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

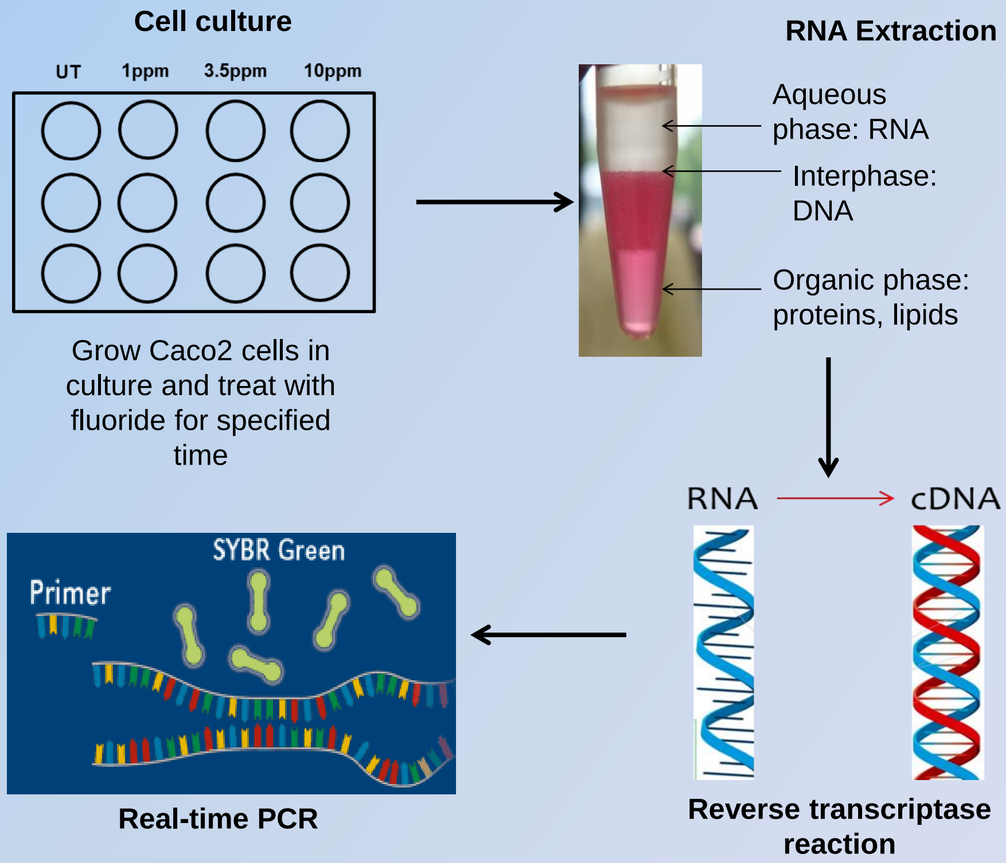
Background

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.

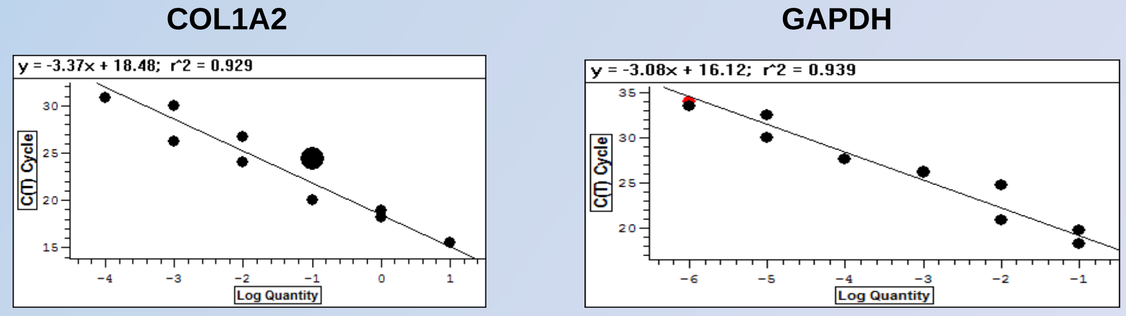
Method



- Caco2 cells were grown in culture and then seeded into three 12-well plates.
- Each row of cells were treated with different fluoride concentrations: Untreated, 1ppm, 3.5ppm and 10ppm. Treatments were for 15 minutes, 30 minutes and 60 minutes.
- After incubation for the specified time, the RNA was isolated and extracted. This was carried out using Trizol reagent and Chloroform.
- A reverse transcriptase reaction was performed on the ssRNA, in order to convert it to ds cDNA.
- This cDNA was then used for real-time PCR, using SYBR Green as the DNA-binding dye. This dye fluoresces as it binds to dsDNA. An increase in DNA product results in an increase in fluorescence intensity, allowing DNA concentrations to be quantified.
- As well as using COL1A2 primers for the real-time PCR, GAPDH primers were also used on duplicates of the sample; to be used as a reference gene.

Reference: Huang, H., et al., *COL1A2 gene polymorphisms (Pvu II and Rsa I), serum calciotropic hormone levels, and dental fluorosis.* Community Dent Oral Epidemiol, 2008. **36**(6): p. 517-22

Results



Efficiency= 98%

Efficiency= 111%

Figure 1. Standard curves for COL1A2 and GAPDH produced from real-time PCR. Standard curves were generated using SYBR green fluorescence and the DNA Opticon 2 (MJ research). Standards for COL1A2 were generated by serially diluting an end point COL1A2 PCR sample and standards for GAPDH were by serially diluting a GAPDH plasmid construct. The mean log concentration of each dilution was plotted against PCR cycle number at which the fluorescence threshold was crossed (Ct). Excluded data points are shown in red. Efficiency of the reaction was calculated and is shown below each figure.

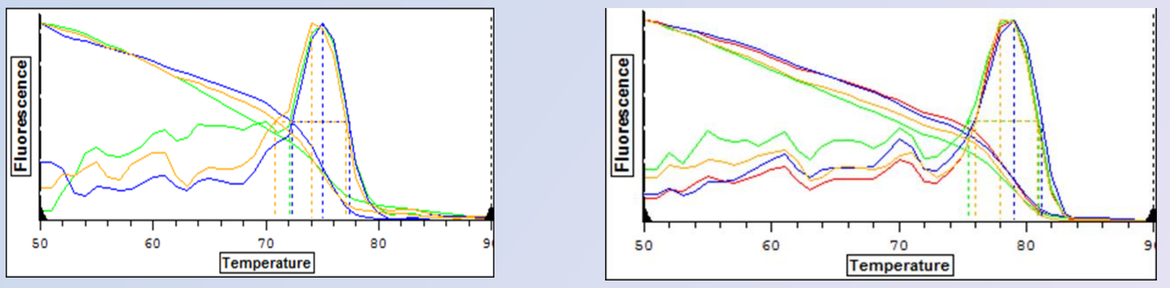


Figure 2. Melting curves showing products for COL1A2 and GAPDH.

Results were calculated as a ratio of COL1A2: GAPDH expression in the same sample and normalised to 1 (for untreated samples; Figure 3).

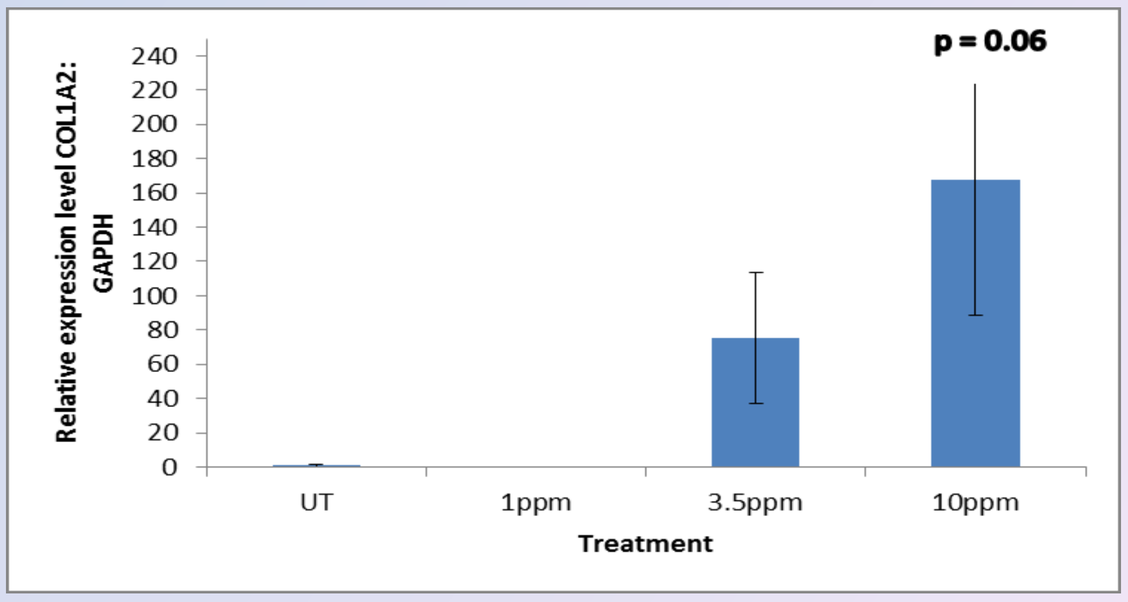


Figure 3. COL1A2 mRNA expression alters with different fluoride concentrations at the 15 minute time point.

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 were 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.