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Original Research

Effect of lactoferrin on some selective immunological parameters in rats immunosuppressed by cyclophosphamide

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Abstract

Eighty male albino rats (350±10 g) 10 to 12 weeks old were conducted in our study. Rats were randomly divided into four, equal groups. The groups treated as following: 1st control group (Gp. A) was given intraperitoneal normal saline (1 mL). 2nd group (Gp. B) was given a single intraperitoneal dose (250 mg/kg body weight) of Cyclophosphamide (CP) on the first day of the experimental period. 3rd group (Gp. C) CP and lactoferrin (LF) treated group. 4th group (Gp. D) administrated LF only (0.5%) in drinking water. Two separate blood samples were collected from heart puncture at end of 1st and 3rd week post treatment for hematological, biochemical and immunological studies. The leukogram of CP treatment group showed severe leucopenia (lymphopenia, neutropenia as well as eozinopenia) as well as decrease total protein and albumin blood level. Immunosuppressive effect of CP is documented in our work by decrease gamma globulin and elevation tumor necrosis factor Alfa (TNF ά). This study revealed that oral treatment with LF can partially reconstitute humoral and cellular immune response in rats given a sub-lethal dose of CP.

INTRODUCTION

Lactoferrin is one of the most exciting immune stimulants drug to come along in nutritional form in recent years. LF is predominantly neutrophil derived but indications are that it may also be produced by other cells. Receptors for LF can be found on intestinal tissue, monocytes/macrophages, neutrophils, lymphocytes, platelets, and on certain bacteria. [1-4].

Cyclophosphamide, a bifunctional alkylating agent, is extensively used as an anticancer chemotherapeutic drug in childhood and adult malignancies, as well as an immunosuppressive agent for autoimmune disorders and other benign diseases [5-8]. CP metabolites targets rapidly dividing cells, disturbing cell growth, mitotic activity, differentiation, and functions via alkylation of DNA and preferentially suppress the immune responses mediated by B-lymphocytes [9].

The aim of this study was to evaluate the immunomodulatory effect of LF in immunosuppressive rat with CP.

MATERIAL & METHODS

Experimental Animals (Rats) Eighty male albino rats of Westar strain (350±10 g,) procured from faculty of veterinary Medicine, Zagazig University were used for the study. Animals were fed with commercially available standard and balanced rat ration and water was provided *ad libitum*. The rats were housed under hygienic controlled conditions and light (12 h light/12 h dark) and had free access to water and food. All animals were acclimatized for 1 week before

experimentation and the experiment extended for 21 days.

Bovine lactoferrin (bLF) was purchased from (Symbiotics Colostrum U.S.A. (lot no. MLF160996;), with a purity of 100% Blf.

Cyclophosphamide (CP) was purchased from Baxter Oncology GmbH, Frankfurt am Main Germany, in the form of dry powder substance, under trade name Endoxan 1gm. Lot # 5D178B.

Rats were randomly divided into four groups; each is consisting of twenty rats. Each group was separated in metal cages. The groups treated as following. 1st control group was given I/P normal saline (1 mL).

2nd group was given a single I/P dose (200 mg/kg body weight) of CP on the first day of the experimental period according to Mythili et al [10]**.** 3rd group CP and bovine LF treated group. 4th group administrated LF only (0.5%) in drinking water during the 21 days according to [11].

Blood Sampling

Five random blood samples were taken from five rats. Samples were collected by heart puncture (under anesthetize effect of diethyl ether) at end of 1st, and 3rd week post treatment. Two separate blood samples were collected from each rat; the first sample was taken in eppendorf tubes at which mixed with EDTA for hematological examination. Second blood sample was kept 30 minutes at room temperature and were centrifuged at 3000 rpm for 10 minutes and the clear serum was separated carefully and storage at -20°C. Total leukocyte count and differential leukocyte count were measurement according to [12,13], respectively. The second blood samples were taken in test tube without anticoagulant. Some selective biochemical parameters (total protein and albumin) were measurement spectrophotometer by using commercial diagnostic kits which were obtained from Human-Germany and Spinreact Spanish).

Regarding to immunological studies, Immuno-electrophoresis of serum protein has been done using cellulose acetate according to Henry et al [14]. Tumor necrosis factor–α (TNF-α) was measurement by Enzyme Amplified Sensitivity Immunoassay (EASIA) performed on microplate. The assay uses monoclonal anti-bodies (MAbs) directed against distinct epitopes of TNF-α according to Beutler and Cerami [15]. The lower detection limit of TNF-α was 3.1 pg/ml and data are presented as pg cytokine/ml serum.

Statistical Analysis

The present results were analyzed by analysis of variance (ANOVA) followed by LSD using SPSS.16 for window. Two groups were significantly different if P was statistically lower than 0.05.

RESULTS

Regarding to the leukogram, the total leukocytes and lymphocytes count were significantly decreased one and three weeks post treatment with CP when compare with control group (Table 1 & 2). In Lactoferrin and CP treated groups, total leukocytes and lymphocytes were significantly increased when compare with CP treated groups. In lactoferrin treated groups, the leukogram was none significant changed in compare with control group.

In the present work, the total proteins, albumin, α-globulin, β-globulin and γ-globulin were significantly increased one and three weeks post treatment with CP, as well as CP and LF group while TNF-α was significant increased when compare with control group (Table 3 & 4).

Table 1. Effect of one week post treatment with lactoferrin (mean ± S.E), on leukogram in immunosuppressed rats with cyclophosphamide.

Group	TLC $10^3/\mu L$	Neutrophils $10^3/\mu L$	Eosinophil $10^3/\mu L$	Basophil $10^3/\mu L$	Lymphocyte $10^3/\mu L$	Monocyte $10^3/\mu L$
Control	7.75^{c} ±0.45	2.74^{b} ±0.25	0.154^{b} ±0.041	0.016 ±0.01	4.39^{c} ±0.31	0.47^{b} ±0.072
CP	3.07^{a} ±0.35	1.185^{a} ±0.19	0.015^{a} ±0.015	0.0	1.65^{a} ±0.34	0.22^{a} ±0.038
LF & CP	4.72^{b} ±0.46	1.34^{a} ±0.17	0.118^{b} ±0.031	0.00	3.02^{b} ±0.32	0.24^{a} ±0.040
LF	8.35^{c} ±0.59	2.52^{b} ±0.29	0.138^{b} ±0.0.39	0.017 ±0.01	5.09^{c} ±0.36	0.56^{b} ±0.071

Means in the same column not followed by the same letter differ significantly (P<0.05).

LF, lactoferrin and CP, cyclophosphamide

Table 2. Effect of three week post treatment with lactoferrin (mean ± S.E), on leukogram in immunosuppressed rats with cyclophosphamide.

Group	TLC $10^3/\mu L$	Neutrophils $10^3/\mu L$	Eosinophil $10^3/\mu L$	Basophil $10^3/\mu L$	Lymphocyte $10^3/\mu L$	Monocyte $10^3/\mu L$
Control	8.35[b] ±0.51	2.53[b] ±0.28	0.165[b] ±0.044	0.016 ±0.016	5.18[c] ±0.36	0.46[b] ±0.051
CP	3.95[a] ±0.24	1.71[a] ±0.19	0.022[a] ±0.016	0.00	1.79[a] ±0.29	0.42[b] ±0.058
LF & CP	6.55[c] ±0.38	2.51[b] ±0.27	0.131[b] ±0.029	0.00	3.53[b] ±0.39	0.39[b] ±0.061
LF	8.91[b] ±0.57	2.98[b] ±0.31	0.178[b] ±0.034	0.089 ±0.09	5.29[c] ±0.34	0.37[b] ±0.065

Means in the same column not followed by the same letter differ significantly (P<0.05).

Table 3. Some immunological parameters one week post treatment with lactoferrin (mean ±S.E) in immunosuppressed rats with cyclophosphamide.

Group	T. Protein g/dl	Albumin g/dl	α-globulin g/dl	β-globulin g/dl	γ-globulin g/dl	TNF-α pg/ml
Control	7.45[b] ±0.41	3.40[b] ±0.25	1.41[b] ±0.18	1.35[b] ±0.17	1.29[b] ±012	21.2[a] ±2.45
CP	5.59[a] ±0.45	2.71[a] ±0.21	1.01[a] ±0.09	0.92[a] ±0.10	0.95[a] ±0.07	42.8[b] ±4.15
LF & CP	5.84[a] ±0.42	2.81[a] ±0.19	1.08[a] ±0.08	0.96[a] ±0.11	0.99[a] ±0.05	39.8[b] ±3.75
LF	7.52[b] ±0.62	3.38[b] ±0.46	1.46[b] ±0.19	1.32[b] ±0.16	1.36[b] ±0.13	20.4[a] 2.01

Means in the same column not followed by the same letter differ significantly (P<0.05).

Table 4. Some immunological parameters three weeks post treatment with lactoferrin (mean ± S.E.) in immunosuppressed rats with cyclophosphamide.

Group	T. Protein g/dl	Albumin g/dl	α-globulin g/dl	β-globulin g/dl	γ-globulin g/dl	TNF-α pg/ml
Control	7.48[b] ±0.48	3.46[b] ±0.23	1.39[a] ±0.16	1.31[a] ±0.17	1.32[b] ±014	18.9[a] ±2.08
CP	5.93b[a] ±0.46	2.89[a] ±0.20	1.09[a] ±0.10	0.96[a] ±0.11	0.99[a] ±0.06	31.9[b] ±3.05
LF & CP	6.17[a] ±0.35	2.88[a] ±0.22	1.12[a] ±0.10	0.99[a] ±0.10	1.18[a] ±0.08	25.2[b] ±3.01
LF	7.52[b] ±0.49	3.38[b] ±0.32	1.34[a] ±0.16	1.32[a] ±0.18	1.48[b] ±0.15	19.4[a] 1.95

Means in the same column not followed by the same letter differ significantly (P<0.05).

DISCUSSION

Cyclophosphamide is an immunosuppressive agent and an anticancer prodrug which requires bioactivation catalyzed primarily by cytochrome P450 enzymes in order to be transformed into its active alkylating compounds [16,17]. Also cyclophosphamide, is extensively used as an anticancer chemotherapeutic drug in childhood and adult malignancies, as well as an immunosuppressive agent for organ transplantation and other benign diseases [5].

Cyclophosphamide known as immunosuppressive drug, where the cyclophosphamide group was showed highly significant decrease of (TLC) all over the experiment period comparing with control group. This could be attributed to severe depression of bone marrow that manifested by significant decrease of all types of blood cells, lymphopenia, neutropenia, eozinopenia and monocytopenia. This result in accordance with**,** Latha et al. [6] who reported leukopenia in mice treated with CP. In addition, Smith et al. [7] recorded leukopenia,

lymphopenia and neutropenia in female rats treated with CP for 30 days. Nygaard and Løvik [8] reported significant lymphopenia in rats treated with a single dose of the CP (250 mg/kg Bw), Zuluaga et al. [18] who reported that I/P injection of female mice with 150 and 100 mg/kg of CP on days 1 and 4, respectively leading to leukopenia, lymphopenia, neutropenia and monocytopenia.

There was increase of total leukocytes count, (TLC) by LF in CP-treated rat group as well as increase of lymphocyte, neutrophil, eosinophil and monocyte blood cells comparing with CP group. Zimecki et al. [2] recorded increase bone marrow neutrophil lineage cell content following 24 h pre-treatment mice with LF. These agree with Artym et al. [3,4] who revealed that treatment of mice with LF induced a strong mobilization/recruitment of myelocytes and band forms in bone marrow. Also LF has been reported that, accelerate neutrophil recruitment in humans and animals [19,20].

In the present work, lymphocytes significantly increased in LF & CP treated group in comparing with CP group. The described activity of LF is in agreement with Sekine et al. [21] who reported enhancement of natural killer (NK) activity and cytokine production by spleen cells in response to mitogen. Artym [3] et al. concluded that oral LF treatment resulted in induced splenocyte proliferation, increased the cellularity of spleens, the content of peritoneal and alveolar macrophages and elevation of leukocytes by LF in CP-immunosuppressed mice. In CP group showed significant decrease of total protein, albumin, alpha and beta globulin comparing with control group. This result may be due to decrease proteins synthesis as a result of liver damage as reported by Senthilkumar et al [22]. Hypoproteinemia was reported in rats administered CP (150 mg/kg bw) for two days [22].

The present result showed significant decreases of γ-globulins in CP group in comparable with control one. The immunosuppressive effect of CP in rats was documented [7,8] also in mice with [21,22].

Gamma globulins were significantly elevated in CP and LF treated group in comparing with CP group. Our result in hand with Zimecki et al. [25] who reported that LF accelerated reconstitution of the immune system function (cellular and humoral immune response) after administration of a sub-lethal dose of CP to mice.

TNF-α is amplify, propagate, and coordinate pro-inflammatory signals, resulting in the synchronized expression of effectors molecules that mediate diverse aspects of innate immunity. TNF is capable of eliciting expression of chemokines and adhesion molecules and thus may be critical to the recruitment of neutrophils from the blood [26]. Our results show significantly increase TNF-α in CP group in compare with control one. The results are in accordance with Cruz-Chamorro et al [27] who re-ported elevated TNF in mice treated with CP. TNF-α was significantly decreased in CP plus LF treated group in compare with CP group. This attributed to immunomodulatory action of LF.

In conclusion CP has significant depression of blood cell production, as well as severe immunosuppressant. Oral treatment with LF can partially reconstitute humoral and cellular immune response in rats given a sub-lethal dose of CP.

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