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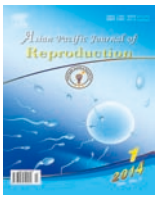

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Effect of sodium fluoride in maternal and offspring rats and its amelioration

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ABSTRACT

High fluoride content is known to cause dental and skeletal abnormalities. In addition, present review indicates that sodium fluoride consumption caused increased number of r=esorptions and dead foetuses. Various skeletal anomalies such as wavy ribs, presence of 14th ribs, lacking 6th sternebrae and incompete ossification of skull occur. All these changes could be due to oxidative stress caused by fluoride consumption. Fluoride-induced changes could be successfully ameliorated by cotreatment with vitamins and calcium.

1. Introduction

Teratogenesis is the formation of congenital defects. Correlation between congenital malformation and chemicals was not suspected to exit because there was a tendency among toxicologists to assume that the natural protective mechanisms such as detoxification, elimination and placental barrier were sufficient to shield the embryo from natural exposure to chemicals. However, the natural protective mechanisms were ineffective against ionizing radiations, viruses and nutritional deficiencies[1].

A teratogen has been defined as a chemical that increases the occurrence of structural and/or functional abnormalities in offspring if administered to either parent before conception, the female during pregnancy or directly to the developing organism. Many chemicals have been shown to cause embryotoxicity of some form. Some agents are predominantly lethal whereas others are predominantly able to produce malformations in the embryo. The difference in the type of embryotoxic effect induced by various chemical agents is mainly in dose requirements and the time during gestation when the foetus is exposed to test compound.

Malformations of the foetus due to chemicals are rare when exposure of the mother to a compound occurs only prior to implantation of the fertilized ovum. Also in early stages of undifferentiated cell multiplication, the cells of the foetus are not susceptible to the teratogenic effects of the chemicals. Thus the stage of the development of the foetus determines susceptibility to teratogenic agent; specific damage occurs readily during organogenesis. When organogenesis completed, the chemical-induced malformation of organs does not occur[1].

Toxic effects on the embryo are experimentally obtained by administering the agent to mother. The embryo relies on the maternal organism for its growth and maintenance. At different times, the placenta may act as a more or less efficient barrier for transfer of an agent from the mother to embryo. If an agent produces toxicity to the mother, such an effect would be expected to influence the intrauterine environment of the foetus.

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In an effort to reduce the incidence of dental caries, fluoride has been added to drinking water. Sodium fluoride (NaF) is commonly used in the fluoridation of drinking water. Now in addition to drinking water, adults, children and pregnant females ingest fluoride with foods and beverages prepared with fluoridated water; and they are also exposed to fluoride that had been anticipated. In many countries fluoride content in drinking water is much higher than safe tolerance limit (>1 μg/mL) causing fluorosis [2,3].

2. Fluoride as a teratogen

Messer *et al.* [4] reported that low levels of fluoride in food rendered mice infertile while a high fluoride diet improved their fertility. Clinical studies revealed that occupational exposure to organic fluoride causes abnormal menstruation and increases the frequency of miscarriages and pregnancy complications among female workers of fluoride factories [5]. Women exposed to high fluoride concentrations in drinking water showed decreased birth rates [6].

Transplacental passage of fluoride in human beings and animals has been shown [7-10] and incorporation into foetal tissues after passage through the placenta has been shown [11]. Maternal -foetal transport of fluoride across the placenta in mothers exposed to high intake of fluoride during pregnancy and subsequent transfer of fluoride through breast milk to new borne has been reported [8].

In the early 1990s, existing NaF studies were reviewed in several reports [12-14] and studies of reproductive effects were considered inadequate to determine potential reproductive or developmental hazards. Thereafter, several studies have been done on the effects of fluoride on the in utero development of rat [15,16] and rabbits [16].

Collins *et al.* [15] tested effect of oral administration of 0, 10, 25, 100, 175 and 250 μg/mL NaF daily throughout gestation in rats. They observed that NaF had no effect on the development of specific bones including sternebrae. A significance increase was seen in average number of foetuses with three or more skeletal variations in the 250 μg/mL group; the number of litters of foetuses with three or more skeletal variations was increased in 250 μg/mL also, but the increase was not significant.

Al-Hiyasat *et al.* [17] studied the toxic effects of different concentrations of NaF (200, 400 and 600 μg/mL) administered in drinking water for 30 days, on the reproductive system of adult female Sprague-Dawley rats. Ingestion of 200 μg/mL NaF had no effect on the pregnancy rate of rats nor on the number of implantations. However, the number of viable foetuses was significantly lower than that in the control group. Furthermore, the pregnant rats with resorptions and the total number of resorptions increases in the NaF-treated groups.

In another experiments, rats and rabbits were exposed to NaF in drinking water for 10 and 14 days of pregnancy respectively. The no observed adverse effect level (NOAEL) for maternal toxicity was about 18 mg/kg body weight / day for both species (150 μg/mL for rats and 200 μg/mL for rabbits) based on decreased water consumption and a reduction in maternal body weight gain. No convincing reproductive effects were seen even at the maximum tested concentrations providing doses of 27 mg/kg body weight for rats and 29 mg/kg body weight for rabbits [16].

Collins *et al.* [18,19] studied the effects of NaF ingestion at 0, 25, 100, 175 and 250 μg/mL in drinking water in rats throughout three generations. Decreased fluid consumption observed at 175 and 250 μg/mL was attributed to decreased palatability and did not affect reproduction. No cumulative effects were observed in three generations. Mating, fertility and survival indices were not affected [18]. Number of corpora lutea, implants, viable foetuses and foetal morphological developments were similar in all groups. However, ossification of the hyoid bone of F2 foetuses was significantly reduced at 250 μg/mL. Because of the decreased ossification of the hyoid bone, 250 μg/mL (25.1 mg/kg body weight) is considered the effect level [19].

However, Ream *et al.* [20] reported that the amount of fluoride crossing the placenta is insufficient to produce morphological changes in bones of weanling rats born to dams given 150 μg/mL of fluoride. Also fluoride hardens and calcifies the blood vessels, thus blood flow to growing foetus is hampered leading to repeated abortions and still birth [21].

Oral administration of NaF (20, 40 & 80 mg/kg body weight/day) orally from day 6 to 19 of gestation in rats significantly lowered body weight gain and feed consumption. No external malformations were observed in any of NaF-treated dams. Increased numbers of resorptions/ dead foetuses were observed in 40 and 80 mg NaF-treated rats. An increase in incidence of skeletal abnormality such as presence of 14th ribs, wavy ribs, dumbbell shaped 6th sternebrae, incomplete skull ossification were observed in foetuses of NaF-treated dams. Visceral abnormalities such as subcutaneous haemorrhage were also observed [22].

3. Fluoride causes oxidative stress

Increased number of resorptions and dead fetuses could be due to oxidative damages [23-25]. High fluoride levels causes accumulation of large amounts of free radicals and peroxides by inhibiting superoxide dismutase and glutathione peroxidase activities causing cell damage in people living in areas endemic to fluorosis [26]. Fluoride causes inhibition of superoxide dismutase, glutathione peroxidase and catalase in the ovary and increased lipid peroxidation causing tissue injury. It mainly causes denaturation of proteins and peroxidation of membrane lipids with increased permeability of cell membrane [27]. Malondialdehyde (MDA) the marker of extent of lipid peroxidation, was elevated in the brain of rats treated with 100 μg/mL fluoride. Also levels of total glutathione, reduced glutathione (GSH) and ascorbic acid were decreased. Increased oxidative stress could be modulating factor in the pathogenesis of fluoride toxicity in the brain and also in the liver [28].

Fluoride content in foetal skeleton and teeth increases with the age of the foetus and with the fluoride concentration in the drinking water consumed by the mother [29]. Fluoride has been reported to decrease the bone quality of femoral shaft and neck in young growing rats in 30 and 60 mg fluoride/L treated young rats of 6 week old [30]. Fluoride affects bone strength replacing hydroxyl ion in bone crystals to form fluoroapatite and increasing both osteoblast and osteoclast activities [31]. Fluoride has a potent effect on bone cell structure, function and strength. Bone in which fluoride ions are incorporated is more resistant to bone remodeling and thus lead to more brittle skeleton as it ages [32]. The presence of 14th rib could be due to increased osteocytic cellularity as reported by Vigorita and Suda [33] and is a pathologic marker of fluoride-induced abnormal bone formation.

Various reports have suggested that fluoride crosses the placenta in a number of species including rats [15], guinea pigs [34], rabbits [35] and Holstein cows [36].

4. Fluoride and hypocalcaemia

The potential of NaF to affect serum cations was assessed in the parental and F_1 generation rats. The sperm positive pregnant female rats received 40 mg NaF/kg body weight/day from day 6 of gestation to either up to 21 days of lactation or only up to gestation followed by withdrawal of the treatment during day 21 of lactation. Blood samples were collected from both P and F_1 generation rats and analyzed for glucose, protein, sodium, potassium, calcium and phosphorus concentrations. While sodium and potassium concentrations were significantly higher in P and F_1 generation rats, glucose, protein [37], calcium and phosphorus concentrations were significantly lower [38] than the controls. Withdrawal of treatment during lactation caused significant amelioration in all these effects.

Many investigators [39, 40] have reported the occurrence of hypocalcaemia during fluorosis. Hypocalcaemia observed in our study [38] might be due to decreased calcium absorption from the gut. With a high fluoride intake, insoluble calcium fluoride is formed in the intestine and excreted in faeces increasing the likelihood of low blood calcium if there is an insufficient dietary intake. In turn, hypocalcaemia may lead to parathyroid stimulation with a secondary hyperparathyroidism, bone matrix resorption, osteoporosis and osteomalacia [41]. The lowered level of phosphorus in NaF- treated P and F_1 generation rats might be due to parathyroid stimulation.

Hypocalcaemia observed in NaF-treated rats might be responsible for skeletal abnormalities. Collins *et al.* [15] observed a significant increase in the average number of foetuses with three or more skeletal variations in sperm-positive female rats given 250 μg/mL (25.1 mg/kg body weight) NaF daily throughout gestation. On the basis of these results we postulated that perhaps prenatal and postnatal exposures of NaF (250 μg/mL or above) could adversely affect the skeletal development.

Sodium and potassium concentrations increased significantly in the serum of NaF-treated p and F1 generations rats [38]. This could be due to fluoride-induced potassium efflux from cells [42]. Wang *et al.* [43] reported leakage of lactate dehydrogenase from cytosol of fluoride exposed cultured myocardial cells even at lowest level studied, of 3.23 μg/mL. High fluoride concentration is likely to inhibit superoxide dismutase and glutathione peroxidase activities resulting in accumulation of large amounts of free radicals and peroxides causing cell damage [23-25, 44]. Marked hypoglycemia was observed in both P and F_1 generation rats treated with NaF [38]. This could be due to reduced feed consumption. Barot [45, 46] also reported a significant decline in blood glucose levels in a fluoride-afflicted human population of North Gujarat, India. However, hyperglycemia was reported in rats administered fluoride [47, 48] and in inhabitants exposed

to environmental fluoride from aluminium smelters [49]. These differences might be due to variations in dose, duration of exposure, sensitivity of species and physiological status of the animals.

Serum protein content was reduced significantly in NaF-treated P-generation females and F_1 generation pups [38]. Fluoride is well known to affect protein synthesis by causing impairment of polypeptide chain initiation [50, 51], weak incorporation of amino acids into protein [52] abnormal accumulation or inhibition of RNA synthesis [53]. Decreased protein synthesis during fluorosis has been reported [54, 55]. Zang *et al*. [41] reported a significant decrease in serum proteins in individuals with poor nutrition and living in high fluoride areas.

5. Amelioration

Withdrawal of NaF treatment during lactation caused significant recovery in serum changes in both P and F_1 generation rat. Partial recovery in serum cations on withdrawal of NaF treatment has been reported by Verma and Sherlin [38] in rat. Meng *et al*. [56] have also reported reversion in osteofluorosis patients after defluoridation of drinking water.

The significant recovery in body weight and feed consumption on co-treatment with vitamins C, D and C+D+E along with NaF could be due to improved protein synthesis [57]. The significant reduction in glucose level in P-generation females treated with NaF and pups born to them was mitigated on cotreatment with vitamins C [58], E (only in P-generation female) and C+D+E. This could be due to significant amelioration in feed consumption by vitamin cotreatment [38]. Basu and Diskerson [59] reported higher levels of vitamin E in the serum of post-partum mothers than in non-pregnant females indicating that there is a transplacental barrier to the transport of vitamin. This could be the reason for nonamelioration of glucose and protein levels in pups and its amelioration in pregnant dams. Chinoy and Sharma [57] also reported complete recovery from fluoride toxicity in reproductive functions in male mice on co-treatment with vitamins E and D alone and in combination.

The significant recovery on co-treatment with vitamins C [58], C+D+E could be attributed to the action of these vitamins as free radical scavengers [38]. Wilde and Yu [60] opined that the toxicity of free radicals is greater if fluoride can impair the production of free radical scavengers such as ascorbic acid and glutathione and this can be prevented by the additional supplementation with vitamins C and E. The antidotal effect of vitamin E is by preventing the oxidative damage caused by fluoride, which decreases peroxides and free radicals of reactive oxygen species in tissues. The protective role of free radical scavenger is by the hydrogen donor ability of tocopherol (vitamin E).

Vitamin E channels the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH) which in turn helps compression of mono and dehydroascorbic acid to maintain ascorbic acid levels. It also has an inhibitory effect in the conversion of free or protein bound -SH to -SS- groups, thus maintaining -SH groups [59]. Vitamin D plays a crucial role by maintaining the serum calcium and phosphorus concentration thereby supporting cellular processes [41]. A combination of vitamins C+D+E was found to be more efficient in the amelioration and could be due to the synergistic action of all the three vitamins.

Poor nutrition is seen to be an important cause of endemic osteomalacia in a high fluoride environment and increasing dietary energy, calcium, protein and vitamin C may help in its prevention especially in pregnant and nursing women and in children [41]. Various studies [61,62] also revealed that the treatment of vitamin C and D and calcium showed significant improvement in skeletal, clinical and biochemical parameters in children consuming water containing 4.5 ppm of fluoride. Susheela [63] reported that consumption of food rich in vitamins C and E, which acts as antioxidants scavenging the free radicals and eliminating them, also reduces the fluoride levels in the body.

The present review indicates that fluoride in higher concentration cause teratogenic effect which could be due to hypocalcaemia and/ or oxidative stress. Administration of calcium as well as vitamins such as vitamin C, D as well as E has ameliorative effect.

Conflict of interest statement

We declare that we have no conflict of interest.

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