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J. Khalili, H. F. Biloklytska

Influence of supplementary vitamins and minerals on lipid peroxidation and redox state in heart, kidney and liver of rats exposed to fluoride

The effect of fluoride (F) and supplementary vitamins and minerals on lipid peroxidation (LPO) and redox state (RS) in heart, kidney and liver of 40 (4 groups of 10) male Wistar rats were studied. One group of rats was left untreated as control, group 1 was received 5 mg/l NaF in their drinking water, group 2 was received 5 mg/l NaF in their drinking water plus vitamins (A, C, and D) in their diet, and group 3 was received 5 mg/l NaF in their drinking water plus vitamins (A, C, and D) and minerals (Mg-, Mn-, Zn-sulfate, and Na-citrate) in their diet. In comparison with the group 2, 3 and controls, elevated malondialdehyde (MDA) content in the group 1 indicated an increase in LPO product. In addition, unsteady ratios of oxidized to reduced nicotinamide adenine dinucleotide (NAD+/NADH) reflected significant alterations in the RS status. These results demonstrate that the combination of vitamins and minerals supplementation proved to restore MDA content and establish steady RS status that has not been previously reported.
Key words: fluoride, vitamins and minerals, lipid peroxidation, redox state, heart, kidney, liver, rats.

INTRODUCTION

Chronic fluorosis can severely damage many systems of the human body, but its pathogenesis is poorly understood [1]. Since this disease is irreversible, by appropriate and timely intervention it's preventable. Therefore, a greater understanding at biochemical and molecular levels of the disease progression is very important. It has been reported that free radicals (FR) and other reactive oxygen species are derived either from normal essential metabolic processes in the human body or from external sources such as certain drugs, environmental pollutants, industrial chemicals, and pesticides [2]. Thus, FR induced lipid peroxidation (LPO), and because of high molecules reactivity, has been implicated in the toxicity of a wide range of compounds, including F toxicity [3]. In this respect, F has been shown to inhibit many enzymes [4,5,6,7]. It is well known that a central facet of "mitochondrial health" revolves around the oxidized to reduced nicotinamide adenine dinucleotide (NAD^+/NADH) relationship. In addition, redox state (RS) is commonly used to describe the balance of NAD^+ to NADH [8]. On the other hand, metabolism is an extremely complex subject in biochemistry. It is usually consists of sequences of enzymatic steps, the so-called metabolic pathways that interact in a complex way in order to allow an adequate regulation. In each pathway, a principal chemical is modified by chemical reactions and often require dietary minerals (for both physiological and biochemical functions), vitamins and other co-factors in order to function properly. There is strong evidence that certain vitamins and minerals act as antioxidants and may protect against tissue damage [9,10]. In the light of above data, for the first time, the present study aimed to examine malondialdehyde (MDA) content by analysing the LPO product and RS by evaluating the ratio of NAD^+/NADH in the heart, kidney, and liver

of rats exposed to F and supplemented with vitamins and minerals.

METHODS

Thirty male Wistar rats approximately 42 ± 5 day of age, each weighing 140 ± 3 grams on average were randomly divided into 3 equal groups and maintained for 30 consecutive days. They were placed in a quiet polypropylene cages with stainless still grill tops, as 5 animals per cage, temperature and humidity controlled room (22 ± 2°C and 60 ± 5 %, respectively) in which a 12 h/12 h light/dark cycle was maintained (lights on: 08:00 h), fed a standard pelleted diet, and given distilled water ad libitum. One group of rats was left untreated as control. The experimental group 1 was given standard diet, distilled water containing 5 mg/l NaF. The group 2 was given standard diet plus 1/50 of adult dose of vitamins (A, C and D) and distilled water containing 5 mg/l NaF. The group 3 was given standard diet plus 1/50 of adult dose of vitamins (A, C and D) and 300 mg/kg minerals (Mg-, Mn-, Zn-sulfate, and Na-citrate) and distilled water containing 5 mg/l NaF. At the termination of experimental period, the animals were sacrificed under light ether anesthesia. The heart, kidney and liver were removed. Then, the samples were aliquoted into storage glass, submerged in liquid nitrogen and kept at -70°C until analysed.

At the time of analysis the samples were pulverized. The content of MDA as an indicator of LPO was assayed, as previously described [11]. Briefly, 1 g of the pulverized sample was homogenized in 3 ml of 0.025 M Tris-HCl and 0.175 M KCl buffer (pH 7.4). The content of MDA was evaluated by the 2-thiobarbituric acid reactive substances procedure at 532 nm using a spectrophotometer (Zeiss MCS 621 UV-VISl Carl Zeiss, Jena, Germany). The content of NAD+ and NADH was assayed, as previously described [12]. Briefly, 1 g of the pulverized sample was homogenized in 4 ml of ice cold 0.5 N $HClO_4$. The NAD^+ and NADH content were analyzed at 340 nm spectrophotometrically. Then, the NAD^+/NADH ratio was calculated.

The content of MDA, NAD^+ and NADH are expressed as micromoles per gram protein. The experiments performed in this study have been carried out according to the rules in the guide for the care and use of laboratory animals adopted by Ministry of Health (Ukraine). This study was approved by the Ethics Committee of National Medical Academy of Postgraduate Education (named after P. L. Shupyk). The values were expressed as mean ± SD. To compare the differences in all parameters between experimental and control groups the data were statistically analyzed by Student's t-test using SPSS 11.5 statistical package (SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

The balance between the production of free radical and antioxidant defense in the body has important health implications: if there are too many free radicals or too few antioxidant for protection, a condition of oxidative stress develops, which may cause chronic and permanent damage [10]. Although, a close association between F toxicity and oxidative stress in human beings [13], experimental animals [14], and cultured cells [15] has been reported. But the mechanism of F induced LPO is not fully clarified and the existing data are not only conflicting but also contradictory. Therefore, prevention of the disease should be the aim, knowing the pathogenesis of fluorosis.

Many investigations indicate that excessive F can enhance LPO and inhibit antioxidative enzymes activity in various organs [16,17]. Although different materials and methods were used, the elevated MDA content in the group 1 observed in this study are thus in accord with previous findings. The MDA content in the heart, kidney and liver of

Table 1. The content of lipid peroxidation product, malondialdehyde (MDA), in the heart, kidney and liver of rats: NaF-intoxicated (group 1), NaF-intoxicated plus vitamins (group2), NaF-intoxicated plus vitamins and minerals (group3), and control. The values are expressed as mean ± s.d; the results are expressed as μmol/g; n = 10 in each group

MDA	Control	Group1	Group 2	Group 3
Heart	51.10 ± 2.50[b]	192.90 ± 0.12[a]	61.63 ± 3.70	51.10 ± 2.50[b]
Kidney	50.21 ± 1.50[b]	296.00 ± 1.20[a]	48.93 ± 0.13	46.54 ± 1.25[b]
Liver	40.74 ± 0.23[b]	297.00 ± 1.52[a]	47.60 ± 1.71	41.10 ± 1.71[b]

[a]Significant differences when compared to groups 2, 3 and control (P<0.001).
[b]No significant differences when compared group 3 to control (P>0.001).

study subjects is shown in Table 1. The differences in the contents of MDA in the group 1 were statistically significant in comparison with the groups 2, 3 and control (P<0.001). No significant differences when compared group 3 to control (P>0.001). Hence, some investigators have reported that F does not impair antioxidant defense systems [18,19]. However, the differences in results are possibly due to many factors such as age, dietary factors, F absorption, methods and materials used for biochemical assay, and sex [20].

An increasing central principle to mitochondrial (and overall) health is the importance of maintaining a favourable ratio of NAD+ to NADH [21,22]. In this respect, NADH oxidase is an enzyme involved in molecular oxygen detoxification and in maintaining a low redox potential inside cells [23]. Therefore, content of NAD^+ and NADH influence by various physiological and toxicological factors. Experimental data show that metabolic alterations strongly affect NAD^+/NADH ratio controlling in such a way carbon flow distribution within the fundamental pathways [24]. There is strong evidence that in diabetic ketoacidosis the more reduced cytoplasmic NAD^+/NADH ratio is accompanied by an oxidized mitochondrial redox state [25,26]. There is a growing body of evidence suggesting that F interferes with hydrogen bonding and inhibits numerous enzymes [27]. Despite the existence of a large amount of data concerning the influence of F compounds on metabolism in various animal species and humans living in a contaminated environment, no studies appear to have been carried out on the relation between F toxicity and content of NAD^+ and NADH, and/or NAD^+/NADH ratio. The content of NAD+ and NADH in the heart, kidney and liver of rats are shown in Table 2. The differences in the content of NAD^+ and

Table 2. The content of oxidized nicotinamide adenine dinucleotide (NAD+) and reduced nicotinamide adenine dinucleotide (NADH) in the heart, kidney and liver of rats: NaF-intoxicated (group 1), NaF-intoxicated plus vitamins (group2), NaF-intoxicated plus vitamins and minerals (group3), and control. The values are expressed as mean ± s.d; the results are expressed as μmol/g; n = 10 in each group

NAD+	Control	Group1	Group 2	Group 3
Heart	15.28 ± 0.43[b]	10.49 ± 0.44[a]	9.04 ± 0.24	12.18 ± 0.21[b]
Kidney	12.31 ± 0.22[b]	5.34 ± 0.21[a]	9.34 ± 0.21	11.45 ± 0.21[b]
Liver	13.56 ± 0.43[b]	5.48 ± 0.19[a]	10.74 ± 0.31	12.32 ± 0.23[b]
NADH				
Heart	21.26 ± 0.18[b]	31.82 ± 0.33[a]	23.32 ± 0.50	20.21 ± 0.30[b]
Kidney	21.80 ± 0.24[b]	29.49 ± 0.43[a]	25.40 ± 0.23	21.19 ± 0.21[b]
Liver	23.08 ± 0.49[b]	32.28 ± 0.36[a]	25.59 ± 0.46	20.24 ± 0.42[b]

[a]Significantly different when compared to group 2, 3 and control (P<0.001).
[b]No significant differences when compared group 3 to control (P>0.001).

NADH in the group 1 were statistically significant in comparison with the groups 2, 3 and control ($P<0.001$). No significant differences when compared group 3 to control ($P>0.001$). The ratio of NAD^+/NADH in the heart, kidney and liver of rats are shown in Figure 1. The observed values for the ratio of NAD^+/NADH in the heart, kidney and liver of the control and three groups of rats were 0.72 ± 0.02, 0.33 ± 0.05, 0.39 ± 0.22, and 0.60 ± 0.03; 0.56 ± 0.01, 0.18 ± 0.02, 0.37 ± 0.02, and 0.54 ± 0.03; 0.58 ± 0.02, 0.17 ± 0.02, 0.42 ± 0.02, and 0.61 ± 0.02, respectively. The differences in the ratio of NAD^+/NADH in the group 1 were statistically significant in comparison with the groups 2, 3 and control ($P<0.001$). No significant differences when compared group 3 to control ($P>0.001$).

It is well known that enzyme activity can be stimulated and potentiated by making the required vitamins and minerals available to the body thus ensuring that essential chemical reactions are maintained. In this respect, it is interesting to note that nutrition appears to play a crucial role in the incidence and severity of fluorosis. A review of the literature clearly supports the suggestion that diet can modify fluoride toxicity and may have some merit. [28]. Three decades or so ago it was decoded to market oral fluoride supplements as part of a vitamin preparation [29,30], a practice that still continues. The vitamins included, in either a liquid suspension or in tablet form, vitamins A, C, and D with or without B vitamins. It was reported that children fluoride in a vitamin preparation consistently excreted about 30% less fluoride in urine that those taking fluoride without vitamins [31]. Recent studies also suggest that the presence of certain trace elements in high concentration in water and food could influence fluoride toxicity, some beneficial and others detrimental [32,33]. To the best of our knowledge, no study conducted to date have evaluated the effect of F and certain vitamins and minerals on LPO and RS in animals. In current study, the lack of significant differences in the values of LPO (MDA content), NAD^+ and NADH content, and NAD^+/NADH ratio between control and group 3 indicate that the combination of supplementary vitamins and minerals proved to reduce oxidative stress (Tables 1 and 2, and Figure 1).

Although the findings obtained for rats cannot be directly referred to the human body. But experimental studies on fluorosis have greatly helped in understanding the disease and provided a rational approach of management of the menace of fluoride toxicity. Therefore, based on the data presented in present study. We suggest that F is very biologically active even at low concentrations also; combination of the vitamins and minerals may prove to be useful in prevention of F toxicity. In summary, the biochemical changes observed in the rats exposed to F clearly demonstrate that increased MDA contents are associated with unsteady RS status and may provide additional advantages in elucidating the pathogenesis of fluorosis. In addition, the vitamins and minerals proved to restore MDA content and establish steady RS status. This novel approach might promote a better understanding for the mechanism of fluorisis and may be a key mechanism for prevention of this disease.

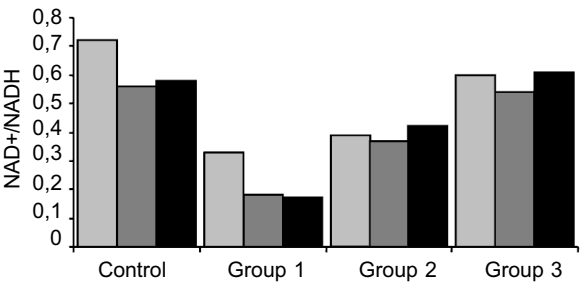

Figure 1. The ratio of the oxidized nicotinamide adenine dinucleotide (NAD+) to reduced nicotinamide adenine dinucleotide (NADH) in the heart, kidney and liver of rats: NaF-intoxicated (group 1), NaF-intoxicated plus vitamins (group2), NaF-intoxicated plus vitamins and minerals (group3), and control. The differences between the controls and groups 1 and 2 were significant at ($P<0.001$). No significant differences when compared group 3 to control ($P>0.001$)

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CORRESPONDENCE

J. Khalili, DMD, MDS. Department of Therapeutic Stomatology, National Medical Academy of Postgraduate Education. No: 1 Blvd. Shevchenko, Kiev, Ukraine. 01004. Email: KhaliliJ@mail.ru

Халілі Джафар, Г.Ф. Білоклицька

ВПЛИВ ДОДАТКОВИХ ВІТАМІНІВ ТА МІНЕРАЛІВ НА ПЕРЕКИСНЕ ОКИСНЕННЯ ЛІПІДІВ ТА РЕДОКС-СТАН У СЕРЦІ, НИРКАХ ТА ПЕЧІНЦІ ЩУРІВ

Ефект фтору (F), вітамінів і мінералів на перекисне окиснення ліпідів (ПОЛ) та редокс-стан (РС) був досліджений у серці, нирках і печінці у 40 (4 групи по 10) щурів-самців лінії Вістар. Тварин контрольної групи утримували на стандартному раціоні та дистильованій воді; в питну воду для щурів 1-ї групи додавали фторид натрію (NaF) 5 мг/л; раціон щурів 2-ї групи, окрім NaF (5 мг/л), був доповнений вітамінами (А, С, Д); до раціону щурів 3-ї групи, окрім NaF (5 мг/л) та вітамінів (А, С, Д), додавали мінерали у вигляді солей (сульфат Mg, Mn, Zn та цитрат Na). У порівнянні з контрольною, 2-ю та 3-ю групами, підвищення вмісту малонового діальдегіду (МДА) у 1-й групі свідчить про підвищення вмісту продукту ПОЛ. Нестійке відношення окисних до відновлених нікотинамідних коферментів ($НАД^+$/НАДН) відображає значні зміни у статусі РС тканин. Результати досліджень свідчать, що додаткове введення в раціон щурів вітамінів і мінералів призводить до відновлення вмісту МДА в тканинах та рівноваги РС, про що раніше не повідомлялося.

Ключові слова: фтор, вітаміни та мінерали, перекисне окиснення ліпідів, редокс-стан, серце, нирки, печінка, щури.

Халили Джафар, Г.Ф. Белоклицкая

ВЛИЯНИЕ ДОПОЛНИТЕЛЬНЫХ ВИТАМИНОВ И МИНЕРАЛОВ НА ПЕРЕКИСНОЕ ОКИСЛЕНИЕ ЛИПИДОВ И РЕДОКС-СОСТОЯНИЕ В СЕРДЦЕ, ПОЧКАХ И ПЕЧЕНИ КРЫС

Эффект фтора (F) и дополнительных витаминов и минералов на перекисное окисление липидов (ПОЛ) и редокс-состояние (РС) был исследован в сердце, почках и печени у 40 (4 группы по 10) крыс-самцов линии Вистар. Животных контрольной группы содержали на стандартном рационе и дистиллированной воде; в питьевую воду крыс 1-й группы добавляли фторид натрия (NaF) 5 мг/л; рацион крыс 2-й группы, кроме NaF (5 мг/л), был дополнен витаминами (А, С, Д); к рациону крыс 3-й группы, кроме NaF (5 мг/л) и витаминов (А, С, Д), добавляли минералы в виде солей (сульфат Mg, Mn, Zn и цитрат Na). В сравнении с контрольной, 2-й и 3-й группами повышение содержания малонового диальдегида (МДА) в 1-й группе свидетельствует о повышении содержания продукта ПОЛ. Нестойкое соотношение окисленных и восстановленных никотинамидных коферментов ($НАД^+$/НАДН) отображает значительные изменения в статусе РС тканей. Результаты исследований свидетельствуют о том, что дополнительное введение в рацион крыс витаминов и минералов способствует восстановлению содержания МДА в тканях и равновесия РС, о чем раньше не сообщалось.

Ключевые слова: фтор, витамины и минералы, перекисное окисление липидов, редокс-состояние, сердце, почки, печень, крысы.

REFERENCES

1. Krishnamachari K.A. Skeletal fluorosis in humans: a review of recent progress in the understanding of the disease // Prog. Food Nutr. Sci. – 1986. – **10**. – P. 279–314.
2. Langseth L. Oxidants and antioxidants: some basic concepts. In: Oxidants, antioxidants and disease prevention. – Belgium: International Life Science Institute Press, 1996. – P. 1–4.
3. Chinoy N.J., Patel D. Influences of fluoride on biological free radicals in ovary of mice and its reversal // Environ. Sci. – 1998. – **6**. – P. 171–184.
4. Carlson J.R., Suttie J.W. Pentose phosphate pathway enzymes and glucose oxidation in fluoride-fed rats // Amer. J. Physiol. – 1966. – **210**. – P. 79–83.
5. Vani M.L., Reddy K.P. Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice // Fluoride. – 2000. – **33**. – P. 17–26.
6. Park S., Ajtai K., Burghardt P. Inhibition of myosin ATPase by metal fluoride complexes // Biochem. Biophys. Acta. – 1999. – **1430**. – P. 127–140.
7. Khalili J., Biloklytska H.F., Pogrebnyak H.V. Acid-base status and fructose diphosphatase activity in rats exposed to fluoride and induced periodontitis // Fiziol. Zh. – 2009. – **55**, № 3. – P. 118–124.
8. Pollak N., Dцlle C., Ziegler M. The power to reduce: pyridine nucleotides – small molecules with a multitude of functions // Biochem J. – 2007. – **402**. – P. 205–218.
9. Palacios C., Joshipura K., Willett W. Nutrition and health: guidelines for dental practitioners // Oral Dis. – 2009. – **15**. – P. 369–381.
10. Abdollahi M., Ranjbar A., Shadnia S. et al. Pesticides and oxidative stress: a review // Med. Sci. Monit. – 2004. – **10**. – P. RA141–147.
11. Stalnaya I.D., Garishvili T.G. The method of deter-

mines the malondialdehyde with the aid of thiobarbituric acid. In: Modern Methods in Biochemistry / Ed. V. N. Orekhovich. – Moscow: Meditsina, 1977. – P. 66–68.
12. Putilina F.E. Determination of oxidized and reduced nicotinamide adenine dinucleotide in tissue. In: Methods of Biochemistry Investigations / Ed. M. I. Prohorova. – Leningrad: Leni Uni Press, 1982. – P. 161–166.
13. Pendrys D.G. Fluoride ingestion and oral health // J. Nutrition. – 2001. – **17**. – P. 979–980.
14. Shanthakumari D., Srinivasalu S., Subramanian S. Effect of fluoride intoxication on the levels of intestinal antioxidants studied in rats // Methods Find Exp. Clin. Pharmacol. – 2007. – **29**. – P. 93–99.
15. Wang A.G., Xia T., Chu Q.L. et al. Effects of fluoride on lipid peroxidation, DNA damage and apoptosis in human embryo hepatocytes // Biomed. Environ. Sci. – 2004. – **17**. – P. 217–222.
16. Ghosh D., Das Sarkar S., Maiti R. et al. Testicular toxicity in sodium fluoride treated rats: association with oxidative stress // Reprod. Toxicol. – 2002. – **16**. – P. 385–390.
17. Zhan X.A., Xu Z.R., Li J.X. et al. Effects of fluorosis on lipid peroxidation and antioxidant systems in young pigs // Fluoride. – 2005. – **38**. – P. 157–161.
18. Kumar T., Mohan E.M., Ramesh N. et al. Toxicity of combination of fluoride and monocrotophos 36% SL to wistar rats. // J. Environ. Biol. – 1998. – **19**. – P. 305–311.
19. Chlubek D., Grucka–Mamczar E., Birkner E. et al. Activity of pancreatic antioxidative enzymes and malondialdehyde concentrations in rats with hyperglycemia caused by fluoride intoxication. // J. Trace. Elem. Med. Biol. – 2003. – **17**. – P. 57–60.
20. Rzeuski R., Chlubek D., Machoy Z. Interactions between fluoride and biological free radical reactions // Fluoride. – 1998. – **31**. – P. 43–45.
21. Suh J.H., Shigeno E.T., Morrow J.D. et al. Oxidative stress in the aging rat heart is reversed by dietary supplementation with (R)-a-lipoic acid // FASEB J. – 2001. – **15**, N 3. – P. 700–706.
22. Williamson J.R., Chang K., Frangos M. et al. Hyperglycemic pseudohypoxia and diabetic complications // Diabetes. – 1993. – **42**. – P. 801–813.
23. Diaz P.I., Zilm P.S., Wasinger V. et al. Studies on NADH oxidase and alkyl hydroperoxide reductase produced by Porphyromonas gingivalis // Oral Microbiol. Immunol. – 2004. – **19**. – P. 137–143.
24. Krebs H.A. Pyridine nucleotides and rate control. In: Rate Control of Biological Processes/ Ed. D. D. Davies. – Cambridge, England: Cambridge University Press, 1973. – P. 241–281.
25. Williamson D.H., Lund P., Krebs H.A. Redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver // Biochem. J. – 1967. – **103**. – P. 514–527.
26. Greenbaum A.L., Gumaa K.A., McLean P. The distribution of hepatic metabolites and the control of carbohydrate metabolism in animals of different dietary and hormonal states // Arch. Biochem. Biophys. – 1971. – **143**. – P. 617–663.
27. Emsley J., Jones D.J., Miller J.M. et al. An Unexpectedly Strong Hydrogen Bond: ab initio Calculations and Spectroscopic Studies of Amide-Fluoride Systems // J. Am. Chem. Soc. – 1981. – **103**. – P. 24–28.
28. Wadhwani T.K. Prevention and mitigation of fluorosis (Endemic) Part II // Ind. Med. Sci. – 1954. – **36**. – P. 64–69.
29. Hennon D.K., Stookey G.K., Muhler J.C. Fluoride Excretion with Sodium Fluoride–Vitamin Tablets // J. Dent. Res. – 1968. – **47**. – P. 710–716.
30. Petrushko O.E., Zhedik A.N. Results of using Vitafluor for dental caries prevention in 1st graders in Brest // Stomatologiia (Mosk). – 1985. – **64**. – P. 84–85 (Article in Russian).
31. Margolis F.J., Nagler R.C., Holkeboer P.E. Short-term fluoride excretion in young children // Am. J. Dis. Child. – 1967. – **113**. – P. 673–676.
32. Reddy D.R., Rao C.R., Nayak R.K. et al. Estimations of trace elements elements in drinking water supplies of Podili, Darsi and Kanigiri areas of Andhra Pradesh. In: Proceedings of the workshop on medical geology, IGCP-454. – Nagpur, 2004. – P. 384–96.
33. Reddy D.R. Some observations of fluoride toxicity // NIMHANS J. – 1985. – **3**. – P. 79–86.

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