Renal Failure

Antioxidative status and lipid peroxidation in kidney tissue of rats fed with vitamin B$_6$-deficient diet

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LABORATORY STUDY

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ABSTRACT

Introduction: The aim of this study was to evaluate lipid peroxidation (LP) and free radical scavenging enzyme activities in kidney tissue of vitamin B$_6$-deficient rats. *Material and Methods*: The rats were divided into control and vitamin B$_6$-deficient groups. After 4 weeks of feeding, animals in all groups were anesthetized by thiopental sodium (50 mg/kg). Thoraces were opened, 2 mL blood samples were taken from aortas, then the rats were killed by cervical dislocation, and kidney tissues were removed. Biochemical measurements in kidney tissue were carried out using a spectrophotometer. *Results*: Total superoxide scavenger activity (TSSA), nonenzymatic superoxide scavenger activity (NSSA), superoxide dismutase (SOD) activities, and antioxidant potential (AOP) values in the vitamin B$_6$-deficient group were significantly lower than those of the control group, whereas glutathione peroxidase (GSH-Px), glutathione reductase (GRD), glutathione-*S*-transferase (GST) activities, and malondialdehyde (MDA) level were significantly higher than those of the control group ($p < 0.05$). *Discussion*: The results show that vitamin B$_6$ deficiency causes an attenuation in antioxidant defense system and an increase in oxidative stress in kidney tissue of rats.

Keywords: lipid peroxidation; antioxidant potential; antioxidant enzymes; vitamin B$_6$ deficiency; vitamin

INTRODUCTION

Oxygen free radicals (OFRs), such as superoxide radical ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet-}$), are highly reactive species generated by biochemical redox reactions as part of normal cell metabolism. Oxygen metabolism in aerobic organisms has some advantages. In view of the generation of OFR, certain adverse effects also occur. Practically, all the essential biomolecules can undergo oxidative reactions mediated by OFR. The study of OFR and their proposed effects on biological systems has become an important area of biomedical research in recent years.[1–3]

Vitamin B$_6$ has been shown to be important for normal cognitive function and in lowering the incidence of coronary heart disease among the elderly.[4–6] In addition, vitamin B$_6$ supplementation has been shown to reduce diabetic complications and incidences of neurodegenerative diseases in varying degrees.[4–8] Vitamin B$_6$ refers to three primary forms of water-soluble vitamins: pyridoxine, pyridoxal phosphate, and pyridoxamine. Some studies have reported antioxidant activities of vitamin B$_6$.[9,10] Kannan and Jain reported that pyridoxine and pyridoxamine inhibit $O_2^{\bullet-}$ generation, reduce lipid peroxidation (LP), and prevent damage to mitochondrial membrane integrity in U937 cells.[8] Vitamin B$_6$ deficiency affects lipid metabolism, modifying fatty acid composition of some tissues and increasing plasma triglyceride and cholesterol levels. LP has been linked with changed membrane structure

and enzyme inactivation. The induction of LP is considered to be important in the etiology of many diseases.[11–15]

Cells have different antioxidant systems and various antioxidant enzymes to defend themselves against free radical attacks. Superoxide dismutase (SOD), the first line of defense against OFR, catalyzes the dismutation of $O_2^{\bullet-}$ into hydrogen peroxide (H_2O_2). Glutathione (GSH)-dependent antioxidant system consisting of reduced GSH and an array of functionally related enzymes plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. Of these enzymes, glutathione peroxidase (GSH-Px) is a selenoprotein that reduces hydroperoxides as well as H_2O_2 but oxidizing GSH. A number of potentially toxic electrophilic xenobiotics (such as certain carcinogens, bromobenzene and chlorobenzene) are conjugated to the nucleophilic GSH by glutathione-S-transferases (GSTs) present in high amounts in cell cytosol. GST can also catalyze reactions reducing peroxides like GSH-Px. Reduction of oxidized glutathione (GSSG) to GSH is mediated by the widely distributed enzyme GSH reductase (GRD) that uses NADPH as the reducing agent.[16–18]

Chronic renal failure and vitamin B$_6$ deficiency share some common features such as peripheral neuropathy, normochromic anemia, depression of the immune system disturbances, improvement with low-protein diet, and increased body oxalate levels.[19]

To our knowledge, there is no study that simultaneously investigates antioxidant potential (AOP), total (enzymatic plus nonenzymatic) superoxide scavenger activity (TSSA), nonenzymatic superoxide scavenger activity (NSSA), GSH-Px, GRD, GST, catalase (CAT), SOD activities, and malondialdehyde (MDA) levels in kidney tissue in vitamin B$_6$-deficient rats and control group. Therefore, in this study, we aimed to investigate the effects on LP and free radical scavenging enzyme activities in kidney tissue of vitamin B$_6$-deficient rats.

MATERIALS AND METHODS

Twenty male rats (4 weeks old, Sprague–Dawley strain) with a weight of 50–60 g were used for the experiment. The animals were randomly divided into two groups of 10 rats each. All animals received humane care in compliance with the guidelines of Ataturk University Research Council's criteria. The composition of the diet was as described in Table 1.[11]

Biochemical analyses

After 4 weeks of feeding, animals in all groups were anesthetized by thiopental sodium (50 mg/kg). Thoraces were opened, 2 mL blood samples were taken from

TABLE 1. The composition of the experimental diet.

Diet (ingredients)	%	Control group (g/kg dry matter)	Vitamin B$_6$ deficient group (g/kg dry matter)
Vitamin-free casein	70	700	700
DL-Methionine	1	10	10
Sucrose	12.3	123	123
Corn oil[a]	10	100	100
Cellulose powder	2	20	20
Choline bitartrate	0.2	2	2
Vitamin mixture (AIN 76)	1	10	–
Vitamin mixture (AIN 76) (without pyridoxine-HCL)	1	–	10
Mineral mixture (AIN 76)	3.5	35	35

Note: All compounds used in the study were analytical grade.
[a]Ulker, Bizim corn oil (Ulker, Istanbul, Turkey).

aortas, then the rats were killed by cervical dislocation, and kidney tissues were removed, washed out from contaminated blood with cold water, and homogenized in 10-fold physiological saline solution by using a homogenizer (Omni Accessory Pack International homogenizer, Warrenton, Virginia, USA). The homogenate was centrifuged at $10,000 \times g$ for 1 h to remove debris. The supernatant was collected and all assays were carried out on this fraction.

The kidney tissue AOPs were measured by a method described by Durak et al.[20] According to this method, in the reaction medium enriched with fish oil, samples (supernatant obtained after centrifugation) were exposed to $O_2^{\bullet-}$ produced by the xanthine–xanthine oxidase system for 1 h. Fish oil was used for this purpose because it is a polyunsaturated oil, which is very sensitive to free radical attack. As known, when there is an inability in the cell to eliminate free radicals, unsaturated free fatty acids would be easily oxidized and then the MDA concentration would be increased. By using this reaction system, it is possible to obtain more precise information about AOP of the samples. MDA levels were measured in the control and sample studies. AOP values were assessed from the difference between MDA levels of control and sample studies. AOP values were expressed as nmol/mg protein·h^{-1}. The kidney tissue MDA levels were measured by the spectrophotometric method of Ohkawa et al.[21] Total thiobarbituric acid-reactive substances were expressed as MDA. MDA levels are also expressed as nmol/mg protein.

TSSA, NSSA, and SOD assays were performed in the samples before and after trichloroacetic acid (TCA) 20% (w/v).[22] GSH-Px, GRD, and GST activities were

measured as described, respectively.[23–25] Protein content was determined by using the Bradford method.[26] Results were expressed in U/mg protein for TSSA, NSSA, SOD, GRD, and GST activities; mIU/mg protein for GSH-Px activity; and nmol/mg protein for MDA levels. One unit of TSSA, NSSA, and SOD was defined as the amount of enzyme protein causing 50% inhibition in nitroblue tetrazolium reduction rate. Biochemical measurements were carried out using a spectrophotometer (CECIL CE 3041, Cambridge, UK). Plasma pyridoxal-5-phosphate levels were measured by high-performance liquid chromatography.

Statistical analysis

The findings were expressed as mean ± SD. Statistical and correlation analyses were undertaken using the Mann–Whitney U-test and Spearman's rank correlation test, respectively. A p-value < 0.05 was accepted as statistically significant. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 11.0, Chicago, Illinois, USA).

RESULTS

All parameters are shown in Table 2. As seen from the table, kidney tissue TSSA, NSSA, SOD activities, AOP, and plasma pyridoxal-5-phosphate levels in the vitamin B_6-deficient group were significantly lower than those of the control group ($p < 0.05$), whereas GSH-Px, GST, GRD activities, and MDA levels were significantly higher than those of the control group ($p < 0.05$).

TABLE 2. Comparison of different variables between the study and control groups.

	Control group $n = 10$	Vitamin B_6-deficient group $n = 10$
GSH-Px (mIU/mg protein)	1.6 ± 0.52	2.1 ± 0.55*
TSSA (U/mg protein)	46.3 ± 5.8	41.5 ± 4.5*
NSSA (U/mg protein)	1.13 ± 0.06	0.96 ± 0.19*
SOD (U/mg protein)	45.2 ± 2.8	40.6 ± 4.4*
GST (U/mg protein)	3.2 ± 1.9	5.3 ± 1.6*
GRD (U/mg protein)	1.2 ± 0.25	1.6 ± 0.33*
AOP (nmol/mg protein·h^{-1})	4.6 ± 0.98	3.8 ± 0.76*
MDA (nmol/mg protein)	2.6 ± 0.5	3.4 ± 0.88*
Plasma pyridoxal-5-phosphate (µg/L)	116.2 ± 19.8	41.8 ± 8.3**
Final body weight (g)	163.4 ± 8.9	82.3 ± 6.8**

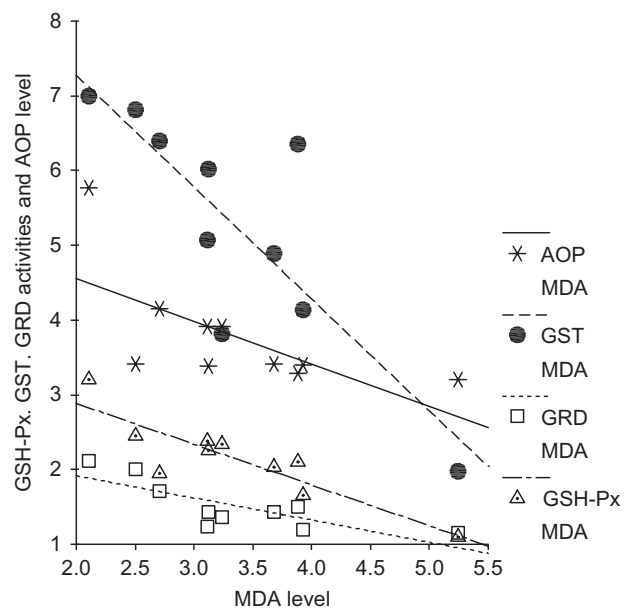Notes: *$p < 0.05$; **$p < 0.001$ vs. control group.

FIGURE 1. Correlations between different variables in the study group.
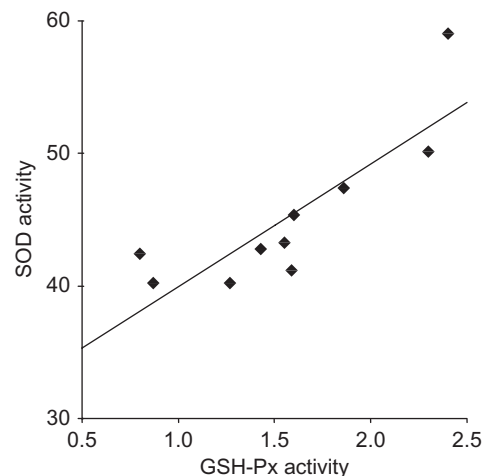

FIGURE 2. Correlations between different variables in the control group.

Correlation analysis revealed significant negative correlations between MDA and GST ($r = -0.82$, $p < 0.005$), MDA and GRD ($r = -0.77$, $p < 0.01$), MDA and SOD ($r = -0.79$, $p < 0.01$), and MDA and AOP ($r = -0.79$, $p < 0.01$) in the vitamin B_6-deficient group (Figure 1). There was a significant positive correlation between GSH-Px and SOD ($r = 0.87$, $p < 0.001$) in the control group (Figure 2). However, no correlation between the groups in same parameters could be found.

DISCUSSION

Oxidative stress is an imbalance between the production of free radicals that contain unpaired electrons and the antioxidant defenses buffering the oxidative damages. Oxidative effects of free radicals are controlled by exogenous antioxidants, such as vitamins E and C, and also by endogenous antioxidants. Under some conditions, increases in oxidants and decreases in antioxidants cannot be prevented, and oxidative–antioxidative balance shifts toward the oxidative stress.[27]

In this study, determination of some oxidative stress parameters has been carried out in the kidney tissue of vitamin B$_6$-deficient rats. The data show that kidney tissue TSSA, NSSA, SOD activities, and AOP values are decreased; GSH-Px, GST, GRD activities, and MDA level are increased in vitamin B$_6$-deficient rats. These results explicitly indicate that vitamin B$_6$ deficiency causes a decrease in antioxidant defense system and an increase in oxidant stress in kidney tissue in rats.

Reactive oxygen species (ROS) have the potential to cause reversible or irreversible damage in all kinds of biochemical materials containing nucleic acids, proteins, free amino acids, lipids, lipoproteins, carbohydrates, and connective tissue macromolecules. Biological membranes and intracellular components, which are rich in polyunsaturated fatty acid, can be easily affected by free radicals. One of the primary events in oxidative cellular damage is the oxidation of membrane lipids. Measurement of the breakdown products such as MDA, a highly toxic molecule, which is used as a biological marker of oxidative stress, is the most common approach to determine the degree of LP induced by ROS.[11] Increased kidney tissue MDA levels in vitamin B$_6$-deficient rats of our study were in concordance with previous reports.[28,29] This confirms the presence of increased oxidative stress in vitamin B$_6$-deficient rats.

The findings showed increased GSH-Px and decreased SOD activities in kidney tissue. Alterations in the antioxidant enzymes in kidney tissue of rats fed with vitamin B$_6$-deficient diet are inconsistent. SOD dismutates $O_2^{\bullet-}$ to H_2O_2. Although H_2O_2 is weakly reactive, its major toxicity derives from its conversion to the highly toxic $OH^{\bullet-}$ through the Fenton or Haber–Weiss reactions. GSH-Px detoxifies H_2O_2 by converting it into water and molecular oxygen. It seems that increased $O_2^{\bullet-}$ radical is not converted to H_2O_2 because of decreased activity of SOD. Positive correlation between SOD and GSH-Px in the control group may suggest collaboration present between these two antioxidant enzymes (Figure 2). However, this collaboration seems to be impaired in the vitamin B$_6$-deficient group. These antioxidant enzymes protect the cell constituents from damage by OFRs.[11,14]

The antioxidant status (composed of enzymatic and nonenzymatic antioxidants) is known to be a barrier (both endogenous and exogenous) against free radical attacks in all body compartments.[26] In this study, we found that TSSA, NSSA, and AOP level in kidney tissue of rats fed with vitamin B$_6$-deficient diet were significantly decreased compared with the control group. AOP reflects the total capacity of the enzymatic and nonenzymatic antioxidant systems. Weakness of AOP indicates impairment in the total defense system. When SOD activity and TSSA are suppressed by increasing oxidant stress in vitamin B$_6$ deficiency, $O_2^{\bullet-}$ radicals may be elevated in the kidney tissues. SOD enzyme is the most important defense mechanism against the $O_2^{\bullet-}$ radicals produced in the cells.[30] Increased $O_2^{\bullet-}$ radicals might be responsible for the oxidative damage reflected as increased kidney tissue MDA levels in vitamin B$_6$-deficient group in our study. Increased GSH-Px activities might be an attempt to lower H_2O_2, a potent toxic metabolite for living cells.[30]

GSH synthesis depends on the availability of cysteine, which is synthesized by vitamin B$_6$-dependent enzymes cystathionine β-synthase and cystathionine γ-lyase from methionine. It has been reported that cysteine synthesis reduced in vitamin B$_6$-deficient rats because of the altered activity of these enzymes, which leads to decreased GSH synthesis.[11,30–32] It might be concluded that increased GRD and GST activities in vitamin B$_6$-deficient rats in this study were related to decreased synthesis of GSH, which has been mentioned above, because of increased oxidative stress in response to vitamin B$_6$ deficiency. GST utilizes GSH for detoxification of a number of xenobiotics whereas reduction of GSSG to GSH is mediated by the widely distributed enzyme GRD.

How vitamin B$_6$ compounds scavenge OFRs and thereby inhibit LP reaction has not been clearly understood yet. However, the potency of a chemical to act as an antioxidant is generally determined by several factors, such as intrinsic chemical reactivity of the antioxidant toward the radical. The phenolic compounds have high reactivity toward the peroxy radical, because a phenolic group can react much faster with the peroxy radical than peroxy radical can react with lipid in the membrane. This reactivity is determined by the bond dissociation energy, such as that of the O–H bond in case of phenolic group, as well as the resonance stabilization of the resulting radical, the redox potential, and the steric hindrance to abstraction of the hydroxyl or amine hydrogen by peroxy radicals. Functional groups such as hydroxyl and amine can generally scavenge oxygen radicals.[8,33] Vitamin B$_6$ groups of compounds have both the hydroxyl and amine groups' substitution on a pyridine ring; those groups can scavenge OFRs. This condition may explain, at least in part, the scavenging action of vitamin B$_6$ compounds. Thus, besides the

scavenging function of vitamin B_6, involvement of other biochemical pathways cannot be ruled out in explaining the mechanism of action of these vitamin compounds.[8]

It has been suggested in this study that vitamin B_6 deficiency disturbs nutrition balance and causes oxidant stress and LP by means of excessive free radical production in kidney tissue in rats.

Declaration of interest: None of the authors has a commercial interest, financial interest, and/or other relationship with manufacturers of pharmaceuticals, laboratory supplies, and/or medical devices or with commercial providers of medically related services.

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