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RESEARCH ARTICLES / BİLİMSEL ARAŞTIRMALAR

Stability and Hemolytic Effect of Parenteral Lorazepam Emulsion Formulations

Filiz ÖNER*,***, Melek YALIN**, A. Atilla HINCAL*

Abstract: *Intravenous lorazepam preparations in the drug market are solutions in water miscible organic solvents. Organic solvent containing formulations show considerable side effects when they are applied via i.v. route. In this study, suitable emulsion formulations for lorazepam have been developed. Considering the stability of the carrier emulsions, three different corn oil emulsions (10 %), which were stabilized with egg lecithin and/or Pluronic F-68 and Pluronic F-88 were selected. The incorporation of lorazepam does not cause the chemical instability of lorazepam and physical instability of the emulsion. Formulations(fresh and eight months aged) containing lorazepam and different emilsifiers were evaluated as a measure of safety of emulsions for parenteral drug carriers. These experimental findings showed that emulsions did not have any significant influence on the hemolysis of red blood cells. Water miscible cosolvents and lorazepam solutions in these cosolvents caused ten times higher hemolytic effect than emulsions.*

Keywords : *Lorazepam, hemolytic effect, parenteral emulsions, stability*

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Parenteral Lorazepam Emülsiyon Formülasyonlarının Stabilitesi ve Hemolitik Etkisi

Özet: *İlaç piyasasında i.v. yoldan verilen lorazepam preparatları, lorazepamın su ile karışan organik çözücülerdeki çözeltileridir. Bu organik çözücüleri içeren formülasyonlar i.v. yoldan verildiğinde ciddi yan etkiler gösterirler. Bu çalışmada lorazepam için uygun su içerisinde yağ emülsiyon formülasyonları geliştirilmiştir. Farklı emülgatörleri içeren en iyi üç formül fiziksel stabilitelerine bakılarak seçilmiştir. Seçilen taşıyıcılar yumurta lesitini, Pluronik F-68 ve Pluronik F-88 ile stabilize edilmiş % 10 mısır yağı emülsiyonlarıdır. Lorezapam eklenmesi ile emülsiyonlarda ve lorazepamın kendisinde bir yıl süre ile bozunma belirtileri görülmemiştir.*

Lorazepam ve farklı emülgatörleri içeren üç farklı formülasyonun taze ve sekiz ay beklemiş örneklerinin neden olduğu hemolizin derecesi emülsiyonların parenteral ilaç taşıyıcı sistem olarak kullanılmalarında bir güvenilirlik ölçüsü olarak değerlendirilmiştir. Deney bulgularına göre alyuvarların hemolizi üzerinde emülsiyonların önemli bir etkileri olmadığı görülmüştür. Su ile karışabilen çözücü yardımcıları ve bunlarla hazırlanan lorazepam çözeltilerinin ise emülsiyonlarına göre on kez fazla hemolitik etkide oldukları bulunmuştur.

Anahtar kelimeler : *Lorazepam, hemolitik etki, parenteral emülsiyonlar, stabilite*

Introduction

Lorazepam is a potent tranquilizer and almost insoluble in water. It is necessary to use water miscible cosolvents in the formulations of intravenous solutions of water insoluble drugs. Commonly marketed intravenous solution of lorazepam contain water miscible cosolvents such as polyethylene glycol 400 and propylene glycol[1].

This type of marketed formulations cause serious side effets, such as hemolysis, pain and tissue damage related to formulation and site of injection. Emulsion formulations of diazepam and physostigmine salicylate have shown decreased side effects, such as hemolysis, pain and tissue damage related to formulation and site of injection. Another disadvantage of this type of intravenous formulation is the probable precipitation risk upon the addition of the cosolvent mixture to intravenous fluid or blood[2]. A more con-

* Department of Pharmaceutical Technology, Faculty of Pharmacy, Hacettepe University, 06100 Hacettepe, Ankara-TURKEY.
** Refik Saydam Centram Institute of Hygiene, Drug, Cosmetic and Research Department, 06100 Sıhhiye, Ankara-TURKEY.
*** Correspondance

venient alternative method of intravenous drug administration involves the formulation of O/W emulsions for lipid soluble drugs[3]. Liposomes can be recommended as alternatives[4]. Intravenous emulsion formulations of physostigmine salicylate[5] and diazepam[6] showed decreased side effects, which are mentioned above.

In vitro test methods for hemolytic effects of solutions, co-colvents and emulsions involves the incubation of red blood cells(from different animals or human[7] with test materials[7,8].

In the present study, formulation of three carrier parenteral emulsions prepared with different emulsifiers as an alternative to the use of water miscible organic solvents in lorazepam administration has been studied in terms of physical and chemical stability and hemolytic activity.

Materials and Methods

Materials

Lorazepam was kindly provided from Wyeth, İstanbul, Turkey. Lorazepam and all other compounds were used as received from the supplier: Corn oil (Komili, İstanbul, Turkey), lecithin(Wako, Japan), polyoxyethylene polyoxypropylene emulsifiers [Pluronic F-68 and Pluronic F-88 (Asaki Denka, Japan)], DL-α tocopherol (Sigma, St. Louis, MO, USA), polyethylene glycol 400(E. Merck, Germany), agar, sodium thioglycolate, and soybean meal(Difco, USA). All other chemicals were reagent grade. Blood was obtained from a volunteer donor and rabbit and citrated to prevent clotting.

Methods

Emulsion Preparation and Evaluation

Different combination of egg lecihtin, Pluronic F-68 and Pluronic F-88 were used in the proportions ranging from 1 to 2.5 %. Egg lecithin was dissolved in corn oil at low temperature. Pluronics were dissolved in water by mixing at room temperature. 2.5 % of glycerine was used to adjust viscosity and DL-tocopherole was used as an antioxidant at 0.05 % concentration. The oil and aqueous phases were combined at approximately 70°C, and a coarse emulsion was formed by means of a hand homogenizer. Emulsification was performed by Branson Model 2000 ultrasonic probe at intensity position 200 for 10 minutes. After the emulsion was cooled rapidly to below 20°C, it was filled into glass vials, sterilized in autoclave at 121°C for 20 minutes and cooled rapidly to below 20°C by mechanical rolling of the container.

Emulsions were kept at 4°C. Droplet sizes were measured by using optical microscope and Coulter Multisizer II (Coulter Electronics Ltd. UK). Viscosity of the emulsions were measured by means of the Brookfield Digital viscometer(Brookfield Engineering Labs., Inc. MA, USA) at room temperature at given time intervals over a one year period.

Lorazepam is incorporated in to these emulsions as a solution in polyethylene glycol at the concentration of 100 $mg.mL^{-1}$ concentration, under laminar air flow in the aseptic conditions.

Each milliliter of lorazepam reference solution contains 2 mg of lorazepam, 0.18 mL of polyethylene glycol 400 and 2 % of benzyl alcohol in propylene glycol. This composition is the same as the marketed formulation of lorazepam($Ativan^R$). Lorazepam amounts in the emulsions were determined by the HPLC based method of Gunavan and Treiman[9] as described in our previous paper[10]. Lorazepam emulsion was diluted with metanol. This mixture was injected into the HPLC by using the mobile phase of metanol: water (70:30) with the flow rate of 1.2 $mL.min^{-1}$ at 230 nm. The column was 10 μm Spherisorb ODS (C-18), (20 cm x 4.6 mm i.d).

Sterility testing (USP XXII method) and partition coefficient measurements, based on Friedman and Benita's method, were carried out using the procedure described elsewhere[11].

pH of the emulsions were adjusted to 8 with 0.1 N NaOH. Auter the sterilization, pH was found to be 7.5, due to the free fatty acids. Determination of hemolytic activity was carried out by the method described by Reed and Yalkowsky[7]. Citrated human and rabbit blood were used freshly. 0.2 mL citrated blood is added to 0.1 mL of test solution or emulsion. After gently mixing they were incubated at 25°C for 2 min, and 5 mL of 0.9 % sodium chloride solution was added. These mixtures were centrifuged at 3000

g for 5 minutes. The supernatant was discarded and pellets were washed with three additional 5 mL portions of sodium chloride 0.9% and supernatants discarded. These washings removed the test solution and any hemoglobin from lysed red blood cells. Pellets consisting of red blood cells and ghosts were dispersed with 4 mL distilled water and they were centrifuged at 3000 g for 5 minutes. 1 mL of the supernatant was diluted with 4 mL of distilled water and the absorbances were read at 540 nm. The absorbance at this wavelength is directly proportional to the hemoglobin concentration.

The 100 % hemolysis level was defined as the absorbance at 540 nm of hemoglobin in the supernatant after the complete hemolysis of erythrocytes in distilled water. All of the studies were carried out with freshly prepared emulsions and with aged emulsions.

Results and Discussion

Emulsion formulations are useful for the parenteral administration of liposoluble drugs. The incorporation of a drug into commercial O/W emulsions may introduce a factor of instability. In the current study, lorazepam was incorporated into three different corn oil emulsion vehicles, which remained stable for a period of one year storage.

After testing a variety of different excipients for physical stability, the best three emulsion formulations were selected as given in Table 1. as the basis of macroscopic and microscopic observations. Although the physical stability of fat emulsions emulsified with lecithin is good, additional surfactants are needed to improve the stability of emulsions. This enhanced stabilization was attributed to the probable formation of a complex film between Pluronics and phospholipid molecules at the oil water interface[12]. Pluronic emulsifiers were found to be less toxic than the other nonionic emulsifiers [13].

Viscosity is an important factor affecting the flow of parenteral emulsions through a needle or a catheter. Takamura et al.[14] have reported that decrease in mean particle size contributes to an increase in viscosity.

Table 1. Composition of the Emulsion Vehicles

	Corn Oil w/w%	Pluronics F-68+F-88 w/w%	Egg Lecithin w/w%	Glycerol w/w%	DL-a-Tocpherol w/w%
FORMULA					
E15	10	-	1.2	2.5	0.05
E17	10	2.5	1.2	2.5	0.05
E18	10	2.5	-	2.5	0.05

Viscosity and accordingly particle size distribution were not changed significantly over a one year period (Fig. 1). Lorazepam incorporation caused a light decrease in the viscosity of emulsions. Lecithin containing emulsions showed lower viscosity values than the other two formulations. pH of the emulsion vehicles and lorazepam emulsions slowly decreased for the first two months and then it has not changed for eight months (Fig. 2).

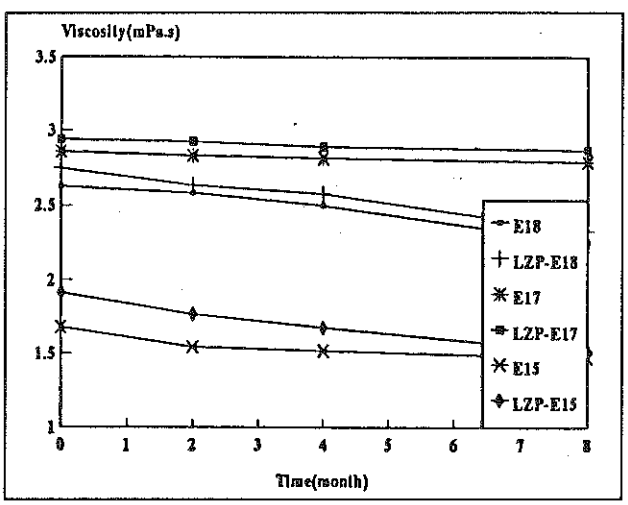

Fig. 1. Plot of the viscosity of emulsions as a function of storage time.

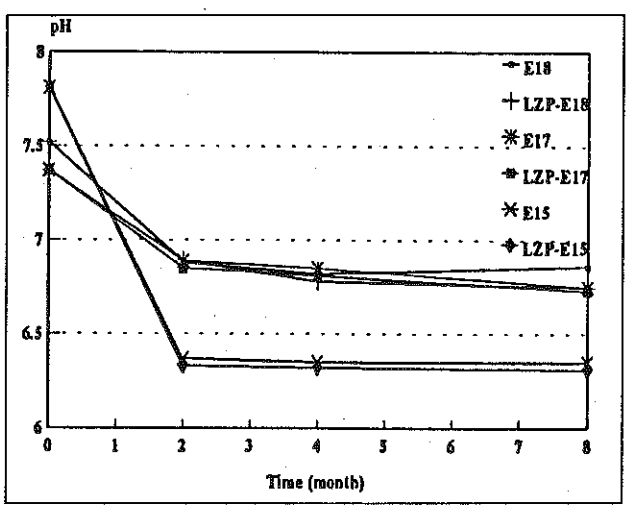

Fig. 2. Plot of the pH of the emulsions as a function of time

Solubilization of lipophilic drugs for intravenous use can be achieved either by pH control or by the use of cosolvents such as ethanol and glycols[15,16]. In this study, pH is found to be effective on the incorporated amounts of lorazepam to the emulsions. The best pH range was between 7 and 8. The highest concentration of the drug that can be safely incorporated into the emulsion was 1.2 mg.mL^{-1} (Fig.3). Average droplet sizes of the emulsion vehicles and lorazepam emulsions were found to be 1 μm.

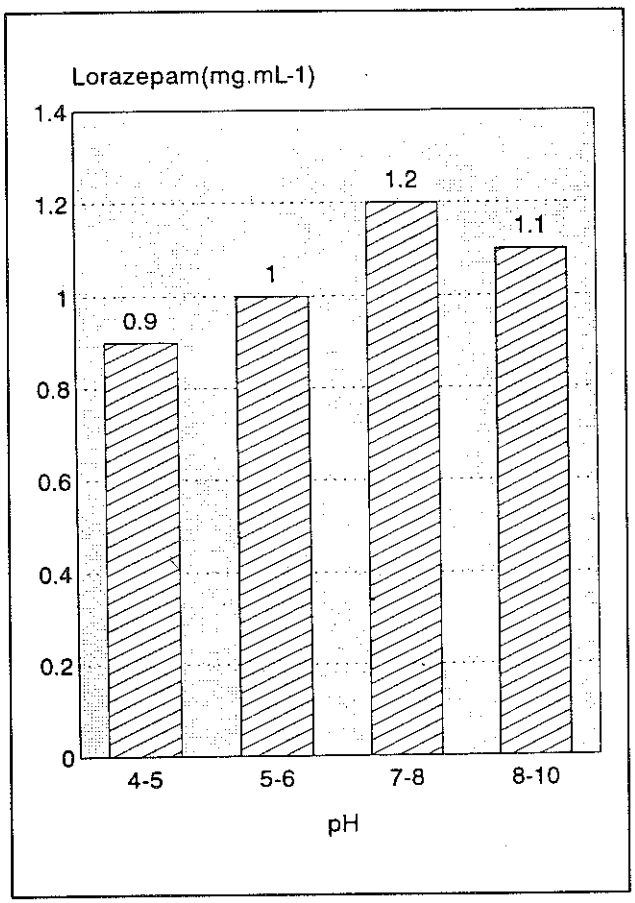

Fig. 3. Effect of pH on the incorporated amount of Lorazepam

Lorazepam addition does not appear to destabilize the emulsion and also lorazepam itself does not appear to degrade over a one year period in this carrier emulsion formulations. No degradation products were observed by HPLC.

This study showed that the fraction of lorazepam residing in the oil phase is between 75-90 %. In the first two months, drug amount in the internal phase decreased to 75 % for one formulation and to 90 % for the other two formulations(Fig. 4). After two months, drug amount in the oil phase has not changed and no degradation products were found by HPLC. The extent of partition of the drug between the oil and water phases would influence the loss of a drug[13]. These results show that some part of the drug can reside in the interface, or in the water phase as micellar solution.

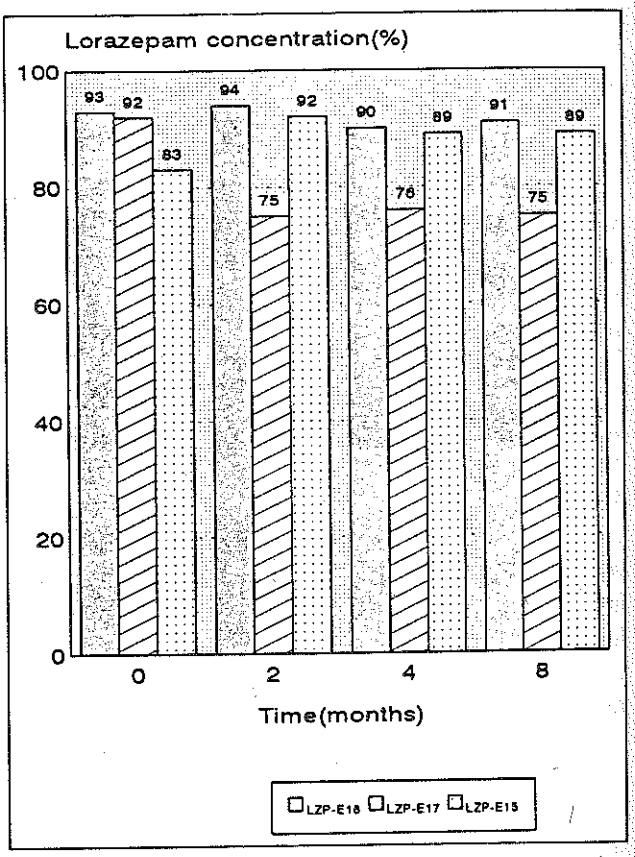

Fig. 4. Effect of storage time on the lorazepam concentration over an 8 month period.

The degree of hemolysis caused by interaction between erythrocytes from human and rabbit with three types of emulsion and organic solvents are shown in Table. 2. Organic solvent solution of lorazepam and organic solvents alone caused cell lysis (Fig. 5 and 6). The lysis of erythrocytes from human and rabbit blood by the three formulations is shown in these figures too. Although higher percentage hemolysis values were obtained with rabbit blood, it is useful for relative evaluations. The formulations containing Pluronics showed no detectable hemoglobin release but emulsions stabilized with lechithin showed hemoglobin release. Lipid composition of

Table 2. Percentage of Hemolysis Induced by Emulsion and Organic Solvent Based Formulations of Lorazepam.

FORMULA	Human Blood		Rabbit Blood	
Lorazepam Solution	95.6%		100%	
Diluted Lorazepam Solution	82.0%		98.1%	
Organic Solvent Vehicle	98.3%		100%	
Diluted Organic Solvent Vehicle	93.5%		100%	
E18	5.2%**	4.7%***	7.9%**	4.9%***
LZP-E18	9.7%**	5.4%***	14.0%**	11.2%***
E17	6.0%**	6.5%***	13.4%**	11.6%***
LZP-E17	8.9%**	7.9%***	17.0%**	16.9%***
E15	15.0%**	11.7%****	17.0%**	11.0%***
LZP-E15	16.4%**	15.3%***	20.0%**	34.2%***

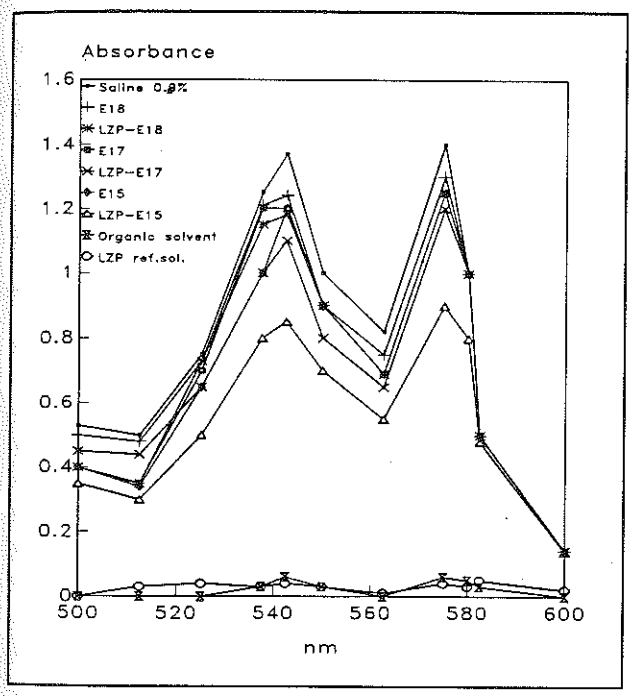

Fig. 5. Absorbance scans of the supernatants from human and rabbit red blood cells.

the lecithin was found as an important factor responsible for the hemolysis[8]. Lecithin is biodegredable, but it changes upon exposure to physical conditions during storage and hemolytic effects can be seen as a result of hydrolysis.

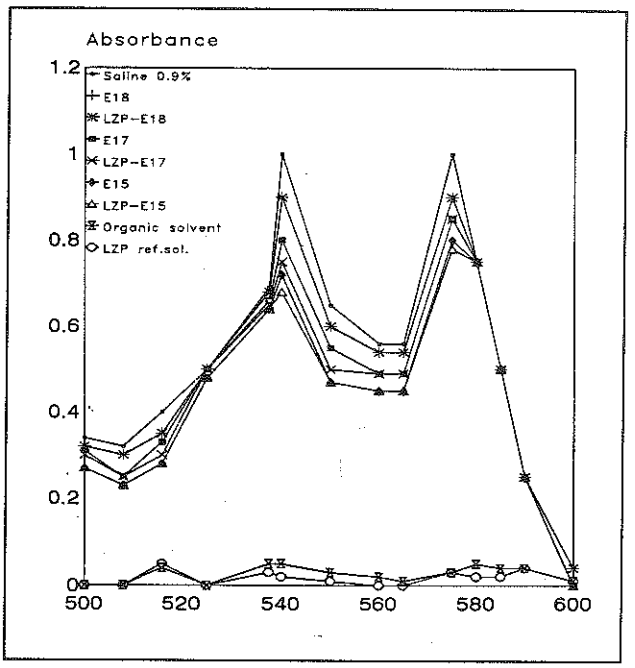

Fig. 6. Absorbance scans of the supernatants from mixtures of human red blood cells and emulsions.

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