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# Fluoride and Lipid Peroxidation: A Comparative Study in Different Rat Tissues

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The toxic effects due to fluoride are not only confined to the skeletal tissues but deleterious effects of fluoride on central nervous system, gastrointestinal tract, liver, kidneys, cardiovascular system, respiratory tract and muscles have also been reported (Zhavoronkov 1977). Alterations in the permeability of membranes and membrane bound enzymes under the influence of fluoride have been reported (Martin et al. 1980). Lipid peroxidation has been suspected to play an important role in wide variety of pathological and degradative conditions (Tappel 1973). Many studies suggest that the influence of various environmental pollutants (Mudd and Freeman 1977) are closely related to lipid peroxidation.

The superoxide anion ($O_2^-$) either generated naturally or by induction from xenobiotics, attack the polyunsaturated fatty acids of biological membranes and initiate the process of lipid peroxidation. Fluoride has been reported to induce metabolic activation in polymorphonuclear leukocytes measured as oxygen uptake and $O_2^-$ production.

The present study was undertaken to investigate the effect of fluoride on lipid peroxidation in liver, brain and intestine of rats, in vitro and in vivo. While liver and brain are ideal tissues to study the effect of fluoride on lipid peroxidation, intestinal epithelium is one of the most exposed tissues to fluoride, since the effect of fluoride on human health stems largely from fluoride taken through oral route.

## MATERIALS AND METHODS

2-Thiobarbituric acid was purchased from E. Merk, Darmstadt,Germany, 5-5'-dithiobis (2-nitrobenzoic acid) was obtained from Kochlight Combrook Bucks, England and 1,1,3,3,tetramethoxy propane was supplied by Sigma Chemical Company, St. Louis, Mo, U.S.A. All other chemicals used during this study, were of analytical grade.

Male albino rats weighing 200-250 gm (for in vitro studies) and of average 100 gm body weight (for in vivo studies) obtained from Industrial

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Toxicology Research Centre animal colony, fed ad libitum on a pellet diet (Hind Lever Laboratory Feeds, India), and maintained under standard laboratory conditions (steel cages and air-conditioned rooms) were used throughout the study.

For in vitro studies rats were sacrificed by decapitation. Liver, brain and intestine (ileum and jejunum portions) were immediately removed and cleared free of blood and extraneous materials. Intestine were everted on a glass rod and the epithelial layer was scraped off with the help of a plain tipped forcep. Scraping was carried out by pulling the everted intestine through the small gap at the tip of the forcep. The tissues were homogenized (Potter Elvehjem type homogenizer fitted with a teflon pestle) to give 10% (w/v) homogenates of liver and brain, and 20% (w/v) of intestinal epithelium, in ice cold 1.15% KCl.

Ten ml fractions from each homogenate with different concentrations of NaF/NaCl were placed in 25 ml Erlenmeyer flasks and incubated at 37°C for 3 hours with continuous shaking (120 cycles/min). 1 ml aliquotes were drawn after 0, 1, 2 and 3 hours of incubation and malonaldehyde (MDA) formation was determined as described by Okhawa et al. (1979). 1,1,3,3,tetramethoxypropane was used as an external standard.

The effect of fluoride on MDA formation by fresh and heated homogenates of intestine, liver and brain was studied. The homogenates were heated in a boiling water bath for 10 minutes and after cooling these were rehomogenized to get a homogenous preparation (Sharma and Krishna Murti 1976). The MDA formation in NaF and NaCl treated homogenates was compared.

For in vivo studies rats were divided into two groups. Group I or the experimental animals were orally administered with 25 mg NaF per kg body weight per day. Group II received NaCl in an identical manner and served as the controls. Six rats from each group were sacrificed after 30 and 60 days of NaF and NaCl administration. Animals were subjected to overnight fasting before sacrifice. Homogenates of different tissues were prepared and lipid peroxides were determined as mentioned above. Free sulphydryl content was determined by Sedlak and Lindsay method (1968).

## RESULTS AND DISCUSSION

Observed effect of fluoride on in vitro lipid peroxide formation varied in brain, liver and intestine. Homogenates were treated with different concentrations of NaF (1-200 mM) and MDA formation was measured. The percent inhibition/activation per three hours was calculated and plotted against different fluoride concentrations as shown in Fig. 1. Significant inhibition of lipid peroxidation in liver and intestine was noticed at 10 mM NaF and further increase in NaF concentration resultant into spontaneous decrease in MDA formation. But the extent of inhibition in intestine was greater than in liver. In brain, fluoride exerted a biphasic effect on MDA formation; from 10-50 mM NaF concentrations, a significant induction and later an inhibitory effect.

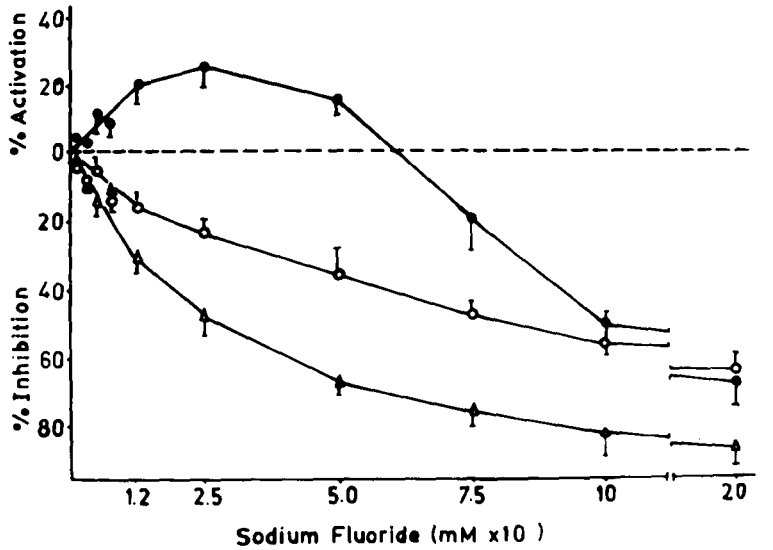


Figure 1. Effect of NaF on the MDA formation in brain (●—●) liver (o—o) and intestine (Δ—Δ) homogenates. MDA formation was measured at 0 hour and after 3 hours incubation at 37°C in shaking water bath. The percent inhibition/activation per three hours was calculated and plotted against different NaF concentrations.

The effect of 50 mM NaF on MDA formation in fresh and heated homogenates of intestine and liver is shown in Fig. 2. A similar pattern of fluoride effect was observed in both cases, i.e. loss of inhibitory effect of fluoride on lipid peroxidation in heated homogenates. Fig. 3 shows the effect of 50 mM (which induced lipid peroxidation in brain) and 100 mM (which inhibited lipid peroxidation in brain) NaF concentrations on MDA formation in fresh and heated homogenates of brain. The induction of MDA formation observed at 50 mM NaF was not affected by heating the homogenate while inhibitory effect observed at 100 mM NaF was lost by heating the homogenate.

Enzymatic induction of $O_2^-$ by fluoride in polymorphonuclear leukocytes of different origin has been reported. Elfrink (1981) reported a biphasic effect of fluoride on $O_2^-$ production in polymorphonuclear leukocytes: a stimulatory effect initially (9-18 mM) and later, an inhibitory effect. Biphasic effect in the present study was observed only in brain homogenates, but here, induction was non-enzymatic in nature while both induction as well as inhibition of $O_2^-$ production in polymorphonuclear

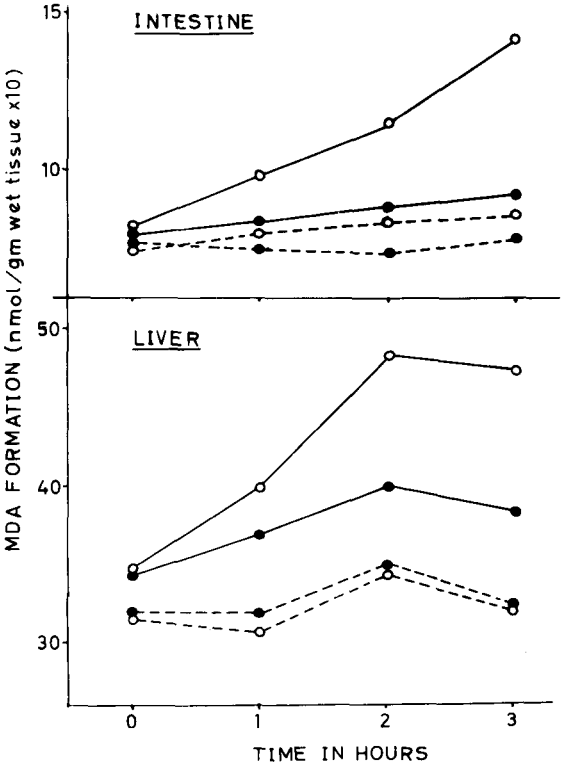


Figure 2. Effect of 50 mM NaF on the MDA formation in heated (o----o) control (●-----●) experimental, and unheated (o——o) control (●——●) experimental, homogenates of rat intestine and liver.

leukocytes have been reported to be enzymatic in nature. The inhibition of $O_2^-$ production could be associated with the inhibition of glycolysis by fluoride, since fluoride inhibits glycolysis by interaction with the enzyme enolase and glycolysis plays an important role in $O_2^-$ production (Cohen and Chovaniec 1978).

As shown in Table 1, the inhibitory effect of luoride on lipid peroxide formation was completely lost in presence of calcium and in presence of iron it was almost doubled in comparison to control. The activation of lipid peroxide formation in brain remained unaffected in presence of calcium but was further enhanced by iron. Induction of $O_2^-$ production by fluoride in polymorphonuclear leukocytes is maximum in presence of calcium but addition of $CaF_2$ instead of NaF and $CaCl_2$ separately failed to induce any effect. Lipid peroxide formation catalysed by ascorbic acid and iron and presumed to be non-enzymatic has been well recognised (Ottolenghi 1959). Membrane bound enzymes such as NADPH cytochrome P-450 reductase which catalyses the transfer of reducing equivalents from co-factor NADPH (Lyakhovich et al. 1976) causing

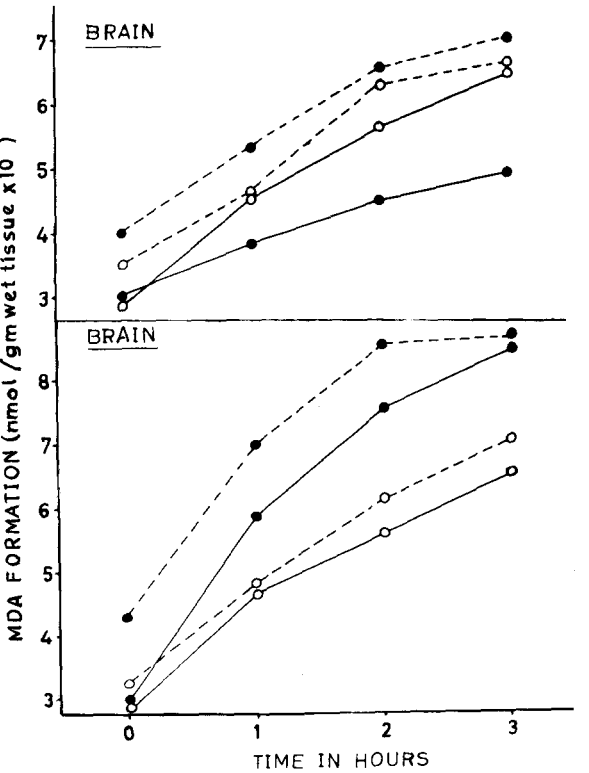


Figure 3. Effect of 100 mM NaF (top) and 50 mM NaF (bottom) on the MDA formation in heated, (o - - - - o) control (● - - - - ●) experimental and unheated (o——o) control (●——●) experimental, homogenates of rat brain.

lipid peroxide formation, are also dependent on iron. Chelation of calcium and iron could be the reason for observed inhibition of lipid peroxide formation. The mechanism of induction of lipid peroxide formation in brain which seems to be non-enzymatic in nature is not at present fully understood. Oral administration of NaF to the rats for a period of 60 days produced significant decrease in the formation of lipid peroxides in the intestine, while no effect was observed in liver and brain (Fig. 4). A significant change in the free sulfhydryl content of intestine was also observed while liver and brain showed little change (Table 2). The reason for inhibition of lipid peroxide formation by fluoride could also be due to stimulation of reducing capacity of tissues by increasing free sulfhydryl content which protect the membrane from oxidative attack of oxygen free radicals under natural conditions (Bus et al. 1976). Liver and brain did not show any change in lipid peroxide and sulfhydryl content. The reason for absence of effect on lipid peroxidation in liver and brain could be the lesser concentrations of fluoride reaching/accumulating in these organs in comparison to intestine which was directly

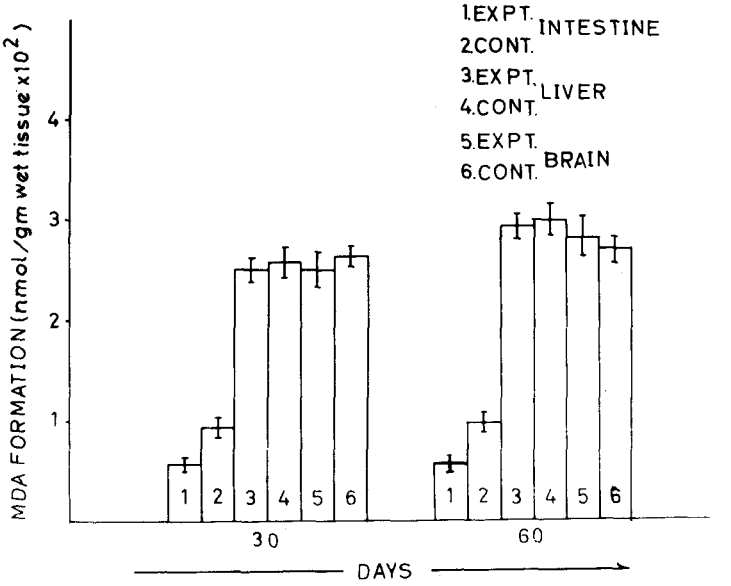


Figure 4. In vivo effect of fluoride (25 mg NaF/kg body wt/day) on the lipid peroxide potential of intestine, liver and brain of rats. P 0.02 in intestine.

Table 1. Effect of fluoride on malonaldehyde formation in intestine, liver and brain homogenates in presence of calcium and iron

| Additions | n moles of MDA formed/gm wet tissue | | |
|---|---|---|---|
| | Intestine | Liver | Brain |
| None (control) | 145±10 | 470±18 | 650±21 |
| +50 mM NaF | 78±07 | 396±12 | 810±25 |
| +50 mM NaF and 10 mM $CaCl_2$ | 152±11 | 402±21 | 830±19 |
| +50 mM NaF and 10 mM $FeSO_4$ | 325±17 | 835±19 | 1002±32 |
| +100 mM NaF | - | - | 510±17 |
| +100 mM NaF and 10 mM $CaCl_2$ | - | - | 616±21 |
| +100 mM NaF and 10 mM $FeSO_4$ | - | - | 112±31 |

10 ml of each homogenate was incubated for 3 hours at 37°C with or without different additions. Values are mean ± S.E. of five observations.

Table 2. Effect of fluoride on the free sulfhydryl content of intestine, liver and brain, in vivo

| Tissue | Days | Free sulfhydryl content* | | % Increase |
|---|---|---|---|---|
| | | Control | Experimental | |
| Intestine | 30 | 113.3±4.8 | 153.5±4.3 | 35** |
| | 60 | 93.3±6.1 | 137.5±4.5 | 47** |
| Liver | 30 | 317.1±3.1 | 327.7±9.1 | 3 |
| | 60 | 289.5±8.5 | 301.5±8.7 | 4 |
| Brain | 30 | 324.0±8.0 | 461.6±10.2 | 9 |
| | 60 | 394.0±11.0 | 416.6±7.5 | 5 |

* µgm of free sulfhydryl content/gm wt tissue value are mean ± S.E. of six rats.
**$P < 0.02$.

exposed to the daily oral administration of fluoride.

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