Genetic toxicity of biomaterial

2. DNA damaging effects of sodium fluoride and other fluoride compounds

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Introduction

In order to inhibit dental caries, sodium fluoride (NaF), diammine silver fluoride ($\text{Ag}(\text{NH}_3)_2\text{F}$), acidulated fluoride solution ($\text{Na}_2\text{PO}_4 \cdot \text{NaF}$), stannous fluoride (SnF) and potassium fluoride ($\text{KF} \cdot 2\text{H}_2\text{O}$) have been used for both topical and general application by adding to drinking water¹⁾.

However, these compounds of fluoride are feared to harm human health because of their possibility of toxicity²⁾.

In the present investigation, therefore, DNA damaging effects of fluoride compounds which are used in the dental field were investigated.

Materials and Methods

This study on whether sodium fluoride and other compounds of fluoride could induce DNA damage was carried out by the Rec-assay system. This system was used to screen out the number of chemicals for their DNA damage or mutagenic potentiality³⁾. If an agent produced damage in DNA, this damage could in most cases be repaired due to cellular recombination capacity. But if cells lack this repairing ability (as well as mutant strains deficient in genetic recombination), these cells (rec^-) could be killed much more completely than wild (rec^+) cells by those chemicals. Therefore, if any chemical inhibits more rec^- than rec^+ cell growth, it would be a DNA attacking agent. The bacterial strains used in this study were H17

(rec^+ , arg^- , try^-) and M45 (rec^- , arg^- , try^-) of *Bacillus subtilis*. Broth medium was used for the bacterial culture. This contained, in 1,000 ml, 10 g meat extract, 10 g yeast extract and 5 g NaCl and this was adjusted to pH 7.0 and autoclaved for sterilization. For preparation of solid medium about 50 ml of heat dissolved agar broth was poured into a Petri-dish (diameter: 9 cm).

Both H17 (rec^+) and M45 (rec^-) strains of *Bacillus subtilis* were streaked like the letter V on the surface of broth agar of the Petri-dish by small pipette. (H17 (rec^+) was streak as \: the left side of letter V and M45 (rec^-) was streaked as /: like the right side of letter V).

On the cross point of two streaks, paper disk (diameter: 12 mm) was placed and liquid examination sample soaked into the paper disk. Sodium fluoride (NaF) and other compounds of fluoride were dissolved in distilled water at a concentration of 2% and 4% respectively. 0.01-0.5 ml of these solutions were soaked into the paper disk. The plate was then stocked at 4°C overnight. During this interval, drugs slowly diffused out from the paper disk. The plate was then incubated at 37°C for 24 hrs.

During the incubation, since drugs could attack the biomechanisms of the bacteria, they inhibit the growth of the two strains of *Bacillus subtilis*. Therefore, no bacterial growth zone (inhibition zone) of the two strains of *Bacillus subtilis* were revealed close to the paper disk. The length of the inhibition zone of H17 (rec^+) and M45 (rec^-) was measured and then, the length

of inhibition zone of M45 (rec⁻) was compared with the length of inhibition zone of H17 (rec⁺) strain of *Bacillus subtilis*.

Results and Discussion

Figure 1 shows results of Rec-assay of sodium fluoride (NaF), diamine silver fluoride ($\text{Ag}(\text{NH}_3)_2\text{F}$), acidurated fluoride solution ($\text{Na}_2\text{PO}_4\cdot\text{NaF}$), stannous fluoride (SnF₂) and potassium fluoride ($\text{KF}\cdot 2\text{H}_2\text{O}$).

0.5 ml of 2%, 4% concentration of sodium fluoride (NaF), 0.5 ml of 4% concentration of potassium fluoride ($\text{KF}\cdot 2\text{H}_2\text{O}$) and 0.01 ml, 0.05 ml of diamine silver fluoride ($\text{Ag}(\text{NH}_3)_2\text{F}$) showed strong effect of DNA damage potentiality (more than 5 mm of their difference of inhibition zone between H17 (rec⁺) and M45 (rec⁻) strains of *Bacillus subtilis* was compared).

Other fluoride compounds such as 0.1 ml of 4% concentration of sodium fluoride (NaF), 0.5 ml of 4% concentration of stannous fluoride (SnF₂) and 0.1 ml, 0.5 ml of 2% concentration of acidurated fluoride solution ($\text{Na}_2\text{PO}_4\cdot\text{NaF}$) showed weak effects of DNA damage potentiality (less than 5 mm and more than 1 mm in their difference of inhibition zone).

Sodium fluoride (NaF) and other fluoride compounds have been used for prevention of dental caries. These fluoride compounds are either utilized by local application on teeth

surface or whole body application by adding to drinking water. Concentration of these fluoride compounds which actually used either local application on the surface of the teeth or applied by adding to drinking water are considered to have no harmful action on human health.

As for genetic toxicity of fluoride compounds, there are opposing opinions that either sodium fluoride (NaF) increased chromosomal aberration⁴⁾ or it did not increase mutation⁵⁾. Besides, there is the opinion that low density of sodium fluoride suppresses chromosomal aberration and high density of sodium fluoride increases chromosomal aberration of human lymphocyte⁶⁾. Furthermore, there are reports that if men usually drink water which contains relatively high density of sodium fluoride, the birth rate of Down's syndrome⁷⁾ and mortality from malignant tumor are increased⁸⁾.

On the other hand, some chemical substances which are usually used would have a potential of weak mutagenicity and weak carcinogenicity. Accumulation of these weak mutagen and carcinogen have a possibility of inducing human genetic toxicity. Therefore, DNA damageable materials such as fluoride compounds must be used carefully.

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Fig. 1 DNA damaging effects of Fluoride compounds by Rec-assay system.

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