56th ORCA Congress

Saturday Afternoon Symposium:
The effective use of effective fluoride toothpastes for caries control
Strengths and limitations of pre-clinical models for measuring effectiveness of fluoride containing toothpastes:

- Bioavailability and F uptake -

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Pre-clinical models
Bioavailability and F uptake

- Mechanism of action of F toothpastes
- F availability in toothpastes
- F bioavailability from toothpastes
- F uptake
- Strengths and limitations
FLUORIDE !!!
F bioavailability in dentifrices

• Bioavailability: “the extent to which a drug reaches its site of action or reaches a biological fluid from which the drug has access to its site of action”

Goodman & Gilman. The Pharmacological Basis of Therapeutics.
Ekstrand & Oliveby, 1999, modified; Cury & Tenuta, 2008
Fig. 2. Mean salivary fluoride clearance curve after use of 1,500 μg F/g Na$_2$FPO$_3$ dentifrice (n = 10). O = Experimental data (bars = SD); --- = computer-fitted model curve.
Site of action:

- Remaining biofilm
- Tooth structure (enamel/dentine)
• Reduce **tooth demineralization during a pH challenge**
• Reduce **tooth demineralization** during a **pH challenge**
• Enhance **tooth remineralization** at resting biofilm pHs
Site of action:

✓ Remaining biofilm
  • Tooth structure (enamel/dentine)
Sound enamel/dentine
New biofilm

Sound enamel/dentine
As a consequence of demineralization and remineralization processes, $F$ concentration in the tooth structure will increase.
F availability X F bioavailability

- F must be chemically soluble in the formulation, as F ion or MFP, to be considered available
F availability in toothpastes

Calcium-based abrasives:

DCPD,
CaCO$_3$
\((\text{CaCO}_3)_n \Leftrightarrow \text{Ca}^{++}\)

+ 

\(\text{NaF} \longrightarrow \text{F}^- \quad \leftrightarrow \quad \text{“CaF}_2”\)  

Insoluble
Effect of time on F availability in a NaF/CaCO₃ dentifrice

Tabchoury & Cury. Rev. Bras. Farm., 1994
NaMFP
Sodium monofluorophosphate

\[
\text{Na}^+ \text{O} - \text{P} - \text{O}^- \text{Na}^+ \quad \iff \quad 2 \text{Na}^+ + \text{O} - \text{P} - \text{O}^- \\
\]

\[
\text{oral phosphatases} \quad \frac{\text{H}_2\text{O}}{	ext{H}_2\text{PO}_4^- + \text{F}^-} 
\]
But with time.....

\[
\text{Na}_2\text{FPO}_3 \quad \text{MFP} \quad \rightarrow \quad \text{FPO}_3^{2-} \quad \rightarrow \quad \text{F}^- \\
\text{H}_2\text{O} \quad + \\
\text{(CaCO}_3\text{)}_n \quad \rightarrow \quad \text{Ca}^{++} \\
\downarrow \\
\text{“Ca}_F^2\text{”} \quad \text{Insoluble}
\]
Effect of time on F availability in a MFP/CaCO$_3$ dentifrice

Tabchoury & Cury. Rev. Bras. Farm., 1994
Concerns about F availability in toothpastes

• Low F availability in toothpastes produced in African and Asiatic countries have been shown recently

van Loveren et al., 2005; Kikwilu et al., 2008
Methods for assessing F availability in toothpastes

• Forms of F available in toothpastes
  – Total F

  Soluble F
  • Ionic F: readily available in aqueous solutions
  • Ionizable F: MFP, will release ionic F in the mouth upon the action of oral phosphatases
  • Insoluble F: “CaF$_2$-like”, will not release ionic F in the mouth
Total F

- Measured after acid dissolution of all F forms, using the ion-specific electrode (Pearce, 1974; Bruun et al., 1984; Cury et al., 1981-2008)

- Measured after F separation from the slurry by microdiffusion (Council on Scientific Affairs, ADA 2005)

- Directly measured using gas chromatography (van Loveren et al., 2005)
Soluble F

- $F^-$ can be directly measured using the $F$ electrode (Pearce, 1974; Cury et al., 1981-2008; Council on Scientific Affairs, ADA 2005)

- MFP:
  - $F^-$ measured after MFP hydrolysis
    - Acid (Pearce, 1974; Cury et al., 1981-2008)
    - Phosphatase (van Loveren et al., 2005)
  - Directly measured by ion cromotography (against MFP standards) (CSA, ADA 2005)
Accelerated aging by high temperature

Tabchoury & Cury. Rev. Bras. Farm., 1994
Concerns about F availability in toothpastes

• Brazil:
  – The leading-sales toothpastes use the MFP/CaCO$_3$ system
  – Numerous studies conducted since 1980 have shown adequate availability and stability of Brazilian F toothpastes, as well as anticaries potential

(Reviewed by Cury et al., 2004)

“Only a method that reaches all heterogeneous population of the different regions of Brazil could be implicated in the caries decline observed all over the country in groups with different socioeconomic backgrounds.”

Cury et al., 2004
✅ Total: 32 milhões de kits odontológicos para apoiar as ações de promoção e prevenção em Saúde Bucal.
Dentífrico com Flúor

Fórmula Básica: 1500 ppm de Flúor como Sodium Monofluorophosphate, Calcium Carbonate, Acqua, Sodium Lauryl Sulfate, Sodium Silicate, Sorbitol, PEG-12, Sodium Saccharin, Methylparaben, Propylparaben, Celullose Gum, Aroma (Menta)

Contém Monofluorofosfato de Sódio.

Instruções de Uso: Escove seus dentes sempre após as refeições e antes de dormir ou conforme orientação do dentista. Mantenha fora do alcance de crianças menores de 6 anos, que devem usar uma pequena quantidade de creme dental.
F availability and stability in a Brazilian F dentifrice supplied by the federal government

*Formulation containing 1200 ppm F. Analysis done at the Biochemistry Lab, Piracicaba Dental School, UNICAMP
F availability X F bioavailability

- Bioavailable F is F available in the oral cavity, within clinically relevant times and considering use protocols (e.g. post-brushing rinse habits), to present an anticaries effect.
F bioavailability

Ekstrand & Oliveby, 1999, modified; Cury & Tenuta, 2008
F bioavailability (and fluorosis)

Ekstrand & Oliveby, 1999, modified; Cury & Tenuta, 2008
The amount of soluble F (and not total F) in dentifrices should be considered to calculate the risk of dental fluorosis in young children.
Fig. 1—Comparison of fluoride levels in unstimulated whole saliva after application of placebo dentifrice (PD), fluoride dentifrice (FD), fluoride rinse (FR), or fluoride gel (FG). Points show geometric means, ± one standard error factor.

Fig. 2—Comparison of fluoride levels in pooled plaque after application of placebo dentifrice (PD), fluoride dentifrice (FD), fluoride rinse (FR), or fluoride gel (FG). Points show geometric means, ± one standard error factor.

Zero et al., J Dent Res 1992;71:1546-1552
Residual* F in the biofilm formed under F dentifrice** use (Mean ± SE)

*18 h after brushing

**MFP/alumina dentifrices used daily for 4 weeks, samples collected once per week

From Duckworth & Morgan, Caries Res, 1991

Dose-response anticaries effect (Stephen et al., Comm Dent Oral Epid, 1988)
Salivary F bioavailability

• Effect of brushing habits
  – Higher toothbrushing daily frequencies result in lower caries increment (Chesters et al., 1992; Chestnutt et al., 1998)
  – Rinsing with water greatly accelerates F elimination from saliva (Sjögren & Birkhed, 1994; Duckworth et al., 1991; Issa & Toumba, 2004) and reduces the anticaries benefit (Chesters et al., 1992; Sjögren & Birkhed, 1993; Chestnutt et al., 1998)
F bioavailability in saliva up to 60 min after brushing with F dentifrices and the effect of a water rinse (Mean ± SE)

- 500 µg F/g dentifrice, water rinse
- 500 µg F/g dentifrice, no water rinse
- 1100 µg F/g dentifrice, water rinse
- 1100 µg F/g dentifrice, no water rinse

Sjögren et al., 1994; van Loveren et al., 2004

F in the fluid phase of a test biofilm
(Mean ± SD, n=12)

*30 min after brushing
**30 min after contact of the test biofilm with treated enamel blocks
Surface hardness loss after a cariogenic challenge

(Mean ± SD, n=12)

Tenuta, Zamataro, Del Bel Cury, Tabchoury, Cury. Caries Res, 2009
Vogel et al., ORCA 2009, Abstract 80

NaF/Baseline Data

Small • and △ are individual NaF or baseline values
Large ○ and △ are the average value of this data

This correlation is significant.
F in plaque-like biofilm formed *in situ* under increasing frequencies of sucrose exposure

The effect of carbohydrate exposure to reduce inorganic ions concentration in the whole biofilm have been consistently shown *in situ* and *in vivo* (Cury et al., 1997, 2000; Nobre dos Santos et al., 2002; Ribeiro et al., 2005; Aires et al., 2006; Tenuta et al., 2006; Vale et al., 2007)

From Ccahuana-Vasquez et al., Caries Res, 2006
Residual* F in plaque-like biofilm formed in situ under F dentifrice** use and controlled exposure to sucrose*** (Mean ± SE)

*10 h after brushing

**NaF/silica, 1100 µg F/g, used 3 times/day

***Exposure to 20% sucrose solution 10 times/day

Cenci, Tenuta, Del Bel Cury, Pereira-Cenci, ten Cate, Cury. Caries Res, 2008
Fig. 1. Total F concentrations in whole saliva during and after brushing with NaF or MFP dentifrices containing different levels of F.

Bruun et al., Caries Research 1984;18:282-288
Fig. 3. Data on fluoride and unhydrolyzed MFP in centrifuged saliva after a NaF or NaMFP rinse containing 228 ppm fluoride. See figure 1 for a description of the symbols and table 1 for a statistical comparison of the rinses.
Fig. 1. Fluoride (---) and unhydrolyzed MFP (---) from plaque fluid recovered from (a) upper or (b) lower molar and premolar sites after a NaF or NaMFP rinse containing 228 ppm fluoride. Error bars: Standard deviation. * = this site (upper or lower) is greater than the corresponding site (upper or lower), p<0.05. See table 1 for statistical comparison of the rinses. Note that the time scale (x-axis) data have been slightly offset for the ‘Fluoride from MFP’ symbols to prevent overlapping of the plotted data points.
Fluoride (as MFP and F ion) concentration in the fluid phase of a test plaque according to dentifrice used and time of plaque collection (mean ± SD, n=10)

- Diffusion through the plaque
- Effect of toothpaste pH

Pearce and Dibdin, 1995

Tenuta et al., ORCA 2009, Abstract 83
Bioavailability and F uptake

“Although it is acknowledged by the scientific community that F uptake by enamel may no longer be accepted as a critical indicator of the mechanism of action of F, it is accepted as an excellent pre-screening tool”

F uptake

• **Loosely-bound** F: will be released and could have an effect on tooth de and remineralization

• **Firmly-bound** F: represents mainly the result of preceding de/re cycles in the presence of F
F uptake

• Method of F extraction:
  – KOH has been used to selectively extract loosely-bound, alkali-soluble F (Caslavska et al., 1975), prior to the extraction of firmly-bound F using acid or by mechanical removal of a tooth sample (by drilling or abrading the surface)
F uptake

• In *in situ*/*in vivo* studies, directly related to F bioavailability
• In *in vitro* studies, directly related to F⁻ availability
  – The *in vitro* reactivity of MFP containing-toothpastes with enamel is a result of ionic F present in the formulation (Bruun & Givskov, 1993; Cruz et al., 1994), and thus is a poor predictor of its anticaries effect
F uptake

• Substrate for F uptake
  – Sound x demineralized substrate
    • F uptake in demineralized enamel is 3-10 times higher than in sound enamel (Raven et al., 1991; Bruun & Givskov, 1993), which is useful to differentiate formulations
    • F bioavailability and clinical significance of this high uptake needs to be further evaluated
Strengths and limitations

• F availability:
  – Soluble F in a toothpaste is an indicator of the amount of anticaries active F
  – Simple and easy
  – Does not represent F bioavailability
Strenghts and limitations

- F bioavailability:
  - Salivary F
    - Good predictor of the anticaries effect
    - F is measured in whole saliva, and not in plaque fluid, the relevant site for the caries process
Strenghts and limitations

- F bioavailability:
  - Plaque F
    - Good predictor of the anticaries effect
    - In plaque fluid is a direct measure of the actual bioavailable F
    - Analysis of F in whole plaque should be interpreted with caution due to possible confounding factors
Strengths and limitations

- F uptake:
  - Can be used as an indirect measure of F- availability \((in \text{ vitro})\) or F bioavailability \((in \text{ situ/in vivo})\)
Strenghts and limitations

• F uptake:
  – There is no clear correlation between F in enamel/dentine and the anticaries effect
  – If a whole sample is collected, it is not able to separate loosely-bound and firmly-bound F
  – Cannot be used to assess MFP dentifrices in vitro
  – The high F uptake in demineralized enamel/dentine may not be relevant to sound surfaces
Conclusions

• Pre-clinical models of F bioavailability and F uptake are good indicators of the anticaries effectiveness of F toothpastes. The guidelines for these models should consider the currently accepted mechanism of action of F toothpastes.
Conclusions

• The choice of the study model should be based on the proposed mechanism of action of the F form (i.e. MFP dentifrices cannot be tested in models which do not simulate its in vivo hydrolysis)
Conclusions

• Models to simulate *in vitro* the anticaries effect of all kinds of F toothpastes (including those containing MFP) and a further understanding of their bioavailability in clinically relevant sites should be investigated.
Thank you!

- Dr. Jaime A. Cury

RESEARCH TEAM!

- Drs. Altair A. Del Bel Cury
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- Post-doc, PhD, MSc and undergraduate students