The in vivo preventive and therapeutic properties of curcumin in bile reflux-related oncogenesis of the hypopharynx

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Background:

Hypopharyngeal cancer is considered one of the most aggressive forms of head and neck malignancies with poor prognosis.[1,2] The prevalence of GERD (gastroesophageal reflux disease) has increased significantly in the United States over the last two decades, ranging from 18.1% to 27.8%. Moreover, biliary reflux, a variant of GERD is not uncommon, is linked to inflammatory and neoplastic process.[3,4]

The carcinogenic effect of acidic bile in hypopharynx was recently documented by our *in vivo* model, with NF-κB playing a central role by mediating the activation of an oncogenic mRNA phenotype.[5,6] Using an *in vitro* model we previously documented that curcumin, a dietary inhibitor of NF-κB, can effectively inhibit the acidic bile-induced cancer-related mRNA phenotype in human hypopharyngeal primary cells[7] similarly to pharmacologic inhibitor of NF-κB.[8,9]

Aim:

Here, we aimed to investigate the *in vivo* preventive and therapeutic properties of curcumin to hypopharynx under acidic bile effect, by blocking NF-κB and its related oncogenic molecular events.

Methods:

We used 48 wild-type mice, Mus Musculus (C57B16J; 24-male, 24-female; 8 mice/group).

We performed topical application of bile at pH 3.0 (**Acidic Bile**) (10 mmol/L in buffered saline), or curcumin (250 μmol/L; three times per day; 10 days; 6-hour interval) 15-min before (**Pre-Cur**), 15-min after (**Post-Cur**) or in combination (**Co-Cur**) with acidic bile to hypopharyngeal mucosa (HM), and controls (saline-treated-HM at pH 7.0, **Saline**; untreated HM, **UC,** as negative control).

We performed histologic staining (hematoxylin and eosin, H&E); immunohistochemical (IHC) analysis for p-NF-κB (S536) and cell proliferation marker Ki67, and gene expression analysis, by qPCR, for *Rela(p65), Bcl2, Il6, Tnf, Egfr, Wnt5a, Rela, Stat3, Ptgs2, Mtor, Akt1.*

Results:

Topically applied curcumin on HM, either before (Pre-Cur), after (Post-Cur) or in combination (Co-Cur) with acidic bile, can effectively

- (i) inhibit the acidic bile-induced NF-κB activation, throughout its mucosal thickness (Figure 1),
- (ii) suppress cell proliferation in HM, as indicated by the decreased levels of Ki67 in its regenerating epithelial cells (Figure 2).
- (iii) Curcumin blocks the acidic bile-induced transcriptional activation of *Rela(p65), Stat3, Bcl-2, Egfr, Tnf, Il6, Ptgs2 Wnt5a* (Figures 3 & 4), as similarly observed by pharmacologic inhibitors.[9]

Results:

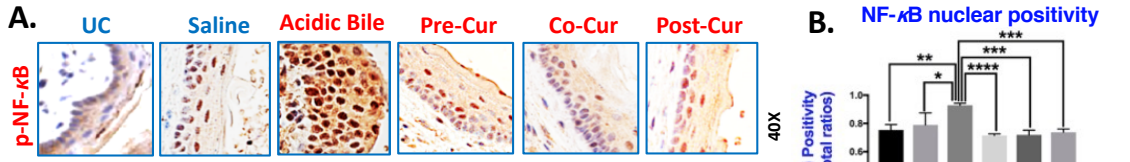

Figure 1: *In vivo* topical application of curcumin inhibits the acidic bile-induced NF-κB activation in 10-day exposed murine HM. **A.** IHC analysis for p-NF-κB (p65 S536). **B.** Graph depicts positivity for p-NF-κB (p65 S536), by IHC (positivity=nuclear p-p65 staining/total number of nuclei; mean±SD bin count of four images; *p <0.05; **p<0.005; ***p<0.0005; ****p<0.00005, by *t* test; multiple comparisons by Holm-Sidak).

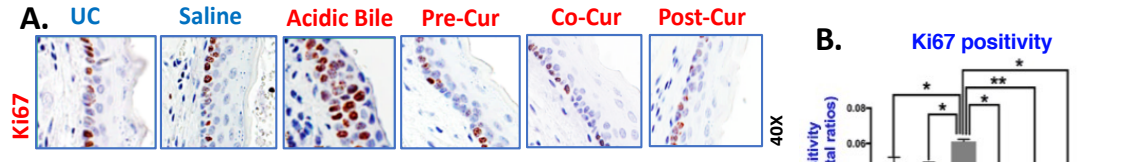

Figure 2: *In vivo* topical application of curcumin inhibits the increased cell proliferation induced by acidic bile in 10-day exposed murine HM. **A.** IHC analysis for Ki67. **B.** Graph depicts nuclear positivity levels for Ki67, by IHC. (positivity=nuclear Ki67 staining/total number of nuclei; mean±SD bin count of four images; *p <0.05; **p<0.005; ***p<0.0005; ****p<0.00005, by *t* test; multiple comparisons by Holm-Sidak).

UC: untreated control
Saline: Saline-DMSO
Acidic Bile: bile at pH 4.0
Pre-Cur: curcumin 15-min before acidic bile
Co-Cur: acidic bile plus curcumin
Post-Cur: curcumin 15-min after acidic bile

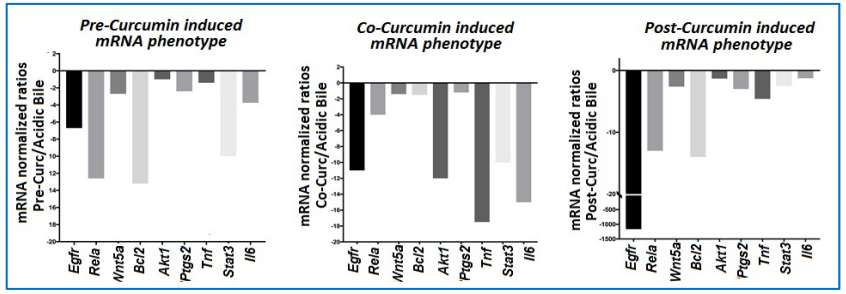

Figure 3. *In vivo* topical application of curcumin suppresses the acidic bile-induced mRNA oncogenic phenotype in murine HM. Columns of graphs depict mRNA phenotype caused by topical exposure to curcumin when it is applied 15 minutes before (Pre-Curcumin), simultaneously (Co-Curcumin), or 15 minutes after (Post-Curcumin) to acidic bile (pH 4.0) vs. acidic bile alone. mRNA phenotypes expression ratios of NF-κB-related genes, by qPCR analysis (reference gene: *Gapdh*).

Results:

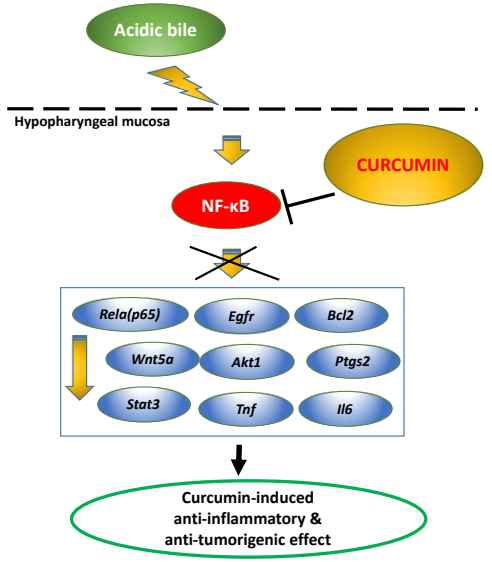

Figure 4: Schematic representation of *in vivo* preventive and therapeutic properties of curcumin on acidic bile-induced hypopharyngeal carcinogenesis.

Conclusions:

- ✓ We provide evidence into the **preventive and therapeutic properties of topically applied curcumin** in acidic bile-induced early oncogenic molecular events in hypopharyngeal mucosa, by inhibiting NF-κB.
- ✓ Our findings shape future translational development of effective targeted therapies using topical **non-pharmacologic NF-κB inhibitors.**
- ✓ It is clear that topical administration of curcumin is overall capable of suppressing the induced oncogenic mRNA phenotype, even if it not precisely synchronized with each reflux event.

References:

1) **Galli J, *et al.*** *Acta Otorhinolaryngol Ital.* **2003**; 23, 377-382.
2) **Langevin SM, et al.** *Cancer Epidemiol Biomarkers Prev.* **2013;** 22, 1061-1068.
3) **Covington MF, et al.** *J Nucl Med Technol.* **2014**; 42, 198-202.
4) **McQuaid KR, et al.** *Aliment Pharmacol Ther.* **2011**; 34, 146-165.
5) **Vageli DP, et al.** *Oncotarget* **2016**; 7, 5892-5908.
6) **Sasaki CT, Doukas SG, Costa, J, Vageli, DP.** *Cancers* **2020**; 12, 1064.
7) **Vageli DP, et al.** *J Cell Mol Med.* **2018**; 22, 4209-4220.
8) **Vageli DP, et al.** *Oncotarget* **2017**; 9, 5876-5891.
9) **Doukas PG, et al.** *Oncotarget* **2019**;10, 3339-3351.

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