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# Less-painful emulsion formulations for intravenous administration of clarithromycin

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**Abstract**

Several emulsion formulations (e.g., mixtures of drug with a nutritional emulsion, e.g., Liposyn®, ready-to-use w/o/w emulsion, and ready-to-use o/w emulsions) were developed and tested for potential use as a less-painful i.v. formulation of clarithromycin. The formulations contained 5 mg/ml drug. Formulations were tested in one or more animal models to estimate the level of pain reduction as compared to an aqueous solution of clarithromycin lactobionate. Most formulations demonstrated 20–80% reduction in pain. However, the nutritional emulsion mixtures and the w/o/w emulsions demonstrated chemical or physical stability problems. On the other hand, a ready-to-use o/w emulsion, composed of 0.5% w/v drug, 2% w/v soybean oil, 5% w/v egg phosphatide, 0.6% w/v oleic acid, 0.3% hexanoic acid and 2.5% w/v glycerin in water, demonstrated adequate physical and chemical stability for clinical development. Additional in vivo studies with this emulsion revealed it to be as efficacious as clarithromycin lactobionate solution and clarithromycin base aqueous suspension in the treatment of *Staphylococcus aureus*, *S. pyogenes* and *Streptococcus pneumoniae* infections. In dogs, the plasma distribution of 100 mg drug infused as emulsion closely compared with clarithromycin lactobionate solution. The data suggest that emulsion formulations may be useful for the parenteral delivery of painful compounds.

*Key words:* Clarithromycin; Injection pain; Emulsion; Animal model; Pain assessment

## 1. Introduction

Clarithromycin is a relatively new macrolide antibiotic with a methoxy group (-$OCH_3$) attached to the $C_6$ position of erythromycin (see Fig. 1). This derivatization makes clarithromycin more acid stable than erythromycin (Kohno et al., 1989; Nakagawa et al., 1992). Clarithromycin is better maintained in blood at high concentrations and is more efficiently excreted into urine than conventional macrolide antibiotics (Fuji and Nishimura, 1988; Ferrero et al., 1990). In addition, it is more effective than erythromycin against streptococci and *Mycobacterium tuberculosis* (Cachet et al., 1987). Clarithromycin is currently marketed in tablet form by Abbott Laboratories as Biaxin®. It is administered intravenously as the lactobionate salt, which is water soluble at pH $\leq 5$. However, in common with other macrolide an-

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tibiotics, clarithromycin lactobionate (CL) has the potential for pain during intravenous (i.v.) administration. Hence, there is considerable interest in the development of a less-painful i.v. formulation of clarithromycin.

Clarithromycin has the dimethylamino moiety as the only ionizable group ($pK_a$ 8.8) and hence its aqueous solubility at pH 7.0 is only about 500 $\mu$g/ml (Nakagawa et al., 1992). Studies in non-aqueous medium have indicated solubility of $\leq 2$ mg/ml in long- and medium-chain triglyceride oils and polyethylene glycol 400 (Abbot Laboratories, data on file). The poor solubility of this compound in aqueous as well as oil medium, at around neutral pH and ambient temperature, has hampered the development of its contemporary less-painful i.v. formulation.

Emulsion formulations have been used in past for the delivery of a variety of therapeutic agents, e.g., amphotericin B (Forster et al., 1987), chemotherapeutic agents (El-Sayed and Repta, 1983; Tarr et al., 1987), clofibride (Santos Magalhaes et al., 1991), cyclosporin (Venkatraman et al., 1990), diazepam (Levy et al., 1991), physostigmine (Pathak et al., 1990), propofol (PDR, 1993), and peptidic compounds (Gasco et al., 1990). Potential applications of emulsion systems for specialized drug delivery have been reviewed frequently (Collins-Gold et al., 1990; Prankerd and Stella, 1990). We hypothesized that the delivery of clarithromycin as an emulsion may reduce the pain response upon i.v. administration. This report summarizes our efforts to test emulsion systems for drug encapsulation and hence develop a less-painful i.v. formulation of clarithromycin. Since there is no standard animal model for testing pain response, the test formulations were screened using one or more animal models which assist in the estimation of pain, and the results compared with aqueous solution(s) of CL.

## 2. Experimental

### 2.1. Materials

Liposyn®, clarithromycin lactobionate, clarithromycin base, soybean oil, medium chain triglycerides (Neobee oil), sodium hydroxide, glycerin USP, sodium chloride, polysorbate 80 (Tween 80), polysorbate 85 (Tween 85), sorbitan monooleate (Span 80), sorbitan trioleate (Span 85) and *N*-methylpyrrolidone (NMP) were obtained from Abbott Laboratories and were reagent grade or USP code materials. Egg phosphatides (low electrolyte) were obtained from Pfansteihl Laboratories (Waukegan, IL). Hexanoic acid, oleic acid and decanoic acid were purchased from Sigma. Chloroform, methanol and dibasic sodium phosphate were procured from Fisher, and disposable 0.2 $\mu$m nylon filters from Nalgene were used.

### 2.2. Equipment

A Silverson® homogenizer (model L2R) was used to obtain crude emulsions. A Microfluidics

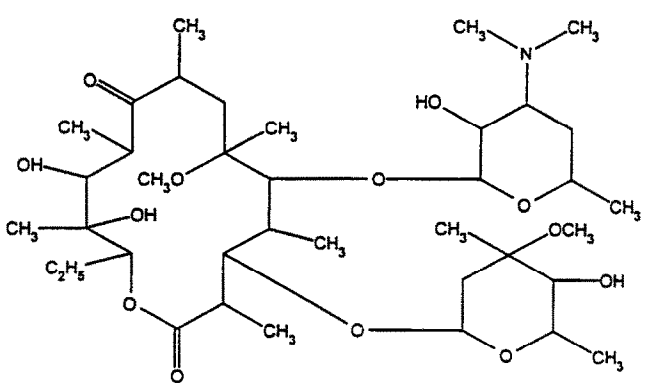


Fig. 1. Chemical structure of clarithromycin.

microfluidizer® or Manton-Gaulen M-15 homogenizer was used to obtain final formulations. A Wescor 5500 vapor pressure osmometer and a laser beam particle sizer (NICOMP) were used to determine osmolarity and particle size distribution of the formulations, respectively. A Waters 5907 pump, Waters 712 WISP automatic sample injector, Spectraflow 783 programmable absorbance detector and Spectra-Physics integrator were used for the HPLC analysis of drug.

### 2.3. Formulation development

#### 2.3.1. Preliminary formulations with clarithromycin-Liposyn® mixtures

A 170 mg/ml solution of clarithromycin in NMP was stirred into commercial Liposyn® to obtain a final drug concentration of 5 mg/ml.

#### 2.3.2. Ready-to-use water-in-oil-in-water (w / o / w) emulsion

The internal water phase consisted of 20–22% of total water, about 50–60% of total hydrophilic surfactant and the drug. The external water phase consisted of the remaining water and hydrophilic surfactant along with sodium chloride to adjust tonicity of the preparation. The oil phase consisted of 33.3% soybean oil along with hydrophobic surfactant. The internal water phase and the oil phase components were warmed to about 60°C, homogenized to obtain a w/o emulsion, which was then added to warm external aqueous phase solution with homogenization to obtain the final w/o/w emulsion (see Table 1).

#### 2.3.3. Ready-to-use oil-in-water (o / w) emulsion

The preparation of o/w emulsions involved three steps: (a) dissolution of clarithromycin base in oil in the presence of fatty acid(s) as lipophilic counterions; (b) preparation of an aqueous phase involving dispersion of egg phosphatides in water with the aid of heat (40–50°C) and stirring, and addition of other non-active ingredients (e.g., glycerin and sodium hydroxide) to it; and (c) incorporation of oil phase into aqueous phase to obtain an o/w emulsion.

Typically, the aqueous phase was microfluidized or homogenized so as to thoroughly disperse its components. When opalescent or near-transparent, the oil phase was incorporated into it with the aid of Silverson homogenizer. After stirring for 15 min, the crude emulsion was transferred either to a Microfluidics microfluidizer to make 15 passes at 10 000–15 000 lb/inch$^2$ or to a Gaulen M-15 homogenizer to make 30 passes at 6000–7000 lb/inch$^2$. In either case, cool water was circulated around the equipment to maintain the temperature of the emulsion at 35–40°C. After this processing, the pH of the formulation was adjusted to 7.4 using 10% w/v aqueous sodium hydroxide solution and its volume made up with distilled water. The small batches of emulsion (i.e., $\leq$ 500 ml) were sterilized using a 0.22 $\mu$m Nalgene® nylon filter and packaged in sterile glass bottles.

A typical investigational batch size was 200 ml. Once the formulations were made, they were visualized under a microscope for possible drug crystallization. Several initial formulations which were not acceptable, either due to physical separation of oil and aqueous phases, or crystal growth over the first few hours, were rejected. Once the formulation development work reached the stage of an acceptable product, particularly in terms of its homogeneity and syringeability through a 0.22 $\mu$m filter, its chemical potency was determined and the possibility of pain reduction investigated using one or more animal model. The mean particle size of this emulsion generally approximated 140 nm.

### 2.4. Drug analysis

A reverse-phase HPLC method was employed for drug analysis. The mobile phase composition was 55% v/v potassium phosphate buffer pH 3.5, 40% v/v acetonitrile and 5% v/v methanol. A 50 × 4.6 mm ODS2 3 $\mu$m 'Little Champ' HPLC column (Regis) was used. The detection wavelength was 214 nm. The injection volume and mobile phase flow rate were set to 25 $\mu$l and 1 ml/min, respectively. Under these conditions, the retention time for clarithromycin was about 6.2 min, and its standard curve between the range of 10 and 500 $\mu$g/ml was linear with $r^2 \geq 0.99$.

The drug potency in emulsion formulations was determined by diluting 0.5 ml samples to 10

ml with HPLC mobile phase, sonicating for 5 min, followed by centrifugation at 10000 rpm for 5 min. The clear supernatant was then directly injected onto the HPLC column to determine the drug concentration.

### 2.5. In vivo studies

#### 2.5.1. Mouse scratch test

In this test, animals in different groups received subcutaneous (s.c.) injections of formulation, a positive control or a negative control. Thereafter, they were monitored for the number of scratches made by their paw over a pre-determined period of time. Typically, the more painful or irritating the formulation, the more scratches the animal makes (Comereski et al., 1986). In most cases, the onset time of scratches was also recorded. Generally, more painful the formulation, the shorter the onset time of scratches. This test is useful for assessing the response due to pain or irritation after injection.

For comparative evaluation of pain response with clarithromycin emulsion formulations, groups of mice ($n = 10$) weighing ~20 g were injected 0.1 ml of test or control formulations. The animal studies were not repeated in additional group of animals. The formulations tested include: (a) clarithromycin lactobionate solution in saline (e.g., 1.0, 2.5, 4.5 or 5.0 mg/ml) as a positive control; (b) 5% dextrose in water (D5W) or saline as a negative control; (c) 5 mg/ml clarithromycin test emulsion.

The initial mouse scratch tests (i.e., for the NMP-liposyn® mixtures and the decanoic acid-neobee oil emulsion) involved counting the total number of scratching episodes during the first 5 min after injection of test solution or 5 mg/ml CL. The test was subsequently modified to establish the dose-response curve and hence improve quantitation of the level of pain reduction with test formulations. In the modified method, the time of initiation of scratches as well as total number of scratches over 10 min were counted. In studies where a series of clarithromycin lactobionate solutions (positive control) were injected in mice, the results were used to construct a standard curve which was subsequently used to correlate pain with test emulsion versus clarithromycin lactobionate solution. This in turn assisted in estimating pain reduction with the emulsions.

#### 2.5.2. Rat paw lick test

In this test, a group of 10 Weanling rats received a single subplantar injection of 0.1 ml test formulation into the footpad of their right hind paw. Thereafter, the number of time the animals licked their paw as well as the total time for which the paw was licked were monitored over a period of 15 min (Celozzi et al., 1980). Once again, a series of clarithromycin lactobionate solutions and D5W served as positive and negative controls, respectively.

#### 2.5.3. Rabbit ear vein test

Here, groups of three rabbits received a single infusion of 5 mg/ml test emulsion formulation, 5 mg/ml clarithromycin lactobionate solution (positive control), or an equivalent volume of D5W (negative control) into their marginal ear vein. The rate of infusion was maintained at 1 ml/min and the total drug dose was 40 mg/kg. Following infusion, visual observations of the site of injection were made over a period of 24 h, particularly in terms of swelling, bruises and/or discoloration of the injected vein and the surrounding tissue.

#### 2.5.4. Rat tail vein irritation test

In this study, groups of six rats received infusion of 5 mg/ml test clarithromycin emulsion or 5 mg/ml clarithromycin lactobionate solution at a dose of 100 mg/kg and rate of 0.3 ml/min over a period of 3 consecutive days. An additional group of animals receiving an equivalent volume of D5W under similar conditions served as a negative control. The site of injection was visualized for vein discoloration, spotting and/or swelling during injection and over a period of 24 h after dosing.

#### 2.5.5. Plasma distribution study in dogs

In a two way cross-over study, two groups of beagle dogs ($n = 4$) received an i.v. infusion of 100 mg clarithromycin as a 5 mg/ml clar-

ithromycin lactobionate solution in D5W and a 5 mg/ml test o/w clarithromycin emulsion. The infusion rate was maintained at 0.25 ml/min, and hence the total dosing time approximated 80 min. After dosing, 3–4 ml blood samples were collected over a period of 24 h, processed to obtain plasma, and assayed for drug concentration using a validated microbiological method. The animals were fasted overnight before the study; however, food was provided after collecting the 12 h time-point blood sample. Except during the dose administration, water was given ad libitum.

*2.5.6. Acute toxicity studies*

The $LD_{50}$ values of selected test emulsion formulations were determined in mice and/or rats. The animals were given i.v. infusion of formulation at a rate of 2 ml/min. The results were reported in ml/kg and converted into mg/kg active component. Although doses > 20 ml CL per animal were considered excessive, in some instances up to 50 ml dose of emulsion were tested.

*2.5.7. Efficacy studies*

Efficacy studies were conducted in *Staphylococcus aureus*, *S. pyrogenes* and *Streptococcus pneumoniae* infected CF-1 female mice, weighing about 20 g, following oral, s.c. or i.v. administration of test formulation. The efficacy was compared with aqueous clarithromycin lactobionate solution or suspension and the results reported as mean mg/kg per day dose required to protect 50% animals ($ED_{50}$) along with 95% confidence limits.

## 3. Results and discussion

### *3.1. Formulation development*

*3.1.1. Preliminary formulations with clarithromycin-Liposyn® mixtures*

As mentioned earlier, clarithromycin has very low solubility in water and parenterally acceptable oils. Hence, its incorporation into a traditional o/w emulsion is difficult. However, it was thought that distribution of drug at oil-water interface, particularly in case of nutritional emulsions due to the presence of phospholipids, may reduce pain due to transient masking of the painful portion of drug. With this goal, an attempt was made to stir clarithromycin base into Liposyn®. Although drug dissolution was achieved after prolonged stirring, allowing syringeability of a 5 mg/ml emulsion through a 0.2 $\mu$m filter, the drug precipitated after a few days. Thereafter, efforts were made to dissolve clarithromycin into an oil-miscible, relatively non-toxic cosolvent (NMP) to be used as a pre-mixture for dilution with Liposyn®. If this approach were to allow pain reduction following i.v. administration, the goal was to market an NMP solution of drug to be diluted in hospitals with Liposyn® at recommended concentrations. Dilution of a 170 mg/ml solution of clarithromycin in NMP with Liposyn® resulted in a physically stable product with 5 mg/ml drug and ~24 mg/ml NMP. No drug crystallization was observed for at least 1 month at room temperature. Based on mouse scratch testings, it demonstrated almost 2-fold reduction in pain as compared to a 5 mg/ml CL solution (see Fig. 2). However, the NMP solution of drug demonstrated chemical stability problems, with

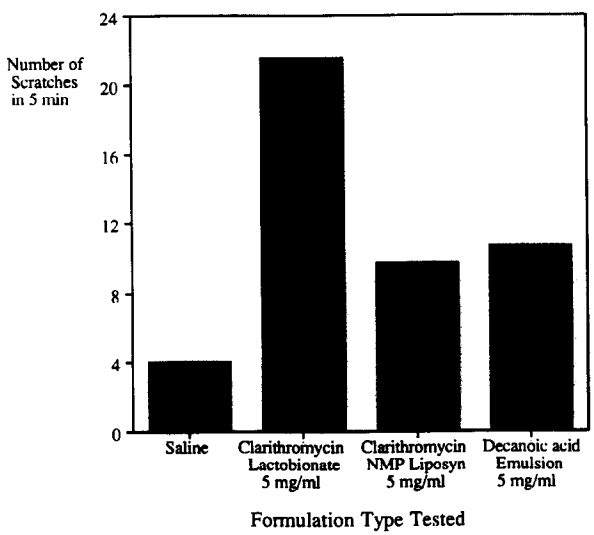


Fig. 2. Mouse scratch results following the injection of saline, and 5 mg/ml clarithromycin formulations (lactobionate solution, NMP-Liposyn® mixture and decanoic acid emulsion).

≤ 90% drug remaining at room temperature after 1 month. Hence, this approach was not further investigated. Similar instability of erythromycin in NMP has been reported (Cachet et al., 1987).

### 3.1.2. Ready-to-use water-in-oil-in-water (w / o / w) emulsion

Table 1 summarizes the composition of two w/o/w emulsions tested in this study. CL, which is soluble in water, was incorporated into internal water phase. Neither emulsion formulation demonstrated drug crystallization for several months at room temperature. In the modified mouse scratch model, a 5 mg/ml emulsion formulation containing Tween 80 and Span 85 resulted in pain response comparable to 4.2 mg/ml CL solution (see Fig. 3), indicating marginal reduction in pain response. The second 5 mg/ml formulation containing Tween 20 and Span 80 resulted in pain response comparable to that of the 1.7 mg/ml CL solution (see Fig. 3), indicating almost 3-fold reduction in pain response upon injection. The latter formulation also demonstrated a scratch onset time of about 2.35 min as opposed to that of 0.75 min with 5 mg/ml CL solution. The higher value of scratch onset time denotes delay in pain response, which once again suggests possible reduction in pain response following i.v. injection. The pain response with placebo formulation was found to be equivalent to ≤ 0.25 mg/ml CL solution. Unfortunately, both clarithromycin w/o/w formulations demonstrated signs of physical instability after 3 months at room temperature, resulting in discontinuation of this formulation approach.

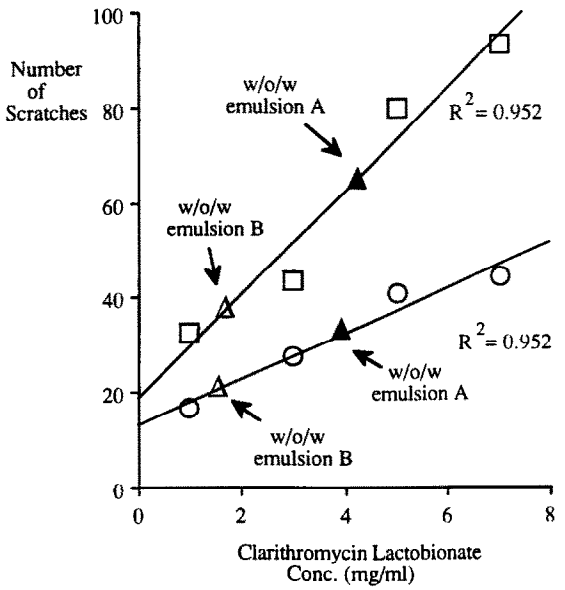


Fig. 3. Dose-response curve in mouse scratch results over the first 5 min (○) and the first 10 min (□) following the injection of 1, 3, 5 and 7 mg/ml clarithromycin lactobionate solution. The number of scratches with two 5 mg/ml w/o/w emulsion formulations is shown for comparison. (▲) w/o/w emulsion A; (△) w/o/w emulsion B.

Table 1
Details of various emulsion formulations

| Ingredients (% w/v) | w/o/w emulsion [a] | | o/w emulsion [b] | |
|---|---|---|---|---|
| | A | B | A | B |
| Clarithromycin[c] | 0.50 | 0.50 | 0.50 | 0.50 |
| Tween 20 | – | 1.55 | – | – |
| Tween 80 | 1.90 | – | – | – |
| Hexanoic acid | – | – | – | 0.30 |
| Oleic acid | – | – | – | 0.60 |
| Decanoic acid | – | – | 1.13 | – |
| Soybean oil | 33.3 | 33.3 | – | 2.0 |
| Neobee oil | – | – | 20.0 | – |
| Egg phosphatide | – | – | 2.4 | 5.0 |
| Span 80 | – | 2.07 | – | – |
| Span 85 | 1.50 | – | – | – |
| Glycerin | – | – | – | 2.5 |
| Water | q.s. | q.s. | q.s. | q.s. |
| NaCl[d] | q.s. | q.s. | – | – |
| NaOH[e] | – | – | q.s. | q.s. |
| pH | 5.5–6.0 | 5.5–6.0 | 7.2–7.6 | 7.2–7.6 |

[a] Clarithromycin lactobionate salt was used in these formulations.
[b] Clarithromycin base was used in these formulations.
[c] The concentration represents base equivalent.
[d] Used to adjust tonicity of formulations to 300 mOsm.
[e] Used to adjust pH of formulations.

### 3.1.3. Ready-to-use oil-in-water (o / w) emulsion

Relatively poor solubility of drug in oils, and its precipitation from Liposyn®, led to an investigation of the effect of lipophilic counterions on solubilization of clarithromycin base in oils. Inclusion of lipophilic counterions was also anticipated to minimize drug partitioning from oil into aqueous phase during and following its emulsifi-

cation, and hence reduce pain upon i.v. administration.

Initially, attempts were made to solubilize clarithromycin base in neobee oil with the aid of 1.13% decanoic acid. The oil phase was then emulsified into water with the aid of 2.4% egg phosphatide (see o/w emulsion A in Table 1 for composition). This formulation was physically and chemically stable at room temperature for at least 6 months, and in the mouse scratch model it demonstrated almost 2-fold reduction in pain as compared to a 5 mg/ml CL solution (see Fig. 2). However, this formulation was found to be more toxic than CL solution (i.e., $LD_{50}$ was 60 and 47 mg/kg in mice and rats, respectively, compared to 140 and 70 mg/kg in these species for CL), indicating unacceptability of decanoic acid and/or neobee oil at these concentrations. Since lower concentrations of decanoic acid were found to be ineffective, alternative lipophilic counterions were assessed.

Safflower and soybean oils contain up to 13 and 26% oleic acid in ester form, respectively (Davis et al., 1985). In addition, sodium oleate is often used in emulsions as an auxiliary emulsifier (Davis et al., 1985). This can be achieved either by adding oleate salt, or adding acid and then forming salt in situ by addition of sodium hydroxide which often also serves to adjust the pH of the preparation to a desirable value. In fact, Lipofundin®, a 10% i.v. lipid emulsion for total parenteral nutrition containing 0.03% w/v sodium oleate, is currently marketed in Europe. In addition, an NDA has been filed by Abbott Laboratories for a medium chain triglyceride based Liposyn® with 0.03% w/v sodium oleate. With this background information, oleic acid was investigated as a possible lipophilic counterion to solubilize clarithromycin base in soybean oil. Due to its low toxicity, hexanoic acid was also examined as a counterion. Hence, subsequent formulations contained oleic acid and/or hexanoic acid as lipophilic counterions, egg phosphatide as an emulsifier to develop o/w emulsion, glycerin to adjust its tonicity, and sodium hydroxide to adjust its pH to about 7.4.

Initial attempts indicated that 1 g of drug can be readily dissolved in 5 g soybean oil, with the aid of heat (55°C), in the presence of about 6 g oleic acid, giving an oil phase containing ~ 8% w/w drug. Efforts to incorporate this oil phase into an aqueous phase containing egg phosphatide, at an inherent pH of about 5.5, did not allow formation of a physically stable emulsion. However, increase in the aqueous phase pH to about 8.5, prior to the incorporation of oil, resulted in a physically stable emulsion. Nonetheless, this preparation indicated the presence of numerous drug crystals after a few days at room temperature, suggesting inefficient encapsulation of drug in the oil phase. The increase in the pH of continuous phase to about 9.5, prior to the addition of oil phase, allowed development of a formulation without drug crystals. Given a requirement of an emulsion containing 5 mg/ml drug, these experiments led to the realization that prevention of crystal growth requires high levels of lipophilic counterions such that the molar ratio of lipophilic counterion: drug needed to be relatively high (e.g., $\gg 1$).

An attempt was also made to develop an o/w emulsion using hexanoic acid alone as a lipophilic counterion; however, a stable emulsion could not be obtained. Hence, a combination of oleic and hexanoic acids was tried. The use of these two counterions at total concentration of 0.9% w/v allowed formation of a stable emulsion (see o/w emulsion B in Table 1). Due to acceptable physical and chemical stability at room temperature for $\geq 3$ months (Gupta et al., 1991), a preparation containing 0.5% w/v drug, 0.3% w/v hexanoic acid, 0.6% w/v oleic acid, 2.0% w/v soybean oil, 5.0% w/v egg phosphatide and 2.5% w/v glycerin in water was evaluated in various animal models for pain response, efficacy, toxicity and plasma distribution in dogs. The formulation was sterilized by filtration through disposable 0.2 $\mu$m filter prior to in vivo testings (Lidgate et al., 1992).

### *3.2. In vivo evaluation of ready-to-use oil-in-water (o/w) emulsion B*

In vivo studies were conducted to assess pain/irritation, plasma distribution, toxicity and efficacy of ready-to-use 5 mg/ml clarithromycin

o/w emulsion B. Wherever possible, appropriate controls were included, e.g., placebo and 5 mg/ml CL solution. The pH of CL solutions was generally $\leq 6.0$ and that of the o/w emulsion B about 7.4. It is realized that the differences in pain and/or irritation monitored may in part be due to the differences in the pH of the formulations. However, due to stability problems, different formulations could not be prepared and tested at same pH. Nonetheless, we believe that the pain/irritation due to drug is probably much higher than that due to differences in the pH of formulations.

### *3.2.1. Mouse scratch test*

The mouse-scratch test measures the number of scratches over a period of time following s.c. administration of a small volume of preparation. The comparison between the number of scratches and onset time of scratches with two formulations provides an estimate of their relative pain upon injection (Comereski et al., 1986). Implicitly, this model is better suited for comparison of pain following local (for example, s.c. or i.m.) administration of formulations. However, it served as a good tool for screening prototype formulations for i.v. administration. As mentioned earlier, the test was modified to establish a good dose-response relationship.

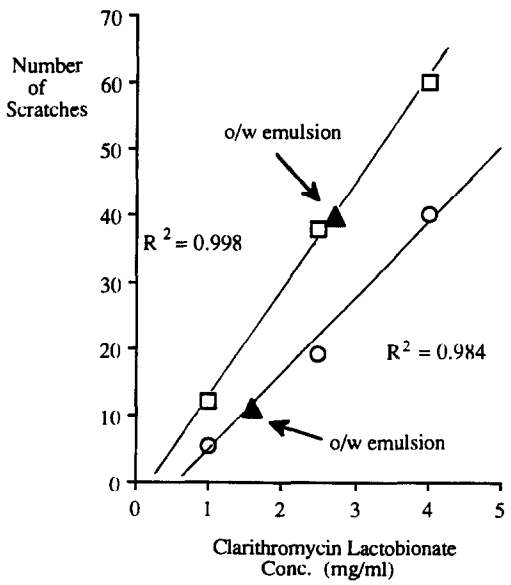


Fig. 4. Dose-response curve in mouse scratch results over the first 5 min (○) and the first 10 min (□) following the injection of 1, 2.5, and 4 mg/ml clarithromycin lactobionate solution. The scratches with the 5 mg/ml o/w emulsion B are shown for comparison.

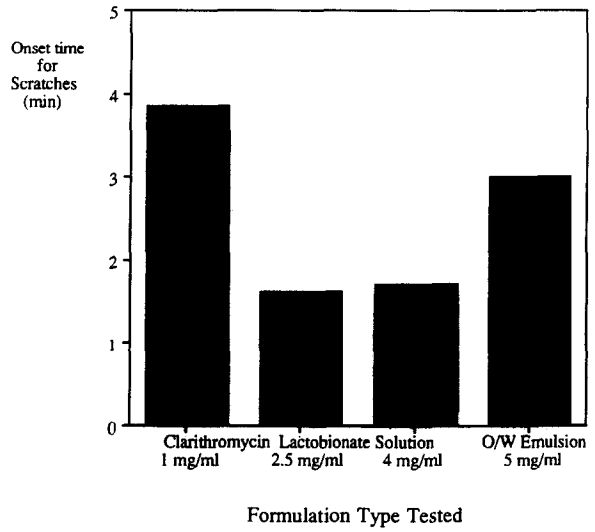


Fig. 5. Effect of the concentration of clarithromycin lactobionate on the time of onset of scratches. The time of onset of scratches with the 5 mg/ml o/w emulsion B is shown for comparison.

As shown in Fig. 4, the mean number of scratches using the modified method over the first 5 and 10 min demonstrated good correlation with the concentration of injected CL solution ($r^2 \geq 0.980$). The pain response with 5 mg/ml test emulsion was found to be comparable to 1.6–2.7 mg/ml CL solution for the 5 and 10 min test periods, respectively, suggesting that the emulsion formulation may be 2–3-fold less painful than the equivalent concentration of CL. Fig. 5 displays the effect of CL solution concentration on the onset time of scratches. It should be noted that at CL concentrations $\geq 2.5$ mg/ml, the onset time for scratches appeared to level off. Interestingly, when 5 mg/ml clarithromycin o/w emulsion was administered, the onset time for scratches approximated 3 min and this value was found to represent CL concentration equivalent

Table 2
Results of rat paw lick test

| Formulation tested | Number of animals licking | Average number of times each rat licked | Average total licking time (s) |
|---|---|---|---|
| 5% dextrose | 10% (1/10) | 1 | 2 |
| Clarithromycin lactobionate in D5W | 100% (10/10) | 16 | 115.1 |
| o/w emulsion B | 70% (7/10) | 7 | 54.4 |

to about 1.3 mg/ml. Once again, these results suggest that the emulsion may be less painful than the comparable concentration of CL.

*3.2.2. Rat paw lick test*

In order to gain better appreciation of the level of pain reduction due to drug delivery as an emulsion, an alternative animal model called rat paw lick was investigated. Once again, this model is traditionally used to compare pain response with formulations intended to be administered s.c. or i.m. The basis of this test is that the more painful the formulation, the more the number of paw licks per animal. In addition, the number of times an animal licks the paw also increases with pain and/or irritation (Celozzi et al., 1980).

Table 2 summarizes the data from rat paw lick study. When D5W was injected, only one out of the 10 animals in that group was found to lick the injected paw for about 2 s. When 5 mg/ml CL solution was injected, all the animals licked their paw. Each rat licked its paw about 16 times which corresponded to a total licking time of about 115 s. However, when 5 mg/ml clarithromycin o/w emulsion was injected, only 70% animals licked their paw and the number of times the rats licked their paw was reduced to 7. This in turn equated to a total licking time of about 54 s. Although linear response between drug concentration and lick response parameters was not established, the test suggests the possibility that the emulsion may be only 50% as painful as a 5 mg/ml CL solution.

*3.2.3. Rabbit ear vein irritation test*

The rabbit ear vein irritation test was used to evaluate the irritation of drug formulations on i.v. injection and the observations compared with D5W and CL solution. While having the advantage of being an i.v. injection, it models erythema, discoloration and other visible damage to the vein used for injection, rather than pain upon injection.

The area around the rabbit ear vein in the group receiving D5W remained normal over the 24 h study period. With 5 mg/ml CL solution, the ears of all three animals were slightly flushed with blood during the dose administration. At 1–4 h after dosing, slight bruises or red discoloration of the site of injection were observed. At 24 h after dosing, all the three animals had slight bruises around the vein at the site of injection. With 5 mg/ml clarithromycin o/w emulsion, one rabbit demonstrated blue discoloration of the vein during dosing while the other two animals remained normal. Between 1 and 4 h, and at 24 h after dosing, slight bruises were observed in two rabbits at the site of injection and one rabbit remained normal throughout the study period. No quantitation of the level of reduction of pain or irritation could be made. Nonetheless, based on the observations, the emulsion formulation was found to be less irritating than the CL solution.

*3.2.4. Rat tail vein irritation test*

The emulsion formulation was compared to D5W and CL solution using the rat tail vein irritation test, which like rabbit ear vein irritation test, evaluates potential for erythema and visible damage to the vein rather than pain upon injection. When D5W was infused, in five out of six animals, slight purple, red and pink spots were observed proximate to the injection site, at various observation time points. The effect was rated moderate in one animal. With 5 mg/ml CL solution in D5W, pink, red and purple areas were observed in all the animals at all observation time points. The area of effect ranged from the immediate injection site to about 50–75% of total tail in 67% (4/6) animals. When 5 mg/ml clarithromycin o/w emulsion was infused, small pink, red and purple spots were observed in five of the six animals, mostly near the site of injection. Also, these spots were observed only at about

half the observation time points over 25–75% of the tail. Based on these observations, the emulsion formulation was found to be only as irritating as D5W. The CL solution was ranked as more irritating than D5W and emulsion. Once again, no quantitation of the level of reduction of irritation with the emulsion formulation could be made.

### 3.2.5. *Plasma drug distribution in dogs*

Since the goal of the study was to develop a formulation which reduces pain upon i.v. administration without altering bioavailability, plasma distribution of 5 mg/ml clarithromycin o/w emulsion was compared with CL solution in dogs. The mean drug concentrations over a period of 24 h in the two groups of animals receiving CL

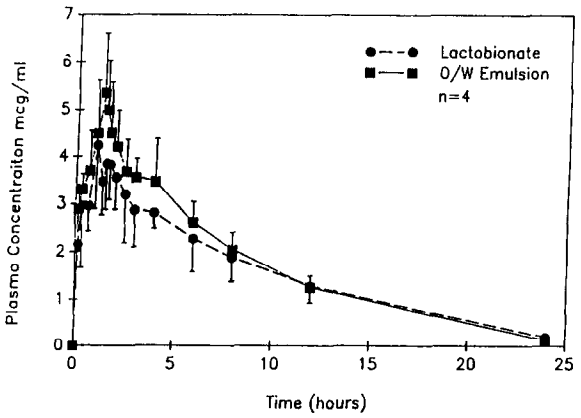


Fig. 6. Plasma drug concentration profile over 24 h following the i.v. infusion of 100 mg drug as a lactobionate solution and o/w emulsion B.

Table 3
Inidividual and mean pharmacokinetic parameters estimated following the infusion of 100 mg clarithromycin as a lactobionate solution and o/w emulsion

| Time (h) | Clarithromycin lactobionate | | | | | o/w emulsion B | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dog 1 | Dog 2 | Dog 3 | Dog 4 | Mean ± SD | Dog 1 | Dog 2 | Dog 3 | Dog 4 | Mean ± SD |
| $T_{max}$ (h) | 1.67 | 2.0 | 1.0 | 1.0 | 1.42 ± 0.50 [a] | 1.0 | 1.33 | 1.33 | 1.33 | 1.25 ± 0.17 [a] |
| $C_{max}$ ($\mu$g/ml) | 3.57 | 3.44 | 6.12 | 4.27 | 4.35 ± 1.24 [b] | 5.52 | 3.87 | 6.86 | 5.73 | 5.49 ± 1.23 [b] |
| $AUC_{0-24}$ ($\mu$g h ml$^{-1}$) | 41 | 35 | 43 | 30 | 37.3 ± 5.9 [c] | 47 | 32 | 43 | 42 | 41.0 ± 6.4 [c] |

Results of statistical comparison using one-way analysis of variance:
[a] $F(1,6) = 0.416$ ($p = 0.5493$); [b] $F(1,6) = 1.710$ ($p = 0.2388$); [c] $F(1,6) = 0.744$ ($p = 0.4303$).

Table 4
Results of toxicity studies

| Lot no. | Formulation composition [a] | Species tested [b] | $LD_{50}$ (95% confidence limits) |
|---|---|---|---|
| A | clarithromycin lactobionate aqueous solution | mouse | 141 mg/kg (130–159 mg/kg) |
| | | rat | 70.30 mg/kg (54.74–79.81 mg/kg) |
| B | placebo emulsion with 1.5% oleic acid | mouse | 15.55 ml/kg (13.5–15.9 ml/kg) |
| C | placebo emulsion with 3% oleic acid | mouse | 6.45 ml/kg (6.22–6.72 ml/kg) |
| D | placebo emulsion with 1.5% oleic acid, 2.5% glycerin | mouse | 15.29 ml/kg (13.63–19.88 ml/kg) |
| | | rat | 12.38 ml/kg (–) [c] |
| E | emulsion with 1% drug, 1.5% oleic acid, 2.5% glycerin | mouse | 96.8 mg/kg (87.9–103.1 mg/kg) |
| | | rat | 45.4 mg/kg (31.4–59.3 mg/kg) |
| F | emulsion with 0.5% drug, 0.6% oleic acid, | mouse | 141.4 mg/kg (122.3–169.3 mg/kg) |
| | 0.3% hexanoic acid, 2.5% glycerin | rat | 113.5 mg/kg (102.1–128.5 mg/kg) |
| G | placebo emulsion of lot no. F | mouse | > 50 ml/kg |
| | | rat | > 50 ml/kg |

[a] All percentages are expressed in terms of w/v.
[b] Male rats and mice were used in all studies.
[c] Not determined.

solution and clarithromycin o/w emulsion are displayed in Fig. 6. The drug concentrations within each group indicated a coefficient of variation of about 10–30%, and the two groups did not demonstrate statistically significant difference in their mean drug concentrations. The selected pharmacokinetic parameters estimated based on this data is summarized in Table 3. Both systems resulted in maximum drug concentration of about 4–5 $\mu$g/ml at 1.25 h after infusion, and the AUC over 24 h was about 38–41 $\mu$g h ml$^{-1}$. The statistical analysis of these parameter values did not reveal any significant difference, thereby suggesting comparable drug availability from CL solution and o/w emulsion in dogs.

*3.2.6. Acute toxicity studies*

The effect of oleic acid content of emulsion on its acute toxicity in mice was assessed during the early phase of this study. At oleic acid concentrations $\geq 3\%$ w/v, the emulsion demonstrated $LD_{50} \leq 7$ ml/kg. However, the reduction in the oleic acid concentrations to $< 1.0\%$ w/v appreciably decreased the toxicity of emulsion. A detailed summary of the toxicity studies with different emulsion formulations is presented in Table 4. Wherever possible, rats as well as mice were used and results expressed along with 95% confidence intervals. The $LD_{50}$ values of the preparations containing $< 1\%$ w/v total lipophilic counterions (e.g., see formulation F in Table 4) was generally found to exceed 20 ml emulsion per kg for both rats and mice, corresponding to $> 100$ mg/kg drug based on 5 mg/ml drug concentration. This value is comparable to that observed with CL solution (see Table 4). Hence, the data revealed the clarithromycin o/w emulsion to be no more toxic than CL solution.

*3.2.7. Efficacy studies*

The efficacy of clarithromycin o/w emulsion (0.5% w/v drug, 2% w/v soybean oil, 5% w/v egg phosphatide, 0.6% w/v oleic acid, 0.3% hexanoic acid and 2.5% w/v glycerin in water) expressing optimal biological properties was determined in mice infected with *S. aureus*, *S. pyogenes* and *Strep. pneumoniae* and the results compared either with aqueous CL solution or clarithromycin base suspension. Oral, s.c. as well as i.v. routes of administration were pursued in these studies and the results are summarized in Table 5. When administered i.v. in animals infected with *S. pyogenes*, the $ED_{50}$ (i.e., effective dose to protect 50% animals from death) with the emulsion formulation was found to compare closely with that of CL solution. In animals infected with *S. aureus*, the $ED_{50}$ with emulsion was found to be higher than that obtained with CL solution,

Table 5
Results of efficacy studies in infected CF-1 mice

| Formulation type | Route [a] | $ED_{50}$ (mg/kg per day) | | |
|---|---|---|---|---|
| | | *Staphylococcus aureus* | *Staphylococcus pyogenes* | *Streptococcus pneumoniae* |
| Clarithromycin lactobionate | i.v. | 12.3 (5.2–28.9) [c] | 0.9 (0.6–1.3) | – [b] |
| Clarithromycin o/w emulsion B | i.v. | 23.2 (15.4–35.1) | 0.9 (0.4–1.9) | – [b] |
| Clarithromycin base suspension | s.c. | 10.0 (7.8–12.8) | – [b] | 2.0 (1.3– 3.2) |
| Clarithromycin o/w emulsion B | s.c. | 9.2 (6.8–12.4) | – [b] | 2.3 (1.4–3.6) |
| Clarithromycin base suspension | oral | 30.2 (20.9–43.7) | – [b] | 13.2 (8.4–20.7) |
| Clarithromycin o/w emulsion B | oral | 28.4 (23.8–41.2) | – [b] | 11.7 (7.3–18.8) |

[a] i.v., intravenous; s.c., subcutaneous.
[b] Not determined.
[c] Value in parentheses refers to 95% confidence limits.

but this difference was not statistically significant suggesting that formulation of drug into o/w emulsion does not alter its potency. Good agreement in the $ED_{50}$ values was also obtained following the oral and s.c. administration of clarithromycin emulsion and clarithromycin base aqueous suspension in mice infected with *S. aureus* or *Strep. pneumoniae*. These data suggested that the processing and administration of clarithromycin as an o/w emulsion do not alter its efficacy.

## 4. Conclusions

This report has summarized efforts towards development of a potential less-painful i.v. formulation for clarithromycin. The solubilization of drug in oil with the aid of lipophilic counterions allowed development of a formulation with characteristics similar to those of a fat emulsion, e.g., Liposyn®. If this formulation were to be developed as a commercial product, it could be sterilized by filtration through a 0.2 $\mu$m filter (Lidgate et al., 1992). Animal models were used to assess the potential of emulsion formulation in reducing pain, and these tests suggest the possibility of 2–3-fold pain reduction with the developed o/w emulsion. Limited clinical trials are warranted to reveal the real potential of this formulation in terms of reducing pain upon i.v. administration. Correlation between the data obtained from the human studies and animal models already investigated should enable further optimization of the formulation, if necessary.

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