Development of Nutraceutical Emulsions as Risperidone Delivery Systems: Characterization and Toxicological Studies

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ABSTRACT: Emulsions are gaining increasing interest to be applied as drug delivery systems. The main goal of this work was the formulation of an oil/water nutraceutical emulsion (NE) for oral administration, enriched in omega 3 (ω3) and omega 6 (ω6), and able to encapsulate risperidone (RISP), an antipsychotic drug widely used in the treatment of autism spectrum disorders (ASD). RISP has low solubility in aqueous medium and poor bioavailability because of its metabolism and high protein binding. Coadministration of ω3, ω3, and vitamin E complexed with RISP might increase its bioavailability and induce a synergistic effect on the treatment of ASD. Here, we developed an easy and quick method to obtain NEs and then optimized them. The best formulation was chosen after characterization by particle size, defects of the oil-in-water interface, zeta potential (ZP), and *in vitro* drug release. The formulation selected was stable over time, with a particle size of around 3 μm, a ZP lower than −20 mV and controlled drug release. To better understand the biochemical properties of the formulation obtained, we studied *in vitro* toxicity in the Caco-2 cell line. After 4 h of treatment, an increase in cellular metabolism was observed for all RISP concentrations, but emulsions did not change their metabolic rate, except at the highest concentration without drug (25 μg/mL), which showed a significant reduction in metabolism respect to the control. Additionally, locomotor activity and heart rate in zebrafish were measured as parameters of *in vivo* toxicity. Only the highest concentration (0.625 μg/mL) showed a cardiotoxic effect, which corresponds to the decrease in spontaneous movement observed previously. As all the materials contained in the formulations were US FDA approved, the NE selected would be good candidate for clinical trials. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:4142–4152, 2015

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INTRODUCTION

The antipsychotic drug risperidone (RISP), 3-[2-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]ethyl]-6,7,8,9-tetrahydro-2-methyl-4hpyrido[1,2-a]pyrimidin-4-one, which belongs to the chemical class of benzisoxazoles, is one of the most widely used drugs in the treatment of autism spectrum disorders (ASD).[1–6] ASD include different neurodevelopmental disorders that manifest mainly in the earlier years of life, affecting the development of language, communication, and reciprocal social interaction,[7–9] and occur in one out of 110 individuals.[7] RISP has low solubility in aqueous and physiological media and, when orally administered, exhibits low bioavailability because of extensive first-pass metabolism and high protein binding (>90%).[10] Moreover, nontargeted delivery usually results in numerous side effects. As the target site of RISP is the brain, it is necessary not only to develop a strategy to improve drug bioavailability, by avoiding first-pass metabolism, but also to achieve the desired drug concentration at the site of action, and thus reduce undesirable side effects.[4,5]

In the last years, several strategies to overcome these issues, particularly the design of nano- and microstructured drug carrier systems, have been proposed.[1–6] However, these kinds of carriers (plain, ultradeformable, stealth or pH-sensitive liposomes, immunoliposomes, nanoparticles, dendrimers, and emulsions) must be carefully designed and/or chosen because their pharmacokinetics, biodistribution, and tissue selectivity will exclusively depend on the carrier's structure.[4,6,11,12]

Alanazi[9] reported that the use of nutraceuticals in ASD management can generate a successful integrative model with the current treatment that may allow achieving the desired results. Nutraceuticals offer several promising benefits that may include promoting a healthy gut, lowering body burdens of toxins, reducing cytotoxicity, improving antioxidant capacity, enhancing immunomodulatory systems, and minimizing stress and environmental contamination/hazards.[9] The nutraceuticals most used in ASD treatment are: multivitamins and mineral complexes, L-carnitine, and polyunsaturated fatty acids.[9]

Almost all autistic patients suffer from a deficiency of essential fatty acids, especially omega-3 (ω3) and omega-6 (ω6) fatty acids.[13] It has been reported that administration of ω3 fatty acids to autistic children for 6 weeks induces improvements in their behavior (i.e., a decrease in their hyperactivity and stereotyped movements), resulting in an efficient and well-tolerated treatment.[14] Furthermore, administration of a commercial ω3 complex to autistic children for 3 months has shown not only improvements in their behavior, but also enhancements at a biochemical level, with an increase in blood fatty acids.[15] Oils

Abbreviations used: ASD, autism spectrum disorders; bpm, beat per minute; dpf, day postfecundation; HF, hydrophobicity factor; HLB, hydrophilic–lipophilic balance; hpf, hours postfecundation; MC540, merocyanine 540; NE, nutraceutical emulsion; o/w, oil in water; PG, propylene glycol; RISP, risperidone; SL, soy lecithin; Smix, mix of surfactants; ZP, zeta potential; ω3, omega 3; ω6, omega 6

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with the highest ω3 and ω6 concentration (% of total ω) are cod liver (animal source, 21.5% ω3 and 8.7% ω6) and canola (vegetable source, 8.8% ω3 and 21.9% ω6).[16] Besides, it has been reported that children with ASD have increased oxidative stress and/or decreased antioxidant defenses, with decreased plasma levels of vitamin E.[17] In a group of 187 autistic children, a combinational supplementation of vitamin E and ω3 fatty acids produced dramatic improvements in speech, imitation, eye contact, coordination, behavior, and sensory function.[18] In addition, it has been demonstrated that ω3 can inhibit hepatic metabolism, increasing the bioavailability of drugs such as cyclosporin A, saquinavir, and midazolam in rats.[19] All this suggests that coadministration of ω3, ω6, and vitamin E complexed with RISP could inhibit the metabolic reactions catalyzed by hepatic enzymes, thus increasing its bioavailability and inducing a synergistic effect on the treatment of autism.

Although there are commercial formulations of microencapsulated RISP (RISPERDAL® CONSTA®), these are given by injection by a healthcare professional every 2 weeks and do not offer nutraceutical properties. The administration through the gastrointestinal tract is the most popular route for drug delivery[20,21] because of the convenience and patient's compliance, especially for treatment in children with multiple applications per day or long-term treatment.[22] Therefore, our objective was to obtain a microencapsulated RISP formulation suitable for oral administration and rich in nutraceuticals.

Oil-in-water (o/w) emulsions are promising as drug delivery systems, as lipids are known oral drug absorption enhancers.[6,23] Moreover, o/w emulsions retain the advantages of traditional colloidal systems[24–29]: enhanced physical stability, protection of drug molecules from degradation in the body, controlled drug release, specific targeting, biocompatibility, and laboratory-to-commercial scalability. Emulsions have been previously used as drug delivery systems for the following hydrophobic molecules: nalbuphine and its prodrugs,[30] paclitaxel,[25,29] resveratrol and doxorubicin,[29] carbamazepine,[31] cheliensisine A,[32] ibuprofene, ketoprofene, tamoxifen, testosterone, tolbutamide, and cyclosporin A.[33] As mentioned before, RISP has low solubility and exhibits extensive first-pass metabolism and high protein binding (>90%). In order to avoid these undesired facts, a drug delivery system is required and emulsions are ideal for this.

On the basis of all the above-mentioned points, the aim of this work was to obtain a drug delivery system for oral administration based on an o/w nutraceutical emulsion (NE) enriched in ω3 and ω6 fatty acids and able to encapsulate RISP (NE-RISP), to improve its bioavailability and reduce secondary effects. The components selected were: canola oil, cod liver oil, vitamin E, Tween 80, soy lecithin (SL) and propylene glycol (PG). Several mixtures with different oil–surfactant ratios were analyzed. Also, several preparation methods were tested. The formulations selected were characterized by studying particle size, hydrophobicity factor (HF), morphology, zeta potential (ZP), and releasing profile. Finally, toxicity was analyzed *in vitro* in a cell line (Caco-2) and *in vivo* in an animal model (zebrafish) for the finally selected formulation.

The Caco-2 cell line was isolated by Fogh et al.[34] from human epithelial cells of colon adenocarcinoma. This cell line is most often used to test compounds to be orally administered.[20] However, it is known that *in vitro* assays in cells lack the complexity present in organisms, and that this complexity is required to test neurotoxicity, teratogenicity, cardiotoxicity, and the functions of the central nervous system. Thus, in several fields of biomedical research such as drug screening, drug safety, and toxicity tests, zebrafish embryos are a powerful alternative model to test toxicity and teratogenicity[35] as well as to study the cardiotoxicity[36] and hepatotoxicity[37] of drugs.

EXPERIMENTAL

Materials

Cod liver oil, canola oil, vitamin E, Tween 80, and SL were acquired from the local pharmacy and were suited for human intake. PG was from BioPack (Zarate, Buenos Aires, Argentina). Commercial solution of RISP (Risperdal®) was from Janssen-Cilag (Beerse, Belgium). Methanol, diethylamine, and dimethyl sulfoxide (DMSO) were from J. T. Baker (Buenos Aires, Argentina). The tetrazolium salt MTT [3-(4,5-dimethyl-thiazol-2-il)-2,5-diphenyl tetrazolium], minimum essential medium (MEM), fetal bovine serum (FBS), glutamine, and antibiotic antimycotic, all used in cell culture, were obtained from Gibco (Waltham, MA, USA).

Preparation of Emulsions

Optimization: Composition

Nutraceutical emulsions were developed by the spontaneous emulsification method.[5] The oily phase (66.55% cod liver oil, 33.28% canola oil, and 0.17% vitamin E) was flushed with nitrogen and stored. The surfactant (Tween 80) and cosurfactants (SL and PG) were mixed in fixed weight ratios as follows: 1:1:1 (A), 2:1:1 (B), 1:1:2 (C), and 4:1:1 (D). To simplify denominations, the mix composed of surfactant and cosurfactants was called Smix. In the optimization process, the drug was replaced by a PG solution in water (1:1), which was added to the oily phase. Briefly, each Smix was added to the oily phase and homogenized under vigorous stirring. The oil–Smix ratio varied as follows: 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (w/w). A constant volume of water was added dropwise at room temperature to each oil–Smix mixture under vigorous stirring. After equilibrium, the samples were observed and those that presented phase separation were discarded. On days 1, 7, 14, and 21 post-preparation, the globule size was determined by light scattering with a Malvern Mastersizer 2000E (Malvern Instruments Ltd., Worcester, UK). Measurements were performed three times. After that, formulations with the best features were selected.

Optimization: Method of Preparation

The emulsions selected were prepared and the method was optimized based on obtaining globule size. During this step, formulations were prepared using an UltraTurrax homogenizer (UltraTurrax IKA T25 Digital with rotor S25-20 NK-186; Labortechnik, Wasserburg, Germany). Stirring speed and time were varied. Globule size was determined and samples that presented stable sizes were selected.

Preparation of NEs and NEs-RISP

Nutraceutical emulsions-RISP were obtained by mixing 1 ml of stock solution of RISP (1 mg/mL) with Smix and oily phase for 1 min at 10,000 rpm, using an UltraTurrax homogenizer. Water was added dropwise with continuous stirring at 20,000 rpm for 3 min. Samples were kept on ice during this process to avoid an increase in temperature. A NE (without RISP) was used as control.