

OXIDATIVE STRESS PARAMETERS IN RATS EXPOSED TO FLUORIDE AND ASPIRIN

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SUMMARY: The action of fluoride (F) and aspirin (acetylsalicylic acid, ASA) administered separately and together on oxidative stress parameters and on F excretion in rats was studied over a period of six weeks. Thirty adult male Wistar rats were divided into five equal groups of six each: (I) controls receiving tap water; (II) controls receiving intragastrically 1 mL of tap water (once a day); (III) animals receiving 25 mg F/L in their drinking water; (IV) animals receiving 35 mg ASA/kg bw/day; (V) animals receiving 25 mg F/L in drinking water and 35 mg ASA/kg bw/day. In rats treated only with fluoride the F excretion in urine significantly increased in an exposure time-dependent manner and decreased both in rats treated with ASA and co-exposed to ASA and F. In animals treated with ASA alone, the activity of glutathione peroxidase (GPx) and the concentrations of glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) increased in brain, liver and blood, whereas the activity of catalase (CAT) decreased in liver and erythrocytes, but increased in liver. In rats exposed to F alone the concentration of GSH and the activities of GPx and CAT decreased, whereas the concentration of TBARS significantly increased in liver, brain, kidney and blood. In rats co-exposed to F and ASA, the concentrations of GSH and TBARS and the activities of GPx and CAT (except in liver) were higher than in animals receiving only F.

Keywords: Antioxidant potential; Aspirin treatment; Fluoride and aspirin; Fluoride in urine; Lipid peroxidation; Rats and aspirin.

INTRODUCTION

People are often exposed to many chemical substances that are deleterious and dangerous to health. Nowadays one pays attention not only to the effects of toxic xenobiotics but also to their interactions with one another.¹⁻² Particularly important are interactions between such toxicants to which exposure is the most common. Examples of such substances are fluoride (F) and aspirin.

In recent years, many studies have indicated that F induces free radical toxicity in humans and animals.³⁻⁶ It is well known that the metabolism and toxicity of F may be modified by a great many factors, including xenobiotics.⁷⁻⁹ Aspirin (2-acetylsalicylic acid, ASA) is the most popular medicine in the world. ASA has been used as an analgesic, anti-inflammatory, and antipyretic substance. In low doses taken for a long time, ASA will have anticoagulant activity.¹⁰⁻¹¹ At present there are several scientific reports about the effect of ASA on antioxidant potential.¹²⁻¹⁴ However, there does not appear to be any information available in the literature about interactions between F and ASA in soft tissues.

The aim of this study was to investigate the impact of F and ASA on free radical mediated parameters and on urinary F excretion in rats when given separately and together in a 6-week experiment

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MATERIALS AND METHODS

Animals and experimental design: The experiment was carried out for 6 weeks. Thirty adult male Wistar rats weighing 189 ± 6.9 g were kept under standard laboratory conditions (temperature $22 \pm 2^\circ\text{C}$ in natural light-dark cycle, humidity 60%). All animals were fed on a standard laboratory pellet diet containing 0.7 mg water-extractable F/kg. The rats were divided into five groups:

I. Controls drinking tap water containing 0.3 mg F/L

II. Controls receiving intragastrically 1 mL tap water once a day by a stomach tube

III. Exposed animals receiving 25 mg F (from NaF)/L in drinking water

IV. Exposed animals receiving 35 mg ASA/kg bw/day (in 1 mL of water once a day by a stomach tube)

V. Exposed animals receiving 25 mg F/L in drinking water and 35 mg ASA/kg bw/day (in 1 mL of water once a day by a stomach tube)

Water consumption by the animals was measured daily; body weight was measured once a week. Every week 4 rats of each group were placed into metabolic cages, and their 24-hr urine samples were collected. After 6 weeks of exposure, the animals of each group were sacrificed, and samples of blood, brain, kidney, and liver were collected. Blood was taken with an anticoagulant by cardiac puncture.

Analytical procedures: The concentration of fluoride in the urine was determined potentiometrically after dilution with equal volumes of TISAB buffer by a F ion-specific electrode (Orion) and Ag/AgCl reference electrode.¹⁵ Urinary creatinine was determined by the method of Folin and Morris.¹⁶ Urinary F is reported as mg F/g creatinine. The accuracy of measurements was assessed with reference materials — Seronorm Control Urine (Nycomed Pharma AC, Oslo, Norway). Mean F recovery was 98.9%. In tissue homogenates and blood (or plasma) the activity of glutathione peroxidase (GPx) was determined according to Paglia and Valentine,¹⁷ the activity of catalase (CAT) according to Aebi,¹⁸ the concentration of glutathione (GSH) by the method of Sedlak and Lindsay,¹⁹ and the concentration of thiobarbituric acid reactive substances (TBARS) according to Rice et al.²⁰ The protein content in plasma, kidney, liver, and brain was determined by the method of Lowry et al.²¹ Hemoglobin content in blood was determined by the method of Drabkin.²²

Statistical analysis: Data are given as means \pm standard error. Statistical analysis was performed using the Fisher-Snedecor and Student's t test.

RESULTS

Results of water consumption and fluoride intake by the five groups of rats are presented in Table 1. In this experiment decreased water consumption was noticed only in the ASA group, whereas in the F and F plus ASA groups water consumption did not change.

Table 1. Water consumption and fluoride intake

Group treatment of animals		Water consumption mL/24 hr Means±SD	F intake mg F/24 hr/rat Means
I	Controls	36.6±2.6	0.011
II	Controls (i.g.)	34.5±2.5	0.010
III	F	35.9±4.1	0.897
IV	ASA	31.8±3.2	0.009
V	F+ASA	37.7±3.9	0.943

F concentration in urine is given in Table 2. Exposure to NaF resulted in a significant increase in F excretion in urine. In animals exposed only to ASA, urinary F excretion decreased markedly. Likewise, co-exposure to F and ASA significantly decreased the F excretion, when compared to animals treated only with F.

Table 2. Fluoride concentration in urine (mg F/g creatinine±SD)

Group treatment of animals	Exposure time in weeks					
	0	1	2	3	4	5
I Controls	2.56±0.168	2.12±0.456	2.54±0.911	2.98±0.378	3.42±0.096	3.04±0.009
II Controls (i.g.)	2.09±0.061	2.47±0.087	2.48±0.349	2.63±0.281	2.87±0.118	2.98±0.418
III F		3.98±0.264	5.36±0.910	6.78±0.491	7.74±1.246	8.49±0.692
IV ASA		1.89±0.034	1.46±0.049	1.68±0.413	1.34±0.061	1.26±0.411
V F+ASA		3.48±1.094	4.34±1.456	5.01±0.986	5.63±1.109	6.27±1.031

Statistical significance (values are for 4 animals in each group):

I vs. III	p<0.01↑	p<0.001↑	p<0.001↑	p<0.001↑	p<0.001↑
II vs. IV	n.s.	p<0.05↓	p<0.05↓	p<0.05↓	p<0.01↓
I vs. V	p<0.05↑	p<0.001↑	p<0.001↑	p<0.001↑	p<0.001↑
II vs. V	p<0.05↑	p<0.01↑	p<0.001↑	p<0.001↑	p<0.001↑
III vs. V	n.s.	p<0.05↓	p<0.01↓	p<0.01↓	p<0.01↓
IV vs. V	p<0.01↑	p<0.001↑	p<0.001↑	p<0.001↑	p<0.001↑

The activity of glutathione peroxidase (GPx) and the concentration of glutathione (GSH) are presented in Table 3 and Table 4, respectively. In this experiment, the GPx activity decreased significantly in brain and blood of animals treated only with F and co-exposed to F and ASA. However, the decrease in co-exposed animals was smaller than in animals intoxicated only with F.

The concentration of reduced glutathione (GSH) decreased significantly in kidney, liver, and blood of rats exposed to F. The decrease was lower in animals co-exposed to F and ASA. On the other hand, in animals given only ASA the concentration of GSH markedly increased.

The activity of catalase (CAT) is presented in Table 5. Catalase activity decreased in kidney, liver, brain, and erythrocytes of animals treated only with F. In rats given only ASA and in co-exposed animals the CAT activity decreased in liver and erythrocytes, but increased in kidney.

Table 3. Activity of glutathione peroxidase in tissues (U/g protein) and blood (U/gHb)

Group treatment of animals		Kidney mean±SD	Liver mean±SD	Brain mean±SD	Blood mean±SD
I	Controls	17.5±2.24	42.0±6.65	3.96±0.60	2.10±0.39
II	Controls (i.g.)	17.8±3.64	39.9±5.81	4.09±0.58	1.98±0.41
III	F	12.0±0.60	35.7±3.04	1.29±0.01	1.35±0.34
IV	ASA	20.9±4.31	44.1±2.88	4.35±0.45	2.29±0.18
V	F+ASA	16.8±1.55	36.4±0.80	2.33±0.30	1.77±0.09

Statistical significance (values are for 6 animals in each group):

I vs. III	p<0.01↓	n.s.	p<0.001↓	p<0.02↓
II vs. IV	n.s.	n.s.	n.s.	n.s.
I vs. V	n.s.	n.s.	p<0.01↓	p<0.05↓
II vs. V	n.s.	n.s.	p<0.01↓	p<0.05↓
III vs. V	p<0.01↑	n.s.	p<0.01↑	p<0.05↑
IV vs. V	n.s.	p<0.001↓	p<0.001↓	p<0.01↓

Table 4. Concentration of glutathione in tissues (µM /g protein) and blood (µM/gHb)

Group treatment of animals		Kidney mean±SD	Liver mean±SD	Brain mean±SD	Blood mean±SD
I	Controls	26.9±2.02	57.7±8.02	30.0±5.62	5.45±0.28
II	Controls (i.g.)	24.6±0.96	54.6±7.41	28.6±5.89	5.09±0.43
III	F	19.3±2.41	41.2±7.92	24.6±4.42	4.03±0.15
IV	ASA	27.4±4.67	82.1±8.42	34.1±2.54	7.02±0.56
V	F+ASA	24.2±0.82	48.3±3.99	27.7±2.07	5.11±0.16

Statistical significance (values are for 6 animals in each group):

I vs. III	p<0.001↓	p<0.01↓	n.s.	p<0.001↓
II vs. IV	p<0.02↑	p<0.001↑	n.s.	p<0.001↑
I vs. V	p<0.02↓	p<0.05↓	n.s.	n.s.
II vs. V	n.s.	p<0.05↓	n.s.	n.s.
III vs. V	p<0.01↑	n.s.	n.s.	p<0.001↑
IV vs. V	n.s.	p<0.001↓	p<0.01↓	p<0.001↓

Table 5. Catalase activity in tissues (k/mg protein) and erythrocytes (k/mg Hb)

Group treatment of animals		Kidney mean±SD	Liver mean±SD	Brain mean±SD	Erythrocytes mean±SD
I	Controls	0.580±0.09	2.58±0.232	7.27·10 ⁻³ ±6.24·10 ⁻⁴	24.3±3.13
II	Controls (i.g.)	0.479±0.04	2.76±0.196	6.94·10 ⁻³ ±6.71·10 ⁻⁴	25.9±3.41
III	F	0.458±0.08	1.03±0.152	1.09·10 ⁻³ ±9.27·10 ⁻⁴	17.5±1.52
IV	ASA	0.794±0.03	1.99±0.377	6.69·10 ⁻³ ±8.24·10 ⁻⁴	18.7±3.06
V	F+ASA	0.625±0.02	0.63±0.076	6.58·10 ⁻³ ±6.78·10 ⁻⁴	18.2±3.41

Statistical significance (values are for 6 animals in each group):

I vs. III	p<0.02↓	p<0.001↓	p<0.001↓	p<0.01↓
II vs. IV	p<0.01↑	p<0.05↓	n.s.	p<0.05↓
I vs. V	n.s.	p<0.001↓	p<0.01↓	p<0.05↓
II vs. V	p<0.01↑	p<0.001↓	n.s.	p<0.05↓
III vs. V	p<0.01↑	p<0.05↓	p<0.001↑	n.s.
IV vs. V	n.s.	p<0.001↓	n.s.	n.s.

Table 6. TBARS concentration in tissues and plasma ($\mu\text{M/g}$ protein)

Group treatment of animals		Kidney mean \pm SD	Liver mean \pm SD	Brain mean \pm SD	Plasma mean \pm SD
I	Controls	4.16 \pm 0.436	5.39 \pm 0.31	16.83 \pm 1.94	9.06 \pm 0.54
II	Controls (i.g.)	4.06 \pm 0.396	5.21 \pm 0.41	15.93 \pm 1.43	8.99 \pm 0.41
III	F	19.85 \pm 4.23	16.25 \pm 1.96	67.14 \pm 4.65	11.27 \pm 0.98
IV	ASA	19.66 \pm 3.89	17.66 \pm 0.41	31.00 \pm 7.50	11.62 \pm 1.15
V	F+ASA	73.46 \pm 3.98	78.24 \pm 9.71	103.39 \pm 12.6	63.67 \pm 6.24
Statistical significance (values are for 6 animals in each group):					
I vs. III		p<0.001 \uparrow	p<0.001 \uparrow	p<0.001 \uparrow	p<0.05 \uparrow
II vs. IV		p<0.001 \uparrow	p<0.001 \uparrow	p<0.01 \uparrow	p<0.05 \uparrow
I vs. V		p<0.001 \uparrow	p<0.001 \uparrow	p<0.001 \uparrow	p<0.001 \uparrow
II vs. V		p<0.001 \uparrow	p<0.001 \uparrow	p<0.001 \uparrow	p<0.001 \uparrow
III vs. V		p<0.001 \uparrow	p<0.001 \uparrow	p<0.001 \uparrow	p<0.001 \uparrow
IV vs. V		p<0.001 \uparrow	p<0.001 \uparrow	p<0.001 \uparrow	p<0.001 \uparrow

Concentrations of TBARS are given in Table 6. TBARS concentrations increased significantly in all investigated tissues of animals exposed to F as well as to ASA. In co-exposed animals the TBARS concentrations markedly increased when compared to animals treated with single substances.

DISCUSSION

The exposure of rats to 25 mg F/L in their drinking water in this study corresponds to human environmental exposure in areas with high F in the drinking water or in F-polluted occupational conditions.⁷

Decreased water consumption was noticed in this study only in the ASA group. ASA as a typical NSAIDS (Nonsteroidal Anti-Inflammatory Drug) is nephrotoxic and consequently may cause even oliguria,²³ whereas in the F and F plus ASA groups water consumption did not change.

As expected, in this study exposure to NaF resulted in a significant increase of F excretion in urine³² The increase changed in an exposure-time-dependent manner. In animals exposed only to ASA, urinary F excretion markedly decreased. This may be caused by the fact that both F and ASA compete for the same anion transport system in the renal tubules.³³ Indeed, in co-exposed animals, F excretion was significantly decreased, when compared to rats exposed only to F.

In this study the activity of GPx and the concentration of GSH in all investigated tissues is the lowest in rats exposed only to F, intermediate in co-exposed animals, and the highest in rats treated only with ASA. These results are consistent with our previous studies performed on animals exposed to F.^{24-25,27} A decrease in the activity of GPx, CAT, and SOD has been found in tissues of experimental animals exposed to F compounds and in people living in endemic areas.³⁻⁶ On the other hand, Kirkova et al.²⁶ ascertained that GPx activity did not change in animals treated with ASA.

The intermediate activities of GP_x and the concentrations of GSH in this study found in co-exposed animals could mean that ASA minimized the toxic effects of F. However, the mechanism of action of aspirin (ASA) with regard to oxidative stress is not fully understood. Available data indicate that it may involve a decrease of superoxide anion production.¹⁴

Also, the activity of catalase in this study in most tissues behaved similarly to that of GPx. However, it increased in kidney of animals treated with ASA. In a previous study we found that CAT activity decreased in rats exposed to F.²⁸ Kirkova et al.²⁶ stated that higher aspirin concentration (5.0 mM) inhibited the activity of this enzyme.

Many researchers independently have confirmed enhanced lipid peroxidation caused by F.^{4-6,29} On the other hand, some authors have reported that F does not impair antioxidant defense systems.^{30,31} Kirkova et al.²⁶ observed that ASA in low concentrations stimulated the formation of malondialdehyde in liver homogenates but in higher concentrations it inhibited this process. However, Steer et al.¹³ affirmed that aspirin both *in vivo* and *in vitro* protects LDL (low-density lipoproteins) against subsequent oxidative modification, thereby providing an additional mechanism whereby aspirin may protect against atherosclerosis. Schwarz et al.¹² reported that ASA intensifies MDA (malondialdehyde) production in hepatic microsomes and mitochondria. In our study we noticed that the TBARS concentration significantly increased in all investigated tissues of animals exposed both to F and ASA. However, combined exposure caused a marked increase of lipid peroxidation in liver, brain, kidney, and plasma.

In conclusion, our study indicates that F and ASA administered both singly and together participate in free radical-mediated reactions and affect antioxidant parameters. The changes in the activity of GPx and CAT and the concentration of GSH suggest that ASA counteracts the toxic effects of F on antioxidant parameters. An interesting further observation was that ASA decreases F excretion in urine.

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