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Effects of hydroxypropyl-β-cyclodextrin on the chemical stability and the aqueous solubility of thalidomide enantiomers

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The aim of this work was to study the effect of hydroxypropyl-β-cyclodextrin on the solubility and stability of thalidomide enantiomers in aqueous solutions for clinical oral administration to be used in HIV-infected children. For this reason racemic thalidomide was added to solutions containing different concentrations of hydroxypropyl-β-cyclodextrin. True complexes were obtained by using hydroxypropyl-β-cyclodextrin and the solubility of both thalidomide enantiomers was increased directly depending on the amount of hydroxylpropyl-β-cyclodextrin in the medium although no enantioselective differences were observed at 37 °C. The chemical stability of thalidomide enantiomers is clearly improved by hydroxypropyl-β-cyclodextrin. No enantioselective degradation of thalidomide was observed in sodium chloride solution (0.9%) samples stored at 6 °C for nine days when hydroxypropyl-β-cyclodextrin was employed as excipient. Therefore a thalidomide solution suitable for oral administration can be prepared by adding hydroxypropyl-β-cyclodextrin at 10% (w/v).

1. Introduction

Thalidomide was introduced as a sedative in 1957 and in 1960 was marketed in 20 countries. It was subsequently withdrawn chiefly because it caused foetal malformations. Nowadays, there is a renewed interest in thalidomide (Keravich et al. 1999) and in its anti-inflammatory and modulator effect on erythema nodosum leprosum and other skin and mucous membrane diseases (Höglund et al. 1998).

Several reports have described the use of thalidomide to treat aphthous ulcers and Kaposi's sarcoma in HIV-infected children (Jibaly et al. 1998; Soler et al. 1996a, b). Thalidomide may also be useful in the management of specific complications of pediatric HIV infection, particularly growth failure.

Thalidomide has a chiral center in its structure and it is synthesized as the racemate. One enantiomer is effective, the other one is teratogenic and causes the teratogenic defects. The enantiomers can interconvert *in vivo*, if a human is given pure (*R*)-thalidomide or (*S*)-thalidomide, both isomers can be found in the serum, therefore, administering only one enantiomer will not prevent the teratogenic effect in humans. Cyclodextrins are excipients which can promote complexation of several poorly soluble drugs, increasing their solubility, bioavailability and improving the chemical stability of labile drugs (Frömming et al. 1994). The oral hydroxypropyl-β-cyclodextrin solution of itraconazole for use in children (Groll et al. 2002) improves the solubility and thereby the gastric absorption of the drug and provides high topical drug concentrations in the oral cavity and the esophagus.

Cyclodextrins are also optical active substances that may interact with chiral drugs and therefore they are useful and currently employed for chiral assay and resolution of racemic drugs (Scheiderman et al. 2000).

Regarding to pharmaceutical formulations, solubility and stability can be probably considered as the two most studied *in vitro* characteristics of inclusion complexes. Hydroxypropyl-β-cyclodextrin-thalidomide inclusion complexes have been described before (Krenn et al. 1992). Although these reported studies were done with no enantioselective assay methods their results may be useful as background of this work.

An enantioselective assay of thalidomide enantiomers by HPLC has been validated and previously published (Álvarez et al. 2000).

The aim of this work was to study the effect of thalidomide enantiomers with hydroxypropyl-β-cyclodextrin on the solubility and stability properties of an oral formulation, in order to be used in HIV-infected children. Moreover, the possible enantioselective effect of the interaction between hydroxypropyl-β-cyclodextrin and thalidomide enantiomers was studied.

The solubility diagrams of hydroxypropyl-β-cyclodextrin with thalidomide were determined in deionized water at 37 °C and the chemical stability study was performed at different temperatures: 37 ± 2 °C, 25 ± 2 °C and 6 ± 2 °C for at least two weeks.

The hydroxypropyl-β-cyclodextrin-thalidomide inclusion complex is a potentially useful alternative to conventional therapies in immunocompromised pediatric patients. However, little is known about the safety and pharmacokinetic

properties of this complex for the treatment of HIV-infected population.

2. Investigations, results and discussion

Figure 1 shows the solubility of thalidomide enantiomers in deionized water. A linear relationship between hydroxypropyl-β-cyclodextrin concentration and drug solubility was observed ($p < 0.05$). The effect of enantiomerism on drug solubility was studied by statistical comparison of the regression lines obtained with each enantiomer. Under our experimental conditions no significant differences were observed either in the slopes or in the intercept of the different regression lines ($p > 0.05$).

Figure 2 shows the stability of thalidomide enantiomers in deionized after two weeks at 37 °C, simulating accelerated conditions. Regarding to thalidomide degradation, a previous work (Krenn et al. 1992) has reported that hydroxypropyl-β-cyclodextrin improved chemical stability. Thalidomide achieved a fast degradation and the storage of the liquid samples was performed at 37 °C for two weeks. In our studies a protective effect of hydroxypropyl-β-cyclodextrin was clearly observed. No significant enantioselective ($p > 0.05$) differences were observed between *R*-thalidomide and *S*-thalidomide results. In order to avoid the use of high amounts of hydroxypropyl-β-cyclodextrin for oral administration a 10% proportion was chosen for further stability experiments in a sodium chloride solution at 0.9% which is an isosmothic vehicle suitable for oral administration in pediatry.

Due to the absence of enantioselective effects on solubility or stability of thalidomide several experiments were performed with a non enantioselective HPLC assay which is also more appropriate for the analysis of the degradation products of thalidomide. Figure 3 shows that good stability results are achieved when samples are stored in the refrigerator at 6 ± 2 °C. The presence of hydroxypropyl-β-cyclodextrin improved thalidomide stability and no degradation was observed in samples stored in the refrigerator for nine days. Good stability results were also reported by Eriksson et al. (2000). Nevertheless, samples prepared without the hydroxypropyl-β-cyclodextrin in our experimental conditions had a significant degradation of approximately 15% after nine days of storage at low temperature.

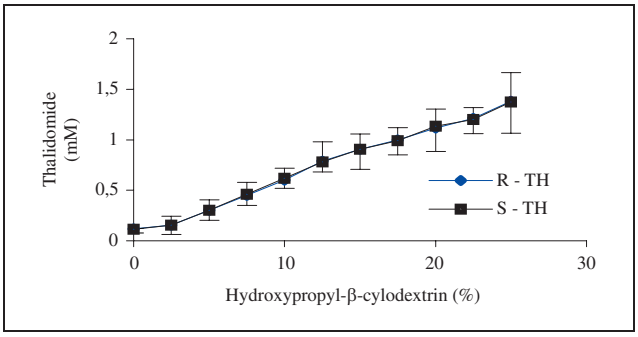

Fig. 1: Phase solubility diagram of R-thalidomide and S-thalidomide in deionized water. Error bars represent standard deviations

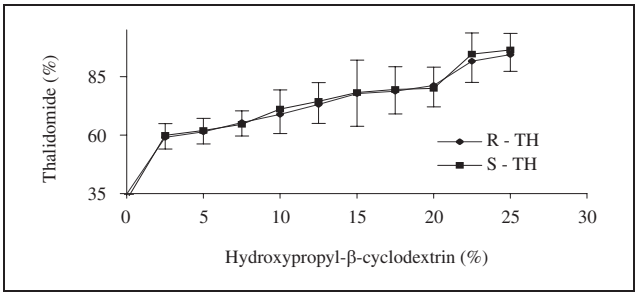

Fig. 2: Thalidomide remaining in the chemical stability study after 12 days of storage in deionized water. Error bars represent standard deviations

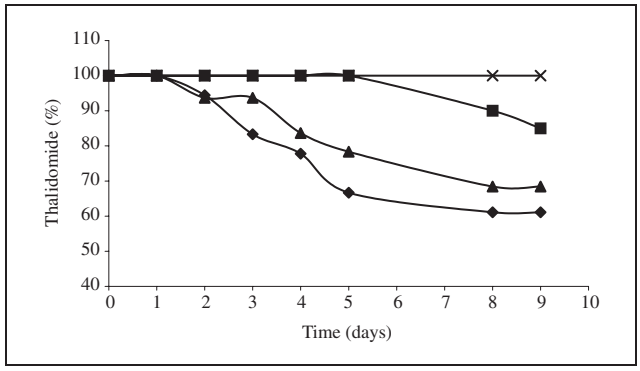

Fig. 3: Thalidomide remaining in the chemical stability study during 9 days at 6 ± 2 °C and 25 ± 2. Keycode: ◆ (without cyclodextrins at 25 ± 2 °C), ■ (without cyclodextrins at 6 ± 2 °C), ▲ (with cyclodextrins at 25 ± 2 °C), × (with cyclodextrins at 6 ± 2 °C)

Meanwhile, for the samples storage at 25 °C (long-term stability conditions) poor stability results were obtained for both formulations, either with and without hydroxypropyl-β-cyclodextrin (see Fig. 3). Degradation at 25 ± 2 °C is about approximately 40% or 30% depending on the absence or inclusion of hydroxypropyl-β-cyclodextrin. These poor stability characteristics of thalidomide samples obtained at temperatures of 25 ± 2 °C (Fig. 3) and 37 ± 2 °C (Fig. 2) are in good agreement with those obtained by Krenn et al. (1992). From this results it is clear that thalidomide stability in aqueous solution is improved when hydroxypropyl-β-cyclodextrin is used as excipient.

The results obtained for the solubility and stability of thalidomide suggest the formation of inclusion complexes of thalidomide in the hydrophobic cavity of the hydroxypropyl-β-cyclodextrin. The formation of the complexes is directly related to the proportion of the hydroxypropyl-β-cyclodextrin added to the deionized water medium at 37 °C. The stability of thalidomide was also improved by the complexation process. The improvement in the stability properties of thalidomide liquid formulations is related to the protector characteristics of hydroxypropyl-β-cyclodextrin.

It can be concluded that it is possible to obtain thalidomide solutions suitable for oral administration in HIV-infected children with good stability characteristics by using hydroxypropyl-β-cyclodextrin as excipient from the pharmaceutical development point of view.

The safety, pharmacokinetics, and pharmacodynamics of this proposed solution in HIV-infected children should be studied.

3. Experimental

3.1. Chemicals

Thalidomide was a gift from Grünenthal (Aachen, Germany) and hydroxypropyl-β-cyclodextrin was obtained from Cerestar (Barcelona, Spain). All other chemicals were of analytical grade.

3.2. Complexes in liquid medium

3.2.1. Phase solubility studies

The studies were elaborated according to Higuchi et al. (1965). Different concentrations of hydroxypropyl-β-cyclodextrin (0, 7, 18, 36, 54, 71, 89, 109, 125, 143, 161 and 179 mM) were employed to prepare solutions in deionized water and an excess of racemic thalidomide was added. The tubes were closely fitted, sonicated for 15 min and then shaken at approximately 100 r.p.m. in a thermostatted bath at 37 °C with an horizontal shaking system (Clifton Shaking Bath, England). Each sample was prepared by triplicate. After equilibrium was attained, the supernatant was filtered through membrane filter (0.45 μm) and assayed by HPLC.

3.2.2. Chemical stability studies

The effect of the use of different hydroxypropyl-β-cyclodextrin concentrations (0–179 mM) on the stability of the thalidomide enantiomers was studied in deionized water. Samples were prepared in the same way as described previously. The samples were filtered and stored for a minimum time of two weeks at controlled temperature of 37 ± 2 °C, simulating accelerated stability conditions.
Several samples were also prepared both in presence and absence of hydroxypropyl-β-cyclodextrin 10% (w/v) with racemic thalidomide (0.03%) and they were stored at long-term conditions of 25 ± 2 °C and at 6 ± 2 °C (samples intended to be storaged in a refrigerator) in sodium chloride solution at 0.9%. Samples were analyzed for nine days.

3.2.3. Chromatographic assay

HPLC methods were used to assay either thalidomide enantiomers or racemic thalidomide. A modular HPLC (Gilson, USA) equipped with a 306 piston pump, 116 UV-VIS detector, SP-4270 integrator and 231 XL sampling injector was employed. In order to isolate and quantify thalidomide enantiomers a chiral AGP column (ChromTech AB, Sweden, 100 mm × 4.0 mm ID) was used. The mobile phase was a mixture of 30 mM ammonium acetate buffer at pH 7.0 containing 0.3% of tetrahydrofuran at a flow rate of 0.9 ml/min.
The column for analyzing racemic thalidomide was a C_{18} Hypersil (250 × 4.6 mm, 5 μ, BDS) supplied by Thermo Hypersil Ltd (Cheshire, England). The mobile phase consisted of a NaH_2PO_4 buffer (50 mM, pH 3.5) and acetonitrile (4 : 1) at a flow rate of 1 ml/min.
For both HPLC methods the injection volume was 20 μl and the wavelength of detection was fixed at 230 nm.

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