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TOPICAL LIPOSOMAL LOCAL ANESTHETICS: DESIGN, OPTIMIZATION AND EVALUATION OF FORMULATIONS

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ABSTRACT

To improve the rate of penetration into the skin, and to develop an effective topical anesthetic product, selected local anesthetic agents, benzocaine, lidocaine, dibucaine, etidocaine and tetracaine were encapsulated into liposomes using the solvent evaporation method. After the pilot experiments, tetracaine was selected for further development. Encapsulation efficiency was determined by centrifugation of liposomes and spectrophotometric analysis of liposome pellets and supernatants. Physical stability and organoleptic properties of the various liposomal tetracaine formulas were monitored visually and by microscopy for 1 year. Tetracaine was found to be suitable for the development of a liposomal drug delivery system with high encapsulation efficiency (60-90%) and physical stability. The results showed that encapsulation efficiency of tetracaine into liposomes can be increased by increasing drug concentration and pH, and including negatively charged stearic acid or unsaturated lipids in the formula. Stability of tetracaine increased with higher encapsulation efficiency, however the shelf life of the product was still short (2 months). In-process and finished product quality control parameters are suggested to facilitate the topical liposomal product development in general.

INTRODUCTION

Many attempts have been made to provide efficient anesthesia of the skin in order to alleviate unpleasant sensations such as pain, itching and burning associated with minor surgical operations, injections as well as that associated with various dermatological disorders. However, several studies and the clinical experience indicated the inefficacy of presently available local anesthetic preparations on intact skin (Dalili and Adriani, 1971). To achieve sufficient anesthetic effect, prolonged application time and high concentration of drug (10-30%) are required (Brechner et al., 1967; Lubens et al., 1974; Akerman, 1978; Olsen and Englesson, 1980). The achievement of a relatively effective topical anesthesia using the eutectic mixture of local anesthetics (EMLA) as 2.5% lidocaine-2.5% prilocaine oil-in-water emulsion, is due to high concentration (20%) of drug in the emulsion droplets (Evers et al., 1985). Recent studies indicated that tetracaine and lidocaine in liposome encapsulated form provides better topical anesthesia, tested by pin-prick, than a conventional cream i.e. the drug incorporated into Dermabase® (Gesztes and Mezei, 1988; Foldvari et al., 1990; Mezei and Gesztes, 1990). Liposomes (microscopic phospholipid vesicles), recognized as potential drug carriers, applied topically were proposed to penetrate into the skin (dermis) through the "lipid channels" of the epidermis and localize the drug within the skin (Foldvari et al., 1990). Due to the multilamellar structure of liposomes sustained release of the encapsulated drug is possible (Mezei, 1987).

This paper describes the encapsulation of various local anesthetic agents into liposomes with the main emphasis on the detailed results of the formulation and characterization of various topical liposomal tetracaine preparations as potential selective drug delivery systems for the anesthesia of the skin. The ultimate aim was to formulate a topical tetracaine preparation which can provide deep anesthesia (sufficient to relieve pain in procedures involving the skin such as venipuncture, lumbar puncture, bone marrow aspirations, phlebotomy, injections, skin biopsy, split skin grafting, dermabrasion to remove tattoos and removal of hair in hirsutism), has a fast onset and long duration of action along with minimal systemic absorption and local side effects.

METHODS

Materials

The various phosholipids for liposome preparation were obtained from Natterman Phospholipide GmbH, Cologne, Germany (Phospholipon 90) and American

Lecithin Company, Atlanta, GA. (soya phosphatidylcholine NC-95 and 95H). Centrolex P was from Central Soya (Fort Wayne, Ind.). Tetracaine, lidocaine and dibucaine (all bases), cholesterol and butylhydroxyanisole (BHA) were from Sigma Chemical Company, St Louis, MO. Etidocaine was provided by Astra Pain Control AB, Sodertalje, Sweden. Stearic acid USP grade was obtained from J.T. Baker Chemical Co., Phillipsburg, NJ. Glyceryl monostearate, polyethyleneglycol-8-stearate, triethanolamine, cetostearyl alcohol, propylene glycol and benzocaine were received from BDH Inc., Toronto, Ontario. Methylcellulose (1500cP) was obtained from Aldrich Chemical Company, Milwaukee, WI.

Formulation of liposomes

Multilamellar liposomes were prepared by the solvent evaporation method of Mezei and Nugent (1984). The lipid phase and the appropriate amount of local anesthetic agent (benzocaine, lidocaine, dibucaine, etidocaine or tetracaine) were dissolved in chloroform: methanol (2:1) in a round bottom flask containing glass beads. The solvent was then removed by rotary evaporation at 25-30 °C (Buchi RE 111 Rotavapor, Buchi Laboratoriums, AG Flawil/Sheiz, Switzerland) such that a thin lipid film was deposited on the wall of the flask and the surface of the beads. Once the thin lipid film was formed the flask was heated to the gel-to-liquid crystalline transition temperature of the lipids (approx. 55 °C). The appropriate aqueous phase (Aqueous phases (%w/w); **A**: NaCl, 0.45; $NaHCO_3$, 0.65; propylene glycol, 7; ethanol, 10; BHA, 0.02; pH, 9.0; **C**: NaCl, 0.45; $NaHCO_3$, 0.65; propylene glycol, 15; ethanol, 10; BHA, 0.02; pH, 8.5; **D**: NaCl, 0.45; $NaHCO_3$, 0.65; propylene glycol, 7; BHA, 0.02; pH, 9.0; **F**: NaCl, 0.9; propylene glycol, 7; ethanol, 10; BHA, 0.02; pH, 5.5; **G**: NaCl, 0.45; propylene glycol, 7; BHA, 0.02; pH 5.5) was then added and the flask hand-shaken vigorously for two minutes to hydrate the lipid film. The flask was then placed into a waterbath at 55 °C and shaken for 20 minutes continuously to complete liposome formation (Girotory waterbath shaker, model G76, New Brunswick Scientific Co. Inc., New Brunswick N.J.).

Drug encapsulation efficiency

The encapsulation efficiency of the drug was determined for several preparations using the spectrophotometric method. About 0.5 g of each of the preparations was placed into Beckman polycarbonate centrifuge tubes and diluted 3 times with their

respective aqueous phase. The samples were centrifuged in a Beckman L8-55 Ultracentrifuge for 90 min at 50,000 rpm at room temperature. The supernatant was removed, leaving the pellet containing the liposomes at the bottom of the tubes. Small amounts of the pellets and the supernatants were dissolved in chloroform/methanol 2:1 and concentration was determined at 307 nm with a Shimadzu UV-Visible spectrophotometer (UV-265). Drug encapsulation was determined as the fraction of drug found in the liposome pellet expressed as percentage of total drug content.

$$\% \text{ Tetracaine Encapsulated} = \frac{m_{pellet}}{m_{pellet} + m_{sup}} \times 100$$

m_{pellet} - amount of tetracaine in pellet/gram product after centrifugation
m_{sup} - amount of tetracaine in supernatant/gram product after centrifugation

Stability studies

The physical stability (shelf-life) of various liposomal preparations was followed for about one year at room temperature. The stability was evaluated using a visual stability rating scale for disperse system products as described by Hanna (1989).

In order to analyze the tetracaine hydrolysis in liposomes a standard solution of the degradation product of tetracaine, 4-butylaminobenzoic acid (4-BABA), was prepared. Tetracaine base was hydrolyzed with 1M NaOH by refluxing for five hours according to Bauer et al. (1984). Then the reaction mixture was cooled and acidified to pH 2 with the addition of concentrated HCl. The precipitated 4-BABA was extracted with chloroform (yield was approx. 70%). The resulting hydrolysis product of tetracaine showed one single spot on TLC plates. The various liposome preparations (stored for various time intervals) were analyzed by TLC using chloroform-methanol (9:1 v/v) as the mobile phase and iodine vapor for identification. The hydrolysis of tetracaine was determined by densitometry of the TLC plates (BioRad Video Densitometer, Richmond, CA).

Microscopic studies

All preparations were viewed in a Microstar optical microscope using polarized light (Cambridge Instruments Canada Ltd., Monteal, Que.). Particle size range was estimated with a calibrated ocular micrometer.

Assessment of the efficacy of various liposome-encapsulated tetracaine formulas in guinea pigs

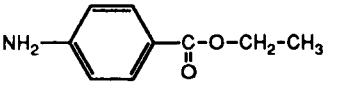
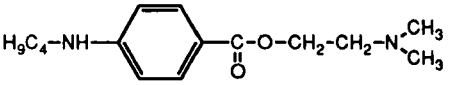
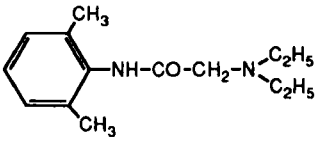
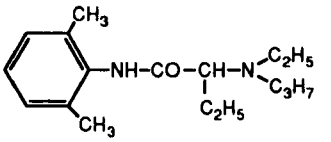
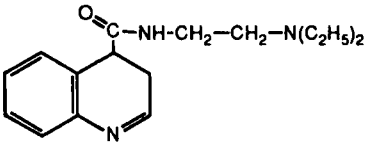
A single dose of 0.2g of the test (containing 4 mg tetracaine) and the control preparations were applied on a 10 cm^2 area to the two sides sides on the shaved back of a guinea pig. The treated area was covered with parafilm and Blenderm^R tape (3M Co, St.Paul, Minnesota) to provide occlusion for 30 min. The pin-prick test was used to assess the local anesthetic effect. The device for the pin-prick test consisted of a surgical pin pushed through a rubber stopper, which prevents the pin from penetrating the skin (Santos et al., 1987). This device has been used by anesthetists for testing of sensory neural blockade during regional anesthesia. The advantage of the device over a needle is more uniform stimulus intensity and prevention of skin injury. Testing was done immediately after removal of the sample and at 15, 30, 45 minutes and 1, 2, 3, 4, 5, 6h. The same guinea pig was used for each experiment to avoid differences in the behaviour of the animals. The guinea pig was allowed to recover after each experiment for at least 24h. "Empty" liposomes (no drug) and intradermal Pontocaine injection (Winthrop Laboratories) where a 4 mg dose was divided to four sites within the 10 cm^2 area, served as controls. All preparations were tested twice on different days.

RESULTS AND DISCUSSION

Encapsulation of Local Anesthetic Agents

Preliminary formulations with various local anesthetic agents (Table 1) indicated that tetracaine can be the most successfully incoporated into liposomes in the required concentration with suitable physical stability. Benzocaine and dibucaine crystallized out from the preparations immediately after manufacturing when concentrations higher than 0.5-1% w/w (a minimum concentration required for anesthesia) were employed. Increasing lipid concentration, changing lipid and aqueous phase composition did not improve encapsulation for benzocaine. In case of dibucaine the use of unsaturated phospholipids only as liposome constituents appeared to prevent crystal formation and 80.6% encapsulation efficiency could be achieved when 0.5% starting concentration was used. Etidocaine also crystallized out from the liposome preparation within one day after preparation, when used at 0.5% concentration. According to our studies with benzocaine, dibucaine, lidocaine and etidocaine, it is difficult to formulate a physically stable liposomal preparation

TABLE 1 Structure and physical properties of local anesthetic agents selected for liposome encapsulation

Structure	Molecular Weight (base)	pKa
Benzocaine 	165.2	2.50
Tetracaine 	264.4	8.39
Lidocaine 	234.4	7.86
Etidocaine 	276.4	7.74
Dibucaine 	343.5	8.15

with high encapsulation efficiency and without crystal formation. Crystal formation in the liposomal formulas is of concern since the unpredictable growth of crystals contributes to phase separation, uncontrolled particle size and drug release and unacceptable application properties.

Tetracaine, one of the most potent local anesthetic with a slow onset and long duration of action, appeared to be the most suited for liposome encapsulation with the highest encapsulation efficiency. This is probably due to its relatively large hydrophobic portion and amphipatic properties. Despite the fact that its chemical stability could be a problem (ester hydrolysis), tetracaine still has the greatest potential for producing the most efficacious topical liposomal product.

Development of Topical Liposomal Tetracaine

I. Physicochemical Parameters

It was shown previously in our laboratory that it is necessary for the drug to be encapsulated into the liposomes (as opposed to a simple mixture of drug and liposomes) to achieve efficient delivery into the skin. In order to optimize the encapsulation efficiency of liposomal tetracaine formulas, the following steps were taken. Tetracaine, as a base, being more lipophilic was used for all formulas, and the drug was incorporated through the lipid phase. Initially the pH of the aqueous phase was adjusted to 8.5-9 to keep the drug in the unionized form, when higher encapsulation into lipid bilayers can be expected. This latter approach can lead to higher encapsulation efficiencies, although for dermatological purposes a pH < 7 would be preferred since the pH of the skin is about 5.5. Therefore, subsequent preparations were prepared with aqueous phases having a pH of 5.5. All liposomal tetracaine formulas with this aqueous phase, however, invariably resulted in preparations having a final pH of 7.3-7.6. Numerous formulas (80-100) were prepared during the optimization process. Since it is impossible to list all of these, only selected formulas are listed in Table 2 for the illustration of the various aspects of liposome formulation and the results of the characterization.

The difference in encapsulation efficiency was noticable at different pH values. For example, the encapsulation efficiency of tetracaine in Formula 1A (pH 9) was 76.0% whereas in Formula 1F (pH 5.5) it was 59.8% (Table 3). It is speculated that mainly the unioinized form of tetracaine is associated with the liposomes, however it is possible that the ionized tetracaine in the lower pH preparations (pH ~ 7.3) can also interact with negatively charged bilayer containing

TABLE 2 Selected formulas of topical liposomal tetracaine preparations.

Basic formula	Modifications	Prep.#	AEE*(%)	Application properties	Physical stability #
SPL-H 7% Chol 1.75% SA 0.7%	Aq.phase/ Drug conc:	1A 1F 1F2 7C	76.0 59.8 80.4 62.0	white, smooth, viscous liquid, slightly tacky white liquid white, slightly viscous liquid, not tacky off-white cream, slightly tacky	stable (8) 10mo; C** separated(5) 2mo separated(5) 2mo stable (8) 5mo; C
	Lipid conc:	1F2(10SPL-H)	84.7	white, viscous liquid, slightly tacky	stable (8) 3mo
	Additive:	1F2-2MC 1F2-3MC	82.8 -	white cream, slightly tacky white cream, slightly tacky	separated (7) 3mo stable (8) 1mo
SPL-H 10% Chol 1.75% SA 1.75%	Aq.phase:	23C2 23F2	86.4 75.6	white, viscous cream white liquid	stable (9) 12mo separated (3) 2w
SPL-H 5% SPL 4% Chol 0.9% SA 0.9%	Aq.phase/ Drug conc:	4A 13D-5Pst 13G2-1G	79.0 - 90.8	yellow liquid yellowish, pearly cream off-white viscous liquid, tacky	stable (7) 17mo stable (8) stable (7)
	Additive:	9A-5Pst 10A-5S3T 12A-5S1Sp	- - -	yellowish cream yellow cream, foamy, gritty, tacky yellow cream, foamy, tacky	not homogeneous not homogeneous stable (7)
SPL-H 7% Chol 1% GM 1%	Additive/ Drug conc:	14A-1.75SA 14A2-1PSt	61.0 89.5	off-white cream, not tacky off-white cream, slightly tacky	stable (8) 6mo; C stable (8) 10mo
SPL-H 5% Plipon 2% Chol 1.75%	Aq.phase/ Drug conc:	42F 42F2	74.3 88.6	yellowish liquid yellowish liquid	separated (6) separated (6)

* Apparent Encapsulation Efficiency (average of 3-5 assays, s.d. was within 5% in each case)
** Uncontrolled crystal growth in the formula
Aqueous phases A, C, D: pH 8.5-9.0; G, F : pH 5.5

Legend for TABLE 2:

Abbreviations:

SPL-H	Soya phosphatidylcholine NC 95H (hydrogenated)
SPL	Soya phosphatidylcholine NC 95 (not hydrogenated)
Plipon	Phospholipon 90
Chol	Cholesterol
SA	Stearic acid
GM	Glyceryl monostearate
PSt	Polyethyleneglycol-8-stearate
T	Triethanolamine
MC	Methylcellulose (1% sol., 1500cP)
S	Stearyl alcohol
G	Gelatin B
Sp	Span 20

Formula Key: (example)

1 F 2 - 3 MC

- 1: formula identification number (reflects lipid composition)
- F: aqueous phase used
- 2: concentration of tetracaine if other than 1%
- 3: concentration of additive
- MC: additive

 # Visual stability rating for disperse system products (Hanna, 1989)

9	No visual separation, completely homogeneous
8	No visual separation, virtually homogeneous
7	Very indistinct separation, no clear layer at bottom or top
6	Indistinct separation, no clear layer at bottom or top
5	Distinct separation, no clear layer at bottom or top
4	Homogeneous top or bottom layer, clear layer at bottom or top
3	Distinct separation, clear layer at bottom or top with no coalescence
2	Distinct separation with slight coalescence
1	Distinct separation with strong coalescence
0	Complete separation and complete coalescence

TABLE 3 Effect of dilution of liposomes on the determination of encapsulation efficiency of tetracaine

Basic Formula: SPL-H 7% Chol 1.75% Tetracaine 1%		Drug concentration in liposome pellet		
SA conc.	Aq. phase	No dilution	10X	20X
0%	C	92.3	11.7	2.9
0.5%	C	96.4	13.9	3.9
0.8%	C	96.2	18.3	5.6
1%	C	97.9	21.5	10.1
0%	bicarbonate buffer	97.5	71.9	34.0
0.5%	bicarbonate buffer	98.9	68.2	21.2

stearate. The presence of stearic acid appeared to increase the amount of tetracaine associated with liposomes. This is consistent with the results of Surewicz and Leyko (1982), who investigated the interaction of tetracaine with liposome bilayers. They showed that the fluorescent of 12-(9-anthroyl) stearic acid incorporated into liposomes was quenched by added tetracaine. This phenomenon can be explained by the ability of tetracaine to be incorporated into the bilayer, in the vicinity of the probe. The fluorescent quenching rate by tetracaine is dependent on pH, being more effective in alkaline medium, indicating that the neutral form of drug binds to the membrane. However, there are certain results which cannot be explained by only the neutral form binding. The binding of tetracaine to phosphatidylserine (negative) membranes is not as dependent on pH as the binding to phosphatidylcholine (neutral) membranes. Surewicz and Leyko (1982) concluded that both charged and uncharged species of tetracaine interacts with membranes.

In addition, Davio and Low (1981) showed that uncharged species of lidocaine preferentially interact with neutral membranes (dipalmitoylphosphatidylcholine and dipalmitoylphosphatidylethanolamine) while charged lidocaine interacted with dimiristoylphosphatidylserine membranes.

In this study the encapsulation efficiency generally increased with increasing initial drug concentration. Increasing initial tetracaine concentration from 1% to 2% increased encapsulation efficiency by 20-40% (Table 2). For example formulation 1F had an encapsulation efficiency of 59.8% compared with that of 80.4% for 1F2 an formulation 42F had an encapsulation efficiency of 74.3% compared with that of 88.6% for 42F2. This means that the concentration of drug in supernatant decreased from 40.2 to 19.6% for 1F to 1F2, and from 25.7 to 11.4% for 42F to 42F2. However, due to twice the initial concentration in the second preparation the amount of drug in supernatant in both preparations (1% or 2%) is fairly similar. It appears therefore that the encapsulation is an equilibrium process and depending on the solubility of drug in the supernatant (i.e. the external phase) the excess drug (because of its lipophilicity) will be associated with lipids. Increasing lipid concentration from 7 to 10% with formula 1F2 increased physical stability and encapsulation efficiency (table 2).

It appeared that the composition of the aqueous phase was also playing an important role in the partitioning of drug into the liposomes (formulas 1A and 7C, Table 2). Aqueous phase C contains twice as high concentration of propylene glycol, than aqueous phase A, therefore it is possible that tetracaine is more soluble in aqueous phase C, which can result in lower encapsulation efficiency .

Fernandez (1980) investigated the effect of tetracaine HCl on the integrity of egg lecithin liposomes at a concentration similar to the critical micellar concentration of tetracaine (i.e. approximately 0.06-0.07 M). Fernandez (1980) found that at this concentration, liposomes become disrupted and lipids are solubilized into tetracaine HCl micelles. In this study the highest concentration of tetracaine used was 2%w/w i.e. 0.0756M. Therefore it is conceivable that micelle formation by tetracaine or mixed micelle formation by tetracaine and lipids can take place. The possible damaging effect of micelle formation on lipid integrity in these preparations is not noticeable because of the high concentration of phospholipids (0.095-0.136 M), hence the great number of liposomes.

However, micellization in the formulas was suspected because of the difficulty in determining encapsulation efficiency in many preparations. The measurement of encapsulation efficiency was based on the determination of drug content in the centrifuged pellet and supernatant. As part of the assay, the liposomes were washed with 3 times their volumes of aqueous phase before centrifugation. Further studies indicated that the concentration of drug in the supernatant increased

with increasing dilution rate (see Table 3). The effect of the volume of washing solution (no solution added, ten times or twenty times dilution) on the encapsulation efficiency of tetracaine in one representative basic formula is shown in Table 3. With preparations containing aqueous phase C a great difference (a 70 to 80% decrease) is noticeable with respect to encapsulation when measured in undiluted and ten times dilution. When bicarbonate buffer was used this difference is much lower (30 to 40%). These results indicate that in these liposome preparations containing bicarbonate buffer or aqueous phase C, partitioning of drug between the lipid bilayers and the aqueous medium and formation of (mixed) micelles will influence the encapsulation of liposomes. Increasing stearic acid concentration in the formula appeared to increase the amount of tetracaine associated with liposomes at ten times and twenty times dilution but not at zero dilution. It is reasonable to assume that upon combining the lipid phase which contains stearic acid and the alkaline aqueous phase C or bicarbonate buffer, the anionic surface active agent sodium stearate may form which can participate in (mixed) micelle formation.

In this regard, these formulas are a unique type of "liposome product" in that they contain liposomes, encapsulated drug, micelles of drug, possibly mixed micelles of drug and lipid, and the dissolved drug in the external aqueous phase.

Although 80-90% encapsulation of tetracaine in some of the liposome formulas was acceptable for further in vivo experiments, one more variable was changed. Boulanger et al. (1980) and Habib and Rogers (1987) found that the presence of unsaturated lipids increases the encapsulation efficiency. Using SPL and Phospholipon the encapsulation efficiency was slightly higher and no crystals developed during storage Table 2). However, when present the viscosity of preparation decreased and the appearance of product was not as appealing. Formulas containing unsaturated phospholipids (SPL, Centrolex P, Phospholipon) showed signs of oxidization (discoloration) within 2-4 months even in the presence of antioxidant BHA. Ascorbyl palmitate was incompatible with each of the local anesthetic agents, causing yellow discoloration in each preparation, therefore it was avoided.

Liposome size distribution in the various formulas was similar, 0.2-5 μm. This size distribution is fairly wide, nevertheless easily reproducible. For topical products very tight size range is not required if other parameters (eg. encapsulation efficiencies and drug release rate) are reproducible (consider that in an o/w emulsion type of cream droplet size distribution is never the sole criterium). Flow

characteristics for one selected liposome preparation was determined. Liposome 1F2 was found to exhibit pseudoplastic flow with no thixotropic features (results not shown).

The composition of various topical local anesthetic liposome preparations, the results of encapsulation efficiency, organoleptic and stability evaluations are shown in detail in Table 2. As indicated, most of the preparations do not possess the optimal degree of viscosity necessary for topical application except for those with high lipid content. This increased amount of lipid usually causes those preparations to be fairly tacky and also causes an increase in product cost.

II. Application Properties

The organoleptic evaluations were performed to describe the appearance and application properties of preparations since these are intended for topical use. These findings allowed the elimination of several formulas which were found unappealing for topical use or had either low viscosity or inadequate physical stability. In certain formulas a third phase such as glyceryl monostearate (1%), PEG-8-stearate (up to 5%), methylcellulose (up to 3%), gelatin (up to 2%) or cetostearyl alcohol (up to 5%) was added after the liposomes had been formed. The purpose of these additives was to improve the viscosity, increase stability of the product or to facilitate application to the skin and improve cosmetic appeal and patient's acceptance.

Preparations with PEG-8-stearate showed good viscosity, however, separation generally occurred within 1 month. Preparations containing cetostearyl alcohol tended to have a gritty texture. The best results were obtained with the addition of gelatin which resulted in smooth viscous preparations.

Among the topical liposomal tetracaine preparations Formulas 1F2, 13G2-1G, 14A2-1PSt, and 23C2 were found to be acceptable for efficacy testing.

III. Chemical Stability of Tetracaine in Liposomes

The liposome formulas were also evaluated for chemical stability. Tetracaine is an ester which is relatively easily hydrolyzed in aqueous medium. Therefore the appearance of its degradation product, 4-BABA, was monitored in the formulas during storage at room temperature. Generally all liposome formulas were stable (i.e. contained less than 10% of total drug in the form of 4-BABA) for 2-3 months. Longer storage resulted in progressive hydrolysis of tetracaine. By 4-8 months 40-

TABLE 4 Hydrolysis of tetracaine in liposomes (Formula 1F2).

Formula	% of 4-BABA after storage			
	2mo	4mo	6mo	8mo
1F2	**4.6**	**38.5**	**55.1**	**59.4**
1F2 pellet	11.8	19.6	49.5	49.5
1F2 supernatant	55.1	74.6	84.9	80.7

60% of tetracaine was converted into 4-BABA (Table 4). The level of 4-BABA was also assessed separately in the supernatants and pellets of selected liposomes. The relative quantities of tetracaine or 4-BABA are expressed as % of total drug in the respective fraction (Table 4). It was found that the liposome encapsulated fraction of tetracaine was protected from hydrolysis to a greater degree when compared to the drug in the supernatant (Table 4).

IV. Efficacy testing in the guinea pig

Preliminary screening of the four selected liposome formulas for efficacy was carried out using the guinea pig model. The liposome formula was applied under occlusion to the shaven back of a guinea pig. Thirty minutes later the effect was tested with the pin prick method. The guinea pig responds to the pin prick with a shivering reflex. The lack of shiver can serve as an indicator of the local anesthetic effect. Fig 1 shows the results of the pin prick tests. The number of painful scores out of ten pricks were counted. For comparison of the various formulas the painful scores versus time were plotted (Fig 1). The anesthetic effect was noticable between 45-60 min after application for each formulas. There was a difference however in the duration of effect. Formulas 14A2-1PSt and 13G2-1G showed fairly good anesthetic effect (score<2) for a short period of time (30 min and 75 min, respectively). Formulas 23C2 and 1F2 showed a longer lasting anesthesia (>3h). Using these latter two formulas skin sensation was not regained for 6h. "Empty" liposomes did not have any effect. After intradermal Pontocaine complete anesthesia was obtained for about 40 min, and 100 min after injection no anesthetic effect was noted.

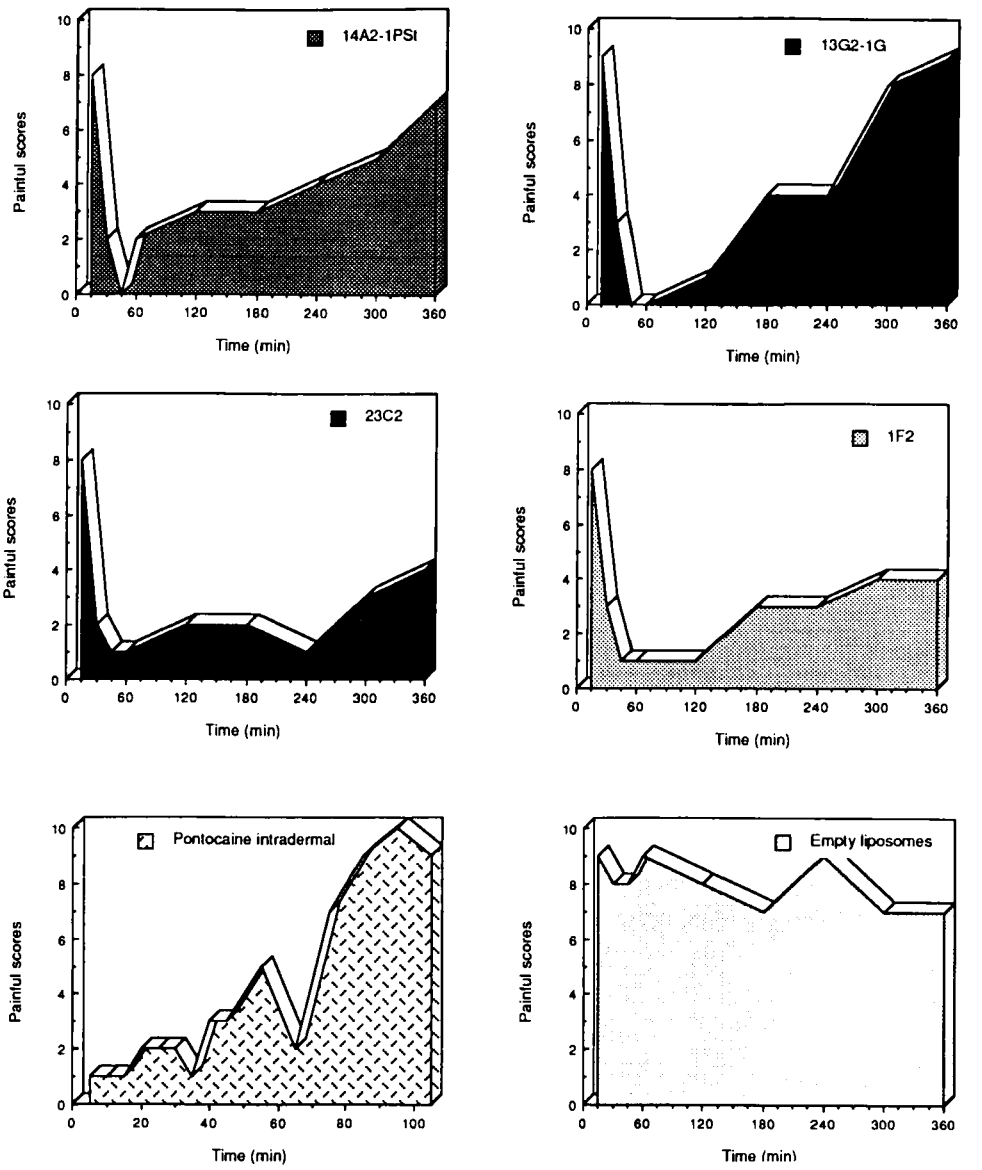


FIGURE 1

Assessment of topical anesthetic effect of selected formulas in guinea pigs. The mean painful scores (n=2) out of ten pinpricks were plotted as a function of time.

TABLE 5 Suggested parameters to be optimized for topical liposomal dosage forms.

Parameters to be optimized	
I.	**Physicochemical parameters.**
	1. Encapsulation efficiency.
	2. Particle (liposome) size distribution.
	3. Viscosity profile.
	4. Stability.
II.	**Application properties.**
III.	**Chemical stability.**
IV.	**Bioavailability parameters.**
	1. Drug release from liposomal vehicle.
	2. Drug/vehicle penetration into skin (in vitro or in vivo).
	3. Efficacy testing.

On the basis of these experiments Formulas 23C2 and 1F2 were selected for further trials in volunteers. The guinea pig was found to be a suitable model for preliminary testing of the topical anesthetic preparations. Certain precautions however have to be taken to obtain consistent and reliable results. The pig should be tested by the same person in a calm environment devoid of sudden movements and noise. Depending on the depth of anesthesia the pig might also respond to touching by shivering even though no pain was experienced. Therefore it is important to distinguish pain sensation from touching.

Topical Liposomal Products

Liposomes as drug delivery systems can be used systemically (iv, im) and topically (on skin and body cavities). These various routes of administration require appropriate liposome formulation. Liposomes intended for use on the skin need to comply with requirements similar to other topical creams. Although there are no firm guidelines for topical products at present , there are certain basic requirements which need to be fulfilled. These are proper consistency, physical stability of the preparation, chemical stability of the drug and acceptable feel on the skin.

For characterization of liposome products, as new type of dosage forms used in skin treatment the proposed requirements are shown in Table 5.

In summary, the results of these formulation experiments (evaluation of Group I, II & III parameters) indicate that a physically stable topical liposomal tetracaine (2%) product can be prepared with good application properties, which however has a relatively short shelf-life. Further work includes stability improvement and the investigation of Group IV Parameters (Table 5), i.e. the bioavailability parameters.

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