ROCZN. PZH, 2002, 53, NR 1

IRENEUSZ P. GRUDZIŃSKI

A PILOT STUDY ON THE HISTOCHEMICAL EVALUATION OF Na/K-ATPase AND SUCCINATE DEHYDROGENASE IN RAT INTESTINAL VILLI

BADANIA PILOTOWE NAD HISTOCHEMICZNĄ OCENĄ ATPazy-Na/K ORAZ DEHYDROGENAZY BURSZTYNIANOWEJ W KOSMKACH JELITA CIENKIEGO SZCZURA

Department of Applied Physiology
Military Institute of Hygiene and Epidemiology
Kozielska 4, 01–163 Warsaw, Poland
Head: prof. dr. hab. med. *J. Faff*

Poisoning of rats with sodium nitrite has been found to decrease Na/K-ATPase and succinate dehydrogenase activity in the small intestinal villi.

INTRODUCTION

Evidence has accumulated in recent years linking exposures to inorganic nitrites to an increased risk of gastric and/or colorectal cancer [2, 15]. It should be noted that the exogenous source of these exposures may be dietary (food and drinking water) or it may be of endogenous origin, as well [6, 16]. Dietary nitrites have been suggested to be precursors of endogenous synthesis of nitrosoamines, and can therefore contribute to human cancer development [1, 24]. These results seem to further support some epidemiological evidences that a risk(s) of stomach and/or colorectal cancer is higher than other type of cancer(s) in human population exposed to nitrites from drinking water and/or diet [15, 25]. Although the association between the higher risk of gastric and colorectal cancers may be due to increased levels of nitrosation processes in the gastrointestinal tract of humans and animals [4, 14, 27, 31], the mechanism(s) of nitrite-mediated toxicity in the gastrointestinal mucosa of laboratory rodents still remain to be completed [12].

Previous studies from our laboratories showed that sodium nitrite and/or N-nitrosodiethylamine caused lipid peroxidation in the small intestine mucosa and liver of rats [7, 8]. Since nitrite also decreased mitochondrial respiration [11] and the intestinal transport of sugars [12], and the agent lowered gastric juice volume, acid and peptide output, as well [3], in the present studies, we have determined Na/K-ATPase and succinate dehydrogenase (SDH) activity in the small intestinal villi of rats pretreated with or without nitrite.

MATERIAL AND METHODS

Male *Wistar* rats (200 ± 20 g) were randomized into nitrite and control groups of 20 animals in each (5 animals per cage). Throughout the experiment, the rats were given a pellet of laboratory chow (Murigran, Motycz, Poland) and water *ad libitum* but they were not allowed to have food for 24 hrs before sacrificing. The animals were treated *per os* with either an aqueous solution of sodium nitrite (10 mg $NaNO_2$/kg body weight) or normal saline (control) daily for 3 or 12 weeks. The rats were sacrificed by cervical dislocation 24 hr after the last nitrite and/or saline dosage and the small intestine were removed from the rats. The mucosal surface of intestinal samples was then gently washed with a solution of normal saline and prepared for Na/K-ATPase (EC 3.6.1.3.) and succinate dehydrogenase, SDH (EC 1.3.98.1.) analysis [5, 19–23]. Briefly, the small pieces of intestine were promptly frozen and subsequently cut on a cryostat into 7–8 μm sections. Frozen (unfixed) intestine sections were further incubated with a solution of *Tris* buffer, pH 7.2 (Na/K-ATPase) containing adenosine 5'-triphosphate di-sodium salt, ATP (2.5 mg/ml), 2% lead phosphate, 0.1 M magnesium sulfate, 0.1 M sodium chlorate, and 0.02 M potassium chlorate for 60 min at 37°C. The specimens were washed with distilled water and fixed with a solution of 1% acetic acid for 3–4 min, and closed in glycerol gelatin. Similarly, the slices were incubated with a solution of phosphate buffer, pH 7.6 (SDH) containing 0.2 M sodium succinate, and Nitro BT, p-Nitrotetrazolium blue (mg/ml) for 3 min at 37°C. Then, the slices were washed with normal saline and fixed with a solution of 10% formic acid for 10 min. After washing with 15% ethanol, the slides were closed in glycerol gelatin. The activity of the enzymes were qualitative evaluated under a light microscope based on the presence of black (Na/K-ATPase) or blue (SDH) color precipitates of lead sulfide or formazan in rat intestinal villi, respectively. All reagents were of the highest quality available and purchased from Sigma Chemical Company (St. Louis, MO, USA).

RESULTS AND DISCUSSION

The histological characteristic of the small intestine specimens taken from the proximal intestine of the nitrite and normal saline (control) rats was shown in figures 1 and 2. Results show that sodium nitrite diminished Na/K-ATPase and SDH activities since a weak enzyme histochemical reaction(s) was evidenced in the small intestinal villi of nitrite-treated rats. In sharp contrast, the small intestine taken from rats pretreated with normal saline showed no such changes (Figs. 1 and 2). These results were found in accordance with those previously reported by *Grudziński* and co-workers, who noted that sodium nitrite and sodium azide, an inhibitor of cytochrome c oxidase, reduced oxygen consumption and ATP production in murine mitochondria [10, 11]. It is generally accepted that Na/K-ATPase occupies a key position in the maintenance of energy-dependent transport of amino acids and/or glucose in the small intestinal mucosa [28]. On the other hand, succinate dehydrogenase (SDH), a member of the tricarboxylic acid cycle catalyzes an oxygen-linked reaction, which mainly leads to energy conservation in cells [29]. Since sodium nitrite has been found to reduce SDH activity in the pyloric stomach of rats [13], and the agent oxidized thiol groups [30], we further hypothesized that the SDH enzyme could be one of the major target(s) of nitrite-induced energy disturbances and/or toxicity in the gastrointestinal tract of rodents. It should be noted that the presence of –SH groups essential for the activity of SDH was previously described by *Singer*, who showed that the substrate and/or competitive inhibitors protect this mitochondrial-linked enzyme from the action of –SH inhibitors [26]. Interestingly, nitrite-induced lipid peroxidation and/or pro-oxidant

shift(s) in the small intestine and liver of rats was recently diminished by an oral supplementation of coenzyme Q10 (ubiquinon), a well-known integral component of the mitochondrial electron transport chain [9].

In summary, pretreatment of rats with a multiple dosage of sodium nitrite has been shown to decrease Na/K-ATPase and succinate dehydrogenase activity in the small intestinal villi. It is generally known that sodium nitrite and its red-ox derivatives, e.g. nitric oxide (NO) and/or peroxynitrite ($ONOO^-$), a product of the reaction between NO and superoxide anion radical ($O_2^{\cdot-}$) are reactive nitrogen species and can plausibly react with secondary amines and/or amides to form N-nitroso compounds; many of which have been found to be carcinogenic in animals, e.g. N-nitrosodiethylamine [17, 18, 27, 31]. Since poisoning with sodium nitrite was previously found to cause severe damages and/or atrophies of murine gastrointestinal mucosa [12, 13], and agent promoted forestomach tumors in rats [14, 31], further studies should be addressed to elucidate a molecular scenario of nitrite-induced toxicity in the gastrointestinal tract of laboratory rodents.

I.P. Grudziński

A PILOT STUDY ON THE HISTOCHEMICAL EVALUATION OF Na/K-ATPase AND SUCCINATE DEHYDROGENASE IN RAT INTESTINAL VILLI

Summary

Effects of poisoning with sodium nitrite on Na/K-ATPase and succinate dehydrogenase (SDH) activity were examined in the small intestine mucosa of male *Wistar* rats. The animals were treated *per os* with either an aqueous solution of sodium nitrite (10 mg $NaNO_2$/kg b.w.) or normal saline (control) daily for 3 or 12 weeks. Histochemical analyses show that sodium nitrite decreased Na/K-ATPase and SDH in rat intestinal villi, and the nitrite side effect(s) was mainly observed in animals treated for 12 weeks.

I.P. Grudziński

BADANIA PILOTOWE NAD HISTOCHEMICZNĄ OCENĄ ATPazy-Na/K ORAZ DEHYDROGENAZY BURSZTYNIANOWEJ W KOSMKACH JELITA CIENKIEGO SZCZURA

Streszczenie

Badano wpływ zatrucia azotynem sodowym na aktywność ATPazy-Na/K oraz dehydrogenazy bursztynianowej (SDH) w błonie śluzowej jelita cienkiego szczurów. Zwierzęta otrzymywały *per os* wodny roztwór azotynu sodowego (10 mg $NaNO_2$/kg m.c.) i/lub 0,9% roztwór chlorku sodowego (kontrola) przez okres 3 i 12 tygodni. Histoenzymatyczna analiza wykazała azotynowe obniżenie aktywności ATPazy-Na/K oraz SDH w kosmkach jelitowych ze szczególnym nasileniem wpływu azotynu w 12 tygodniu zatrucia zwierząt.

REFERENCES

1. *Bartsch H.*: N-nitroso compounds and human cancer: where do we stand? IARC. Sci. Publ. 1991, 105, 1–10.
2. *Boeing H.*: Epidemiological researches in stomach cancer: progress over the last ten years. J. Cancer Res. Clin. Oncol. 1991, 117, 133–143.

3. *Cazottes C.H., Blanquat G., Fritsch P., Derache R.*: Effect of nitrites and nitrates on irrigation and secretion of gastric mucosa in rats. Naunyn-Schmiedeberg's Arch. Pharmacol. 1979, 309, 64–75.
4. *Furihata C., Oka M., Amin S., Krzemiński J., Weisburger J.H., Kobayashi K., Tatematsu M.*: Effect of 2-chloro-4-methylthiobutanoic acid in a rapid bioassay for gastric carcinogens. Cancer Lett. 1996, 108, 129–135.
5. *Gomori G.*: An improved histochemical technique for acid phosphatase. Stain Technology 1950, 25, 81–85.
6. *Green L.C., Ruiz L.K., Wagner D.A., Rand W., Istafan N., Young V.R., Tannenbaum S.R.*: Nitrite biosynthesis in man. Proc. Natl. Acad. Sci. USA 1990, 78, 7764–7768.
7. *Grudziński I.P., Frankiewicz-Jóźko A., Gajewska A., Szczypka M., Szymanski A.*: Effects of whole-body γ-irradiation on lipid peroxidation and anti-oxidant enzymes in the liver of N-nitrosodiethylamine-treated mice. Polish J. Environ. Studies 2000, 9, 385–390.
8. *Grudziński I.P., Frankiewicz-Jóźko A.*: Antioxidative effects of putrescine in poisoning with sodium nitrite. Polish J. Environ. Studies 1999, 8, 153–157.
9. *Grudziński I.P., Frankiewicz-Jóźko A.*: Effects of oral coenzyme Q10 supplementation on sodium nitrite-induced lipid peroxidation in rats. Roczn. PZH 2001, 52, 213–218.
10. *Grudziński I.P., Szymański A.*: Metabolizm tlenowy mitochondriów błony śluzowej jelita cienkiego szczurów zatruwanych azotynem sodowym. Proceedings of the Symposium „Jakość zdrowotna żywności i żywienia" Białystok, Poland, 1999, 16–17, Sep., p. 89 (abstract).
11. *Grudziński I.P.*: Studies on the toxic action of sodium nitrite on intestinal absorption in rats. Arch. Environ. Contam. Toxicol. 1991, 21, 475–479.
12. *Grudziński I.P., Szymański A.*: The effect of subchronic poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose. Arch. Environ. Contam. Toxicol. 1991, 21, 447–452.
13. *Grudziński I.P., Szymański A.*: The effect of acute poisoning with potassium nitrate and sodium nitrite on the processes of intestinal absorption of D-xylose. Arch. Environ. Contam. Toxicol. 1991, 21, 453–461.
14. *Kitano M., Takada N., Chen T., Ito H., Nomura T., Tsuda H., Wild C.P., Fukushima S.*: Carcinogenicity of methylurea or morpholine in combination with sodium nitrite in rat multi-organ carcinogenesis bioassay. Jpn. J. Cancer Res. 1997, 88, 797–806.
15. *Knekt P., Jarvinen R., Dich J., Hakulinen T.*: Risk of colorectal and other gastrointestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: a follow-up study. Int. J. Cancer. 1999, 80, 852–856.
16. *Korenekova B., Kottferova J., Korenek M.*: The fat of added nitrate used in the manufacture of Emmental cheese. Food Addit. Contam. 2000, 5, 373–737.
17. *Lijinsky W.*: N-nitroso compounds in the diet. Mut. Res. 1999, 443, 129–138.
18. *Lijinsky W.*: Lifespan and cancer: the induction time of tumours in diverse animal species treated with nitrosodiethylamine. Carcinogenesis 1993, 14, 2373–2375.
19. *Nachlas M.M., Tsou K.C., De Souza E., Cheng C.S., Sseligman A.M.*: Cytochemical demonstration of succinate dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. J. Histochem. Cytochem. 1975, 5, 420–436.
20. *Novikoff A.B.*: Enzyme localization with *Gomori* type lead phosphate procedures: real or artifact. J. Histochem. Cytochem. 1970, 18, 366–367.
21. *Omar M.S., Raoof A.M.*: *Onchocerca fasciata*: histochamical demonstration of succinate and NADH dehydrogenase. J. Helmintol. 1996, 70, 47–51.
22. *Peagler F.D., Redman R.S.*: Enzyme histochemical localization of Na/K-ATPase and NADH-DE in the developing rat parotid gland. Anat. Rec. 1999, 256, 72–77.
23. *Pears A.G.*: Histochemistry. Theoretical and applied. Vol. 2., Third edition, Edinburg and London, Churchill Livingstone, 1972.

24. *Pignatelli B., Malaveille C., Rogatko A., Hautefeuille A., Thuillier P., Munoz N., Moulinier B., Berger F., De Montclos H., Lambert R.*: Mutagens, N-nitroso compounds and their precursors in gastric juice from patients with and without pre-cancerous lesions of the stomach. Eur. J. Cancer 1993, 29, 2031–2039.
25. *Pobel D, Riboli E, Cornee J, Hemon B, Guyader M.*: Nitrosamine, nitrate and nitrite in relation to gastric cancer: a case-control study in Marseille, France. Eur. J. Epidemiol. 1995, 11, 67–73.
26. *Singer T.P.*: Flavoprotein dehydrogenases of the respiratory chain. In: Comprehensive biochemistry, eds. Florkin M., Stotz E.H., Vol. 14, Biological oxidation, Elsevier, Amsterdam, London, New York, 1966, pp. 127–198.
27. *Tepsuwan A., Furihata C., Rijanapo W., Matsushima T.*: Genotoxicity and cell proliferative activity of a nitrosated Oroxylum indicum Vent fraction in the pyloric mucosa of rat stomach. Mutat. Res. 1992, 281, 55–61.
28. *Therien A.G., Blostein R.*: Mechanisms of sodium pump regulation. Am. J. Physiol. Cell. Physiol. 2000, 279, C541-C566.
29. *Vladutin G.D., Hefner R.R.*: Succinate dehydrogenase deficiency. Arch. Pathol. Lab. Med. 2000, 124, 1755–1758.
30. *Wei M., Wanibuchi H., Yamamoto S., Iwai S., Morimura K., Nnomura T., Takayama R., Fukushima S.*: Chemopreventive effects of S-methylcysteine on rat hepatocarcinogenesis induced by concurrent administration of sodium nitrite and morpholine. Cancer Lett. 2000, 161, 97–103.
31. *Yoshida Y., Hirose M., Takaba K., Kimura J., Ito N.*: Induction and promotion of forestomach tumors by sodium nitrite in combination with ascorbic acid or sodium ascorbate in rats with or without N-methyl-N'-nitro-N-nitrosoguanidine pre-treatment. Int. J. Cancer 1994, 56, 124–128.

Otrzymano: 2001–03–29