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(Article begins on next page)
A BIOSIMILAR MUCUS MODEL TO EVALUATE THE DIFFUSION OF DRUGS FOR MORE EFFICIENT CYSTIC FIBROSIS THERAPIES

Cosmin Butnarasu, A Maura Vallaro, A Daniela Pacheco, B Paola Petrinii, B Livia Visai, C and Sonja Visentin D

Aims

The orally taken systemic drugs must pass through the gastrointestinal mucus barrier, whereas inhaled drugs must pass through airway mucus and their pulmonary deposition to reach their targets. Due to the wide variety of functional groups present in the mucus structure (Figure 2a), many interactions can be established with molecules of hydrophilic as well as hydrophobic nature [4] and association $(K_A)$ and dissociation $(K_D)$ can be obtained (Figure 2b).

Therefore, the design of effective cys- tic fibrosis drugs must take into account the interaction of the potential candidates with mucin, and the diffusion across the mucus layer. The need to characterize drug behavior in a rapid, simple and reproducible manner has urged the development of airway mucus models. In this work, we investigate the affinity of some drugs to mucin and an airway mucus model composed by alginate and mucin, which aim to model both composition and rheological properties of the pathologic CF-mucus, is developed.

MUCIN-ANTIMICROBIC DRUGS INTERACTION

Since mucin is the mainly expressed glycoprotein within mucus, the protein-drug interaction may have an important role on the drug pharmacokinetics as a strong bond with mucin may result in a reduced drug absorption, hence a low drug efficacy. The determination of the extent of the interaction between the dataset drugs with a mucin solution was measured using two spectrophotometric methods: UV-VIS absorption and fluorescence spectra of mucin upon increasing concentration of drugs were recorded. $K_A$ and $K_D$ constants were calculated by using the Stern-Volmer equation (Figure 3).

Ceftazidime, aztreonam, CFTR(inh)-172 and levofloxacin showed to interact with mucin whereas no interaction was detected in presence of rifampin, tobramycin and ampicillin. The values of association constant are all in the same order of magnitude. Contrary to what was expected, the charge of the molecule seems to play such a fundamental role upon interaction with mucin, in fact positively charged molecules at pH 7.4, such as tobramycin, have no interaction, whereas negatively charged drugs such as CFTR(inh)-172 or aztreonam can interact. This suggest that the binding to mucin could be due only to electrostatic interactions but other factors as lipophilicity or H-bonding may play an important role. All the tested drugs have high hydropicity, in fact the values of $K_D$ are all negative, (except for CFTR(inh)-172 and rifampin). CFTR(inh)-172 which has exhibited the higher affinity is also the most hydrophilic at pH 7.4. The biosimilar mucin was employed also in PAMPA test, and the permeability of some drugs was measured in absence and in presence of mucin. Compared to a highly permeable compound (propanolol) the drugs we tested are low permeable already in absence of mucus and consequently the permeability is less influenced by mucus if compared to propanolol (Figure 6).

Results

Ceftazidime, aztreonam, CFTR(inh)-172 and levofloxacin showed to interact with mucin whereas no interaction was detected in presence of rifampin, tobramycin and ampicillin. The values of association constant are all in the same order of magnitude. Contrary to what was expected, the charge of the molecule seems to play such a fundamental role upon interaction with mucin, in fact positively charged molecules at pH 7.4, such as tobramycin, have no interaction, whereas negatively charged drugs such as CFTR(inh)-172 or aztreonam can interact. This suggest that the binding to mucin could be due only to electrostatic interactions but other factors as lipophilicity or H-bonding may play an important role. All the tested drugs have high hydropicity, in fact the values of $K_D$ are all negative, (except for CFTR(inh)-172 and rifampin). CFTR(inh)-172 which has exhibited the higher affinity is also the most hydrophilic at pH 7.4. The biosimilar mucin was employed also in PAMPA test, and the permeability of some drugs was measured in absence and in presence of mucin. Compared to a highly permeable compound (propanolol) the drugs we tested are low permeable already in absence of mucus and consequently the permeability is less influenced by mucus if compared to propanolol (Figure 6).

Conclusions

Even though some of the antibiotics herein investigated (ceftazidime, aztreonam, levofloxacin) can interact with mucin, the order of magnitude of $K_A$ is quite low (Table 1) while tobramycin showed no affinity to mucin. All these data could in part explain why these antiobiotics are the most employed antibiotics in CF. The low affinity of the tested drugs could be in part explained by their high hydropilicity. However, in order to obtain a structure-affinity relationship a broader database should be investigated.