

研究简报

氟中毒大鼠肝肾自由基代谢及硒对其影响

边建朝 赵力军 叶 平 相有章 王 林 杨晓霞 咸树梅 刘永平

(山东省地方病防治研究所, 济南 250014)

摘要 为探讨硒对氟中毒大鼠肝、肾自由基代谢的影响, 两组 Wistar 大鼠饮 1.58 mmol/L 和 2.63 mmol/L 高氟水; 饮高氟水的同时加饲 2.0 mg/kg 硒饲料; 饮氟水 7 个月后加饲硒饲料. 实验 14 个月时用低温电子自旋共振 (ESR) 技术测其肝、肾活性氧自由基 (FR) 含量; 同时测氟 (F)、硒 (Se) 含量; 谷胱甘肽过氧化物酶 (GSH-Px)、超氧化物歧化酶 (SOD) 活性和脂质过氧化物 (LPO) 含量. 结果: 氟中毒大鼠在肝、肾氟升高的同时, FR 和 LPO 上升, GSH-Px、SOD 下降. 在氟中毒不同时期投硒, 大鼠肝、肾氟降低, FR 和 LPO 减少, 抗氧化酶活性恢复. 表明硒不但可拮抗大鼠体内的高氟, 还可纠正高氟造成的自由基代谢紊乱.

关键词 氟中毒, 自由基代谢, 硒, 电子自旋共振

近年来有关氟中毒发病过程中的自由基代谢受到广泛关注. 有研究表明氟中毒人群和动物抗氧化酶类发生变化, 但其实验结果存在差异, 同时关于高氟状态下自由基产生与否及产生的途径意见尚不一致^[1-4]. 本文应用电子自旋共振技术直接测定水型氟中毒大鼠肝、肾中活性氧自由基, 同时测肝、肾抗氧化酶类和脂质过氧化产物. 又以硒作为干预因素, 观察氟中毒大鼠自由基代谢变化及硒对其影响.

1 材料与方法

1.1 实验动物分组与处理因素

Wistar 雄性大鼠 70 只, 体重 90~110 g (山东医科大学实验动物中心提供), 分为 7 组. 对照组 (I): 饮 0.44 mmol/L 氟正常自来水, 饲基础饲料; 低氟组 (II): 1.58 mmol/L 氟自来水, 基础饲料; 低氟加硒组 (III): 1.58 mmol/L 氟水, 基础饲料加 2.0 mg/kg 硒; 高氟组 (IV): 2.63 mmol/L 氟水, 基础饲料; 高氟加硒组 (V): 2.63 mmol/L 氟水, 饲料加 2.0 mg/kg 硒; 低氟 7 个月后加硒组 (VI): 1.58 mmol/L 氟水

7 个月后, 饲料中加 2.0 mg/kg 硒; 高氟 7 个月后加硒组 (VII): 2.63 mmol/L 氟 7 个月后, 饲料中加 2.0 mg/kg 硒. 实验期 14 个月. 饲料来源、饲料配方参考文献 [5].

1.2 自由基含量测定

大鼠用氨基甲酸乙酯 (NH₂COOH₅ A.R. 上海曹杨冲化工厂) 1.0 g/kg 腹腔注射麻醉, 剖腹取肝、肾脏, 2 min 内剪碎, 装直径为 4 mm 塑料管, 置液氮瓶内, 2 h 内用低温电子自旋共振技术测自由基 (FR) 含量. 仪器为德国 Bruker 公司 ESP-300 型电子自旋共振波谱仪 (北京医科大学天然和仿生药物国家重点实验室).

1.3 抗氧化酶和脂质过氧化物测定

肝、肾谷胱甘肽过氧化物酶 (GSH-Px) 活性用 DTNB 法; 超氧化物歧化酶 (SOD) 活性用黄嘌呤氧化酶法; 脂质过氧化物 (LPO) 含量用 TBA 荧光法测定.

1.4 氟和硒含量测定

饮水、饲料、肝和肾氟 (F) 含量用氟离

子选择电极法测定; 饲料、肝和肾硒 (Se) 含量用 2, 3-二氨基萘荧光法测定.

2 结 果

2.1 肝、肾氟和硒含量

投 Se 组 (III、V、VI、VII) 肝、肾 Se 含量较对照组 (I) 和未投 Se 组 (II、IV) 升高. 6 组实验组肝、肾 F 含量显著高于 I 组. 4 组投 Se 组肝、肾 F 含量较两组未投 Se 组降低 (表 1).

表 1 氟中毒大鼠肝、肾脏 F、Se 含量变化及 Se 的影响

分组	肝 脏		肾 脏	
	Se 含量/ $\text{mg}\cdot\text{kg}^{-1}$	F 含量/ $\text{mg}\cdot\text{kg}^{-1}$	Se 含量/ $\text{mg}\cdot\text{kg}^{-1}$	F 含量/ $\text{mg}\cdot\text{kg}^{-1}$
I	0.42 ± 0.03	0.24 ± 0.03	0.49 ± 0.04	0.41 ± 0.05
II	0.44 ± 0.04	$0.56\pm0.04^{1)}$	0.50 ± 0.03	$0.69\pm0.06^{1)}$
III	$0.87\pm0.17^{1)2)}$	$0.43\pm0.06^{1)2)}$	$0.90\pm0.20^{1)2)}$	$0.53\pm0.04^{1)2)}$
IV	0.45 ± 0.05	$0.68\pm0.06^{1)}$	0.48 ± 0.04	$0.77\pm0.05^{1)}$
V	$0.89\pm0.19^{1)3)}$	$0.53\pm0.04^{1)3)}$	$0.91\pm0.17^{1)3)}$	$0.63\pm0.04^{1)3)}$
VI	$0.81\pm0.15^{1)2)}$	$0.42\pm0.05^{1)2)}$	$0.87\pm0.16^{1)2)}$	$0.52\pm0.05^{1)2)}$
VII	$0.84\pm0.13^{1)3)}$	$0.52\pm0.05^{1)3)}$	$0.86\pm0.19^{1)3)}$	$0.64\pm0.04^{1)3)}$

注: ¹⁾ $P<0.001$ 与 I 组比较; ²⁾ $P<0.001$ 与 II 组比较; ³⁾ $P<0.01$ 与 IV 组比较. $\bar{x}\pm s$, $n=10$.

2.2 肝、肾活性氧 FR 含量

ESR 波谱显示 3 个信号峰 g 值为 2.040、2.004 和 1.950, 分别与代表烷氧和超氧 FR O 峰的 $g_{\parallel}$ 、 $g_{\perp}$ 值和代表过渡金属 T 峰的 g 值相符. 以各峰高度代表 FR 相对浓度. II、IV 组肝 ESR 波谱 $g_{\parallel}$ 高于 I 组; 投 Se 组则降低,

III、VI 组与 II 组比较有差异; V、VII 组显著低于 IV 组. 肾 ESR 波谱变化基本同肝, II、IV、V 组 $g_{\parallel}$ 较 I 组升高, 4 组投 Se 组 $g_{\parallel}$ 较未投 Se 组降低, 均有显著性差异. 肝、肾 $g_{\perp}$ 各组间无差异 (表 2).

表 2 氟中毒大鼠肝、肾脏 FR 含量变化及 Se 的影响

分组	肝 脏		肾 脏	
	$g_{\parallel}$	$g_{\perp}$	$g_{\parallel}$	$g_{\perp}$
I	11.67 ± 2.83	114.23 ± 20.32	13.0 ± 2.29	115.49 ± 15.47
II	$18.11\pm4.31^{1)}$	110.32 ± 18.02	$18.0\pm3.61^{4)}$	117.39 ± 19.24
III	$11.33\pm2.45^{2)}$	112.24 ± 17.14	$14.56\pm2.96^{8)}$	113.12 ± 18.14
IV	$18.0\pm3.67^{1)}$	115.0 ± 21.32	$19.78\pm2.81^{1)}$	116.44 ± 15.27
V	$13.78\pm2.22^{3)}$	111.58 ± 14.78	$15.22\pm2.11^{7)6)}$	116.42 ± 18.17
VI	$14.0\pm2.34^{5)}$	110.85 ± 19.12	$13.11\pm2.09^{5)}$	117.14 ± 20.18
VII	$14.22\pm2.81^{9)}$	113.69 ± 20.43	$13.88\pm1.83^{3)}$	114.12 ± 20.30

注: 自由基含量以相对信号强度 (mm) 表示. ¹⁾ $P<0.001$, ⁴⁾ $P<0.01$, ⁷⁾ $P<0.05$, 与 I 组比较; ²⁾ $P<0.001$, ⁵⁾ $P<0.01$, ⁸⁾ $P<0.05$, 与 II 组比较; ³⁾ $P<0.001$, ⁶⁾ $P<0.01$, ⁹⁾ $P<0.05$, 与 IV 组比较. $\bar{x}\pm s$, $n=10$.

2.3 肝、肾 GSH-Px、SOD 活性和 LPO 含量

II、IV 组肝、肾 GSH-Px 活性低于 I 组, 4 组加 Se 组则高于 I、II、IV 组. II、IV 组肝 SOD 活性、II~VII 组肾 SOD 活性低于 I

组, 4 组加 Se 组高于未加 Se 组. II~VII 组肝 LPO 含量和 II、IV、V、VII 组肾 LPO 含量高于 I 组. 投 Se 组肝、肾 LPO 含量则较未投 Se 组降低, 均有显著性差异 (表 3).

表 3 氟中毒大鼠肝、肾脏 GSH Px、SOD 活性, LPO 含量变化及 Se 的影响

分 组	肝 脏			肾 脏		
	GSH-Px / U · g ⁻¹	SOD / NU · g ⁻¹	LPO / nmol · g ⁻¹	GSH-Px / U · g ⁻¹	SOD / NU · g ⁻¹	LPO / nmol · g ⁻¹
I	5. 10 ± 0. 60	110. 50 ± 18. 40	37. 40 ± 5. 64	3. 62 ± 0. 35	75. 20 ± 8. 19	22. 67 ± 4. 13
II	4. 40 ± 0. 73 ⁷⁾	93. 80 ± 10. 60 ⁷⁾	61. 30 ± 7. 34 ¹⁾	2. 93 ± 0. 59 ¹⁾	52. 93 ± 8. 48 ¹⁾	30. 41 ± 5. 48 ¹⁾
III	6. 45 ± 0. 87 ¹⁾²⁾	109. 70 ± 19. 13	53. 40 ± 5. 37 ¹⁾⁵⁾	4. 48 ± 0. 24 ¹⁾²⁾	63. 9 ± 6. 13 ⁴⁾⁵⁾	25. 31 ± 4. 24 ⁸⁾
IV	4. 39 ± 0. 70 ⁷⁾	94. 30 ± 12. 34 ⁷⁾	60. 63 ± 6. 73 ¹⁾	2. 96 ± 0. 29 ¹⁾	40. 89 ± 4. 44 ¹⁾	35. 1 ± 3. 43 ¹⁾
V	6. 32 ± 0. 69 ¹⁾³⁾	105. 30 ± 13. 04	51. 20 ± 6. 31 ¹⁾⁶⁾	4. 38 ± 0. 58 ¹⁾³⁾	50. 30 ± 4. 97 ¹⁾³⁾	29. 40 ± 5. 35 ¹⁾⁹⁾
VI	6. 34 ± 0. 73 ¹⁾²⁾	106. 40 ± 13. 08	52. 48 ± 6. 43 ¹⁾⁵⁾	4. 53 ± 0. 30 ¹⁾²⁾	64. 80 ± 7. 36 ⁴⁾⁵⁾	25. 80 ± 4. 52 ⁸⁾
VII	6. 41 ± 0. 69 ¹⁾³⁾	105. 60 ± 10. 08	51. 45 ± 5. 31 ¹⁾⁶⁾	4. 48 ± 0. 31 ¹⁾³⁾	51. 10 ± 3. 78 ¹⁾³⁾	29. 90 ± 5. 15 ¹⁾⁹⁾

注: ¹⁾ $P < 0. 001$, ⁴⁾ $P < 0. 01$, ⁷⁾ $P < 0. 05$, 与 I 组比较; ²⁾ $P < 0. 001$, ⁵⁾ $P < 0. 01$, ⁸⁾ $P < 0. 05$, 与 II 组比较; ³⁾ $P < 0. 001$, ⁶⁾ $P < 0. 01$, ⁹⁾ $P < 0. 05$, 与 IV 组比较. $\bar{x} \pm s$, $n = 10$.

3 讨 论

实验大鼠饮高氟水 14 个月, 其肝、肾氟含量显著升高, 表明实验动物已处于氟中毒状态. 此时应用低温 ESR 技术测肝、肾自由基含量, 代表烷氧自由基和/或超氧自由基 O 峰的 $g_{\parallel}$ 显著升高, 提示大鼠在高氟状态下肝、肾活性氧自由基升高, 与此同时肝、肾 GSH-Px、SOD 等抗氧化酶活性降低, 此导致进一步继发性脂质过氧化反应, 使 LPO 大量堆积. 抗氧化酶系失衡和自由基代谢紊乱是造成氟中毒时肝、肾损伤的重要因素之一.

在一定浓度范围内的硒具有拮抗生物体内高氟的作用. 氟中毒人群应用亚硒酸钠, 可使尿氟排泄增加, 血氟下降^[6]. 实验大鼠饲用氟、硒或投氟 7 个月后再饲用硒, 其肝、肾中氟含量均显著降低, 表明在氟中毒不同时期投硒均可降低动物体内氟的含量. 体内氟含量减少, 可减轻对组织的原发性脂质过氧化损伤. 亚硒酸钠抑制多形核白细胞 (PMN) 呼吸爆发, 清除其产生的超氧阴离子 ($O_2^{\cdot -}$)^[7], 又可直接清除自由基^[8]. 同时亚硒酸盐掺入使 GSH-Px 活性升高. 上述诸因素均增强肝、肾抗氧化能力, 使活性氧自由基 $g_{\parallel}$ 信号减弱及脂质过氧化产物减少.

参 考 文 献

1 边建朝, 咸树梅, 叶 平等. 氟病区人群微量元素水平

对血液抗氧化酶类的影响. 中国地方病学杂志, 1992, **11** (6): 332~ 338

2 Li J X, Cao S R. Recent studies on endemic fluorosis in China. Fluoride, 1994, **27** (3): 125~ 128

3 Shayiq R M, Raza H, Kidwai A M. Fluoride and lipid peroxidation: A comparative study in different rat tissues. Bull Environ Contam Toxicol, 1986, **37** (1): 70~ 76

4 李健学, 全笑江, 吴云鹏. 氟对大鼠肝、肾、脑细胞活性氧代谢的影响. 地方病通报, 1995, **10** (2): 10~ 13

5 边建朝, 宋术亮, 杨晓霞等. 氟中毒大鼠心肌细胞电生理变化及硒的影响. 中国地方病学杂志, 1996, **15** (6): 346~ 349

6 边建朝, 咸树梅, 叶 平等. 氟中毒患者口服亚硒酸钠对体内氟及抗氧化酶类的影响. 营养学报, 1994, **16** (1): 56~ 60

7 王春霞, 王子健, 彭 安等. 用自旋捕集技术研究硒化合物对活性氧自由基的清除作用. 生物化学与生物物理学报, 1994, **26** (6): 585~ 589

8 徐辉碧. 硒化合物与脂质过氧化自由基作用的基础研究. 华中理工大学学报, 1993, **17** (4): 193~ 197

Metabolism of Free Radicals and Effects of Selenium in Liver and Kidney of Rats with Chronic Fluorosis. BIAN Jianchao, ZHAO Lijun, YE Ping, XIANG Youzhang, WANG Lin, YANG Xiaoxia, XIAN Shumei, LIU Yongping (Shandong Institute for Prevention and Treatment of Endemic Disease, Jinan 250014, China).

Abstract In order to explore effects of senium on metabolism of active oxygen radicals in liver and kidney of rats with fluorosis, 2 groups of Wistar rats were fed with normal fodder and high concentration of NaF water (1.58, 2.63 mmol/L fluorine, F). 2 groups were fed

with selenite fodder (2.0 mg/kg selenium, Se) and high F. At the same time, 2 groups were fed with normal fodder and high F for 7 months, then 2.0 mg/kg Se were supplemented in fodder. The contents of free radicals (FR) in liver and kidney were demonstrated by means of the technique of electronic spin resonance (ESR), the contents of Se and F, the activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and the contents of lipid peroxidase (LPO) in liver and kidney were tested in

14 months. The results showed that there was an increase of F, FR and LPO and a decrease of GSH-Px and SOD in rats with fluorosis. When Se was given in the different time of fluorosis, F lowered, the signal of FR weakened, LPO lessened, GSH-Px and SOD recovered. It indicated that Se not only could antagonize high F but could rectify metabolic disorders of FR in rats with fluorosis.

Key words fluorosis, free radical metabolism, selenium, electronic spin resonance

(上接第 520 页, Continued from page 520)

- 7 Pratt S A, Scully N F, Shur B D. Cell surface $\beta 1$, 4-galactosyltransferase on primary spermatocytes facilitates their initial adhesion to Sertoli cells *in vitro*. Dev Biol, 1993, **156**: 470 ~ 482
- 8 Miller D J, Macek M B, Shur B D *et al.* Complementarity between sperm surface $\beta 1$, 4-galactosyltransferase and egg-coat ZP3 mediates sperm-egg binding. Nature, 1992, **357** (18): 589~ 593
- 9 Bayna E M, Runyan R B, Scully N F *et al.* Cell surface galactosyltransferase as a recognition molecule during development. Mol Cell Biochem, 1985, **72**: 141~ 151
- 10 Romagnano L, Babiarz B. The role of murine cell surface galactosyltransferase in trophoblast: laminin interactions *in vitro*. Dev Biol, 1990, **141**: 254~ 261
- 11 Armant D R. Cell interactions with laminin and its proteolytic fragments during outgrowth of mouse primary trophoblast cells. Biol Reprod, 1991, **45**: 664~ 672
- 12 Begovac P C, Hall D E, Shur B D. Laminin fragments E8 mediates PC12 cell neurite outgrowth by binding to cell surface $\beta 1$, 4-galactosyltransferase. J Cell Biol, 1991, **113**: 637~ 644
- 13 Dean J W, Chandrasekaran III S, Tanzer M L. A biological role of the carbohydrate moieties of laminin. J Biol Chem, 1990, **265**: 12553~ 12562
- 14 Huang Q L, Shur B D, Begovac P C. Overexpressing cell surface $\beta 1$, 4-galactosyltransferase in PC12 cells increases neurite outgrowth on laminin. J Cell Sci, 1995, **108**: 839~ 847
- 15 Gong X H, Doboies D H, Miller D J *et al.* Activation of a G protein complex by aggregation of $\beta 1$, 4-galactosyltransferase on the surface of sperm. Science, 1995, **269**: 1718~ 1721
- 16 Hinton D A, Evans S C, Shur B D. Altering the expression of cell surface $\beta 1$, 4-galactosyltransferase modulates cell growth. Exp Cell Res, 1995, **219**: 640~ 649

Cell Surface β -1, 4-Galactosyltransferase and Its Biological Functions. ZHANG Chunyu, DUAN Enkui, ZENG Guoqing, LIU Yixun (State Key Laboratory of Reproductive Biology, Institute of Zoology, The Chinese Academy of Sciences, Beijing 100080, China).

Abstract β -1, 4-galactosyltransferase (GalTase) can be divided into short and long forms by its mRNA. The short form GalTase within the *trans*-Golgi compartment participates in the biosynthesis of glycoconjugates. The long form on cell surface mediates cell-cell and cell-matrix interactions by binding to appropriate glycoside substrates on adjacent cell surfaces or in the extracellular matrix, including spermatogenesis, sperm-egg binding, early embryo cell adhesion, secondary trophoblast giant cell migration and neurite outgrowth, and functions as a signal-transducing receptor for extracellular oligosaccharide ligands to affect G protein signal cascades. Surface GalTase also delivers a growth inhibitory signal by modulating the ability of the EGF receptor to transduce EGF-dependent signals, and plays an important role during cell growth.

Key words β -1, 4-galactosyltransferase, extracellular matrix, attachment, migration, signal