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SYNTHESIS AND THERMAL STUDY OF THE PRODRUG OF OXAMNIQUINE

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Oxamniquine polymeric prodrug with potential antischistosomal activity was prepared using dextran T-70 as a carrier, which was analysed by ^{1}H NMR, ^{13}C NMR and IR spectroscopy. The formation of the oxamniquine salt was confirmed by thermogravimetric analysis (TG) and differential scanning calorimetry (DSC) which showed a different thermal behaviour when compared to the physical mixture.

Keywords: *DSC, oxamniquine, prodrug, TG*

Introduction

Schistosomiasis, an important disease in Brazil, is caused by a trematode of the genus *Schistosoma*. It is a very debilitating disease with a high socioeconomic importance and its distribution goes around 52 countries of South America, Caribbean, Africa and Oriental area of Mediterran [1–4]. Only two drugs are available in the Brazilian market: oxamniquine and praziquantel. *S. mansoni* is very susceptible to oxamniquine (OXA), but this drug is used as second drug of choice, due to its side effects, mainly those associated to CNS [3, 5]. These effects, in association with the high incidence of the disease in our country and the risk of the development of resistance, increase the importance of the development of new schistosomicides agents with more selectivity and lower toxicity [6–8]. Prodrug synthesis is one of the most promising methods of molecular modification in order to obtain new drugs. The use of a polymeric molecule as a carrier can provide or delay the time of action and can improve the bioavailability of the drug which is linked [9, 10]. In this context, the aim of this work is to synthesize an oxamniquine polymeric prodrug, using dextran T-70 as a carrier, with this, we hope to improve the oxamniquine action and minimize its side effects. The preliminary test of biological activity of this compound was carried out in the Fundação Oswaldo Cruz – BA and presented similar activity when compared to the commercial oxamniquine (Mansil®).

Experimental

Materials

Oxamniquine hydrochloride

A mixture of oxamniquine (1.0 g) and anhydrous dimethyl sulfoxide (100.0 mL) was bubbling on gaseous hydrochloride acid over a period of 10 min.

Carboxymethyl dextran sodium salt (CMD)

Dextran (T-70; 5.0 g) was dissolved in 11.5 M NaOH aqueous solution and chloroacetic acid (10.0 g) was added after. The mixture was stirred at 60–70°C for 1 h. Then, the mixture was transferred to a dialysis tube (12.000 molecular mass cut-off) and dialyzed against distilled water thoroughly. The dialysate was freeze-dried. ^{1}H NMR ($CDCl_3$) δ: 4.87 (d, H-1), 3.46 (dd, H-2), 3.61 (t, H-3), 3.41 (t, H-4), 3.80 (ddd, H-5), 3.88 (dd, H-6a), 3.65 (dd, H-6b). ^{13}C NMR ($CDCl_3$) δ: 96.9 (C-1), 70.6 (C-2), 72.6 (C-3), 68.8 (C-4), 69.3 (C-5), 64.6 (C-6), 72.3 (CH_2-7), 177.6 (C=0).

Prodrug dextran-methylcarboxylate of oxamniquine

The oxamniquine hydrochloride was added to carboxymethyl dextran sodium salt (2.0 g) dissolved in anhydrous dimethyl sulfoxide (100.0 mL) and was stirred at room temperature for 24 h. Then, the mixture was transferred to a dialysis tube (12.000 molecular mass cut-off) and dialyzed against distilled water thoroughly. The dialysate was freeze-dried.

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The determination of the replacement rate was performed by spectrophotometry at UV range, in a HITACHI Spectrophotometer model U-2001. In order to obtain the calibration curve were aqueous solutions of oxamniquine used in concentrations ranging from 2.5 to 50 μg mL^{-1}. To calculate this concentration of oxamniquine on the CMD was used regression analyses method that gave the slope and interception as:

$$Y=0.056029X+0.029079$$

where Y is the absorbance in 250 nm and X is concentration of oxamniquine.

The correlation coefficient was 0.9996. The replacement rate of dextran was 20.9%.

The physical mixture (oxamniquine and CMD)

The mixture was prepared in the same proportion that was used with the prodrug dextran-methylcarboxylate of oxamniquine.

Methods

Instrumental methods

TG curves were obtained with the Shimadzu model TG-50, on nitrogen atmosphere at a flow rate of 25.0 mL min^{-1}, at a heating rate of 10.0°C min^{-1}, up to 600.0°C. The sample mass was 6.400–8.300 mg.

DSC curves were obtained with the Shimadzu model DSC-50 calorimeter, on nitrogen atmosphere at a flow rate of 25.0 mL min^{-1}, at a heating rate of 10.0°C min^{-1}, up to 600.0°C. The sample mass was 7.600–8.029 mg.

All NMR spectra were recorded on a Brüker DRX400 (9.4 Tesla) spectrometer equipped with a 5 mm inverse probe, using $CDCl_3$ solutions, TMS as an internal standard and a 298 K constant temperature. Chemical shifts are reported in δ (ppm) and coupling constants in Hz.

The infrared absorption data were obtained in the range 4000–400 cm^{-1} in KBr pellets using a Spectrophotometer Shimadzu FTIR-8300, at room temperature.

Biological test

The biological evaluation was performed on male 'Swiss' mice, weighing 38–47 g, infected subcutaneously with 100 *Schistosoma mansoni* Feira de Santana cepa cercareae. For confirmation of infection, fecal analysis by the direct egg method was performed 70 days after inoculation. The mice were distributed into two groups of 7 animals:

- Mansil group – 7 infected mice which received commercial Mansil® 50 mg mL^{-1} syrup.
- Prodrug dextran-methylcarboxylate of oxamniquine group – 7 infected mice treated with prodrug dextran-methylcarboxylate of oxamniquine in water.

The prodrug was suspended in water, administered per os, in single dose, by gastric sond, 100 mg kg^{-1} mass, representing the ED_{99} as is often used in biological tests. Each animal was sacrificed 7 days after treatment. The adult worms were quantified by a method of perfusion of the mesenteric and portal veins. From each mouse two thin gut fragments (about 1 cm) were removed for quantification of the number of eggs and verification of stages of development-quantitative oogram method [11].

The experimental protocol were conducted according to ethics principles of the Brazilian College of Animals' Experimentation – (COBEA).

The quantitative oogram showed oviposition suppression, concluding that the prodrug presented equivalent results comparing to the group that received the standard treatment (Mansil®, Pfizer).

Using the perfusion technique, the search of adult worms also confirmed the pharmacological activity of the prodrug dextran-methylcarboxylate of oxamniquine.

The results show that the biological activity resembles the oxamniquine. Toxicological studies will have to be performed to verify the advantages of the use of the prodrug when compared to oxamniquine.

Results and discussion

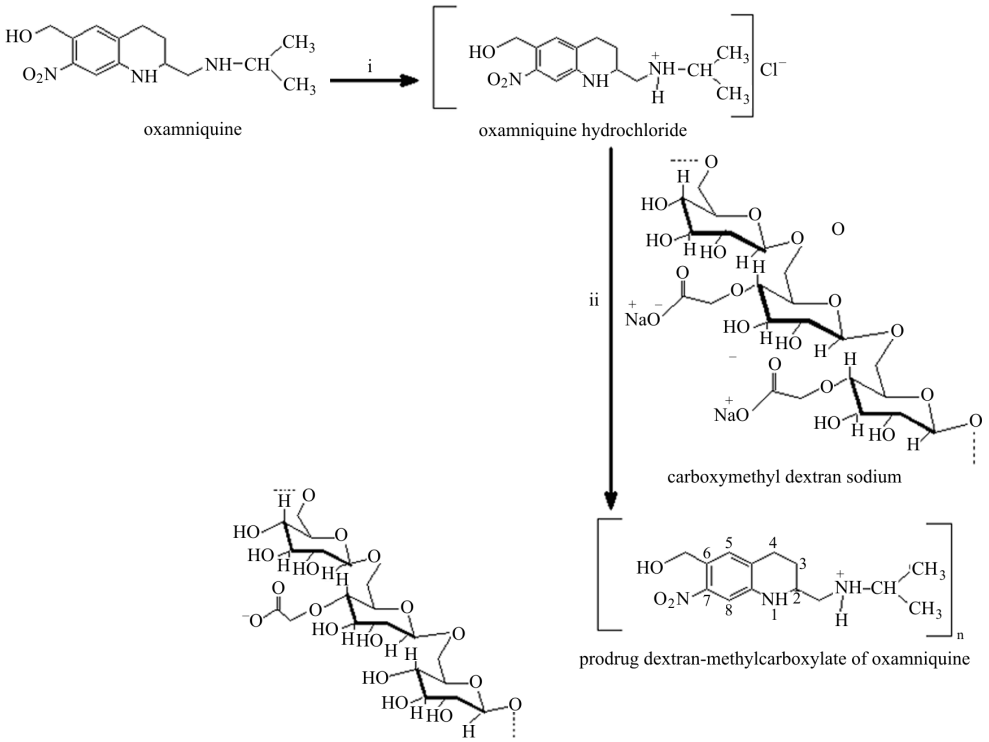
In order to prepare the synthesis of the prodrug dextran-methylcarboxylate of oxamniquine (Scheme 1), the first step was to prepare oxamniquine hydrochloride and the carboxymethyl dextran sodium salt (CMD). Thus, oxamniquine hydrochloride was added to carboxymethyl dextran sodium salt, which was dissolved an anhydrous dimethyl sulfoxide medium.

Thermoanalytic techniques have been applied in the identification and characterization of drugs and medicines [5, 12–14]. The most widely used techniques are thermogravimetry (TG), differential thermal analysis (DTA) and differential scanning calorimetry (DSC). Through suchlike techniques it is possible to compare if the prodrug is a salt or just a physical mixture of the compounds [15–17].

The prodrug of oxamniquine was identified by DSC and TG curves, once the thermal behavior of the original compounds is known (Figs 1 and 2).

Thermal behavior of oxamniquine

The curve a on the Fig. 1 presents an endothermic peak at 149.2°C referred to the fusion of the compound and two exothermic peaks at 276.3°C and a small one at 409.5°C attributed to the thermal decom-



Scheme 1 Synthesis of the prodrug dextran-methylcarboxylate of oxamniquine

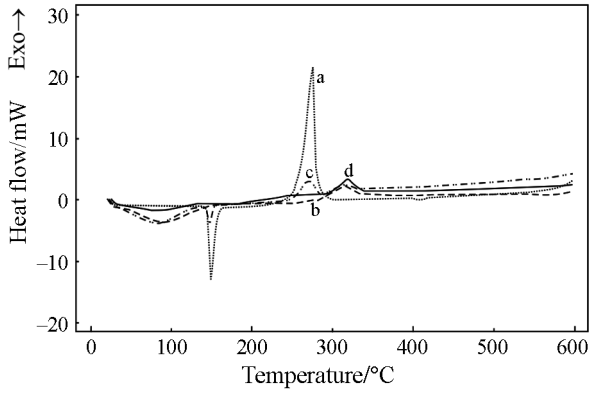


Fig. 1 DSC curve obtained on nitrogen atmosphere (25 mL min^{-1}), sample mass around 8 mg, alumina crucible and heating rate of 10°C min^{-1}; a – oxamniquine, b – CMD, c – physical mixture, d – prodrug dextran-methylcarboxylate of oxamniquine

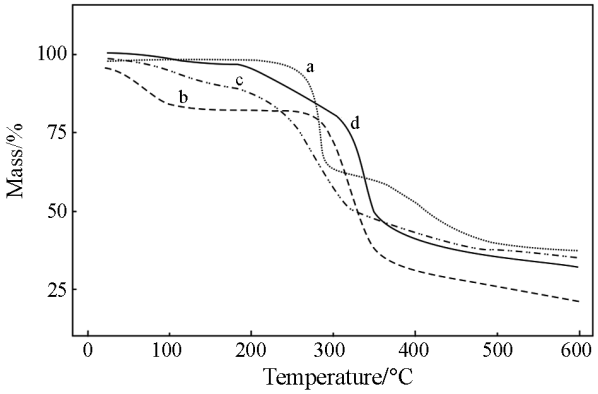


Fig. 2 TG curve obtained on nitrogen atmosphere (25 mL min^{-1}), sample mass around 7 mg, platinum crucible and heating rate of 10°C min^{-1}; a – oxamniquine, b – CMD, c – physical mixture, d – prodrug dextran-methylcarboxylate of oxamniquine

position of oxamniquine, which is related in Fig. 2 (curve a) in which we observe two mass losses between 200 and 350°C (39.8%) and between 350 and 600°C (20.5%), respectively.

Thermal behavior of CMD

In the DSC curve b, Fig. 1, we have observed an endothermic peak at 90.5°C and an exothermic peak at 315.9°C with two mass losses in the TG curve b, Fig. 2, which was determined by two mass losses between 40–150°C (13.9%) and between 200–430°C (55.4%), those peaks are attributed to the dehydration reaction and to the thermal decomposition of the compound, respectively.

Thermal behavior of the physical mixture (oxamniquine and CMD)

The same peaks of each individual reactants, e.g., an endothermic peak at 147.7°C due to the fusion of oxamniquine, an endothermic peak due to the dehy-

dration of CMD and two exothermic peaks, at 272.2 and 313.0°C, respectively due to the thermal decomposition of the original compounds, curve c on Fig. 1 were observed.

The curve c (Fig. 2) presents three mass losses steps referred to the dehydration between 40 and 150.0°C (9.8%) and two others attributed to the thermal decomposition that occurs between 150 and 290°C (15.2%) and between 290 and 450.0°C (40.1%), respectively.

The synthesized prodrug dextran-methylcarboxylate of oxamniquine, shows different thermal behavior when compared to the physical mixture.

DSC curve d, Fig. 1, presents an endothermic peak around 90°C and two exothermic peaks at 180 and 300°C, which are described as the dehydration and the thermal decomposition of only one compound, respectively. The absence of an endothermic peak of the fusion of oxamniquine is an indicative that this substance have reacted with CMD.

TG curve d, Fig. 2, shows three mass losses steps, between 40 and 160°C (4%), 160 and 260°C 10.5%) and finally 260 and 430°C (48.8%) corresponding to the mentioned peaks in DSC curve.

This data suggests that the synthetized compound is probably the oxamniquine-CMD salt.

NMR spectra

The CMD identification was obtained from ^{1}H, gCOSY, gHSQC and gHMBC spectra. From the spectra analysis we can attribute all de ^{1}H and ^{13}C chemical shifts as well as the coupling constants. The presence of carboxyl groups were confirmed by the ^{1}H δ 3.95–4.05 and ^{13}C δ 177.6 chemical shift using gHSQC correlations. All the ^{13}C chemical shifts were observed by gHSQC and gHMBC spectra because the sample amount does not allow direct detection in the ^{13}C channel. The ^{1}H NMR analysis of prodrug demonstrated that a possible esterification reaction involved CH_2OH group from oxamniquine and carboxylate from carboxymethyl dextran didn´t occur, because we could not see any chemical shifts displacement for the oxamniquine methylene in δ 4.6 and 98 ppm in ^{1}H and ^{13}C spectra respectively when we compare with pure oxamniquine spectra data. This data was corroborated by the prodrug thermical study.

The infrared absorption data were obtained in the range 4000–400 cm^{-1} in KBr pellets using a spectrophotometer Shimadzu FTIR-8300, at room temperature.

IR spectra

The prodrug dextran-methylcarboxylate of oxamniquine was also analysed by infrared spectroscopy (FTIR). Figure 3 shows the bands that contribute to the characterization of the compounds. Analysing the oxamniquine spectrum (Fig. 3a), characteristic bands are observed in 3328 cm^{-1} (ν_{N-H} and ν_{O-H} free), 2964 cm^{-1} (ν_{C-H} aromatic), 2883–2846 cm^{-1} (ν_{C-H}, ν_{CH_2} and ν_{CH_3} symmetric and asymmetric), 1620 cm^{-1} ($\nu_{C=C}$ aromatic), 1517 cm^{-1} (ν_{NO_2}), 1332 cm^{-1} (ν_{NO_2} symmetric), 1288 cm^{-1} (ν_{C-O}) and 1051 cm^{-1} (ν_{O-H} primary alcohol and ν_{C-N}). Figure 3b shows the absorption bands of CMD: 3346 cm^{-1} (ν_{O-H}), 2925 cm^{-1} (ν_{C-H}, ν_{CH_3} symmetric), 1649 cm^{-1} ($\nu_{C=O}$ asymmetric), 1419 cm^{-1} ($\nu_{C=O}$ symmetric), 1012 cm^{-1} (ν_{C-O}). Figure 3c shows the absorption bands of the physical mixture (oxamniquine and CMD), where it is possible to observe characteristics from oxamniquine and CMD: in 3446 cm^{-1} (ν_{O-H} overlapped with ν_{N-H}), 1620 cm^{-1} ($\nu_{C=C}$ aromatic), 1517 cm^{-1} ($\nu_{C=C}$ aromatic and ν_{NO_2}), 1328 cm^{-1} (ν_{NO_2} symmetric), 1051 cm^{-1} (ν_{C-O}). In the spectrum of prodrug dextran-methylcarboxylate of oxamniquine (Fig. 3d), some bands can be shown, like in 3408 cm^{-1} (ν_{N-H} and ν_{O-H}), 1620 cm^{-1} ($\nu_{C=C}$ aromatic), 1517 cm^{-1} ($\nu_{C=C}$ aromatic and ν_{NO_2}) and 1008 cm^{-1} (ν_{C-O}).

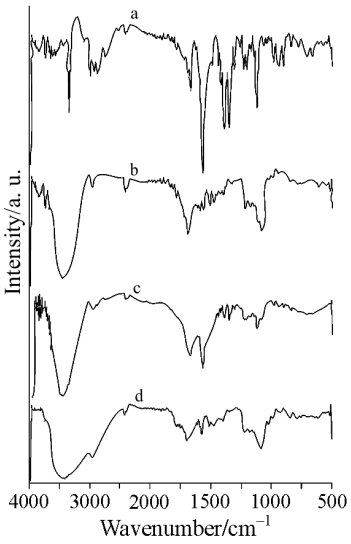


Fig. 3 IR spectra of a – oxamniquine, b – CMD, c – physical mixture, d – prodrug dextran-methylcarboxylate of oxamniquine

Conclusions

The analysis of ^{1}H NMR was insufficient for the structural confirmation, of the drug because it was only observed the presence of the original compounds with chemical shifts displecement. The thermal study has demonstrated the presence of a new compound and not the mixture of the original ones. The data shows the importance of the use of TG and DSC in the structural identification as a complement to the already used methods.

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