Development of a sustained-release form of dexamethasone for the local treatment of sensorineural hearing loss

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1. INTRODUCTION

Liposomes are phospholipid vesicles capable of encapsulating an active molecule. As smart nanocarriers, liposomes can deliver the active molecules to their site of action and control their release. In the case of sensorineural hearing loss, the therapeutic target is the hair cells of the inner ear. However, the inner ear is a difficult to access area due to various physiological barriers. Therefore, the use of liposomes combined with transtympanic administration is proposed (Figure 1 – A). The active principle will be deposited in the middle ear, in contact with the round window membrane (RWM ; Figure 1 – B) and will diffuse through this membrane to reach the cochlea (Figure 1 – C). The development of a sustained-release formulation would allow a stable concentration of active ingredient in the cochlea while reducing the number of administrations.

The lipid composition of the liposome determines its physicochemical properties (stability, size, encapsulation rate, release rate,…) and therefore its effectiveness. Thus, the first step of this research is the optimization of this lipid composition.

Biocompatibility and cellular penetration will be improved by incorporating into the formulation various components such as phosphatidylcholine (PC), phosphatidylglycerol (PG), dipalmitoylPC (DPPC), ceramides, surfactant proteins,... Sustained release will be modified by using saturated lipids (dipalmitoylPC or distearoylPC) or by changing the percentage of cholesterol that stabilizes the membrane (Figure 2).

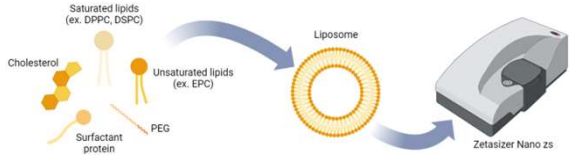

Figure 2. Optimization of lipid composition, production by rapid mixing and DLS analysis [1].

2. OBJECTIVES

The first objective is to select a formulation allowing a sustained release of the encapsulated active ingredient. Different combinations of lipids and cholesterol will therefore be tested, and the percentages of these components will be modulated. The parameters selected to produce liposomes by rapid mixing will also be optimized in order to obtain suitable sizes and polydispersity (PdI).

3. MATERIALS & METHODS

a. Liposomes formulation

- Egg phosphatidylcholine (EPC), Cholesterol (Chol), DSPE-PEG_{2000} : 60/35/5 (% m/m)
- Dexamethasone : 2,1 mg/mL

b. Rapid mixing process (Figure 3)

- Lipids and Chol were all placed in the ethanolic phase. TFR : 1 mL/min & FRR : 1/1.
- Syringe pumps (Chemyx Fusion 200-X, KR Analytical Ltd, Sandbach, UK) connected by a T-junction (PEEK™ Tee, ThermoFisher Scientific (Walthman, MA, USA)) and Tube Peek (1/16'' OD, 0.010'' ID, 20 cm between the pump and the T-junction; 1/16'' OD, 0.020'' ID, 30 cm after the T-junction).

c. Physicochemical properties

Size was characterized right after production and 24 hours later, using Dynamic Light Scattering (DLS ; n=3). Liposomes were then purified and characterized again (= 48 hours after production).

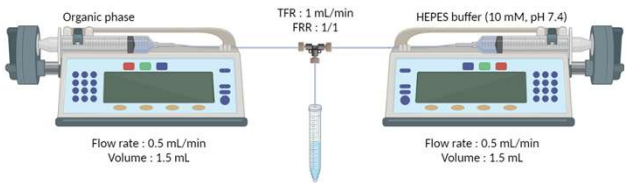

Figure 3. Production of liposomes by rapid mixing [1].

4. RESULTS

First results are presented in Table 1. Three batches of liposomes were produced. A first post-production DLS analysis showed promising size and polydispersity results, with a target size being around 150 nm. 24 hours later, a second analysis was performed, and the results showed some short-term stability. The liposomes were then dialyzed before being analysed a third time by DLS. The results obtained show that the purification process does not impact the size and polydispersity of the liposomes.

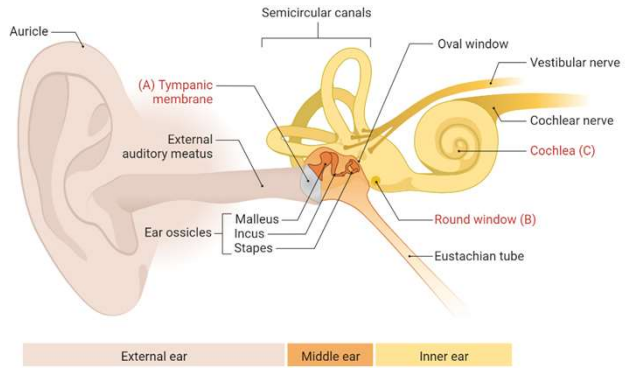

Figure 1. Middle and inner ear anatomy [1].

Table 1. Size and PdI measurements showing good stability through purification steps.

		After production (t = 0h)	t = 24h	After dialysis (t = 48h)
1st batch	Size (nm)	154,4	143,7	155,4
	PdI	0,192	0,162	0,166
2nd batch	Size (nm)	134,5	142,1	162,2
	PdI	0,162	0,131	0,143
3rd batch	Size (nm)	142,0	149,2	154,8
	PdI	0,180	0,120	0,123

5. CONCLUSION

Currently, the parameters selected for the optimization of the rapid mixing method allow us to produce liposomes with size and polydispersity results suitable for transtympanic administration.

6. PERSPECTIVES

- The next step will therefore be to test different formulations and study their sustained release effect.
- The biocompatibility, safety and efficacy of the developed formulation will then be tested *in vitro*.
- A method using supercritical CO_2 will be optimized and applied to produce and sterilize liposomes in one step.
- Liposomes will be incorporated in an optimized hydrogel to increase their residence time in the middle ear and their passage towards RWM.
- *In vivo* studies will finally evaluate the cochlear biodistribution, the sustained release effect and the efficiency.

7. REFERENCES

[1] Created with BioRender.

8. ACKNOWLEDGMENTS

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