111 – 113

Original research

Effects of fluoride on immune system function

Sheila LM Gibson

Summary

The fluoridation of public water supplies was introduced over 40 years ago in the belief that it was beneficial to teeth. More recent evidence, however, reveals no lasting benefits and suggests that fluoride may be harmful to many physiological systems. The present study investigates effects of fluoride on the immune system and demonstrates that low concentrations inhibit the migrational ability of leucocytes.

In 1945 the fluoridation of public water supplies was introduced as a public health measure aimed at reducing tooth decay. Though some initial results were encouraging other trials showed little or no effect and more recent studies show that tooth decay rates have fallen equally in both fluoridated and non-fluoridated areas world-wide[1-6].

Fluoride is one of the most toxic inorganic chemicals in the Earth's crust, but it is believed that at a concentration of one part per million, or one microgram per millilitre (1μg per ml) in public water supplies, and at the concentrations used in dental preparations, it is both safe and beneficial to teeth. However, with increasing experience, doubts about both safety[7-9] and efficacy[1-6] have arisen. While there is evidence of harm to the stomach[10-12], kidneys[13], thyroid[13], bones[14-21] and teeth[22 23] at higher concentrations, evidence for harm at concentrations around 1g/ml is controversial. A possible link between fluoridation of public water supplies and an increase in the cancer death rate has been debated for over 20 years and there is now no doubt that fluoride can cause genetic damage[24-29]. Disagreements in this field revolve around the level at which damage manifests[29].

Inhibitory effects of fluoride on different enzyme systems have been demonstrated[30 31]. However, one aspect of metabolism which has, to date, received scant attention with respect to possible toxic effects of fluoride is the immune system.

Materials and methods

The macrophage migration inhibition test studies aspects of cell mediated immunity[32 33]. Measuring the ability of leucocytes to migrate under different experimental conditions, it was originally developed as a test for allergy and cytotoxicity, and has been adapted to cancer research[34 35]. In the present study it has been used as a test for cytotoxicity.

One hundred heparinised blood samples (10-15 ml) were collected from patients undergoing venepuncture for other investigations and from volunteering members of staff. They were prepared by the method of Cochran *et al*[34 35]. Six 200-μlitre aliquots of the cells suspended in Eagle's medium containing 10% foetal calf serum and 1% antibiotic/antimycotic solution were prepared. Four of these aliquots were incubated with sodium fluoride to give final concentrations of fluoride ion of 0.5, 1.0, 2.0 and 20.0μg/ml, and 2 were control tubes incubated with medium only. In 15 cases, four additional aliquots containing mercury and paraquat at concentrations of 2.0 and 20.0μg/ml were used for comparison with known toxins. All aliquots were incubated for 3 hours at 37°C.

At the end of the incubation period, quadruplicate samples were taken from each 200-μlitre aliquot using microcapillary tubes and prepared for migration by the method of Cochran[34 35]. The cells were left to migrate at 37°C for 18 hours, after which time the migration areas were drawn using a light microscope with a drawing arm attachment. The migration areas were measured using a planimeter, and the quadruplicate values meaned. The migration index was calculated by dividing the means of the test samples by the means of the two controls. From these figures the percentage inhibition or enhancement of migration was calculated.

The results were analysed by the Student *t* test, the matched pairs, signed ranks test and McNemar's test[36].

Results

Table 1 shows the mean percentage inhibition of migration of the cells when incubated for 3 hours at 37°C with the four concentrations of fluoride. The mean percentage inhibition obtained for all four concentrations of fluoride was statistically significant at the $p < 0.01$ level. With the exception of 0.5 and 1.0μg/ml which were not measurably different, the means for the different concentrations also differed significantly ($p < 0.01$). There was a dose-dependent relationship between the concentration of fluoride and the mean percentage inhibition obtained. A plot of the logarithm of the dose against mean

Table 1:

Percentage inhibition of migration at the different concentrations of fluoride in 100 samples

	concentration (μg/ml)			
	0.5	*1.0*	*2.0*	*20.0*
Fluoride				
Mean	9.46	10.92	16.42	26.86
SD	11.17	13.89	12.57	20.88

percentage inhibition approximated to a straight line relationship over the range of 0.5 to 20.0μg/ml. (Regression equation: percent inhibition = 12.3 + 11.3 log concentration; r = 0.99; p = 0.007) (See *Figure 1*).

Figure 1:

Graph of logarithm of fluoride concentration against mean percentage inhibition of migration

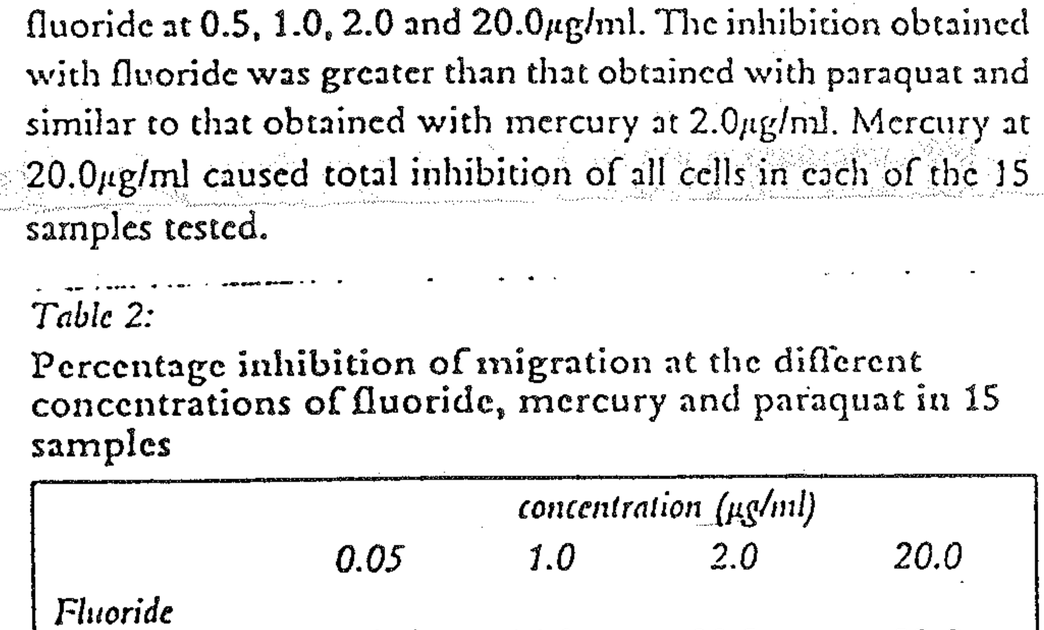

Table 2 shows the mean percentage inhibition of migration obtained in the 15 cases in which cells were incubated with mercury and paraquat at 2.0 and 20.0μg/ml in addition to fluoride at 0.5, 1.0, 2.0 and 20.0μg/ml. The inhibition obtained with fluoride was greater than that obtained with paraquat and similar to that obtained with mercury at 2.0μg/ml. Mercury at 20.0μg/ml caused total inhibition of all cells in each of the 15 samples tested.

Table 2:

Percentage inhibition of migration at the different concentrations of fluoride, mercury and paraquat in 15 samples

	concentration (μg/ml)			
	0.05	*1.0*	*2.0*	*20.0*
Fluoride				
Mean	10.0	14.3	22.9	33.8
SD	10.9	9.2	18.4	26.1
Paraquat				
Mean	–	–	12.1	25.2
SD	–	–	16.6	30.1
Mercury				
Mean	–	–	22.4	100
SD	–	–	11.3	0.0

Discussion

These results show that concentrations of fluoride of 0.5, 1.0, 2.0 and 20.0μg/ml significantly inhibit the ability of leucocytes to migrate after incubation for 3 hours at 37°C. This inhibition may be due to a direct toxic effect of fluoride on the leucocytes, but it is possible that fluoride could trigger the release of cytokines from the T-lymphocytes, which then act to inhibit macrophage activity.

Fluoride reacts with hydrogen bonds in biological molecules to form hydrogen-fluoride bonds thus distorting the hydrogen bonding responsible for the normal configuration of proteins and the active sites of enzymes[30]. The finding that fluoride has adverse effects on many aspects of body function is therefore not surprising. It has been claimed[29] that fluoride does not have adverse physiological effects below a concentration of 10μg/ml. It is, however, more likely that fluoride affects cellular metabolism at all concentrations but that in some systems this effect is not detectable until doses in excess of 10μg/ml are reached. The present series of experiments clearly demonstrate effects of fluoride as low as 0.5μg/ml.

Many of the experiments purporting to show evidence of harm have been carried out with concentrations in excess of those used in the fluoridation of public water supplies. These concentrations, however, are all much lower than those used in fluoridated toothpastes, topical gels and mouth rinses, which are often swallowed, particularly by young children. These experiments are therefore relevant to the general thesis that the toxic hazards of these dental measures have yet to be fully evaluated[8]. The present study covered the concentrations used in public water supplies and demonstrated that the ability of leucocytes to migrate was significantly impaired even at the lowest concentrations.

The immune system is our first line of defence against attack whether from the outside from bacteria, viruses and other parasites, or from within, from the spontaneous generation of potentially cancerous cells. Any agent which affects the ability of the immune system to function efficiently either by a direct toxic effect or by interfering with the release of cytokines will tend to reduce the resistance of the population to infection as well as increasing the susceptibility to cancer and immune depressed states such as the post-viral fatigue syndrome and AIDS. The effect on individuals already suffering from such immune-depressed conditions is likely to be serious.

In this study the cells were exposed to the various concentrations of fluoride for a period of 3 hours. Where fluoridation of public water supplies is undertaken, exposure will be continuous and life-long. Reported values for plasma fluoride range from 0.7 to 2.4μg/l[18 37], the conditions reproduced by the lower levels of fluoride used in this study. While some of the 100 blood samples showed little or no inhibition at the concentrations of fluoride used, some were very obviously affected, and the mean effect was a significant inhibition at all concentrations. A section of the population may therefore be at risk of compromised immune system function from water fluoridation schemes.

All recent large-scale surveys have shown minimal benefits to teeth from fluoridation programmes[1-6]. On the other hand, chronic exposure to fluoride at 1μg/ml could have a long-term detrimental effect on the general health of the population. Over the past 20 to 30 years there has been a substantial and unexplained rise in a number of conditions such as allergy, auto-immune diseases and the post viral fatigue syndrome. The common factor in these conditions is an alteration in the efficiency of the immune system.

Sheila LM Gibson *MD BSc MFHom*
Research Physician
Glasgow Homoeopathic Hospital

Acknowledgement
The author wishes to thank Dr Harper Gilmour of the Department of Public Health, University of Glasgow, for statistical advice.

References

1. Colquhoun J. Influence of social class and fluoridation on child dental health. *Comm Dent Oral Epidemiol* 1985; 13: 37-41.
2. Diesendorf M. The mystery of declining tooth decay. *Nature* 1986; 322: 125-129.
3. Gray AS. Fluoridation. Time for a new base line? *J Canad Dent Assoc* 1987; 53: 763-765.
4. Yiamouyiannis JA. Water fluoridation and tooth decay: results from the 1986-1987 national survey of US schoolchildren. *Fluoride* 1990; 23: 55-67.
5. Colquhoun J, Mann R. The Hastings fluoridation experiment: science or swindle? *Ecologist* 1986; 16: 243-248.
6. Hildebolt CF, Elvin-Lewis M, Molnar S *et al.* Prevalences among geochemical regions of Missouri. *Am J Phys Anthrop* 1989; 78: 79-92.
7. Smith GE. Is fluoride a matagen? *Sci Total Environ* 1988; 68: 79-96.
8. Whiford GM. The physiological and toxicological characteristics of fluoride. *J Dent Res* 1990; 69 (special issue): 539-549.
9. Diesendorf M, Sutton PRN. Fluoride: new grounds for concern. *Ecologist* 1986; 16: 237-242.
10. Spak C-J, Sjostedt S, Eleborg L *et al.* Tissue response of gastric mucosa after ingestion of fluoride. *Br Med J* 1989; 298: 1686-1687.
11. Fujii A, Tamura T. Deleterious effect of sodium fluoride on gastrointestinal tract. *Gen Pharmacol* 1989; 20: 705-710.
12. Spak C-J, Sjostedt S, Eleborg L *et al.* Studies of human gastric mucosa after application of 0.42% fluoride gel. *J Dent Res* 1990; 69: 426-429.
13. Rose D, Marier JR. In *Environmental Fluoride*. National Research Council of Canada, 1977 (no 16081).
14. Hedlund LR, Gallacher JC. Increased incidence of hip fracture in osteoporotic women treated with sodium fluoride. *J Bone Min Res* 1989; 4: 223-225.
15. Orcel Ph de Vernejoul MC, Prier A, Miravet L *et al.* Stress fractures of the lower limbs in osteoporotic patients treated with fluoride. *J Bone Min Res* 1990; 5 (supplement 1): s191-194.
16. Boivin G, Chavassieux P, Chapuy M-C *et al.* Skeletal fluorosis: histomorphometric findings. *J Bone Min Res* 1990; 5 (supplement 1): s185-189.
17. Bayley TA, Harrison JE, Murray TM *et al.* Fluoride-induced fractures: relation to osteogenic effect. *J Bone Min Res* 1990; 5 (supplement 1): s217-222.
18. Gutteridge DH, Price RI, Kent GN *et al.* Spontaneous hip fractures in fluoride-treated patients: potential causative factors. *J Bone Min Res* 1990; 5 (supplement 1): s205-215.
19. Okuda A, Kanehisa J, Heersche JNM. The effects of sodium fluoride on the resorptive activity of isolated osteoclasts. *J Bone Min Res* 1990; 5 (supplement 1): s115-120.
20. Carter DR, Beaupré GS. Effects of fluoride treatment on bone strength. *J Bone Min Res* 1990; 5 (supplement 1): s177-184.
21. Grynpas MD. Fluoride effects on bone crystals. *J Bone Min Res* 1990; 5 (supplement 1): s169-175.
22. Dooland MB, Wylie A. A photographic study of enamel defects among South Australian school children. *Aust Dent J* 1989; 34: 470-473.
23. Holt RD, Winter GB, Fox B, Askew R. Enamel opacities in children whose mothers took part in a dental health education scheme. *Comm Dent Oral Epidemiol* 1990; 18: 74-76.
24. Tsutsui T, Suzuki N, Ohmori M, Maizumi H. Cytotoxicity, chromosome aberrations and unscheduled DNA synthesis in cultured human diploid fibroblasts induced by sodium fluoride. *Mutat Res* 1984; 139: 193-198.
25. Thomson EJ, Kilanowski FM, Perry PE. The effect of fluoride on chromosome aberration and sister-chromatid exchange frequencies in cultured human lymphocytes. *Mutat Res* 1985; 144: 89-92.
26. Cole J, Muriel WJ, Bridges BA. The mutagenicity of sodium fluoride to L5178Y (wild-type and TK+/-((3.7.2c))) mouse lymphoma cells. *Mutagenesis* 1986; 1: 157-167.
27. Li Y, Dunipace AJ, Stookey GK. Genotoxic effects of fluoride: a controversial issue. *Mutat Res* 1988; 195: 127-136.
28. Aardema MJ, Gibson DP, Le Boeuf RA. Sodium fluoride-induced chromosome aberrations in different stages of the cell cycle: a proposed mechanism. *Mutat Res* 1989; 223: 191-203.
29. Scott D, Roberts SA. Extrapolation from in vitro tests to human risk: experience with sodium fluoride clastogenicity. *Mutat Res* 1987; 189: 47-58.
30. Edwards SL, Poulos TL, Kraut J. The crystal structure of fluoride-inhibited Cytochrome c Peroxidase. *J Biol Chem* 1984; 259: 12984-12988.
31. Rigalli A, Ballina JC, Roveri E, Puche RC. Inhibitory effect of fluoride on the secretion of insulin. *Calcified Tissue Int* 1990; 46: 333-338.
32. Soborg M, Bendixen G. Human lymphocyte migration as a parameter of hypersensitivity. *Acta Med Scand* 1967; 181: 247-256.
33. Wolberg WH. Inhibition of migration of human autogenous and allogeneic leukocytes by extracts of patients' cancers. *Cancer Res* 1971; 31: 798-802.
34. Cochran AJ, Spilg WGS, Mackie RM, Thomas CE. Postoperative depression of tumour-directed cell-mediated immunity in patients with malignant disease. *Br Med J* 1972; iv: 67-70.
35. Cochran AJ, Mackie RM, Ross CE *et al.* Studies of the immunology of melanoma patients. *Pigment Cell* 1976; 2: 182-190.
36. Siegel S. *Non-parametric Statistics for the Behavioural Sciences*. Tokyo: McGraw Hill Kogakusha.
37. Shen YW, Taves DR. Fluoride concentrations in the human placenta and maternal and cord blood. *Am J Obstet Gynecol* 1974; 119: 205-207.