Potential of Roupya Bhasma as a Bioenhancer

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Abstract

The high expense of treatment linked to drug dosage has particularly propelled the search for new methodologies to reduce drug dosage. Poor bioavailable drugs remain "sub-therapeutic" because a major portion of the dose never reaches the plasma and hence does not exert its pharmacological effects until a very large dose is used. In general, of the total drugs and chemicals, 20–50% of the drug dosage accounts for the unutilized drug that remains in the body, and result in unwanted side effects as well as higher costs of the treatment. The early bio-enhancers were of herbal origin and can be dated back to a period between the 7th century B.C. and the 6th century A.D. For thousands of years, the traditional medical system of Ayurveda has utilized formulations including metal powders (bhasma) to treat a large number of health issues. Without the support of modern science, these methodologies are largely ignored throughout the western world. Recent studies have begun to examine the mode of action of these materials, with initial results indicating their viability as bioenhancers. Our hypothesis was that the roupya bhasma particles act on the cellular tight junctions resulting in a reversible disruption or rearrangement of tight junction associated proteins in order to increase translocation of drug molecules; the bhasma particles facilitate the absorption of drug molecules into systemic circulation thereby reducing the dosage and subsequent side effects. Interaction of these nanoparticles with the different types of cellular systems were studied at different levels. We used several microscopic techniques in order to monitor morphological and cellular transcytosis response through marker molecules. Information from this study will help in exploiting bhasma as a potential bio enhancer for the delivery of different drug molecules.

Hypothesis

To study the changes in drug translocation in presence or absence of bhasma across different types of epithelial cell monolayers to examine the potential of bhasma as a bioenhancer.

Literature Research

- Most of the drugs available in the market are formulated to be administrated to patients via the oral route as a result of it convenience and cost effective administration.
- The drugs taken via oral route need to thus be formulated keeping in mind the plethora of barriers faced while going through the GI tract.
- Several approaches have been proposed to overcome these barriers, including enhancing drug potency through the use of bioenhancers.

- Bioavailibity may be defined as "a substance which in combination with a drug or nutrient provides more availability of the drug thereby reducing the amount of active molecule that is required".

- Bioenhancers involve increasing the transport of drugs by disrupting the TJ structure, facilitating paracellular transport of hydrophilic drugs across tight junctions.

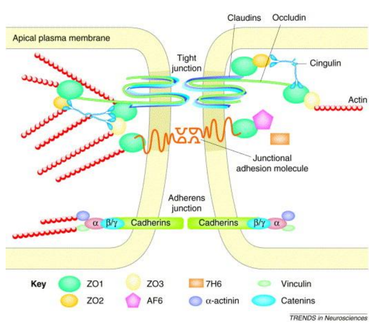


Fig. 1. Structure of TJ. Transmembrane proteins such as occludin and claudin (tetraspan) and JAM (single span) seal the paracellular spaces between adjacent epithelial cells. Plaque proteins, such as the ZO family proteins, act as adaptors that connect transmembrane proteins to the perijuntional actomyosin ring.

- The concurrent application of bio-enhancers with pharmaceuticals is not a new development, but has been used for thousands of years.
- The traditional medical system used in India (Ayurveda) has utilized formulations including metal powders (bhasma) to treat a large number of health issues.
- Preliminary studies carried out below indicate the potential of these powders to act as non-reactive bio-enhancer for drug delivery.

Methods

- ***Sample preparation:*** HT-29 cells were grown on glass coverslips in a 37 °C / 5% CO2 humidified incubator in recommended media containing 10% FBS. Cells were washed with PBS, and air dried. Roupya bhasma from Baidyanath was used for all of our studies.

- ***MTT Assay :*** The MTT assay was carried out by replacing the spent media with either serum free media or serum free media containing appropriate concentrations of bhasma, or ethanol for 24 h. 10 µl of MTT reagent was added and incubated until a purple precipitate was observed (2-4 hours). Once a precipitate was observed, 100 µl of detergent was added to the well and swirled gently to mix the contents. The plate was covered and left in the dark overnight before it was read at 570 nm.

- ***Confocal Microscopy:*** Coverslips coated with cells were treated with media containing 300nM Alexa-488 DrBoNT (recombinant botulinum neurotoxin) in the presence and absence of 1mg/ml bhasma containing media; on a Carl Zeiss LSM 700. Cells were washed with PBS, fixed with 4 % paraformaldehyde for 20 mins, and membrane was stained with membrane dye for 15min.

- ***Incubation of HBE cells with DrBoNT in (a) absence of bhasma and (b) presence of bhasma at 37 ºC.*** Cells grown on transwell inserts were incubated with AlexaFluor 488 labeled DrBoNT (green) on the apical side for 32 h, washed with HBSS and labeled plasma membrane with WGA-AlexaFluor 594 (red), washed, fixed and mounted for confocal microscopy as described above.

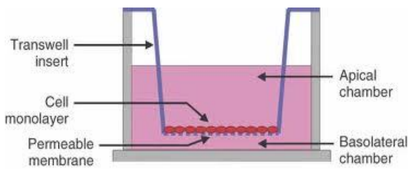


Fig. 2. Schematic diagram of a transwell system

- ***Atomic Force Microscopy:*** Coverslips coated with cells were scanned in air in contact or non contact modes. Images were acquired with non contact (spring constant – 42 N/m) on a Park Systems XE 100 atomic force microscope are shown below.

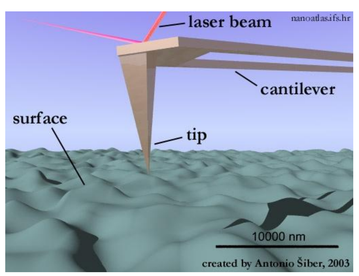
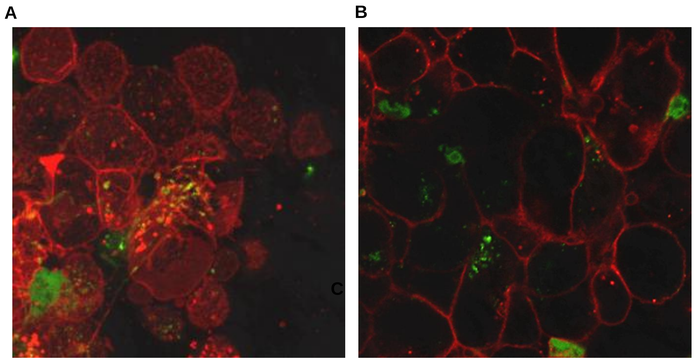


Fig. 3. Atomic Force Microscope (AFM): *AFM is a powerful technique that can be used for high resolution live cell imaging in buffer or growth media without the need for potentially interfering fluorescent labels or fixing cells.*

Results

(A) Confocal images- Cell Surface image of untreated HBE intestinal epithelial cells at 32 hours. (B) Membrane Surface of untreated HBE intestinal epithelial cells at 32 hours



(C) Confocal images- Cell Surface image of bhasma treated HBE intestinal epithelial cells at 32 hours. (D) Membrane Surface of bhasma treated HBE intestinal epithelial cells at 32 hours

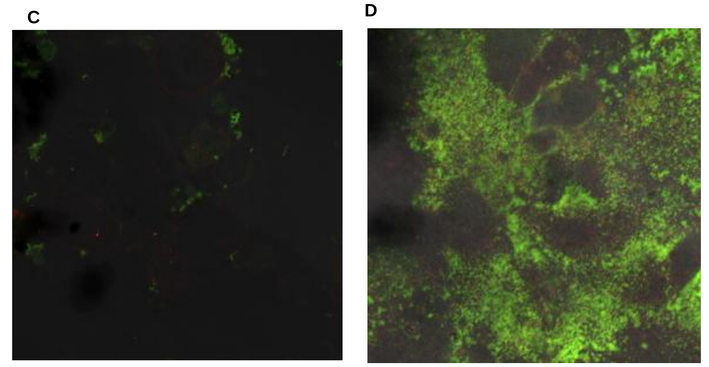


Fig 4. Incubation of HBE cells with DrBoNT Above images were obtained using a 63X oil immersion objective. Images in Fig. 4(a) show Alexa488-DrBoNT , only on the cell surface at 32 h in absence of bhasma, while Fig 4(b) shows accumulation closer to the insert membrane. Fig 4 (c &d) shows accumulation of Alexa 488-DrBoNT in the cell surface and membrane surface respectively, when treated with 1mg/ml of bhasma.

AFM Non-Contact mode image – Intestinal epithelial cells (HT-29)

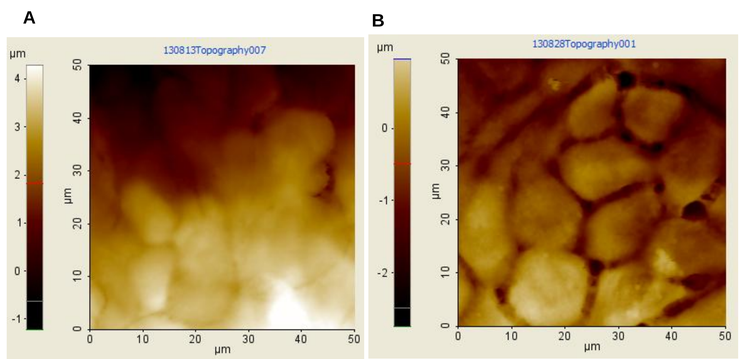


Fig. 5. AFM topography imaging of HT-29 cells: AFM images of HT-29 cells was carried out in the non-contact mode using a cantilever of 0.01N/m spring constant. (panel a)50µmx50 µm area of the untreated HT-29 cells (panel b)50 µm x50 µm section of HT-29 cell monolayer after 2 hour treatment;

Conclusion

- We tested the toxicity of bhasma at various concentrations in a physiological system. The cell viability ranged from 90.5% to 86.7% for 1 mg/ml to 4mg/ml of bhasma , respectively when compared to the untreated cells.

- The confocal images of intestinal epithelial cells treated with bhasma and incubated with Alexa-488 DrBoNT showed increased trafficking of the marker molecule upon treatment with bhasma.

- The AFM images show increased separation between cells in the monolayer.

- The results imply the ability of bhasma to potentially increase the rate of paracellular transportation of co-incubated molecules.

- Further studies need to be conducted to determine the exact mechanism by which bhasma interacts with the cell membrane in order to cause the increase in the rate of paracellular transportation in cells

- Information from these studies will help in exploiting bhasma as a potential bio enhancer for the delivery of different drug molecules.

Future Work

- Identify alterations in structural arrangement and levels of tight junction proteins due to bhasma treatment.

- Study alteration of rate of paracellular trafficking of molecules of different sizes across tight junction.

- Differences in the tight junctions at different time points will be assessed and bhasma concentrations.

- Carry out animal studies to study efficacy of bhasma as a bioenhancer

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