**In Vitro/in Vivo** Characterization of a Tramadol HCl Depot System Composed of Monoolein and Water

Hugues MALONNE,*a,b Jeanine FONTAINE,*b and André MOEs*a

Laboratoire de Pharmacie Galénique et de Biopharmacie,*a Campus Plaine CP 207 and Laboratoire de Physiologie et de Pharmacologie Fondamentales,*b Campus Plaine CP 205–7, Institut de Pharmacie, Université Libre de Bruxelles, 1050 Bruxelles, Belgium. Received October 14, 1999; Accepted January 26, 2000

Various monoolein–water systems containing tramadol HCl, a potent analgesic, were formulated to obtain sustained-release dosage forms which could be administered by subcutaneous, intramuscular or intrathecal injections. They were examined for their *in vitro* drug-release profiles and *in vivo* analgesic properties in rats in a 14 h period following intramuscular administration. In order to obtain a lower viscosity, we have substituted a part of monoolein by oleic acid and phospholipids. Both binary (monoolein–water) and quaternary (oleic acid–phospholipid–monoolein–water) formulations exhibited controlled drug-release profiles which were accelerated by surfactant adjunction. This surfactant action was probably due to structural changes in the lipid arrangement and was much more pronounced for the modified formulations. According to the results obtained *in vitro*, formulations with slower drug release (*i.e.*, the native formulation and the modified one without surfactant) were selected for assessment of their *in vivo* properties. Both formulations demonstrated prolonged analgesic activities in the rat tail flick test manifested by stable pain relief during more than 10 h compared with the 3 to 4 h analgesia obtained with the commercially available tramadol HCl solution. The sustained-release capabilities were evaluated by using a modified half value duration (HVD) ratio and all sustained-released formulations exhibited a HVD ratio equal or superior to 3.9.

**Key words** injectable sustained-release; tramadol HCl; lyotropic phase; rat tail flick test; polar lipid; monoolein

Tramadol HCl, (1RS,2RS)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)-cyclohexanol HCl, is a small molecule freely soluble in water and methanol (1 g/ml), slightly soluble in acetone and practically insoluble in petrol ether. It’s exact molecular weight and pKa values are 263.38 and 8.3, respectively. It displays weak affinity for the µ- and δ-opioid receptors and an even weaker affinity for the κ subtype, although its unique active metabolite, O-desmethyl tramadol (M1), shows a slightly greater affinity for the µ-opioid subtype. However, these relatively weak affinities are not sufficient to explain its analgesic action which has been also attributed to an effect on the descending inhibitory pathways and interference with the neuronal release and reuptake of serotonin (5HT) and noradrenaline (NA).2,3 Interestingly, tramadol is much more rapidly metabolised in animals than in humans with an approximate apparent elimination half-life of 3 h in rat compared to 6 h in human.4 The pharmacokinetics and safety profiles of this drug permit slow-release formulations and some of them, for oral administration, have already been marketed in various countries.7

In the present study, we focused our research on a depot system for subcutaneous, intramuscular or intrathecal administration. Monoolein (glyceryl monooleate), a common food additive, belongs to the water-insoluble amphiphilic lipids which form different lyotropic liquid crystals in the presence of water.6 Monoolein/water mixtures spontaneously form a transparent and stiff gel-like structure called cubic (Q) phase when placed in aqueous medium.7 The water uptake of monoolein follows second-order swelling kinetics and can rapidly reach as much as 50% w/w content.6 Since the cubic phase is composed of 50–60% of monoolein and 40–50% water and since its bilayer structure gives rise to a large interfacial area (around 400 m² per gram of cubic phase),9 one can expect large flexibility in the system which could therefore promote the incorporation of both hydrophilic and hydrophobic drugs. In fact, various works have been published on the incorporation (dissolution or dispersion) of drugs such as lidocaine,10 clonazepam,9 vitamin E polyethylene glycol succinate, hydroxychloroquine sulfate, diclofenac sodium and acetylsalicilic acid.11 These experiments have demonstrated the weak influence of the pH variation and composition (pancreatic enzymes and peptic) of the gastrointestinal fluids on drug-released profiles. They have also showed the possible incorporation of drug crystals or large molecular weight drugs. The drug release from the classical formulation (monoolein–water system) generally follows a square root of time dependence8,11 according to the diffusion matrix theory.12,13 The partial substitution of monoolein by polar lipids has shown slower drug release profiles probably by modifying the lipid aggregate organization. If we now consider the *in vivo* degradation of the system, the biodegradation of monoolein to glycerol and oleic acid by lipases has already been demonstrated. This reaction is completed in a few days since the cubic phase possesses a very large interface and since lipolysis needs a large oil/water interface to take place efficiently.4

The aim of the present work was to formulate a monoolein–water system which could be handled and injected at room temperature. In order to obtain this kind of formulation and also to modify the drug-release profile, we have incorporated oleic acid, propylene glycol, phospholipids or surfactants to the classical binary mixture.

**MATERIALS AND METHODS**

**Chemicals** Monoolein (Myverol 18–99®) was manufactured and kindly provided by Eastman Chemicals, Kingsport, USA. Its exact monoglyceride composition is shown in Table 1. Pluronic F68® was a gift of BASF, Parsippany, U.S.A. Oleic acid of analytical grade was provided by

---

* To whom correspondence should be addressed. e-mail: hmalonne@ulb.ac.be

© 2000 Pharmaceutical Society of Japan
UCB (Braine-l’Alleud, Belgium), propylene glycol by Federa (Brussels, Belgium) and Phospholipon 90® by Nattermann Phospholipid GMBH (Köln, Germany). Tramadol HCl and its commercial injectable solution (immediate release) was obtained from Therabel Research (Brussels, Belgium). The water used was double-distilled and pyrogen-free.

**Animals** Male Wistar rats (Iffa-Credo, Paris, France), 150—200 g were used. They were housed under suitable conditions of temperature (21 °C), relative humidity (60%) and light (12 h) throughout the experiments. They had free access to food and water.

**Sample Preparation** Components were melted at 42 °C and vigorously mixed. Tramadol HCl was dissolved in water prior to the preparation of the various gels. An opaque and uniform paste was obtained under stirring. Monoglycerides were thickened to a viscous gel in contact with water. To avoid water evaporation, samples were prepared in vials which after being sealed were left standing until equilibrium was reached (a few days). In order to lower the viscosity obtained with the classical mixture (monoolein–water–drug), we substituted partly the monoolein by oleic acid and Phospholipon 90 PG®, a commercial solution of phosphatidylcholine in propylene glycol (50% w/w) in various proportions. The exact compositions of each formulation used are listed in Table 2. To evaluate the effect of surfactant on the drug-release profile of the classical and modified formulations, we have incorporated Pluronic F68®, a copolymer formed by a central hydrophobic nucleus (polyoxypropylene) surrounded by hydrophilic sequences (polyoxethylene), to the A and B formulations (Table 3).

**Drug Release Studies** The release of tramadol HCl from the various formulations was carried out by using the USP XXIII n7 (paddle) apparatus with a stirring rate of 60 rpm. The dissolution medium was a 0.05 M phosphate buffer warmed at 37 ± 0.1 °C. The volume and pH of the dissolution medium were 500 ml and 7.4, respectively. A weighed quantity of mixture (containing 100 mg tramadol HCl) was poured into gelatin capsules. The capsules were introduced into metal serpentine and launched in the dissolution medium which was withdrawn continuously from the vessels with a Gilson minipuls 2 peristaltic pump (Villiers-Le-Bel, France), passed through a filter (Prolabo No. 07179504, 10 μm porosity, Vel, Brussels, Belgium), then through a multi-cell Philips 8620 Series spectrophotometer (Philips Analytical, Cambridge, U.K.) and assayed at 271 nm. All of these operations were controlled by the Philips PUB 620 Tablet Dissolution System.

**In Vivo Experiments** The analgesic activity of our formulations was assessed by means of the Tail Flick Analgesia Meter type 33 from IITC Life Science Instruments (Woodland Hills, CA, U.S.A). The tail flick test was evoked by placing the rat’s tail over a slit on which a quartz projection bulb was focused. When the lamp was turned on, the timer was simultaneously started. When the rat felt pain and flicked its tail, the light fell on the photocell and automatically stopped the light and the timer. The light beam intensity was adjusted to yield a basal response between 5 and 6 s. However, some animals did not respond in this time range and were excluded from the experiments. According to D’Amour and Smith, 15 who first described the method, an adapted cut-off time of 20 s was used to avoid tissue damage. Tail flick latencies to thermal stimulation were determined before and at various times after a single intramuscular injection in the left hind limb. The injected volumes were around 0.1 ml depending on the formulation and the exact animal weight. The experiment was stopped when pain thresholds returned to control (pre-administration) values. A time–response curve was constructed for each rat. Administered doses were selected to obtain latency times under the cut-off time (20 s) throughout the experiment and by this means, avoid any undesirable ceiling effects.

**Data and Statistical Analysis** All groups were composed of six animals, the tail flick response latencies were recorded and converted to percentage of the maximal possible effect (MPE) 16 following the equation:

\[
\% MPE = \frac{\text{Post drug latency} - \text{pre drug latency}}{\text{Cut off time} - \text{pre drug latency}} \times 100
\]

Table 1. Myverol 18-90® Monoglyceride Composition as Provided by the Manufacturer

<table>
<thead>
<tr>
<th>Fatty acid distribution (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Glyceryl monoleate</td>
<td>C18:1 60.9%</td>
</tr>
<tr>
<td>Glyceryl monooleate</td>
<td>C18:2 21.0%</td>
</tr>
<tr>
<td>Glyceryl monolinooleate</td>
<td>C18:3 8.8%</td>
</tr>
<tr>
<td>Glyceryl monopalmitate</td>
<td>C16:0 4.1%</td>
</tr>
<tr>
<td>Glyceryl monostearate</td>
<td>C18:0 1.8%</td>
</tr>
<tr>
<td>Glyceryl monogadoleate</td>
<td>C20:1 1.0%</td>
</tr>
</tbody>
</table>

Table 2. Composition of the Slow Release Formulations

<table>
<thead>
<tr>
<th>System</th>
<th>Composition (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Myverol/water/tramadol</td>
</tr>
<tr>
<td>B</td>
<td>Myverol/oleic acid/water/phospholipon/tramadol</td>
</tr>
<tr>
<td>C</td>
<td>Myverol/oleic acid/water/phospholipon/tramadol</td>
</tr>
</tbody>
</table>

Table 3. Formulations Modified by Surfactant Adjunction

<table>
<thead>
<tr>
<th>System</th>
<th>Composition (% w/w)</th>
<th>Pluronic F68®</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>Myverol/water/tramadol 72/14/14</td>
<td>+0.4%(w/w)</td>
</tr>
<tr>
<td>A2</td>
<td>Myverol/water/tramadol 72/14/14</td>
<td>+0.8%(w/w)</td>
</tr>
<tr>
<td>B1</td>
<td>Myverol/oleic acid/water/phospholipon/tramadol 25/25/16.7/16.7/16.7</td>
<td>+0.4%(w/w)</td>
</tr>
<tr>
<td>B2</td>
<td>Myverol/oleic acid/water/phospholipon/tramadol 25/25/16.7/16.7/16.7</td>
<td>+0.8%(w/w)</td>
</tr>
</tbody>
</table>
Fig. 1. *In Vitro* Drug Release from Classical (A) and Modified (B,C) Monoolein–Water Systems
Each point represents the mean of 3 experiments realized in duplicate.

Fig. 2. Surfactant Incorporation Effect on *in Vitro* Drug Release from the Classical Monoolein–Water System
Each point represents the mean of 3 experiments realized in duplicate.

All data are presented as means±S.E.M. The parametric ANOVA and Student’s *t*-test were used in the comparison between groups and the criterion for significance was *p*<0.05.

RESULTS AND DISCUSSION

**Sample Preparation and *in Vitro* Drug Release Kinetics**

The aim of our investigation was to evaluate *in vitro* and *in vivo* formulations which can act as depot systems. They should therefore be biocompatible and biodegradable. Furthermore, they should display low viscosity at room temperature in order to be syringable. Once injected, they should also undergo a rapid lamellar-cubic phase transition to insure that a slow release of the drug could be achieved. Myverol 18—99° a commercially available source of monoolein was chosen for our experiments. When a small amount of water is added to monoolein (around 5% w/w), relatively low viscosity reverse micellar structures are formed but further addition of water generally leads to lamellar-cubic phase transition characterised by a rapid increase in viscosity. However, the incorporation of the highly water soluble drug, tramadol HCl, in the same proportion of water (1 g/1 g) permitted the formation of mixtures with a water content as high as 15% w/w which conserved relatively low viscosity and allowed their expression through a 25G×5/8" needle used for the *in vivo* administration. The *in vitro* release of Tramadol from the classical formulation (A) and the modified ones (B and C) is shown in Fig. 1, and the surfactant’s effect on drug re-
lease is also shown in Figs. 2 and 3. Pluronic F68® proportionally accelerated the release in both formulation types (A and B) but showed a more pronounced effect on the modified ones (B). The partial substitution of myerol 18–99® by oleic acid in formulation B demonstrated a slower drug release but further substitution (formulation C) resulted in a drug release profile close to that of the native formulation (A). These changes could probably be attributed to structural changes in the arrangement of lipids (Fig. 1).

**In Vivo Activity** To assess their analgesic activities, formulations A, B and C were administered intramuscularly in rats and tail flick responses to radial heat were recorded over a 14 h period. Activity was compared to the analgesia obtained by intramuscular injection of the commercially available aqueous solution of tramadol HCl (immediate release). Three control groups received one of the unloaded vehicles (i.e. formulations A, B and C without drug) and no significant analgesic activities were registered (data not shown). A first series of experiments comparing the aqueous solution (60 mg/kg) with formulations A and B (120 mg/kg) is illustrated in Fig. 4. The commercial solution exhibited a rapid onset of analgesia (15 min after administration) and reached a maximum of 85% MPE after 90 min but also demonstrated a rapid decrease in analgesia characterised by a return to control values (pre drug latency) within 4 h. Conversely, the sustained-release formulations demonstrated a slightly less rapid onset of action (30 to 45 min) but a much more pronounced duration. A stable plateau of approximately 50% MPE was
obtained with both formulations and was maintained during at least 10 h. Of note, the slight difference in drug-release kinetics between formulation A and B obtained in the in vitro experiments were not distinguishable in vivo. A second series of experiments were designed to compare the second modified formulation (C) with the native one (A) at a 140 mg/kg dose (see Fig. 5). The slightly higher dose showed the same onset of action but a longer duration in analgesia (12 h). As in the first set of experiments, no significant difference could be detected between sustained-release formulations. A more accurate way to compare the slow release formulations to the aqueous solution is to evaluate the half value duration (HVD) of each formulation and to calculate the HVD ratio (RHVD) obtained from the time–response curves. 17 We adapted these parameters to our results as follows: HVD was calculated for each formulation as the time during which the analgesia level (the plasma level for classical HVD) is superior or equal to the half value of the analgesia peak (the plasma peak concentration for classical HVD). The RHVD was obtained by dividing the HVD of the sustained-release formulation by the HVD of the aqueous solution. Calculated HVD and RHVD values are listed in Table 4.

In conclusion, our formulations demonstrated a RHVD highly superior to 2 which is the general criterion to accept a sustained-release dosage form. 17 These formulations should therefore be considered as interesting depot systems for parenteral administration able to maintain stable pain relief during more than 10 h.

Acknowledgement We gratefully acknowledge Therabel Research for their financial support and the gift of tramadol HCl.

REFERENCES