EFFECTS OF SODIUM FLUORIDE INGESTED BY LACTATING MICE ON SOME HAEMATOLOGICAL PARAMETERS IN SUCKLING PUPS AND DAMS

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SUMMARY: Because it accumulates in bones, fluoride (F) could conceivably affect the formation of haematopoietic cells in bone marrow cavities. The present study was undertaken to assess the overall effect of sodium fluoride (NaF) on haematopoietic cells in mice. Exposure of pregnant female Wistar mice to 500 ppm NaF (226 ppm F–) in their drinking water from the 15th day of pregnancy until day 14 after delivery induced in their suckling pups and the mothers a reduction in some haematological parameters such as red blood cell number (RBC) and haemoglobin (Hb) concentration, by 59% and 30%, respectively. However, haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) remained unchanged. Consequently, anaemia in our findings was typically normocytic, which may be due to induced deficiencies of folic acid, vitamin B12, iron, or copper, thereby causing disruptive effects on erythropoiesis. Moreover, it has been demonstrated that F causes enhanced production of superoxide radicals and lipid peroxidation that lead to alterations in erythrocyte cell membrane function and structure. In fact, our study revealed a decrease in red blood cell activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which act as antioxidants to scavenge free radicals and lipid peroxidation products.

Keywords: Anaemia; Folic acid; Haemoglobin; Iron; Sodium fluoride; Suckling mice; Vitamin B12.

INTRODUCTION

Fluoride (F) is a major environmental pollutant, entering the biosphere from such sources as iron and steel operations, coal power production, aluminium smelting, and phosphate fertilizer manufacturing.1 In previous papers we reported that 500 ppm sodium fluoride (226 ppm F–) in drinking water, caused impairment of soft tissues in mice: kidney,2 liver3 and thyroid functions,4-6 retarded growth, and altered bone.6 Structural bone changes may also occur under chronic F exposure. Since F ion accumulates in bone tissues,7,8 it could conceivably affect the formation of haematopoietic (blood-forming) cells in bone marrow cavities.1 In fact, F ion can inhibit or activate various functions in blood cells. Neutrophils affected by F ion exhibit increased oxygen intake and production of superoxide anion along with decreased phagocytic activity.9 F ion affects erythrocyte membrane transport systems e.g inhibits K+/Cl– co-transport10 in erythrocyte suspended in hypo-osmotic medium. Alteration in cation pump activity, caused by F, occurs as a direct inhibition of Na+/K+ -ATPase.11 Moreover, F in vitro and in vivo has enhanced generation of superoxide radicals (O2–) and lipid peroxidation in polymorphonuclear leucocytes and in tissues of fluorosed animals leading to alteration in cell membrane function and structure.12-14
In addition, development of anaemia has been also demonstrated in human and in experimental animals chronically exposed to toxic amounts of F. Anaemia is one of the most prevalent health problems in the world and the high risk groups are pregnant women and young children.

Although environmental F pollution has been linked to increased morbidity from haematological diseases, the influence of F on human haematopoiesis has not yet been sufficiently investigated and appears to be lacking for newborn mice.

The purpose of this study was to investigate effects of NaF ingested by pregnant and lactating mice on haematopoietic parameters of dams and their pups during the suckling period.

**MATERIALS AND METHODS**

The same protocol was followed as in our previous papers. Twenty pregnant mice were randomised into two groups of ten animals in each group. The first group of animals did not receive supplemental fluoride and was considered as the untreated or control group. The second group was administered 500 ppm of NaF (226 ppm F⁻) in their drinking water from day 15 of pregnancy until day 14 after delivery. The study was approved by the local Ethical Committee for Experimental Use at the Faculty of Sciences, Sfax University.

All pups (n = 96) and their mothers (n = 12) were sacrificed on postnatal day 14. After fatal anaesthesia by intra-abdominal injection with chloral hydrate, blood samples were collected from the brachial artery in 5 mL test tubes containing EDTA.

On the same day blood samples were collected, the following blood cell parameters were assessed: Hb, Ht, RBC, and cell volume of MCV (mean corpuscular volume), MCH (mean corpuscular haemoglobin), and MCHC (mean corpuscular haemoglobin concentration). A hematology analyser Coulter MAXM (Beckman Coulter, Inc. Fullerton, USA) was used to determine these parameters.

Other blood samples were collected into heparinized tubes. The blood was centrifuged at 2200 g for 10 min and plasma was removed and kept at −20°C until analysis of iron and copper by colorimetric method (BIOMERIEUX kit, France, ref: 61075) and by atomic absorption, respectively, and of folate and vitamin B₁₂ measurements by immuno-electro-chemiluminescence analysis (Elecsys Folate Immunoassay and Elecsys B₁₂ Immunoassay for Elecsys 2010 System; ROCHE diagnostics, USA).

The erythrocytes were carefully sampled from the bottom of the tubes to minimize contamination with leukocytes. They were then washed four times with isotonic saline solution and lysed by addition of double distilled water (1V/4V). Antioxidant enzymes (AOEs) were measured on the same day of collecting. SOD activity was measured with RANSOD kits (Randox Laboratories, Crumlin, UK, ref: SD 125). GSH-Px activity was determined with RANSEL kits (Randox Laboratories, Crumlin, UK, ref: RS 505).

Comparisons of mean values between treated and control animals were made using Student’s t test. Statistical significance was defined as a P value of less than 0.05.
RESULTS

Compared to the controls, the NaF-treated mothers and their pups showed a significant decrease in the number of red blood cells (–11 and –59%, respectively) and in haemoglobin concentrations (–12 and –30%, respectively). Other erythrocyte parameters such as Ht and cell volume of MCV, MCH, and MCHC did not change significantly after NaF treatment (Table).

Table. Effect on erythrocyte parameters of adult mice and their pups administered NaF in the drinking water (0.5 g/L) from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth.

<table>
<thead>
<tr>
<th>Erythrocyte parameters</th>
<th>Mother (Mean ± SD)</th>
<th>Offspring (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RBC count (10^6/mm³)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (6)</td>
<td>7.753±0.517</td>
<td>4.317±0.531</td>
</tr>
<tr>
<td>NaF groups (6)</td>
<td>6.914±0.141†</td>
<td>1.766±0.444†</td>
</tr>
<tr>
<td><strong>Haemoglobin (g/dL)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Controls (6)</td>
<td>13.233±0.825</td>
<td>8.461±0.661</td>
</tr>
<tr>
<td>NaF groups (6)</td>
<td>11.601±0.254*</td>
<td>5.951±1.261†</td>
</tr>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (6)</td>
<td>36.93±1.86</td>
<td>25.11±3.15</td>
</tr>
<tr>
<td>NaF groups (6)</td>
<td>34.52±2.52</td>
<td>27.01±5.01</td>
</tr>
<tr>
<td><strong>MCV (mm³/RBC)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (6)</td>
<td>49.86±2.63</td>
<td>57.91±3.17</td>
</tr>
<tr>
<td>NaF groups (6)</td>
<td>48.01±2.01</td>
<td>57.33±3.17</td>
</tr>
<tr>
<td><strong>MCH (pg/RBC)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (6)</td>
<td>15.96±0.56</td>
<td>18.91±1.12</td>
</tr>
<tr>
<td>NaF groups (6)</td>
<td>16.71±0.31</td>
<td>17.01±2.11</td>
</tr>
<tr>
<td><strong>MCHC (g/dL)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (6)</td>
<td>32.01±0.52</td>
<td>31.93±1.66</td>
</tr>
<tr>
<td>NaF groups (6)</td>
<td>34.61±1.81</td>
<td>30.71±2.81</td>
</tr>
</tbody>
</table>

RBC : Red blood cell.
MCV : Mean corpuscular volume.
MCH : Mean corpuscular haemoglobin.
MCHC : Mean corpuscular haemoglobin concentration.

Significant differences: NaF vs Controls: * p≤0.01, † p≤0.001.
Exposure of mothers to 500 ppm NaF also altered haematopoietic factors in plasma, especially folic acid and vitamin B\textsubscript{12}, which, compared to their controls, decreased by 36 and 47\% in dams and by 30 and 54\% in their pups, respectively (Figure 1).

**Figure 1.** Effect on plasma folic acid and vitamin B\textsubscript{12} levels of adult mice and their pups administered NaF in the drinking water (0.5 g/L) from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth. Significant differences: NaF vs Controls: *\(p \leq 0.01\); †\(p \leq 0.001\).
Plasma levels of iron and copper decreased in NaF-treated dams by 54 and 43%, respectively, and in their pups by 50 and 24%, respectively (Figure 2).

**Figure 2.** Effect on plasma iron and copper levels of adult mice and their pups administered NaF in the drinking water (0.5 g/L) from the 15th day of pregnancy until sacrifice of the pups on the 14th day after their birth. Significant differences: NaF vs Controls: *p ≤ 0.01; †p ≤ 0.001.
At the same time, SOD and GSH-Px activities, in red blood cells of F-treated mice, decreased significantly by 38 and 49% in dams and by 28 and 42% in their pups, respectively (Figure 3).

**DISCUSSION**

In this study, mice that were administered F showed abnormalities in some blood cell parameters. We observed a significant decrease in RBC counts and haemoglobin content as found previously in adult rats,19,20 weanling rats,21 rabbits,22 dogs,23 and children24 exposed to lower doses of F. Our results concerning other erythrocyte parameters (Ht, MCV, MCH, and MCHC) were in agreement with other findings on human red blood cells incubated in F solution.25 The anaemia that developed in these studies was typically normocytic. The anaemia observed in our study and in another report26 may result either from inhibition of globin synthesis by F or from reduced food consumption by dams.
treated with NaF, as it has been found in our previous study.6 This last hypothesis is confirmed by data of Harris et al.,27 who explained anaemia as the result of reduced intake of dry matter after excessive F ingested by cattle and sheep.

Moreover, genesis of anaemia could also be due to deficiency in some hematopoietic factors (iron, folic acid, and vitamin B12) as shown by our results and as suggested by previous findings of Hoogstratten et al.28 and Sahashi et al.29 These factors are essential for normal haematopoiesis which is critical for DNA synthesis and cellular division.30 In fact, folate serves as a coenzyme in single-carbon transfers in the metabolism of nuclei and amino acids. It is required for the synthesis of purines and pyrimidines that are needed for DNA production and erythropoiesis. A deficiency of folate causes abnormal cell replication, particularly in the erythropoietic system, and results in megaloblastic anaemia.30 This type of anaemia also results after vitamin B12 deficiency, since this vitamin is required for normal erythrocyte production.31

In addition, the present investigation revealed a marked decrease in plasma iron. This finding concurs with results showing that F in excess interacts with iron and alters its metabolism.19 The main function of iron in the body is in erythropoiesis.32 It serves as a functional component of iron-containing proteins including haemoglobin and myoglobin.31 In pregnant women a deficit of iron often coexists with deficiencies of vitamin B12 and/or folate,33 which are vital nutrients for cell division and DNA synthesis. Their deficiency can lead to megaloblastic anaemia.34

An interesting finding throughout the present study is that the anaemia associated with folic acid and vitamin B12 deficiency was typically normocytic. Similar results have been reported by Arinzon et al.,35 in psychogeriatric patients, in whom megaloblastosis appeared about 19 weeks after administration of a folate-deficient diet and may be masked by iron deficiency, as also found here.

On the other hand, iron deficiency anaemia can also be due to copper deficiency. Copper is crucial for iron absorption and therefore for haemoglobin formation and prevention of anaemia.36,37 Copper depletion is known to affect not only the transport of iron but also its utilization for the synthesis of haemoglobin.38 Low haematological levels of copper are associated with deficiency of ceruloplasmin and cytochrome C oxidase, which are copper-dependent enzymes required for iron metabolism.39 Previous studies indicate that reticulocytes from copper-deficient swine assimilated iron poorly, and heme synthesis was impaired.40 The data clearly showed that animals and humans needed copper to utilize iron.41 There is ample evidence that copper deficiency decreases erythrocyte half-life, possibly due to lipid peroxidation of the red blood cell membrane, resulting in the accumulation of free radicals by reduced antioxidant activities,39 in agreement with many studies showing that F induces excessive production of oxygen free radicals42,43 and causes injury by lowering activities of certain antioxidants44 that scavenge free radicals and lipid peroxidation products.45 Our results support these findings.

A decrease in the activity of free radical scavenging enzymes, SOD and GSH-Px, was also found in people living in areas of endemic fluorosis.46 Likewise, Shivarajashankara et al.,43 recorded increased lipid peroxidation and
decreased SOD activity and GSH level in erythrocytes of children with skeletal fluorosis. In experimental animals decreased levels of GSH and reduced activities of GSH-Px in erythrocytes\(^47\) and of SOD in other tissues\(^48\) were observed.

We conclude that F, ingested by lactating mice, has a toxic effect on bone marrow haematopoietic cells in both dams and their suckling pups. Our study showed a normocytic anaemia in pups and in their mothers. High F intake in the early stage of life appeared to have a pronounced toxic effect on haematopoietic cells. This may be explained by transplacental transfer of F from the mother to its foetus during the pregnancy period.

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