Technical Report:

Evaluating the Effects of LoxaSperse® Using an *In Vitro* Model of Human Respiratory Tract Tissue

Abstract: LoxaSperse™ is an excipient manufactured by PCCA that can be used in compounding as a dispersing or solubilizing agent for active pharmaceutical ingredients (APIs) in nasal nebulizations or irrigations. It can help improve the solubility and therefore potential bioavailability of poorly water soluble drugs or combinations of drugs used in the treatment of respiratory and pulmonary diseases. The *in vitro* toxicity profile of LoxaSperse was evaluated using normal tracheal/bronchial epithelial cells in an assay which closely resembles the epithelial tissue of the respiratory tract. At concentrations of 0.01 µg/µL, 0.1 µg/µL, and 1 µg/µL, LoxaSperse was shown to be substantially less toxic than the positive control, Polysorbate 20 NF, with mean percent cell viabilities of 96%, 98%, and 69% for LoxaSperse compared to 65%, 28%, and 7% for the toxicant Polysorbate 20 NF, respectively. In addition, LoxaSperse exhibited a low toxicological profile similar to that of a known nontoxic respiratory agent, Monohydrate Lactose inhalation grade (negative control). The results of this study suggest a positive safety profile for the use of LoxaSperse as an excipient for compounding with APIs used in the treatment of respiratory and pulmonary diseases.

Purpose:

The objective of this study is to compare the toxicity profile of LoxaSperse against a known toxic (Polysorbate 20 NF) and commercial nontoxic (Monohydrate Lactose inhalation grade) agent in the pulmonary system using an *in vitro* model of the human respiratory tract tissue.

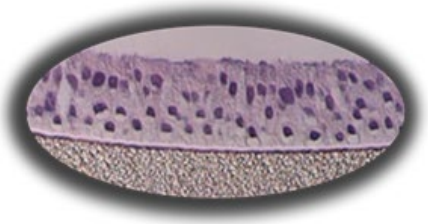
Introduction:

Local delivery of medication to the sinuses and lungs is highly desirable, especially in patients with specific sinus and pulmonary diseases such as cystic fibrosis, asthma, chronic sinus and pulmonary infections, and lung cancer. The principal advantages include reduced systemic side effects and higher doses of the applicable medication at the site of drug action (Harvey & Schlosser, 2009; Pilcer & Amighi, 2010).

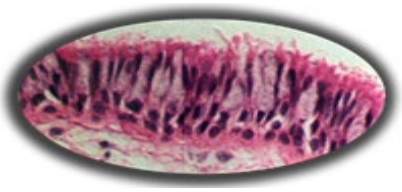
Many existing APIs and an increasing number of new drugs are often poorly water soluble (Zhang et al., 2011). Drug insolubility, regardless of the administration route, commonly generates bioavailability or efficacy problems. Different techniques exist to increase drug dissolution and/or solubility, which often require the use of specific excipients. The excipients used in nasal nebulizations and irrigations should be chemically and physically stable, inert to the API used with them, and exhibit no side effects (Duret *et al*., 2012).

LoxaSperse is a proprietary excipient manufactured by PCCA for use in compounding as a dispersing or solubilizing agent for APIs in nasal nebulization and irrigation formulations. It consists of a blend of micronized xylitol and poloxamers. This combination is designed to be mixed with APIs in order to improve the drugs' water solubility and dispersability (PCCA, 2013). Xylitol is a 5-carbon sugar with low transepithelial permeability and is poorly metabolized by bacteria (Durairaj *et al*., 2007). Poloxamers are a series of synthetic block copolymers of poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) or PEO–PPO–PEO with varying molecular weights and block ratios. They are nonionic amphiphilic surfactants possessing excellent wetting, antifoaming, and solubilizing properties (Moebus *et al*., 2009). The use of xylitol and poloxamers in nasal nebulizations and irrigations is thoroughly referenced in the literature, and there is evidence of their safety (Durairaj *et al*., 2007; Jagannath *et al*., 1995; Plataki *et al*., 2011; Zabner *et al*., 2000). LoxaSperse is an excipient base used in compounding that allows for the preparation of non-sterile capsules and powder sachets that are added to sterile water or water for injection by the patient at the time of administration (PCCA, 2013).

This study was conducted to determine the toxicological profile of LoxaSperse in human-derived tracheal/bronchial epithelial cells (TBE) which have been cultured to form a multilayered, highly differentiated model which closely resembles the epithelial tissue of the respiratory tract (Figure 1). Histological cross-sections of both the *in vitro* tissue and a normal human bronchiole reveal a pseudostratified epithelial structure. The LC50 (the concentration at which the survival of the exposed cells is 50% of the maximum value) was determined for LoxaSperse (test compound), Polysorbate 20 NF (positive control), and a commercial Monohydrate Lactose inhalation grade (negative control) as a comparative measure of toxicity among the compounds.



In vitro human respiratory tract tissue



Normal human bronchiole

Figure 1. Histology – *in vitro* human respiratory tract tissue and normal human bronchiole. Formalin fixed, paraffin embedded and H&E stained (100x).

Methodology:

Materials: Polysorbate 20 NF and a commercial Monohydrate Lactose inhalation grade were selected due to their known toxic and nontoxic effects in the respiratory tract tissue, respectively. Given that LoxaSperse is designed to be an excipient for respiratory drugs, low doses of LoxaSperse were used in this study. LoxaSperse (Lot: 6212182), Polysorbate 20 NF (Lot: C162820), and a commercial Monohydrate Lactose inhalation grade (Lot: 10765667) were provided by PCCA (Houston, TX, USA) as powders and were dissolved in sterile water to make a 1 µg/µL stock solution for each compound. The stock solution was then diluted to make the 0.1 µg/µL (1:9 dilution) and 0.01 µg/µL (1:99 dilution) solutions. All compounds were prepared on the day of the assay.

Methods: In the *in vitro* model, the human tracheal/bronchial epithelial cells were cultured on specially prepared cell culture inserts using serum free media to form ciliated, bronchiole-like structures, which resemble the human epithelial tissue of the respiratory tract. This multilayered, highly differentiated 3D model was used to determine the toxicological profile of LoxaSperse in the respiratory tract tissue.

The human tracheal/bronchial tissue inserts were incubated on a 6-well plate with each compound at concentrations of 0.01 µg/µL, 0.1 µg/µL, and 1 µg/µL for 3 hours with 5% CO_2 at 37°C and ≥ 90% humidity. After the 3-hour incubation period, each tissue insert was individually removed from its plate, decanted into a waste beaker, and rinsed 3 times with phosphate buffered saline (PBS). After the final rinse, excess liquid was shaken off and each treated tissue insert was dosed with 300 µL of MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyltetrazolium bromide). MTT is a water soluble, yellow tetrazolium salt which is reduced by succinate dehydrogenase in the mitochondria of viable cells to a purple, insoluble formazan derivative. Toxic substances that damage the mitochondrial enzyme, succinate dehydrogenase, inhibit the reduction of MTT; therefore, the amount of reduced MTT is proportional to the number of viable cells. Following a 3-hour incubation with MTT, each insert was removed and gently rinsed with PBS. Once residual MTT and PBS have been removed, the inserts were placed into one 24-well extraction plate, which was immersed into 2 mL of extraction solution. The plate was then sealed in a plastic bag and stored overnight at room temperature.

After the extraction period, the liquid within each insert was decanted back into the 6-well plate. The remaining extractant solution was then agitated, and a 200 µL aliquot of each extract was removed for evaluation. A Molecular Device SpectraMax M5 Microplate Reader was used to determine the absorbance of each extract at 570 nm – the wavelength that corresponds to the amount of reduced MTT or cell viability.

Data Analysis: The percent cell viability for each well was measured from the absorbance or optical density (OD) reading while using the mean defined absorbance of the diluent (sterile water) as control. Toxicological profiles as a function of log concentration were generated using GraphPad Prism 5 (La Jolla, CA, USA). The LC50s were interpolated from a sigmoidal dose response curve fitting analysis. All results were expressed as mean ± standard error of the mean.

Results and Discussion:

Using a nonlinear regression model, the toxicological profiles of LoxaSperse, Polysorbate 20 NF (positive control), and a commercial Monohydrate Lactose inhalation grade (negative control) were measured as shown in Figure 2.

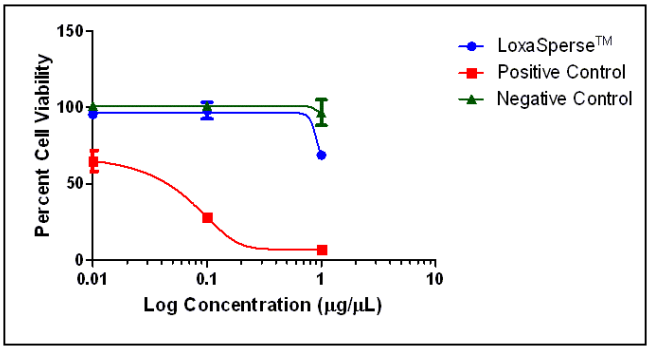


Figure 2: Graph shows the toxicological profiles of LoxaSperse, Polysorbate 20 NF (positive control), and a commercial Monohydrate Lactose inhalation grade (negative control).

At concentrations of 0.01 µg/µL, 0.1 µg/µL, and 1 µg/µL, LoxaSperse was shown to be substantially less toxic than the positive control, Polysorbate 20 NF, with mean percent cell viabilities of 96%, 98%, and 69% for LoxaSperse compared to 65%, 28%, and 7% for the toxicant Polysorbate 20 NF, respectively. In addition, no clinically significant differences in toxicological profiles were observed between LoxaSperse and the nontoxic agent, Monohydrate Lactose inhalation grade, indicating very minimal toxicity of LoxaSperse in the human respiratory tract tissue. Unlike the toxic agent, Polysorbate 20 NF, percent cell viabilities for LoxaSperse and Monohydrate Lactose inhalation grade did not decrease to less than 50%; thus, the predicted LC50s for LoxaSperse and Monohydrate Lactose inhalation grade were at least 7-fold higher than that of Polysorbate 20 NF (predicted LC50 for LoxaSperse = 7.95 µg/µL; predicted LC50 for Monohydrate Lactose inhalation grade = 7.64 µg/µL; LC50 for Polysorbate 20 NF = 1.11 µg/µL). Altogether, these results indicate that exposure of human tracheal/bronchial epithelial cells to LoxaSperse produced very minimal toxicity, with LoxaSperse exhibiting low toxicological profile similar to that of a known nontoxic respiratory agent.

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Conclusions:

LoxaSperse is an excipient used to enhance the water solubility and dispersibility of APIs, leading to increased bioavailability, which can potentially improve drug efficacy and therapeutic outcome. Using LoxaSperse as an excipient in compounded preparations allows for APIs with low water solubility and combinations of APIs to be delivered locally to the area of treatment. LoxaSperse improves the dissolution of poorly water soluble drugs into hydrophilic and permeable tissue compartments such as the oral and nasal cavities. This study showed that LoxaSperse as an excipient produced very minimal toxicity in the human respiratory tract tissue. The results of this study suggest a positive safety profile for the use of LoxaSperse as an excipient for compounding with APIs used in the treatment of respiratory and pulmonary diseases.

Financial Disclosure: PCCA contracted Consumer Product Testing Company, Inc. (Fairfield, NJ) to conduct this study. Consumer Product Testing Company, Inc. has no proprietary or financial interests in the test products or equity interest in PCCA, the sponsor of the study.

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