International Journal of Pharmaceutics 541 (2018) 224–233

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

# Aqueous injection of quercetin: An approach for confirmation of its direct *in vivo* cardiovascular effects

Elena Piera Porcu[a], Massimo Cossu[a], Giovanna Rassu[a], Paolo Giunchedi[a], Guido Cerri[b], Jana Pourová[c], Iveta Najmanová[d], Thomas Migkos[c], Veronika Pilařová[e], Lucie Nováková[e], Přemysl Mladěnka[c,*], Elisabetta Gavini[a,*]

[a] Department of Chemistry and Pharmacy, University of Sassari, Sassari, Italy
[b] Department of Architecture, Design and Urban Planning – GeoMaterials Lab, University of Sassari, Sassari, Italy
[c] Department of Pharmacology and Toxicology, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[d] Department of Biological and Medical Sciences, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic
[e] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Czech Republic

ARTICLE INFO

*Keywords:*
Solid dispersion
Cardiovascular effects
Quercetin
Polyvinylpyrrolidone
Solubility enhancement
Injectable aqueous solution

ABSTRACT

Potential positive effects of flavonol quercetin on humans were suggested by many studies. However, it is not clear if these effects are mediated by quercetin or its metabolites. The *in vivo* confirmation of quercetin effects is largely hindered by its low water solubility and thus impossibility to test directly its impact. Therefore, a solid dispersion of quercetin with polyvinylpyrrolidone (PVP) was developed to prepare an injectable formulation of water-soluble quercetin. The optimized formulation provided a 20,000-fold increase in quercetin solubility. This formulation was tested on conventional and spontaneously hypertensive rats; it lowered their blood pressure in both short- and long-term basis. Pharmacokinetic data are also provided. This study reports for the first time an injectable water-soluble formulation of quercetin suitable for confirmation of its vascular effect *in vivo*.

## 1. Introduction

Flavonoids always attracted a great attention due to their presence in common diet and their possible positive effects on humans (Kumar and Pandey, 2013; Mladěnka et al., 2010). The major flavonol in the human diet is quercetin and indeed, most flavonoid studies have been performed with it. There is a huge number of studies showing its positive effects, and in particular, some are claiming lowering arterial blood pressure (Larson et al., 2010). However, notwithstanding such enormous quantity of articles, there are still no definite proofs if quercetin has clearly positive effects on human being. The major controversies arise from the facts that orally given quercetin has very low bioavailability (Li et al., 2009; Rothwell et al., 2016), and its *in vitro* effects were commonly observed in quite large concentrations which are hardly, if at all, achievable after oral intake. Another possible explanation is based on the fact that although quercetin is poorly absorbed, it is extensively metabolized by human microflora into a number of small phenolic compounds which can have significant biological effects in humans (Del Rio et al., 2013). For example, our group recently demonstrated than at least one of these metabolites lowers arterial blood pressure in rat (Najmanová et al., 2016). In order to confirm or refute the direct effect of quercetin on arterial blood pressure, a water-soluble formulation of quercetin is needed since only biologically friendly solvents are fully compatible with biological systems and can be applied intravenously without producing inadvertent additional effects.

In general, the use of quercetin in pharmaceutical area is still limited due to its poor solubility in water, which limits formulation strategies. Because of limited hydrophilicity, quercetin shows low dissolution rate, and consequently minimal quercetin absorption occurs in the gastrointestinal tract (Li et al., 2009; Puerta et al., 2017). For this reason, several efforts have been made to improve the aqueous solubility and therapeutic effects of quercetin.

Various approaches such as polymeric nanoparticles (Wang et al., 2016), cocrystals (Smith et al., 2011), lipid nanoparticles (Bose et al., 2013; Li et al., 2009; Kumar et al., 2016), complexation (Jullian et al.,

* Corresponding authors at: Department of Chemistry and Pharmacy University of Sassari, via Muroni 23/a, 07100 Sassari, Italy (Elisabetta Gavini), Department of Pharmacology and Toxicology, Faculty of Pharmacy, Charles University, Akademika Heyrovského 1203, 500 05 Hradec Králové, Czech Republic (Přemysl Mladěnka).
*E-mail addresses:* elena.piera1988@gmail.com (E.P. Porcu), mcossu@uniss.it (M. Cossu), grassu@uniss.it (G. Rassu), pgiunc@uniss.it (P. Giunchedi), gcerri@uniss.it (G. Cerri), pourova@faf.cuni.cz (J. Pourová), najmanoi@faf.cuni.cz (I. Najmanová), migkost@faf.cuni.cz (T. Migkos), pilarovv@faf.cuni.cz (V. Pilařová), novakoval@faf.cuni.cz (L. Nováková), mladenkap@faf.cuni.cz (P. Mladěnka), eligav@uniss.it (E. Gavini).

https://doi.org/10.1016/j.ijpharm.2018.02.036
Received 18 December 2017; Received in revised form 14 February 2018; Accepted 19 February 2018
Available online 21 February 2018
0378-5173/ © 2018 Elsevier B.V. All rights reserved.

2007; Sri et al., 2007), micelles (Gao et al., 2012), emulsions (Gao et al., 2009; Tran et al., 2014; Hädrich et al., 2016), liposomes (Caddeo et al., 2016) and solid dispersion have been developed to enhance the solubility, dissolution rate and bioavailability of quercetin. Solid dispersions refer to a solid product made up of at least two different components, generally a hydrophilic and inert matrix and one or more hydrophobic drugs. In these systems, the drug solubility and its dissolution profile can be improved through the amorphous solid state and by reducing the particle size of the drug. Additionally, the use of hydrophilic carriers increases the wettability of hydrophobic drugs (Park et al., 2016). In particular, various polymers have been employed to increase quercetin solubility such as polyethylene glycols (PEGs) (Otto et al., 2013; Park et al., 2016), cellulose derivatives (Sansone et al., 2011; Li et al., 2013a,b; Gilley et al., 2017) and polyvinylpyrrolidone (Povidone, PVP) (de Mello Costa et al., 2010; Kakran et al., 2011; Yan et al., 2014).

PVP is one of the most commonly used carriers for solid dispersions due to its amphiphilic properties, nontoxicity and biocompatibility. Indeed, PVP is known to form water-soluble complexes with several drugs including carbamazepine (Sethia and Squillante, 2004), lansoprazole (Zhang et al., 2008), efavirenz (Alves et al., 2014), atorvastatin (Jahangiri et al., 2015) and others. In all cases, the aqueous solubility of the product is largely improved.

PVP is available in different grades based on molecular weights (Kadajji and Betageri, 2011). The mean molecular weight of PVP is characterized by the K-value (e.g. Povidone K-12, Povidone K-17, Povidone K-25, Povidone K-30, Povidone K-90) (Folttmann and Quadir, 2008). PVPs with low K-value are suitable solubilizing agents particularly for injectables (e.g. rifampicin, sulfonamide, melphalan, metronidazole, trimethoprim formulations) due to their low viscosity.

The solid dispersions represent a profitable strategy because of its simplicity and efficacy for enhancing the solubility and bioavailability of poorly soluble drugs. This aspect has led to an increase in their applications in the area of cosmetics and pharmaceuticals. Solid dispersions can be prepared by different techniques (Vasconcelos et al., 2007; Sareen et al., 2012). Solvent evaporation method is particularly suitable for PVPs due to their good solubility in most solvents. In addition, co-solvent technique is particularly important for parenteral dosage forms. Due to this method, it is possible to incorporate a large quantity of a drug in small volume of liquid, as required for injections (Soni et al., 2014).

Although there are many papers in the literature regarding the formulation improving delivery and solubility of quercetin in different delivery systems, very few studies have investigated the possibility of preparing quercetin formulations for injection (Yuan et al., 2006; Date and Nagarsenker, 2008; Sun et al., 2011); particularly scarce is the literature concerning the development of injectable aqueous solutions. These systems may be of significant utility not only for a possible therapeutic application but also for studying the real direct effects of quercetin *in vivo,* through a simple system enabling easy dose modification. For these reasons, the objective of the present work was to prepare an aqueous formulation of quercetin for i.v. injection to evaluate its antihypertensive effects *in vivo*. For this purpose, a solid dispersion powder of quercetin with PVP suitable for injections was developed by using the co-solvent method.

## 2. Materials and methods

### 2.1. Materials

Quercetin (purity ≥ 95%) and polyvinylpyrrolidone (PVP10, Mw = 10000 g/mol, K-value 13–19) were purchased from Sigma Aldrich (Milan, Italy). Saline (0.9% NaCl injectable solution) was supplied by Eurospital (Trieste, Italy). Ethanol 96% was purchased from Carlo Erba Reagents (Italy). Analytical grade solvents were used.

**Table 1**
Qualitative and quantitative composition of all formulations.

| FORMULATION | QUERCETIN (mg) | PVP10 (mg) | Weight Ratio QUERCETIN/ PVP10 (w/w) | Theoretical Drug Content QUERCETIN (%) |
|---|---|---|---|---|
| D3L | 25 | 62.5 | 1/2.5 | 28.5 |
| D5L | 25 | 125 | 1/5 | 16.7 |
| D10L | 25 | 250 | 1/10 | 9.1 |
| D11L | 25 | 275 | 1/11 | 8.3 |
| D12L | 25 | 300 | 1/12 | 7.7 |
| D15L | 25 | 375 | 1/15 | 6.3 |
| D20L | 25 | 500 | 1/20 | 4.8 |
| D12E | 25 | 300 | 1/12 | 7.7 |

The code L indicates powders obtained by evaporation and freeze-drying, whereas the code E denotes the formulation obtained by evaporation until dryness.

### 2.2. Preparation of solid dispersions

Solid dispersions of quercetin with the different weight ratios of quercetin/PVP10 were obtained by using the co-solvent evaporation technique. Briefly, the solutions were prepared by dissolving 25 mg of quercetin in 12.5 ml of ethanol and variable amounts of PVP10 in 40 ml of deionized water (Table 1).

The solutions were then mixed under magnetic stirring to produce a transparent yellow mixture. The solvent was evaporated until it reached 15–20 ml using a rotary evaporator under reduced pressure at 95 °C. Finally, the resulting solutions were freeze-dried at − 54.5 °C under vacuum (0.909 mbar) for approximately 8 h, without the addition of cryoprotectants, using a Lio 5P Cinquepascale (Trezzano sul Naviglio, Italy). The freeze-drying process was carried out for 8 h. The formulation having a weight ratio quercetin/PVP10 of 1:12 (D12L) was selected as the leader one.

Moreover, a solid dispersion with the same quercetin/PVP10 weight ratio was also prepared evaporating by rotary evaporator, under reduced pressure at 95 °C, until dryness. The crumbly powder obtained was denoted as D12E.

### 2.3. Solubility studies

Excess amounts of pure quercetin or quercetin solid dispersions were dispersed into 2 ml of deionized water and the samples were kept shaking in a water bath at 20 °C. Powder of solid dispersion was added to water solution until clouding solution was observed, in order to verify the maximum solubility. The suspensions were filtered through a 0.22 µm syringe filter and an aliquot of 600 µl was mixed with the same volume of 20 mM water solution of NaOH.

Due to the chemical changes of quercetin in basic medium over time (Yang et al., 2010), quantification of the drug was performed after 40 min to allow stabilization of solution. According to Yang et al. (2010), the absorption bands of the reaction solution of quercetin and sodium hydroxide changed during the reaction due to the formation of intermediate and final products. After 40 min only the absorption band of the final product at 350 nm appeared.

The concentration of quercetin was determined by measuring the absorbance using UV–VIS spectrophotometer (Thermo Spectronic, Helios Gamma, England) at 350 nm. The calibration curve was linear in the range of 1–20.0 mg/L ($R^2 = 0.9999$).

### 2.4. In vitro characterization of solid dispersions D12L and D12E

#### 2.4.1. Drug content

The amount of quercetin entrapped within the solid dispersions was determined spectrophotometrically by the same way as reported above. Briefly, 10 mg of each formulation were weighed accurately and

Download English Version:

https://daneshyari.com/en/article/8520082

Download Persian Version:

https://daneshyari.com/article/8520082

Daneshyari.com