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Novel carvedilol paediatric nanomicelle formulation: in-vitro characterization and in-vivo evaluation

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Abstract

Objectives Carvedilol (CAR) is a poorly water-soluble beta-blocker. Its encapsulation within nanomicelles (NMs) could improve drug solubility and its oral bioavailability, allowing the development of a paediatric liquid CAR formulation with commercially available copolymers: D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) and poly(vinyl caprolactam)-poly(vinyl acetate)-poly (ethylene glycol) (Soluplus®).

Methods Drug-loaded NMs were prepared by copolymer and CAR dispersion in distilled water. Micellar size and morphology were characterized by dynamic light scattering and transmission electron microscopy, respectively. In-vitro drug permeation studies were evaluated by conventional gut sac method. In-vivo CAR oral bioavailability from NMs dispersions and drug control solution was evaluated in Wistar rats.

Key findings Carvedilol apparent aqueous solubility was increased (up to 60.4-folds) after its encapsulation within NMs. The micellar size was ranged between 10.9 and 81.9 nm with a monomodal size distribution. There was a significant enhancement of CAR relative oral bioavailability for both copolymers vs a micelle-free drug solution ($P < 0.05$). This improvement was higher for TPGS-based micelles (4.95-fold) in accordance with the in-vitro CAR permeation results.

Conclusions The present investigation demonstrates the development of highly concentrated CAR liquid micellar formulation. The improvement on drug oral bioavailability contributes to the potential of this NMs formulation to enhance CAR paediatric treatment.

Introduction

According to the World Health Organization (WHO), cardiovascular diseases remain as the first cause of death worldwide (31% of global deaths).[1] Particularly, for heart failure treatment in adults and children, a nonselective beta-adrenergic blocker with vasodilating properties as carvedilol (CAR) has been employed. It has been reported a reduction on morbility and mortality in adults and better tolerability compared with other beta-blockers.[2,3] On paediatric field, studies have shown that the oral administration of CAR to the heart failure standard therapy denoted an enhancement on ventricular function, being these studies of clinical relevance due to the lack of clinical reports employing beta-blockers in children and the data extrapolation from clinical trials in adults.[4–6] Further, CAR is also employed in paediatric hypertension treatment.[7]

In terms of paediatric pharmacotherapy, children therapeutics needs (e.g. pharmacokinetics parameters,

administration routes and even taste preferences) are different from those of adults.[8] Unfortunately, many drugs employed in children are 'off label' or 'unlicensed' (30–90%).[9] Thereafter, medicines should be adapted for children size, an initiative promoted by WHO.[10] Paediatric treatment outcomes depend on formulation acceptance, taking into account that children (≤5 years old) are unable to swallow solid formulations as capsules or tablets. Therefore, the development of oral liquid formulations as solutions, suspension, syrups and powder/granules for reconstitution becomes an excellent strategy to improve the dose-per-weight adjustment in children.[11,12] In this framework, CAR (class II drug according to the BCS) presents a (bio)pharmaceutical limitation considering that its poorly water solubility (10 μg/ml, 25°C)[13] makes more difficult the development of liquid paediatric formulations. Moreover, the only CAR commercial formulation is a solid dosage form (3.125–50 mg, oral tablets).

Nanotechnology represents an attractive alternative for the development of liquid formulations to overcome the (bio)pharmaceutical limitations of hydrophobic/unstable drugs.[14] For instance, nano-sized micelles, also known as nanomicelles (NMs), have been studied for encapsulation of water-poorly soluble/unstable drugs.[15,16] Different studies have focused on the development of novel micelle-based paediatric formulations.[17–20]

A variety of biomaterials have been evaluated to obtain micellar dispersions. For instance, amphiphilic triblock copolymers composed of poly(ethylene glycol)-poly(propylene oxide)-poly(ethylene glycol) represent well-investigated micelle-forming biomaterials.[21] Among these derivatives, poloxamer 407 or Pluronic® F127 (F127) is a commercially available copolymer approved by the Food and Drug Administration (FDA) as an inactive ingredient.[22,23] Different F127-based NMs have been explored where special focus was made on paediatric antiretroviral therapy improvement.[24]

Recently, a water-soluble form of the natural vitamin E known as D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) has been on the spot as an excellent pharmaceutical excipient.[23,25] It is a commercially available biomaterial which has been approved by the FDA as a generally regarded as safe (GRAS)-listed oral supplement. Due to its amphiphilic nature, TPGS micelles (alone or mixed with other biomaterials) have been developed to encapsulate hydrophobic drugs.[26–30]

Other novel micelle-forming biomaterial is represented by the graft copolymer of poly(vinyl caprolactam)-poly (vinyl acetate)-poly(ethylene glycol) denoted as Soluplus®. This is a biomaterial recently investigated for the development of both polymeric micelles and solid dispersions (even employing CAR) where its low critical micellar concentration value provides high micellar stability under dilution.[31-35] Also, Soluplus® improved the intestinal absorption of various drugs and reduce the activity of the P-glycoprotein.[36,37]

Further, a nanotechnological strategy as the development of NMs colloidal dispersions based on a variety of polymeric biomaterials could overcome the CAR (bio)pharmaceutical limitations. Surprisingly, only a few studies have been focused on the development of nano-sized CAR liquid formulations. For instance, a CAR nanosuspension for oral administration and a micellar CAR formulation based on noncommercially available copolymers for intranasal administration have been explored.[11,38]

In this context, the novelty of our investigation is focused on the development of a novel liquid oral CAR micellar dispersion employing FDA approved-biomaterials, to get a CAR concentration of clinical relevance, according to the drug paediatric dose for heart failure (0.3–0.7 mg/kg per day) and hypertension (0.1–0.5 mg/kg per day).[7] Special focus was made on the development of a simply and highly concentrated CAR oral liquid formulation without the addition of commonly pharmaceutical additives such as propylene glycol as an attempt of avoiding middle- to long-term side effect in children.[9,12]

Materials and Methods

Materials

Carvedilol (CAR) was provided by Parafarm® (Argentina). D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) was supplied by Eastman Chemical Company (Kingsport, Tennessee, USA). Poly (vinyl caprolactam)-poly(vinyl acetate)-poly(ethylene glycol) (Soluplus®) and Pluronic F127 were donated by BASF (CABA, Argentina). Solvents of analytical or HPLC grade were used as received.

Preparation of NMs

NMs (1–10% w/v) were prepared by dissolving the appropriated amount of polymer (TPGS, F127 and Soluplus®) in distilled water under magnetic stirring (100 rpm, 25°C). Samples were equilibrated (24 h, 25°C) before use.

Preparation and characterization of CAR-loaded NMs

A CAR excess was added to the micellar dispersions (1, 3, 5, 7 and 10% w/v) under magnetic stirring (100 rpm) over 48 h. Samples were filtered (0.45-μm cellulose nitrate membranes; Sartorius Stedim Biotech GmbH, Goettingen, Germany), and aliquots (10–1000 μl) were diluted with methanol (10 ml). CAR concentration was determined by UV–visible spectrophotometry (λ: 241 nm, 25°C, UV-260,

UV–visible Recorder Spectrophotometer, Shimadzu, Japan). Drug-free micellar dispersions were used as blanks. The linearity range was established between 0.0016 and 0.02 mg/ml (R^2: 0.9998). CAR solubility factors (f_s) were calculated according to the equation:

$$f_s = \frac{S_a}{S_i} \quad (1)$$

where S_a and S_i are the apparent solubility of CAR in the NMs dispersions and the intrinsic aqueous drug solubility at pH 5.2 and 7.0. Results were assessed by triplicate ± SD.

Size and size distribution (polydispersity index, PDI) of CAR-loaded (1 mg/ml) NMs (5% w/v) were determined by dynamic light scattering (DLS, scattering angle of θ = 173° to the incident beam, Zetasizer Nano-Zs; Malvern Instruments, Worcestershire, UK) at 25°C. Samples were equilibrated for 5 min at 25°C before measurements. Results were expressed as the average of five measurements (±SD).

The morphology of the CAR-loaded (1 mg/ml) NMs (5% w/v) was characterized by transmission electron microscopy (TEM, Philips CM-12 TEM apparatus; FEI Company, Eindhoven, The Netherlands). Aliquots (5 µl) were placed onto a grid and covered with Formvar film. They were negatively stained with 5 µl of phosphotungstic acid solution (1% w/v), washed with distilled water and dried in a silica gel container.

Finally, drug-loaded (1 mg/ml, 50 µl) micellar dispersions (5% w/v, TPGS and Soluplus®) were collected at 0, 7, 14, 21 and 28 days, and they were diluted to 10 ml with methanol in a volumetric flask. Then, the percentage of CAR (CAR %) in solution was determined by UV–visible spectrophotometry (λ: 241 nm) as previously described. CAR-free micellar dispersions were used as blanks. Results were assessed by triplicate ± SD.

In-vitro permeation studies

In-vitro CAR permeation profiles were evaluated by an adapted conventional gut sac method.[39] The main objective of this assay was the drug permeation comparison between NMs formulations. Briefly, bovine duodenum was divided into pieces (6 cm each), washed with normal saline solution (NaCl 0.9% w/v) and maintained into NaCl 0.9% w/v until use. CAR (1 mg/ml, 2 ml) micellar formulations (5% w/v, TPGS and Soluplus®) prepared in distilled water (pH 7.0 for TPGS NMs and pH 5.2 for Soluplus NMs) were placed inside the intestine, and each sac piece was kept into the external media (phosphate buffer pH 6.8 USP 30, 25 ml, 37°C) in addition to polysorbate 80 (0.5% w/v). At different time points (1, 2 and 3 h), external media was completely replaced by the same amount of fresh media (37°C). CAR concentration was assessed by RP-HPLC.[40] The analytical method consisted in a Spherisorb ODS column 5 µm, C18, 250 × 4.6 mm (Waters Spherisorb, Wexford, Ireland) and a mobile phase (distilled water: acetonitrile: triethanolamine, 55:45:0.2 v/v), adjusted to pH 3.0 with phosphoric acid. Detection was performed using a fluorescence detector (FL-3000, excitation (238 nm) and emission (350 nm) wavelengths; Thermo Finnigan, Villebon-sur-Yvette, France). Drug retention time was 6.4 min, the flow rate was 1 ml/min and the linearity range was 2–2000 ng/ml. Results were assessed by triplicate ± SD.

Oral pharmacokinetics

Plasma CAR concentration vs time profiles after drug oral administration was investigated in fasted (12 h) male Wistar rats (300–350 g). Animal experiments and animal care were approved by the Animal Care Committee of School of Pharmacy of the University of Buenos Aires (EXP-UBA N°0062949/2015) and were in line with the published Guide for the Care and Use of Laboratory Animals (NIH, 8° Ed., 2011). Animals were maintained on a 12-h light/dark routine (22 ± 2°C) receiving standard rodent diet (69% starch, 20% proteins, 6% minerals, 3% fat, 2% fibre (w/w), vitamin supplements, Asociación Cooperativas Argentinas, San Nicolas, Argentina).

Animals were divided into three groups (n = 6) and CAR (1 mg/ml, 200 mg/kg) formulations (polymer concentration 5% w/v) evaluated were (i) drug-loaded TPGS micelles, (ii) CAR-loaded Soluplus® micelles and (iii) a micelle-free CAR solution (prepared as previously described).[41] Formulations were orally administered by gavage, and blood aliquots (70 µl) were collected from the tail vein at different time points (5, 10, 15, 30, 60, 90, 120, 180, 240 and 300 min). Then, aliquots were centrifugated (10,000 rpm, 10 min, 4°C), the supernatants (40 µl) were deproteinized with acetonitrile (55 µl) and zinc sulphate solution 10% w/v (10 µl) and they were centrifuged (13,000 rpm, 2 min, 4°C). Drug concentration was determined by RP-HPLC as previously described (Section 'In-vitro permeation studies').

Finally, the oral pharmacokinetic parameters: (i) the maximum plasma concentration (C_{max}), (ii) the time to the maximum plasma concentration (t_{max}), (iii) the area-under-the-curve between the administration time and 2 h (AUC_{0-2}), (iv) the area-under-the-curve between the administration time and infinity ($AUC_{0-\infty}$) and half-life of elimination ($t_{1/2}$) were estimated by noncompartmental analysis of CAR plasma concentrations profiles using TOPFIT 2.0 program (Dr Karl Thomae Gmbh, Schering AG, Germany). Results were log transformed for statistical analysis.

Drug relative bioavailability (Fr %) after oral administration of CAR-loaded NMs and drug control solution was calculated according to the equation:

$$\mathrm{Fr}\,(\%) = \frac{\mathrm{AUC_{NMs}}}{\mathrm{AUC_{sol}}} \times 100 \qquad (2)$$

where AUC_{NMs} and AUC_{sol} are the $AUC_{0-\infty}$ of NMs dispersion and control solution, respectively.

Statistical analysis

Statistical analysis was performed using the analysis of variance ANOVA (Kruskal–Wallis) test. Then, it was evaluated with post hoc, that is Dunn's test, by considering probability value (P value) <0.05 as significant (GraphPad Prism version 5.02 for Windows® San Diego, CA, USA).

Results

Drug encapsulation within NMs

Carvedilol encapsulation within NMs-based on different commercially available biomaterials represents one of the main objectives of the present investigation.

First of all, we determined the drug S_i values at pH values of 5.2 and 7.0. Results showed that the CAR S_i values were 1.15 and 0.05 mg/ml at pH 5.2 and 7.0, respectively. Thereafter, these S_i values were used to calculate each f_s according to the polymer employed (Table 1).

In second place, we investigated the NMs capacity to encapsulate the hydrophobic CAR. Each polymer (F127, TPGS and Soluplus®) was employed between 1 and 10% w/v to obtain the aqueous micellar dispersions. Then, after CAR encapsulation, clear dispersions were observed where the S_a and f_s values are summarized in Table 1. There was an increase in CAR S_a values as the polymer concentration increases from 1 to 10% w/v. For instance, it was observed a sharp increase in CAR aqueous solubility (up to 6.57 mg/ml) for Soluplus® NMs at 10% w/v (pH 5.2). Then, the S_a values observed for TPGS and F127 (at the same polymer concentration) were 3.02 and 0.63 mg/ml (pH 7), respectively (Table 1).

Interestingly, the S_a values observed for each biomaterial were statistically different ($P < 0.05$) at every polymer concentration assayed. Moreover, the lowest S_a values were observed for F127 NMs dispersions (Table 1). For example, at a polymer concentration of 5% w/v, the S_a values observed for Soluplus®, TPGS and F127 micelles were 3.68, 1.35 and 0.10 mg/ml, respectively. A similar trend was observed for the other concentrations assayed (Table 1).

Taking into account the f_s values, the highest increment on drug solubility was observed for TPGS-based NMs. Indeed, the f_s value obtained for a polymer concentration of 10% w/v was 60.4 in comparison with its counterparts Soluplus® and F127 where the f_s values observed were 5.7 and 12.6, respectively (Table 1). Further, with a polymer concentration of 1% w/v, CAR was encapsulated within TPGS micelles (0.29 mg/ml, f_s: 5.8); however, no drug encapsulation was observed for Soluplus® and F127 micelles as the S_a values for both biomaterials were lower than the S_i values at pH 5.2 and 7.0 (Table 1).

As the CAR concentration of 1 mg/ml exhibits clinical relevance (according to the dose-per-weight adjustment), this concentration was obtained employing at least a polymer (TPGS) concentration of 5% w/v (Table 1). Thereafter, this biomaterial concentration was chosen for further studies.

Characterization of CAR-loaded NMs

Micellar size and morphology

The Dh and size distribution (PDI) of the CAR (1 mg/ml)-loaded NMs (5% w/v) were characterized by DLS. Results showed that the polymeric micelles within the hydrophobic drug were in the nanoscale range. For TPGS-based NMs, it was observed a monomodal size distribution with a Dh value of 10.9 ± 0.8 nm (PDI: 0.123). Following a similar trend, Soluplus®-based NMs demonstrated a Dh value of 81.9 ± 5.6 nm along with an unimodal size distribution (PDI: 0.232).

Table 1 CAR apparent solubility (S_a) and solubility factors (f_s) for NMs dispersions employing three biomaterials in distilled water at 25°C. Data represent mean ± standard deviation (SD), $n = 3$

Polymer concentration (% w/v)	Soluplus®		TPGS		F127	
	S_a (mg/ml) (±SD)	f_s[a]	S_a (mg/ml) (±SD)	f_s[b]	S_a (mg/ml) (±SD)	f_s[b]
1	0.66 (0.08)	0.6	0.29 (0.04)	5.8	0.01 (0.01)	0.2
3	1.28 (0.19)	1.1	0.83 (0.09)	16.6	0.04 (0.01)	0.8
5	3.68 (0.10)	3.2	1.35 (0.17)	27.0	0.10 (0.01)	2.0
7	4.12 (0.18)	3.6	1.97 (0.14)	39.4	0.22 (0.02)	4.4
10	6.57 (0.31)	5.7	3.02 (0.42)	60.4	0.63 (0.02)	12.6

CAR, carvedilol; NMs, nanomicelles. [a]Calculated based on S_i value of 1.15 mg/ml (pH 5.2). [b]Calculated based on S_i value of 0.05 mg/ml (pH 7.0).

Furthermore, the morphology of the CAR-loaded NMs dispersion was evaluated by TEM as shown in Figure 1. For TPGS NMs, it was observed a spherical morphology with only one size population (Figure 1a). As opposite, for Soluplus® NMs, rod-shaped micelles were visualized (Figure 1b).

In-vitro physical NMs stability

To evaluate micelle physical stability in aqueous media, CAR-loaded (1 mg/ml) NMs (5% w/v) were prepared as described in Section 'Preparation and characterization of CAR-loaded NMs', stored at room temperature and the drug percentage (%) was determined at different time points. Data demonstrated that micellar formulations remain stable up to 28 days, for both copolymers, where the CAR content was 104.7 and 108.3% for TPGS and Soluplus®-based NMs, respectively (Figure 2). Further, no drug precipitation was observed.

In-vitro CAR permeation

The in-vitro drug permeation through a biological membrane was assessed employing bovine duodenum at different time points (1, 2 and 3 h). As it is shown in Figure 3, the amount of CAR permeated from TPGS NMs was 2.43 μg over 1 h. Then, the drug permeation was decreased where the drug amount permeated was 1.11 and 1.25 μg at 2 and 3 h, respectively. An opposite behaviour was observed for Soluplus® NMs. In this case, it was observed an increment on drug permeation over 3 h. For instance, the amount of CAR permeated was 1.36 and 2.45 μg at 1 and 3 h, respectively, representing an increment of 1.8-fold (Figure 3). It is worth stressing that the difference on drug permeated was significant ($P < 0.05$) only at 3 h.

Oral pharmacokinetics

Carvedilol oral pharmacokinetic parameters were obtained by noncompartmental analysis of plasma concentrations at different time points (Table 2). The pharmacokinetic profiles of the CAR plasma concentration vs time after oral administration are represented in Figure 4. In-vivo data showed that there was a significant increment ($P < 0.05$) in the AUC_{0-2} values for TPGS NMs in comparison with Soluplus® NMs and the micelle-free CAR solution. Indeed, the AUC_{0-2} values were 120.1, 61.3 and 40.6 ng/ml per hour for TPGS NMs, Soluplus® NMs and micelle-free drug solution, respectively. Further, the CAR relative oral bioavailability encapsulated within TPGS NMs was increased up to 4.95-fold (Table 2), being these results clearly demonstrated in Figure 4. Conversely, there was only a significant increase in the $AUC_{0-\infty}$ values for TPGS micellar dispersion in contrast to the micelle-free CAR solution, as shown in Table 2.

A similar behaviour was observed with the C_{max} values, where a significant increase ($P < 0.05$) was observed for TPGS NMs (108.6 ng/ml) vs Soluplus® NMs (51.8 ng/ml) and the micelle-free CAR solution (56.9 ng/ml) (Table 2).

For Soluplus® NMs, AUC_{0-2} and C_{max}, were not significantly higher ($P > 0.05$) than those values obtained for the micelle-free drug solution. However, it was observed an

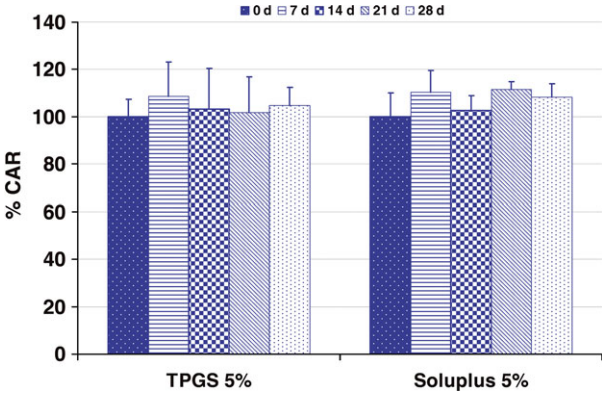

Figure 2 CAR percentage (%) in solution of drug-loaded NMs at 25°C over 28 days. Data represent mean ± standard deviation (SD), $n = 3$. CAR, carvedilol; NMs, nanomicelles. [Colour figure can be viewed at wileyonlinelibrary.com]

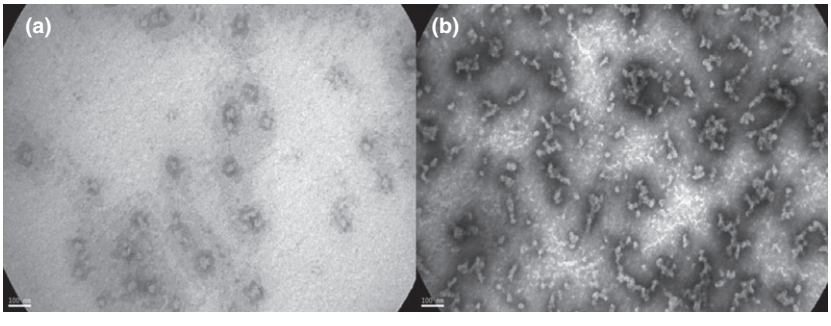

Figure 1 TEM micrographs of CAR-loaded (1 mg/ml) NMs (5% w/v) based on (a) TPGS and (b) Soluplus®. CAR, carvedilol; NMs, nanomicelles.

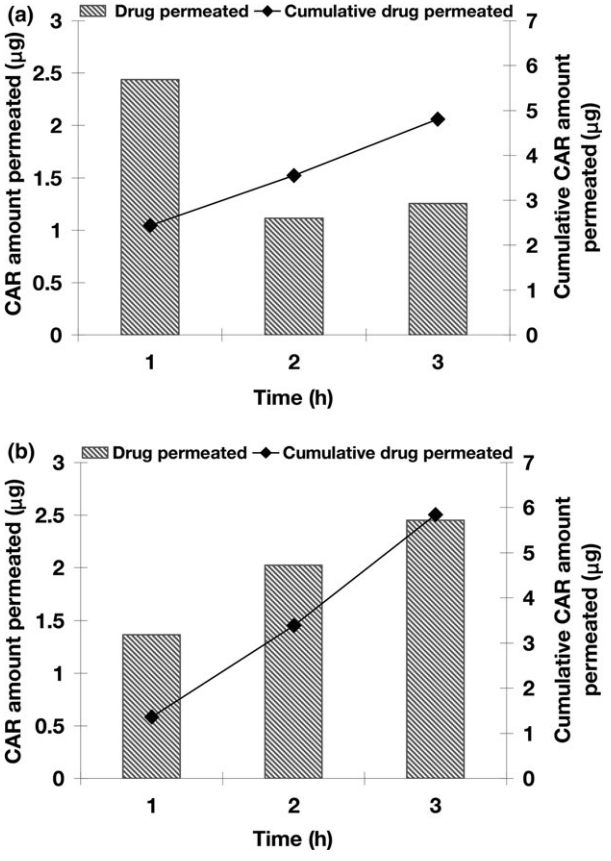

Figure 3 In-vitro permeation profiles of CAR-loaded (1 mg/ml) NMs dispersions (5% w/v) of (a) TPGS and (b) Soluplus® at 37°C over 3 h. Data represent mean ± standard deviation (SD), $n = 3$. Receptor medium was changed every hour. CAR, carvedilol; NMs, nanomicelles.

Table 2 Pharmacokinetic parameters of CAR formulations (1 mg/ml) administered orally. Results are expressed as mean ± standard error (SE), $n = 6$

Pharmacokinetic parameter	TPGS NMs	Soluplus® NMs	Control solution
$t_{1/2}$ (min)	120.8 ± 25.4	202.5 ± 27.4*	78.1 ± 16.4
AUC_{0-2} (ng/ml per hour)	120.1 ± 20.4*	61.3 ± 14.1	40.6 ± 6.9
$AUC_{0-\infty}$ (ng/ml per hour)	308.6 ± 69.5*	159.1 ± 46.6	62.3 ± 15.1
C_{max} (ng/ml)	108.6 ± 16.7*,#	51.8 ± 8.0	56.9 ± 8.8
t_{max} (min)	10.0 ± 1.9	45.0 ± 14.9	10.1 ± 1.3
Fr (%)	495	255	100

AUC, area-under-the-curve between 0 and 2 h; AUC, area-under-the-curve between 0 and ∞; CAR, carvedilol; C_{max}, the maximum plasma concentration; t_{max}, the time to the maximum plasma concentration; $t_{1/2}$, Half-life of elimination; Fr (%), relative bioavailability; NMs, nanomicelle dispersion. *$P < 0.05$ vs Control solution, #$P < 0.05$ vs Soluplus® micelles.

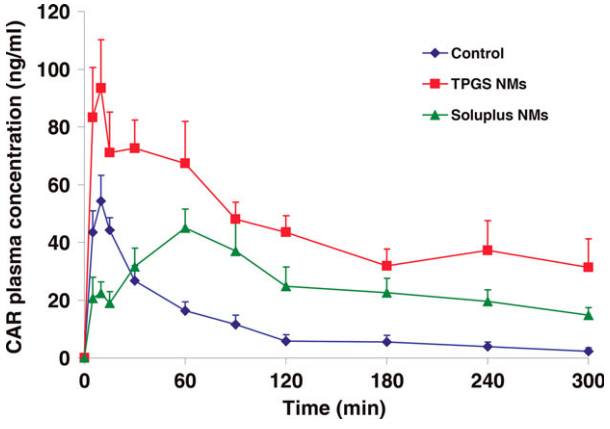

Figure 4 CAR plasma concentrations upon oral administration of drug control solution and CAR-loaded NMs. Results are expressed as mean ± standard error (SE) of the mean ($n = 6$). CAR, carvedilol; NMs, nanomicelles. [Colour figure can be viewed at wileyonlinelibrary.com]

increment in CAR oral relative bioavailability of 2.55-fold. Interestingly, the t_{max} value was higher (45 min) than that observed for the micelle-free CAR solution and TPGS dispersion (10 min). A similar behaviour was observed for the $t_{1/2}$ values (Table 2).

Discussion

One of the main goals of the present study was the development of a novel oral CAR formulation employing commercially available biomaterials with special focus on paediatric field. Particularly, CAR presents a (bio)pharmaceutical limitation due to its poorly aqueous solubility (10 µg/ml, 25°C),[13] which hampers liquid formulation development. Our research group previously studied the stability of CAR paediatric solutions and one suspension, employing pharmaceutical additives including polyvinyl pyrrolidone, propylene glycol, glycerine and sorbitol 70% w/v.[41] Further, we aimed to develop a more simple CAR liquid formulation in the absence of common pharmaceutical excipients, as an attempt to avoid middle- to long-term side effects associated with these additives in children.[9,12,42]

To obtain the NMs dispersions, we employed three different polymers (F127, TPGS and Soluplus®), which could self-assemble in water to form polymeric micelles by simple aqueous dissolution of the polymer without the incorporation of organic solvents. This represents a main advantage on the development of a paediatric formulation as no traces of any organic solvents could be expected.

Initially, CAR S_i values were determined in different pH media as CAR solubility at pH < 5 and at pH 7.4 is reported to change from 0.1 to 0.02 mg/ml in aqueous buffer solutions, respectively.[13] Hence, CAR S_i values were determined at pH 5.2 (corresponding to the pH value of

drug-loaded Soluplus® micellar dispersions) and 7.0 (corresponding to the pH value of drug-loaded TPGS and F127 micellar dispersions).

Secondly, CAR was efficiently encapsulated within NMs, where the drug aqueous solubility was increased up to 60.4-fold. Interestingly, after CAR encapsulation with NMs, it was observed an increment in CAR aqueous solubility as the polymer concentration was increased, probably due to the incorporation of the hydrophobic drug within the nanocarrier.[43–45]

These results suggest the interaction between CAR and the NMs hydrophobic micellar core based on different polymers. It has been demonstrated that drug encapsulation within polymeric micelles strongly depends on the micellar core nature. Indeed, the cohesive forces between the drug and the polymer hydrophobic block could affect not only the drug loading but also the drug release profile from the micellar system.[46] In this study, results clearly demonstrated that CAR could be efficiently encapsulated by TPGS micelles, where the highest f_s values were observed at every polymer concentration assayed.

Although the highest S_a values were observed for Soluplus® NMs, the f_s values were lower than those for TPGS micelles. These results could be related to the S_i values obtained for the different drug-loaded micellar dispersions where S_i values for Soluplus® NMs were higher (1.15 mg/ml, pH 5.2) than those observed for TPGS and F127-based NMs (0.05 mg/ml, pH 7.0).

Finally, the f_s values for F127 micellar dispersions were also lower than those observed for TPGS, supporting the idea that the drug interaction with the micellar core results not as efficiently as with TPGS hydrophobic core. Similar results were observed with paclitaxel (a hydrophobic antineoplastic drug) and mixed micelles of TPGS and Soluplus®.[29]

Overall, CAR solubility enhancement obtained with TPGS and Soluplus® NMs demonstrates paediatric clinical relevance, taking into account the drug dose for heart failure (0.3–0.7 mg/kg per day) and hypertension (0.1–0.5 mg/kg per day) usually used in children.[7] As our NMs dispersions result in highly concentrated CAR, only a little amount of micellar dispersion could be administered. For instance, a 5-year-old child (~18 kg)[47] would require only between 1.8 and 12.6 ml of CAR formulation (1 mg/ml), being in good accordance with the dose volumes recommended according to the child age. Moreover, these dose volumes could be easily oral administered using currently available dosing devices as droppers, oral syringes or moulded plastic medicines cups. Also, novel 'child-friendly' delivery systems as the Medibottle® (especially for newborn and infants) could enhance the delivery of paediatric oral formulations.[48,49] Moreover, our nanotechnological formulation avoids the employment of common cosolvents as propylene glycol, glycerine and sorbitol. For further studies, we assayed CAR-loaded (1 mg/ml) NMs dispersions based on Soluplus® and TPGS at a polymer concentration of 5% w/v to get a drug concentration clinically relevant and reduce the total amount of biomaterial daily administered.

Another key parameter to be evaluated is the micellar size and its distribution, as it has been demonstrated that nano-sized carriers <300 nm could efficiently overcome mucociliary clearance after an oral administration.[50] In the present study, both CAR micellar formulations assayed demonstrate Dh values lower than 300 nm which could enhance the drug bioavailability after an oral administration. Moreover, to gain further insight into NMs morphology, TEM analysis revealed that TPGS-based NMs were spherical. This kind of morphology, for a block copolymer, was expected as the length of the TPGS hydrophilic domain is longer than its hydrophobic portion composed of vitamin E succinate.[51] By contrast, for Soluplus® NMs, a non-spherical morphology was observed. In this case, this graft copolymer exhibits a hydrophilic portion shorter than its hydrophobic one, where other micellar morphologies, rather than spherical, could be expected. Similar results were observed for poly(acrylic acid)-graft-poly(propylene oxide) amphiphilic copolymer.[52]

Further, for the development of a liquid formulation, the physical stability of the colloidal system in aqueous media over time represents a relevant parameter. Under regular storage conditions, variations on the micellar critical concentration of the micelle-forming biomaterials could lead to aggregation and CAR precipitation over time. In this context, our nanotechnological platform exhibited excellent in-vitro micellar stability for 28 days, regardless the biomaterial employed, where no drug precipitates were observed.

Special focus was made on the development of a micellar dispersion with improved CAR oral bioavailability. It is well-known that polymeric micelles are dynamic colloidal systems, and their stability can be affected by different physiological conditions. In-vivo data results are crucial to estimate the real potential of polymeric micelles as drug delivery systems. In this context, CAR showed an absolute bioavailability after oral administration of only 20–24% due to high degree of first-pass elimination.[40,53,54] Hence, we aimed to develop a micellar CAR formulation with better in-vivo performance than a drug formulation currently employed on a paediatric hospital (Garrahan Pediatric Hospital, Argentina).[41] Then, the improvement on drug oral bioavailability was related to the oral bioavailability of the drug formulation currently employed in children (relative oral bioavailability). In-vivo data revealed that oral pharmacokinetics of CAR encapsulated within NMs based on TPGS and Soluplus® show an enhancement on relative bioavailability, especially for TPGS micelles (4.95 fold).

Moreover, a faster absorption rate for TPGS NMs was shown by both, the higher C_{max} and shorter t_{max} values, observed with respect to Soluplus® NMs. These data correlate with the in-vitro permeation studies, as CAR permeation over 1 h was higher for TPGS-based micelles in comparison with their counterparts based on Soluplus®. Also, a good relationship was found between the previous results and the micellar size observed for both biomaterials. It has been described that absorption through intestinal epithelium depends on structural features as the nanocarrier size. As TPGS NMs demonstrated smaller Dh values than Soluplus® micelles, it could be expected a faster absorption rate as it was observed on the in-vivo studies. Similar results were observed with efavirenz and polymeric micelles.[24]

Conclusions

In the present study, the water-poorly soluble CAR was successfully solubilized by its encapsulation within TPGS and Soluplus® NMs. CAR aqueous solubility was increased promoting the development of a liquid drug formulation (1 mg/ml) of clinical relevance according to the CAR dose for heart failure (0.3–0.7 mg/kg per day) and hypertension (0.1–0.5 mg/kg per day) used in children.[7] Further, preclinical comparative evaluation demonstrated an improvement on drug oral bioavailability from micellar dispersions in comparison with a drug control solution without the addition of a common pharmaceutical additive as propylene glycol.

Hence, the results reinforce the potential use of CAR-loaded TPGS and Soluplus® NMs as a novel drug delivery system to enhance paediatric CAR therapy in terms of dose adjustment per weight and easy swallowing.

Declarations

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