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FLUORIDE AND OXIDATIVE STRESS

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INTRODUCTION

Biological reduction of molecular oxygen (O_2) generates products collectively termed reactive oxygen species (ROS). By accepting a single electron, O_2 is transformed into the superoxide radical anion $\cdot O_2^-$, which plays a key role in biological systems. Superoxide radicals are generated under natural conditions during mitochondrial respiration, by UV-B radiation, and in phagocytosis of cells engaged in immune response.[1] The superoxide radical anion is the substrate for the most reactive form of ROS — the hydroxyl radical (OH·) generated in the Haber-Weiss and Fenton reactions.[2]

ROS exhibit a wide spectrum of pathogenic properties. Their uncontrolled overproduction has been implicated in atherosclerosis, diabetes, and inflammatory disorders.[3,4] They react with methylene groups of polyunsaturated fatty acids (PUFA), initiating the peroxidation of membrane lipids and producing malondialdehyde (MDA) as one of the end products. Determinations of MDA levels provide a good measure of peroxidation,[5] which is among the chief mechanisms of cell damage leading to necrosis or apoptosis.[6]

Living organisms possess several antioxidative species and mechanisms protecting them against the harmful action of ROS. These include the enzymes superoxide dismutase (SOD, EC 1.15.1.1), glutathione peroxidase (GSH-Px, EC 1.11.1.9), and catalase (CAT, EC 1.11.1.6), together with nonenzymatic antioxidants, like selenium compounds, vitamins A, E, and C, and compounds containing thiol groups. Imbalance between ROS and antioxidants is referred to as oxidative stress.

In recent decades extensive information has accumulated on the role of fluoride in cellular respiratory processes and associated free radical reactions.[1] Fluoride is also known to be an inhibitor/activator of numerous enzymes.[7,8] Although the relationship in both human and animal fluorosis between free radical generation, lipid peroxidation, and antioxidant defense systems has been investigated extensively, these various studies have produced conflicting results.

CONTRARY FINDINGS

Soni *et al* studied the influence of sodium fluoride (NaF) intoxication at 5 and 20 mg/kg body mass on some tissues of the rat.[9] The lower dose was accompanied by increased peroxidation of lipids in all examined tissues, *i.e.*, liver, kidneys, lungs, intestine, and testes. With the higher dose, peroxida-

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tion continued in the kidneys and intestine, but was inhibited in the liver, lungs, and testes.

Interesting results were reported by Jain from studies on the peroxidation of lipids in human erythrocyte membranes incubated in hyperosmotic solutions of glucose (experimental hyperglycemia).[10] When glucose was used alone, peroxidation was faster, but when erythrocytes were preincubated with fluoride ions, peroxidation was inhibited. A similar protective action of fluoride was observed by de Ferreyra *et al* in rat hepatocytes exposed to carbon tetrachloride, a well-known stimulator of peroxidation.[5] The authors suggested a rise in reduced glutathione levels caused by fluoride (as phenylmethylsulphonyl fluoride — PMSF) with subsequent removal of hydrogen peroxide and oxygen free radicals by glutathione peroxidase, and, in effect, inhibition of peroxidation.

In a report by Chlubek *et al*, the effect of increasing concentrations of NaF (2.5, 50, and 500 μM) on lipid peroxidation in the mitochondrial fraction from human placenta was described.[11] Incubation with fluoride induced MDA formation, but higher concentrations of NaF were less potent in raising levels of MDA. The strongest effect (highest MDA levels) was observed with the lowest fluoride concentrations, normally found in plasma of humans unexposed to environmental contamination with fluorine compounds. These data support the view that fluoride at relatively low concentrations stimulates lipid peroxidation, but at high and very high concentrations may act as inhibitor of MDA generation.

In a study by Gardner and Fridovich, incubation of *E. coli* mutants with fluoride protected 6-phosphogluconate dehydratase against the action of superoxide radicals.[12] Practically 100% protection was observed at a fluoride concentration of 10 mM, whereas at a concentration of 0.2 mM, fluoride protection decreased to around 75% after one hr. It is interesting that *E. coli* deprived of SOD are unable to grow in an aerobic environment, because there is then a fall in the activity of 6-phosphogluconate dehydratase. Results of this study show that even in the absence of the major antioxidative enzyme SOD, fluoride is able to protect living cells against the action of ROS.

A stimulatory activity of fluoride on human erythrocyte CAT was reported by Zawierta *et al.*[13] Erythrocytes from healthy subjects were incubated with 0.25 or 2.5 mM NaF. Changes in CAT activity were observed with the lower fluoride concentration, while the higher concentration significantly reduced MDA levels. No influence of fluoride on SOD and GSH-Px activity was found.

In a recent study, Chlubek *et al* elicited hyperglycemia in rats exposed to 50 or 100 ppm fluoride in drinking water during four months and studied the effect on pancreatic antioxidative systems.[14] Cytoplasmic Cu-Zn SOD ac-

tivity was reduced by 50%, with little effect on mitochondrial Mn-dependent SOD. No change was observed in GSH-Px activity and MDA levels in pancreatic homogenates. Even stronger evidence has been presented by Reddy *et al.*[15] They reported finding no changes in lipid peroxides, GSH, and vitamin C levels, as well as in SOD, GSH-Px, and CAT activities in red blood cells of fluorotic humans and fluoride-intoxicated rabbits.

EVIDENCE FOR INVOLVEMENT OF ROS

In contrast to the above reports, a number of studies on oxidative stress in fluorotic humans and fluoride-intoxicated animals indicate that generation of ROS and lipid peroxidation (MDA formation) can be directly induced by fluoride. Moreover, there is evidence that both ROS and lipid peroxides play an important role in fluorosis. Shivarajashankara *et al* showed that rats receiving 100 ppm fluoride (as NaF) in drinking water for four months have increased levels of MDA and glutathione (GSH) and higher activity of GSH-Px in erythrocytes, brain, and liver, but decreased activity of erythrocyte SOD.[16]

Another study by Shivarajashankara *et al* on fluorotic children revealed the following changes: elevated levels of MDA, decreased GSH levels, increased GSH-Px activity and decreased SOD activity in red blood cells.[17] Earlier studies by others reported increased lipid peroxidation,[18] increased[19] or unaltered levels of GSH,[20] decreased activity of GSH-Px and unaltered activity of SOD in erythrocytes of fluorotic humans.[20]

In a study published earlier this year, a significant increase in MDA and enhanced SOD and GSH-Px activities in liver were observed by Guo *et al* in rats receiving 50, 100, and 150 ppm fluoride in their drinking water.[21]

Chinoy and Patel administered 10 mg of NaF/kg body mass during 30 days to female mice and found that cerebral levels of GSH and ascorbic acid decreased, as well as the activities of SOD, GSH-Px, and CAT.[22] These effects correlated with increased levels of lipid peroxides. Administration of vitamins C, E, and calcium fully reversed these changes.

Studies reported by Vani and Reddy carried out on mice treated with NaF (20 mg/kg body mass) for 14 days revealed decreased SOD, CAT, and glutathione transferase (GST) activities in brain and gastrocnemius muscle.[23] The effect of fluoride on muscle enzymes was comparatively larger, evidently owing to a greater accumulation of fluoride in muscle than brain.

CURRENT STATUS AND FUTURE NEEDS

The appended Tables 1–6 summarize most of the published studies on fluoride and oxidative stress in humans and animals. In these tables, special attention has been paid to the influence of fluoride on the activity of SOD and GSH-Px (major antioxidative enzymes) and MDA concentrations (indicator of lipid peroxidation).

There is no doubt that the available results, which often differ significantly from one another, depend on many important factors. Among these factors are: water fluoride levels in fluorotic areas, ages of children and adults exposed to fluoride in drinking water, animal species, kind of tissue examined, dose and mode of fluoride exposure, time of exposure, and methods for biochemical assay. In her Editorial in the previous issue of *Fluoride*, Chinoy proposed that the diet of fluorotic populations, medications, daily consumption of water, differential sensitivity of different tissues to fluoride, and fluoride levels in blood should be also taken into consideration.[24] The importance of these factors is unquestionable. However, to understand and explain the various differing results concerning oxidative stress in fluoride intoxication will require further investigation. At least the following five areas are worthy of attention:

1. Aging: Kasapoglu and Ozben investigated the correlation between oxidative stress and aging by determinations of lipid peroxidation expressed as thiobarbituric acid reactive substances (TBARS; MDA), and activities of SOD, GSH-Px, and CAT in a sample of 100 healthy men and women ranging in age from 20 to 70 years.[25] From their results, these authors suggest there is an age-related increase in lipid peroxidation expressed as MDA, and that aging is not linked to a decline in antioxidant enzyme activity, except for GSH-Px.

Similarly, Rikans and Hornbrook believe increased lipid peroxidation and decreased antioxidant protection frequently occur but are not universal features of aging.[26] Instead, age-dependent changes in these parameters appear to be species-, strain-, sex- and tissue-specific. Potential correlations between lipid peroxidation and declining antioxidant protection were obscured by the contradictory nature of the findings.

Palomero *et al* studied changes in liver glutathione and antioxidant enzymes of 1-, 2-, 4-, and 24-months-old rats.[27] The hepatic content of GSH increased with aging, peaked at four months, and decreased in senescent rats. By contrast, SOD, CAT, and GSH-Px activities were higher in the oldest rats than in the youngest ones.

2. Antioxidants as pro-oxidants: Jacob and Burri claim that most antioxidants can act as pro-oxidants under certain conditions, and clearly more research is needed to determine the occurrence and importance of this effect *in vivo*.[28]

3. Hypersensitivity to fluoride: Some individuals may experience hypersensitivity to fluoride-containing agents. Lee described an increase in the serum bilirubin concentration in patients with Gilbert's disease, which was due

solely to fluoride-containing tablets.[29] An enzyme-inhibiting action by fluoride was considered to be the most likely mechanism involved.

4. Diseases: Devi *et al* reported that plasma lipid peroxidation products in untreated leukemia patients were in the normal range.[30] Red cell Cu-Zn SOD and GSH-Px activities were significantly increased and showed no correlation with the hemoglobin content. Although superoxide generation was high, lipid peroxide levels were normal in these patients. This might be due to the increased activities of the antioxidant enzymes SOD and GSH-Px which counteract lipid peroxidation. Increased free radical generation, especially superoxide anion in leukemia patients and increased antioxidant defense enzymes, which is an adaptive protective response, are indicative of mild oxidative stress. These results conflict with the opinion that increased oxidative stress must be followed by enhanced lipid peroxidation and a decline in antioxidative enzymes activities.

5. Fluoride as a competitive inhibitor: In their valuable study on fluoride and the earthworm *Eisenia fetida*, Lawson and Yu paid attention to fluoride as a competitive inhibitor of SOD.[31] Their proposed mechanism for inhibition of SOD by fluoride involves its binding to the active site of Cu on SOD, thus displacing water. In their view the binding of fluoride to the active site of SOD is not readily, or spontaneously, reversible, and that the reaction rate for fluoride binding is fairly constant, reaching an equilibrium within a very short period of time. This could explain why the activity of SOD may be decreased even without enhanced lipid peroxidation expressed by normal level of MDA.[14]

According to Chlubek *et al*, a decrease in SOD activity can be attributed to a direct action of fluoride on the enzyme rather than to increased generation of free radicals induced by fluoride intoxication.[14] Moreover, it seems logical that increased production of the superoxide radical, which serves as a substrate for SOD, should be followed by increased rather than decreased activity of the enzyme. This point of view is confirmed by Kale *et al*, who measured pyrethroid-induced lipid peroxidation and the antioxidant system in rat erythrocytes.[32] Their results showed that lipid peroxidation increased within three days after pyrethroid treatment. The increased oxidative stress resulted in an increase in the activity of antioxidant enzymes such as SOD and CAT, which together with increased GSH content in erythrocytes may probably be an initial adaptive response to increased oxidative stress in intoxicated rats.

Gumuslu *et al* observed similar changes in the activity of antioxidative enzymes in rats exposed to sulfur dioxide.[33] Exposure to SO_2 stimulated lipid peroxide formation in the lung as indicated by an increase in the level of thiobarbituric acid reactive substances (TBARS). Lung SOD, GSH-Px,

and GST activities were also increased in response to SO_2. The authors conclude that the increase in the activities of the antioxidant enzymes in lung could be interpreted as a positive feedback mechanism in response to increased lipid peroxidation.

Results of the above-mentioned studies show that increases in the level of oxidative stress may be explained by numerous factors other than ROS that contribute to this process. These factors can influence one another and must also be considered in fluoride intoxication.

ADDENDUM TABLES

The following six tables are concerned with the effects of fluoride on fluorotic humans and fluoride-intoxicated animals with respect to:

- Superoxide dismutase (SOD) activity (Tables 1 and 2).
- Glutathione peroxidase (GSH-Px) activity (Tables 3 and 4).
- Malondialdehye (MDA) formation and concentration (Tables 5 and 6).

Table 1. Influence of fluoride on superoxide dismutase (SOD) activity in fluorotic humans (in order of publication)

Area: water fluoride level	Length of exposure	Tissue	Effect	% of control activity	Method	Reference
Suicheng, Guizhou Province, China: Level ?	? (children)	Plasma	None	100%	?	20
Kheru Nayak Thanda, Gulbarga District of Karnataka, India: 0.5 – 12.6 ppm	3-10 yrs. (children)	RBC	Inhibition	94.3% p<0.001	34	17
Edavalli, Nalgonda District, India: > 5 ppm	> 15 yrs. (adults)	RBC	None	99.7%	35	15

Table 2. Influence of fluoride on superoxide dismutase (SOD) activity in fluoride-intoxicated animals (in order of publication)

Species	Dose	Exposure length	Tissue	Effect	% of control activity	Method	Reference
Mice	10 mg NaF/kg	30 days	Liver	inhibition	?	?	36
Mice	10 mg NaF/kg	30 days	Kidney	inhibition	?	?	36
Mice	5 mg NaF/kg	?	Ovary	inhibition	?	?	37
Rat	150 ppm NaF drinking water (DW)	6 months	Liver	inhibition	80% p<0.05	38	39
Rat	150 ppm NaF drinking water	6 months	Kidney	inhibition	63.7% p<0.01	38	39
Rat	150 ppm NaF + 75 ppm As_2O_3 DW	6 months	Liver	insignificant*	98% NS	38	39
Rat	150 ppm NaF + 75 ppm As_2O_3 DW	6 months	Kidney	inhibition	74.9% p<0.01	38	39
Mice	20 mg/kg/d i.p. injections	14 days	Brain	inhibition	87.5%	40	41
Mice	20 mg/kg/d i.p. injections	14 days	Gastrocnemius muscle	inhibition	86.8%	40	41
Rat	100 ppm NaF drinking water	4 months	RBC	inhibition	84.8% p<0.001	34	16
Rat	50 ppm NaF drinking water	4 months	Pancreas CuZn-SOD	inhibition	54.3% p<0.001	42	14
Rat	100 ppm NaF drinking water	4 months	Pancreas CuZn-SOD	inhibition	54.7% p<0.001	42	14
Rat	50 ppm NaF drinking water	4 months	Pancreas Mn-SOD	insignificant*	89.7% NS	42	14
Rat	100 ppm NaF drinking water	4 months	Pancreas Mn-SOD	Insignificant*	91.8% NS	42	14
Rabbit	150 ppm NaF drinking water	6 months	RBC	insignificant*	91.2% NS	35	15
Rat	50 ppm NaF drinking water	3 months	Liver	insignificant*	95% NS	43	21
Rat	100 ppm NaF drinking water	3 months	Liver	inhibition	85.5% p<0.05	43	21
Rat	150 ppm NaF drinking water	3 months	Liver	inhibition	83.2% p<0.01	43	21
Earthworm	0.1 mM in vivo	24 hs	CuZn-SOD	inhibition	36%	44	31
Earthworm	0.1 mM in vivo	48 hs	CuZn-SOD	inhibition	67%	44	31
Earthworm	0.1 mM in vivo	72 hs	CuZn-SOD	inhibition	69%	44	31
Earthworm	1.0 mM in vivo	24 hs	CuZn-SOD	inhibition	16%	44	31
Earthworm	1.0 mM in vivo	48 hs	CuZn-SOD	inhibition	17%	44	31
Earthworm	1.0 mM in vivo	72 hs	CuZn-SOD	inhibition	23%	44	31
Earthworm	5.0 mM in vivo	24 hs	CuZn-SOD	inhibition	6%	44	31
Earthworm	5.0 mM in vivo	48 hs	CuZn-SOD	inhibition	12%	44	31
Earthworm	5.0 mM in vivo	72 hs	CuZn-SOD	inhibition	15%	44	31
Earthworm	1.0 mM in vitro	20 min	CuZn-SOD	inhibition	32% p<0.05	44	31
Earthworm	5.0 mM in vitro	20 min	CuZn-SOD	inhibition	33% p<0.05	44	31

*inhibition

Table 3. Influence of fluoride on glutathione peroxidase (GSH-Px) activity in fluorotic humans (in order of publication)

Area: water fluoride level	Exposure length	Tissue	Effect	% of control activity	Method	Reference
Suicheng, Guizhou Province, China: Level ?	?	plasma	inhibition	?	?	20
Kheru Nayak Thanda, Gulbarga District of Karnataka, India: 0.5 – 12.6 ppm	3-10 years (children)	RBC	activation	134.3% p<0.001	45	17
Edavalli, Nalgonda District, India: >5 ppm	>15 years (adults)	RBC	none	104.9%	35	15

Table 4. Influence of fluoride on glutathione peroxidase (GSH-Px) activity in fluoride-intoxicated animals (in order of publication)

Species	Dose	Exposure length	Tissue	Effect	% of control activity	Method	Reference
Mice	10 mg NaF/kg	30 days	Liver	inhibition	?	?	36
Mice	10 mg NaF/kg	30 days	Kidney	inhibition	?	?	36
Mice	5 mg NaF/kg	?	Ovary	inhibition	?	?	37
Rat	150 ppm NaF drinking water	6 months	Liver	inhibition	63.8% p<0.01	46	39
Rat	150 ppm NaF drinking water	6 months	Kidney	inhibition	63.6% p<0.01	46	39
Rat	150 ppm NaF+75 ppm As_2O_3 drinking water	6 months	Liver	insignificant inhibition	98.9% NS	46	39
Rat	150 ppm NaF+75 ppm As_2O_3 drinking water	6 months	Kidney	inhibition	54.7% p<0.01	46	39
Rat	100 ppm NaF drinking water	4 months	RBC	activation	159.3% p<0.001	45	16
Rat	100 ppm NaF drinking water	4 months	Brain	activation	387.9% p<0.001	47	16
Rat	100 ppm NaF drinking water	4 months	Liver	activation	180% p<0.001	47	16
Rat	50 ppm NaF drinking water	4 months	Pancreas	insignificant inhibition	91.3% NS	45	14
Rat	100 ppm NaF drinking water	4 months	Pancreas	none	98.5% NS	45	14
Rabbit	150 ppm NaF drinking water	6 months	RBC	none	104.7% NS	35	15
Rat	50 ppm NaF drinking water	3 months	Liver	insignificant inhibition	86.8% NS	46	21
Rat	100 ppm NaF drinking water	3 months	Liver	insignificant inhibition	70.3% NS	46	21
Rat	150 ppm NaF drinking water	3 months	Liver	insignificant inhibition	54.3% NS	46	21

Table 5. Influence of fluoride on malondialdehyde (MDA) formation in fluorotic humans (in order of publication)

Area: water fluoride level	Exposure length	Tissue	Effect	% of control concentration	Method	Reference
Kheru Nayak Thanda, Gulbarga District of Karnataka, India: 0.5 – 12.6 ppm	3-10 years (children)	RBC	increase	116.7% p<0.001	48	17
Edavalli, Nalgonda District, India: >5 ppm	>15 years (adults)	RBC	insignificant increase	146.2% NS	35	15

Table 6. Influence of fluoride on malondialdehyde (MDA) concentration in fluoride-intoxicated animals (in order of publication)

Species	Dose	Exposure length	Tissue	Effect	% of control concentration	Method	Reference
Mice	10 mg NaF/kg	30 days	Liver	increase	?	?	36
Mice	10 mg NaF/kg	30 days	Kidney	increase	?	?	36
Mice	5 mg NaF/kg	?	Ovary	increase	?	?	37
Rat	150 ppm NaF drinking water	6 months	Liver	insignificant increase	135% NS	49	39
Rat	150 ppm NaF drinking water	6 months	Kidney	insignificant inhibition	141.1% NS	49	39
Rat	150 ppm NaF + 75 ppm As_2O_3 drinking water	6 months	Liver	none	106.5% NS	49	39
Rat	150 ppm NaF + 75 ppm As_2O_3 drinking water	6 months	Kidney	insignificant increase	134.2% NS	49	39
Rat	100 ppm NaF drinking water	4 months	RBC	increase	127.8% p<0.001	48	16
Rat	100 ppm NaF drinking water	4 months	Brain	increase	177.4% p<0.001	48	16
Rat	100 ppm NaF drinking water	4 months	Liver	increase	205.2% p<0.001	48	16
Rat	50 ppm NaF drinking water	4 months	Pancreas	insignificant decrease	61.3% NS	48	14
Rat	100 ppm NaF drinking water	4 months	Pancreas	insignificant decrease	62.1% NS	48	14
Rabbit	150 ppm NaF drinking water	6 months	RBC	insignificant decrease	95% NS	35	15
Rat	50 ppm NaF drinking water	3 months	Liver	insignificant increase	222.2% NS	50	21
Rat	100 ppm NaF drinking water	3 months	Liver	insignificant increase	431.7% NS	50	21
Rat	150 ppm NaF drinking water	3 months	Liver	increase	812.7% p<0.01	50	21

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