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Effects of Vitamin B6 Supplement on Serum Transaminase and Total Glutathione in Rats with Nonalcoholic Steatohepatitis (NASH)

Samuhasaneeto S, M.Sc.[1]
Thong-Ngam D, M.D.[1]
Kulaputana O, M.D.[1]
Klaikeaw N, M.D.[2]

ABSTRACT

Aim: To determine the effects of vitamin B6 supplement on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels and total glutathione in rats with nonalcoholic steatohepatitis (NASH).

Material and Method: Male Sprague-Dawley rats were randomly divided into three groups. Group 1: fed ad libitum with regular dry rat chow for 6 weeks (control group, n = 8). Group 2: fed ad libitum with 100% fat diet for 6 weeks to induce NASH (NASH group, n = 8). Group 3: fed ad libitum with 100% fat and added 2 mg/kg/day of vitamin B6 orally (NASH+B6 group, n = 10) for 6 weeks. All rats were sacrificed to collect blood at the end of the study.

Results: Serum ALT and AST were significantly decreased in NASH group as compared with control (ALT; 23.00 ± 1.92 U/L vs 40.13 ± 2.35 U/L, AST; 53.63 ± 9.31 U/L vs 86.75 ± 4.28 U/L, $p < 0.05$), respectively. Total glutathione (GSH) was significantly increased in NASH group as compared with control group (2066.67 ± 93.81 μM vs 1337.54 ± 31.48 μM, $p < 0.05$). Vitamin B6 treatment significantly increased serum ALT and AST level when compared to NASH group (ALT; 76.30 ± 6.21 U/L vs 23.00 ± 1.92 U/L: AST; 97.80 ± 9.35 U/L vs 53.63 ± 9.31 U/L $p < 0.05$), respectively.

Conclusion: Vitamin B6 treatment involves in synthesis of aminotransferase that could response for the high serum ALT and AST and improves total GSH in rats with NASH.

Key words : Vitamin B6, Transaminase, Glutathione (GSH), Nonalcoholic steatohepatitis (NASH)

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Address for Correspondence: *Duangporn Thong-Ngam, M.D., Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand. thongngam007@yahoo.com*
Telephone: +662-256-4267, Fax: +662-256-4267

[1]*Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand*
[2]*Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand*

INTRODUCTION

Nonalcoholic steatohepatitis (NASH) is a liver disease characterized by finding macrovesicular steatosis, hepatocyte necrosis, inflammation, Mallory bodies, and fibrosis[1]. NASH is a component of the metabolic or insulin resistance syndrome[2]. This is a cluster of disorders, such as obesity, diabetes mellitus, dyslipidemia, arteriosclerosis and hypertension, with insulin resistance as a common feature[3]. In initial phases, during which fat accumulates in the liver, no clinical symptoms are evident. In advanced stage, fibrosis is detectable (eventually progressing to cirrhosis in some patients)[4].

There are many models of NASH-like liver injuries in animal as the genetic model of ob/ob mice[5], the methionine and choline deficient diet model[6,7], and a model with high-fat liquid diet in which 71% of energy is derived from fat, 11% from carbohydrate, and 18% from protein[8]. Our previous studied showed that feeding rat with 100% fat diet exhibited pathology of NASH[9,10]. It was interesting that serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels significantly decreased in NASH group. These were probably due to nutritional deficiency of pyridoxal phosphate (vitamin B6) which is a cofactor for both ALT and AST to catalyze the transfer of the α-amino group from alanine or aspartate to α-ketoglutarate with made the release of pyruvate, oxaloacetate, and glutamate[11]. Apart from its function as a cofactor, antioxidant activities of vitamin B6 are reported. Vitamin B6 compounds seemed to quench singlet oxygen at a rate comparable with vitamin C[12]. Furthermore, vitamin B6 supplementation may have antioxidant effects in stroke disease that is independent of a homocysteine-lowering effect[13].

In the present study, we have examined the effect of vitamin B6 supplement on serum ALT, AST and total glutathione levels in rats with nonalcoholic steatohepatitis.

MATERIAL AND METHOD

Animal Preparation

This study was approved by the Ethics Committee of the Faculty of Medicine, Chulalonghorn University, Bangkok, Thailand. Male Sprague-Dawley rats weighing 220-260 grams from the National Laboratory Animal Center, Mahidol University, Salaya, Nakorn Pathom were used. The animals were allowed to rest for a week after arrival at the Animal Center, Department of Physiology, Faculty of Medicine, Chulalongkorn University before used in the experiment. They were kept in a controlled temperature room at 25 ± 1 ˚C under standard conditions (12 hours dark:12 hours light cycle), fed regularly with dry rat chow ad libitum, and had freely access to drinking water.

Experimental Protocols

Rats were randomly divided into three experimental groups. Group 1: fed ad libitum with regular rat chow for 6 weeks (control group, n = 8). Group 2 : fed ad libitum with 100% fat diet for 6 weeks to induce NASH (NASH group, n = 8). Group 3 : fed ad libitum with 100% fat diet and gavages 2 mg/kg/day of vitamin B6 orally (NASH+B6 group, n = 10) for 6 weeks.

All rats were weighed weekly. At the end of the study, rats were anaesthetized using intraperitoneal injection of an overdose (45 mg/kg BW) of sodium pentobarbital and then the abdominal walls were opened. Blood were drawn by cardiac puncture for total glutathione levels and biochemical assay.

Serum ALT and AST Measurement

Blood were allowed to clot and sera were separated by centrifugation at 3500 rpm for 10 min. ALT and AST activities in serum were determined at the Laboratory Center, King Chulalongkorn Memorial Hospital using the International Federation of Clinical Chemistry (IFCC) method.

Total Glutathione Determination

Total glutathione levels were measured using Cayman's GSH assay kit. This assay uses glutathione reductase for the quantification of GSH. The sulfhydryl group of GSH reacts with DTNB (5,5'-dithiobis-2-nitrobenzoic acid, Ellman's reagent) and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (between GSH and TNB) that is concomitantly produced, is reduced by glutathione reductase to recycle the GSH and produce more TNB. The rate of TNB production is directly proportional to this recycling reaction which is in turn directly proportional to the concentration of GSH in the sample. Measurement of the absorbance of TNB at 405 nm provides an accurate estimation of GSH in the sample.

Table 1 Serum biochemical parameters in all groups

Parameter (mean ± SEM)	Control (n = 8)	NASH groups (n = 8)	NASH+B6 groups (n = 10)
ALT (U/L)	40.13 ± 2.35	23.00 ± 1.92	76.30 ± 6.21*†
AST (U/L)	86.75 ± 4.28	53.63 ± 9.31	97.80 ± 9.35†

* P<0.05 when compared with control.
†P<0.05 when compared with NASH group.

Statistical Analysis

The data were expressed as mean ± SEM using the SPSS version 11.5 for Windows program. Statistical comparison between groups was analyzed by ANOVA and post hoc comparison was done with LSD. p <0.05 was considered significantly.

RESULTS

Body Weight and General Condition

The body weight at 6 weeks of the NASH group and NASH+B6 group were decreased when compared to the control (197.00 ± 8.07 g, 200.20 ± 6.80 g vs 438.38 ± 9.70 g, p <0.05), respectively. Despite weight loss, the general condition of NASH group and NASH+B6 group remained good condition throughout the experiment.

Serum Biochemical Parameters

Serum biochemical parameters in experimental groups are showed in Table 1. Serum ALT and AST were decreased significantly in the NASH group as compared with control (ALT; 23.00 ± 1.92 U/L vs 40.13 ± 2.35 U/L, AST; 53.63 ± 9.31 U/L vs 86.75 ± 4.28 U/L, p <0.05), respectively. Vitamin B supplement group significantly increased serum ALT level when compared to the control group or NASH group (ALT; 76.30 ± 6.21 U/L vs 40.13 ± 2.35 U/L or 23.00 ± 1.92 U/L, p <0.05), respectively. Additionally, serum AST level was significantly higher in NASH+B6 group than NASH group (97.80 ± 9.35 U/L vs 53.63 ± 9.31 U/L p <0.05).

Total Glutathione Level in Whole Blood

The total glutathione level in whole blood in the NASH group was increased significantly as compared with control group (2066.67 ± 93.81 μM vs 1337.54 ± 31.48 μM, p <0.05) while the total GSH in the NASH+B6 group was decreased significantly as compared with the NASH group (1682.35 ± 80.69 μM vs 2066.67 ± 93.81 μM, p <0.05), respectively. (Figure 1)

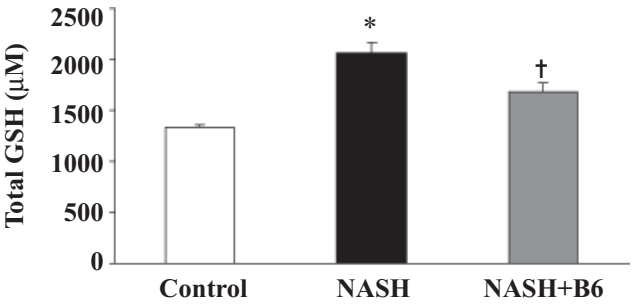

Figure 1 Effect of vitamin B6 supplement on total GSH level in rats with NASH (mean ± SEM)
*P <0.05 when compared with control.
†P <0.05 when compared with NASH group.

DISCUSSION

Vitamin B6 in the form of pyridoxal phosphate (PLP) is function as a coenzyme for ALT and AST to catalyze the transfer of the α-amino group from aspartate or alanine to α-ketoglutarate with made the release of pyruvate, oxaloacetate, and glutamate[11]. These enzymes are the useful screening tests for detecting liver injury[11]. They are found in hepatocytes and can't diffuse out of the cells in normal situation. When the hepatocytes are injured, plasma membranes will be disrupted and the leakage through extracellular fluid of the enzymes will occur. We can detected the abnormal liver function tests in the serum[14]. Recently, feeding rats with 100% fat diet to induce NASH, we found the reduction of serum ALT and AST[9]. These may be due to inadequate of dietary intake that caused a deficiency of vitamin B6 to synthesis of transaminase activity. In this study, we proved this hypothesis by supplementing vitamin B6 in NASH group for 6 weeks. Therefore, we found the increased of serum

ALT and AST levels when compared with NASH group.

Glutathione (GSH) is the major intracellular non-protein antioxidant and plays a crucial role in the detoxification of free radicals[15,16]. Serum glutathione was found increasing in patients with NASH or in 100% fat diet feeding rats in NASH model[9,17]. It could be explained by the compensatory protection mechanism against oxidative stress[9]. In this study, we found that supplement with vitamin B6 could decrease the elevation of total GSH in whole blood. This data indicated the role of vitamin B6 as an antioxidant agent.

In conclusion, vitamin B6 is important as a coenzyme for aminotransferase synthesis, that responds for the high serum ALT and AST and improves total GSH in rats with NASH.

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