

The protective role of resveratrol on serum total sialic acid and lipid-bound sialic acid in female rats with chronic fluorosis

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ABSTRACT

In the present study, the effect of resveratrol on serum total sialic acid (TSA) and lipid bound sialic acid (LSA) was investigated in the rats exposed to chronic fluoride.

The study was administered using 32 female Sprague Dawley rats weighing 200-250 g. Rats were divided into four groups (n=8/group). Group I comprised the control group, group II was treated with sodium fluoride (NaF) (10 mg/L/day), group III was treated with resveratrol (50 mg/L/day) and group IV was treated with NaF+resveratrol for 90 days period. Total sialic acid (TSA) and lipid-bound sialic acid (LSA) were determined in serum samples.

Statistical analysis showed that the NaF group was significantly higher than the control group with regards to LSA (17.59±2.734 mg/dL, 12.61±2.013 mg/dL) and TSA (87.86± 8.34 mg/dL, 71.47± 8.57 mg/dL) levels (p<0.01 and p<0.05 respectively). Whereas the Resveratrol group was also significantly lower than the NaF group regarding LSA (13.21±2.848 mg/dL, 17.59±2.734 mg/dL) and TSA (72.44± 10.43 mg/dL, 87.86± 8.34 mg/dL) levels (p<0.05 and p<0.05 respectively). Moreover, no significant differences in LSA (14.62±1.85 mg/dL, 12.61±2.013 mg/dL) and TSA (81.19 ±10.24 mg/dL, 71.47± 8.57 mg/dL) levels were observed in the Resveratrol + NaF groups, as compared to the control group (p>0.05).

The present study demonstrated slight positive and beneficial effect of resveratrol on the concentration levels of LSA and TSA in serum.

Key Words: TSA, LSA, fluorosis, resveratrol, rat

Introduction

Fluoride is one of the several required rare earth elements to perform certain functions in body (1). Approximately 95% of fluoride in the body is in the form of fluorapatite in teeth or skeleton. Rest of fluoride is stored in the soft tissues (2). Main source of fluoride is drinking waters even though fluoride can be found all around the world. Excessive intake of fluoride for long period can cause fluoride toxicity that can be called fluorosis. Primer symptoms of fluoride are stain on teeth and osteosclerosis in the skeleton system. Chronic fluorosis has effect on hepatic, respiratory, cardio vascular system (CVS), immunologic, neurologic systems in the body (1).

Resveratrol is a plant phenol known as phytoalexins. Resveratrol, naturally occurring in some various foods, grapes and berry skins (3). Resveratrol is believed to have the various biological abilities such as antioxidant and

anti-inflammatory effects (4). Previous experiments have shown that resveratrol is able to scavenge free radicals directly by hydrogen atom transfer and sequential proton loss electron transfer (5,6). Resveratrol has the anti-tumor effect against various cancer types due to inhibition of cell proliferation, induce apoptosis and suppress metastasis and invasion in a number of cell lines. Also resveratrol has cardioprotective effects (7,8).

Sialic acids are carbon derivatives of neuraminic acid and terminal component comprise oligosaccharide chains of many glycoproteins and glycolipids (9). Total sialic acid (TSA) has mainly two types of isoforms: one of the isoform binds to proteins and second binds to lipids (LSA) (10). Sialic acids take part in several cellular and molecular events such as bounding or pushing substance with positive charge due to having negative charge (10,11). Serum sialic acid is used as parameter of inflammation (12). Increase of

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serum sialic acid levels has been demonstrated in several pathologic situations in several studies. For instance; increase of LSA and TSA levels have been demonstrated on several cancer types (13). Studies have also shown that serum total sialic acid is elevated in cardiovascular disease (14).

Purpose of our study is to investigate effect of resveratrol on LSA and TSA levels in chronic fluorosis.

Materials and methods

Animals and study protocols: 32 female Sprague Dawley rats weighing 200-250 grams were used. This study was approved by animal experiments ethical committee with its decision date on 28.02.2013 and numbered 2013/-02/.

Rats were divided into four groups (n=8/group). Group I: comprised the control group, group II: was treated with NaF (10 mg/L/day), group III: was treated with resveratrol (50 mg/L/day) and group IV: was treated with NaF+resveratrol (10 mg/L/day +50 mg/L/day), for 90 days period. Rats were sacrificed after 90 days and serum samples were collected and Total sialic acid (TSA) and lipid-bound sialic acid (LSA) were determined in serum samples.

Serum analyses: Serum TSA concentration was determined by using the method described by Sydow (15). Briefly, a mixture of 0.2 mL serum and 1.5 mL of 5% perchloric acid (HClO₄) was incubated for 5 min at 100 °C, cooled down, and centrifuged at 500 g for 4 min. Then, 0.2 mL Ehrlich's reagent was added to 1.0 mL of the clear supernatant and heated for 15 min at 100 °C. After cooling, 1.0 mL distilled water was added to this mixture and the optical density was measured at 525 nm in a spectrophotometer. The amount of TSA was determined by the use of a standard curve developed from a standard sample of n-acetyl neuraminic acid.

Serum LSA concentration was determined by using the method described by Katopodis et al. (16). Briefly, 44.7 µL of serum were transferred with a capillary pipet to 150 µL distilled water. The contents were vortexed for 5 s. The tube was transferred to crushed ice. Three milliliters of cold (4-5°C) 2: 1 (v/v) chloroform: methanol were added to the tube, and the mixture was vortexed for 30 s. To this mixture, 0.5 mL cold distilled water was added, and the tube was capped. Then, the contents were mixed by repeatedly inverting the tube for 30 s. After centrifuging the tube for 5 min at room temperature at 500 g, 1 mL of the upper layer was transferred into another tube. Fifty microliters of phosphotungstic acid solution (1 g/mL) were added, and after mixing,

the tube stood at room temperature for 5 min. The tube was centrifuged for 5 min at 500 g, and the supernatant was removed by suction. One milliliter of distilled water was added and the tube was vortexed until the precipitate was in suspension without grossly visible particles. One milliliter of the resorcinol reagent was added, and the tube was mixed and placed in boiling water for exactly 15 min. Immediately after the 15 min, the tube was transferred to an ice and water bath, and left for 10 min. To the ice-cold tube, 2 mL of 85:15 (v/v) butyl acetate:n-butyl alcohol were added at room temperature, and the tube was vortexed and centrifuged for 5 min at 500 g. The extracted blue color was read at 580 nm. The amount of LSA was determined by use of a standard curve developed from a standard sample of n-acetyl neuraminic acid.

Statistical analysis: Data are presented as $X \pm SEM$ (standard error of mean). Differences in biochemical parameters were statistically evaluated using one-way analysis of variance (Anova) followed by Tukey multiple comparison test.

Results

Statistical analysis showed that the NaF group was significantly higher than the control group with regards to LSA (17.59 ± 2.734 mg/dL, 12.61 ± 2.013 mg/dL) and TSA (87.86 ± 8.34 mg/dL, 71.47 ± 8.57 mg/dL) levels ($p < 0.01$ and $p < 0.05$ respectively). Whereas, the Resveratrol group was also significantly lower than the NaF group regarding LSA (13.21 ± 2.848 mg/dL, 17.59 ± 2.734 mg/dL) and TSA (72.44 ± 10.43 mg/dL, 87.86 ± 8.34 mg/dL) levels ($p < 0.05$ and $p < 0.05$ respectively) Moreover, there was no any significantly difference between LSA (14.62 ± 1.85 mg/dL, 12.61 ± 2.013 mg/dL) and TSA (81.19 ± 10.24 mg/dL, 71.47 ± 8.57 mg/dL) levels in the Resveratrol + NaF group, as compared to the control group ($p > 0.05$). Also there was no any significantly difference between resveratrol + NaF group and NaF group with regards to LSA (14.62 ± 1.85 mg/dL, 17.59 ± 2.734 mg/dL) and TSA (81.19 ± 10.24 mg/dL, 87.86 ± 8.34 mg/dL) levels ($p > 0.005$) (Table 1, Figure 1,2).

Discussion

In this study, we have investigated effects of resveratrol which have anticancer, antioxidant effect on LSA and TSA levels in chronic fluorosis.

Fluorine mainly stores in strong tissues such as teeth and bones in the body. Therefore various pathological effects occur such as change of color in teeth and

Table 1. Average concentrations of TSA and LSA levels in serum samples of the Control, NaF, Resveratrol and NaF+Resveratrol groups

Groups	LSA	TSA
Control (n=8)	12.61±2.013 (mg/L) ^b	71.47± 8.57 (mg/dL) ^c
NaF (n=8)	17.59±2.734 (mg/dL) ^{bc}	87.86± 8.34 (mg/dL) ^{c,c1}
Resveratrol (n=8)	13.21±2.848 (mg/dL) ^c	72.44± 10.43 (mg/dL) ^{c1}
NaF+Resveratrol (n=8)	14.62±1.85 (mg/dL)	81.19 ±10.24 (mg/Ll)

The groups with same letter are statistically significant (b: p<0.01, c,c1: p<0.05)

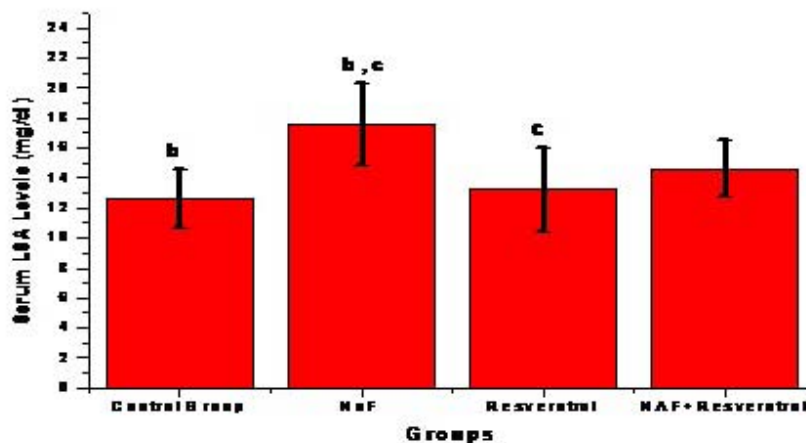


Fig. 1. LSA levels in serum samples of the Control, NaF, Resveratrol and NaF+Resveratrol groups.

The groups with same letter are statistically significant (b: p<0.01, c,c1: p<0.05).

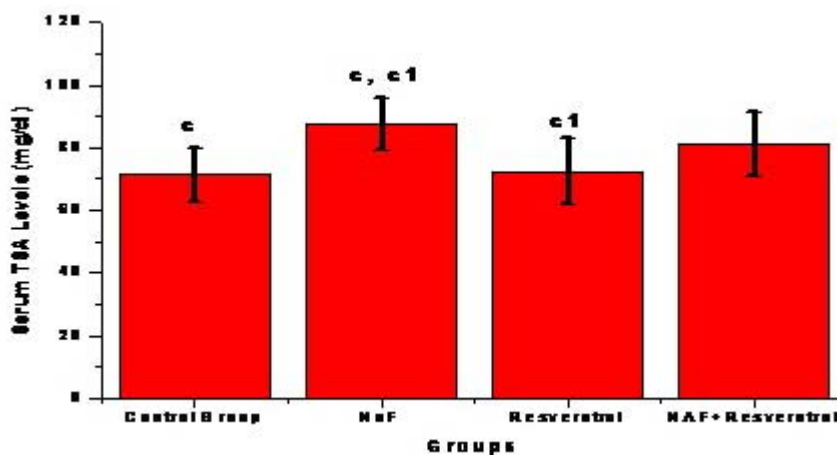


Fig. 2. TSA levels in serum samples of the Control, NaF, Resveratrol and NaF+Resveratrol groups.

The groups with same letter are statistically significant (b: p<0.01, c,c1: p<0.05).

osteosclerosis of bones as a result of fluorosis. Furthermore it has been reported that damage in soft tissue can occur in CVS and liver (17,18). Studies showed that chronic fluorosis also cause toxicity on renal, muscle, thyroid, GIS and alter erythrocyte membrane (1). Especially toxic effects of fluorine in soft tissues because of free radicals and mitochondrial damage due to oxidative stress (19,20). Sialic acids,

which is found in surface of several cells and its responsible for antigenic characteristic, should be an important component of glycolipids and glycoproteins (21). It is found in several fluids such as plasma, gastric secretion, urine and also tissues such as erythrocyte, leucocytes, colon, and stomach in the body (14). Serum sialic acid level was increased in certain diseases and it might be used as a tumor

marker in several diseases. For example it is increased in CVS, especially TSA level was increased due to cell damage after myocardial infarction (MI) and therefore was asserted as prognostic indicator in acute coronary syndrome (ACS) (9). Moreover serum sialic acid level was increased in many cancer types (21). Also TSA was increased in several inflammatory diseases such as rheumatoid arthritis (RA). Oxidative damage was created by free radicals that is playing important role in inflammatory diseases. Free radicals also cause increase of serum sialic acid level that leads to lipid peroxidations and cellular damage (12). Both LSA and TSA levels were significantly increased in NaF group than control group in our study ($p < 0.01$ and $p < 0.05$ respectively). Our results are consistent with mentioned studies above. These results support to other results from several studies as where fluorosis was claimed to cause oxidative damage and serum sialic acid level increase and this increase can be used as a tumor marker in several pathologic situations such as cancer and inflammation.

Resveratrol is widely known as an antioxidant. It was claimed that this effect is going through from different mechanism. For instance resveratrol causes activation of anti-oxidant transcription factor (Nrf2) which translocates into the nucleus and create anti-oxidant environment when oxidative stress occurs (22). Furthermore resveratrol activates peroxisome proliferator-activated receptor gamma (PPAR γ) which has property as a free radical scavenger in several studies (23). Resveratrol has different effects on apoptosis. For example it has proapoptotic effect in certain cancer types interestingly even though it was reported with antiapoptotic effects in several diseases such as stroke. This antiapoptotic effects were demonstrated to active several antiapoptotic proteins (24,25). It also has effect on anti-inflammatory, antiproliferative, cardioprotective and antiaging mechanisms (7). LSA and TSA levels were significantly decreased in resveratrol group compared to control group but there was no any significant change between NaF+resveratrol and other groups in our study. According to our results, resveratrol is relatively consistent with same studies and this study demonstrated that there is no direct effect of resveratrol on chronic fluorosis which causes TSA and LSA increase.

Consequently this study demonstrates that chronic fluorosis cause toxic effect leading to increase of LSA and TSA levels and a slight positive and beneficial effect of resveratrol on the concentration levels of LSA and TSA in serum.

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