Evaluation of aprotinin-loaded microemulsion formulations for parenteral drug delivery: In vitro release studies

H. Yeşim Karasulu[1], **Neslihan Üstündağ Okur[1],** Derya İlem[2], Nevin Oruç[3], Hayal Özkılıç[4], Makbule Aşıkoğlu[2], İ. Eren Akçiçek[3], Zeynep Ay Şenyiğit[1], Funda Yılmaz[5], Ömer Özütemiz[3], Tamer Güneri[1]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Ege, Izmir, Turkey
[2]Department of Radiopharmacy, Faculty of Pharmacy, University of Ege, Izmir, Turkey
[3]Department of Gastroenterology, Faculty of Medicine, University of Ege, Izmir, Turkey
[4]Nuclear Medicine, Faculty of Medicine, Ege University, Izmir, Turkey
[5]Department of Pathology, Faculty of Medicine, University of Ege, Izmir, Turkey
neslihanustundag@yahoo.com

ABSTRACT

Microemulsions (M) are thermodynamically stable, transparent and low-viscosity colloidal dispersions consisting of microdomains of oil and/or water stabilized by an interfacial film of alternating surfactant and cosurfactant molecules [1].

Aprotinin is a monomeric globular polypeptide derived from bovine lung tissue; it has a molecular weight of 6512. It is a Kunitz protease inhibitor and has a wide action with particular against trypsin, chymotrypsin and kallikrein, making it theorically attractive in ameliorating the effects of acute pancreatitis [2].

The aim of this study was to formulate appropriate w/o and o/w microemulsion formulations for parenteral aprotinin administration to use in pancreatitis therapy.

To investigate in vitro release profile of aprotinin from newly developed microemulsion formulations, aprotinin was radiolabeled with ^{99m}Tc and comparative in vitro release studies have been performed with radiolabeled complex loaded microemulsions.

Preparation of aprotinin-loaded microemulsion formulations

The microemulsion systems (M1-2-3) were formulated with various compositions of oleic acid (OA), isopropyl myristat (IPM), Labrasol, Cremophor EL (Cr-El), ethanol, isopropyl alcohol (IPA) and 0.9 % NaCl solution. Drug-loaded microemulsions were prepared by dissolving 2 mg of aprotinin.

Preparation of ^{99m}Tc-Aprotinin

Aprotinin was dissolved in glycine phosphate alkaline buffer (GPB). While stirring, freshly prepared stannous chloride solution was added under a nitrogen atmosphere. The mixture was mixed well and filtered from a cellulose acetate filter (0.22 µm) into a vial. $^{99m}TcO_4^-$ in saline was added to this solution and allowed to stand for 20 minutes at room temperature prior to radiochemical analysis.

Quality Control and the Stability of ^{99m}Tc-Aprotinin

The radiochemical purity of the product was analyzed by paper and Thin Layer Chromatography (TLC), using Whatman 3MM papers in acetone (100%) and Albumin-impregnated ITLC-SG strips in ammonia/ ethanol/water (1/2/5) [3]. Radiochemical purity and stability of ^{99m}Tc-Aprotinin were analyzed by TLC scanner (Bioscan AR2000) at room temperature up to 6h.

Preparation of ^{99m}Tc-Aprotinin loaded microemulsion

2 mg of aprotinin included ^{99m}Tc-Aprotinin solution was added into M1-M2 and M3 formulations.

In Vitro Release Profile of Aprotinin

^{99m}Tc-Aprotinin from microemulsions (M1-3) and solution were examined using the dialysis tube method. Dialysis tubes (Dialysis sacks, width 35 mm, diameter 21 mm, molecular weight grater than 12.000) were purchased Spectro-por and appropriate closures were purchased from Spectrum (Los Angeles, CA, USA). Inbrief, a dialysis tube containing 1 mL ^{99m}Tc-Aprotinin-microemulsion (M1-3) or solution was immersed in 50 mL of PBS (pH 7.4) as receiver. In vitro release studies were performed at 37±0.5°C and 600 rpm during the experiment. Then 100 µL of sample was withdrawn at an appropriate time intervals and an equal volume of the PBS was added. For determination of radioactivity, samples were placed in to a gamma counter (Sesa Uniscaler I/S).

RESULTS AND DISCUSSION

The optimum microemulsion formulations according to phase diagrams are shown in Table 1.

Table 1: Composition of the microemulsion formulations

	M1	M2	M3
Oil phase	OA	OA	IPM
	33.92%	9.34%	10.18%
Surfactants/	Cr- EL/	Cr-EL /	Labrasol/
co-surfactants	Ethanol	IPA	IPA
	51.62%	54%	66.58%
Water phase	0.9% NaCl	0.9% NaCl	0.9% NaCl
	14.46%	36.66%	23.25%

Radiolabeling and Quality Control and the Stability of ^{99m}Tc-Aprotinin

Radiolabeling efficiency of ^{99m}Tc-Aprotinin was found to be greater than 95%. The TLC studies indicated that ^{99m}Tc-Aprotinin is stable for up to 6 hrs at room temperature (

Figure **1**).

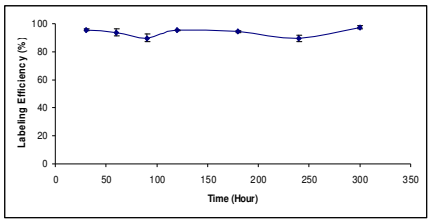

Figure 1: Stability of the ^{99m}Tc-Aprotinin at room temperature

In Vitro Release of M-Aprotinin

The in vitro release behavior of ^{99m}Tc-Aprotinin from microemulsions (M1-3) and solution is shown in Figure 2. As clearly seen in Figure 2, the release behavior of Aprotinin from M2 formulation exhibited a slower and continuous release for 6 hrs compared wih the other formulations and solution. Therefore, this suggests that release rate of Aprotinin from microemulsion could be controlled by this formulation.

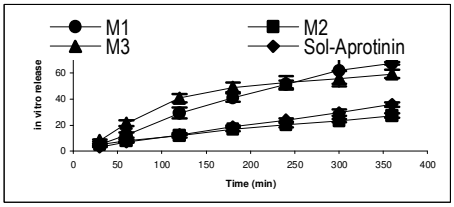

Figure 2: Release profiles of ^{99m}Tc-Aprotinin from M1-2-3 and Sol-Aprotinin (n = 3). Each value represents the mean ± S.D.

CONCLUSION

The results of in vitro release studies showed that release rate of ^{99m}Tc-Aprotinin from microemulsion could be controlled by microemulsion formulations.

REFERENCES

1- Jadhav KR, Shaikh, IM, Ambade KW, Kadam VJ. Applications of microemulsion based drug delivery system. Curr Drug Deliv 3: 267-273, 2006.

2- Smith M, Kocher HM, Hunt BJ. Aprotinin in severe acute pancreatitis. Int J Clin Pract, in press, 2009.

3- Smyth DR, Tsopelas C. 2005, An improved ^{99m}Tc-aprotinin kit formulation: quality control analysis of radiotracer stability and cold kit shelf life. Nuclear Medicine and Biology 32 (2005) 885–889.

Acknowledgements

This study was supported by The Scientific and Technological Research Council of Turkey (Tubitak-108 S 083). The authors also would like to thank to the T.R. Prime Ministry State Planing Organization Foundation (Project Number: 09DPT001).