Proceeding Paper

Anti-Inflammatory Drug Repurposing for Intranasal Delivery: Ketoprofen Nanoemulgel Development for the Treatment of Glioma [†]

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Abstract: The anti-inflammatory drug ketoprofen has shown promising results in the field of drug repurposing for the treatment of brain cancer, but currently developed formulations are for invasive administration (intravenous) and have very limited drug strength. Hence, the purpose of this work was to develop an intranasal oil-in-water nanoemulgel, with drug strength maximization, for non-invasive, more effective and safer treatment of glioma. The developed formulations were made of Capryol® 90 (hydrophobic surfactant), Tween® 80 (hydrophilic surfactant), Transcutol® (co-solvent and permeation enhancer), Pluronic® F-127 (surfactant and gelling agent) and ketoprofen. Droplet size, polydispersity index, in vitro drug release and accelerated stability were measured. Results showed that the addition of Pluronic to a preliminary optimized nanoemulsion led to a significant droplet size and PDI reduction (176 to 22 nm, and 0.3 to 0.1, respectively). Achieved drug strength was 4 mg/mL, which is more than 50 times higher than ketoprofen's aqueous solubility. The developed formulations also appeared to have high stability, with instability indexes between 0.130 and 0.265, and high cumulative drug release percentage, varying between 78 to 93% after 24 h. Formulations also showed a controlled release profile, fitting a Korsmeyer-Peppas kinetic model, with low AIC (43.84 to 54.67) and high R^2 (0.9725 to 0.9971) values, depicting non-Fickian diffusion (n between 0.7 and 0.8). Hence, high drug strength, high stability and high drug release ketoprofen-loaded nanoemulgels were successfully prepared. Future in vitro cytotoxicity evaluation in glioma cells will assess the true potential of the developed formulations for the treatment of brain cancer.

Keywords: brain cancer; drug repurposing; glioma; intranasal; ketoprofen; nanoemulsion; nanoemulgel

1. Introduction

A new drug molecule takes more than 10 years and costs more than 1 billion euros to reach the pharmaceutical market. On the other hand, drug repurposing, which is the redirecting of marketed drugs for therapeutical purposes different from the ones they were first approved for, takes about half of that time and also costs about half, making it a much less time-consuming and expensive strategy. Moreover, the fact that the repurposed drug molecule's characteristics are already known (chemical properties, biodistribution, safety) can be a significant advantage in all stages of pharmaceutical product development. Due to the high potential of this approach, drug repurposing is being

performed for several diseases, such as neurological diseases (Alzheimer's disease, Parkinson's disease), infectious diseases (bacterial, fungal or viral infections), and oncologic diseases, including colorectal, prostate, ovarian, breast, lung, pancreatic, gastric, skin and brain cancer. Specifically, for cancer treatment, drug repurposing could be the way to find less expensive treatments, that also have less side effects [1–5].

Ketoprofen, an anti-inflammatory and analgesic drug, has shown promising results in the field of drug repurposing for the treatment of brain cancer, namely glioma [6,7]. Although the exact mechanisms by which it has antitumor effect in brain cancer have yet to be elucidated, it is thought to exhibit its anticancer properties due to cyclooxygenase inhibition, caspase activation and cell cycle arrest, which will ultimately lead to cancerous cell death [8–12].

Nevertheless, formulations that have already been developed are for invasive administration only (intravenous), and have very limited drug strength [6,7]. Hence, the purpose of this work was to develop an intranasal oil-in-water nanoemulgel, with drug strength maximization, for non-invasive, more effective and safer treatment of glioma.

2. Materials and Methods

Ketoprofen, Tween® 80 (polysorbate 80) and Pluronic® F-127 (poloxamer 407) were purchased from Sigma-Aldrich (Steinheim, Germany). Capryol® 90 (propylene glycol monocaprylate, type II) and Transcutol® (diethylene glycol monoethyl ether) were gift samples from Gattefossé (Lyon, France).

The preliminary oil-in-water nanoemulsions were prepared by spontaneous emulsification. The first step was adding and mixing different ratios of Capryol 90 (oil and hydrophobic surfactant), Tween 80 (hydrophilic surfactant), and Transcutol (co-solvent, cosurfactant and permeation enhancer), with ratios varying from 1 to 6 parts Capryol, 1 to 6 parts Tween, and 3 to 5 parts Transcutol, to make a preconcentrate. After this, the drug was solubilized in the prepared preconcentrate, and only after was the water added and mixed, giving origin to preliminary nanoemulsions. Lead nanoemulsions were transformed into nanoemulgels by adding a Pluronic® F-127 (surfactant and gelling agent) aqueous solution to the preconcentrate, instead of water.

Both preliminary nanoemulsions and final nanoemulgels were characterized, for droplet size and polydispersity index (PDI), zeta potential, in vitro drug release and accelerated stability. Droplet size and PDI were measured by dynamic light scattering, and zeta potential was measured by electrophoretic light scattering, using a Zetasizer Nano ZS apparatus (Malvern, United Kingdom).

In vitro drug release assays were performed in Franz diffusion cells (PermeGear, Pennsylvania, United States of America), using synthetic membranes (cellulose dialysis tubing membranes, Sigma-Aldrich, Steinheim, Germany), with temperature being kept at mean nasal temperature (35 °C). The receptor compartment was filled with 5 mL of nasal simulant fluid pH 6.5 (monobasic sodium phosphate 7 mM, dibasic sodium phosphate 3 mM, potassium chloride 30 mM, sodium chloride 107 mM, calcium chloride 1.5 mM, magnesium chloride 0.75 mM, and sodium hydrogen carbonate 5 mM), and 200 μL of the test formulations were placed on the donor compartment, on the upper side of the membrane. The experiments were performed under constant stirring, and 300 μL samples were collected at predetermined times (5, 10, 20, 40, 60, 120, 240, 360 and 1440 min), followed by volume replacing with new buffer solution. Drug sample quantification was done by absorbance reading at 300 nm, in 96-wells polystyrene plates (flat bottom, Orange Scientific, Braine-l'Alleud, Belgium), using a microplate reader (xMark spectrophotometer, Bio-Rad, Japan).

Formulation accelerated stability was assessed by photocentrifugation (LUMiSizer, LUM GmbH, Berlin, Germany), with transmission profiles being at a wavelength of 865 nm (CCD-line detector), recorded in 5 s intervals, for a total of 1000 profiles, in capped disposable polycarbonate cells, at 4000 rpm. The formulations' instability index was determined by the equipment software (LUMiSizer SEPView®, Berlin, Germany), and an

adimensional number between 0 and 1 was attributed to each preparation, with 0 meaning no formulation physical destabilization, and 1 meaning complete phase separation.

3. Results and Discussion

In order to maximize drug strength in the final formulation, preliminary preformulation studies focused on nanoemulsions with a 1:1 preconcentrate-to-water ratio. In these emulsions, after varying the Capryol-to-Tween ratio (and maintaining Transcutol at high amounts in the preconcentrate, namely 30, 40 and 50%, for increased drug solubilization), it was evident that a lower ratio led to smaller droplet size and lower PDI values (Figure 1A,B). These results were expected, since over the years many studies have proven that a higher hydrophilic surfactant concentration will lead to smaller and more homogeneous nanosized droplets, due to more decreased interfacial tension [13–15].
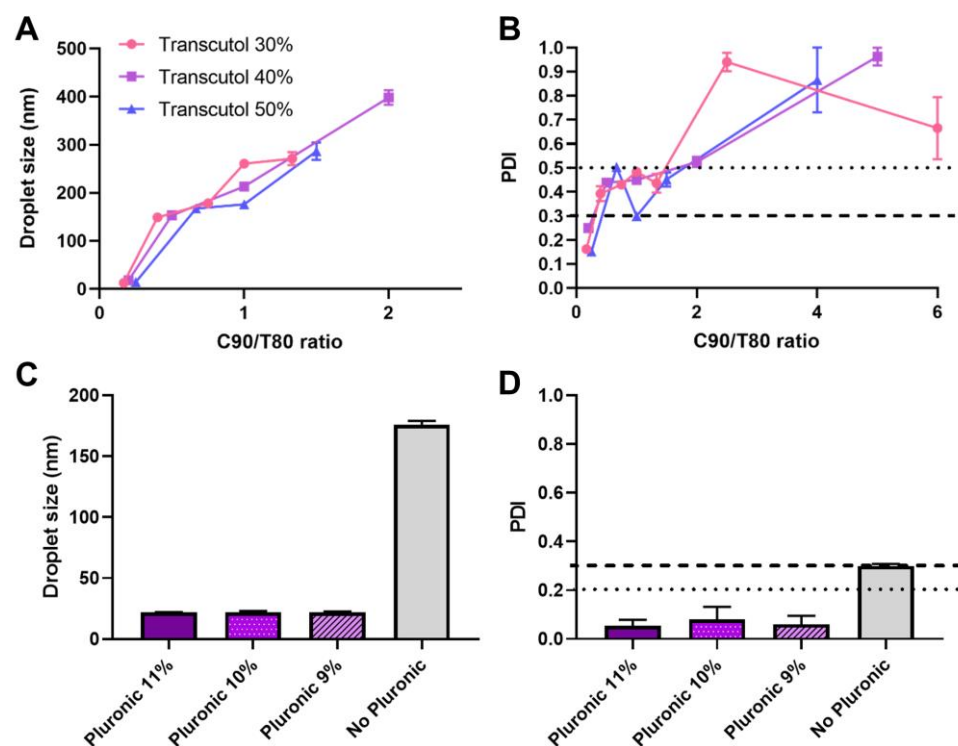

Figure 1. A and B–Droplet size (**A**) and PDI (**B**) variation with different Capryol 90 (C90) to Tween 80 (T80) ratios, at 30, 40 and 50% Transcutol (percentage in emulsion preconcentrate); C and D– Effect of Pluronic addition, at different concentrations, to a preliminary nanoemulsion, in droplet size (**C**) and PDI (**D**) values.

Additionally, Pluronic addition to the external phase of a selected preliminary nanoemulsion, at various concentrations, led to a significant droplet size and PDI reduction (Figure 1C,D), from around 176 to 22 nm, and from around 0.3 to 0.1, respectively. This was due to the fact that, aside from being a thermosensitive gelling polymer under the right circumstances, Pluronic F-127 is also a potent surfactant, which led to a further drop in interfacial tension.

Nevertheless, even when attempting to increase Pluronic F-127 concentration in the formulations up to levels where is in known to gellify around room temperature [16], we were not able to produce true nanoemulgels (fluid consistency even under heating up to 40 °C). Thereby, the amount of preconcentrate in the final formulation was decreased, and the amount of Pluronic solution was increased, until nanoemulgels that had good droplet size and PDI, and were gels at mean nasal temperature (35 °C) and fluid under refrigeration (4 °C), were obtained. After several attempts, it was not possible to make formulations

that were fluid under refrigeration and at room temperature (20 or 25 °C), and gels at mean nasal temperature (35 °C), but it was possible to develop nanoemulgels that were fluid under refrigeration and gels both at room temperature and mean nasal temperature. Hence, it would be possible to administer these formulations immediately after refrigeration, and when reaching the nasal cavity they would form a gel quickly, hence decreasing mucociliary clearance and increasing nasal retention time, giving more time for the drug to be absorbed.

Three final formulations were obtained, with a preconcentrate-to-Pluronic F-127 solution proportion of 1:9 or 2:8, and with Pluronic F-127 either at 8, 10 or 11% (*w/w*%). Drug incorporation into these formulations did not significantly change their characteristics (droplet size and PDI), and final achieved drug strength was 4 mg/mL, which at least more than 50 times higher than ketoprofen's aqueous solubility [17,18]. Zeta potential values were essentially neutral (between −10 and +10 mV) [19,20], which was also expected since all used excipients were also neutral (uncharged).

Additionally, the developed nanoemulgels appeared to have good physical stability, with instability indexes between 0.130 and 0.265 in accelerated stability studies (Figure 2). Drug incorporation into the formulations did not seem to have a significant effect in formulation stability (vehicle vs. drug loaded preparations).
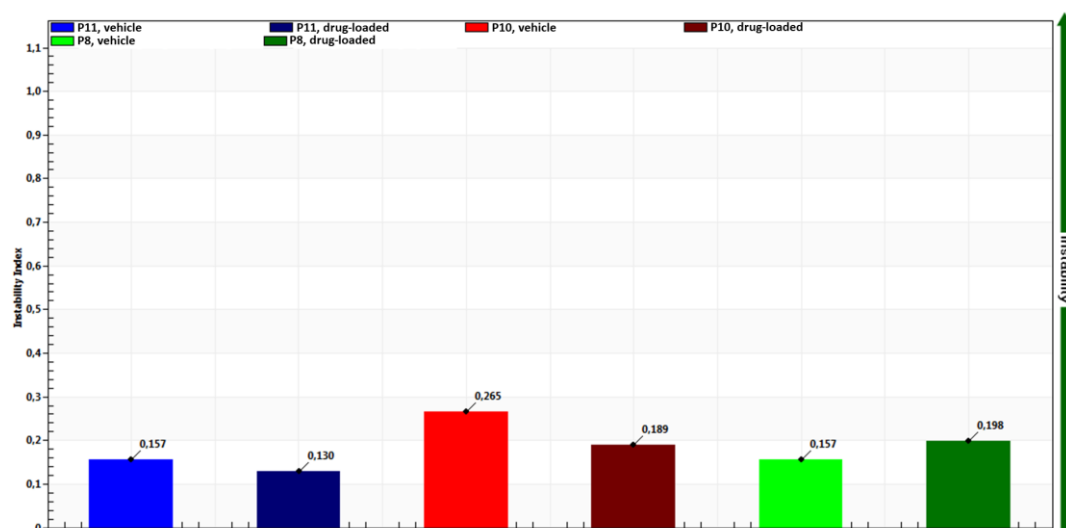

Figure 2. Instability indexes in accelerated stability studies of nanoemulgels with 11 (P11), 10 (P10) or 8% (P8) Pluronic, encapsulating ketoprofen (drug-loaded) or not (vehicle).

The selected formulations were evaluated for in vitro drug release, and all led to high cumulative drug release percentage after 24 h, varying between 78 to 93%, and being comparable to the positive control, a drug solution (Figure 3). Formulations also showed a controlled release profile, fitting a Korsmeyer-Peppas kinetic model, with low AIC (43.84 to 54.67) and high R^2 (0.9725 to 0.9971) values, depicting non-Fickian diffusion (n between 0.7 and 0.8), which was indicative that the drug was released from the developed formulations in a combination of both diffusion from within the oil-phase droplets, and slow erosion of the droplets themselves, which is in accordance with previous studies [21].
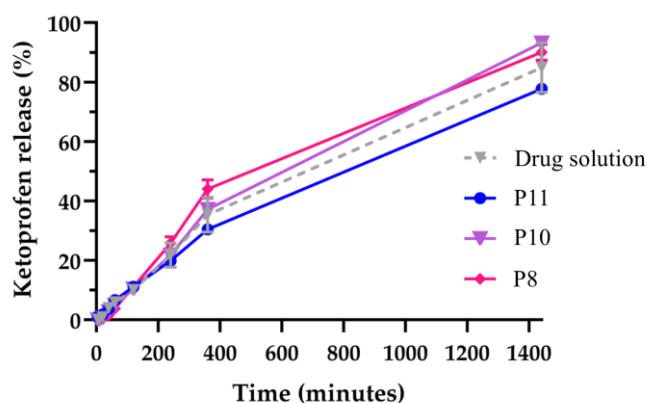
Figure 3. In vitro drug release percentage (cumulative release) of nanoemulgels P11 (11% Pluronic), P10 (10% Pluronic) and P8 (8% Pluronic), containing Capryol 90, Tween 80, Transcutol and ketoprofen, and positive control (drug solution, with ketoprofen dissolved in Transcutol), during 24 h (1440 min).

4. Conclusions and Future Prospects

High drug strength, high stability and high drug release ketoprofen-loaded oil-in-water nanoemulgels were successfully prepared, for intranasal delivery, for the treatment of glioma. Future studies will include the assessment of in vitro cytotoxicity in glioma cells to further assess the potential of these developed formulations as new technological strategies for the treatment of brain cancer.

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