Dexamethasone Attenuates Cyclophosphamide-induced Hepatotoxicity in Albino Rats

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Abstract. Cyclophosphamide (CP) has appreciably improved survival rate in cancer patients, but the occurrence of hepatotoxicity may undermine its use. This study assessed the potential of dexamethasone (DEXA) to prevent CP-induced hepatotoxicity in albino rats. Thirty-six adult male albino rats were randomised in to six groups of n = 6. Group I (Control) was treated with a dose of normal saline (0.3mL) intraperitoneally (i.p.) for 24 hr. Group II was treated with a dose of DEXA (1mg/kg) ip for 24 hr. Group III was treated with a dose of CP (150mg/kg) i.p for 24 hr. Group IV was treated with a dose of CP (150 mg/kg) for 24hr before treatment with a dose of DEXA (1mg/kg) ip for 24 hr. Group IV was treated i.p with a dose of CP (150 mg/kg) for 24hr before treatment with a dose of DEXA (1mg/kg) for 24 hr. Group V was co-treated with a dose of DEXA (1mg/kg) and a dose of CP (150mg/kg) i.p for 24 hr. Group VI was pre-treated with a dose of DEXA (1mg/kg/ day) for 24 hr before treatment with a dose of CP (150mg/kg) ip for 24 hr. The rats were anesthetized after treatment; liver samples were excised and evaluated for histology and biochemical markers. Sera were obtained from blood samples and assessed for liver function markers. Liver glutathione, catalase, superoxide dismutase and glutathione peroxidase levels were decreased significantly (p < 0.001) whereas malondialdehyde levels were increased significantly (p < 0.001) in CP-treated rats when compared to control. Significant (p < 0.001) elevations in serum and liver aminotransferases, alkaline phosphatase, gamma-glutamyl transferase, conjugated bilirubin, lactate dehydrogenase and total bilirubin levels occurred in CP-treated rats when compared to control. The liver of CP-treated rats showed hepatocyte necrosis. Interestingly, the aforementioned alterations were significantly reversed in rats post-treated (p < 0.05), co-treated (p < 0.01) and pre-treated (p < 0.001) with DEXA when compared to CP. DEXA may be re-purposed as treatment for CP associated hepatotoxicity.

Keywords: Cyclophosphamide, Liver, Toxicity, Dexamethsone, Rat

1. Introduction

Cyclophosphamide (CP) is a derivative of alkylating agents which has been used over the years as an effective anti-neoplastic agent. It is also effective against non-neoplastic diseases such as systemic lupus erythematosus and rheumatoid arthritis [1, 2]. The use of CP has been characterized by array of toxicities including hepatotoxicity, pulmonary fibrosis, cardiotoxicity, nephrotoxicity and urotoxicity [3, 4]. Hepatotoxicity is a major adverse effect associated with the use of CP. It is metabolized in the liver by hepatic cytochrome p450 to its active metabolites (acrolein and phosphoramidemustard) [5]. Acrolein is highly reactive and reduces hepatic glutathione (GSH) sulphydryl groups’ thus inducing oxidative stress (OS) and causing hepatic damage [6]. CP can damage mitochondria and impairs cellular respiration [8]. Mitochondria is a repertoire for reactive oxygen species (ROS) hence, damage will increases more ROS attack, distortion of hepatic intracellular oxidant/antioxidant balance and increase OS [8]. In addition, CP causes up-regulation in lipid peroxidation (LPO) indices and nuclear transcription factor kappa-B (NF-κB) which have been associated with the stimulation of
2. Materials and Methods

2.1. Drugs, chemicals and animals. The rats were sourced from the animal unit of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria. The rats were placed in metal cages under natural condition and fed with rat diet and water \textit{ad libitum}. The rats were allowed for two weeks prior to the experiment for acclimatization and were handled according to the guideline on animal handling by National Academy of Science. DEXA (Ranbaxy Laboratory Ltd, India) and CP (Biochem Pharmaceutical Industries Ltd, India).

2.2. Dose selection and animal treatment.

- This study used CP (150 mg/kg) [15] and modified dose of DEXA (1mg/kg ) [16] diluted with normal saline
- Thirty-six adult male albino rats (200 ± 50g) randomised into six groups (I-VI) of n = 6 were used.
- Group I (Control) was treated with a dose of normal saline (0.3mL) intraperitoneally i.p for 24 hr
- Group II was treated i.p with a dose of DEXA (1mg/kg) for 24 hr
- Group III was treated i.p with a dose of CP (150 mg/kg) for 24 hr
- Group IV (Post-treatment) was treated i.p with a dose of CP (150 mg/kg) for 24hr before treatment with a dose of DEXA (1mg/kg) for 24 hr.
- Group V (Co-treatment) was co-treated i.p with a dose of DEXA (1 mg/kg) and a dose of CP (150 mg/kg) for 24 hr.
- Group VI (Pre-treatment) was pre-treated i.p with a dose of DEXA (1 mg/kg) for 24 hr before treatment with a dose of CP (150 mg/kg) for 24 hr.

2.3. Sacrifice of animals and biochemical analyses. After treatment, the rats were exposed to diethyl ether in a chamber and blood samples were taken from the heart. Blood samples were allowed to clot, centrifuged and serum samples were separated for biochemical analyses. Liver specimens were collected rinsed in cold saline and homogenized in buffered (pH 7.4) 0.1 M Tris-HCl solution. The homogenates were centrifuged at 2000 g for 20 min and the supernatants were decanted and used for biochemical analyses. Alanine aminotransferase, lactate dehydrogenase, total bilirubin, aspartate aminotransferase conjugated bilirubin, gamma-glytamyltransferase activities were determined using diagnostic laboratory test kits (Randox Laboratories Ltd., Crumlin, UK). Liver glutathione (GSH) was measured as described by Sedlak and Lindsay, 1968 [17]. Superoxide dismutase (SOD) was measured as described by Sun and Zigman, 1979 [18]. Glutathione peroxidase (GPx) was evaluated according to Rotruck et al. 1973 [19]. Catalase (CAT) was assessed according to Aebi, 1984 [20]. Protein was determined as reported by Gonall et al. 1949 [21].Malondialdehyde (MDA) was assayed as described by Buege and Aust, 1978 [22].

2.4. Histological assessment of the liver. Liver specimens were collected, rinsed and fixed in 10% neutral buffered formalin for 24hr. Liver specimens were dehydrated in ascending degree of ethanol. Liver tissues were processed, paraffin embedded and 5 µm sections were obtained using a microtome. The sections were stained with haematoxylin and eosin dye and assessed for histological changes using a light microscope

2.5. Statistical analysis. All data were analyzed with Graph Pad Prism (Version 5.0, Graph Pad Software Inc., La Jolla, California, U.S.A.) and values were presented as mean ± SEM. Differences between the control and experimental groups were analysed using one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test. Probability value less than 0.05, 0.01 and 0.001 was considered significant.
Table 1: Effect of dexamethasone on serum liver biochemical indices of cyclophosphamide-treated rats.

| Treatments        | AST (U/L) | ALP (U/L) | ALT (U/L) | LDH (U/L) | GGT (U/L) | TB (g/dL) | CB (g/dL) |
|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| CONTROL           | 32.0 ± 2.08a | 34.0 ± 2.11a | 35.3 ± 3.03a | 37.8 ± 3.05a | 13.5 ± 1.65a | 4.52 ± 0.19a | 3.63 ± 0.19a |
| DEXA              | 31.3 ± 2.11a | 33.8 ± 2.25a | 31.8 ± 3.17a | 35.3 ± 3.32a | 12.8 ± 1.09a | 4.50 ± 0.29a | 3.57 ± 0.19a |
| CP                | 121.5 ± 14.0c | 128.5 ± 13.3c | 115.4 ± 12.4c | 120.5 ± 10.2c | 49.3 ± 4.29c | 19.3 ± 1.25c | 18.3 ± 1.35c |
| DEXA+CP (Post)    | 85.5 ± 7.90d* | 81.3 ± 8.35d* | 79.0 ± 7.87d* | 80.8 ± 8.03d* | 34.3 ± 3.18d* | 15.0 ± 1.27d* | 14.0 ± 1.36d* |
| CP+DEXA (Cotr)    | 62.3 ± 6.21e* | 61.0 ± 6.96e* | 56.5 ± 4.41e* | 61.8 ± 5.18e* | 23.5 ± 2.25e* | 10.5 ± 0.53e* | 10.5 ± 0.65e* |
| CP+DEXA (Pre)     | 40.8 ± 3.37f* | 52.8 ± 4.32f* | 41.8 ± 3.44f* | 51.5 ± 5.45f* | 17.0 ± 1.13f* | 7.54 ± 0.65f* | 6.88 ± 0.48f* |

Data presented as mean ± SEM, n = 6, CP: Cyclophosphamide, DEXA: Dexamethasone, Post: Post-treatment, Cotr: Co-treatment, Pret: Pre-treatment, AST: Aspartate aminotransferase, CB: Conjugated bilirubin, GGT: Gamma glutamyl transferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, TB: Total bilirubin, ALP: Alkaline phosphatase. Values with different alphabets down the column differ at p < 0.05, *p < 0.05 in comparison to CP, #p < 0.001 in comparison to CP.

Table 2: Effect of dexamethasone on liver tissue biochemical indices of cyclophosphamide-treated rats.

| Group             | AST (U/L) | ALT (U/L) | ALP (U/L) | GGT (U/L) | LDH (U/L) |
|-------------------|-----------|-----------|-----------|-----------|-----------|
| Control           | 200.3 ± 14.7a | 227.7 ± 16.2a | 191.9 ± 15.0a | 30.3 ± 2.12a | 175.1 ± 13.7a |
| DEXA              | 198.1 ± 16.3a | 210.6 ± 19.0a | 185.4 ± 17.5a | 28.7 ± 2.00a | 160.7 ± 10.8a |
| CP                | 891.3 ± 23.2c | 811.1 ± 24.8c | 760.8 ± 22.7c | 107.9 ± 10.3c | 780.3 ± 20.2c |
| CP+DEXA (Post)    | 650.6 ± 17.7d* | 610.8 ± 28.5d* | 510.6 ± 20.1d* | 70.8 ± 6.33d* | 560.8 ± 16.1d* |
| CP+DEXA (Cotr)    | 530.7 ± 19.1e* | 500.6 ± 17.1e* | 370.7 ± 14.3e* | 50.4 ± 4.32e* | 400.1 ± 15.0e* |
| DEXA+CP (Pret)    | 390.1 ± 18.9f* | 370.5 ± 13.8f* | 240.1 ± 12.0f* | 33.5 ± 2.37f* | 251.4 ± 11.2f* |

Data presented as mean ± SEM, n = 6, CP: Cyclophosphamide, DEXA: Dexamethasone, Post: Post-treatment, Cotr: Co-treatment, Pret: Pre-treatment, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, LDH: Lactate dehydrogenase. Values with different alphabets down the column differ at p < 0.05, #p < 0.05 in comparison to CP, *p < 0.001 in comparison to CP.

Table 3: Effect of dexamethasone on liver oxidative stress indices of cyclophosphamide treated rats.

| Treatments        | SOD (U/mg protein) | CAT (U/mg protein) | GSH (µmole /mg protein) | GPx (U/mg protein) | MDA (nmol /mg protein) |
|-------------------|---------------------|--------------------|-------------------------|-------------------|------------------------|
| Control           | 20.0 ± 2.00a        | 31.9 ± 2.70a       | 15.0 ± 1.00a            | 15.4 ± 1.33a      | 0.15 ± 0.07a           |
| DEXA              | 21.1 ± 2.22a        | 33.7 ± 2.42a       | 16.1 ± 1.27a            | 15.9 ± 2.21a      | 0.13 ± 0.09a           |
| CP                | 8.70 ± 0.63c        | 12.0 ± 1.20c       | 4.10 ± 0.20c            | 4.13 ± 0.32c      | 0.89 ± 0.01c           |
| CP+DEXA (Post)    | 11.8 ± 0.57d*       | 17.1 ± 2.01d*      | 6.51 ± 0.19d*           | 6.00 ± 0.25d*     | 0.55 ± 0.06d*          |
| CP+DEXA (Cotr)    | 14.9 ± 1.33e*       | 22.8 ± 2.42e*      | 9.75 ± 0.17e*           | 9.32 ± 0.06e*     | 0.32 ± 0.07e*          |
| DEXA+CP (Pret)    | 19.4 ± 1.27f*       | 30.7 ± 2.31f*      | 14.6 ± 1.54f*           | 13.0 ± 1.32f*     | 0.20 ± 0.04f*          |

Data presented as mean ± SEM, n = 6, CP: Cyclophosphamide, DEXA: Dexamethasone Post: Post-treatment, Cotr: Co-treatment, Pret: Pre-treatment, SOD: Superoxide dismutase, MDA: Malondialdehyde, GSH: Glutathione, GPx: Glutathione peroxidase, CAT: Catalase. Values with different alphabets down the column differ at p < 0.05, #p < 0.05 in comparison to CP, *p < 0.01 in comparison to CP, #p < 0.001 in comparison to CP.
Figure 1: Normal hepatocytes (G) were observed in the liver of control rat (Figure A) whereas hepatocyte necrosis (P) (Figure B) and inflammatory cell infiltration (H) (Figure C) were observed in the liver of rat treated with CP (150mg/kg). Hepatocyte necrosis (L) and inflammatory cell infiltration (M) were observed in the liver of rats post-treated with DEXA (Figure D) whereas inflammatory cell infiltration (E) was observed in the liver of rat co-treated with DEXA (Figure E). Normal liver hepatocytes (K) were observed in the liver of rat pre-treated with DEXA (Figure F).
3. Results

3.1. Result on liver biochemical indices. DEXA treatment had no significant (P > 0.05) effects on serum ALT, AST, , CB, TB, LDH, GGT, TB, ALP and CB levels in relation to control (Table 1). However, the aforementioned parameters were elevated significantly (P < 0.001) in CP-treated rats in relation to control (Table 2). In contrast, ALT, AST, CB, TB, LDH, GGT, TB, ALP and CB levels were significantly decreased in rats post-treated (P < 0.05), co-treated (P < 0.01), and pre-treated (P < 0.001) with DEXA when compared to CP (Table 2). Furthermore, liver AST, ALP, ALT, GGT and LDH levels were normal (P > 0.05) in DEXA-treated rats, but were significantly (p < 0.001) elevated in CP-treated rats when compared to control (Table 3). However, the aforementioned parameters were decreased significantly in rats post-treated (P < 0.05), co-treated (P < 0.01), and pre-treated (P < 0.001) with DEXA when compared to CP (Table 3).

3.2. Results on liver oxidative stress markers and liver histology. Normal (P > 0.05) liver antioxidants (SOD, GSH, CAT, GPX) and MDA levels were observed in DEXA-treated rats in relation to control. In contrast, liver antioxidant levels were reduced significantly (p < 0.001) whereas MDA levels were increased significantly (p < 0.001) in CP-treated rats in relation to control (Table 4). However, liver antioxidant levels were elevated whereas MDA levels were reduced significantly in DEXA post-treated (P < 0.05), co-treated P < 0.01), and pre-treated (P < 0.001) rats when compared to CP (Table 4). Furthermore the liver of control rat showed normal histology (Figure A). The liver of treated with CP-treated rats showed hepatocyte necrosis (Figure B) and inflammatory cell infiltration (Figure C). The liver of rats post-treated with DEXA showed hepatocyte necrosis and inflammatory cell infiltration (Figure D). The liver of rats co-treated DEXA showed inflammatory cell infiltration (Figure E). On the other hand, normal liver histology was observed in rat pre-treated with DEXA (Figure F).

4. Discussion

Hepatotoxicity caused by drugs is responsible for about 5% of admissions in hospitals and 50% of cases of liver failure. About 75% of hepatotoxicity caused by drugs result to transplantation of liver or death [23] hence, the need for continuous screening of new substances for possible hepatoprotective property. Established drugs can also be assessed for possible repurposing as hepatoprotective agents. This study assessed the potential of DEXA to be repurposed as a hepatoprotective agent against CP-induced hepatotoxicity in albino rats. The alterations in the physiologic and metabolic functions of the liver accompanied by elevations in serum ALT, AST, ALP, LDH, GGT, CB and TB levels are basic features of hepatotoxicity caused by CP[24]. In this study, notable hepatotoxicity occurred in CP-treated rats marked by remarkable elevations in serum and liver levels of the aforementioned parameters. However, the effects of CP on ALT, AST, ALP, LDH, GGT, CB and TB were down-regulated in rats post-treated and co-treated with DEXA with most down-regulation observed in rats pre-treated with DEXA. One of the primary functions of antioxidants is to protect biomolecules from assaults caused by free radicals. However, assaults may become inevitable in the face of insurmountable pressure from free radicals especially ROS leading to OS and decreased antioxidant concentrations [25]. OS causes LPO which has been associated with some disease states and drug-induced hepatotoxicity. MDA is among the low-molecular-weight by-products produced as a result of LPO. Its measurement gives vivid idea on the participation of LPO in biological processes [25]. This study observed decreased hepatic antioxidants (GPx, CAT, GSH, SOD, and GSH) with increased MDA levels in CP-treated. This observation is in unison with earlier reports [26, 27]. However, hepatic antioxidants were up-regulated whereas MDA levels were down-regulated in rats post-treated and co-treated with DEXA with most remarkable effects observed in rats pre-treated with DEXA. Histological examination is one of the techniques used to ascertain the impact of drugs on the architecture of the liver. Histological assessment of CP-treated rats showed hepatocyte necrosis and inflammatory cell infiltration. This observation supports earlier reports [28]. On the other hand, CP-induced changes in hepatic structure were attenuated in rats post-treated, co-treated and pre-treated with DEXA. The observation in this study correlates with the reported hepatoprotective effect of DEXA against Yondelis (ET-743) a promising antitumor drug in rats [29]. The mechanism by which CP causes hepatotoxicity has been speculated to be associated with acrolein produced during its hepatic activation and biotransformation [30]. Acrolein is a highly reactive metabolite that triggers the production of ROS. ROS can provoke LPO, protein carbonylation and the oxidation of DNA and other biomolecules. Acrolein can also activate pro-inflammatory mediators and multiple signalling pathways that can facilitate cell death [31]. Furthermore, acrolein can bind to cellular antioxidants, leading to their depletions thereby increasing vulnerability to assault by ROS [32]. The mechanism by which DEXA attenuates CP-induced nephrotoxicity may not be far from its anti-inflammatory effect. Also, through its antioxidant effect because it has been associated with increased antioxidant expression and decreased LPO in humans and animals [33, 34]. Conclusion: DEXA attenuates CP-induced hepatotoxicity in rats with most attenuation observed in rats pre-treated with DEXA. This study suggests that DEXA may be effective against cyclophosphamide-induced hepatotoxicity.

Competing Interests

The authors declare no competing interests.
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