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Research Article

SODIUM FLUORIDE INDUCED TOXIC EFFECT ON TUMOR PROTEIN 53 FORMING GENE IN SWISS ALBINO MICE

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ABSTRACT
The Albino mice have been subjected to fluoride intoxication for 14 days by administering the dose of aqueous NaF (30 mg/kg/body weight/day). At the end of the 14 day treatment, the animals were sacrificed by cervical dislocation, and the liver is dissected out, blotted free of blood, transferred to trays maintained at ice-cold conditions and used for isolation of DNA. Genomic DNA was isolated using standardized protocol and quantified on spectrophotometer to check its quality and then run on 8 % gel.Methods validated for the PCR Amplification and run for the gene under study. The amplified PCR products wproducts for both the cases. The PCR products obtained were further sequenced and matched but there were not any differences in their sequences. Only histopathological and enzyme reaction changes were observed.

Key Words: *Fluoride, Toxicity, Tumor Protein 53, Gene, Albino Mice*

INTRODUCTION
Fluorosis caused by excess intake of fluoride is a slow, progressive degenerative disorder, known to affect predominantly the skeletal systems, teeth and also the structure and function of skeletal muscle, brain and spinal cord. Recent studies have shown accumulation of fluoride in the hippocampus of the brain causing degeneration of neurons and decreased aerobic metabolism and altered free-radical metabolism in the liver, kidney, and heart. However, the effect of fluoride on neuromuscular tissue is far from clear. Fluoride in drinking water is easily absorbed by the intestines and is quickly distributed throughout the body. Fluoride easily crosses membranes and enters tissues, thus affecting every phase of metabolism. Bones and teeth especially are the sink for fluoride, which accumulates in them and causes fluorosis. Only limited work has been done, however, on the toxicity of fluoride on soft tissues, *viz* liver, kidney, muscles and testes. The aim of the present study, therefore, was to examine the effects of Fluoride on genes coding for above mentioned proteins in Swiss albino mice.

Review of Litterature
Most of the following reversible ill effects caused by fluoride were first recognized among aluminum workers in the 1930s by the Danish health officer Dr. Kaj Roholm. Not all the Symptoms are necessarily present at the same time. Their severity and duration(often episodic) depend on a person's age, nutritional status, environment kidney function amount of fluoride ingested' genetic background tendency to allergies and other factors.To test for fluoride intoxication, the following procedure must be rigorously followed Avoid all fluoridated water (substitute distilled or other non fluoridated low-fluoride water), fluoridated Beverages, fluoride-rich foods (tea, ocean fish, gelatin, skin of chicken etc.), fluoridated toothpastes and any other source of environmental fluoride, including cigarette smoke and industrial pollution.

Clinical manifestations in Fluorosis reveal severe involvement of dental and skeletal tissues (Shikone *et al.*, 1994). However, it has been reported that Fluorosis is not merely a disease of bone and tooth, but it also affects the non-ossues tissues (Rao *et al.*, 1979). Collagen, one of the structural constituents of both osseus and non-osseus tissues, appears to be severely affected due to Fluoride intoxication (Susheela, 1980). The constitution of nascent collagen protein in fluoride toxicity has been reported to be defective. The major defect has been localized in the absence of low molecular weight peptides which normally are known to fabricate the collagen fiber (Susheela, 1980). Most of the following reversible ill effects caused by fluoride were first recognized among aluminum workers in the 1930s by the Danish health officer Dr.

Kaj Roholm. Not all the Symptoms are necessarily present at the same time. Their severity and duration (often episodic) depend on a person's age, nutritional status, environment kidney function amount of fluoride ingested' genetic background tendency to allergies and other factors. To test for fluoride intoxication, the following procedure must be rigorously followed Avoid all fluoridated water (substitute distilled or other non fluoridated low-fluoride water),fluoridated Beverages fluoride-rich foods (tea, ocean fish, gelatin, skin of chicken, etc.), fluoridated toothpastes and any other source of environmental fluoride, including cigarette smoke and industrial pollution.

Leukemias originate from hematopoietic stem cells at certain stages of their maturation and differentiation. It is well established that acute leukemias originate from immature hematopoietic stem cells that have the capacity to undergo self-renewal, whereas less aggressive leukemias such as chronic leukemias seem to originate from the more mature committed hematopoietic progenitor cells. Potential toxic effect of sodium fluoride on early progenitor and stem cells has been described previously (Machalinska, 2001). Surprisingly, few investigations have examined the effects of fluoride on human leukemic cells. According to Kawase *et al.,* (1996), sodium fluoride (NaF) at a concentration of 0.5 mM for up to four days inhibited proliferation of humanpromyelocytic HL-60 cells. Anuradha *et al.,* (2001) reported that NaF induced apoptosis in HL-60 cells by caspase-3 activation.

Fluoride is an essential trace element in humanbodies and is highly correlated with the metabolism of bone and tooth. But excessive exposure to fluoride for a long term leads to bone damage with complicated pathological changes such as osteoporosis and osteopetrosis. Arsenide is an environmental toxicant and a known carcinogen (National research council, 1999). Inorganic arsenide is highly accumulated *in vivo*. Arsenate deposits in skeleton because it takes the place of phosphate in the apatite crystal of bone. Fluoride and arsenide in groundwater, air, and food in some areas of China as a result of specific geographical and geological environment and living habits of local people have tremendous impact on health of local residents. Great attention has been paid to the possible combined effect of fluoride and arsenide (Huang *et al.,* 1992). There are different reports about the role of arsenide in the bone damage caused by fluoride (Li *et al.,* 1996). Both osteoblasts and osteoclasts are involved in bone damage. Studies indicate that the proliferation, differentiation, and maturity of osteoclasts are dependent on the existence of osteoblasts (Takahashi, 1998). Osteoprotegerin (OPG) and osteoclast differentiation factor (ODF) are secreted by osteoblasts. The former is responsible for inhibiting osteoclastogenesis, while the latter plays an important role in stimulating the differentiation and maturity of osteoclasts, activating mature osteoclasts and inhibiting their apoptosis. So OPG and ODF may combine the functions of osteoblasts and osteoclasts, which is of great help to the study of the influence of toxicants on bone remodeling.

Fluoride Toxicity

Fluoride in drinking water is easily absorbed by the intestines (Carlson *et al.,* 1960) and is quickly distributed throughout the body. Fluoride easily crosses membranes and enters tissues, thus affecting every phase of metabolism. Bones and teeth especially are the sink for fluoride, which accumulates in them and causes fluorosis (Singh *et al.,* 1962). Only limited work has been done, however, on the toxicity of fluoride on soft tissues, *viz* liver, kidney, muscles and testes.

Although artificial fluoridation of water supplies is practiced in many parts of the world in an effort to reduce the incidence of dental caries, there is growing evidence that the resulting increased exposure to fluoride (F) may cause serious toxic effects (Spittle, 2009). Several clinical investigations and animal experiments suggest that F has adverse impacts on male reproductive function (Chinoy *et al.,* 1997), including structural and functional defects in spermatozoa(Chinoy et.al,1998) a decrease in sperm count (Ghosh et al., 2002), disturbances in the levels of reproductive hormones, alterations in the epididymis and accessory reproductive glands, and reduced fertility. Spermatogonia undergo various processes to ultimately fertilize an oocyte, including spermatogenesis, capacitation, and the acrosome reaction. F has been shown to impair all three of these processes.

Research Article

p53 (also known as protein 53 or tumor protein 53), is a transcription factor that in humans is encoded by the *TP53* gene. p53 is important in multicellular organisms, where it regulates the cell cycle and thus functions as a tumor suppressor that is involved in preventing cancer. As such, p53 has been described as "the guardian of the genome," "the guardian angel gene," and the "master watchman," referring to its role in conserving stability by preventing genome mutation. The name p53 is in reference to its apparent molecular mass: it runs as a 53 kilodalton (kDa) protein on SDS-PAGE. But based on calculations from its amino acid residues, p53's mass is actually only 43.7kDa. This difference is due to the high number of proline residues in the protein which slow its migration on SDS-PAGE, thus making it appear heavier than it actually is. This effect is observed with p53 from a variety of species, including humans, rodents, frogs, and fish.

p53 Pathway

In a normal cell p53 is inactivated by its negative regulator, mdm2. Upon DNA damage or other stress, various pathways will lead to the dissociation of the p53 and mdm2 complex. Once activated, p53 will either induce a cell cycle arrest to allow repair and survival of the cell or apoptosis to discard the damage cell. How p53 makes this choice is currently unknown.Activated p53 binds DNA and activates expression of several genes including WAF1/CIP1 encoding for p21. p21 (WAF1) binds to the G1-S/CDK (CDK2) and S/CDK complexes (molecules important for the G1/S transition in the cell cycle) inhibiting their activity. p53 has many anticancer mechanisms, and plays a role in apoptosis, genetic stability, and inhibition of angiogenesis.When p21(WAF1) is complexed with cdk2 the cell cannot pass through to the next stage of cell division. Mutant p53 can no longer bind DNA in an effective way, and as a consequence the p21 protein is not made available to act as the 'stop signal' for cell division. Thus cells divide uncontrollably, and form tumors. Recent research has also linked the p53 and RB1 pathways, via p14ARF, raising the possibility that the pathways may regulate each other. When p53 expression is stimulated by sunlight, it begins the chain of events leading to tanning.

Regulation

p53 becomes activated in response to myriad stress types, which include but is not limited to DNA damage (induced by either UV, IR, or chemical agents such as hydrogen peroxide), oxidative stress, osmotic shock, ribonucleotide depletion and deregulated oncogene expression. This activation is marked by two major events. Firstly, the half-life of the p53 protein is increased drastically, leading to a quick accumulation of p53 in stressed cells. Secondly, a conformational change forces p53 to take on an active role as a transcription regulator in these cells. The critical event leading to the activation of p53 is the phosphorylation of its N-terminal domain. The N-terminal transcriptional activation domain contains a large number of phosphorylation sites and can be considered as the primary target for protein kinases transducing stress signals.

The protein kinases that are known to target this transcriptional activation domain of p53 can be roughly divided into two groups. A first group of protein kinases belongs to the MAPK family (JNK1-3, ERK1-2, p38 MAPK), which is known to respond to several types of stress, such as membrane damage, oxidative stress, osmotic shock, heat shock, etc. A second group of protein kinases (ATR, ATM, CHK1 and CHK2, DNA-PK, CAK) is implicated in the genome integrity checkpoint, a molecular cascade that detects and responds to several forms of DNA damage caused by genotoxic stress. Oncogenes also stimulate p53 activation, mediated by the protein p14ARF.

In unstressed cells, p53 levels are kept low through a continuous degradation of p53. A protein called Mdm2 (also called HDM2 in humans) binds to p53, preventing its action and transports it from the nucleus to the cytosol. Also Mdm2 acts as ubiquitin ligase and covalently attaches ubiquitin to p53 and thus marks p53 for degradation by the proteasome. However, ubiquitylation of p53 is reversible. A ubiqiutin specific protease, USP7 (or HAUSP), can cleave ubiquitin off p53, thereby protecting it from proteasome-dependent degradation. This is one means by which p53 is stabilized in response to oncogenic insults.

Phosphorylation of the N-terminal end of p53 by the above-mentioned protein kinases disrupts Mdm2-binding. Other proteins, such as Pin1, are then recruited to p53 and induce a conformational change in p53 which prevents Mdm2-binding even more. Phosphorylation also allows for binding of trancriptional coactivators, like p300 or PCAF, which then acetylate the carboxy-terminal end of p53, exposing the DNA binding domain of p53, allowing it to activate or repress specific genes. Deacetylase enzymes, such as Sirt1 and Sirt7, can deacetylate p53, leading to an inhibition of apoptosis9Kawase T.et.al.,1996) Some oncogenes can also stimulate the transcription of proteins which bind to MDM2 and inhibit its activity.

MATERIALS AND METHODS

Sample Preparation

Twelve healthy, adult female albino mice, *Mus musculus* of Swiss strain, each weighing about 30 ± 2 g, were obtained from the Animal house.

Body weight and organo-somatic index: The body weight of each animal was noted before treatment and also on day 15. The weight of liver of respective groups of animals was recorded.

Estimation of Fluoride: Fluoride levels of control and treated mice were determined by the method of Birkeland with required modifications and are expressed as g of fluoride/g dry tissue.

The liver is dissected out, pooled, homogenized, and dried for 24 hours at 105°C. About 200 mg of the dry sample was dissolved in an acid mixture (equal parts of 11.6 M perchloric acid and 14.3 M nitric acid) and neutralized with citrate buffer to a pH 5.5 with an alkaline mixture of 7.8 M sodium hydroxide and 1.0 M trisodium citrate. The process was carried out in a closed compartment. The sample thus obtained was used after appropriate dilutions for recording the fluoride content on a fluorimeter (Orion R 94-09). The values were calculated from a standard curve

The mice were housed in polypropylene cages with stainless steel grill tops and were fed a standard pellet diet and given distilled water.

p53	Forward primer: 5′-GCGTCTTAGAGACAGTTGACT-3′ Reverse primer: 5′-GGATAGGTCGGCGGTTCATGC-3′	458 base (bp)	pairs

Table 1: PCR cycling conditions for p53

Steps		**Temperature**	**Time**	**No of Cycles**
Step I	Initial Denaturation	95°C	10 min	1 cycle
Step II	Denaturation	95°C	30 Sec	30 cycles
	Annealing	55°C	30 Sec	
	Extension	72°C	30 Sec	
Step III	Final Extension	72°C	5 min	1 cycle
Hold at 4°C				

The animals were allowed to acclimatize to the laboratory conditions for four days before experiments began. The mice were randomly divided into two groups of six each; the first groups served as controls and were given mammalian physiological saline. The second group animals were injected i.p. with aqueous NaF (20 mg/kg/body weight/day) selected on the basis of the LD50 value of fluoride, which is 51.6 mg/kg body weight/day in female mice and maintained for 14 days.

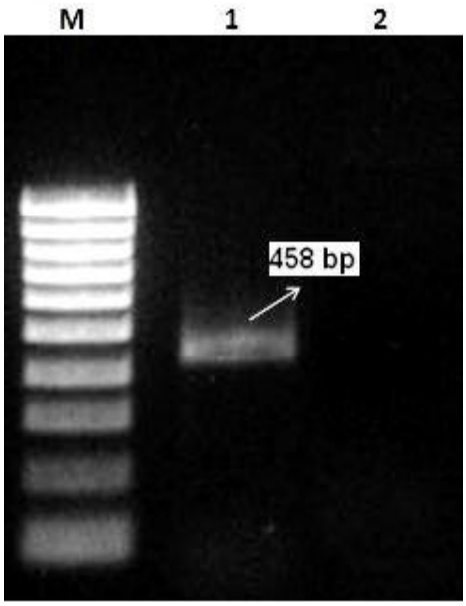

Figure 2: PCR product run on 2.0 % agarose gel and was stained with EtBr (10 mg/ml)
Lane M: 100 bp DNA Ladder; Lane 1: PCR product

The NaF solution (30 mL) was prepared fresh each day in double distilled water. At the end of the 14 day treatment, the animals were sacrificed by cervical dislocation, and the liver is dissected out, blotted free of blood, transferred to trays maintained at ice-cold conditions and used for analysis.

RESULTS AND DISCUSSION

Methods validated for the PCR Amplification and run for the four genes under study. The amplified PCR products were run on 2% Agarose gel with the 100 bp DNA ladder the check the amplified product size. The results obtained for the TP53 gene under study did not vary for the normal and test mice; we get the same size of amplified products for both the cases. The PCR products obtained were further sequenced and matched but there were not any differences in their sequences.

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