Porcine Mammary Papilla is a Suitable In-vitro Model to Study Transmammary Drug Delivery through Human Mammary Papilla

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ABSTRACT SUMMARY

This study was aimed at developing an in-vitro model for studying transmammary drug delivery through human mammary papilla. Two model compounds 5-fluorouracil (5FU) and estradiol (EST) were used in the study. In-vitro transport studies were conducted using Franz diffusion cells. The drug transport was studied through mammary papilla with and without keratin plug. Further the permeation was compared to the surrounding breast skin. The results showed that porcine mammary papilla can be used as a model to study the transmammary delivery of drugs through human mammary papilla. Further the study also showed that the keratin plug removal with a simple alcohol swab can significantly increase the transport through mammary papilla.

INTRODUCTION

Breast cancer is the 2nd leading cause of cancer related deaths in women world. Currently used chemotherapy and chemopreventive therapies are severely limited by their systemic side effects, thus warranting alternative approaches. Majority of breast cancers originate in the epithelial cells lining the ducts in the breast and therefore localized delivery of chemopreventive/chemotherapeutic agents would be a promising approach for treatment of breast cancer. The multiple duct openings on the surface of the mammary papilla (nipple) offers a potential route for direct drug delivery to the breast tissue. Further, the epidermis in the mammary papilla is thinner compared to the surrounding breast skin. The main goal of this study is to develop an in-vitro model for testing drug delivery across human mammary papilla. To this end the in-vitro transport of model compounds were compared between porcine and human mammary papilla.

EXPERIMENTAL METHODS

Porcine mammary papillae were procured from the local slaughterhouse and female human mammary papillae were obtained from human tissue bank (NDRI, Philadelphia, PA). The in-vitro transport studies were conducted using mammary papilla with or without the keratin plug (removed by gently wiping the surface of the mammary papilla with an alcohol swab). The removal of the keratin plug was confirmed using a stereomicroscope. Estradiol (EST) and 5-fluorouracil (5FU) were used as model hydrophobic and hydrophilic compounds respectively. For comparison the permeation of these compounds was also studied separately using the surrounding breast skin.

Drug transport studies were carried out in Franz diffusion cell. The tissues were treated with saturated solution of 5FU (spiked with ^14C-5FU) and EST (spiked with ^3H-EST) in ethanol: water (50:50). The receptor medium was phosphate buffer (pH 7.4) for 5FU while 20% ethanol was added to the receptor medium for EST. The transport studies were performed for 48 h. Samples were withdrawn from the receptor compartment at different time points. At the end of study, the mammary papilla was cut and was digested using tissue solubilizer. The drug concentrations were determined by liquid scintillation counting.

RESULTS AND DISCUSSION

Permeation profile of 5FU via both, human and porcine, mammary papillae is shown in Fig.1A When the study was done with intact keratin plug the human mammary papilla showed significantly lower permeation than porcine mammary papilla. However when the keratin plug was removed the permeation profile was comparable between porcine and human mammary papillae. Interestingly the removal of keratin plug did not have a significant influence on the transport of 5FU in porcine tissues. The difference in permeation between porcine and mammary papillae may be attributed to the difference in the keratin content in these two tissues.

Figure 1. Profile of 5FU (A) and EST (B) permeation across human and porcine mammary papillae with keratin plug removal (KR) and without keratin plug removal. Each data point is represented as mean ± SEM (n=3-4).

In contrast, for the hydrophobic EST, the permeation profile was comparable between porcine and human mammary papillae irrespective of whether the keratin
plug was intact or removed. However, the keratin plug formed a significant transport barrier for the permeation of hydrophobic EST in both porcine and human tissues. The permeation of EST was 10 fold lower in presence of keratin plug (Fig. 1B). As evident from Fig. 1, removal of keratin plug reduced the lag time and increased the flux of 5FU and EST in both, porcine and human, mammary papillae.

Drug retention in mammary papilla for both 5FU and EST was higher in porcine mammary papilla compared to human mammary papilla. (Fig. 2). This could be due to the difference in the keratin content in porcine and human mammary papilla. The tissue retention was higher after removal of keratin plug for both the drugs and the tissue retention was comparable between porcine and human mammary papillae.

**Figure 2.** Retention of 5FU and EST in human and porcine mammary papillae with keratin plug removal (KR) and without keratin plug removal after 48 h treatment. Each value is represented as mean ± SEM (n=3-4).

Fig. 3 shows the comparative permeation profile between human mammary papilla and surrounding breast skin. Both the drugs showed higher permeability through mammary papilla after the removal of keratin plug compared to the surrounding breast skin. However the difference was not statistically significant for EST. The results show that the hydrophilic drugs may face a lesser transport barrier through the mammary papilla compared to the surrounding breast skin. On the other hand, the permeation of both the drugs through mammary papilla with intact keratin plug was significantly lower compared to surrounding breast skin (Fig. 3).

It is important to note that the triplicate data for human mammary papilla is from three different donors and as a result, higher variability was observed. Given the limited number of mammary papilla that can be obtained from human donors only triplicate experiments were conducted. However additional studies will be conducted to reduce the variability.

**CONCLUSION**

The findings from the study demonstrate that porcine mammary can be used as an in-vitro model for human mammary papilla. Further, the study also showed that the keratin plug can pose a barrier for drug transport through the mammary papilla. Overall the findings from the study can be used to test and develop localized delivery strategies for breast cancer and other breast diseases.

**REFERENCES**


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