

## THE INFLUENCE OF SODIUM FLUORIDE ON THE CLONOGENECITY OF HUMAN HEMATOPOIETIC PROGENITOR CELLS: PRELIMINARY REPORT

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**SUMMARY:** Since fluoride accumulates not only in bones but also in bone marrow cavities where hematopoiesis occurs, a preliminary study was undertaken of the potential toxicity of sodium fluoride (NaF) against early human myeloid (CFU-GM, Colony Forming Unit of Granulocyte-Macrophages) and erythroid (BFU-E, Burst Forming Unit of Erythrocytes) progenitors. CD34<sup>+</sup> cells isolated from human umbilical cord blood were exposed for 30 and 120 min at 37°C or 4°C to increasing concentrations of NaF (0, 1, 10 and 50 mM). At 1 mM NaF, a detectable but not statistically significant stimulatory effect was observed in 7 of the 8 different sets of experiments. At 10 and 50 mM, however, NaF was significantly toxic against cord blood CFU-GM and BFU-E progenitors. Fluoride may therefore be potentially toxic toward early human hematopoietic cells.

Keywords: Clonogenicity, Cord blood CD34<sup>+</sup> cells, Hematopoiesis, Fluoride toxicity.

### INTRODUCTION

Fluorine compounds belong to an important group of chemicals that accumulate in our environment with increasing industrialization. Many different negative effects of fluorine compounds, particularly on growing organisms, have been reported.<sup>1-3</sup> Since fluoride (F<sup>-</sup>) accumulates in bone tissues,<sup>4,7</sup> it could conceivably affect the formation of hematopoietic (blood-forming) cells in bone marrow cavities. Although environmental fluoride pollution has been linked to increased morbidity from hematological diseases,<sup>2</sup> the influence of fluoride on human hematopoiesis has not yet been sufficiently investigated.

In this research we evaluated the effect of sodium fluoride (NaF) on the clonogenic growth of early human hematopoietic cells: CFU-GM (Colony Forming Unit of Granulocyte-Macrophages) and BFU-E (Burst Forming Unit of Erythrocytes).

### MATERIALS AND METHODS

*Cells:* Umbilical cord blood (60-120 mL) was obtained immediately after delivery of the placenta from five consenting healthy women. Normal light-density mononuclear cells (MNC) were depleted of adherent cells and T lymphocytes (A-T-MNC) as described.<sup>8</sup> CD34<sup>+</sup> cells were enriched from the A-T-MNC population by incubation with an anti-CD34 antigen (anti-HPC1) murine monoclonal antibody (Becton-Dickinson, Mountainview, CA) and subsequent immunoselection of antibody-labeled cells with magnetic beads according to the manufacturer's protocol (Dynal, Oslo, Norway). Briefly, 2x10<sup>7</sup> A-T-MNC were incubated in 1.5-mL Eppendorf tubes for 1 h in 1 mL of Iscove DMEM

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(Gibco, USA) + 5% Bovine Calf Serum (BCS) (Hyclone, USA) with 50  $\mu$ L of anti-CD34 antibodies (anti-HPC1) at 4°C on a rotating rack. The cells were then washed 3 times by centrifugation in Iscove DMEM + 5% BCS and again resuspended in 1 mL of Iscove DMEM + 5% BCS. Then 150  $\mu$ L of polystyrene beads (Dynal, Oslo, Norway) covered with sheep antimouse antibodies was added. The cells were incubated again for 45 min at 4°C on a rotating rack. They were then transferred to 5 mL polystyrene plastic tubes and collected for 2 min by employing a magnetic field (Dynal, Oslo, Norway). The magnetic separation was repeated 3 times. During each separation, the medium with the cells not attached to the tube walls was removed by gentle aspiration. After the third separation, the cells were resuspended in Iscove DMEM + 10% BCS. Their viability was assayed by a trypan blue exclusion test. The cells were counted with a hemocytometer and subsequently used for further experiments.

*Exposure to sodium fluoride (NaF):*  $2 \times 10^4$  CD34<sup>+</sup>A<sup>-</sup>T<sup>-</sup> cells were resuspended in 1 mL of PBS + 5% BCS and then were exposed to different concentrations of NaF: 0 mM (control), 1 mM, 10 mM and 50 mM for 30 and 120 min at 37°C and 4°C. After incubation the cells were washed twice in Iscove DMEM and subsequently used for clonogenic assays.

*Cell cultures:* CD34<sup>+</sup> cells ( $2 \times 10^4$ ) were resuspended in 0.4 mL Iscove DMEM and mixed with 1.8 mL methylcellulose medium MethoCult HCC-4230 (StemCell Technologies Inc., Vancouver, Canada) supplemented with L-glutamine (0.125 mM). The appropriate growth factors were added to the mixture, which was then transferred to 3.5-cm plastic petri dishes and incubated (37°C, 95% humidity, 5% CO<sub>2</sub>) for the period of time appropriate for the formation of the particular colonies.

Growth factors employed for colony stimulation were as follows: Colony Forming Units of Granulocytes-Macrophages (CFU-GM): interleukin-3 (IL-3, 20 U/mL) (Genetics Institute, USA) + granulocyto-macrophagopoietin (GM-CSF, 5 ng/mL). Burst Forming Units of Erythrocytes (BFU-E): erythropoietin (Epo; 5 U/mL) (Amgen, USA) + kit ligand (KL; 100 ng/mL) (Immunex, USA). Recombinant human growth factors were employed in all experiments. Colonies were counted with an inverted microscope on day 11 (CFU-GM) and on day 14 (BFU-E). Cultures were performed in quadruplicate.

*Statistical analysis:* Arithmetic means and standard deviations were calculated on an IBM computer using MS Excel v. 97. Data were subjected to statistical analysis using the Student t-test for unpaired samples. Statistical significance was defined as  $p < 0.01$ .

## RESULTS

*Effect of incubating CD34<sup>+</sup> cells with NaF at 37°C on the CFU-GM and BFU-E colony formation:* Figures 1 and 2 show the effect of incubating cord blood CD34<sup>+</sup> cells with increasing levels of NaF on the clonogenic growth of CFU-GM and BFU-E. The cells were exposed to NaF at 37°C for 30 (Figure 1) and

120 min, respectively (Figure 2). After incubation, the cells were washed twice with PBS and plated in the methylcellulose based cloning medium.

As shown in Figures 1 and 2, the lowest concentration of NaF (1 mM) caused little or only a weak stimulatory effect on the growth of human CD34<sup>+</sup> cells. In contrast, the higher concentrations of NaF (10 and 50 mM) damaged CFU-GM and significantly decreased the cloning potential of these cells. We found that the growth of CFU-GM was inhibited similarly after 30 and 120 min of exposure to NaF. At the same time, BFU-E growth was also inhibited, particularly after a prolonged exposure to the highest concentration of NaF (Figures 1 and 2).

*Effect of incubating CD34<sup>+</sup> cells with NaF at 4°C on the CFU-GM and BFU-E growth:* Figures 3 and 4 show the influence of increasing NaF concentrations on colony formation by human cord blood CD34<sup>+</sup> cells exposed to NaF at 4°C for 30 (Figure 3) and 120 min respectively (Figure 4). After exposure, cells were washed twice with PBS and plated in the methylcellulose cultures.

As shown in Figures 3 and 4, low levels of NaF (1mM and 10 mM) did not significantly inhibit the growth of CFU-GM and BFU-E progenitors, which have been exposed to NaF before plating at 4°C. Colony formation by CFU-GM and BFU-E was at first slightly inhibited after exposure to the highest concentration of NaF (50 mM). Nevertheless, even after exposure to the highest levels of NaF, the inhibition of colony formation by CFU-GM and BFU-E was not statistically significant.

**Figure 1.** Influence of 30-min NaF exposure on myeloid and erythroid colony formation (CD34<sup>+</sup> cells incubated at 37°C before plating)

**Webmaster's note:  
charts not available on the internet**

**Figure 2.** Influence of 120-min NaF exposure on myeloid and erythroid colony formation (CD34<sup>+</sup> cells incubated at 37°C before plating)

**Figure 3.** Influence of 30-min NaF exposure on myeloid and erythroid colony formation (CD34<sup>+</sup> cells incubated at 4°C before plating)

**Figure 4.** Influence of 120-min NaF exposure on myeloid and erythroid colony formation (CD34<sup>+</sup> cells incubated at 4°C before plating)

#### DISCUSSION

Because environmental fluoride pollution has increased due to industrial emissions, the potential influence of fluoride on humans is a very important issue. One of the most important mechanisms responsible for damage to living cells exposed to NaF is the inhibition of the respiratory enzymes in the mitochondria.<sup>9</sup> Cells which show intensive metabolism are particularly affected by this mechanism.<sup>2,9</sup> The majority of publications describe the influence of the fluorides on the metabolism of the hard tissues.<sup>6,7,10</sup> In contrast, the direct effect of the NaF on human hematopoiesis has not been extensively studied. In one publication, children living in industrial regions polluted by fluoride showed a decrease in hemoglobin levels and an increase in the number of erythrocytes in peripheral blood.<sup>1</sup> Similarly, it has been reported that NaF in relatively low doses (5 mM) significantly inhibits phagocytosis of mature neutrophils.<sup>11</sup>

Since hematopoiesis takes place in bone marrow cavities, NaF could conceivably directly interfere with the proliferation and differentiation of hematopoietic cells. Unfortunately, the influence of fluoride on human hematopoietic progenitors has not yet been addressed in the literature. We were especially interested in examining the influence of fluoride on the proliferation of human hematopoietic progenitors. Since the most primitive early hematopoietic progenitors possess few mitochondria, they show much lower metabolic activity than more mature hematopoietic cells.<sup>12</sup> On the other hand, they express P-glycoprotein on the surface, which is responsible for active elimination of toxic substances from the cytoplasm.<sup>12</sup>

Our findings show that a low concentration of NaF (1 mM) may have a mild but not significant stimulatory influence on the clonogenicity of CB CD34<sup>+</sup> cells. However, at higher concentrations (10 and 50 mM) NaF was found to be toxic against CB myeloid (CFU-GM) and erythroid (BFU-E) progenitors. These results are in agreement with data obtained on mature fibroblasts and granulocytes, in which NaF at a concentration of 20 mM significantly inhibited the biological functions of these cells.<sup>13</sup> The toxic influence of NaF on the growth of early human hematopoietic progenitors could partially explain hematological changes described in children exposed to NaF.<sup>1</sup>

We conclude that prolonged exposure to NaF may be potentially toxic against early human hematopoietic cells. Therefore, the influence of fluoride on human hematopoiesis needs further studies in appropriate experimental models.

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