Circulating haptoglobin of rabbits after acute and chronic fluoride toxicity

P. Jethanandani, K. Sharma and A.K. Susheela

Fluoride and Fluorosis Research Laboratories, All India Institute of Medical Sciences, New Delhi, India

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Introduction: Tissue damage due to fluorosis/fluoride toxicity is well documented [1–3]. Several *in vitro* studies have shown that fluoride stimulates cells involved in inflammation, such as poly-morphonuclear neutrophils, platelets and mast cells, causing the release of histamine, prostaglandins, eicosanoids, proteases and superoxide anions [4–6].

From these reports it seems that fluoride may induce an inflammatory reaction in the body. This is supported by the fact that fibrinogen, an acute phase reactant, is raised in experimental fluoride toxicity [7]. The levels of the seromucoid fraction are also raised in experimental fluoride toxicity [8]. Nedeljkovic *et al.* [9] have reported a decrease in body weight, decrease in plasma albumin (levels decrease in inflammation) and an increase in plasma levels of globulins and fibrinogen in rabbits administered 2–20 mg fluoride in drinking water for 35 days. Pillai *et al.* [10] studied the effect of sub-acute dosage of fluoride on male mice and observed a decrease in body weight gain and a decrease in serum total protein and albumin levels.

Earlier studies have shown that serum haptoglobin and C-reactive protein levels are raised in skeletal fluorosis patients as compared to individuals consuming the same water as patients but not exhibiting clinical manifestations of skeletal fluorosis as well as compared to individuals consuming water containing permissible levels of fluoride [11].

The present study deals with the quantitation of haptoglobin levels in an animal model of fluoride toxicity. We quantified haptoglobin levels in rabbits after a single large dose of fluoride, and, after prolonged administration of fluoride.

Materials and methods: Ten male rabbits weighing 1,000–1,200 g (obtained from Experimental Animal Facility, A.I.I.M.S., New Delhi) were randomly chosen and divided into two groups for the acute study. Rabbits were housed in individual cages and fed a pelleted diet (Hindustan Lever Ltd, Bombay) and water (containing < 0.5 ppm fluoride) *ad libitum*.

After an overnight fast, one group of five rabbits was orally administered 20 mg NaF kg^{-1} body weight as a 2% NaF solution in deionised water (NaF obtained from Qualigens Fine Chemicals, Glaxo India Ltd, Bombay). Rabbits were bled from the orbital plexus at 0, 2, 4, 6, 8, 12, 16, 21, 25, 37, 49 and 72 h after fluoride administration. The control group was also bled simultaneously at the same time intervals. Serum was separated and stored at −70°C.

For the chronic study, a separate group of 14 rabbits (800–1,000 g) were divided equally into two groups. Rabbits were housed in similar conditions in individual cages and fed the same diet and water as above. One group of seven rabbits was orally administered 10 mg NaF kg^{-1} body weight daily for a period of 18 months. Thereafter, control and treated rabbits were bled from the orbital plexus. Serum was separated and stored at −70°C.

Haptoglobin levels were quantitated according to the method of Roy *et al.* [12]. Standard haptoglobin was not available. Hence inflammation was induced in two normal rabbits by subcutaneous injection of 1 mL of turpentine oil in the dorso-lumbar region. Blood was drawn from the orbital plexus 48 h after turpentine oil treatment and pooled.

The haptoglobin-binding capacity of this 'standard serum' was determined by adding increasing amounts of this serum to a fixed amount of haemoglobin solution containing known amount of haemoglobin and determining the 'saturation point' of haemoglobin binding to the haptoglobin present in the 'standard serum'. From the saturation point, the haemoglobin-binding capacity of the 'standard serum' was calculated. From the net optical density of the samples their equivalence to x mL of 'standard serum' was obtained by extrapolation on the calibration curve and haemoglobin-binding capacity was calculated.

Fluoride levels in serum were determined according to the method of Hall *et al.* [13] using an ION85 ion analyser (Radiometer, Copenhagen).

The significance of the difference between the mean haptoglobin and fluoride levels of short-term fluoride-treated rabbits as compared to control rabbits was determined by the Freidman test with multiple range analysis. The significance of the difference between mean haptoglobin and fluoride levels of the long-term fluoride-treated rabbits as compared to controls was estimated using the Wilcoxon Rank sum test.

Results: The kinetics of fluoride after administration of a single dose of fluoride to rabbits are shown in Figure 1. Fluoride levels peaked at 4 h after fluoride adminstration ($p < 0.001$). The difference after 6 h of fluoride administration was significant at $p < 0.01$ and remained at this level up to 49 h after fluoride administration. At 72 h the difference was not statistically significant.

Figure 2 shows the kinetics of haptoglobin after administration of a single dose of fluoride. Haptoglobin levels of fluoride-treated rabbits started increasing 8 h after fluoride administration ($p < 0.01$), peaked at 37 h ($p < 0.01$) and remained significantly raised up to 72 h after fluoride administration ($p < 0.01$).

Table 1 shows the haptoglobin and fluoride levels of rabbits treated for prolonged periods with fluoride. There was a significant difference in the serum haptoglobin ($p < 0.01$) and fluoride ($p < 0.01$) levels between fluoride-treated and control animals using the Wilcoxon Rank sum test.

Discussion: Haptoglobin levels in the serum of short-term and long-term fluoride-treated rabbits were raised as compared with controls. The time taken for the haptoglobin to increase after a single dose fluoride administration, indicates that haptoglobin synthesis is increased [14]. Fluoride is a toxic ion and causes tissue damage. Hence, an inflammatory reaction leading to increased synthesis of haptoglobin seems likely.

Haptoglobin prevents loss of iron through the formation of

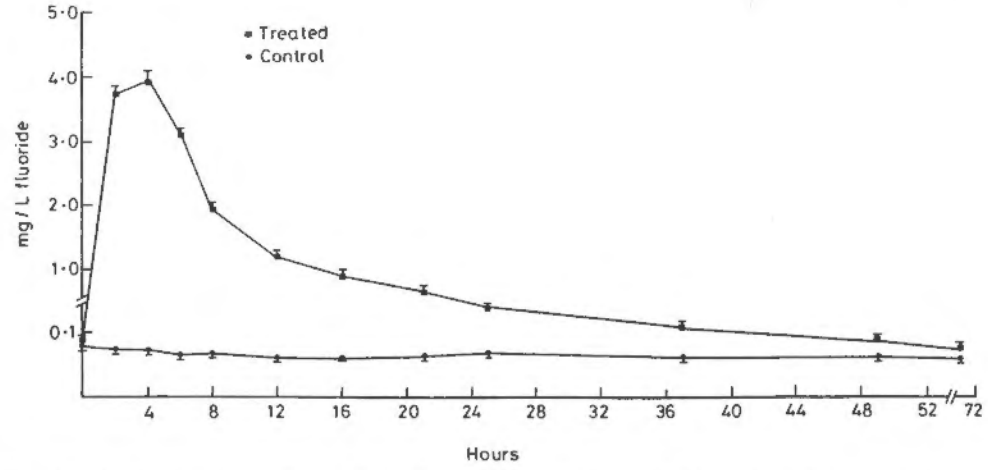

Figure 1: Fluoride kinetics in rabbits given a single dose of 20 mg NaF kg^{-1} body weight and in age- and sex-matched controls.

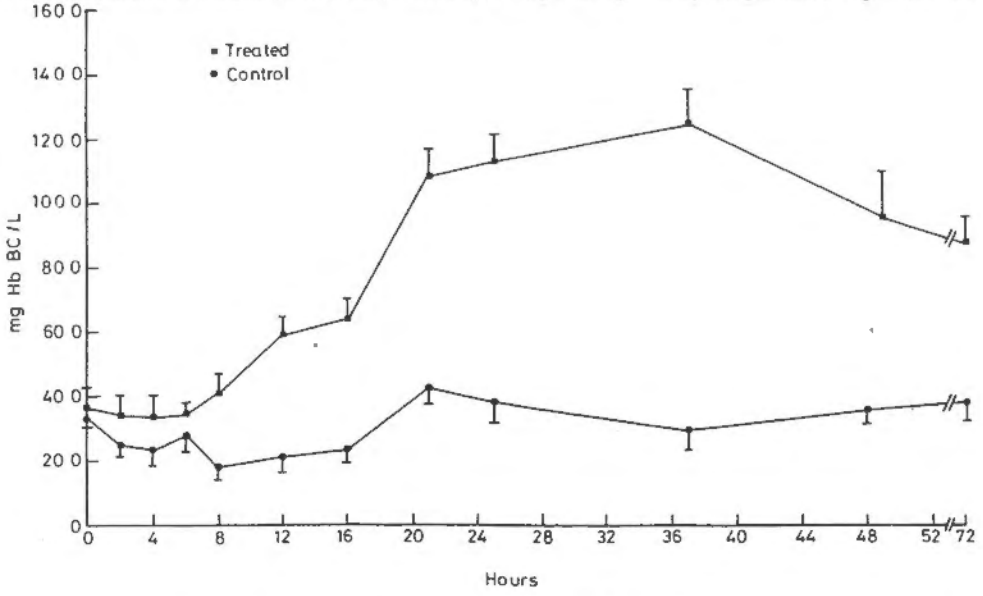

Figure 2: Haptoglobin kinetics in rabbits given a single dose of 20 mg NaF kg^{-1} body weight and in age- and sex-matched controls.

Table 1: Haptoglobin levels (mg HbBC L^{-1}) and fluoride levels (mg L^{-1}) in the sera of rabbits given 10 mg NaF kg^{-1} body weight daily for 18 months and in controls (means ± SD).

	Fluoride	Haptoglobin
Control (n = 7)	0.09 ± 0.02	355.2 ± 79.3
Treated (n = 7)	0.78 ± 0.12*	708.4 ± 109.0*

* *As compared with control,* $p < 0.01$. *HbBC = haemoglobin-binding capacity.*

the haptoglobin–haemoglobin complex [15] and also protects the kidney from damage caused by haemoglobin through the formation of the haptoglobin–haemoglobin complex. However, the exact role for haptoglobin in inflammatory conditions is not known. Haptoglobin has been reported to be an endogenous inhibitor of prostaglandin synthetase [16].

Haptoglobin also has anti-proteinase activity and may thus prevent secondary tissue damage caused due to an excessive action of proteinases [17]. Haptoglobin is also reported to be an anti-oxidant and prevents iron-stimulated formation of oxygen radicals [18].

Haptoglobin has recently been reported to have angiogenic properties [19] and may thus help in tissue repair. The haptoglobin–haemoglobin complex but not haptoglobin or haemoglobin in isolation has been reported to stimulate collagen synthesis [20]. From these properties, a role in the restoration of homeostasis can be ascribed to haptoglobin.

Fluoride causes a disturbance in bone metabolism. Low doses of fluoride intake for prolonged periods, result in osteosclerosis when calcium intake is adequate, whereas high fluoride intakes coupled with low calcium intake are associated with osteoporotic changes [21]. Haptoglobin has recently been reported to have a role in bone metabolism. Lerner and Frohlander [22] have reported that haptoglobin is capable of resorbing bone. Interleukins and growth factors are synthesised by osteoblasts [23, 24], and regulate bone metabolism. Haptoglobin and other plasma proteins are sequestered in the bone matrix [25]. These plasma proteins are released during bone resorption. Hence, local high concentrations of haptoglobin/interleukins/growth factors may interact and be involved in bone metabolism regulation during fluoride toxicity.

In conclusion, the results of this study demonstrate that fluoride toxicity is associated with increased serum levels of haptoglobin. The results confirm our earlier observations on an inflammatory condition being associated with patients of skeletal fluorosis.

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Reprint requests to: Professor A.K. Susheela, Department of Anatomy, All India Institute of Medical Sciences, New Delhi 110 029, India.

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