

## Short Communication

# The Influence of Sodium Fluoride on the Growth of Ameloblasts and Kidney Proximal Tubular Cells

(sodium fluoride / ameloblast cells / amelogenin / Bcl-2 / Caspase-3)

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**Abstract.** Fluoride has toxic potential particularly for teeth, bones, and kidney. This study was aimed to investigate the NaF exposure effects on the growth of ameloblasts and kidney proximal tubular cells. Adult male healthy rats were used as experiment models, divided into control and NaF-induced groups. The expression of amelogenin, Bcl-2, and caspase-3 were significantly different in the control and NaF-induced group ( $P < 0.05$ ). There was no correlation among these proteins in the control group but significant correlation in the NaF-induced group ( $r = 0.694$ ). There was a significant correlation in proximal tubular cells, as seen from the increase of caspase-3 in the NaF-induced group ( $r = 0.715$ ).

## Introduction

Fluoride is a trace element which is widely found in nature and commonly consumed by the community in the form of fluoride salt such as natrium fluoride (NaF). The most common impact of fluoride consumption is damage and death of ameloblast cells as the main element of tooth enamel. An excessive exposure to fluoride during the development of tooth enamel will have the potential to damage the tooth enamel (McDonald et al., 2005). Previous studies have found that excessive absorption of fluoride during the dentition would result in

retention and declines of protein release and mineral contents in the tooth enamel. It is stated that fluoride ion has a potential of damaging the process of hydroxyapatite deposition that in the course of mineralization forms the tooth enamel (Agalakova and Gusev, 2012).

The ameloblast cell damage and death affecting formation of the tooth enamel is known as enamel hypoplasia or dental fluorosis. The prevalence of dental fluorosis resulting from excessive fluoride exposure from drinking water consumption in Indonesia is relatively high. Two studies conducted in 2011 in two villages in Asembagus District, Situbondo Regency found that the prevalence of dental fluorosis in these two villages was 98.33 % and 78.75 %, with Community Fluorosis Index (CFI) average of 1.60 and 0.80, respectively (Wahluyo, 2012).

Many latest research findings suggest that excessive exposure to fluoride damages not only tooth enamel, but also other organs such as bones, kidney, liver, and brain (Xiong et al., 2007). Rather than exploring the influence of fluoride exposure on the existence of kidney tissue, some studies focus merely on the influence of fluoride on the function of the kidney. As an element with high electronegativity, fluoride is supposed to be able to stimulate production of superoxide radical in cells. The fluoride exposure results not only in fluorosis in teeth and bones, but also in apoptosis that may damage the kidney (Song et al., 2013).

Kidney is a vital organ for decreasing the influence of fluoride on the human body. It has been found that 50–60 % of fluoride intake by a healthy individual will be excreted by the kidney. The proximal tubule of the kidney is one of the important organs since its function is to absorb substances that later will be actively transported from tubular cells to the proximal tubule. From here, the substances will move to the intercellular space and the basal channel that cause the increase of osmotic pressure of peritubular capillaries to become hyperosmotic. If minerals are excessively absorbed, the physiological functions of the kidney change. Therefore, an individual with kidney disease will be vulnerable to the toxicity

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Abbreviations: ACUC – Animal Care and Use Committee, Bcl-2 – B-cell lymphoma 2, CFI – Community Fluorosis Index, HPF – high-power field, IHC – immunohistochemistry, MMP-20 – matrix metalloproteinases 20, MPTP – mitochondrial permeability transition pore, NaF – natrium fluoride, VDAC – voltage-dependent anion channel.

effects of the fluoride such as renal osteodistrophy (DenBesten et al., 2002).

Some histological changes also occur in experimental animals exposed to fluoride such as incidence of interstitial nephritis, which will usually deteriorate with the increase of fluoride content. In this case, there will be changes in the proximal tubule resulting in either hypertrophy or hyperplasia (Xu, 2006). These disorders may occur due to molecular changes caused by the cytotoxicity of the fluoride in the kidney. In this stage, the fluoride in the kidney will induce specific proteins that stimulate cell death in the kidney and affect the structure of DNA (Song et al., 2014).

Considering the fact that exposure to fluoride brings impacts to vital organs, we performed a study of the influence of fluoride exposure on ameloblast cells and kidney cells of Wistar rats.

## Material and Methods

This study was an experimental one using the randomized post-test only control group design. In this study, we used 40 male rats (*Rattus Norvegicus*) from the Department of Medical Biochemistry, Faculty of Medicine, Airlangga University-Indonesia, aged 10–11 weeks and weighing 150–170 g, as a model (Isroi, 2010). These rats were then randomly divided into two groups, 20 rats in each group. The first group, as the control group, was exposed to 2 ml of sterile distilled water, and the second group was exposed to NaF (MERCK, Kenilworth, NJ, product reg. number 1.06449.0250), 6.75 mg in 2 ml of sterile distilled water (Mathieu and Pelletier, 2010; Catani et al., 2010). The dose of fluoride to which these animals were exposed conformed to the National Research Council of the United States (2005). After 28 days of induction, the analysis was conducted by monitoring the expression of caspase-3 and B-cell lymphoma-2 (Bcl-2) protein using anti-caspase-3 and anti Bcl-2 monoclonal antibodies (Bioworld Technology Inc., St. Louis Park, MN, catalogue number: BS-1518; Rat/FITC product by Bioscience, San Diego, CA, catalogue number: 11-6992-41) measured by calculating the number of ameloblast cells and proximal tubular epithelial cells of the rat kidneys that positively reacted to the induction.

The influence of fluoride particularly on the ameloblast cells was investigated by analysing the expression of amelogenin proteins (Primer antibody from Santa Cruz Biotechnology, Dallas, TX, catalogue number: SC-32892). The expression of these three proteins was determined by the immunohistochemistry (IHC) colouring technique, which was then calculated per 10 High Power Fields (HPF) and observed through an optical microscope with 400× magnification (Taylor et al., 2010). All procedures were approved by the research ethic board (Animal Care and Use Committee/ACUC) at the Faculty of Veterinary Medicine, Airlangga University, based on the Ethical Clearance No. 191-KE.

## Results and Discussion

The fluoride exposure causes reduction of amelogenin proteins, so that there is a delay in the mineralization phase of the tooth enamel. This affects the quality of the tooth, as there are a number of these proteins in the enamel structures. The proximal tubular epithelial cells are also affected due to some physiological changes of the kidney function. Fluoride also causes damage and death of ameloblast cells and proximal tubular epithelial cells, inducing the process of apoptosis. Formation of apoptosis-induced mitochondrial channel is regulated by Bcl-2 and caspase-3. Our study shows that there are differences in the expression of amelogenin protein and Bcl-2 and caspase-3 proteins in both groups, as seen in Tables 1 and 2.

Based on the results of the data normality test, the data in this study show a normal distribution ( $P > 0.05$ ). The results of the performed statistical tests between the control and experimental groups in particular show that the expressions of amelogenin, Bcl-2, and caspase-3 proteins are significantly different ( $P < 0.05$ ). The correlational test indicates that, on the one hand, there is no correlation among amelogenin, Bcl-2, and caspase-3 in ameloblast cells of the control group. On the other hand, there is a significant correlation among these proteins in ameloblast cells in the experimental group with NaF induction ( $R = 0.694$ ). The correlational test in proximal tubular epithelial cells of the rat kidneys with NaF exposure indicates that there is a significant correlation ( $r =$

Table 1. Expression of amelogenin, Bcl-2 and caspase-3 protein in ameloblast cells

	N	Amelogenin		Bcl-2		Caspase-3	
		Mean ± SD	t-test (P)	Mean ± SD	t-test (P)	Mean ± SD	t-test (P)
Normal	15	6.01 ± 2.07	0.027	12.07 ± 1.99	0.017	3.85 ± 1.03	0.014
Treated	15	14.87 ± 2.48		3.49 ± 1.07		11.97 ± 2.68	

Table 2. Expression of Bcl-2 and caspase-3 proteins in proximal tubule epithelial cells

	N	Bcl-2		Caspase-3	
		Mean ± SD	t-test (P)	Mean ± SD	t-test (P)
Normal	15	7.29 ± 2.09	0.029	4.07 ± 1.19	0.021
Treated	15	2.21 ± 0.17		17.02 ± 3.08	

0.715), as seen from the increase of the expression of caspase-3 protein.

The IHC analysis of the expression of amelogenin proteins in ameloblast cells in rat teeth is shown in Fig 1A and Fig 1B.

Fig. 1A, B depicts groups with a number of cells expressing the amelogenin protein. The comparison of the number of cells expressing amelogenin between the control group and the NaF-induced group can be seen in Table 1.

Fig. 1A shows that the expression of amelogenin in the control group was lower than that in the experimental group exposed to NaF. The expression of Bcl-2 protein in the experimental group was lower than that in the control group. In contrast, the expression of caspase-3 in the NaF-induced group was higher than that in the control group. Similar findings are shown by the expression of caspase-3 protein in the proximal tubules of the kidney, which was lower in the control group than that in the experimental group with NaF exposure.

The results of this study revealed that the expression of amelogenin in the control group was lower than that in the NaF-induced experimental group. This can be explained by the fact that during the normal phase of amelogenesis without any influence from external factors, amelogenin is degraded into smaller fragments through the hydrolytic process by matrix metalloproteinase 20 (MMP-20) activity occurring at the C-terminus of the amelogenin protein. The amelogenin hydrolysis occurs when pH in the environment of enamel matrix ranges between 7.2–7.3 (DenBesten et al., 2002; Uskokovic et al., 2008).

Previous research indicated that excessive absorption of fluoride during dentition would cause retention and reduction of protein release as well as decline of the mineral content of enamel (Aoba and Fejerskov, 2002). Fluoride ion has been proved to display destructive effects on hydroxyapatite deposition during the mineralization process to form enamel. The main characteristics of fluoridated enamel are accumulation of the protein content and slow release of certain proteins. These findings support the results showing that fluoride induction reduces MMP-20 activity, which is the mediator of enamel matrix remodelling. This inactivation is caused by rapid binding of the electronegative ion  $F^-$  with three amino acid components in the amelogenin protein

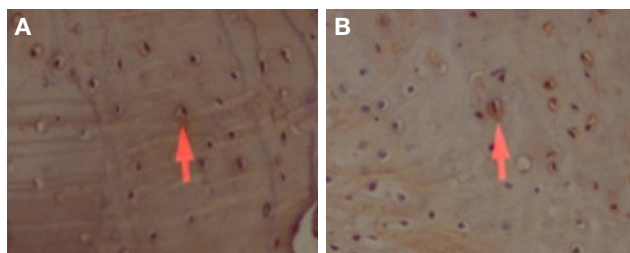


Fig. 1. IHC colouring technique with 400× magnification. Positive expression of amelogenin is shown by the arrows; brownish colour on the cytoplasm. **A** – control group, **B** – NaF-induced group.

through ionic hydrogen bonds. The fluoride element has a highly electronegative nature, and as it binds to histamine and histidine, the alkaline properties of glutamine will change the alkaline properties of the amino acid residues.

The reduction of MMP-20 activity occurs when pH ranges between 5.5–5.6, while the optimum required pH for MMP-20 activity ranges between 7.2–7.3. The former condition would hinder MMP-20 to degrade amelogenin, and therefore delay the reduction of amelogenin in the enamel matrix. This delay affects the structures and composition of the enamel matrix, which are dominated by the amelogenin protein, and therefore the enamel matrix structure is not optimal at the time of maturation and frequent enamel porosity is found.

Bcl-2 proteins are essential in mitochondrial regulation of cell death. Stimuli from fluoride mediate a pro-apoptotic signal by Bax, Bak, and Bid proteins. Fluoride induction would give pressure to mitochondria that leads to damage and death of ameloblast cells through the process of apoptosis, and in turn, influence formation and growth of the tooth enamel (Kubota et al., 2005). These events can further be explained through two processes: **(a)** NaF induction released cytochrome-c from mitochondria, and this was followed by the increase of cytochrome-c in the cytosol that activated caspase-3 as executor of the apoptosis process; **(b)** NaF induction increased expression of the voltage-dependent anion channel (VDAC) as the main component of mitochondrial permeability transition pores (MPTP), and this increased the cytochrome-c content in the cytosol that activated caspase-3. These findings are in line with those by Agalakova and Gusev (2012).

The process of apoptosis can either be intrinsic or mitochondrial. Mitochondria play a crucial role in intrinsic apoptosis. The external induction will lead to changes of permeability of the mitochondrial membranes. Several mitochondrial proteins can activate the apoptotic programme, and these proteins are normally located in the mitochondrial membrane. Apoptotic stimuli will lead to the release of proteins into the cytoplasm or nucleus (Wang, 2001).

The kidney proximal tubule is one of the vital organs to absorb substances, and later these substances are actively transported from the tubular cells to the proximal tubule. From here, they move to the intercellular space and the basal channel, resulting in the increase of osmotic pressure of peritubular capillaries to become hyperosmotic. Excessive absorption of certain minerals will certainly change the physiological functions of the kidney. Kidney is also a vital organ to suppress the impact of fluoride on the human body, as 50–60 % of fluoride intake by a healthy individual will be excreted by the kidney (Kiyatno, 2009).

In proximal tubular epithelial cells of a rat kidney, the process of cell death or apoptosis due to fluoride exposure requires pro-apoptotic and anti-apoptotic proteins. It is theoretically argued that the fluoride exposure will lead to hydropic degeneration of epithelial cells and

cause moderate dilatation of tubules that can also be observed in the kidney proximal tubule. Mononuclear cell infiltration is also observed in several tubular and perivascular regions accompanied with the narrowed Bowman's capsule. These lead to severe degenerative glomerular changes that cause deterioration of the kidney and vascular congestion (Karaoz et al., 2004).

The proximal tubule of the kidney helps reabsorb substances needed after glomerular filtration. The tubule has low columnar epithelium, with a luminal surface covered with a brush border and a basolateral surface. The functions of proximal tubular epithelial cells are mainly absorption and distribution. The existence of a chronic lesion will impair the function of the epithelium. The damaged epithelium will fill the lumen kidney tubule that influences transportation. The chronic glomerular pressure will cause necrosis and apoptosis executed by the caspase-3 protein (Wang and Niu, 2015).

As a result of fluoride exposure in general, 21–24 % of proximal tubular epithelial cell damage of the rat kidney occurs depending on the dose, concentration and length of the exposure (Xu, 2006).

### Conclusion

Fluoride exposure causes cell damage and cell death, and thereby affects the growth of ameloblast cells in the rat teeth and the existence of proximal tubular epithelial cells in the rat kidneys.

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### Discloser of conflict of interest

The authors have no conflict of interest.

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