საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 18, №4, 2024
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 18, no. 4, 2024

Medical Science

Liposomal and Polymeric Nanoparticle Technology of Amphicezine Formulation

Aliosha Bakuridze[*], Irakli Nadiradze[**], Nodar Chigogidze[§], Alexander Boroda[§§]

[*] *Department of Pharmaceutical Technology, Tbilisi State Medical University, Tbilisi, Georgia*
[**] *Georgia-Israel Clinic Gidmedi, Tbilisi, Georgia*
[§] *Tbilisi State Technical University, Tbilisi, Georgia*
[§§] *I.M. Sechenov First Moscow State Medical University, Moscow, Russia*

(Presented by Academy Member Ramaz Khetsuriani)

Amphicezine is a state-of-the-art formulation consisting of a multiply-charged anion-active compound containing negative, multi-charged, long-chain organic ions and alkali metals (Cs, RB). Amphicezine liposomes were obtained by hydration of the lipid layer. For the preparation of liposomes, we took Amphicezine, soy lecithin (Lipoid, Germany), cholesterol (Sigma-Aldrich, Japan) in the following molar ratio: 1:50:10 and dissolved in chloroform. The obtained solution was transferred to a round-bottomed flask and evaporated the solvent under rarefaction conditions at a temperature of 50°C. A semi-transparent plate was formed, which was dried until complete removal of chloroform also under rarefaction conditions. We hydrated the plate by adding distilled water to it, resulting in a multi-layered liposome with a concentration of 5 mg/ml of amphicezine. The liposomal mass was treated with an ultrasonic sonicator (1400 rpm) and filtered through a membrane with pores of 0.2 μm in diameter (Whatman, Great Britain). Sucrose Isomalt (chemically pure, Great Britain) was selected as a cryoprotectant. Liposomes dimensions were determined using an electron emission microscope. We judged the quality of the prepared samples by the size of the vesicles and its Zeta-potential. The particles were rectangular in shape with a smooth surface, and no aggregation of particles was observed. The size of the particles was within 200-230 nm. *© 2024 Bull. Georg. Natl. Acad. Sci.*

amphicezine, soy lecitin, liposome, nanoparticle technology

During the surgical intervention for any malignant tumor, there is a substantially increased risk of shedding cancer cells into the circulation. Surgical trauma induces local and systemic inflammatory responses that can also contribute to the accelerated growth of the residual and micrometastatic disease. The cancer cell follows a stream of blood and lymph and its sedimentation occurs in the microcirculation bed of any distant organ by the means of a fibrin membrane. Fibrin plays an important role promoting cell migration by providing a matrix for tumor cell migration and by interactions with

adhesive molecules and integrins. Vascular endothelial growth factor bound to fibrin promotes angiogenesis. Fibrin interacts with platelets and leukocytes, and promotes their respective carcinogenic properties. Amphicezine is a state-of-the-art formulation consisting of a multiply-charged anion-active compound containing negative, multi-charged, long-chain organic ions and alkali metals (Cs, RB). The compound blocks interaction between fibrin and tumor cells and as the result, prevents protective fibrin enveloping of cancer cells detached from the primary tumor lesion during surgery. Thus, Cs and Rb cations are adsorbed by tumor cells, penetrate inside and cause alkalization of the intracellular environment, which leads to the inhibition and death of metastatic cells. In this paper, we addressed the application of liposomal and polymeric nanoparticle technology to Amphicezine formulation [1-5].

Study Design and the Results

Three technological schemes were analyzed according to the stage of incorporation of cryoprotector into liposomal forms. The results are shown in Table 1.

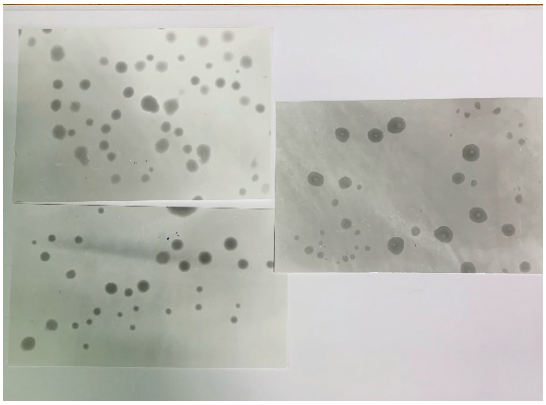

Fig. 1. Microscopic images of monolayer liposomes.

Electron emission microscopy images of the final product (Fig. 1) showed that during liposomal nanoparticles formation particles had a smooth surface with a sphere or elliptical shape, however no aggregation of particles was detected. It should be noted that the microscopic results are consistent with the results obtained with the nanosizer, in particular, the size of the particles is within 140-210 nm. The presence of separate, relatively large particles is due to the specifics of the preparation of samples for microscopy, which includes the impact of a high-speed electron flow on the sample under vacuum and the encapsulation of individual nanoparticles. All this leads to coalescence of particles.

Table 1. Comparative analysis of the technologies for obtaining the liposomal form of amphicezine

Characteristics		Technology		
		1	2	3
Stage of application of cryoprotector		Hydration of the lipid plate	Lipid plate after hydration	After sonication and filtration
Quality indicators of multilayered liposomes	Sizes of vesicles, nm	210±10	315±17	460±20
	Zeta potential, mv	-(21.4±1.0)	-(25.2±1.3)	-(22.9±1.6)
Quality indicators of monolayer liposomes	Sizes of vesicles, nm	145±8	1860±12	174±18
	Zeta potential, mv	-(17.9±1.4)	-(20.0±1.5)	-(19.1±1.3)

It can be seen from Table 1 that the inclusion of cryoprotectant has a significant effect on the quality indicators of liposomes. Optimal results are obtained when the cryoprotectant is introduced at the stage of hydration of the lipid plate. In this case, the sizes of multi-layered and single-layered liposomes are 190 and 163 nm, respectively.

At the next stage, we prepared polymer nanoparticles of Amphicezine. The technological process consists of 4 consecutive stages:

1. Preparation of polylactic acid solution: 125 mg polylactic acid is placed in the 100 ml volume flask, added 25 ml of acetone and mixing using magnetic mixer at the room temperature.

Table 2. Comparative analysis of the technologies for obtaining polymeric nanoparticles of amphicezine

Characteristics		Technology			
		1	2	3	4
Stage of application of cryoprotector		Polylactic acid solution preparation	Surfactant solution preparation	Obtaining a suspension containing nanoparticles	Dispersion of suspension containing nanoparticles_
Polymer nanoparticle quality indicators	Sizes of nanoparticles, nm	230 ± 16	136 ± 12	255 ± 2 0	346 ± 24
	Zeta potential, mv	- (7.6 ± 1.4)	- (4.6 ±1.2)	- (8.3 ±1.6)	- (12.4 ±1.8)
Polymeric nanoparticle grade badgers after cryoprotectant application	Sizes of nanoparticles, nm	210 ± 9	125 ± 7	235 ± 10	290 ± 15
	Zeta potential, mv	- (6.5 ±1.5)	- (4.0 ± 1.0)	- (7.1 ±1.2)	- (8.7 ± 1.5)

2. Preparation of surfactant solution 125 mg of poloxamer 188 (co-polymer of ethylene oxide with propylene glycol) is transferred to a 200 ml flask, 50 ml of distilled water is added and dissolved by stirring on a magnetic stirrer at room temperature.

3. Obtaining a suspension containing nanoparticles 5 mg of Amphicezine is dispersed in an acetone solution of polylactic acid under the conditions of mixing on a magnetic stirrer (100 rpm). An aqueous solution of poloxamer 188 is added in a thin stream to the obtained acetone solution under stirring conditions.

4. Dispersion of suspension containing nanoparticles.

The suspension containing nanoparticles is treated with an ultra sonicator (1400 rpm). We selected sucrose (chemically pure, Turkey) as a cryoprotectant. We determined the nanoparticles size using an electron emission microscope and judged the quality of the prepared samples by the size and zeta-potential of the polymer nanoparticles. According to the stage of inclusion of cryoprotectant (sucrose) in the polymer mass, 4 technological schemes were analyzed. The results are shown in Table 2.

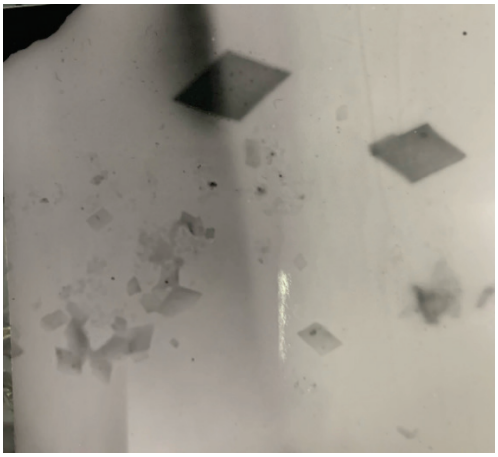

Fig. 2. Microscopic image of a polymer nanoparticle.

Microscopic image (Fig. 2) confirms the formation of nanoparticles. The particles are rectangular in shape with a smooth surface, and no aggregation of particles is observed. It should be noted that the size of the particles is within 200-230 nm.

Conclusion

We developed stable Amphicezine nanoparticles, which were rectangular in shape with smooth surfaces. No particle aggregation was observed, and the particle size ranged from 200 to 230 nm.

მედიცინის მეცნიერება

ამფიცეზინის ლიპოსომური და პოლიმერული ნანონაწილაკების ტექნოლოგია

ა. ბაკურიძე*, ი. ნადირაძე**, ნ. ჩიგოგიძე§, ა. ბოროდა§§

* *თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ფარმაცევტული ტექნოლოგიის ფაკულტეტი, თბილისი, საქართველო*
** *საქართველო-ისრაელის ერთობლივი კლინიკა „გიდმედი“, თბილისი, საქართველო*
§ *საქართველოს ტექნიკური უნივერსიტეტი, თბილისი, საქართველო*
§§ *მოსკოვის ი. სეჩენოვის სახ. სამედიცინო უნივერსიტეტი, მოსკოვი, რუსეთი*

(წარმოდგენილია აკადემიის წევრის რ. ხეცურიანის მიერ)

ამფიცეზინის ბიოქიმიური ფორმულა უახლესია და წარმოადგენს მრავალმუხტიანი გრძელჯაჭვიანი ანიონაქტიური მაკრომოლეკულებისა და ტუტე მეტალების (Cs, RB) ნაერთს. ამფიცეზინის ლიპოსომები მიღებულ იქნა ლიპიდური ფენის ჰიდრატაციით. ლიპოსომების მოსამზადებლად გამოვიყენეთ ამფიცეზინი, სოიოს ლეციტინი (Lipoid, გერმანია), ქოლესტერინი (Sigma-Aldrich, იაპონია) შემდეგი მოლარული თანაფარდობით - 1:50:10 და გავხსენით ქლოროფორმში. მიღებული ხსნარი გადავიტანეთ მრგვალი ფსკერის მქონე კოლბაში და გამხსნელი ავაორთქლეთ ვაკუუმის პირობებში 50°C ტემპერატურაზე. წარმოიქმნა ნახევრად გამჭვირვალე ფირფიტა, რომელიც გავაშრეთ ქლოროფორმის მთლიან მოცილებამდე ასევე ვაკუუმის პირობებში. ფირფიტის ჰიდრატაცია განხორციელდა მასზე დისტილირებული წყლის დამატებით, რის შედეგადაც მიღებულ იქნა მრავალშრიანი ლიპოსომა ამფიცეზინის კონცენტრაციით 5 მგ/მლ. ლიპოსომური მასა დამუშავდა ულტრაბგერითი სონიკატორით (1400 rpm) და გაფილტრულ იქნა 0.2 მიკრომეტრის დიამეტრის მქონე ფორებიანი მემბრანის (Whatman, დიდი ბრიტანეთი) საშუალებით. კრიოპროტექტორად შეირჩა საქაროზის იზომალტი (ქიმიურად სუფთა, დიდი ბრიტანეთი). ლიპოსომების ზომები განისაზღვრა ელექტრონული ემისიური მიკროსკოპით. მიღებული ნიმუშების ხარისხი შეფასდა ვეზიკულების ზომისა და მათი ზეტა-პოტენციალის მიხედვით. ნაწილაკები მართკუთხედი ფორმისაა, გლუვი ზედაპირით, მათი აგრეგაცია არ აღინიშნება. ნაწილაკების ზომა 200-230 ნმ ფარგლებშია.

REFERENCES

1. Dmitreva M., Shprak Z., Orlova O., Ignatyeva E., Lantsova A., Nikolaeva L., Krasniuk I. (2020) Selection of the composition of a liposomal dosage form of a Russian somatostatin analogue with antitumor activity. *International Journal of Applied Pharmaceuticals,* **12**(6): 65-68.
2. Nsairat H., Khater D., Sayed U., Odeh F., Al Bawab A., Alshaer W. (2022) Liposomes: structure, composition, types, and clinical applications. *Heliyon,* **8**(5): e09394.
3. Duong TT., Isomäki A., Paaver U., Laidmäe I., Tõnisoo A., Yen TTH., Kogermann K., Raal A., Heinämäki J., Pham TM. (2021) Nanoformulation and evaluation of oral berberine-loaded liposomes. *Molecules,* **26**(9): 2591. doi: 10.3390/molecules26092591. PMID: 33946815; PMCID: PMC8125214.
4. Kim J., Kang D., Kang S., Kim BH., Park J. (2022) Coalescence dynamics of platinum group metal nanoparticles revealed by liquid-phase transmission electron microscopy. *Science,* **25**(8): 104699. doi: 10.1016/j.isci.2022.104699. PMID: 35880046; PMCID: PMC9307684.
5. Xu H., Li S., Liu YS. (2022) Nanoparticles in the diagnosis and treatment of vascular aging and related diseases. *Signal Transduct Target Ther.*, **7**(1): 231. doi: 10.1038/s41392-022-01082-z. PMID: 35817770; PMCID: PMC9272665.

Received October, 2024