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Evaluating the Effect of a Taxane-Based Anti-Cancer Drug on the Adult Taste Organ Using a Mouse Model

Archana Kumari
Rowan University

Lucre'ce Estropia
Rowan University

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School of
Osteopathic Medicine

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Lucre'ce Estropia and Archana Kumari
Department of Cell Biology and Neuroscience, Stratford, NJ

Background

Chemosensory alteration is one of the major side effects of chemotherapy that can negatively impact the quality of life of cancer patients.[1] Decreased appetite and food aversions can consequently lead to substantial reductions in food intake and thereby malnutrition and poor patient outcomes. The chemotherapeutic agent, taxane (docetaxel), is an effective choice of treatment for breast cancer, gastric cancer, or prostate cancer.[2-5] Despite its major effects as an anti-cancer medication, patients under taxane treatments have reported taste alterations.[6,7]

Essentially, the drug disrupts normal microtubule growth by inhibiting microtubule depolymerization. By binding to ß-tubulin, a major component of mitotic spindles, docetaxel (DTX) hyper-stabilizes the microtubule structure, rendering it incapable of properly shortening and elongating.[5] This intrinsic property is especially crucial for chromosome segregation during cell division. DTX also causes apoptosis and peripheral neuropathy.[5] Further, microtubules are essential components of cilia, where Hedgehog (HH) signaling takes place. HH signaling plays a critical regulatory role in taste organ formation and maintenance, while its deregulation can severely disrupt taste perception.[8]

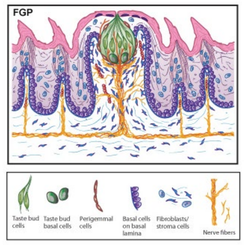
Importantly, taxane-based therapies have demonstrated an upregulating effect on HH signaling, but in combination therapies such as with sodium butyrate, it exhibits a down regulating effect.[2,4] It might be possible that the DTX is causing taste alterations via changes in HH signaling. Alternatively, disruption of cell division can also result in changes in taste cell turnover.

Research Question

The aim of this study is to elucidate whether DTX has an indirect or direct effect on the taste organ and whether it alters HH signaling.

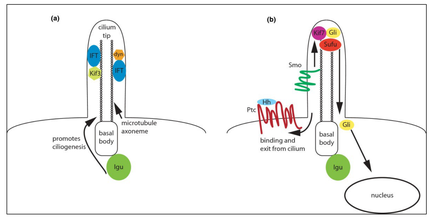
Methods and Materials

1. Animals and treatment: HH signaling reporter, *Gli1lacZ* was used to gavage DTX. Oral gavage was performed on mice every 2nd or 3rd day for 3 weeks. Mice received either solvent alone or solvent with 25 mg/ml DTX.
   Tissue Preparation: Tongues were harvested afterwards, cryopreserved, and embedded.
2. Cryo-sectioning: Tongues were cryo-sectioned at 10 µm and processed for immunostaining.
3. Immunostaining: Tongue sectioned were washed, blocked and incubated with primary antibodies for over 18 hours at 4°C. Following incubation, slides were washed thrice in 1X PBS for 10 minutes at RT. Secondary antibodies were added and slides were incubated for 1 hour at RT in the dark. Following incubation, slides were washed thrice in 1X PBS for 10 minutes at RT while in the dark. Slides were air dried in the hood for 10 minutes and mounted with vectashield with DAPI for imaging.
4. Analysis: Images were analyzed on ImageJ to quantify following
   - HH signaling: X-gal staining of *Gli1LacZ* revealed presence, reduction, or absence of the *Gli1* in the fungiform papilla epithelium.
   - Innervation: Antibody staining of NF+ nerves in the fungiform papilla connective tissue area was measured.
   - Taste bud perimeter analysis. Using antibody K8, the perimeter of taste bud was measured.
   - Proliferation: Antibody Ki67+ proliferating cells were counted in fungiform papilla basal cell epithelium and in perigemmal cell around the circumference of the K8+ taste bud.



Adapted from Mistretta and Kumari, 2017

Figure 1. Diagram of tissue architecture for the fungiform (FGP) papilla. Within the tongue, FGP is a complex organ that are home to specialized collections of taste cells, the taste buds, and are composed of an epithelium covering stromal cell compartments, with fibroblasts, nerves, and blood vessels in the lamina propria. The FGP (illustrated in sagittal section) in mouse and rat accommodates one apical taste bud and is innervated by lingual and taste bud-specific, chorda tympani nerves. Spinous filiform, nontaste papillae surround the FGP, innervated by the lingual nerve.



Adapted from Wilson and Stainier, 2010

Figure 2. Vertebrate Hh signaling is linked to the primary cilium. (a) The primary cilium is composed of microtubule doublets extending from the basal body and proteins required for its construction include intraflagellar transport (IFT) proteins and Iguana (Igu). Kinesin-3 (Kif3) transports IFT particles towards the cilium tip, and dynein (dyn) returns IFT cargo to the base. (b) When Hh ligands bind to Patched (Ptc), the Hh-Ptc complex appears to exit the primary cilium, allowing translocation and activation of Smoothened (Smo). Smo signals to the Gli transcription factors, possibly through modulation of Kinesin-7 (Kif-7) and suppressor of Fused (Sufu). Gli proteins exit the cilium and enter the nucleus to promote transcription of Hh target genes after undergoing conversion to an activated state at an undefined point in this process.

Results

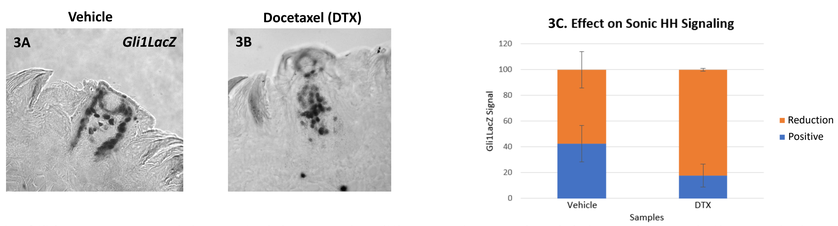


Figure 3. X-Gal staining of Gli1lacZ mouse tongue. A. Vehicle. B. DTX. C: Vehicle group sample size (28). DTX-treated group sample size (57). There is a reduction in the Gli1LacZ signal in the DTX-treated group.

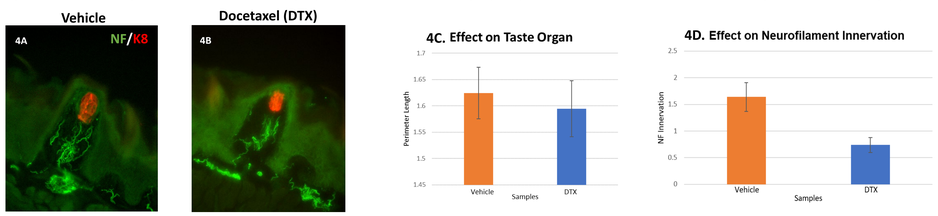


Figure 4. Taste organ and innervation after DTX treatment. K8 is a taste bud marker. NF is a marker for neuronal identity. A. Vehicle. B. DTX. C: Vehicle group sample size (62). DTX-treated group sample size (36). There is a slight reduction in perimeter length of the K8 signal, which represents the taste bud of the fungiform papillae. D. Vehicle group sample size (27). DTX-treated group sample size (15). There is a reduction in the percentage of area covered by NF in the connective tissue area of the fungiform papillae in the DTX-treated group

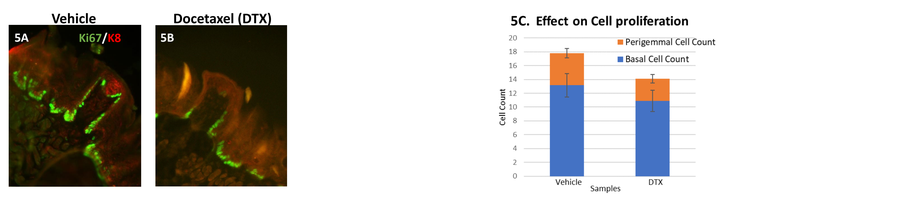


Figure 5. Proliferation quantifications after DTX treatment. Ki67 (arrow) is a proliferation marker. A. Vehicle. B. DTX. C: Vehicle group sample size (20). DTX-treated group sample size (21). There is a reduction in both the basal and perigemmal proliferative cells in the DTX-treated group.

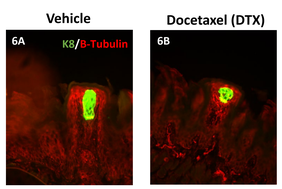


Figure 6. B-tubulin after DTX treatment. A. Vehicle. B. DTX. There is an increasing trend in ß-tubulin in the DTX group

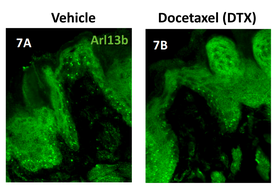


Figure7. Arl13b (cilia) after DTX treatment. A. Vehicle. B. DTX. There is a stabilization of Arl13b+ cilium in the DTX group

Discussion

Conclusions:

- A notable reduction in the *Gli1LacZ* signal in the DTX-treated group was discerned. Thus, DTX reduced HH signaling in the taste fungiform papilla.
- There was a slight reduction in the size of the taste bud after DTX treatment.
- There was a reduction in NF+ nerves in DTX-treated animal tongues. Hence, DTX exposure can result in lingual nerve damage.
- In the DTX-treated group, there was notable reduction in proliferating cells in the basal cells along the epithelium and perigemmal cells circumscribing the taste bud itself. This reduction in cell proliferation might have contributed to the loss of the taste organ.
- Furthermore, the preliminary data on increase of ß-tubulin and presence of cilia in the DTX-treated group indicates that DTX had stabilized the ß-tubulin.

Future Directions:

Moving forward, extended studies with increased treatment duration will have to be conducted to conclude whether there is a partial or complete loss of the taste organ.

Acknowledgements

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