The effect of Fluoride concentration on COL1A2 gene expression

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Aim
Examine the effect of different fluoride concentrations on COL1A2 gene expression at the mRNA level

Background
Fluoride plays a significant role in oral hygiene and is considered to be “one of 10 great public health achievements of the 20th century”. Fluoride is artificially added to many sources such as drinking water, dental products and dietary supplements. After fluoride is ingested, it is rapidly absorbed in the gastrointestinal tract and is either renally cleared or can become associated with calcified tissues such as bones and teeth. Although Fluoride can be beneficial as it can help prevent tooth decay, it can also be harmful in excessive amounts; causing dental and skeletal fluorosis. The latter is a bone disease elicited by excessive consumption of fluoride and its accumulation in the bone, resulting in weakening of the bones. COL1A2 (type 1 collagen alpha 2) is a gene within the body that binds to COL1A1 (type 1 collagen alpha 1), forming the collagen molecule. Collagen is an important fibrous protein; adding tensile strength to bones.

The involvement of genetic determinants in F metabolism have been implicated in several studies. A number of epidemiological studies of populations around the world living in areas with naturally high levels of F in the water suggest that there is considerable variation in fluorosis among and within these populations. Responsiveness to F cannot be correlated with the total bioburden of F as assayed in urine samples. In humans, the possible involvement of gene-environment interaction has been investigated by examining the effect of genotype on susceptibility to fluorosis at two different levels of F exposure. This study identified an association between polymorphisms in the COL1A2 gene and dental fluorosis in high F exposed populations (Huang et al, 2008).

Our aim is to further research and provide evidence for COL1A2 and fluoride interaction. We studied the effects of different fluoride concentrations (0ppm, 1ppm and 3.5ppm) on COL1A2 expression at the mRNA level. This study was carried out in vitro, using a CaCo-2 cell line model of the small intestine. These cells were chosen so that the level of COL1A2 gene could be easily quantified following fluoride administration.

Method

RNA Extraction

Real-time PCR

Dilution curve of COL1A2

Validation of the dilution curve

Figure 1

Figure 2

Figure 3

Results

Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>COL1A2</th>
<th>GAPDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>UT</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1ppm</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>3.5ppm</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>10ppm</td>
<td>1.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Relative expression levelCOL1A2:

UT: untreated
1ppm: 1 ppm fluoride
3.5ppm: 3.5 ppm fluoride
10ppm: 10 ppm fluoride

p = 0.06

Discussion

Untreated cells remained unchanged.
Results indicated that there was a significant increase in COL1A2 mRNA expression at the higher fluoride concentrations at the 15 minute time point (Figure 3). At longer time points, no difference in COL1A2 mRNA expression between samples was observed.
After 30 and 60 minutes, COL1A2 expression appeared to decrease back to normal values. This is most likely due to the fluoride levels decreasing as it is used by the cells.

Conclusion

Results indicate that COL1A2 mRNA expression increases at higher extracellular fluoride concentrations after 15 minute exposure, however after longer exposure times, COL1A2 mRNA expression returns to a similar level to that seen in untreated cells. Fluoride absorption is a fast process and fluoride can be detected in the blood less than 20 minutes after ingestion. This may account for the fast response observed in our experiments. Further research needs to be carried out, to see whether the observed increase in COL1A2 expression observed in our cell line model is replicated in human subjects living in naturally high fluoride areas.