RESVERATROL ATTENUATED THE INCREASED LEVEL OF OXIDATIVE STRESS IN THE BRAINS AND THE DEFICIT OF LEARNING AND MEMORY OF RATS WITH CHRONIC FLUOROSIS

Xiao-Xiao Zeng,[a,b,c] Jie Deng,[b,c] Jie Xiang,[a,b] Yang-Ting Dong,[b,c] Kun Cao,[a,b] Xian-Hong Liu,[b,c] Dan Chen,[a,b] Long-Yan Ran,[a,b] Ye Yang, [b,c] Zhi-Zhong Guan[a,b,c,*]

Guiyang, Guizhou Province, People's Republic of China

ABSTRACT: The protective effect of resveratrol (RSV), a stimulator of the silent mating type information regulation 2 homolog (SIRT1), on the neurotoxicity of rat brain induced by chronic fluorosis was investigated. Thirty-two Sprague-Dawley (SD) rats were divided randomly into four groups: (i) a control group; (ii) a RSV treatment group; (iii) a fluoride-exposed group (50 ppm F^- in drinking water); and (iv) a fluoride plus RSV group. The experimental period was 7 months. The protein levels of SIRT1 in the cortex and the hippocampus of the rat brains were determined by Western blotting, the SOD activity and the MDA content in the brains by biochemical methods, and the 8-OHdG content by ELISA. The spatial learning ability and the memory of the rats was examined by the Morris Water Maze test. The results showed chronic fluorosis caused changes to the brains of the rats with a reduced SIRT1 protein, elevated MDA and 8-OHdG contents, inhibited SOD activity, and, in addition, decreased learning and memory. Interestingly, RSV pretreatment attenuated the reduced SIRT1, the raised level of oxidative stress, and the lowered ability for learning and memory resulting from the chronic fluorosis. The results indicate that RSV may have a neuroprotective effect on fluoride toxicity.

Key words: Fluorosis; Oxidative stress; Rat brain; Resveratrol; Silent mating type information regulation 2 homolog.

INTRODUCTION

A great deal of evidence suggests that the elevation of free radicals and the inhibition of antioxidant defenses may be involved in the pathogenesis of chronic fluorosis, which induces a vast array of symptoms and pathological changes in many tissues and organs.[1,2] Fluoride can cross cell membranes and affect various soft tissues leading to impairment of organ functions.[3] In addition, chronic fluorosis leads to dysfunction of the central nervous system (CNS) with a high level of oxidative stress, inhibited neuronal receptor expression, and a lowered ability of learning and memory.[3-5] Many studies have reported that antioxidants may play an important role in protecting neurons from the injury of chronic fluorosis.[6-10] Resveratrol (3,5,4'-trihydroxystilbene, RSV), a polyphenol found in a wide variety of plants, has been demonstrated to regulate cellular protection against oxidative stress in many disease states, including neurodegeneration, aging, cardiovascular disorders, diabetes, and cancer.[11] It has been found that RSV treatment can down-regulate the damage in rat brains induced by fluorosis to protein and DNA.[10] Furthermore, a wide range of pharmacological activities could be achieved by treatment with RSV, which might be obtained, at least in part, by its direct or indirect antioxidant defense mechanism.[12]

Silent mating type information regulation 2 homolog (SIRT1) has been attracting a great attention from scientists because it is involved in the physiological processes of

[a]Department of Pathology at the Affiliated Hospital of Guizhou Medical University; [b]Key Laboratory of Endemic and Ethnic Diseases of the Ministry of Education of People's Republic China (Guizhou Medical University); [c]Key Laboratory of Medical Molecular Biology, Guiyang 550004, Guizhou, People's Republic of China; *For correspondence: Zhi-Zhong Guan, Department of Pathology at the Affiliated Hospital of Guizhou Medical University, Guiyang 550004, Guizhou, People's Republic of China; E-mail: 1457658298@qq.com or zhizhongguan@yahoo.com

many neurons and it has an important role in the antioxidant effects in neurological diseases.[13-15] RVS may regulate SIRT1 by a mechanism concerning an indirect antioxidant defense. Overexpression of SIRT1 deacetylates Ac-p53 in LS8 cells to attenuate fluoride-induced cell growth inhibition, mitochondrial damage, DNA damage, and apoptosis.[16]

Based on previous research results, in the present study we employed rats with chronic fluorosis to investigate whether prolonged exposure to fluoride can alter the expression of SIRT1 in rat brain and whether RSV, via activating SIRT1, may attenuate the neurotoxicity resulting from chronic fluorosis.

MATERIALS AND METHODS

Materials: RSV (Sigma-Aldrich, USA); mouse monoclonal anti-SIRT1 and anti-β-actin antibodies, and anti-mouse IgG conjugated with horseradish peroxidase (Abcam, United Kingdom); Hyper Performance Chemiluminescence (HPC) film and Electrochemiluminescence (ECL) Plus reagent (Amersham, Sweden); kits for the activity of total-superoxide dismutase (T-SOD) and the content of malondialdehyde (MDA) (Nanjing Jiancheng Bioengineering Institute, China); sodium fluoride (NaF, analytical grade reagent), DNA/RNA Oxidative Damage ELISA Kit (Cayman, USA); and all other general chemicals (Sigma Aldrich, USA) were purchased from the sources indicated.

Animal model with chronic fluorosis: Thirty-two SD (Sprague Dawley) rats (clean grade) weighing 120–150 g were provided by the Animal Experimental Center of Guizhou Medical University of China. These animals were divided randomly into four groups (8 for each group with half male and half female): (i) a control group with normal tap-water (containing <0.5 ppm F^-); (ii) a RSV treatment group with 50 mg RSV/kg/ once daily by gavage; (iii) a fluoride-exposed group with 50 ppm F^- (prepared with NaF) in drinking water; and (iv) a fluoride exposure plus RSV group with 50 ppm F^- and 50 mg/kg RVS (gavage). The experimental period for the rats in the control and the fluoride groups was 7 months; while, for the RVS alone or the fluoride plus RVS groups, after 4 months of the experiment, the rats in the RVS group were treated for three months with either (i) no exposure of fluoride, or (ii) exposure to fluoride (50 ppm F^- in drinking water). Each group was fed standard nutrient food containing <6 ppm of fluoride. The experiments were pre-approved by the Ethical Committee of Guizhou Medical University, China.

Fluoride contents in brain and bone: After the rats were sacrificed, the cerebral cortex and hind limb femur were taken, ashed and ground into powder, and dissolved in 0.25 mol/L of hydrochloric acid. The fluoride content in the brain and bone were then determined by using a CSB-FI fluoride ion electrode (Changsa Analysis Instrumentation, China).[17]

Protein level of SIRT1 in brains determined by Western blotting: The total protein was extracted from the ipsilateral side of the cerebral cortex using RIPA lysis buffer (Thermo Scientific, USA) and the protein concentrations of the supernatants were determined by using the BCA protein assay kit (Thermo Scientific, USA). Protein extracts were separated on a 10% SDS-PAGE gel and transferred electrophoretically to polyvinylidene difluoride membranes. After blocking for 1 hr, the membranes

were incubated with rabbit polyclonal anti-SIRT1 (1:500 or 1:1,000 dilution), or anti-β-actin antibodies (1:5000 or 1:10,000 dilution) overnight at 4ºC in Tris-buffered saline with Tween 20. After incubation with a goat anti-mouse secondary antibody conjugated to horseradish peroxidase for 1 hr, the membranes were visualized with ECL and the signals thus obtained visualized by exposure to HPC film.[14]

The activity of T-SOD and the contents of MDA and 8-OHDG in brain tissues: The brain tissues of the rats were homogenized in normal saline (1:100 dilution). Homogenates were centrifuged to get supernatant. The protein concentrations of supernatants were determined by using the BCA protein assay kit (Thermo Scientific, USA). The activity of T-SOD and the content of MDA were measured by using kits for these parameters. The contents of 8-OHdG were measured by a DNA/RNA Oxidative Damage ELISA Kit.

The ability of spatial learning and memory of rats: The spatial learning and memory ability of the rats was evaluated by using the Morris Water Maze test.[18] The maze consists of a circular pool (180 cm in diameter) with dark walls and is filled with tap water colored by dark ink. An escape platform (9 cm in diameter) made of stainless steel with dark walls is submerged 0.5 cm below the surface of the water. Each rat was subjected to four trials each day, with a 5 to 7 min interval of rest between trials, for a training period of 4 days. The movement of the rats was monitored with a Videotrack software (Viewpoint). During the navigation test, the time required to locate the escape platform (escape latency) was determined, and after locating this platform, the animal was allowed to sit on it for 2 sec. Rats who failed to find the platform within 60 sec were guided to the platform and then allowed to remain on it for 2 sec as well, in which case the escape latency was recorded as 60 sec. The four trials on each individual day were averaged for statistical analysis. Furthermore, on day 5 when the platform was removed, the time of first crossing the site where the original platform located was recorded. All of the behavioral tests were conducted in a quiet environment with subdued lighting.

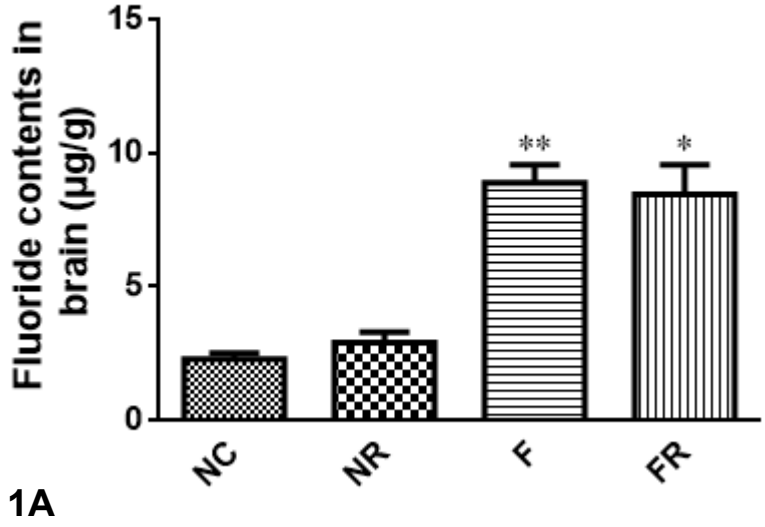
Data analysis and statistics: All data are presented as means±SD, and analysis was conducted by using GraphPad Prism 5.0 and SPSS 22.0 (SPSS Inc., USA). Multiple comparisons between the vehicle and the treatment groups were performed by one-way analysis of variance (ANOVA) followed by the Bonnferoni test or the two-paired Student's *t* test. Differences were considered statistically significant when *P* values were less than 0.05.

RESULTS

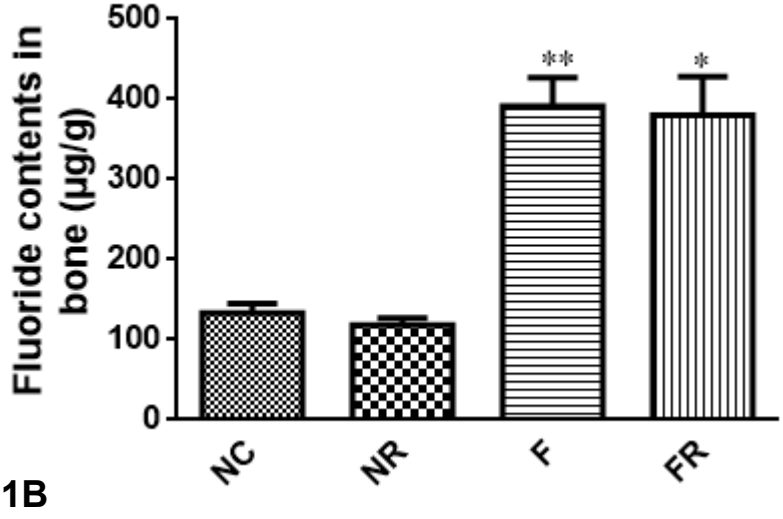
Fluoride contents in brains and bones: At the end of the 7 month experimental period, the contents of fluoride in the brain (Figure 1A) and the bone tissue (Figure 1B) of the rats in the fluoride-exposed and the fluoride plus RSV groups were significantly increased as compared to the control group. No significant differences were present in the fluoride content of the fluoride-exposed and the fluoride plus RSV groups.

SIRT1 expressions in the brains: As shown in Figures 2A and 2B, the protein levels of SIRT1 were significantly decreased in the cortex (Figure 2A) and the hippocampus (Figure 2B) of the rats with chronic fluorosis as compared with the controls. Interestingly, the expressions of SIRT1 at the protein level were significantly

enhanced in both the cortex and hippocampus of the rats treated with RSV alone. In addition, the decreased level of SIRT1 in the brains of the rats by the treatment of fluoride was significantly attenuated by the treatment with fluoride plus RSV as compared with the rats treated with fluoride exposure alone.

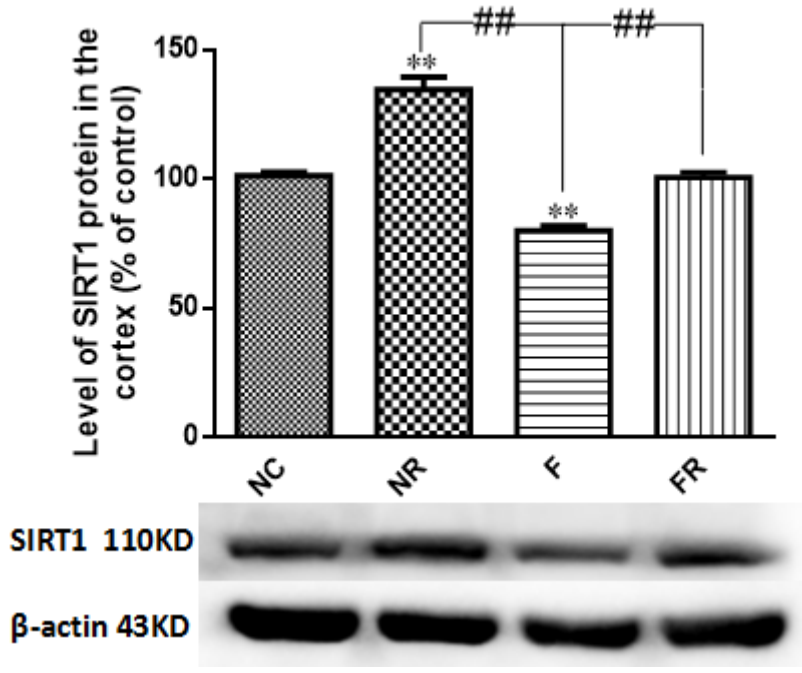


1A

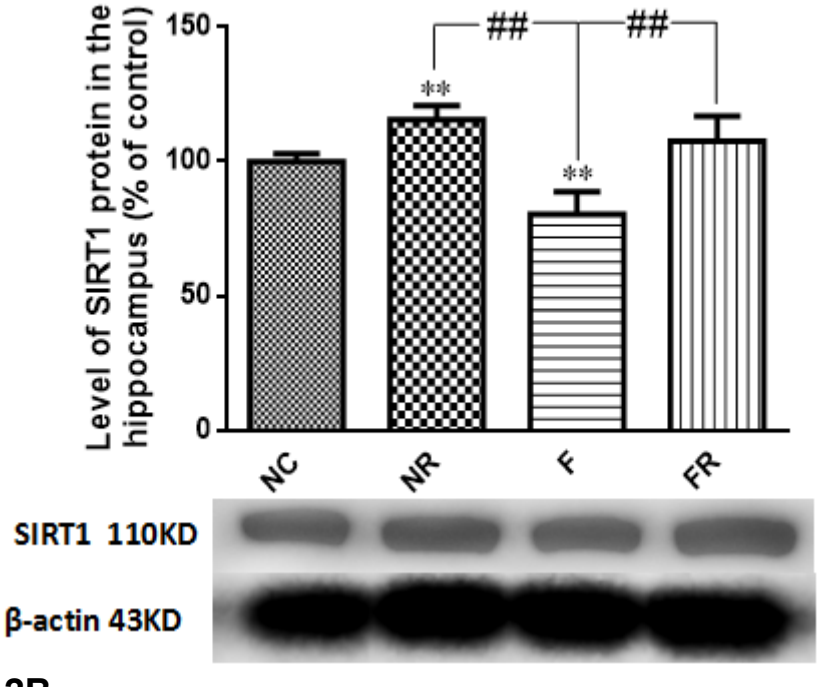


1B

Figures 1A and 1B. The contents of fluoride in the brain (1A) and bone (1B) of rats in the different groups. NC=Normal control; NR=Normal plus RSV; F=fluoride; and FR= fluoride plus RSV. Results are expressed as the means±SD. *p<0.05 and **p<0.01 in comparison to the normal control group as determined by the analysis of variance (ANOVA), followed by the Student-Newman-Keul's test.



2A

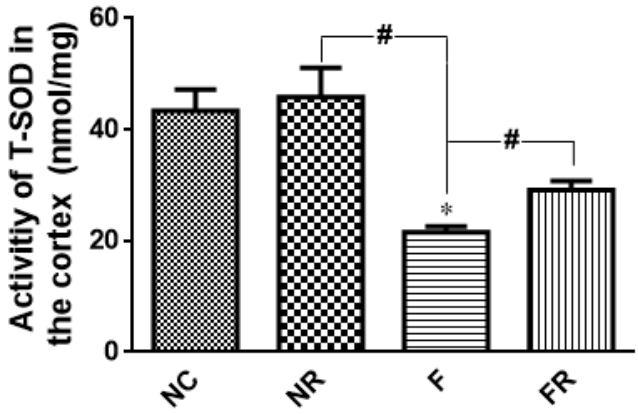


2B

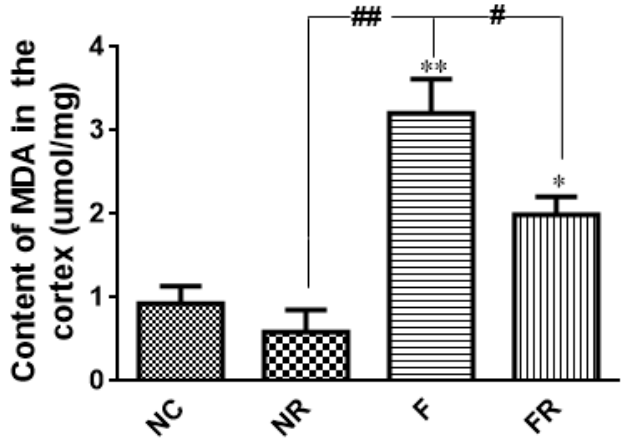
Figures 2A and 2B. Expression of Sirt1 at the protein level in the frontal cortex (2A) and the hippocampus (2B) of rat brains in different groups. 2A, the frontal cortex and 2B, the hippocampus; NC=Normal control; NR=Normal plus RSV; F=fluoride; and FR= fluoride plus RSV. The values are shown as the means±SD. **p<0.01 in comparison to the control group, #p<0.05 and ##p<0.01 in comparison to the fluorosis group as determined by the analysis of variance (ANOVA), followed by the Student-Newman-Keul's test.

The activity of T-SOD, and the content of MDA and 8-OHdG in the brains: Compared to the control group, the fluoride-exposed rats had a significantly decreased activity of T-SOD, and an increased content of MDA, and 8-OHdG. RVS attenuated the high level of oxidative stress induced by chronic fluorosis by significantly ameliorating these changes in T-SOD, MDA, and 8-OHdG (Figures 3A-3C).

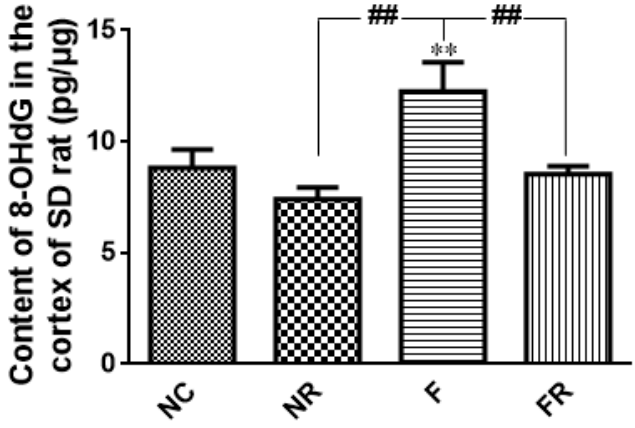
3A



3B

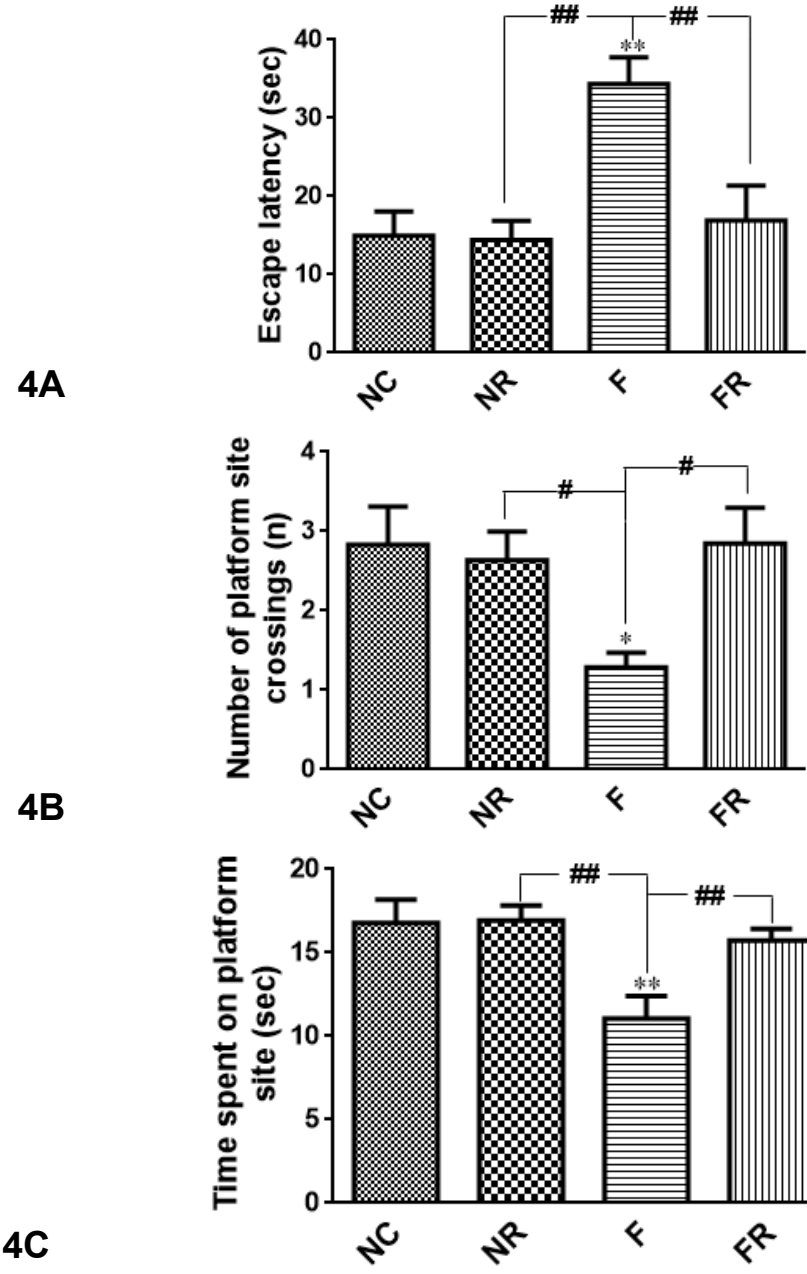


3C



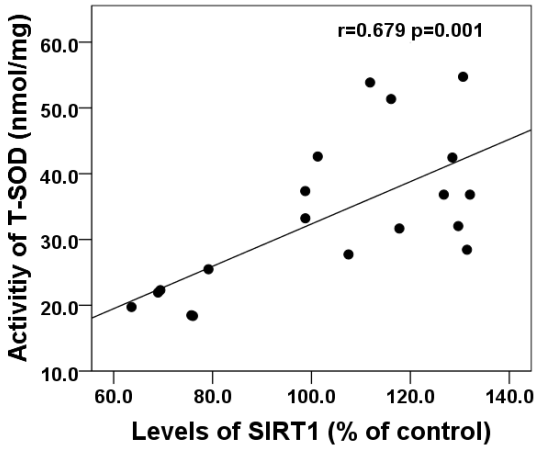
Figures 3A-3C. The activity of T-SOD (3A), and the contents of MDA (3B), and 8-OHdG (3C) in the brains of rats from the different groups. NC=Normal control; NR=Normal plus RSV; F=fluoride; and FR= fluoride plus RSV. The activity of T-SOD and the content of MDA were measured by biochemical method; and the content of 8-OHdG was measured by ELISA. The results are expressed as the means±SD. *p<0.05 and ** p<0.01 in comparison to the control group, #p<0.05 and ##p<0.01 in comparison to the fluorosis group as determined by the analysis of variance (ANOVA), followed by the Student-Newman-Keul's test.

The ability of spatial learning and memory of rats: Compared to the control group, the fluoride-exposed rats with chronic fluorosis had an increased escape latency time, a decreased number of crossings of the platform site, and a decreased time of staying on the site of the platform. The treatment by RVS alone did not induce an increase of learning and memory of the rats, but RVS attenuated the decreased ability for learning and memory in the rats with chronic fluorosis (Figures 4A-4C).

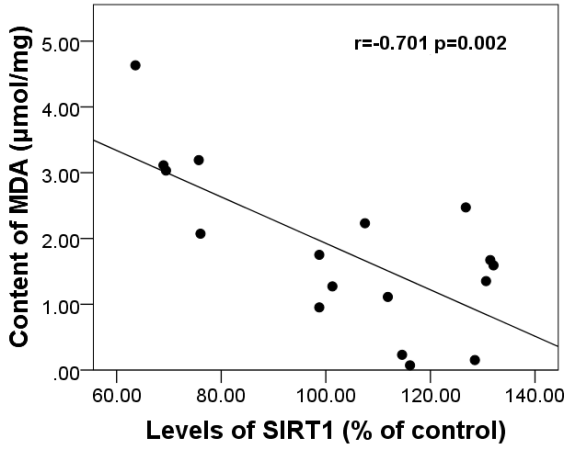


Figures 4a-4C. Escape latency (4A), the number of crossing the original site of the platform (4B) and the time spent staying on the original site of the platform (4C) of the rats in the different groups. NC=Normal control; NR=Normal plus RSV; F=fluoride; and FR= fluoride plus RSV. Values in the bar graphs are expressed as means±SD. *p<0.05 and ** p<0.01 in comparison to the control group, #p<0.05 and ##p<0.01 in comparison to the fluorosis group as determined by analysis of variance (ANOVA), followed by Student-Newman-Keul's test.

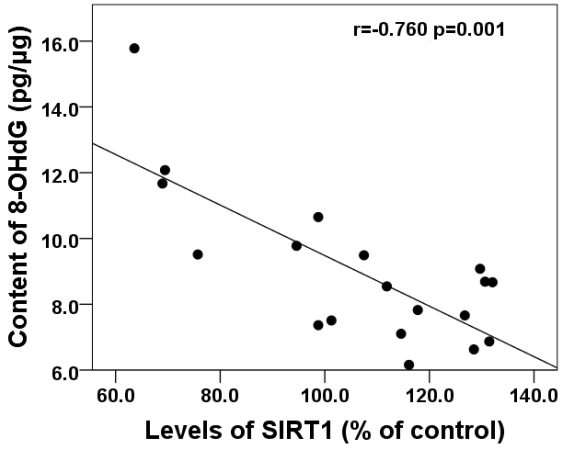
The correlation between oxidative stress and the level of SIRT 1 in the rat brains with the treatment of high fluoride plus RVS: There was a significant correlation between the level of oxidative stress and the expression of SIRT1 in the brains of the rats with fluoride exposure plus RSV treatment (Figures 5A-5C).



5A



5B



5C

Figures 5A-5C. The correlations between SIRT1 and oxidative stress in the rat brains. There were significantly positive-linear correlations between the activities of T-SOD (5A), the contents of MDA (5B), and 8-OHdG (5C) in the brains of rats treated by fluoride plus RVS as revealed by the Pearson correlation test.

DISCUSSION

Oxidative stress, an unbalance between the production of free radicals and the level of antioxidants, has been detected in many tissues and organs in chronic fluorosis, especially in the CNS. Chronic fluorosis leads dysfunction of the CNS, which results in lethargy, insomnia, behavioural change, depression, and deterioration of learning and memory, and aggravation of neural damage in rodents as well as in humans.[19,20] Numerous investigations carried out *in vivo* and *in vitro* have confirmed that prolonged exposure to excessive fluoride can promote oxidative stress in the brains of rats. As a free radical inducer, high F^- results in increased ROS and lipid peroxidation and a decreased level of chemical elimination of them in neurons and glial cells, which may therefore have an important role in the pathogenesis of fluorosis.[5,8,9,21] Moreover, long-lasting oxidative stress leads to an increasing concentration of lipid peroxidation, protein oxidation, and DNA damage.[10,22,23] In the present study, the higher levels of MDA and 8-OHdG, and the decreased activities of T-SOD in the brain tissues of the rats with chronic fluorosis is similar to previous findings,[16,24] suggesting that the high level of oxidative stress in the rat brains was induced by fluoride. At the same time, the deteriorated learning and memory in rats with chronic fluorosis is associated with alterations in the oxidative stress and the cholinergic system.[5,8,9]

A short-term increase in ROS can induce SIRT1 activation, which in turn causes a decrease in ROS, whereas long-term high levels of ROS cause a decrease in SIRT1 activity.[25] SIRT1 may interfere with the reduction of ROS through modulation of several cellular antioxidant pathways, including FOXO and the proliferator-activated receptor gamma coactivator 1 to directly up-regulate the expression of antioxidant enzymes (such as catalase and Mn-SOD) to promote cellular resistance to oxidative stress.[26,27]

The results of several investigations concerning interventions by using compounds or drugs with an antioxidant effect to fight against chronic fluorosis indicate that using different types of antioxidants can inhibit the toxicity induced by a high level of fluoride through attenuating the level of oxidative stress.[7,28,29]

RSV is a natural polyphenolic compound that can reduce oxidative stress. Several lines of evidence indicate that treatment with RSV is beneficial in ameliorating the toxicity induced by fluoride exposure in both *in vivo* and *in vitro* conditions.[10,30,31] The antioxidant defense by RSV could be achieved, at least in part, by its ability as a direct antioxidant, which is able to scavenge both free radicals and non-free radicals.[12] RSV confers an ability to down-regulate the lipid peroxidation represented with MDA, the protein oxidation shown with the carbonyl group, and the DNA oxidation indicated by 8-OHdG.[32,33]

Our results here support the previous findings, in which the levels of MDA and 8-OHdG in the brains of rats exposed to high fluoride were clearly higher than those of controls, while the activity of T-SOD was lower. Interestingly, RSV decreased the production of MDA and 8-OHdG and raised the activity of T-SOD in the brains of rats with the treatment of fluoride plus RSV as compared to the rats exposed to fluoride alone. The mechanism by which RSV protects against the neurotoxicity of

fluoride may involve stimulating SIRT1 since it can ameliorate the symptoms of neurodegeneration and regulate numerous forms of neuroprotection.[34,35]

CONCLUSION

In conclusion, exposure to a high level of fluoride reduced the protein level of SIRT1 and the activity of T-SOD, and elevated the levels of MDA and 8-OHdG in rat brains, and inhibited the abilities of learning and memory of the rats. Interestingly, pretreatment with RSV attenuated the reduced SIRT1 and the raised level of oxidative stress in the rat brains with chronic fluorosis and the lower ability of learning and memory of the animals, suggesting that the stimulatory effect of RSV may play a neuroprotective effect against the toxicity of fluoride.

ACKNOWLEDGMENTS

This work was financed by grants from the Natural Science Foundation of China (81760571) and the Foundation of Guizhou Province of China (2014-06, 2014-6008, 2016-4001).

REFERENCES

1 Guan ZZ, Yang PS, Yu ND, Zhuang ZJ. An experimental study of blood biochemical diagnostic indices for chronic fluorosis. Fluoride 1989;22:112-8.

2 Guan ZZ, Xiao KQ, Zeng XY, Long YG, Cheng YH, Jiang SF, et al. Changed cellular membrane lipid composition and lipid peroxidation of kidney in rats with chronic fluorosis. Arch Toxicol 2000;74:602-8.

3 Gao Q, Liu YJ, Guan ZZ. Decreased learning and memory ability in rats with fluorosis: increased oxidative stress and reduced cholinesterase activity in the brain. Fluoride 2009;42:277-85.

4 Kant V, Srivastava AK, Raina R, Verma PK, Singh P. Negligible amelioration by aluminium sulphate on subacute fluoride-induced enzymatic alterations in goats. Fluoride 2010;43:246-52.

5 Liu YJ, Guan ZZ, Gao Q, Pei JJ. Increased level of apoptosis in rat brains and SH-SY5Y cells exposed to excessive fluoride-a mechanism connected with activation JNK phosphorylation. Toxicol Lett 2011;204:183-9.

6 Blaylock RL. Excitotoxicity: a possible central mechanism in fluoride neurotoxicity. Fluoride 2004;37:301-14.

7 Nabavi SM, Nabavi SF, Moghaddam AH, Setzer WN, Mirzaei M. Neuroprotective effects of silymarin on sodium fluoride-induced oxidative stress. Fluorine Chem 2012;142:79-82.

8 Dong YT, Wei N, Qi XL, Liu XH, Chen D, Guan ZZ. Deficit in learning and memory of rats with chronic fluorosis correlates with the decreased expressions of M1 and M3 muscarinic acetylcholine receptors. Arch Toxicol 2015;89:1981.

9 Dong YT, Wei N, Qi XL, Liu XH, Chen D, Zeng XX, et al. Attenuating effect of vitamin e on the deficit of learning and memory of rats with chronic fluorosis: The mechanism may involve muscarinic acetylcholine receptors. Fluoride 2017;50:354-64.

10 Pal S, Sarkar C. Protective effect of resveratrol on fluoride induced alteration in protein and nucleic acid metabolism, DNA damage and biogenic amines in rat brain. Environ Toxicol Pharmacol 2014;38:684-99.

11 Betul C, Saime B, Yavuz T, Kartal ON. Resveratrol: french paradox revisited. Front Pharmacol 2012;3:141.

12 Truong VL, Jun M, Jeong WS. Role of resveratrol in regulation of cellular defense systems against oxidative stress: cellular defense systems against oxidative stress. BioFactors 2017;44:36-49.

13 Li XH, Chen C, Tu Y, Sun HT, Zhao ML, Cheng SX, et al. Sirt1 promotes axonogenesis by deacetylation of Akt and inactivation of GSK3. Mol Neurobiol 2013;48:490-9.

14 Dong YT, Cao K, Tan LC, Wang XL, Qi XL, Xiao Y, et al. Stimulation of SIRT1 attenuates the level of oxidative stress in the brains of APP/PS1 double transgenic mice and in primary neurons exposed to oligomers of the amyloid-β peptide. J Alzheimers Dis Jad 2018;63:283.

15 Qin W, Yang T, Ho L, Zhao Z, Wang J, Chen L, et al. Neuronal SIRT1 activation as a novel mechanism underlying the prevention of Alzheimer disease amyloid neuropathology by calorie restriction. J Biol Chem 2006;2:S100-1.

16 Suzuki M, Ikeda A, Bartlett JD. Sirt1 overexpression suppresses fluoride-induced p53 acetylation to alleviate fluoride toxicity in ameloblasts responsible for enamel formation. Arch Toxicol 2018;92:1283.

17 Hall LL, Smith FA, Lopez OHD, Gardner DE. Direct potentiometric determination of total fluoride in biological fluids. Clinical Chem 1973;18:1455-8.

18 Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984;11:47-60.

19 Dec K, Kukomska A, Maciejewska D, Jakubczyk K, Baranowskabosiacka I, Chlubek D, et al. The influence of fluorine on the disturbances of homeostasis in the central nervous system. Biol Trace Elem Res 2017;177:224-34.

20 Mullenix PJ, Denbesten PK, Schunior A, Kernan WJ. Neurotoxicity of sodium fluoride in rats. Neurotoxicol Teratol 1995;17:169-77.

21 Zhang KL, Lou DD, Guan ZZ. Activation of the AGE/RAGE system in the brains of rats and in SH-SY5Y cells exposed to high level of fluoride might connect to oxidative stress. Neurotoxicol Teratol 2015;48:49-55.

22 Guan ZZ, Wang YN, Xiao KQ, Dai D, Chen Y, Liu J, et al. Influence of chronic fluorosis on membrane lipids in rat brain. Neurotoxicol Teratol 1998;20:537-42.

23 Atmaca N, Atmaca HT, Kanici A, Anteplioglu T. Protective effect of resveratrol on sodium fluoride-induced oxidative stress, hepatotoxicity and neurotoxicity in rats. Food Chem Toxicol 2014;70:191-7.

24 Shanmugam T, Abdulla S, Yakulasamy V, Selvaraj M, Mathan R. A mechanism underlying the neurotoxicity induced by sodium fluoride and its reversal by epigallocatechin gallate in the rat hippocampus: involvement of NrF2/Keap-1 signaling pathway. Journal of Zoology 2018;79:17.

25 Li H. Sirtuin 1 (SIRT1) and oxidative stress. In: Laher I, editor. Systems biology of free radicals and antioxidants. Berlin Heidelberg: Springer-Verlag; 2014. pp. 418-31.

26 Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, et al. Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. Science 2004;303:2011-15.

27 Olmos Y, Francisco JS, Wild B, Nieves G, Sofía C, Lamas S, et al. SirT1 regulation of antioxidant genes is dependent on the formation of a FoxO3a/PGC-1α complex. Antioxid Redox Signal 2013;19:1507-21.

28 Yüksek V, Dede S, Taşpınar M, Çetin S. The effects of vitamins A, D, E, and C on apoptosis and DNA damage in sodium fluoride-treated renal and osteoblast cell lines. Fluoride 2017;50:300-13.

29 Nalagoni CSR, Karnati PR. Protective effect of resveratrol against neuronal damage through oxidative stress in cerebral hemisphere of aluminum and fluoride treated rats. Interdiscip Toxicol 2016;9:78-82.

30 Sharma C, Suhalka P, Bhatnagar M. Curcumin and Resveratrol rescue cortical-hippocampal system from chronic fluoride induced neurodegeneration and enhance memory retrieval. Int J Neurosci 2018;1-32.

31 Tu W, Zhang Q, Liu Y, Han L, Wang Q, Chen P, et al. Fluoride induces apoptosis via inhibiting SIRT1 activity to activate mitochondrial p53 pathway in human neuroblastoma SH-SY5Y cells. Toxicol Appl Pharmacol 2018;347:60-9.

32 Pandey KB, Rizvi SI. Resveratrol up-regulates the erythrocyte plasma membrane redox system and mitigates oxidation-induced alterations in erythrocytes during aging in humans. Rejuvenation Research 2013;16:232-40.

33 Aydın S, Şahin TT, Bacanlı M, Taner G, Başaran AA, Aydın M, Başaran N. Resveratrol protects sepsis-induced oxidative DNA damage in liver and kidney of Rats. Balkan Med J 2016;33:594-601.

34 Jesko H, Wencel P, Strosznajder RP, Strosznajder JB. Sirtuins and their roles in brain aging and neurodegenerative disorders. Neurochem Res 2017;42:876-90.

35 Cao W, Dou Y, Li A. Resveratrol boosts cognitive function by targeting SIRT1. Neurochem Res 2018;43:1705-13.