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Cefpodoxime-proxetil protection from intestinal lumen hydrolysis by oil-in-water submicron emulsions

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Abstract

Cefpodoxime proxetil is an orally active, broad spectrum, third generation cephalosporin ester. This prodrug was previously found to be hydrolyzed in vitro both in rabbit and human duodenal washing by a cholinesterase. The objective of this work was to find a formulation which can protect the prodrug from enzymatic attack. In order to protect the prodrug from enzymatic hydrolysis, the objective was to include it into the oil phase of an oil-in-water (o/w) emulsion. Somehow, cefpodoxime proxetil posed specific problems related to the solubilization. The solubilization was obtained with a mixed medium-chain-triglycerides (MCT)/blends of mono-, di- and triglycerides oil phase and the optimal ratio was defined to be 60:40 (w/w) in order to obtain emulsification. The emulsifier was a soybean lecithin alone or in mixtures with polysorbate 20. This non-ionic surfactant was chosen since it was found to directly inhibit the hydrolysis of cefpodoxime proxetil in vitro using duodenal washings. The o/w submicron emulsions were proven to be effective in protecting the prodrug from enzymatic attack in rabbit duodenal washings compared with a micellar solution and an aqueous solution of cefpodoxime proxetil. An o/w submicron emulsion incorporating polysorbate 20 was found to be the most protective, which can corroborate the inhibitory role of polysorbate itself.

Keywords: Cefpodoxime proxetil; Oral oil-in-water submicron emulsion; Polysorbates; Cholinesterase; Intestinal lumen

1. Introduction

Cefpodoxime proxetil is an orally absorbed, broad spectrum, third generation cephalosporin

Abbreviations: MCT, medium-chain triglycerides; o/w, Oil-in-water; PCS, photon correlation spectroscopy.

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ester. This prodrug ester is hydrolyzed in vivo into its active metabolite, cefpodoxime. In human, the absolute bioavailability of cefpodoxime proxetil administered as a 132 mg tablet (equivalent to 100 mg of cefpodoxime) is about 50% (Borin, 1991). Moreover, in the feces, cefpodoxime proxetil accounted for less than 0.5% of the administered dose, most of it being recovered as free cefpodoxime. This suggests that a degradation of the prodrug ester occurs in the intestinal lumen.

In our previous studies, cefpodoxime proxetil was found to be hydrolyzed in vitro by cholinesterase both in rabbit and human duodenal washings (Crauste-Manciet et al., 1997a,b). These findings can explain its incomplete absorption.

Moreover, cefpodoxime proxetil is a poorly water-soluble drug. At neutral pH the solubility is 0.4 mg/ml (Hamaura et al., 1995). Dissolution was found to be the rate-limiting factor in intestinal absorption of poorly water-soluble drugs (Finsher, 1968; Nishihata et al., 1988).

The objective of this work was to find a formulation that will increase drug solubility and decrease enzymatic attack by cholinesterase in intestinal washings.

An oil-in-water (o/w) submicron emulsion was selected since inclusion of the prodrug inside the triglycerides oil phase could potentially protect it from cholinesterase attack. This hypothesis could be supported by the fact that cholinesterases are unable to hydrolyze triglycerides (La Du and Snady, 1971). A micellar solution of the prodrug was prepared in order to compare the effects observed with the emulsion but in the absence of the triglyceride oil phase.

The potential use of o/w emulsions to enhance gastrointestinal absorption of poorly-absorbed drugs has already been reported (Carrigan and Bates, 1973). It has been shown that incorporation of orally administered drugs in o/w emulsions significantly increased their intestinal absorption compared with the equivalent aqueous suspension of the drug (Palin et al., 1986; Kimura et al., 1989; Santos Magalhaes et al., 1993)

2. Materials and methods

2.1. Materials

Cefpodoxime proxetil was kindly provided by Roussel Uclaf, France. Purified soybean oil was purchased from Société Industrielle des Oléagineux (Saint-Laurent-Blangy, France), and medium-chain triglycerides (MCT) oil of caprylic and capric acids (Labrafac lipophile® WL 1349) from Gattefossé (Saint Priest, France). Blends of mono-(50%), di- (40%) and triesters (8%) of caprylic and capric acids (Imwitor® 742) was purchased from Hüls (Marl, Germany). Soybean lecithins (Lipoïd S40®, Lipoïd S75®) were purchased from LipoïdKG (Ludwigshafen, Germany). Diethylene glycol monoethylic ether (Transcutol®), Gattefossé (Saint Priest, France), polysorbate 20 (Montanox 20®), polysorbate 40 (Montanox 40®), polysorbate 80 (Montanox 80®), and polysorbate 85 (Montanox 85®) were purchased from Seppic (Paris, France). All other ingredients used were of pharmaceutical grade.

2.2. Methods

2.2.1. Cefpodoxime proxetil solubility

The concentration of the drug was selected to be at least 132 mg/15 ml. This concentration can be related to the actual tablet dosage form and corresponds to one oral administration. We have considered the 15-ml administration to be the maximum allowable volume for one administration in clinical practice.

The above concentration (8.8 mg/ml) of cefpodoxime proxetil was tested in solvents (water, alcohol and Transcutol®) and in oil phases: soybean oil, MCT, blends of mono- and diglycerides (Imwitor® 742) and mixed oil phases: soybean oil/mono- and diglycerides and MCT/mono- and diglycerides in 60:40 and 80:20 (w/w).

2.2.2. Emulsion preparation

The cefpodoxime proxetil was first dissolved in a cosolvent (alcohol or Imwitor®742) and the mixture was dissolved in soybean or MCT oil. The non-ionic emulsifier polysorbate 20, when used, was dissolved in the aqueous phase.

Table 1
Composition (w/w) of the investigated o/w emulsions incorporating cefpodoxime proxetil

	A1	A2	B1	B2	C1	C2	D1	D2	E1	E2
Soybean oil	20	20	—	—	—	—	—	—	—	—
Alcohol	10	10	—	—	—	—	—	—	—	—
Labrafac lipophile® WL1349	—	—	—	—	16	16	12	12	12	12
Imwitor® 742	—	—	20	20	4	4	8	8	8	8
Lipoïd S40®	1.2	0.6	1.2	0.6	1.2	0.6	1.2	0.6	—	—
Lipoïd S75®	—	—	—	—	—	—	—	—	1.2	0.6
Polysorbate 20	—	0.25	—	0.25	—	0.25	—	0.25	—	0.25

The prodrug concentration being 8.8 mg/ml.

Lecithins were dissolved in oil phases when used at 0.6% and in both oil and aqueous phases when used at 1.2%. The oil and aqueous phases were heated at 60°C. Both phases were then mixed and emulsified by a phase inversion method (Becher, 1965) using a high shear mixer (Ultraturrax, Ika Werk, Staufen, Germany). The emulsion was then rapidly cooled and homogenized using a two-stage high pressure valve homogenizer Rannie (Mini-lab, Wesfalia separator, Chateau-Thierry, France) in order to obtain a submicron emulsion.

The lack of cefpodoxime-proxetil degradation after preparation was confirmed by HPLC by measuring the concentration of both cefpodoxime released, and that of the prodrug. In order to verify the inhibitory effect of polysorbate 20, all formulations were prepared with and without this emulsifier. The composition of the investigated o/w emulsions are listed in Table 1.

2.2.3. Micellar solution preparation

Cefpodoxime proxetil was first solubilized in diethylene glycol monoethylic ether (Transcutol®). The mixture was then added to the polysorbate 85. A micellar solution was made at 60°C by adding the final mixture dropwise in the aqueous phase while stirring with a magnetic mixer (Bicasa, Milano, Italy). The composition of the micellar solution in w/w was Transcutol®, 5; polysorbate 85, 15; and water, 80.

2.2.4. Emulsion characterization

Visual observations were made in order to determine macroscopic behavior, possible creaming, coalescence, and phase separation.

2.2.4.1. Particle size analysis. The mean droplet size and size distribution were determined by photon correlation spectroscopy (PCS) (NICOMP 370, Pacific Scientific, Palaiseau, France). For PCS, each emulsion sample was diluted to the appropriate concentration with water before measurement at 25°C.

2.2.4.2. ζ-Potential. The charge on emulsion droplet was measured using the moving boundary electrophoresis technique. Each emulsion sample was diluted with 10mM HEPES buffer (1:2000) prior to examination. ζ-Potential values from several emulsion samples were examined using the Malvern zetasizer (Malvern, Orsay, France). The pH of the emulsion samples was measured using a pH meter (HI 9318, Hanna, Prolabo, Paris, France)

2.2.5. Rabbit intestinal washings

Male New Zealand rabbits weighing 2.5–3 kg were fasted overnight before being sacrificed by i.v. injection of 2 ml/kg sodium pentobarbital. After an abdominal mid-line incision, the small intestine was exposed and a 20 cm segment beginning 5 cm distal to the pylorus was ligatured at both ends. The segment was filled with 5 ml of washing buffer which was left inside for 10 min. The intestine was then evacuated, the washing was spun down at $3800 \times g$ for 10 min to remove particular material, aliquoted and frozen at -22°C.

2.2.6. Hydrolysis studies in rabbit duodenal washings

The enzymatic preparation (5 μg protein) was preincubated at 37°C for 5 min in 200 μl of 10 mM HEPES buffer pH 7, 300 mM mannitol. The reaction was started by the addition of 200 μl of cefpodoxime-proxetil aqueous solution (final concentration of 100 μM). After a 30-min incubation, the reaction was stopped by addition of trifluoroacetic acid (TFA), the samples were centrifuged at $10000 \times g$ for 15 min, and the amount of cefpodoxime released was measured by HPLC analysis of the supernatant.

The effect of polysorbates 20, 40 and 80 on the hydrolysis of cefpodoxime proxetil was measured by preincubating the enzymatic preparation with the above-mentioned compound at three final concentrations, 100, 10 and 1 mg/ml for 15 min at 37°C.

The protective effect of cefpodoxime proxetil formulations was evaluated by replacing the aqueous solution of the drug with the o/w emulsion or micellar solution and following the above-mentioned methodology. In order to compare the results with those obtained with the cefpodoxime-proxetil solution, the formulations were diluted with deionized water to obtain a 100 μM final concentration in the reaction medium. The absence of any physical changes in the emulsions upon dilution was confirmed by visual observation and mean droplet size measurement using PCS.

2.2.7. HPLC analysis of cefpodoxime

The enzymatic incubation media were diluted with a 2% trichloroacetic acid solution. The dilution was analyzed on an optimized system composed of a Supelcosil LC18 (250×4.6 mm, 5 mm particle size) column (Supelco, St. Germain en Laye, France), and a ternary mobile phase (acetate buffer 0.05 M, pH 3.8/methanol/acetonitrile 87:10:3 (v/v)). The flow rate was 1 ml/min and separation was carried out at ambient temperature and monitored at 235 nm. Linearity, and both between- and within-day reproducibility, were assessed. Interassay coefficients of variation were within the range 8.3–3.5% for cefpodoxime concentrations between 0.2 and 2 mM. The quantification limit was 0.05 mM.

3. Results

3.1. Prodrug solubility and emulsion selection for in vitro valuation

Cefpodoxime proxetil solubility in the oil phase was the limiting factor for the formulation of emulsions. Cefpodoxime proxetil (8.8 mg/ml) was neither soluble in soybean oil nor in MCT oil. At this concentration, cefpodoxime proxetil was not soluble in water but was soluble in solvents alcohol and diethylene glycol monoethylic ether (Transcutol®). The solubility found in alcohol allows the emulsion to be formulated using the cosolvent method (Hansrani et al., 1983).

Moreover, the prodrug was soluble only in blends of mono- and diglycerides (Imwitor®742). The solubility in mixed MCT oil and blends of mono-/diglycerides was obtained only with mixed MCT and mono-/diglycerides at 60:40 and 80:20 (w/w). Cefpodoxime proxetil was not soluble when the oil phase used was soybean oil, even when soybean oil was mixed with mono-/diglycerides at 60:40 and 80:20 (w/w). The emulsion formulations used were based on the low solubility of cefpodoxime proxetil in an oil phase.

Moreover, in order to verify the inhibitory effect of polysorbate 20, all formulations were prepared with and without this emulsifier. (Table 1).

All of the above formulations were analyzed by visual immediate observation. In the presence of phase separation or cefpodoxime-proxetil precipitation, the corresponding emulsions were discarded.

The A formulations which used alcohol as a cosolvent for the cefpodoxime proxetil solubilization was homogenous, but prodrug precipitation was observed immediately after the homogeneization step and have been discarded from further investigations.

The B and C formulations where the prodrug was solubilized in blends of mono- and diglycerides or in mixed 80:20 MCT and mono-/diglycerides, respectively, were unstable, and phase separation was observed immediately after the homogeneization step, so that they were discarded from further investigations. The D and E formulations where the prodrug was solubilized in

Table 2
Oil-in-water submicron emulsion characteristics

	D1	D2	E1	E2
pH	6.2	7.0	6.0	6.3
Mean droplet size (nm)	163 ± 2.5^a	201 ± 4.0^a	211 ± 1.7^a	237 ± 1.8^a
ζ-Potential (mV)	-71 ± 2.0^a	-60 ± 1.0^a	-45 ± 1.0^a	-35 ± 1.0^a

[a] Mean ± S.D. of three measures.

mixed 60:40 MCT and mono-/diglycerides were visually stable and therefore retained for further investigations.

3.2. Particle size and ζ-potential of the investigated emulsions

Table 2 summarizes particle size and ζ-potential data of freshly prepared emulsions D and E. The mean droplet size was low for the four formulations. D formulations exhibited better values in mean droplet size and ζ-potential compared with E formulation. The better ζ-potential of D formulations can be related to the lecithin nature (Lipoïd® S40 in place of Lipoïd® S75). Moreover, formulations incorporating lecithins (D1 and E1) as the only emulsifier exhibited better values in ζ-potential compared with those obtained with formulations incorporating lecithin-polysorbate 20 mixtures, D2 and E2, respectively. These differences can be attributed to the lecithin concentration used which is higher in the D1 and E1 formulations. The ζ-potential is a predictive factor for emulsion stability (Washington, 1996). Then the D formulation should be more stable than the E formulation. Consequently, D formulations have been selected for the hydrolysis assay.

3.3. Prodrug hydrolysis inhibition by polysorbates and emulsion formulations

All tested polysorbates show inhibition of hydrolysis of the cefpodoxime proxetil. The best results in terms of inhibition were obtained with polysorbate 20. (Table 3)

All formulations have a significantly ($P < 0.001$) smaller cefpodoxime-proxetil hydrolysis rate compared with the aqueous control solution (Fig. 1). The cefpodoxime-proxetil hydrolysis rate was significantly ($P < 0.001$) lower with the two emulsion formulations (D1 and D2) when compared with the micellar solution. Moreover, the cefpodoxime proxetil hydrolysis rate in emulsion was significantly ($P < 0.001$) lower when polysorbate 20 was included in the formulation (D2). All formulations exhibited a protective effect against the cholinesterase attack in the rabbit duodenal washings. The protective effect of the formulation which can be defined as the ratio between hydrolysis rate found for the tested formulation and the hydrolysis rate found for the aqueous control solution was 38, 79 and 86% for micellar solution, D1 emulsion and D2 emulsion, respectively. The most protective formulation was the o/w emulsion incorporating polysorbate 20.

Table 3
Effect of polysorbates on the cefpodoxime proxetil hydrolysis rate (%) in rabbit duodenal washings

Polysorbate	% Prodrug hydrolysis versus control		
	I	II	III
20	10.0 ± 0.01	48.0 ± 0.02	100 ± 0.01
40	10.7 ± 0.01	58.3 ± 0.01	100 ± 0.01
80	18.3 ± 0.02	61.7 ± 0.05	100 ± 0.01

Polysorbate final concentration was (I) 100 mg/ml, (II)10 mg/ml, and (III) 1 mg/ml, respectively. Cefpodoxime proxetil hydrolysis rate (%) was determined from the release of free cefpodoxime in the incubation media compared with the control without polysorbates. The amount of protein in the reaction mixture was 5 μg and the final concentration of cefpodoxime proxetil was 100 μM. Results are mean ± S.D. of three observations.

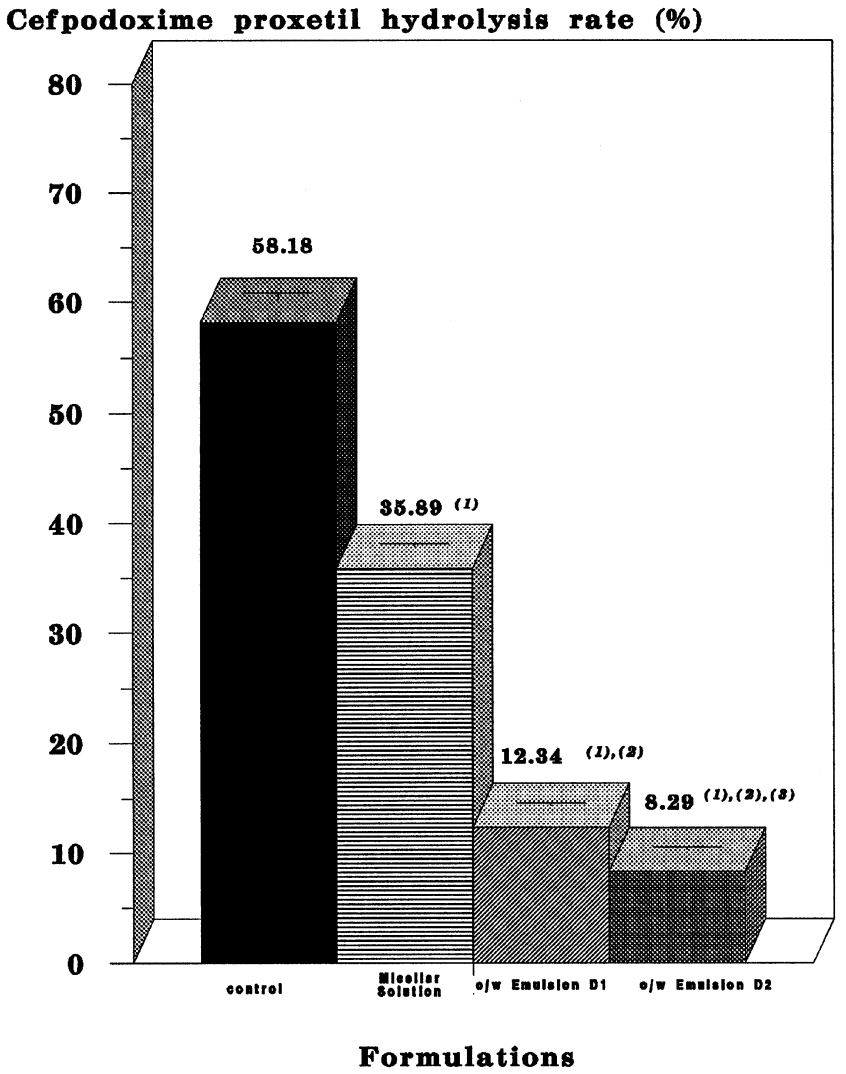

Fig. 1. Cefpodoxime-proxetil hydrolysis rate (%) was determined from the release of free cefpodoxime in the incubation medium related to the initial concentration of cefpodoxime proxetil. The amount of protein in the reaction mixture was 5 μg. The final concentration of cefpodoxime proxetil was 100 μM. Results are mean with error bar of six observations. (1) Significantly different ($P < 0.001$) from paired control; (2) significantly different ($P < 0.001$) from paired micellar solution; and (3) significantly different ($P < 0.001$) from paired o/w emulsion D1.

4. Discussion

4.1. Prodrug formulation and physical characterization

In order to protect prodrug from enzymatic hydrolysis, the drug has to be inside the oil phase. This can be supported by the fact that cholinesterases did not hydrolyze triglycerides (La Du and Snady, 1971). The objective was to include the prodrug into the oil phase and to ensure that the drug was not at the droplet interface. Somehow, the inclusion of the hydrophobic drugs in the oil phase poses specific problems related to the solubilization of the drug. Cefpodoxime proxetil was not solubilized in the two oil phases tested

(soybean oil and MCT). Thus, Cefpodoxime proxetil can be classified in class III (Washington, 1996) because this drug is poorly soluble both in water and in oil. Class III drugs can only be loaded into an emulsion by adsorbing to the droplet interface. Then, our objective was to solubilize the prodrug into the oil phase and not at the droplet interface in order to obtain a class II drug with regards to the Washington classification.

The first method used was cosolubilization. The commonly used method is to cosolubilize the drug in the alcoholic solution of the emulsifier before adding it to the other components of the homogenization mixture (Hansrani et al., 1983). This method was not appropriate for the cefpodoxime proxetil o/w emulsion formulation because the alcohol water solubility induces a migration of the cefpodoxime proxetil in aqueous phase and leads to its precipitation.

The other method we used was to find a hydrophobic solubilizer for cefpodoxime proxetil, which is insoluble in water, to prevent water migration of the prodrug, and which can be incorporated in the oil phase before the homogenization process. The hydrophobic solubilizer used, Imwitor® 742 (blends of mono- and diglycerides of caprylic and capric acids), ensures the incorporation of the drug in the innermost phase of the emulsion in order to successfully exploit the advantages of an emulsion dosage form. Moreover it is water-insoluble which prevents drug migration in water. Moreover, unsaturated fatty acids such as oleic acid and linoleic acid are able to promote intestinal absorption of poorly absorbed drugs (Muranushi et al., 1980).

Inclusion into oil phase of hydrophobic drugs is the main methodology used to take advantage of the lipidic formulation (Lovell et al., 1994; Igarashi et al., 1996; Mizushimata, 1996; Ohmukai, 1996; Takenaga, 1996; Yokoyama and Watanabe, 1996).

The emulsions tested were submicronized for two reasons. First to optimize the stability (Singh and Ravin, 1986; Santos Magalhaes et al., 1991). Second, because absorption of the emulsion in the gastrointestinal tract after oral administration was correlated to the droplet size of the formulation. The smaller droplet size of the emulsion causes a greater absorption (Toguchi et al., 1990). The emulsion formulations tested exhibit low droplet size and high negative ζ-potential. These characteristics were predictive factors for good stability.

A phase volume ratio at 20% was a trade-off to increase the amount of drug which could be incorporated in the oil phase of the emulsion and to increase the mean droplet size. A sharp and parallel increase in mean droplet size value and viscosity was observed in emulsions containing 30% or more oil phase (Levy and Benita, 1989). Then, in order to increase the amount of liposoluble drug which can be incorporated, and to obtain a satisfactory droplet size, we used a 20% oil phase.

Mineral oils were excluded because they are not absorbable by intestinal tract (Mulley, 1974). Two oil phases were tested, soybean oil and MCT of caprylic and capric acids (Labrafac lipophile® WL 1349). Vegetable oils are commonly used for emulsion formulations. Soybean oil was already used to formulate an oral o/w clofibride emulsion (Santos Magalhaes et al., 1991). Moreover, because of its innocuity, soybean oil is commonly used for parenteral emulsion formulation (Hansrani et al., 1983). In the case of cefpodoxime-proxetil emulsion, soybean oil was not appropriate for drug solubilization even when mixed with the hydrophobic solubilizer. Solubilization was obtained with mixed MCT, blends of mono- and diglycerides (Imwitor®742). The ratio MCT/mono- and diglycerides required for emulsification was 60/40 (w/w).

Moreover, MCTs were also investigated because they were found to enhance absorption enterally, rectally and orally of poorly absorbed drugs (Palin et al., 1986; Beskid et al., 1988; Constantinides et al., 1996; Sekine et al., 1985a,b,c; Yamahira et al., 1979, 1980).

Emulsions are intrinsically thermodynamically unstable and require stabilization with emulsifying agents. Parenteral submicron emulsions are stabilized with an emulsifier which is lecithin, at 1.2% (Hansrani et al., 1983). On this basis we used soybean lecithins: Lipoïd S40 and Lipoïd S75. In regard to the ζ-potential, the emulsion carried out with the Lipoïd S40 gives the best value (high negative potential). This can be re-

lated to the presence of more phospholipids with negative charge in S40 than in S75 lecithin.

The ζ-potential is known to be a predictive factor for the stability of the emulsion (Washington, 1996) and oriented our choice for the Lipoïd® S40 formulations.

In our hydrolysis assay we found a direct protective effect of polysorbates on cefpodoxime hydrolysis in rabbit intestinal lumen, the best one being observed with polysorbate 20. In order to improve the action of polysorbate itself, one emulsion was formulated with the couple lecithin–polysorbate and the other with only lecithin. The effect of polysorbate 20 itself is confirmed by our results. The emulsion with polysorbate 20 was statistically more protective than the emulsion without polysorbate 20. In terms of characteristics, the two emulsions were comparable.

4.2. Protective effect of the emulsions on cefpodoxime-proxetil hydrolysis

The hydrolysis assay carried out in rabbit seems to confirm a protective effect of the emulsions. The emulsion formulations were efficient in decreasing the in vitro cefpodoxime-proxetil hydrolysis rate as compared with the control solution. The inhibition rate was significantly higher when polysorbate 20 was included in the formulation. The role of inclusion of prodrug in triglyceride oil phase is evident by the significantly lower decrease in hydrolysis rate with the micellar solution. Micellar solution exhibits some protective effect which can be correlated with the inclusion of the prodrug into micelles and with the cholinesterase inhibitory intrinsic effect of polysorbate itself. Thus, the prodrug inclusion into the oil phase is significantly more protective than the inclusion into micelles.

Oil-in-water emulsions are already recognized to be very interesting formulations for oral administration of poorly water soluble drugs in terms of bioavailability (Wagner et al., 1966; Carrigan and Bates, 1973; Bates and Carrigan, 1975; Bates and Sequeira, 1975) because of the enhancement of the intestinal absorption (Palin et al., 1986; Drewe et al., 1992; Kararli et al., 1992; Constantinides et al., 1994; Constantinides, 1995) and can therefore enhance activity (Sasaki et al., 1984). The enzymatic protective effect of the formulation as an o/w emulsion could be a new factor to be taken into consideration in prodrugs investigation of the formulation.

In conclusion, cefpodoxime proxetil can be protected from the cholinesterase action by incorporation into the oil phase of a submicron o/w emulsion. Thus, emulsion formulations can both solubilize the prodrug and protect it against hydrolysis. This work also emphasizes the need to identify a suitable lipophilic solvent of the prodrug in order to prevent water migration and subsequent drug precipitation. It will be of interest to investigate this formulation in vivo to confirm if it enhances the oral bioavailability of the drug.

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