Pulmonary Delivery of Bevacizumab (Avastin™) in Sheep

[1]Steven A. Giannos, MS, [1]Edward R. Kraft, [1]Jinping Yang, MD, [1]Mary E. Schmitz-Brown, BS,
[1]Kevin H. Merkley, MD MBA, [2]Aristides P. Koutrouvelis, MD

[1] University of Texas Medical Branch, Department of Ophthalmology & Visual Sciences, Galveston, TX
[2] University of Texas Medical Branch, Department of Anesthesiology, Galveston, TX

CONTACT INFORMATION: sgiannos@yahoo.com

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PURPOSE

Pulmonary diseases may benefit from nebulized antibody delivery. However, nebulization damages biopharmaceuticals. Antibody formulations also need to be adjusted to prevent antibody degradation. The purpose of this study was to assess *in vitro* and *in vivo* feasibility of nebulizing and delivering bevacizumab, an anti-VEGF mAb, to the lungs using an anti-aggregation formula (AAF).

INTRODUCTION

Lung cancers, asthma and chronic obstructive pulmonary disease (COPD) and possibly COVD-19 therapy may benefit from direct antibody pulmonary delivery. Although ultrasonic and jet nebulizers are acceptable for small molecule delivery into deep lung tissue, they damage proteins and biopharmaceuticals.[1] Additionally, the formulation carrier is an important factor in maintaining antibody binding capacity.[2] Common carriers, saline and phosphate buffered saline (PBS), reduce active antibody monomers.[3] An anti-aggregation carrier formulation (UTMB AAF, Giannos et al. 2018), protected bevacizumab during aerosolization; providing near 100% intact functional antibodies.[4] We compared the best known previously re-ported vibrating mesh nebulization method (LMU 2, Hertel et al. 2014) [5] with AAF in a pulmonary delivery sheep model

In Vitro Nebulizer Testing

Methods: Three carrier compositions with bevacizumab (100 mg/ml, 10 ml) were tested using an Aerogen Solo vibrating mesh nebulizer: normal saline (negative control), LMU 2 and AAF to determine the recovery of intact, functional antibodies before and after aerosolization compared to a non-nebulized positive control in AAF. Recovered samples were analyzed by SE-HPLC and correlated with ELISA.

Results: Results are shown in Figure 1. Non-nebulized bevacizumab standard in AAF had a slope of 0.2276. LMU 2 pre-nebulization had a slope of 0.2316 and post-nebulization of 0.1974. UTMB AAF pre-nebulization had a slope of 0.2347 and post nebulization of 0.1906. Bevacizumab in saline pre-nebulization had a slope of 0.0044 and post-nebulization of 0.0001.

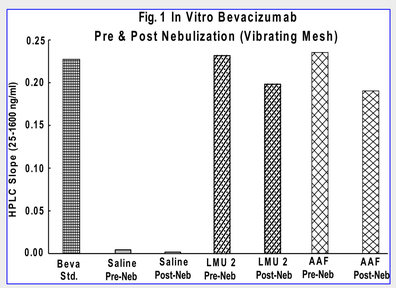
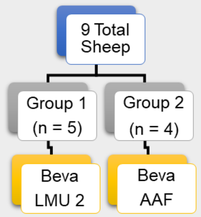


Figure 1. Results from *in vitro* testing.

METHODS

- **Compare 2 compositions containing bevacizumab (100 mg/ml, 10 ml) using Aerogen Solo vibrating mesh nebulizer in sheep.**
- All procedures were approved by the UTMB Institutional Animal Care and Use Committee.
- All chemicals were pharma grade, and solutions filtered with a Steriflip® 0.22 µm filter to assure purity and sterility.
- Nine sheep intubated for nebulization.
- Sheep received bevacizumab (10 mg/ml, 10 ml) using an Aerogen Solo vibrating mesh nebulizer with LMU 2 (n=5) or AAF (n=4) under isoflurane anesthesia and mechanical ventilation for about 30 minutes.
- After nebulization, blood was taken, sheep were euthanized, and lungs removed for tissue sampling.
- One-gram samples of deep lung tissue were obtained from upper, middle and lower lobes of each lung and extracted with AAF overnight at 4°C.
- Lung sample antibody concentration quantified by ELISA.



LMU 2 (Hertel et al. 2014)

Components	% w/w (v/w)
Histidine	0.3103
NaCl	0.4383
Manitol	1.3333
Sucrose	2.6667
Polysorbate 20 (v/w)	0.04
Water (v/w) (Ultrapure MilliQ) (pH 6.5)	95.21

UTMB AAF (Giannos et al. 2018)

Components	% w/w (v/w)
αα,Trehalose Dihydrate	7.5
Sodium Phosphate Dibasic	1.105
Sodium Phosphate monobasic	0.1697
Sodium Chloride	0.3
L-arginine	0.174
Polysorbate 80 (v/w)	0.04
Water (v/w) (Ultrapure MilliQ) (pH 7.4)	90.71

RESULTS

Table 1. Average antibody concentration (mg) detected in each sheep lung.
Antibody was not detected in any serum sample

LMU 2 n=5				UTMB AAF n=4		
	Ave ng/gm ± SD	Lung Wt gm ± SD	Total mg Delivered	Ave ng/gm ± SD	Lung Wt gm ± SD	Total mg Delivered
L Center	12,164 ± 2,163			11,747 ± 3,202		
L Upper	10,089 ± 2,928			9,061 ± 1,380		
L Lower	8,347 ± 2,776			6,075 ± 2,897		
L Lung Ave	**10,200 ± 2,622**	165.2 ± 17.0	**1.68**	**8,961 ± 2,493**	169.8 ± 25.5	**1.52**
R Center	11,086 ± 6,491			10,358 ± 2,442		
R Upper	3,123 ± 1,326			3,179 ± 849		
R Lower	10,638 ± 4,036			7,042 ± 2,870		
R Lung Ave	**8,282 ± 3,951**	225.2 ± 20.6	**1.86**	**6,860 ± 2,054**	245.1 ± 28.9	**1.68**
LMU 2 Ave Total mg Delivered Both Lungs			3.55	AAF Ave Total mg Delivered Both Lungs		3.20

RESULTS (CONT.)

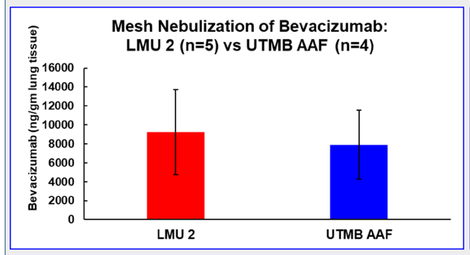


Figure 2. Results of Beva delivery in each lung using LMU 2 or AAF.

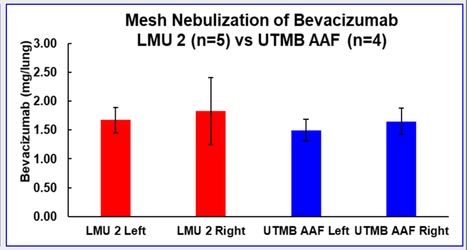


Figure 3. Results of lung tissue delivery of Beva in LMU 2 or AAF.

CONCLUSIONS

Mesh nebulization of bevacizumab, using LMU 2 formula or UTMB AAF, allowed an average of 1.69 mg active bevacizumab to be delivered to each lung. This translates to greater than 3% transfer efficiency of active bevacizumab to deep lung tissue for the whole animal.

In Vitro:

- Nebulization confirmed loss of antibody in saline.
- Both formulas equally protected the antibody during *in vitro* nebulization.
- Microchannel nebulizer [6] (data not shown) delivers 20% more active antibody than vibrating mesh nebulizer.

In Vivo:

- Both formulas equally protected the antibody during *in vivo* nebulization.
- Anti-VEGF antibodies can be nebulized, paving the way for improved devices [6] & therapies.

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