Effects of Two Different Doses of Vitamin B2 and a Single Dose of Vitamin B12 Against Cyclophosphamide Induced Nephrotoxicity

Waleed K. Ghanim[1], Nada N. Al-Shawi[2], Muhsin S. G. Al-Moziel1

[1]MSc. Researcher, Department of Pharmacology and Toxicology, College of Pharmacy, University of Basra, Basra, Iraq, [2]Prof. Dr.Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Abstract

Cyclophosphamide, a medication that is used for the treatment of different types of cancer; however its' use associated with numerous adverse effects. Vitamin B2 and vitamin B12 suggested having nephroprotective effect. This work is designed to investigate the nephroprotective effect of both vitamins against cyclophosphamide induced nephrotoxicity. One hundred adult rats of both sexes were used in this study. The animals were randomly enrolled into ten groups of 10 rats each. On day eight, animals were sacrificed and blood collected for the measurement of serum superoxide dismutase 1, glutathione peroxidase; and kidney extracted for histological examination. Vitamin B2 and vitamin B12 significantly (P<0.05) increase superoxide dismutase 1, glutathione peroxidase; and the combination of vitamins produce significant (P<0.05) increase in superoxide dismutase 1 and glutathione peroxidase compared to the corresponding levels in other groups; and improve histopathological changes compared to cyclophosphamide-treated rats. In conclusion both vitamins may have nephroprotective effects against cyclophosphamide-induced nephrotoxicity.

Key words: *Cyclophosphamide, Vitamin B2, Vitamin B12, Nephrotoxicity, Rats.*

Introduction

Cyclophosphamide (CPA), an alkylating agent that widely used either alone or in combination with other agents for the treatment of different types of cancers and also as immunosuppressant [(1)], however its' use associated with varying adverse effects including nephrotoxicity [(2)]. Authors reported that the nephrotoxicity induced by CPA is due to active metabolites [(3)]. Furthermore, the nephrotoxicity caused by CPA can lead to variable reduction in glomerular filtration rate (GFR) along with tubular dysfunction [(4)]. Histologically in CPA-treated rat, kidneys showed glomerular nephritis, interstitial oedema and cortical tubular vacuolization in addition to that lysosomal enzymes activities were decreased and protein contents were increased with renal damage was consequently produced [(5)].

Vitamin B2 (Riboflavin) is a water soluble vitamin [(6)] which present in a wide sources of foods [(7)]. Such vitamin is important precursor for two active cofactors which are flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) which participate in a wide range of redox reactions which in turn protect the body against variety of oxidative stress conditions [(8)]. Vitamin B12 is a generic name for a specific group of cobalt-containing corrinoids [(9)]. Cobalamin acts as cofactor for enzymatic conversion of homocysteine to methionine and also for conversion of methylmalonic acid (produced when proteins in the body are broken down) to succinyl-CoA [(10)].

Aim of this study to investigate the effects of vitamin B2 and vitamin B12 on cyclophosphamide induced nephrotoxicity.

Corresponding author:
Waleed K. Ghanim
ph_wkg81@yahoo.com

Materials and Method

Experimental study:

One hundred healthy adult albino rats of both sexes, weighing 180-220gm were used in this study; they were obtained from and maintained in the Animal House of the College of Pharmacy, Baghdad University under conditions of controlled temperature. The animals were fed commercial pellets and tap water *ad libitum* throughout the experiment period.

Drugs

Cyclophosphamide vial (500 mg) was purchased from Baxter, USA. Vitamin B2 capsule (400 mg) was purchased from Amazing nutrition, USA. Vitamin B12 tablet (1 mg) was purchased from TQ pharma, Japan.

Experimental protocol

The experimental rats were randomly divided into ten groups (10 rats/group) as follows:

Group I: Rats IP injected 1ml/kg/day normal saline for 7days; as control group. **Group II:** Rats IP injected with single dose of cyclophosphamide (CPA) (150 mg/kg). **Group III:** Rats orally-administered 10 mg/kg/day vitamin B2 for 7 days. **Group IV:** Rats orally-administered 40 mg/kg/day vitamin B2 for 7 days. **Group V:** Rats orally-administered 0.1 mg/kg/day vitamin B12 for 7 days. **Group VI:** Rats orally-administered 10 mg/kg/day vitamin B2 for 7 days and a single IP injection of 150 mg/kg of CPA at day 7. **Group VII**: Rats orally-administered 40 mg/kg/day vitamin B2 for 7 days and a single IP injection of 150 mg/kg of CPA at day 7. **Group VIII:** Rats orally-administered 0.1mg/kg/day vitamin B12 for 7 days and a single IP injection of 150 mg/kg of CPA at day 7. **Group IX**: Rats orally-administered a combination of 10 mg/kg/day vitamin B2 and 0.1 mg/kg/day vitamin B12 for 7 days and a single IP injection of 150 mg/kg of CPA at day 7. **Group X:** Rats orally-administered a combination of 40 mg/kg/day vitamin B2 and 0.1 mg/kg/day vitamin B12 for 7 days and a single IP injection of 150 mg/kg of CPA at day 7.

24 hour after the end of the treatment duration (i.e. at day 8), rats were euthanized by diethyl ether. 8 ±1 ml of blood was obtained by intracardiac puncture and was collected in gel and clot activator tubes to obtain serum for the determination of SOD1, and GP levels.

Histological examination

Kidney of each rat was prepared for histological examination according to the method of Junqueira [11].

Statistical Analysis

Data were expressed as the mean values, mean±standard error of the mean (SEM). Unpaired Student t-test was used for testing the significant difference between two groups. The statistical significance of the differences among various groups was determined by one-way analysis of variance (ANOVA). Differences were considered statistically significant for *P*-value less than 0.05.

Results

Effects on serum superoxide dismutase1 (SOD1)

There were non-significant differences *(P*<0.05) in serum SOD1 level in groups of rats orally-administered vitamin B2 for one week (**Groups III,** and **IV**) respectively and vitamin B12 alone for one week (**Group V**) compared to that level in control (**Group I**) rats. Mean±SEM of serum SOD1 levels were respectively, 2.93±0.004, 2.94± 0.005, 2.95±0.009, and 2.92 ± 0.005. Furthermore, rats IP injected with CPA at day 7 (**Group II**) caused significant reduction (*P*<0.05) in serum SOD1 level compared to that level in rats of **Group I**. Mean±SEM of serum SOD1 levels were respectively, 0.95±0.011 and 2.92±0.016. Table 1

Moreover, there were significant elevation (*P*<0.05) in serum SOD1 level in **Groups VI, VII, VIII, IX,** and **X** of rats each compared to **Group II** rats. Mean±SEM of serum SOD1 levels were respectively, 1.15±0.003, 1.36±0.03, 1.58±0.003, 1.78±0.007, 2.04±0.034, and 0.95±0.011. Furthermore, table 1 showed that there were significant elevation (*P*<0.05) in serum SOD1 level in **Groups IX**, and **X** compared to the corresponding serum level in rats of **Groups VI, VII and VIII**. Mean±SEM of serum SOD1 levels were respectively, 1.78±0.007, 2.04±0.034, 1.15±0.003, 1.36±0.03 and 1.58±0.003.

Effects on serum glutathione peroxidase (GP) levels

Table 1 showed that there were non-significant differences *(P*<0.05) in serum GP level in groups of rats orally-administered vitamin B2 for one week (**Groups III,** and **IV**), and vitamin B12 for one week (**Group V**) each compared to the corresponding serum level in control (**Group I**) rats. Mean±SEM of serum GP levels were respectively, 331±0.611, 332±0.689, 332±0.465 and 330±0.527. Furthermore, rats IP injected with CPA at day 7 (**Group II**) caused significant reduction (*P*<0.05) in GP serum level compared to the corresponding serum enzyme level in control (**Group I**) rats. Mean±SEM of serum levels of GP were respectively, 169±0.603 and330±0.527. Moreover, there were significant elevation (*P*<0.05) in serum GP levels in **Groups VI, VII**, **VIII**, **IX**, and **X** rats compared to the corresponding serum levels in **Group II** rats. Mean±SEM of serum levels of GP were respectively, 216±0.588, 239±0.997, 243±0.683, 269±0.662, 297±0.741, and 169±0.603. Furthermore, table 1 showed that there were significant elevation (*P*<0.05) in serum GP level in Groups **IX**, and **X** of rats compared to the corresponding serum level in rats of **Groups VI, VII and VIII**. Mean±SEM of serum GP levels were respectively, 269±0.662, 297±0.741, 216±0.588, 239±0.997 and 243±0.683.

Table 1. Effects of various treatments on serum superoxide dismutase 1 and glutathione peroxidase levels in rats

Group/Treatment	Superoxide dismutase1(SOD1) ng/ml	Glutathione peroxidase (GP) pg/ml
Group I	2.92± 0.005a	330±0.527a
Group II	0.95± 0.011g	169±0.603g
Group III	2.93 ± 0.004a	331±0.611a
Group IV	2.94 ± 0.016a	332±0.689a
Group V	2.95 ± 0.003a	332±0.465a
Group VI	1.15 ± 0.011f	216±0.588f
Group VII	1.36 ± 0.030e	239±0.997e
Group VIII	1.58 ± 0.011d	243±0.683d
Group IX	1.78 ± 0.007c	269±0.662c
Group X	2.04 ± 0.034b	297±0.741b

Each value represents mean ± standard error of means (SEM).

Values expressed in small letters (a, b, c, d, e, f, and g) are significantly different (*P*<0.05). Number of animals in each group=10.

Histological examination of rats' kidney tissue

Rats IP injected with 1ml normal saline (**Group I**, control), orally-administered vitamin B2 (**Group III** and **Group IV**, respectively), and orally-administered vitamin B12 (**Group V**) each for 7 days showed normal kidney section, that characterized by thin glomerular basement membrane, cellularity and patent capsular space surrounding proximal and distal convoluted tubules. Figures (1-A, 1-B, 1-C and 1-D) respectively.

The kidney section from **Group II** rats IP injected with CPA showed dilatation of bowman space and renal tubules with fibroid tissue and massive apoptosis. Figure (1-E).

The kidney section from **Group VI** rats' orally-administered vitamin B2 for 7 days prior to IP injection of CPA at day 7 showed those histological changes; where, normal glomeruli with dilatation of bowman capsule space, degeneration of renal tubules and numerous apoptosis. Figure (1-F).

The kidney section from **Group VII** rats' orally-administered vitamin B2 for 7 days prior to IP injection of CPA at day 7 showed that atrophy of glomeruli with degeneration of renal tubules and vacoulation with numerous apoptosis compared to G**roup VI** rats. Figure (1-G).

The kidney section from **Group VIII** rats' orally-administered vitamin B12 for 7 days prior to IP injection of CPA at day 7 showed an atrophy of glomeruli, degeneration of renal tubules and numerous apoptosis. Figure (1-H).

While, kidney sections from **Group IX and Group X** rats orally-administered combination of each of vitamin B12 dose with vitamin B2 respectively for 7 days prior to IP injection of CPA; there were atrophy of glomeruli with dilatation of bowman capsule in addition to mild degeneration of renal tubules, and limited number of apoptotic cells. Figures (1-I and 1-J) respectively.

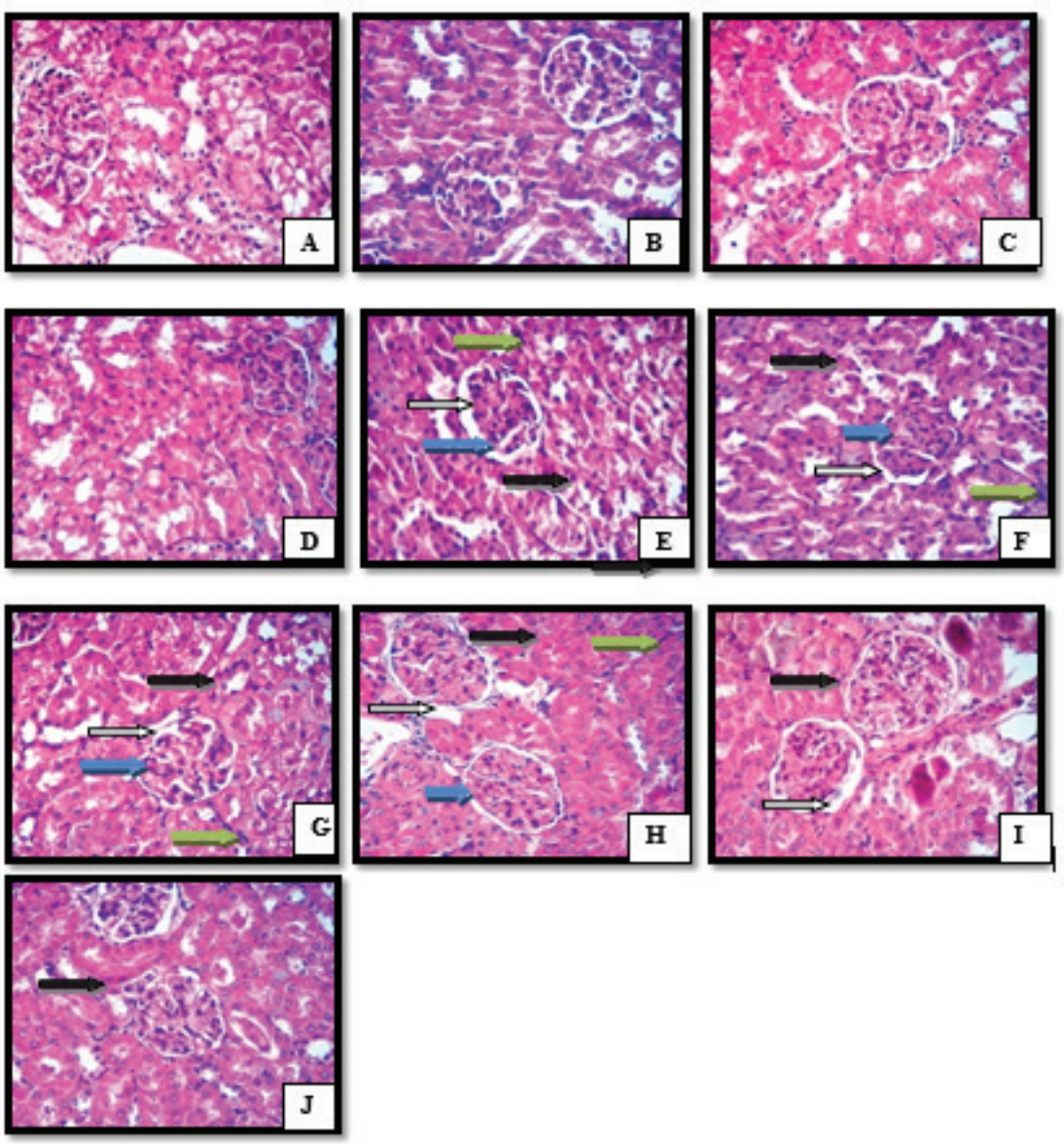

Figure 1. Histopathological sections of kidney in various experimental rats' groups; (Hematoxylin and eosin; X40). Atrophy of glomeruli (blue arrow), dilatation of bowman capsule (white arrow), apoptotic cells (black arrow), fibroid tissue (green arrow).

Discussion

In this study rats IP injected with CPA at day 7 (**Group II**) caused significant reduction in both serum SOD1 and GP levels (P<0.05) compared to that levels in control (**Group I)** rats; these reductions may be due to the oxidative stress (OS) formation, which may responsible for CPA-induced nephrotoxicity; results of this study are coinciding with the work of Singh *et al* (2011) where, the OS was reported to be a possible pathological mechanism of nephrotoxicity-induced by CPA that can reduce both SOD1 and GP levels in serum [12].

Also, results of this study showed that orally-administered vitamin B2 in dose-dependent manner prior to CPA (**Group VI and Group VII**) significantly (P<0.05) elevate serum SOD1 and GP levels compared to such levels in CPA-treated rats (**Group II**); furthermore, the combination of different doses of vitamin B2 with fixed dose of vitamin B12 prior to CPA (**Group IX and Group X**) caused significant (P<0.05) elevations in serum SOD1 and GP enzymes levels compared to those levels in CPA-treated rats (**Group II**); moreover, there were significant (P<0.05) elevations in serum levels of

SOD1 and GP enzymes in rats of **Group IX and Group X** compared to corresponding enzymes levels in rats of G**roup VI, Group VII and Group VIII**; these effects could be explained that the antioxidant function of riboflavin could be attributed to the glutathione redox cycle, the reduction-oxidation reactions of riboflavin itself, and the riboflavin effects on antioxidant enzymes activities [13, 14]. Reduced glutathione (GSH), the active form of this antioxidant during its antioxidant activity, can be oxidized (GSSG), that mean inactive, thus, requiring a reduction through glutathione reductase (GR) to regain its antioxidant activity [15]. This enzyme requires the flavin adenine dinucleotide (FAD) coenzyme form of vitamin B2 for this reduction reaction; thus, emphasizing the important role of vitamin B2 in the formation of reduced, active (GSH); in fact, Dey S and Bishayi B (2016) reported that GSH levels were reduced following a decrease in riboflavin intake [16].

Also in this study, orally-administered vitamin B12 prior to CPA (**Group VIII**) produced significant (P<0.05) elevations in serum SOD1 and GP levels compared to those levels in **Groups II**, **VI and VII** of rats; these effects could be explained that vitamin B12 may act as antioxidant; where, its antioxidant function could be attributed to the following mechanisms: the enzymatically-processed vitamin B12 acts as a direct superoxide scavenger [17]; furthermore, vitamin B12 may indirectly stimulate ROS scavenging by preservation of glutathione, which likely involves an intricate network of reactions that has not been fully elucidated [17]; moreover, vitamin B12 might protect against (low-grade) inflammation-induced OS by modulating the expression of cytokines and growth factors [18, 19].

In this study, histopathological examination of kidney section of rats IP injected with CPA at day 7 **(Group II)** confirmed the nephrotoxicity; where kidney section of such rats showed renal cell disorganization and vacuolated glomeruli and renal tubules in addition to degeneration of renal tubules with fibroid tissues and massive apoptosis were observed; moreover, marked renal cells degeneration with frequent nuclear pyknosis, irregular darky-stained cells with pyknotic nuclei that are surrounded with halos arrows were prominent in figure (1-E). These findings are coinciding with the work of Hamdi A *et al* (2016) [20].

Concerning histological examination of kidney section of rats of **Groups VI, VII**, **IX** and **X**; there were improvement of the histopathological kidney lesions in all treated groups mentioned above [figures (1-F, 1-G, 1-I and 1-J)] compared to **Group II** (CPA-treated) rats [figure (1-E)]. Results of this study are in agreement with the study of Hajihashemi S *et al* (2017); where, a protective effect of vitamin B2 against nephrotoxicity was observed by histopathological examination [21]. Concerning histological examination in rats' kidney section performed in the present study, the effect of vitamin B12 orally-administered prior to CPA (**Group VIII**), showed that there were improvements of the histopathological kidney lesions in rats of (**Group VIII**) [figure (1-H)]compared to sections of rats' kidney of (**Group II**) (CPA-treated) figure (1-E). In this study, results are also in agreement with those performed others; where, a protective effect of vitamin B12 against nephrotoxicity was observed by histopathological examination [21]. In conclusion both vitamins may have nephroprotective effects against cyclophosphamide-induced nephrotoxicity.

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