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Effects of NADH Supplementation on Hematological and Blood Biochemical Parameters and Tissue Oxidant/Antioxidant Status in Rats

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Abstract: Investigation of possible effects of NADH supplementation on hematological and blood biochemical parameters and on oxidant/antioxidant status in some tissues including liver, kidney and heart was aimed in this study. Fourteen Sprague-Dawley type rats of 2 months old were used throughout the study. Seven rats were fed on NADH containing foods for a month and the others were fed on the same food diet except NADH. After a month, blood samples were obtained before animals were sacrificed. Their liver, heart and kidney tissues were removed to be used in the analyses of oxidant/antioxidant parameters. In the NADH group, lowered erythrocyte, leukocyte, platelet, hemoglobin and hematocrit values were observed as compared with control group. Furthermore, increased malondialdehyde and oxidation sensitivity values were measured in the heart tissues from NADH supplemented group. Serum LDH activity was also found to increase in the study group. In conclusion, NADH supplementation causes anemia and leukopenia and leads to increased peroxidation reactions in the heart tissue.

Key words: NADH, oxidation, toxicity

INTRODUCTION

Some researchers have suggested that NADH may be valuable adjunctive therapy in the management of some problems like chronic fatigue syndrome[1,2], Parkinson's disease[3], dementia of Alzheimer type[4] and depression[5] through triggering ATP production. They also reported that no severe adverse effects were observed related to the drug[1]. However, they made no detailed analysis in this regard. Before reaching this conclusion, we think that it should be performed some toxicological, biochemical, hematological and pathological analyses. Unfortunately, they did not perform such analyses to establish toxicological perspective of NADH.

This study was performed to establish possible effects of NADH supplementation on hematological and blood biochemical parameters in order to evaluate its possible toxic potential and, to establish its effects on oxidant/antioxidant status of several tissues as it is previously supposed to have high antioxidant potency and ATP triggering power[1].

MATERIALS AND METHODS

The study was approved by the Ethic Committee of Yüzüncü Yil University, Medical Faculty for the care of animal subjects and that the care and handling of the animals were in accord with guidelines for ethical animal research. Fourteen male Sprague-Dawley type rats of 2 months old were used in the study. Blood samples were obtained from the rats, seven of them were fed on NADH (1 mg kg^{-1} body weight/day) containing foods for a month and others were fed on the same food diet except NADH. After a month, blood samples were obtained from the rats and collected in citrated tubes for hematological and normal tubes for blood biochemical parameters. Then, the animals were sacrificed; their livers, hearts and kidneys were removed to be used in the analyses of oxidant/antioxidant parameters.

Hematological and blood biochemical analyses were measured in auto-analysers (Beckman-Coulter LH 750 for hematological and Technicon DAX-96 system for blood biochemical parameters). For the oxidant/antioxidant parameters, heart, liver and kidney tissues were first washed with distilled water, homogenized in physiologic saline solution, centrifuged at 5000 rpm for 15 min and then, upper clear layer was removed to be used in the analyses[6]. Antioxidant potential (AOP), malondialdehyde (MDA), xanthine oxidase (XO), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and oxidation sensitivity (OS) measurements were made as described, respectively[7-13]. In the activity

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calculations, extinction coefficients of uric acid, hydrogen peroxide and NADPH were used for XO, CAT and GSH-Px, respectively. One unit for SOD activity was expressed as the enzyme protein amount causing 50% inhibition in NBT reduction rate. Protein measurement was made by Lowry's method[14]. Parts of the tissues were sent to pathology department for the histopathological examination.

In the statistical analysis of the results paired t and Mann-Whitney U tests were used appropriately. P values lower than 0.05 were judged statistically significant.

RESULTS AND DISCUSSION

Results showed that leukocyte, erythrocyte, hemoglobin, hematocrit and platelet values are significantly lowered in NADH-supplemented group (Table 1). LDH activity is, however, higher in the study group (Table 2). As to the oxidant/antioxidant status, meaningful differences are only found in the heart tissue (Table 3). MDA level and OS value are higher in the heart tissues from NADH-supplemented animals. In the histopathological examination, spleen and lungs were seemed to be enlarged, pericardium hardened and liver adhered to peritoneum in NADH-supplemented group.

Although NADH is an important cofactor for several biochemical reactions, in particular for oxidation-reduction reactions in the body, it may lead to some problems when given orally in chemical form. In this regard, anemia seems of importance. It is possible that anemia is resulted from inhibition of glycolysis since NADH is an inhibitor for some key enzymes in glycolysis like glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase. Inhibition of glycolysis may lead to decreased ATP synthesis in erythrocytes which may then result in hemolysis. Histopathological observation demonstrating that spleen was enlarged in the study group might also support this hypothesis. We think that distended lung may be a compensatory mechanism against NADH-induced anemia. This kind of inhibition in energy production mechanisms of the leukocytes might be a reason of leukopenia in the study group.

With regard to oxidant/antioxidant status, accelerated oxidative reactions were observed in the heart tissue. Since heart tissue is the one in which ATP need is much more than the other tissues, it can be suggested that electron transport chain reactions are accelerated in the heart tissue more than others due to increased NADH concentration. This may also lead to increased free radical production which then causes accelerated oxidation reactions in this tissue. The changes observed in the histopathological examination of the pericardium in the animals of the study group might result from these oxidative reactions. Increased blood LDH activity may also result from the induced oxidation reactions due to high NADH in the heart tissue.

Table 1: Mean±SD of hematological parameters before and after the study (n=7)

		After the study	
Parameters	Before the study	Control group	NADH group
Leukocytes (10^3 μL^{-1})	8900.00±1750	9200.00±3260	6000.00±1770*
Erythrocytes (10^6 μL^{-1})	9.02±0.80	7.52±1.22	6.87±0.97**
Hemoglobin (g dL^{-1})	17.20±1.50	15.60±2.00	13.30±3.30*
Hematocrit (%)	49.00±4.30	43.20±4.40*	420.00±5.10*
MCV (fL)	54.40±1.60	58.00±4.90	61.70±7.60*
MCH (pg)	19.00±0.70	20.80±1.40	19.20±3.90
MCHC (g dL^{-1})	35.00±0.60	35.90±1.20	31.10±5.50
Platelet (10^3 μL^{-1})	771.00±2250	680.00±262	506.50±125.7*
Lymphocyte (%)	76.00±8.20	75.20±6.10	82.20±7.90

* p<0.05 (Paired t-test) ** p<0.01 (Paired t-test)
MCV: Mean corpuscular volume
MCH: Mean corpuscular hemoglobin
MCHC: Mean corpuscular hemoglobin concentration

Table 2: Mean±SD of blood biochemical parameters before and after the study (n=7)

		After the study	
Parameters	Before the study	Control group	NADH group
Glucose (mg dL^{-1})	105.00±15.3	118.00±19.1	87.00±11.20**
Total bilirubin (mg dL^{-1})	0.12±0.03	0.15±0.03	0.20±0.06*
Direct bilirubin (mg dL^{-1})	0.04±0.01	0.04±0.01	0.05±0.04
Indirect bilirubin (mg dL^{-1})	0.08±0.03	0.11±0.03	0.15±0.07
Triglyceride (mg dL^{-1})	91.00±20.7	127.00±43.7	83.00±16.5
Cholesterol (mg dL^{-1})	52.00±7.70	57.00±10.1	50.00±2.90
HDL cholesterol (mg dL^{-1})	39.00±5.30	44.00±6.70	36.00±2.80
VLDL cholesterol (mg dL^{-1})	18.00±4.20	25.00±8.60	17.00±3.30
ALP (U L^{-1})	458.00±122	449.00±75.0	35.50±83.6
AST (U L^{-1})	117.00±27.3	159.00±32.8	137.00±19 0
ALT (U L^{-1})	46.00±13.3	58.00±9.00	52.00±8.4 0
LDH (U L^{-1})	1265.00±709	1283.00±103	2269.00±904*

* p<0.05 (Paired t-test) ** p<0.01 (Paired t-test)

Table 3: Mean±SD of oxidant/antioxidant parameters in various tissues of the rats (n=7)

	Parameters	Control group	NADH group
Liver	Superoxide dismutase (U mg^{-1})	59.04±3.25	64.05±3.87
	Glutathione peroxidase (m IU/mg)	16.56±1.49	16.73±5.06
	Catalase (IU/mg)	81.22±15.58	86.47±7.13
	Xanthine oxidase (m IU/mg)	0.89±0.09	0.90±0.16
	Malondialdehyde (nmol/mg)	0.72±0.10	0.65±0.13
	Oxidation sensitivity (nmol/mg h)	0.09±0.14	0.15±0.04
Heart	Superoxide dismutase (U mg^{-1})	36.31±7.01	35.50±7.65
	Glutathione peroxidase (m IU/mg)	3.67±1.73	4.55±3.06
	Catalase (IU/mg)	28.01±6.05	29.61±6.68
	Xanthine oxidase (m IU/mg)	0.12±0.03	0.12±0.02
	Antioxidant potential (U mg^{-1})	1.90±0.01	1.89±0.01
	Malondialdehyde (nmol/mg)	0.51±0.15	0.86±0.13**
	Oxidation sensitivity (nmol/mg h)	0.88±0.16	1.19±0.15*
Kidney	Superoxide dismutase (U mg^{-1})	38.94±6.38	43.49±4.75
	Glutathione peroxidase (m IU/mg)	15.40±3.23	22.23±5.79*
	Catalase (IU/mg)	74.63±6.28	79.04±6.29
	Xanthine oxidase (m IU/mg)	0.56±0.07	0.59±013
	Antioxidant potential (U mg^{-1})	1.86±0.02	1.85±0.01
	Malondialdehyde (nmol/mg)	0.80±0.28	0.79±0.24
	Oxidation sensitivity (nmol/mg h)	0.39±0.09	0.37±0.14

* p<0.05 (Mann-Whitney U test) ** p<0.01 (Mann-Whitney U test)

To sum up, NADH supplementation may give some beneficial results for people with some diseases by triggering ATP synthesis, it was observed that it might also cause adverse effects like anemia, leukopenia and accelerated oxidation reactions in some tissues, all of which may limit its use significantly.

REFERENCES

1. Forsyth, L.M., H.G. Preuss, A.L. MacDowell, L. Chiazze, G.D. Birkmayer and J.A. Bellanti, 1999. Therapeutic effects of oral NADH on the symptoms of patients with chronic fatigue syndrome. Ann. Allergy Asthma. Immunol., 82: 185-191.
2. Bellanti, J.A., L.M. Forsyth, A.L. MacDowell-Carnerio, G.D. Birkmayer and H.G. Preuss, 1998. NADH and chronic fatigue syndrome (CFS): A new therapeutic approach. Ann. Allergy Asthma. Immunol., 80: 82.
3. Birkmayer, J.G.D., C. Vrecko, D. Vole and W. Birkmayer, 1993. Nicotinamide adenine dinucleotide [NADH]-new therapeutic approach to Parkinson's disease, comparison of oral and parenteral application. Acta Neurol. Scand., 87 Suppl., 146: 32-35.
4. Birkmayer, J.G.D., 1996. Coenzyme nicotinamide adenine dinucleotide, new therapeutic approach for improving dementia of the Alzheimer type. Ann. Clin. Lab. Sci., 26: 1-9.
5. Birkmayer, J.D. and W. Birkmayer, 1991. The coenzyme nicotinamide adenine dinucleotide (NADH) as biological antidepressive agent experience with 205 patients. New Trend in Clin. Neuropharm., 5: 75-86.
6. Durak, İ., T. Güven, M. Birey, H.S. Öztürk, O. Kurtipek, M. Yel, B. Dikmen, O. Canbolat, M. Kavutcu and M. Kacmaz, 1996. Halothane hepatotoxicity and hepatic free radical metabolism in guinea pigs: The effects of vitamin E. Can. J. Anaesth., 43: 741-748.
7. Durak, I., N.K. Bingöl, A. Avci, M.Y.B. Cimen, M. Kacmaz, L. Karaca and H.S. Öztürk, 2000. Acute effects of smoking of cigarettes with different tar content on plasma oxidant/antioxidant status. Inhal. Toxicol., 12: 641-647.
8. Dahle, L.K., E.G. Hill and R.T. Hollman, 1962. The thiobarbituric acid reaction and the autoxidations of polyunsaturated fatty acid methyl esters. Arch. Biochem. Biophys., 98: 253-261.
9. Hashimato, S., 1974. A new spectrophotometric assay method of xanthine oxidase in crude tissue homogenate. Anal. Biochem., 62: 425-435.
10. Aebi, H., 1974. Catalase. Methods of Enzymatic Analysis. H.U. Bergmayer, New York and London, pp: 673-677.
11. Durak, I., O. Canbolat, M. Kavutcu, H.S. Öztürk and Z. Yurtaslani, 1996. Activities of total, cytoplasmic and, mitochondrial superoxide dismutase enzyme in sera and pleural fluids from patients with lung cancer. J. Clin. Lab. Anal., 10: 17-20.
12. Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169.
13. Dasgupta, A. and T. Zdunek, 1992. *In vitro* lipid peroxidation of human serum catalyzed by cupric ion: Antioxidant rather than prooxidant role of ascorbate. Life Sci., 50: 875-882.
14. Lowry, O., N. Rosenbrough, L. Farr and R. Randall, 1951. Protein measurement with folin phenol reagent. J. Biol. Chem., 182: 265-275.