 5 days (12.63 \pm 1.95). The control rats have

the least weight loss which were not significant (p>0.05) as compared to the initial weight. There was significant difference (p<0.05) in body weight when compared with control and dosage period.

Lipid peroxidation estimation

TBARS concentration increased statistically with time of exposure (Table 2). After 5 days of treatment, the level of TBARS increased by 103.56% with exogenous oxidant and by 141.54% without oxidant, as compared to the respective control. On day 7, TBARS level in the liver was approximately 90.75% higher than in the control group with oxidant and 117.03% higher than the control without oxidant. Proportionally, the highest increase in TBARS, as compared to the control was observed on day 7 of CAP administration (without exogenous oxidant). There was no significant difference in rats either in the presence or absence of oxidants.

Effect of chloramphenicol on protein level content, microsomal lipid hydroperoxide formation and catalase levels

Table 3 shows the effect of chloramphenicol on protein level content, microsomal lipid hydroperoxide formation and Catalase levels. There were decreases in microsomal protein content of the liver in the drug-treated rats when

Table 3: Effect of chloramphenicol treatments on liver protein content in rats.

Treatment	Liver protein level content (Mg Protein/ml)	Microsomal Lipid hydroperoxide ($\mu\text{mol}/\text{mg}$ protein)	Catalase levels in hepatocytes ($\mu\text{mol}/\text{mg}$ protein)
Control (5 days)	40.50 \pm 2.12 ^a	10.90 \pm 0.14 ^a	3.30 \pm 0.89 ^a
Chloramphenicol (5days)	36.00 \pm 1.41 ^b	12.60 \pm 0.57	2.78 \pm 1.50 ^b
% Change	11.11%	-15.59%	15.75%
Control (7days)	40.50 \pm 2.12 ^a	11.50 \pm 0.71 ^a	3.20 \pm 1.06 ^a
Chloramphenicol (7 days)	38.75 \pm 1.06 ^b	21.75 \pm 3.89 ^b	2.63 \pm 2.21 ^b
% change	4.32%	-89.13%	17.81%

Values in the same column with different letters are significantly different ($P < 0.05$), */**denotes significance across dosage days ($P < 0.05$, 0.01).

compared with the control rats. Administration of chloramphenicol for 5 days resulted in a decrease of 11.11% (from 40.50 \pm 2.12 to 36.00 \pm 1.41 mg protein/ml), while administration for 7 days resulted in a decrease of 4.32% (from 40.50 \pm 2.12 to 38.75 \pm 1.06 mg protein/ml). The decrease in the microsomal protein were significant ($P < 0.05$) for both 5 and 7 days treatment.

Lipid hydroperoxide level increased after treatment with chloramphenicol. Five (5) days dosage treatment increased by 15.59% when compared with control, while 7 days dosage treatment increased by 89.13% when compared with control. Analysis of variance of the dosage days showed that there was significant difference between the 5 and 7 days treatment ($p < 0.05$).

Results showed that Chloramphenicol treatment has effect on cytosolic catalase activities. It decreases catalase levels by 15.75% in 5 days dosage treatment and 17.81% in 7 days dosage treatment, respectively. There were significance differences ($P < 0.05$) in the treated and control group irrespective of the days of dosage. The catalase level in hepatocyte in 5 and 7 days group was significant at $p < 0.01$.

DISCUSSION

The effect of Chloramphenicol (CAP) administered singly on rat hepatic catalase and lipid peroxidation in 5 and 7 days dosage treatment was evaluated in this study. The desired medical activity of an antibiotic is to destroy or prevent the growth of offending pathogenic bacteria, but yet, these drugs still have impact on the host in an injurious manner (Barnhill et al., 2012). The results of the present study revealed a dose dependent increase in the production of Malondialdehyde (MDA) upon treatment with chloraphenicol. The production of MDA is estimated by the Thiobarbituric Acid Reactive Substances assay (TBARS) (Marnett, 1999). Malondialdehyde is used for the estimation of damage by Reactive Oxygen Species (ROS). MDA is a major reactive aldehyde resulting from the

peroxidation of biological membranes. It is also used as an indicator of tissue damaged by a series of chain reactions (Siddique et al., 2012).

The antibiotic chloramphenicol was established to be oxidized by cytochrome P450(CYP) monooxygenase to chloramphenicol oxamyl chloride formed by the oxidation of the dichloromethyl moiety of chloramphenicol followed by elimination of hydrochloric acid (Macherey and Dansette, 2008). The reactive metabolite reacts with the -amino group of a lysine residue in CYP (Halpert et al., 1985) and inhibits the enzymatic reaction progressively with time. This type of inhibition is a time-dependent inhibition or a mechanism based inhibition or inactivation, and the substrate involved historically is called a suicide substrate because the enzymatic reaction yields a reactive metabolite, which destroys the enzyme (Fontana et al., 2005; Macherey and Dansette, 2008).

Total microsomal protein was found to decrease in the drug-treated animals as compared with the controls for the different treatment dosage days ($p < 0.05$). This could be as a result of oxidation of amino acids especially by combined action of H_2O_2 and Fe^{2+} which may result in the formation of carbonyl derivative. Carbonyl modification of proteins has been shown to cause enzyme inactivation and likely enhance proteolysis (Sohal and Weindreich, 1996). The results also indicate that chloramphenicol administration decreased catalase activities. Generally, hydrogen peroxide (H_2O_2) can be detoxified by catalase by removing it when present at high concentration (Faronmbi, 2001). The reduction in the level of catalase by the treatment may make the liver more susceptible to hydrogen peroxide and hydroxyl radical-induced oxidative stress (Faronmbi et al., 2002). The oxygen reduction products are highly reactive entities that attack all the cellular components, especially when their normal degradation systems (superoxide dismutase, glutathione peroxidase and catalase) are overburdened. The polyunsaturated lipids are sensitive to these attacks because they are susceptible to a membrane-degrading peroxidation (Macherey and Dansette, 2008).

According to Lutz et al. (1998), damage to

polyunsaturated fatty acids tends to reduce membrane fluidity which is essential for the integrity and proper functioning of biological membrane (Adesanoye et al., 2014), which ultimately alters both the physical properties of microsomes and intrinsic enzyme activities (Taratino et al., 2009).

Conclusion

In conclusion, it was evident that treatment of rats for 5 and 7 days with the therapeutic doses of Chloramphenicol altered the antioxidant system and the intensity of effects was concentration/dosage days dependent; it resulted in membrane lipid peroxidation, protein damage and inhibition of microsomal catalase due to increased generation of free radicals, reactive oxygen species and reactive nitrogen species. The moderate use of chloramphenicol should be mandated and great care taken as regards the use of antibiotics (drugs generally). Self-medication should be avoided totally by patients without pre-testing. Investigation of ROS, nitric oxide and antioxidant enzymes should be performed in order to detect patients with different responses to chloramphenicol, taking into consideration the haematological alterations after therapy with this antibiotic may be a consequence of oxidative damage.

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