Development of an In Vitro Permeation Test to Predict the In Vivo Performance of Naloxone Hydrochloride Nasal Spray Products

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Introduction

Naloxone hydrochloride (HCl) is a standard medication to reverse opioid-related respiratory depression. In 2015, U.S. FDA approved the first intranasal (IN) spray of naloxone HCl. Afterwards, clinical studies were conducted using different IN formulations of naloxone HCl. This study employed the previously published clinical data to make inferences about the critical drug product attributes and conditions related to drug administration that may affect the nasal permeability of naloxone. This information will help in developing a biopredictive and biorelevant in vitro permeation test (IVPT) protocol.

Materials and Methods

Literature search was conducted for clinical studies of IN naloxone HCl using PubMed and ClinicalTrials.gov databases. Data were collected from 14 published clinical studies[2-7] involving 13 IN naloxone HCl formulations (**Table 1**). When data for comparisons were available, statistical analysis (t-test, $\alpha = 0.05$) was performed on each formulation to determine the significance impact of the following on the nasal bioavailability (C_{max} and $AUC_{o\text{-}inf}$): administering drug to one nostril versus two, volume of formulation administered (100 µL versus 200 µL) , pH of formulation (3.5-5.6), and differences in formulation composition (**Table 1**).

Table 1. Summary of clinical studies grouped by study number, formulation number, number of nostrils used, formulation volume administered, dose administered, pH, and other critical formulation ingredients. NR = value not reported.

Study Number	Formulation Number(s)	Number of Nostrils Used	Volume Administered (µL)	Dose (mg)	pH	Critical Ingredient(s)
1	1	2	100	2	4.5	Benzalkonium Chloride
			200	4		
2	2	1	100	2	4.5	
		2		4		
	3	1	100	4	4.5	
		2		8		
3	4	2	200	8	5.6	
	5	2	200	16	5.6	
4	6	1	100	1	4.25	Benzyl Alcohol
		2		2		
	11	2	1000	2	3.5	
5	6	2	100	2	4.5	Benzyl Alcohol
			200	4		
6	7	1	100	0.8	4.3	Glycerin, Polyvinylpyrrolidone (PVP)
		2		1.6		
7	9	2	200	2	NR	Polysorbate 20, Sodium Lauryl Sulfate
	10	2	100	2	NR	
8	2	1	100	2	4.5	Benzalkonium Chloride
	3	1	100	4	4.5	
	11	2	1000	2	3.5	
			2000	4		
9	12	2	900	0.72	3.8	
10	2	1	100	2	4.5	Benzalkonium Chloride
		2		4		
11	8	1	100	2	4.3	Glycerin, PVP
12	3	1	100	4.58	4.5	Benzalkonium Chloride
13	13	1	100	1.4	NR	Glycerin, PVP
			200	2.8		
14	1	1	100	1	4.5	Benzalkonium Chloride
	2	1	100	2	4.5	
		2		4		

In vitro permeation experiments using a mucociliary tissue model [EpiAirway, Mattek] in an Ussing chamber system were performed to determine the effect of benzyl alcohol (BA) and benzalkonium chloride (BC) on the permeability coefficient of a 4 mg/mL naloxone solution. These in vitro samples were analyzed using a published UPLC-UV method.[8]

Results and Discussion

Effect of Number of Nostrils Used for Administration

No significant impact on C_{max} was found when drug was administered to one nostril versus two ($p \geq 0.2522$). The $AUC_{o\text{-}inf}$ showed a similar trend ($p \geq 0.2583$).

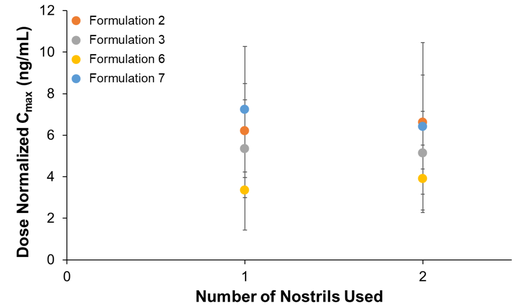

Figure 1. Effect of number of nostrils used for drug administration (change of surface area) on C_{max} of four IN naloxone HCl formulations. Mean C_{max} was dose normalized (to 4 mg naloxone). Formulation 7 has no error bars, study reported mean with 95% confidence interval (CI) but no standard deviation (SD).

Effect of Volume of Formulation Administered

No significant impact on C_{max} was found when the volume of administration was 100 µL versus 200 µL ($p \geq 0.2226$). $AUC_{o\text{-}inf}$ showed a similar trend ($p \geq 0.1024$). **Note:** Formulation 11 was not included due to high variability resulting from the large volume delivered.

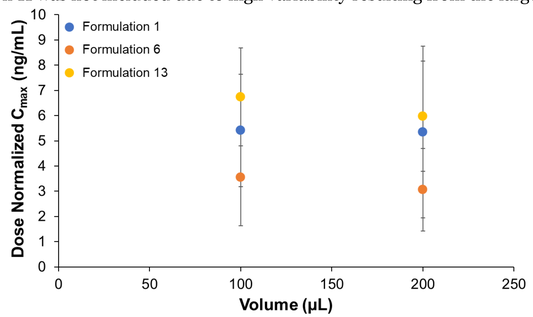

Figure 2. Effect of changing volume or concentration of drug on the dose normalized C_{max} of IN naloxone HCl formulations. Mean C_{max} was normalized to 4 mg naloxone.

Effect of pH Change

- pH change from 3.5 to 5.6 significantly impacted the C_{max} ($p \leq 0.0015$). $AUC_{o\text{-}inf}$ showed a similar trend ($p < 0.0001$).

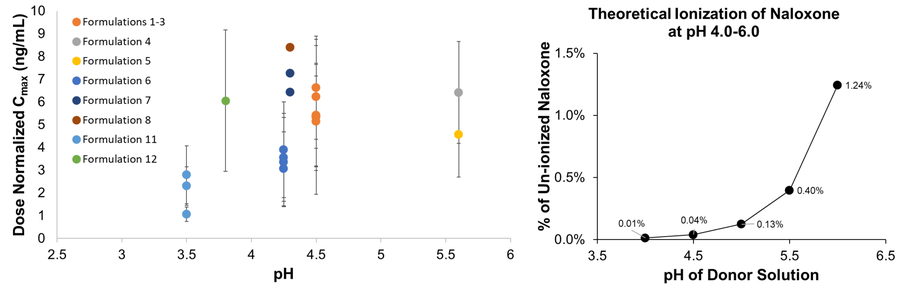

Figure 3. Effect of pH change on C_{max} of seven IN naloxone HCl formulations and Formulations 1-3 (left); the theoretical ionization of naloxone HCl at various pH values (right). Mean C_{max} was normalized to 4 mg naloxone. **Note:** Error bars were not included for formulations that did not report SD.

Effect of Formulation Ingredients

- Compared to Formulations 1-3 (containing BC) C_{max} trendline, Formulation 6 (containing BA) had significantly lower C_{max} values compared to the expected value at each dose (all p-values ≤ 0.013). The $AUC_{o\text{-}inf}$ showed a similar trend ($p \leq 0.0123$).
- Buffer in Formulation 6 resulted in slow IN absorption due to slower neutralization effect.
- In vitro permeation study shows BA is a weaker permeation enhancer than BC that may lead to lower C_{max}.

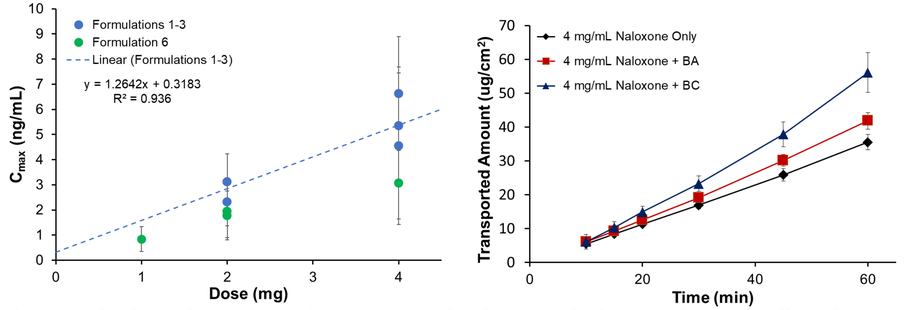

Figure 4. The observed C_{max} of Formulation 6 compared to the expected value using the C_{max} trendline of Formulations 1-3 (left); permeability experiment showing the in vitro amount of drug transported for a 4 mg/mL naloxone HCl formulation alone or containing BA or BC (right).

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Conclusion

Using one or two nostrils or drug dose volume does not appear to impact nasal bioavailability of IN naloxone HCl products. In contrast, a change in formulation pH and choice of permeability enhancer (specifically BA and BC) appear to impact the nasal bioavailability of naloxone in clinical studies. This impact is supported by in vitro permeability experiments. Knowing drug product attributes and drug administration conditions that impact the nasal bioavailability of naloxone HCL will help in the development of a biopredictive IVPT method for the assessment of naloxone HCl.

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